January 2018

Carbazole Based Multifunctional Dopamine Agonists And Related Molecules As Potential Symptomatic And Disease Modifying Therapeutic Agents For Parkinson's Disease

Asma S.mohamed Elmabruk
Wayne State University, Fc9851@wayne.edu

CARBAZOLE BASED MULTIFUNCTIONAL DOPAMINE AGONISTS AND RELATED MOLECULES AS POTENTIAL SYMPTOMATIC AND DISEASE MODIFYING THERAPEUTIC AGENTS FOR PARKINSON'S DISEASE

by

ASMA ELMABRUK

DISSERTATION

Submitted to the Graduate School

of Wayne State University,

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

2018

MAJOR: PHARMACEUTICAL SCIENCES

Approved By:

Advisor Date

DEDICATION

This work is dedicated to the spirit of my father, to my mom, my husband, my siblings, my children, and my friends who have always been there for me.

ACKNOWLEDGMENTS

It is such a great honor and pleasure for me to have the privilege to work with all the lab crews in order to accomplish this mission. I am very delighted to get the opportunity to convey my gratitude to all the members who assisted with this research.

I would like to show my deep appreciation to my advisor Dr. Aloke Dutta. I will not be able to make it this far if it was not for Dr.Dutta's advice, wisdom, and guidance. My journey with him was full of fun and excitement. In addition, I got the opportunity to imbibe from this wonderful professor golden strategies and techniques of how to run a research study in the future. Dr. Dutta is an inspiration and a role model of a great scholar.

I also want to express my profound gratefulness to my committee members Dr. David Pitts, Zhihui Qin, and Ladisalau Kovari for their supervision and crucial support, which made them a vital part in my desertification. Furthermore I want to thank our department chair George Corcoran and helper Dr.Randall Commissaris. Dr. Pitts and Dr. Corcoran were there for me when my father passed. When I was grieving over my father, they gave me moral support that made me stronger to overcome any sort of hardship that I faced during my journey.

I greatly acknowledge the benefits that I gained from their assistance of our present and past lab members Dr. Dan Luo, Tran Vo, Dr. Banibrata Das, Liping Xu, Dr. Bidyut Dinda, Pranay Ravipati , Dr. Deepthi Yedlapudi, Dr. Somava Santra Dr. Horrick Sharma, Dr. Seenuvasan Vedachalam, Dr. Chandrashekhar Voshavar , and Fahd Dholkwala.

I sincerely want to show my praise and appreciation towards, NINDS Grant (NS 047198, AKD) for providing financial support for this research, and the department of

pharmaceutical sciences at the Wayne State University for giving me the opportunity to pursue my graduate study here.

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CHAPTER 1

INTRODUCTION

1.1. Parkinson's Disease.

Parkinson's disease (PD) is a chronic, progressive, neurodegenerative disorder that affects 2-3% of the population with onset age more than 65 years old (J. Z Igmond and E. B Urke 2018; Arora and Fletcher 2013). The neurological hallmarks of PD are the depletion of dopamine in the basal ganglia with an extensive loss of dopaminergic neurons in the midbrain of the substantia nigra pars compacta (SNpc) accompanied by accumulation of an intraneuronal presynaptic filamentous protein called Lewy bodies (LBs). LBs may be responsible for triggering the degeneration of the dopaminergic neuron (**Figure 1**) (Dauer and Przedborski 2003; Sulzer 2007). The loss of SNpc neurons found to be responsible for the main symptoms of PD. In the 1960 researchers discovered that the pathological features of PD appear when the patient losses 75% of neuromelanin, a dark colored pigment formed within the dopamine and noradrenaline neurons of the SNpc and the locus coeruleus in the human brain (Dauer and Przedborski 2003; Michel, Hirsch, and Hunot 2016).

PD was first described by the general physician James Parkinson in London in 1817 in his classic monograph *"An Essay on the shaking palsy"* (Dauer and Przedborski 2003; Hurwitz 2014). Early in the 19th century Charcot gave credit to James for his discovery and named the disease ''Maladie de Parkinson' (Jankovic 2007). Charcot also diagnosed another type of PD (slowing in movement) and distinguished from muscle weakness which Parkinson discovered earlier. In 1919 Parkinson's patients were recognized with losing cells in the substantia nigra. After this discovery, in1957; Carlson

and his colleagues in Sweden realized dopamine as a putative neurotransmitter. In 1960 Ehringer and Hornykiewicz noticed that patient with PD has a low level of dopamine in the striatum (Hurwitz 2014) (Goetz 2011).

There are several symptoms linked to the development of PD. These symptoms classified into motor and non-motor symptoms. There are four cardinal signs associated with the development and progression of PD. The first sign is rest tremor, involuntary movement, which considered as an early sign of the disorder and the most common warning for having PD. 30% of PD people do not have tremor as a first indicator and appear later (Goetz 2011). The second motor symptom of PD is akinesia without motion, bradykinesia or hypokinesia means slow motion and this happens at an initial stage of the disease (slowness and difficulty of movement). Rigidity is the third motor sign of PD, the muscle stiffness movement caused by increased muscle tone, and it may appear in any part of the body (Schapira 2009a). Rigidity may associate with pain, and at the later stage rigidity may affect the whole body and reduce the ability to move. The fourth common motor indicator of PD is the postural instability, the impaired balance and frequency of falls that may lead to secondary bone fracture (Poewe et al. 2017). Some patients do not show all the cardinal manifestations of PD; there may be only one or two symptoms. Other clinical features that are associated with PD include secondary motor symptoms such as hypomimia, dysphagia, micrographia, shuffling, dystonia, glabellar reflexes. The most common non-motor symptoms in PD include anxiety, depression, dementia and sleep disturbance (Jankovic 2007; Poewe et al. 2017). There are several medications to treat PD, the current therapy relieves only the symptoms (Tanzi 2005).

Studies showed that people with PD are more susceptible to depression (Jinling Liu 2013). Researchers also reported that depression is more prevalent in PD patients than in the general population (Cui et al. 2017). It is unclear if depression is an independent risk factor for PD or if it is a primary sign of the neurodegenerative disease (Cui et al. 2017). Researchers suggested that the onset of depression in the elderly people could be one of the risk factors in PD (Zhu, van Hilten, and Marinus 2016). Antidepressant medications also found to increase the chance of developing PD (Reference?). Although the pathogenesis of PD found to be multifactorial, the mechanism of the disease remains unknown (Moore et al. 2005).

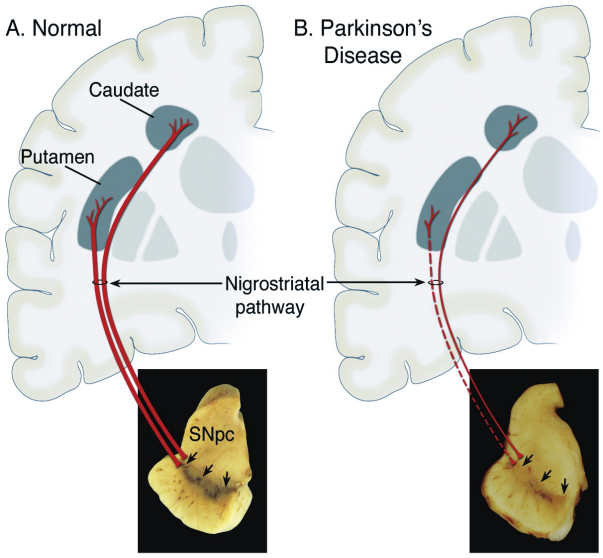


Figure 1. Schematic diagram showing the normal and the diseases nigrostriatal pathways (Sulzer 2007).

The selective vulnerability of the nigral neurons in the PD neurodegenerative process could be explained by the sensitivity of these neurons to a specific stressful such as high physiological levels of excitation and intracellular Ca^{+} loads, genetic background, preexisting conditions (e.g., diabetes), aging and predisposing factors; such as chronic

consequences of lesions, injuries from previous infections, and chronic consequences of stress and environmental toxins (**Figure 2**) (McKee et al. 2009) (Saxena and Caroni 2011).

Several stressful factors such as individual biases, local environmental factors, stress susceptibility, and sensitivity to misfolding-prone proteins cause specific neurodegenerative disease (NDDs). Neuronal connectivity and excitability may have a significant role in determining the intrinsic sensitivity to stress causing dysfunction and decease to the neurons. The loss of the neurons could be due to the accumulation of toxic protein species that could spread to other exposed cells (Arora and Fletcher 2013).

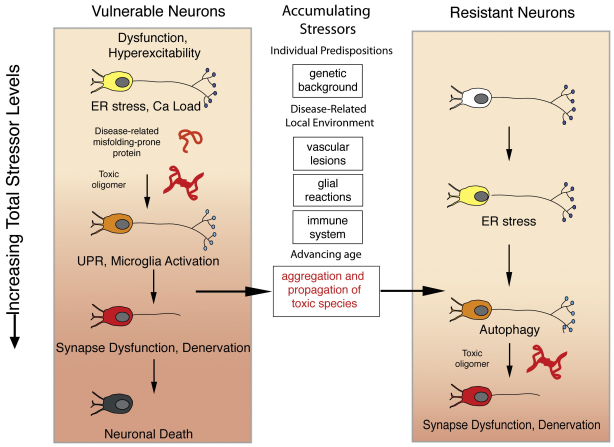


Figure 2: Schematic of How Gradually Increasing Stress in Affected and Selectively Vulnerable Neurons May Underlie the Etiology and Progression of NDDs (Saxena and Caroni 2011).

1.2. Prevalence of PD.

PD is the second most neurodegenerative disease in the worldwide after Alzheimer's disease (AD). The lifetime risk of the disorder is 2% (Schapira 2009a). PD is an age-related disease, it affects around 2% of the population over the age of 60 and 5% of people over age 85 making aging the most significant risk factor for PD. The failure of cellular compromised mechanisms and the accumulation of age-related somatic cells due

to aging contribute to dopamine neuron demise in the PD rather than the disease onset (Hindle 2010; Collier, Kanaan, and Kordower 2011). Minor cases of PD are due to genetic mutation (Zhang et al. 2018). Over 1 million people in the united states alone are affected by this disabling disorder (Schapira ; Schapira 2009a). The prevalence of PD by geographical area was seen only in patients ages 70 to 79 years old (Pringsheim et al. 2014). PD attacks 50% more men than women. The incidence of occurrence of PD in the world reported up to 190 per 100,000 people (Ratner and G. Feldman 2004). According to the American Parkinson's Disease Association, the incidence of PD ranges from 8.6-19 per 100,000 people (Benjamin C.L. Lai 2001). Approximately 50,000 new cases are diagnosed in the U.S. annually. The prevalence of undiagnosed PD cases is expected to be about 3-4 million people or 1.10%.

The socioeconomic factors can easily affect the prevalence of the disorders, both direct and indirect cost for the treatment of PD can influence the incidence, severity, and course of progression pose a significant burden on those who suffer from it (Céu Mateus 2013). The national economic burden of PD exceeds $14.4 billion in 2010. The indirect cost of loss of productivity estimated to be $6.3 billion (MD 2013). The frequency of PD is a predicted to double or triple according to the current demographic trends as the size of elderly population grows. Most cases of PD are sporadic, the familial instances estimated around 1-2 % (Fernandez-Espejo 2004). The cost related to the treatment of PD in the United States for individual patient each year is staggering. The cost of Medication for a single patient average $2,500 a year and the cost of Curative surgery can be up to 100,000 dollars per patient. Such projections give an incentive for the need of innovative treatment for PD.

1.3. Factors implicated in the pathogenesis of the disease:

Although age is the most substantial risk factor involved in the development and progression of PD, numerous factors found to be implicated and contribute to its pathogenesis. These factors could include but not limited to oxidative, and nitrative stress, protein aggregation, genetics, inflammation, and environmental factors (J. Z Igmond and E. B Urke 2018). Protein aggregation, mitochondrial dysfunction, and oxidative stress are considered the most common pathological risk factors (Samluk, Chroscicki, and Chacinska 2018). The process of the interrelated events of these factors causes the degeneration of neurons (Jenner and Olanow 2006). It was not determined if PD is a single disorder with common pathogenic causes or it is a group of disease with different pathological mechanisms (Jenner and Olanow 2006). PD is considered to be a sporadic disorder like other neurodegenerative diseases, but in rare cases, it can be linked to familial genetic factors. These genetic mutations contribute to the pathogenesis of the dopaminergic neuron death (Przedborski 2005).

Based on current data, it appears that the loss of dopaminergic neurons results from the convergence of several pathogenic factors (Przedborski 2005; Shoichet MS 2008). Although several studies have been done to understand the mechanism of neuronal death in PD, several critical questions remain unanswered (Heman-Ackah et al. 2013; Warner and Schapira 2003). Oxidative stress happens due to some etiologic illnesses which increases the production of ROS with consequent compromization of the protective mechanism or the repair system (Rahal et al. 2014). Understanding of the pathogenesis of PD which distinguishes the factors that initiate the disease and the

factors lead to the progression and the development of the illness would be helpful to develop new treatment agents (Jenner and Olanow 2006).

1.3.1. Mitochondrial dysfunction and oxidative stress.

The intracellular membrane-enclosed organelles found in most eukaryotic cells is the Mitochondria, it plays many vital roles in these cells such as pyruvate oxidation, Krebs cycle, metabolism of proteins, lipids, hormones, and, many other functions. The most crucial role is the generation of energy such as the production of adenosine triphosphate (ATP) which is utilized by the mitochondrial electron-transport chain and the oxidative-phosphorylation system (Perier and Vila 2011). This evidence explained the mitochondria as a place where oxidative phosphorylation takes place, and it is an essential source for reactive oxygen species (ROS) (Rezende Figueira et al. 2012).

Recent studies showed that Mitochondria is involved either directly or indirectly in the pathogenesis of PD (Jaimes 2013; Rimessi et al. 2016). Mitochondrial programmed cell death found to be related to the diminishing of neurons in the SN, the protein involved in familial PD also found to be associated with mitochondria. The alteration of mitochondrial DNA with aging is also linked to PD (Jayaraj et al. 2013). Defects or abnormalities in mitochondria include elevated ROS and decreased ATP levels, especially in the complex I, can lead to neurodegeneration (Vila, Ramonet, and Perier 2008; Jayaraj et al. 2013).

As a regular function of mitochondria, the molecular oxygen is reduced to water at complex IV of the ETC during the process of oxidative metabolism. Other redox centers at a site of electron leak in complex I of the ETC may reduce non-enzymatically a small fraction of oxygen to superoxide ($O_2^{\cdot}$) and H_2O_2 (Turrens 2003). Any deficiency in

complex-I can enhance the production of reactive oxygen species (ROS). Neurotoxin causing Parkinsonian syndrome found to inhibit mitochondrial respiration. Studies showed that several neurotoxic agents, such as rotenone, paraquat, and MPP + can have different mechanisms inside dopaminergic neurons in the mitochondria, and produce toxicity: by concentrating inside the mitochondria and causing toxicity, interacting with the cytosolic enzymes or uptaking via the vesicular monoamine transporters (VMAT) into the synaptic vesicles (Dauer and Przedborski 2003). MPP+ inhibits complex I, augments ROS production, and decreases the synthesis of ATP (Dauer and Przedborski 2003). Local ROS can also damage complex I (Wim Mandemakers 2007). The pathogenic role of mitochondria is still unclear (Haddad and Nakamura 2015). Glucolipotoxicity is considered the causative risk factor in mitochondrial dysfunction; the other aspects are the mutation of mitochondrial DNA, the exposure to an environmental toxin, the high-calorie intake and the homeostasis unbalance (Jha et al. 2017). Moreover, researchers found a link correlated between the mitochondrial dysfunction and the oxidative stress in PD (Martins Branco et al. 2010; Hastings 2009). Mitochondrial dysfunction leads to the deficit in energy supply and generation of oxidative stress. Exposure of the mitochondria to DA oxidized products resulted in mitochondrial respiration dysfunction occurs due to the imbalance in the antioxidant mechanisms **(Figure 3)** (Müller et al. 2010; Shahul Hameed 2011).

The two mechanisms generated in the mitochondria for PD states: (1) Primary mitochondrial dysfunction involved in the generation of endogenous or exogenous toxic metabolites in dopaminergic neurons. (2) The secondary mitochondrial dysfunction happens due to genetic mutation toxicity (Exner et al. 2012; Hastings 2009). Besides DA,

its metabolite products may enhance the degeneration of neurons in PD patients (Martins Branco et al. 2010). Although several factors have contributed to the development and pathogenesis of PD, oxidative stress has been found to have a significant implication for the production of free radical (Siegfried Kösel 1999; Shankar J. chinta 2008). Reactive nitrogen species such as NO and its metabolite peroxynitrite (PN) may also play a primary role in the etiology of PD. NO is known to inhibit complexes I and IV of the mitochondrial electron transport chain (Shankar J. chinta 2008).

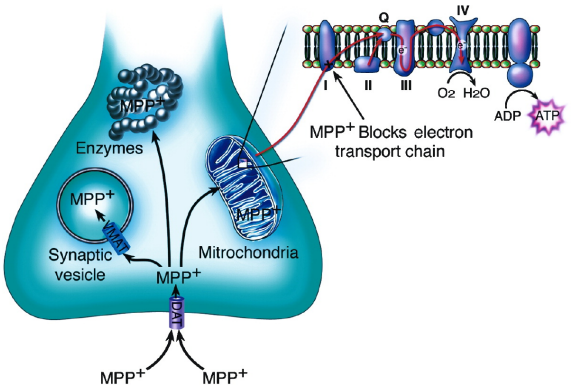


Figure 3. Schematic representation of MPP+ intracellular pathways (Müller et al. 2010.

1.3.1.1. Enzymatic and auto-oxidative DA metabolism.

Studies showed that presence of dopamine, decreased antioxidants (ex; low glutathione level), and increase of iron level are the primary causes of the dopaminergic neurons death due to the induction of ROS generation (Chinta and Andersen 2008). The selectivity of the demise of DA neurons at the SNpc may belong to oxidative stress (Blesa et al. 2015). Dopamine (DA) is a monoamine compound released by neurons and function as a neurotransmitter. DA undergoes auto-oxidation to form dopamine quinones, and free radicals **(Figure 4**). Dopamine is synthesized in the brain from tyrosine which undergoes hydroxylation by thyroxin hydroxylase to DOPA and then decarboxylation to Dopamine **(Figure 4)** (Meiser, Weindl, and Hiller 2013). Dopamine, therefore, is stored in synaptic

vesicles in the presynaptic region after uptake by the vesicular monoamine transporter 2 (VMAT2) (Wimalasena 2011).

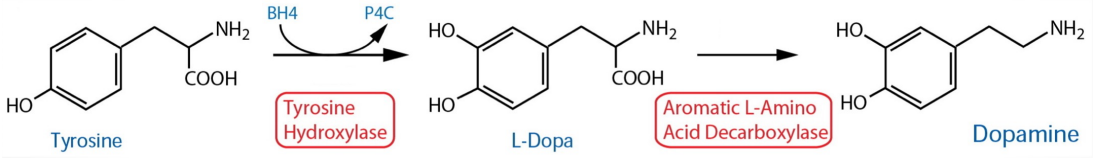


Figure 4. Dopamine synthesis in neurons (Meiser, Weindl.

The excess amount of DA which is not stored in the VMAT2 would undergo hydroxylation by MAO, this process called auto-oxidation(Wimalasena 2011; Chinta and Andersen 2008). Auto-oxidation of dopamine may be amplified in the first stages of the disorder (Zhou, Huang, and Przedborski 2008). Monoamine oxidase is an enzyme involved in the degradation of primary, secondary and tertiary amine such as catecholamines. Monoamine oxidase, specifically, MAO-B catalyzes the oxidative deamination of dopamine in the nerve terminal (substantia nigra and striatum) to form the oxidized dopamine (dopamine-quinone, dopamine-semiquinone and neuromelanin), and free radicals (hydrogen peroxide), H_2O_2 is the common product in this process (Jenner 2003).

In mitochondria, catecholamines including dopamine get metabolized enzymatically by MAO-B and MAO-A, flavin based protein, to produce hydrogen peroxide, superoxide, and hydroxyl radical (Jr. 2012). Moreover, oxidation of dopamine by MAO mainly by MAO-B produces 3,4-dihydroxyphenylacetaldehyde and anionic semiquinone flavin. Quinones and semiquinones are also as toxic as hydrogen peroxide to the cells as they can bind to DNA, lipids, and proteins in the cells **(Figure 5)**. The anionic semiquinone Flavin interacts with oxygen to make superoxide which attacks DNA (Jr. 2012). Autoxidation in PD is a mechanism of cell loss leading to apoptosis (programmed cell

death) (Stanley and Gerald 1992; Jellinger 2000). Dopamine oxidation by MAO found to be the dominant risk factor involved in the progression of PD (Meiser, Weindl, and Hiller 2013). Studies showed that the level of MAO-B enzyme increases with aging that would increase H_2O_2 production in the glial cells, H_2O_2 can cross into the nearby dopaminergic cells, and influence the dopaminergic neuron (Hussain et al. 2018). It is understood that oxidative stress plays a specific role in the degeneration of dopaminergic neurons in PD (Dias, Junn, and Mouradian 2013). The autoxidation of dopamine and its regulation are essential factors in determining the loss of the dopaminergic neurons, which are characterized in humans by the presence of neuromelanin **(Figure 6)** (Olanow 1999).

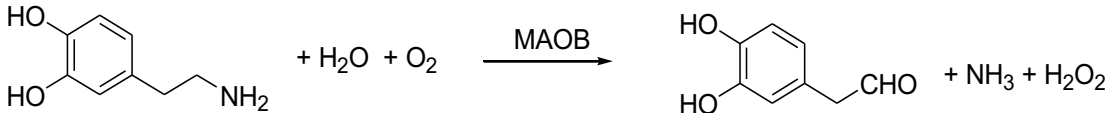


Figure 5. Enzymatic oxidation of DA by MAO-B, yielding toxic metabolites.

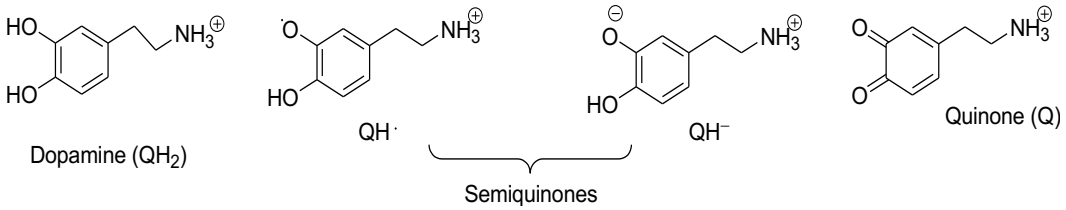


Figure 6. Auto-oxidative metabolism of DA.

Nitric oxide (NO) plays a vital role in cell functions through the signal transduction pathway (Zhang, Dawson, and Dawson 2006). It also involves in the pathogenesis of the neurodegenerative diseases. While the mechanism of implication of NO in PD is not fully understood, studies showed that NO might cause neuronal death, DNA damage, protein aggregation, and misfolding. In the substantia nigra of Parkinson's patients, high level of nitric oxide (NO) was measured (Aquilano et al. 2008). Nitric oxide (•NO) is produced either from the inducible form of nitric oxide synthase (iNOS) or from the neuronal form

(nNOS). NO then participates in the cascade of events which leads to the degeneration of dopamine-containing neurons (**Figure 7)** (Ahlawat et al. 2014).

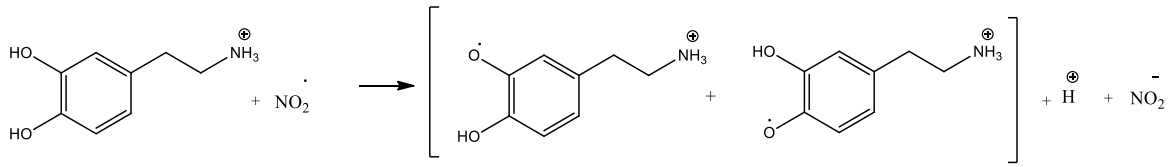


Figure 7. Proposed pathway to form quinones from free radical nitric oxide.

Homeostasis of iron is essential in the normal functioning of the nervous system. Human iron metabolism is the set of chemical reactions maintaining human homeostasis of iron at both systemic and cellular level to maintain the redox-signaling proteins functions in neuron cells and the neuronal survival (Willis and Sandyk 1992; Liu et al. 2017). Iron-mediated Fenton reaction potentially reacts with dopamine and produces a toxic hydroxyl radical which ultimately increases the oxidative stress that can damage the cells, the loosely bound iron Fe^{3+} or iron bound to neuromelanin gives the reduced form Fe^{2+} (Jenner 2003). Since the substantia nigra is rich in iron, it makes the area susceptible to free radical production in the presence of melanin (Hwang 2013). Which explains the significant role of iron in oxidative stress in PD brain. When the iron content of the SN is higher in the PD brain, it enhances the conversion of H_2O_2 to hydroxyl radical via Fenton reaction. **(Figure 8)** (Pichler et al. 2013).

$$H_2O_2 + Fe^{2+} \longrightarrow OH^- + OH\cdot + Fe^{3+}$$

$$Fe^{3+} + O_2^{\cdot -} \longrightarrow Fe^{2+} + O_2$$

$$O_2^{\cdot -} + H_2O_2 \longrightarrow O_2 + OH^- + OH\cdot$$

Figure 8. Oxidative Stress: Increased Iron Content.

1.3.1.2. Compromised antioxidant defense pathways

Oxidative stress has strongly been linked to the pathogenesis of PD due to the production of H_2O_2 and free radicals (Hwang 2013). Numerous neuroprotective strategies have been identified to attenuate ROS in the dopaminergic neurons, one of these strategies is the use of Fruits and antioxidants to reduce the damage caused by the free radicals. Vitamins C, E, and GSH are also essential antioxidants in the body that can be reutilized by antioxidant lipoic acid (Liu et al. 2017). Lipoic acid is another example of antioxidants in human cells, its mechanism of action to protect neurons against both oxidative stress and inducers that cause mitochondrial dysfunction (Ahmadinejad et al. 2017). The antioxidants mechanism is achieved by increasing the GSH generation or diminishing the lipid peroxide level in the brain which results in elevating the production of ATP and improvement of the motor function as a result of neuroprotection (Abramov 2012). Other examples of antioxidants are; glutathione peroxidase, catalase, and superoxide dismutase. The functions of these systems are scavenging of ROS, preventing ROS formation and repairing damage caused by ROS (Borut Poljsak 2013). In case of PD, the level of H_2O_2 elevated due to the reduction of glutathione and catalase enzymes levels in the Substantia Nigra region of PD patients (Mythri et al. 2011).

An appropriate amount of antioxidant is essential for the normal functioning of the cells. **(Figure 9).** Antioxidants scavenge the reactive oxygen species, prevents their formation as well as repairs the damage caused by them (Liu et al. 2017; Drummond et al. 2017). The antioxidant system is a complicated system consisting of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase), glutathione, glutathione reductase, glucose-6-phosphate dehydrogenase (Chinta and Andersen 2008). Superoxide dismutase (SOD) catalyzes the conversion of two molecules of

superoxide to H_2O_2 and O_2, then catalase and glutathione peroxidase Convert H_2O_2 to H_2O (Stanley and Gerald 1992; Liu et al. 2017). The glutathione redox cycle should function well in cells to prevent the degeneration of neurons caused by reactive oxygen species (Schafer and Buettner 2001).

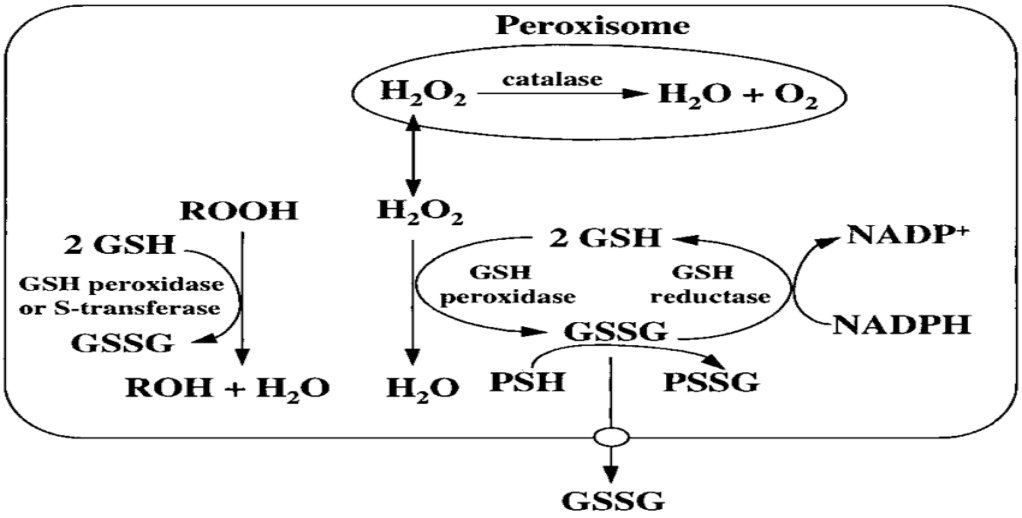


Figure 9. Oxidative stress: reduced antioxidant level (Lu 1999).

1.3.2. Protein aggregation.

Several genetic and environmental factors have been implicated in protein misfolding and aggregation (Soto 2003). Abnormal protein aggregation is a crucial feature in aging and several neurodegenerative diseases in which cells lose their ability to handle the misfolded protein (Takalo et al. 2013). Abnormal formation and aggregation of intracytoplasmic rounded inclusion or extracellular aggregates named Lewy bodies (LBs) are considered one of the pathologic hallmarks of PD (Takalo et al. 2013). α-synuclein (ASN) is the primary component of Lewy bodies, and it is highly expressed in the mammalian brain. Although the exact physiological function of α-synuclein is still unclear, it is found to be involved in the pathophysiology of PD. α-synuclein, a protein consists of 140 amino acids, belongs to the synuclein family of helically folded tetramer that resists

aggregation **(Figure 10**) (Spinelli et al. 2014). The synuclein family includes α-synuclein, β-synuclein, and γ-synuclein. Although all three members of the synuclein family are neuronal proteins, only α-synuclein is implicated in neurodegenerative diseases. **(Figure 10)** (Spinelli et al. 2014; Chan et al. 2012; Xu and Chan 2015).

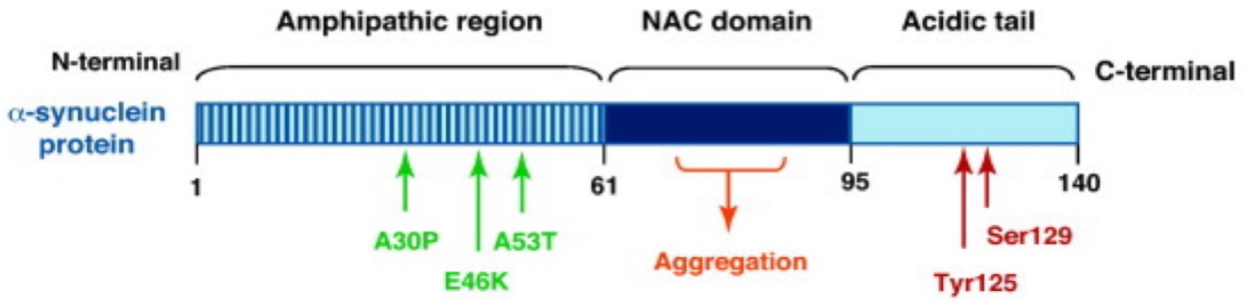


Figure 10. α-synuclein protein domain structure. α-synuclein protein is composed of three distinct regions: (1) an amino acid terminal, from 1-60 is the amphipathic region which has a positive charge and contains Apolipoprotein which gives α-helical structure on the membrane binding, (2) a central hydrophobic region called NAC (non-Aβ component), residue of 61-95, responsible for oligomerization and fibrillization and gives a β-sheet structure, and (3) a carboxyl terminal, the rest of the protein 96- 140 is the acidic feature region, which has a negative charge, and this part is unstructured (Xu and Chan 2015).

In the normal state, alpha-synuclein is unfolded and exists as a monomer. Under the pathological condition, the protein undergoes misfolding, and also with aging, the cells lose the ability to handle misfolded protein. Mutant α-synuclein is more prone to aggregation and causes cell death in PD (Chan et al. 2012). Genetic abnormalities and environmental factors may accelerate the process (Irwin, Lee, and Trojanowski 2013). In normal condition, the cells have a specific system to reverse and prevent the misfolding of proteins; this system includes chaperones, ubiquitin proteasomes, and phagosome-lysosome. In pathological conditions, the systems are overwhelmed by oligomeric species of α-syn (Irwin, Lee, and Trojanowski 2013). However, it is not known which pathogenic species of α-syn is toxic to neurons. Studies showed that the synthetic α-syn fibrils

precipitation in neurons alone could transmit PD between neurons to cause cell death (E. Burke et al. 1999).

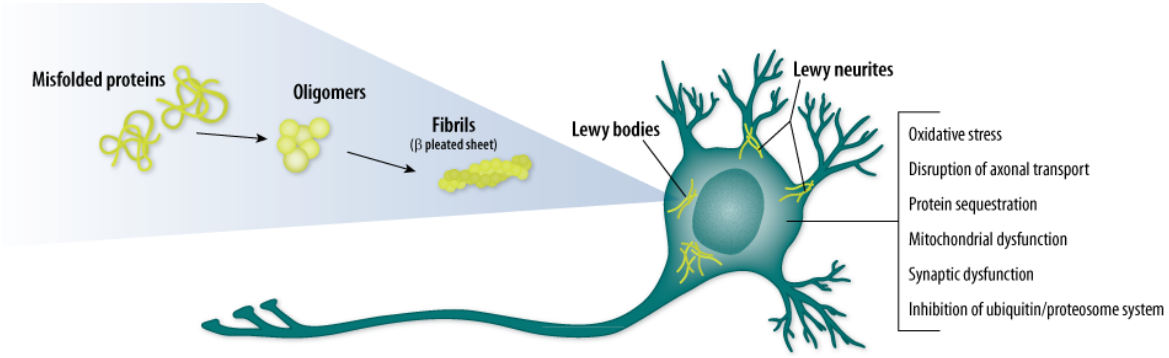


Figure 11. Misfolded alpha-synuclein proteins. ASN in pathological conditions is misfolded into abnormal beta-sheets (dimers, trimers, and oligomers) instead of alpha-helices, that further aggregate and bend to form protofibrils, the protofibrils then further precipitate to form fibrils (amyloid fibrils), and eventually transformed into Lewy bodies. Deposition of α-synuclein protein in Lewy bodies is a hallmark for PD diagnosis. (Lee, V.M-Yet al, 2006)

1.3.3. Genetic factors.

Genetic mutations or variants are potential biomarkers in the diagnosis and identification of persons at the risk of PD (Jankovic 2007; Klein and Westenberger 2012). Although the cause of dopaminergic degeneration in idiopathic PD is unclear, most cases of PD are sporadic (Fernandez-Espejo 2004) in nature. Genetic factors are usually associated with familial PD (Zhang et al. 2018). Although several genes have been associated with PD, genetic influences are responsible for only rare cases (Massano and Bhatia 2012). The estimated incidence of familial PD is 1-2%. Genetic components play a dominant role in the pathophysiology of the disorder with early and late onset, and also involved in the nigrostriatal loss of the dopaminergic neurons (Massano and Bhatia 2012; Klein and Westenberger 2012). Family history with PD leads to increase in susceptibility to develop the syndrome (Ratner and G. Feldman 2004). Also variant genes such as *SNCA* over-expression and *parkin* gene mutation have been involved in the pathogenesis

of PD; they accounted for a small fraction of the overall the frequency of the disease (Dick et al. 2007).

1.3.4. Environmental factors.

Environmental factors are other pathogenic aspects connected to the etiology of PD, such as pesticides, herbicides, and insecticides (Olanow 1999; Ratner and G. Feldman 2004). In the absence of identified genetic causes, environmental factors appear to have a role in the cause of the disease. In another word; people who develop PD due to exposure to the environmental influences are less likely to have a family history of the disorder (Hancock et al. 2008). According to the epidemiologic studies, exposure to environmental toxicants is related to increase the risk of PD. Example of environmental toxins are; pesticides, solvents, metals, and other pollutants (Goldman 2014). Nigral degeneration may occur by binding of oxidative stress and other metabolites to glutathione which happens through the exposure to the industrial toxic chemicals (**Figure 11)** (Ratner and G. Feldman 2004). The amount of pesticide and the degree of exposure that may lead to the development of PD still unknown, and the identification of the agent that causes the disorder is challenging (Dick et al. 2007). In necropsy studies; the level of the organochlorine pesticide found to be high in patients with PD (Dick et al. 2007).

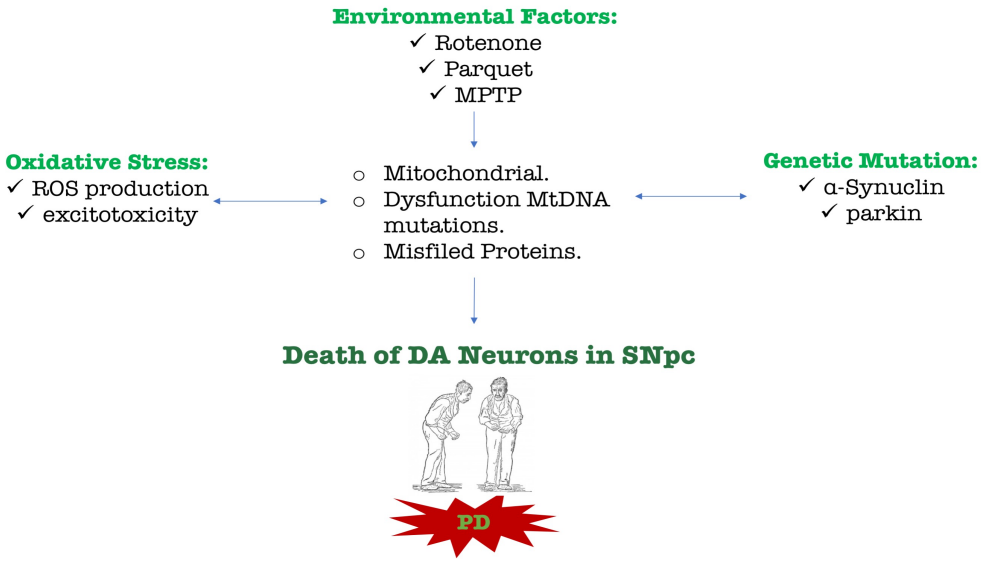


Figure 11. Factors contributed in the pathogenesis of PD.

1.4. Therapeutic strategies in PD.

Several significant advances have been made to understand the etiology, pathology and clinical phenomenology of PD that have underlined the development of symptomatic treatment and also the possibility to extend the intervention of medications that might stop or slow the progression of the disease (Schapira 2009b). This section explains the different treatment strategies that could be used to treat Parkinson's patients. According to researchers, two types of treatment could be applied: symptomatic and neuroprotective treatments.

1.4.1. Symptomatic therapy.

Understanding of the clinical manifestation in PD is necessary for the diagnosis of the disease to choose the proper medication and treatment (Oscar Bernal-Pacheco 2012). From the symptomatic treatment point of view, enormous progress has been made in the treatment of PD over the past decades; Levodopa remains the gold standard in controlling the symptoms of PD. Before choosing the first line medication for PD, the level

of impairment and the precise diagnosis must be done (Jankovic and Aguilar 2008). The treatment plan for each PD patient should depend on each individual, the medications alleviate the symptoms in Parkinson's patients (Chen et al. 2016). Drugs that produce symptomatic relief in PD act either by elevating the regional dopamine levels such as levodopa, monoamine oxidase (MAO) Inhibitors, catechol-o-methyl-transferase (COMT) inhibitors or by stimulating dopamine receptors as dopamine agonists (DA), inhibiting the effect of cholinergic afferents (anticholinergics), or inhibiting glutaminergic NMDA receptors (amantadine) **(Figure 12, 13)** (Chen et al. 2016; Jankovic and Aguilar 2008; 'Parkinson Disease: Neurologic Pathways & Drug Targets' 2015).

1.4.1.1. Levodopa Therapy.

L-Dopa is a DA precursor (L-3,4-dihydroxyphenylalanine), it was introduced in the late 1957 to reverse Parkinson-like akinesia. In 1967 it was reported that oral administration of L-DOPA improved the rigidity and akinesia in PD. The improvement was significant but with short duration of action (McDowell and Lee 1970). Levodopa is the first pharmacological approved drug to treat PD, and considered the gold standard in PD treatment (Nagal and Singla 2012). Although, L-dopa reduces the motor symptoms of PD; it does not show any effect on the non-motor symptoms nor halt the progression of the disease (Mercuri and Bernardi 2005). Levodopa is given in combination with carbidopa (DOPA decarboxylase inhibitor) to prevent the premature conversion of L-DOPA into dopamine in the peripheral nervous system (PNS). This combination reduces the side effect of L-dopa on the periphery cardiovascular effects such as nausea and vomiting as well as and increases its pharmacological effects on the CNS. Levodopa in

the CNS is converted by the DOPA-decarboxylase enzyme to DA (Nagal and Singla 2012). Long-term use of levodopa causes irreversible dyskinesia and motor fluctuations

1.4.1.2. Dopamine Agonist.

Dopamine agonists act directly on dopamine receptors at the postsynaptic region. Many physicians recommended the use of dopamine agonist as a first-line of treatment in PD. Besides, giving dopamine agonist as monotherapy, it is also used as adjuncts therapy to levodopa. For example, bromocriptine and pergolide have been used with levodopa to reduce its motor complications (Mercuri and Bernardi 2005). Dopamine agonist is used in PD to delay the need for levodopa; such delay is recommended to delay or prevent levodopa-induced complications. Pramipexole, a dopamine agonist, was given to PD patients in combination with levodopa, to reduce the severe side effects associated with levodopa (Amos D. Korczyn 2004; Murata 2009). Although this combination reduces the motor complication of levodopa, it causes increased somnolence and hallucination. Pramipexole and ropinirole are other examples of dopamine agonists, and they are expected to have a lower risk of complications than other dopamine agonists because of non-ergolines. Pramipexole found to be safe and effective in the early stage treatment of PD when used as monotherapy (Jankovic and Aguilar 2008). Ropinirole has also exhibited to be safe in a specific dose and effective in the early PD.

1.4.1.3. Monoamine Oxidase Inhibitors (MAO-Is).

Monoamine oxidase inhibitors are used in the treatment of symptoms of PD. They work by blocking the degradation of dopamine by inhibiting the monoamine oxidase enzyme (Schapira 2011). Current treatments of PD involves the use of selective MAO-B inhibitors that addresses the deficiency of dopamine (Edmondson and Binda 2018). MAO-

Is have also been studied for possible neuroprotective properties as a result of decreasing the oxidative stress (Edmondson and Binda 2018; Schapira 2011). They are used as an alternative therapy to treat PD. Selegiline and safinamide are currently selective MAO-B inhibitors used in the market (Edmondson and Binda 2018). Selegiline was developed to address the symptoms in the early stage of PD (Schapira 2011), it also showed mild antidepressant effect. Studies suggested that selegiline might have neuroprotective properties. Selegiline develops some sympathomimetic side effects such as heartburn, nausea, dry mouth, dizziness, confusion, as well as hallucination (H and S 2006). Safinamide demonstrates glutamatergic antagonist effect, studies showed that selegiline and safinamide might be less effective to treat the motor complication compared with previous PD therapy (Schapira 2011; Blair and Dhillon 2017). When applied as monotherapy, MAO-B inhibitors provide a modest effect, but the motor function was significantly improved, MAO-Is can also delay the demand for levodopa (Teo and Ho 2013). Combination of selegiline or safinamide with dopamine agonist showed significant improvements in the treatment of early stage of PD (Teixeira et al. 2018; H and S 2006). MAO-Is showed good efficacy and less side effect, so they are recommended as monotherapy in the early stage of treatment of PD (Löhle and Reichmann 2011). MAO-Is are used as an add-on to levodopa in the advanced stage of PD, and as monotherapy in the early stage of the disorder (Dézsi and Vécsei 2014). MAO-B inhibitors significantly reduced off-time and were comparable in efficacy to COMT inhibitors (Livia and Laszlo 2017).

1.4.1.4. Catechol-O-methyl transferase inhibitors (COMT-Is)

Catechol-*O*-methyl transferase COMT is a primary enzyme in the metabolism of catecholamine compounds such as dopamine, norepinephrine (Nissinen and Männistö 2010). Entacapone and tolcapone are examples of COMT-inhibitors**,** they are given in combination with levodopa to increase its bioavailability and efficacy. They may also be used for individuals who are not tolerant to dopamine agonist and are experiencing wearing off (Rinne, Ulmanen, and Lee 2003). Clinically used COMT inhibitor, Entacapone has been used as an adjunct therapy with levodopa to treat PD who do not suffer from motor fluctuations (Rinne, Ulmanen, and Lee 2003).

1.4.1.5. NMDA glutamate type receptor

To overcome the motor complication side effects associated with previous PD medications, an NMDA-type glutamate receptor antagonist know as amantadine was developed (Chan et al. 2013). Amantadine acts by increasing dopamine release and blocking dopamine reuptake in the presynaptic region (Amos D. Korczyn 2004). Despite no clinical evidence of the effect of Amantadine in the treatment of motor function complication in PD, it is currently used to reduce dyskinesia (Nagal and Singla 2012). Amantadine may also cause a higher risk of psychiatric adverse effects in progressed PD patient (Nagal and Singla 2012; Bédard et al. 2011; M Goldenberg 2008; Chan et al. 2013).

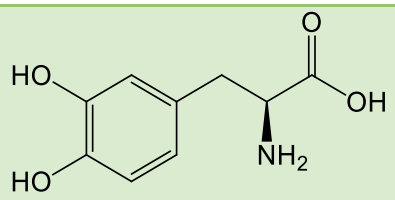
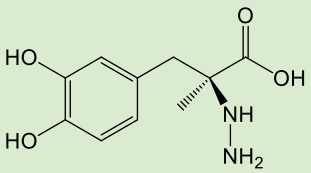
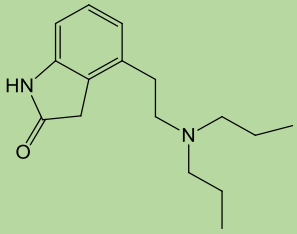
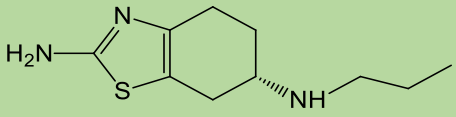
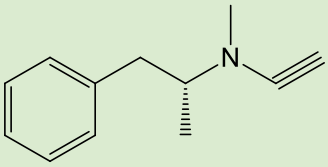
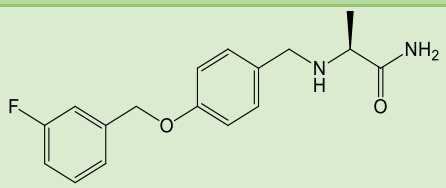
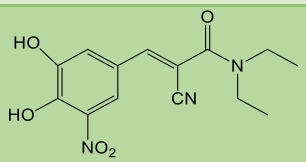
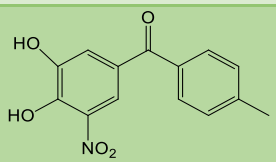
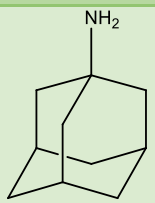
DA replacement therapy	 Levodopa  Carbidopa
Dopamine agonists	 Ropinirole  pramipexole
MAO-inhibitors	 Selegiline  Safinamide
COMT inhibitors	 Ropinirole  Pramipexole
NMDA-Glutamate type receptor	 Amantadine

Figure 12. Chemical structures of pharmacological treatments for PD.

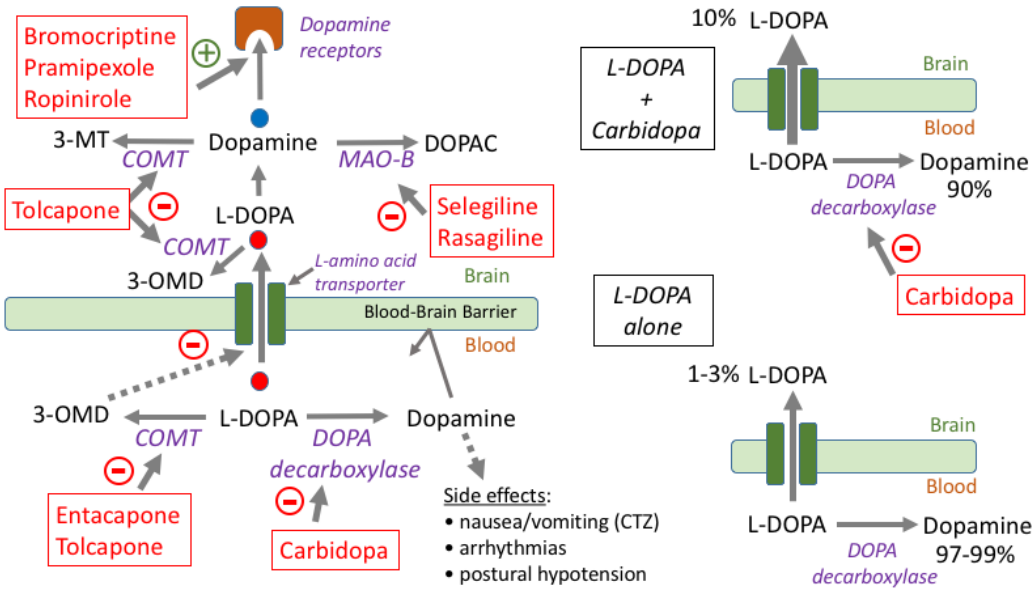


Figure 13. Treatment mechanisms and strategy available for PD. Abbreviations: COMT, catechol-O-methyl-transferase; 3-OMD, 3-O-methyldopa; DA, dopamine; 3-MT, 3-methoxytramine; MAO, monoamine oxidase; HVA, homovanillic acid; DOPAC, 3,4 dihydroxy phenylacetic acid 'Parkinson Disease: Neurologic Pathways & Drug Targets' 2015)

1.4.2. Neuroprotective Therapy.

The idea behind the neuroprotection treatment strategy in PD is to delay or stop the progression of the disease with or without alleviating the symptoms. To develop a novel neuroprotective agent for PD, we need to understand the mechanism and the etiology of the disease. (Zhang, Dawson, and Dawson 2006). Several pathogenic factors have been strongly linked to the death of the neuronal cells in PD. These factors are oxidative and nitrative stress, mitochondrial dysfunction, protein misfolding, excitotoxicity, and inflammation. In PD, the dopaminergic neurons at the SNpc exposed to oxidative stress, which leads to the injury of the neurons. Using neuroprotective agents that will restore the damaged neurons or stop the damages of the neurons would be expected to provide neuroprotective effect (Sarkar, Raymick, and Imam 2016). L-Dopa is used to provide sympathetic relive by increasing the level of dopamine in the synaptic region, but

it could not modify the progression of the disease. Moreover, long-term use of L-dopa administration enhances the neuroprotection of neurons at the SNpc (Calabresi et al. 2015). Several dopamine agonists have been proven to exhibit neuroprotection properties such as pramipexole (Hall et al. 1996).

1.4.2.1. Mechanisms of Neuroprotection.

Since PD is considered as multifactorial, an ideal neuroprotective agent should have the ability to act by various mechanisms to counteract the disease progression. Several strategies have been applied acting on the various possible mechanisms incorporated in agents to act as neuroprotective compounds. Neuroprotection may act by numerous different mechanisms; increase the level of dopamine, reduce the need for L-dopa, activation of DA auto-receptors, antioxidant properties of DA agonists, and also as an antiapoptotic agent (Ossig and Reichmann 2013). The strategy of neuroprotection was proposed to address the deficiency of dopaminergic neurons in the substantia nigra either by using a replacement of dopamine as levodopa or by using MAO-I and COMT-I to prevent the oxidation of dopamine to increase the level of dopamine at the synaptic region and relief the symptoms of PD (Sarkar, Raymick, and Imam 2016). Another strategy of neuroprotection is the use of dopamine agonist to act directly on the dopamine receptor, the example of that is pramipexole which has an antioxidant mechanism, it can bind to the dopamine receptor at the postsynaptic region. Several antioxidant agents could be used to inhibit both oxidation of dopamine and formation of free radicals (Schapira 2009b).

1.5. Overview of the dopamine receptor system.

Dopamine neurons and their receptors are known to be implicated in the pathogenesis of PD (Hisahara and Shimohama 2011). Dopamine receptor is classified as a member of biogenic amine receptors belonging to G protein–coupled receptors (GPCRs) family (Butini et al. 2016). Dopamine is one of the monoamine neurotransmitters produced in several areas of the brain including the substantial nigral and the ventral tegmental area. This neurotransmitter activates five types of the G-protein coupled dopamine receptors, and they are classified into two groups: D1 like receptors: D1, D5, and D2 like receptors: D2, D3, D4. The D1 like receptors activate adenylate cyclase leading to increase the cyclic adenosine monophosphate (CAMP) while D2 like receptors inhibits the adenylate cyclase to reduce the production of CAMP **(Figure 14)** (Levant 1997) Goodman & Gilman's).

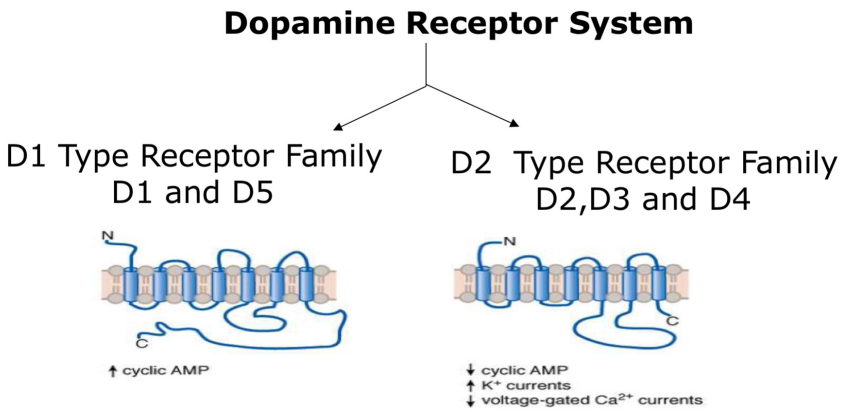


Figure 14. Dopamine receptor system, (Goodman & Gilman's the pharmacological Basis of therapeutics).

1.6. The clinical trial of neuroprotection in PD with dopamine receptor agonists.

To develop a single neuroprotective therapy for Parkinson's patients, it is essential to have a proper clinical diagnosis to find the common pathogenic factors (Jankovic

2007). In 1970 the first major neuroprotective clinical trial was designed is the DATATOP study. It was designed to test the neuroprotection in Parkinson's patients using deprenyl (MAO-I) and Tocopherol (vitamin E). Deprenyl is a Type B monoamine oxidase inhibitor used in a dose of 10mg/day; MAO-B metabolizes the catecholamine including dopamine in the brain to its oxidized forms with the production of H_2O_2. H_2O_2 reacts with iron to produce hydroxyl radical resulting in oxidative stress. By giving seligiline, it will prevent the oxidation of dopamine and block the formation of ROS (Riederer and Youdim 1986). α-Tocopherol is a biologically active component of vitamin E, its mechanism of action to diminish the effect of lipid peroxidation by trapping free radical (Hassan, Stohs, and Murray 1985). The onset of disability was the primary endpoint as the indication to administrate L-dopa. The results showed that no beneficial effect of tocopherol and no interactions have seen between tocopherol and deprenyl. The endpoint time was determined to calculate the required time for L-dopa treatment. Deprenyl demonstrated a strong and significant effect on delaying the onset of disability required L-dopa treatment. The patients showed improvement in the first three months of treatment, and the motor symptoms became worse after the withdraw of the medication. The purpose of this clinical trial was to test if deprenyl or tocopherol can extend the length of the time before the patient require to administrate levodopa (group 1989).

In conclusion, there is no current treatment available for Parkinson's patients that would either stop or slow the progression of the disease or restore the dopaminergic neuronal loss to the normal condition. The available therapy of PD in the market is classified as a symptomatic treatment, they only reduce the disorder symptoms to improve the patient life temporarily. By designing a combination therapy of symptomatic

and neuroprotective treatment we might improve the patient quality of life by diminishing or stopping the progression of the disease or restoring the loss of the damaged neurons as well as alleviating the symptoms of the disease. Example of that a combination has dopamine agonist property as well as antioxidant property. Using selegiline in this experiment can diminish the symptomatic symptoms in PD patients, while deprenyl has antioxidant property. The mechanism of action of selegiline is not fully understood. A studied was carried out to understand the exact mechanism of selegiline if its effect is due to its symptomatic influence or it has a neuroprotection outcome. The result of this study showed that selegiline has a neuroprotective effect.

CHAPTER 2

The unmet need to develop symptomatic and disease modifying therapeutics to treat Parkinson's disease.

Parkinson's disease is multifactorial in nature arising from the involvement of several pathogenic factors (Kalia and Lang 2015). It has been proposed that treatment of PD with a single target drug may not be adequate due to the complex pathogenesis of the disease process, therefore, multiple medication drugs (MMDs) could be a reasonable treatment approach due to their effectiveness to engage more than one targets at the same time (Youdim and Buccafusco 2005). MMDs is usually composed of two or three different drugs that could target various targets implicated in the pathogenesis of the disease (Morphy and Rankovic 2005). However, multiple medications with different pharmacokinetic, bioavailability and metabolism properties could be challenging and can cause serious problems especially for elderly people and people with chronic disease (Hague, Klaffke, and Bandmann 2005). If a patient has other issues, it could be possible to develop several side effects with MMDs. Another strategy is the combination of different medications in the same formulation to reduce the dosing time regimen of the drug and also improve the patient compliance (Andrea Cavalli 2008).

The new strategy and idea which has become the focus of the researcher lately is the designing and development of single multifunctional molecules (MFM) which can potentially target multiple pathogenic pathways involved in the pathogenesis of the disease (Andrea Cavalli 2008; Van der Schyf 2011). By combining different functional groups in the same molecule to introduce multifunctional properties that could provide a new avenue to treat the complex diseases where several factors are responsible for the

pathogenesis of the disease process. Therefore, by interacting with multiple targets at the same time could provide pharmacokinetic advantages over using MMDs as well as less toxicity and more compliance. (Youdim 2010; Morphy and Rankovic 2005; Bansal and Silakari 2014, JP et al. 2011, Youdim 2010). There is an urgent need for such multifunctional single drug (MFM) to treat PD that could bring a new avenue to address PD (Youdim 2010).

2.1. Previous work on development of multifunctional agents to treat neurodegenerative diseases.

Several studies have been done to develop single molecules with multifunctional activities to treat neurodegenerative diseases. The designing of such MFD was based on combination of two or three pharmacophore structures acting on multiple neuronal and biochemical targets (Youdim and Buccafusco 2005). This new model that addresses the pathogenicity and complexity of PD by using a multi-targeted-single-ligand approach strategy has been introduced and recognized for its potential to offer better outcomes (Van der Schyf and Geldenhuys 2011). Researchers also use the MFM strategy to target neurotransmitter receptors or enzymes within the neurons. For example, examples of such targets are monoamine oxidase, acetylcholinesterase and catechol-*O*-methyltransferase enzymes which are responsible for degrading catecholamine neurotransmitter, degrading acetylcholine, and for the metabolism of catecholamine neurotransmitters, respectively, (Youdim and Buccafusco 2005; Bansal and Silakari 2014). Examples of the single drug with two or more functional properties are shown in Figure 14 in chapter 1.

2.1.1. Combination of anticholinesterases and muscarinic M_2 receptor antagonism.

JWSUS-C75IX is a bifunctional compound is a potent AChE inhibitor and high affinity muscarinic M_2 receptor antagonist and is designed to be used as a cognitive enhancing drug (Fig 15?) (Van der Schyf, Geldenhuys, and Youdim 2006; Youdim and Buccafusco 2005). This combination exhibited the ability to enhance the release of acetylcholine leading to a decisive mnemonic action, and also showed a better safety profile than drugs that have only AChE inhibition activity by improving the pharmacokinetic properties as well by addressing the potential side effects.

2.1.2. Combination of anticholinesterases and brain-selective MAO inhibitors.

Ladostigil (TV3326) is a combination of two moieties, carbamate (cholinesterase inhibitory activity) and propargyl (a selective inhibitor of the brain MAO) moieties (Fig 15?). The selectivity of Ladostigil to inhibit the central MAO in the brain provides the compound with potential antidepressant activity due to increasing the level of serotonin in the brain. The neuroprotective effects has been attributed to the propargylamine moiety which activates the mitochondrial Bcl_2 family of proteins, protein kinase, C-mitogen-activated protein MAP (PKC-MAP) and the downregulation of Bad and Bax. (Youdim and Buccafusco 2005).

2.1.3. Combination of iron chelating and MAO inhibitors.

HLA20, M30, and M31 are neuroprotective compounds with bifunctional properties for being an iron chelator and MAO inhibitor (Van der Schyf 2011). The propargyl amine possesses neuroprotective activity by inhibiting the MAO enzyme which results in decreasing the production of H_2O_2 and the antioxidant iron-chelator group is present as a 8-hydroxyquinoline derivative. By combining the two pharmacophore groups led to the

development of neuroprotective agents with a potential activity to treat both Alzheimer's (AD) and PD (Youdim and Buccafusco 2005). HLA20 demonstrated selective inhibition of MAO-B, while M30 was found to be a highly potent inhibitor for both MAO-A and MAO-B (Van der Schyf 2011). Ladostigil is a bifunctional medication used to treat dementia, depression in neurodegenerative diseases, and multifunctional antioxidant iron chelator group (Bansal and Silakari 2014).

2.1.4. Combination of anticholinesterases and NMDA receptor antagonism.

Donepezil (anticholinesterase inhibitor) and Amantadine (NMDA receptor antagonist) are used in a combination to treat dementia in moderate to severe Alzheimer's disease by enhancing the memory with a potential neuroprotective property (Blanpied, Clarke, and Johnson 2005, Matsunaga, Kishi, and Iwata 2015, Gasparini et al. 2013). The mechanism of action of amantadine is to block the ion channels of NMDA receptor while the mechanism of action of donepezil as a reversible inhibitor is to block the breakdown of acetylcholine by inhibiting the acetylcholinesterase enzyme leading to increasing the level of acetylcholine at the cholinergic synaptic region (Bruno et al. 2012; Gasparini et al. 2013; Blanpied, Clarke, and Johnson 2005). There is no evidence if this combination could treat PD even though it improves the ability of thought process, functioning and behavior (Blanpied, Clarke, and Johnson 2005). By adding a L-dopa to the combination, the compound can be used to treat Lewy body dementia DLB (Youdim and Buccafusco 2005; Blanpied, Clarke, and Johnson 2005).

2.1.5. Combination of MAO-B and COMT inhibitors.

MAO-B inhibitors (selegiline and rasagiline) and COMT inhibitors (entacapone and tolcapone) are used in a combination as multifunctional medications to increase the level

of L-dopa in the brain (Youdim and Buccafusco 2005). MAO oxidase enzyme mainly MAO-B in the brain reduces the level of dopamine in the synaptic region via oxidation. Thus, MAO-B inhibitor prevents the MOA-B from breaking down dopamine. COMTs work by inactivating L-dopa through a methylation which block the conversion of L-dopa to dopamine. Therefore, inhibiting the action of COMT leads to increase the availability of dopamine.

2.1.6. Combination of neuroleptic D_2 receptor antagonist and an SSRI.

Eltropzine a neuroleptic dopamine D_2 receptor antagonist and an SSRI like fluvoxamine, fluoxetine, or citalopram are used in combination as antidepressant and antipsychotic drugs to improve the negative symptoms of schizophrenia as well as to diminish depression, without worsening the extrapyramidal side effects (Bansal and Silakari 2014).

2.1.7. Combination of squalene synthase inhibitor and anti-inflammatory.

A biphenyl morphine derivative (squalene synthase inhibitor) and naproxen (anti-inflammatory) are used in combination as potent anti-atherosclerotic agent. To build the pharmacophore of this compounds, the biphenyl ring of the morphine derivative was replaced with naphthalene part of naproxen to have an MFM which works as squalene synthase inhibitor to diminish the level of cholesterol and triglyceride and has antioxidant activity. (Bansal and Silakari 2014).

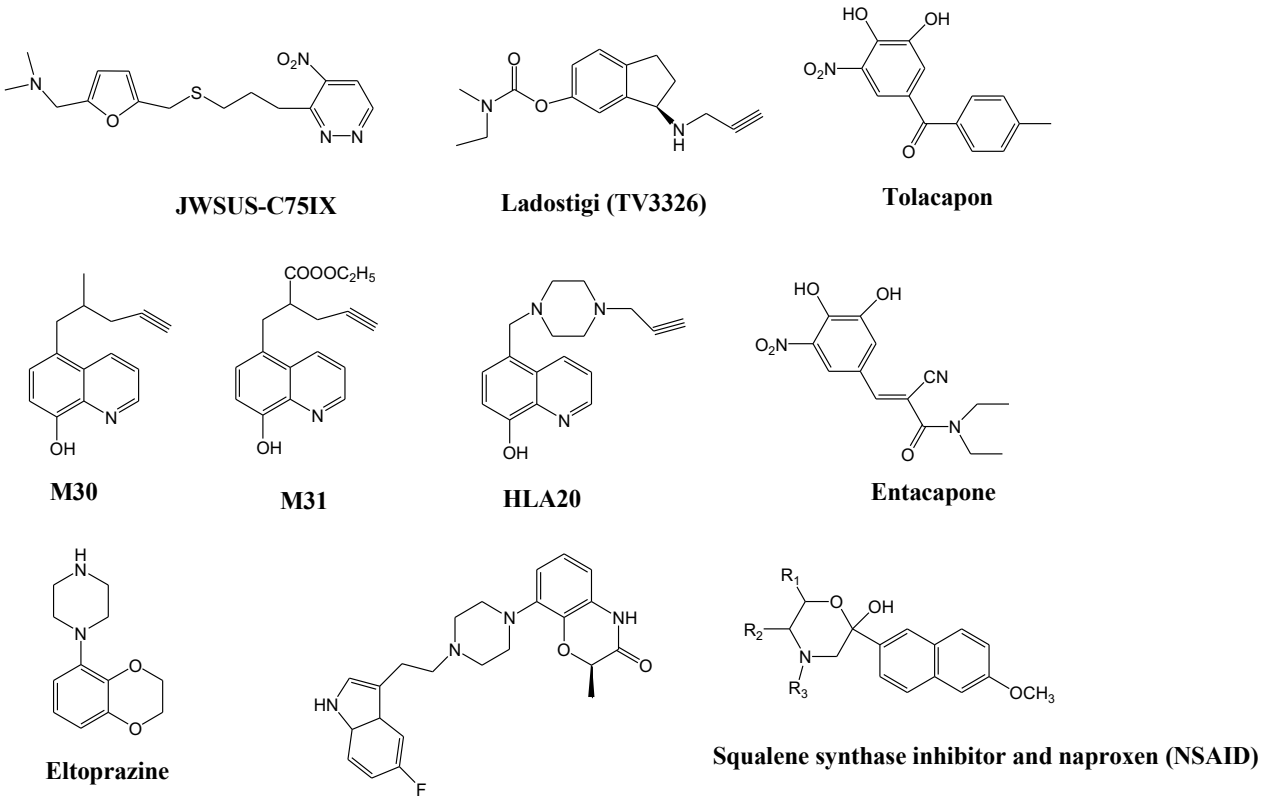


Figure 15. Structure of multi-functional drugs possess two or more pharmacological properties.

2.2. Development of multifunctional ligands to treat PD.

As was mentioned previously, the multifunctional compounds have become the focus of the researchers today to develop medications that may potentially address the multifactorial nature of PD by incorporating appropriate pharmacological activities targeting multiple pathogenic factors implicated in PD. There is an unmet need for multifunctional agents to treat PD because of the complex pathophysiology present in this disorder. Our research group has been working to develop MFD based on the hybrid template that was established earlier in the lab (Dutta, Fei, and Reith 2002). Our molecular template was designed to address several pathogenic factors implicated in the pathogenesis of PD (Johnson et al. 2012). The synthesized lead compounds based on

the hybrid template have demonstrated full agonist activities at both D_2 and D_3 receptors; in addition to the high binding and functional activities for both receptors (Johnson et al. 2012). The synthesized compounds were tested in *vivo* and *vitro assays*. In *vivo* studies showed the ability of our lead compounds to penetrate the CNS and reverse the hypolocomotion of reserpinized rats in a PD animal model (Shah, Rajagopalan, Xu, Voshavar, Shurubor, Beal, Andersen, et al. 2014; Santra et al. 2013). The overall goal of designing and developing multifunctional small dopamine agonist molecules is to treat PD symptoms along with slowing or stopping the progression of the disease (Santra et al. 2013; Gogoi et al. 2011). A series of compounds based on the hybrid template structure was developed earlier by our group to target the critical factors involved in the pathogenesis of PD (Dutta, Fei, and Reith 2002; Johnson et al. 2012). The agonist head group was connected via a piperazine linker to other molecular moieties to produce various biological activities (Dutta, Fei, and Reith 2002; Johnson et al. 2012).

2.2.1. Development of multifunctional ligands to treat PD with neuroprotective properties.

Studies have reported that carbazole compounds exert their effects by enhancing the formation of neurons in the subgranular zone of the dental gyrus (Bashir et al. 2015; Głuszyńska 2015). Aminopropyl carbazole compounds P7C3 (**Figure 16**) was found to protect newborn neurons from apoptosis to enhance neurogenesis by stabilizing the mitochondrial membrane potential. P7C3 was also found to improve the function of the hippocampus, exhibit pro-neurogenic and show potent anti-oxidative activity (Głuszyńska 2015; Yin et al. 2014; Wang et al. 2014; Blaya et al. 2014). Carbazol compounds showed to block the degeneration of the neuronal cells in models of neurodegenerative diseases

(Yin et al. 2014). P7C3 was modified by introducing a benzophenone and alkyne groups to able the compound to bind to nicotinamide phosphoribosyltransferase (NAMPT) (Wang et al. 2014). It was assumed that the protective properties of this derivative could be due to their ability to activate the phosphoribosyl transferase, which is the rate-limiting step in the salvage of nicotinamide adenine dinucleotide, by converting nicotinamide into nicotinamide adenine dinucleotide (Yin et al. 2014; Loris, Pieper, and Dietrich 2017b). Animal experiments results showed that these derivatives have the ability recover the intracellular levels of NAD which lost due to the administration of doxorubicin (Wang et al. 2014). The lead compound, P7C3-S243 was reported to block the axonal cell death and conserve the normal synaptic activity which correlated with the motor coordination (Yin et al. 2014). It was also reported that these carbazole molecules and their derivatives exhibit the ability to regenerate neurons in the substantial nigra in neurodegenerative diseases.

Studies displayed that P7C3A20 carbazole derivative analogs of P7C3 displayed greater efficacy and affinity than P7C3 (De Jesús-Cortés et al. 2012; Shurubor, Beal, Andersen Julie, et al. 2014). The compound also inhibited the mature neuronal degeneration and, enhanced the perinatal neurogenesis in neurodegenerative and acute injury models (Wang et al. 2014). P7C3A20 also demonstrated to produce significant decline in the atrophy of the cortical, and hippocampal neurons (Loris, Pieper, and Dietrich 2017b). Given the importance of the development of neuroprotection therapies for neurodegenerative disease, different strategies to achieve such goals have become the focus of researchers in this area.

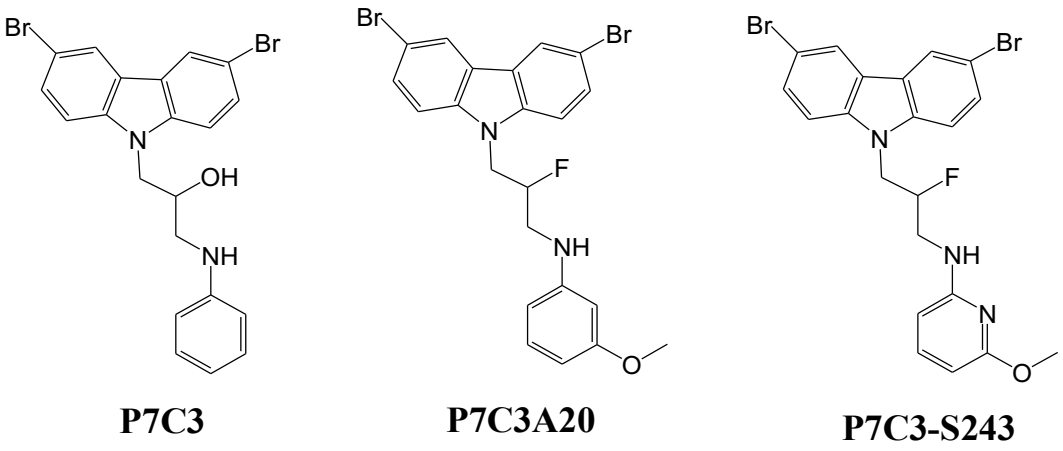


Figure 16. Molecular structure of lead carbazole compounds based neuroprotective property.

2.2.2. Development of multifunctional ligands with MAOI property as neuroprotective therapeutic agents for PD.

Monoamine oxidase (MAO) is a family of a flavin adenine dinucleotide (FAD)-dependent enzyme responsible for the metabolism of catecholamine neurotransmitter molecules (the biogenic amines: serotonin, norepinephrine, and dopamine) in the outer membrane of the mitochondria in the neurons (Garbis and McElhatton 2007). Two isoforms of MAO enzyme have been discovered: MAO-A and MAO-B. These isoforms differ in their distribution in the body and the substrate they metabolize. Serotonin and norepinephrine are the substrates of MAO-A while benzylamine is the substrate of MAO-B (Strydom et al. 2010). MAO-A and -B inhibitors are used as therapeutic medications in the treatment of neurological diseases. MAO-AI has been used in treatment of Depression, and MAO-BI is used in the treatment of PD. Some MAO-Is belong to the earlier class of drugs used in the treatment of PD (Strydom et al. 2010). The rationale behind the use of MAOIs in PD is to inactivate the metabolism of the neurotransmitters from being degraded to increase their level within the presynaptic neuron to escape into

the synaptic space to the site of action (Mongeau, Blier, and de Montigny 1997; Eisenhofer, Kopin, and Goldstein 2004). MAO-Is can also be classified into selective and non-selective inhibitors. Selective MAO-A inhibitors are effective in treating major depression while selective MAO-B inhibitors are effective in treating Parkinson disease (H and S 2006, Happe 2007; Dinesh et al. 2012). MAO-Is are used as a monotherapy or adjunct therapy to levodopa (L-DOPA) in the treatment of PD (Malco et al. ; Goldenberg 2008). The non-selective MAO-Is have restricted use in the treatment of depression in PD due to their adverse effects (Riederer and Laux 2011). MAO-Is are either reversible or irreversible. Reversible MAO-A inhibitors including tranylcypromine are recommended in the treatment of depression in PD. Selective irreversible MAO-B inhibitors including selegiline and rasagiline are preferred for the treatment of akinesia and motor fluctuations in PD (Finberg 2014; Entzeroth and Ratty 2017, J. Rojas et al. 2015) . Although, MAO-Is inhibit the MAO enzyme, they do not inhibit MAO synthesis pathway (H and S 2006). MAO-Is can also inhibit enzymes other than MAO, such as dopamine-β-oxidase, diamine-oxidase, amino-acid decarboxylase and choline dehydrogenase (H and S 2006). The non-selective inhibitory property of MAO-Is appears only with very high doses of the inhibitors, example: tranylcypromine has limited use in the treatment of depression in PD because of its non-selectivity, on the other hand; the selective, reversible MAO-A inhibitors are promoted due to their more straightforward medical management (Finberg 2014). Medications that augment the release and production of serotonin such as tricyclic antidepressants, selective serotonin reuptake inhibitors may cause the serotonin syndrome if they are administered with the MAOIs, even at therapeutic doses ('Antidepressants' ; Michael-Titus, Revest, and Shortland 2010). The toxicity of MAOIs

may be caused by the biogenic amines such as tyramine resulting in hypertensive crises (Santos 1996; M Gardner et al. 1996). MAO enzymes play an important role in the protection of the intestinal and the hepatic systems while by inhibiting the MAO enzymes, the protective role is eliminated due to increase the level of tyramine which releases noradrenaline from the presynaptic vesicles causing a significant effect in increasing the blood pressure (Foley et al. 2000; Noreddine 2016). MAO-Is lose their selectivity if they are given in high dose combination therapies (H and S 2006). Having a MAO-Is with multifunctional activity could be an avenue to overcome the side effects associated with the current MAO-Is, (Morphy, Kay, and Rankovic 2004). Several studies were done in this field in an effort to design and develop MFD with MAO-I activity to treat neurodegenerative diseases specially PD, examples: MT-19, MT-20, KW-6002, and CSC **(Figure 17)** (Bansal and Silakari 2014; Youdim and Buccafusco 2005).

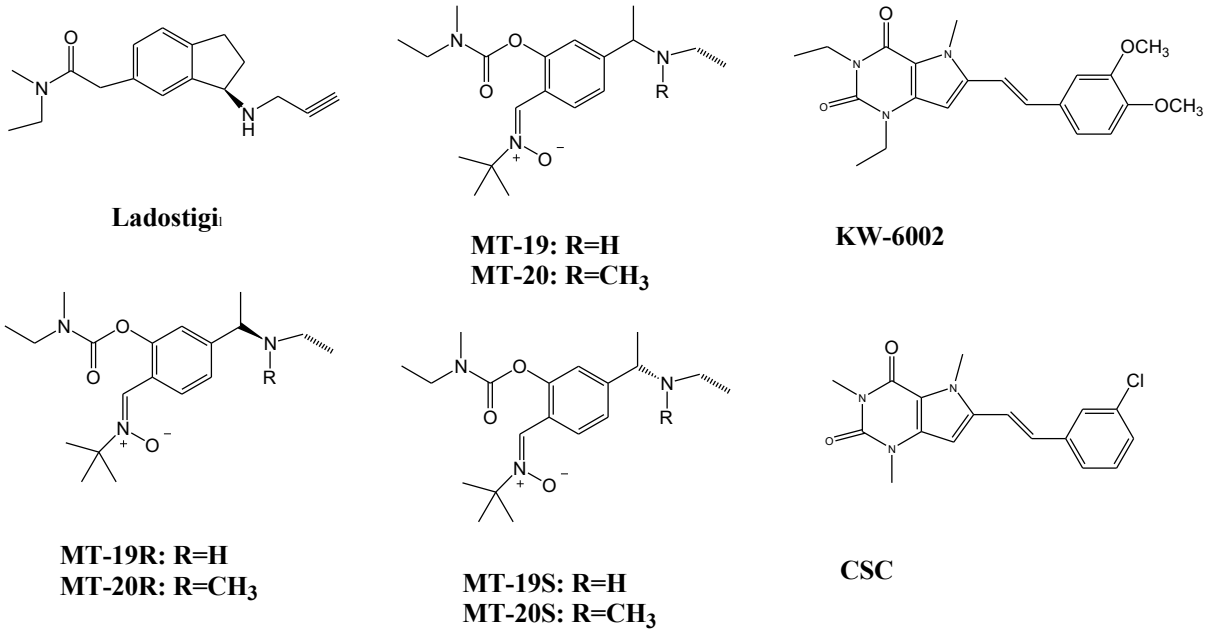


Figure 17. Structures of multimodal cholinesterase-monoamine oxidase inhibitor-iron chelator radical scavenger, and MAO-B and adenosine 2A receptor antagonists.

2.2.3. Development of multifunctional ligands to treat depression associated with PD.

According to the national institute of mental health, depression has been defined as a common severe mental disorder that affects the lives of people from the point of feeling, thinking, handling the regular activities including sleeping, eating, and working. People with depression categorized with the loss of pleasure and interest of activity (Slattery and Cryan 2012). Depression affects a high percentage of the population (between 15-20%) in the United States, and around 450 million people in the world (Anxiety and Depression Association of America). Depression can be categorized according to the severity, the period of the disease, and the way it develops under certain circumstances ('Neurocognitive Disorders').

According to the World Health Organization (WHO), depression is an enervating disease which classified as the second-most cause of disability worldwide. People who suffer from depression are more susceptible to committing suicide (Gopishetty et al. 2011c). According to the National Alliance of Mental Illness (NAMI), the cause of depression is not fully understood, it could be due to the combination of biological, genetical and environmental factors. Other aspects also could be involved in the implication of depression such as the experiences of trauma in the early age.

Although medications are approved in the market to treat depression, it is estimated that 30-40% of patients do not respond sufficiently to them. The therapies for depression include tricyclic antidepressants (TCA), and monoamine oxidase inhibitors (MAO-I) (Oestergaard and Møldrup 2011). Tricyclic antidepressant and monoamine oxidase inhibitors medications were the first used as antidepressant drugs in the market.

Some of the examples include imipramine, amitriptyline, nortriptyline, phenelzine, and tranylcypromine **(Figure 18)** (Lopez-Munoz et al. 2007). However, although antidepressant drugs have been used for the treatment of depression over decades, they were plagued by their nonspecific interactions that cause severe side effects that may be due to their nonspecific binding (Cohen et al. 1982; Sarker et al. 2010; Taylor et al. 2005). The first-generation antidepressant medications were subsequently replaced by selective serotonin reuptake inhibitor (SSRI), selective norepinephrine reuptake inhibitor (SNRI), and dual reuptake inhibitor (SSRI/SNRI) which are currently in use such as fluoxetine, trazodone, and venlafaxine **(Figure 18)** (Lopez-Munoz et al. 2007; Hansen et al. 2005). The second generation antidepressant drugs were developed to overcome the challenges of the first generation by enhancing the effectiveness and decreasing the undesirable outcomes (Hansen et al. 2005). A significant proportion of individuals suffering from depression continue to suffer from their symptoms under the current therapies. Dual reuptake inhibitors still exhibit slow onset of action, low rate of response and side effects (Stahl et al. 2004). Dopaminergic activity has not been included in the current antidepressant therapy, when dopamine has been implicated strongly in depression (Gartlehner G ; Santra, Gogoi, Gopishetty, Antonio, Zhen, Reith, et al. 2012). Preclinical and clinical studies, demonstrated anhedonia as a central component of depression, which develops due to deficit in dopamine activity (Dichter 2010). As the mesolimbic dopamine is associated with reward-related behavior (anhedonia), dopaminergic drug should address anhedonia in depression (Papp, Klimek, and Willner 1994). Therefore, triple uptake inhibitor which includes dopaminergic activity should address the unmet need to treat depression in PD (Sharma, Santra, and Dutta 2015).

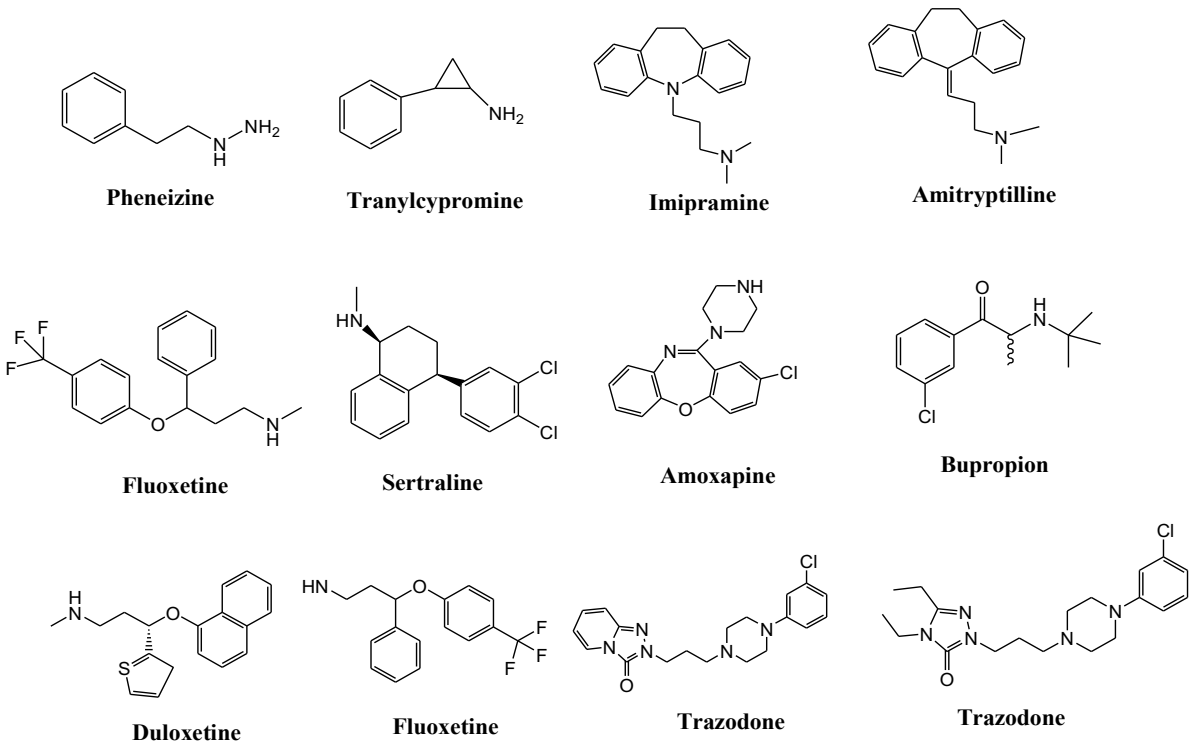


Figure 18. Major classes of antidepressant drugs.

Evolution of triple reuptake inhibitors as the next-generation to treat depression would be more efficacious (Liang and Richelson 2008; Sharma, Santra, and Dutta 2015). Triple reuptake inhibitor is a novel approach to develop new generation antidepressant which has been introduced recently to overcome the constraint associated with the current antidepressant drugs (Bruno, Mostafa El, and Pierre 2009). Researchers have found that treating depression in Parkinson's patients in addition to treating the motor symptoms would improve the overall quality of life (Titova and Chaudhuri 2017). Indeed, researchers have also assumed that people with PD might have a higher number of reuptake pumps for the serotonin, the brain chemical messenger, than normal people (De Jesús-Cortés et al. 2012; 'Depression and Parkinson's disease: a review' 1992; Marsh 2013; Cummings 1992). Studies showed that Parkinson's people have high risk of developing depression than normal population. Developing medications to treat

depression in PD would be a novel approach (Hemmerle, Herman, and Seroogy 2012). Our group has been actively embarked on the development of 3,6-disubstituted and 2,4,5-trisubstituted pyran derivatives targeting monoamine transporters (Santra, Gogoi, Gopishetty, Antonio, Zhen, Reith, et al. 2012). These pyran derivatives showed higher affinity for NET and SERT and moderate affinity for dopamine transporter (DAT) when compared with the piperidine counterparts (Zhang et al. 2005a). One of the explanations of the lower affinity for DAT could be due to the replacement of N-atom in the piperidine analogous by a less basic O-atom (Zhang et al. 2005a). The lead TUIs D-142, D-161 were designed and found to be effective in animal models of depression to show antidepressant-like activity (Santra, Gogoi, Gopishetty, Antonio, Zhen, Reith, et al. 2012; Gopishetty et al. 2011a). D-473 is another TUIs was tested in animal for its penetration to the brain, rat forced swim and locomotor activities. The compound showed good brain penetration and effective activity in rat forced swim test but did not exhibit any locomotion activity (Dutta et al. 2014). D-142 exhibited potent antidepressant activity by inhibiting the reuptake of serotonin, norepinephrine and dopamine, it also showed to diminish the immobility in the mouse tail suspension test (**Figure 19**) (Dutta et al. 2011).

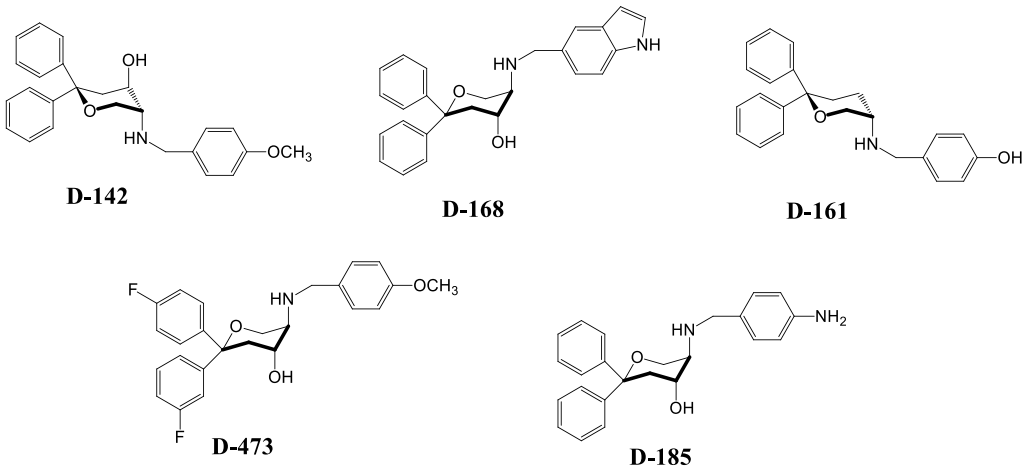


Figure 19. Molecular structure of lead pyran-molecule based triple uptake inhibitors.

Chapter 3- Hypothesis and Specific aims.

Parkinson's disease is the second most common neurodegenerative disorders that is considered as an age-related disease affecting a worldwide population (Hindle 2010; Collier, Kanaan, and Kordower 2011). The symptoms of PD are categorized into motor and non-motor symptoms (Visanji and Marras 2015). According to the current Parkinson's theory (Braak's hypothesis) , the non-motor symptoms may appear early before the motor symptom, resulting in an early diagnosis of the disease that might help to prevent the progression of the disease (Visanji et al. 2013). The existing treatments for PD are classified into monotherapy and multitherapy (a combination of two medications), the monotherapy includes levodopa, monoamine oxidase B (MAO-B), inhibitors and dopamine agonists while the multitherapy includes catechol-O-methyl transferase (COMT) inhibitor with levodopa or dopamine agonists with levodopa (Amos D. Korczyn 2004). However, these medications are only able to improve the symptoms of the disease. Additionally, they produce severe side effects such as dyskinesia and motor fluctuation from long-term therapy with L-DOPA (Dushanova 2012; Sheikh et al. 2012).

3.1. Hypothesis

Parkinson's disease (PD) is a chronic progressive neurodegenerative disease with multiple pathogeneic factors (Sheikh et al. 2012; Facecchia et al. 2011; Alves et al. 2008). To address the complexity of the disease process, compounds targeting several pathogenic features relevant to PD were developed to offer an advantage to treat the illness over a single targeting drug. The overall goal and hypothesis of this work is to design and develop multifunctional D_2/D_3 dopamine agonists to treat not only motor dysfunction symptoms in PD but also to provide disease modifying effects to slow the

progression of the disease. By addressing multiple pathogenic factors implicated in the pathogenesis of PD using multifunctional compounds, the progression and the symptomatic aspects of Parkinson's disease could be potentially reduced or slowed. The proposed compounds are multifunctional dopamine D2/D3 receptors agonists with neuroprotective, antioxidants properties. Carbazole derivatives and MAO inhibitors have been demonstrated to exhibit neuroprotective properties and as such incorporation of these moieties in our multifunctional dopamine agonist template will address not only symptomatic aspect of the disease but also should provide neuroprotective properties (Głuszyńska 2015? ; Gopishetty et al. 2011b?). The other goal of this work is to develop multifunctional small molecules to block the monoamine transporter based on unique pyran template that has been previously developed and established to produce monoamine transporter blocking activity which would not only treat symptomatic aspects of the disease but would also have the potential to treat depression, non-motoric symptom, accompanied with PD as a promising approach for new generation to treat PD (Gopishetty et al. 2011b).

3.2. General aim.

The general aim of this project is to design a library of D_2/D_3 dopamine agonist compounds by linking D_2/D_3 agonist moiety to other pharmacophore moieties with different functional activities to produce neuroprotective and antioxidant activities to treat PD. The synthesis of the lead compounds was relied on the hybrid template that previously established and developed to conduct a structure-activity relationship (SAR) study by linking D_2/D_3 agonist moiety to either novel carbazole moiety or pharmacophore of MAO-inhibitors. Another approach focussed on the development of unique pyran

template that has been designed and established to produce monoamine transporter blocking activity and the lead compounds were found to be efficacious both *in vitro* and *in vivo* assays.

3.2. Specific aims.

To accomplish the goal in this project, the following specific aims were proposed:

a. Design and synthesize of novel carbazole based multifunctional dopamine D_2/D_3 agonists that can potentially exhibit neuroprotective and antioxidant activities. The molecules are designed by linking carbazole moieties with D_2/D_3 agonist moiety through a piperazine linker based on the hybrid molecular template. A SAR study has been carried out to assess the effect of different molecular attachment of carbazole moiety at different position with the different D_2/D_3 agonist moieties.

b. Design and synthesis of D_2/D_3 agonists that can inhibit monoamine oxidase enzyme. The molecules were designed by linking the propargyl containing group to D_2/D_3 agonist through a piperazine linker based on hybrid molecular template. A SAR study has been carried out to evaluate the effect of MAO inhibiton activity by linking propargyl moiety to the different D_2/D_3 agonist moieties.

c. Design and synthesis of D_2/D_3 agonists that could potentially block the monoamine reuptake transporters by incorporating the novel pyran moiety to build the structure-activity relationship study.

3.2.1. *In Vitro* binding Studies (Radioligand binding assay).

The synthesized compounds have been assessed for their binding affinity and selectivity towards the human D_2 and D_3 dopamine receptors using *in vitro* competitive radioligand binding assays.

3.2.2. *In Vitro* Functional Studies ([^{35}S] GTPγS Binding *in vitro* functional assay).

Based on the binding affinity, the Selected compounds were further evaluated for their functional activity through the *in vitro* functional assay.

3.2.3. *In vitro* neuroprotection study.

Selected lead compounds containing carbazole moiety have been also evaluated for their neuroprotective effect to protect PC12 cells from toxicity induced by 6-hydroxydopamine (6-OHDA) in vitro cellular neurotoxin-based model for PD.

3.2.4. *In vitro* Antioxidant study.

In addition to the neuroprotection assay, selected lead compounds containing the carbazole moiety have been assessed for their ability to reduce the reactive oxygen species generated by 6-OHDA in PC12 cells.

3.2.5. *In vitro* Monoamine oxidase inhibitors assay.

Selected lead compounds containing propargyl moiety have been tested for their ability to inhibit MAO enzyme activities using *in vitro* enzymatic assays.

3.2.6. *In vivo* assay with rat model of PD.

Selected lead compounds have been further evaluated for their efficacy and potency to reverse the hypolocomtion in reserpine-treated rat model, a PD animal model for symptomatic activity.

*** CHAPTER 4 & 5 CONTAIN MATERIAL FROM PUBLISHED WORK IN WHICH I WAS THE FIRST AUTHOR. THE CO-AUTHORS OF THESE PUBLICATIONS AGREE TO THE USE OF THE PUBLISHED DATA IN THIS DISSERTATION.**

Chapter 4- Result and discussion

4.1. Overview.

This project has three main objectives. The first objective is to design multifunctional D_2/D_3 agonist molecules with neuroprotection and antioxidant properties to address multiple pathogenic factors of PD. Design of such hybrid molecular template by combining D_2/D_3 agonist head groups to carbazole moiety that might be suitable to modulate the pathogenic pathway of PD to produce neuroprotective properties. Lead compounds were identified from *in vitro* receptor binding and functional assays. Based on the D_2/D_3 binding assay, compounds were selected and subjected to the GTPγS functional assay. Compounds with agonist activity were selected for further *in-vitro* neuroprotection and antioxidant assays. Subsequently, lead compounds were subjected to *in-vivo* assay using a well-established Parkinson's disease (PD) animal model. The second goal is to develop and design a series of multifunctional D_2/D_3 receptor agonist with monoamine oxidase B (MAO-B) inhibitory activity. The development was based on introduction of a propargyl group into the hybrid template. The third objective is to design and synthesize small molecules as Triple reuptake transporter inhibitors that can have the potential to treat depression, symptomatic aspects and non-motoric symptoms of PD based on the pyran template.

This chapter describes the chemistry involved in generating the library, the data from the in vitro binding and functional receptors assay, neuroprotection and antioxidants activities

of selected compounds as well as the in vivo efficacy. The details of all the experiments and procedures will be explained in chapter 5.

4.2. Design, Synthesis and Pharmacological Characterization of Carbazole Based Dopamine Agonists as Potential Symptomatic and Neuroprotective Therapeutic Agents for Parkinson's Disease.

In continuing our work to design multifunctional dopamine D_2/D_3 receptor agonists to address multiple pathogenic factors of PD and the symptomatic aspects involved led us to embark upon a drug discovery approach focused on novel multifunctional dopamine D_2/D_3 agonist molecules. Thus, we have shown from our recent studies that lead molecule like D-512 and D-607 **(Figure 20)** not only to have the potential to provide robust symptomatic effect but also produced potent neuroprotective effects in various in vitro and in vivo experiments (Shah, Rajagopalan, Xu, Voshavar, Shurubor, Beal, Andersen, et al. 2014; Johnson et al. 2012; Das, Kandegedara, et al. 2017; Das, Rajagopalan, et al. 2017). In our current study, we carried out our structure activity relationship (SAR) study on carbazole based molecules that have previously been identified to have neuroprotective properties (Gluszynska 2015; Wu et al. 2017; Wang et al. 2016; MacMillan et al. 2011). Specifically, our hybrid structure strategy **(Figure 21)** which combines D_2/D_3 agonist head groups with other moieties that are suitable to modulate the pathogenic pathway of PD, led to development of molecules to validate our proof of concept (Das, Kandegedara, et al. 2017; Biswas et al. 2008; Das et al. 2015; Modi et al. 2014; Luo et al. 2016; Johnson et al. 2012). Previous studies have shown that carbazole containing compounds exert neuroprotective properties by enhancing the formation of neurons in the subgranular zone of the dental gyrus (Pieper et al. 2010; Loris, Pieper, and

Dietrich 2017a). Moreover, scientists have reported that carbazole molecules and their derivatives exhibit ability to regenerate the neurons in the substantia nigra in neurodegenerative disease (Wang et al. 2016; Yoon et al. 2013; Loris, Pieper, and Dietrich 2017a). Based on these findings, we designed and developed a number of multifunctional molecules by covalently attaching D_2/D_3 agonist head groups such as pramipexole and 5-OH-DPAT to various carbazole moieties through a piperazine linker **(Scheme 1-3).**

In our current work, a series of compounds were synthesized, and the selected compounds were characterized by *in vitro* binding and GTPγS functional assays to examine the affinity and potency at both D_2 and D_3 receptors. The selected compounds were subjected to further *in vitro* experiments to evaluate the neuroprotection and antioxidants properties. In addition to the *in vitro* evaluation, PD animal model study was established to assess *in vivo* activity in the reserpinized rats.

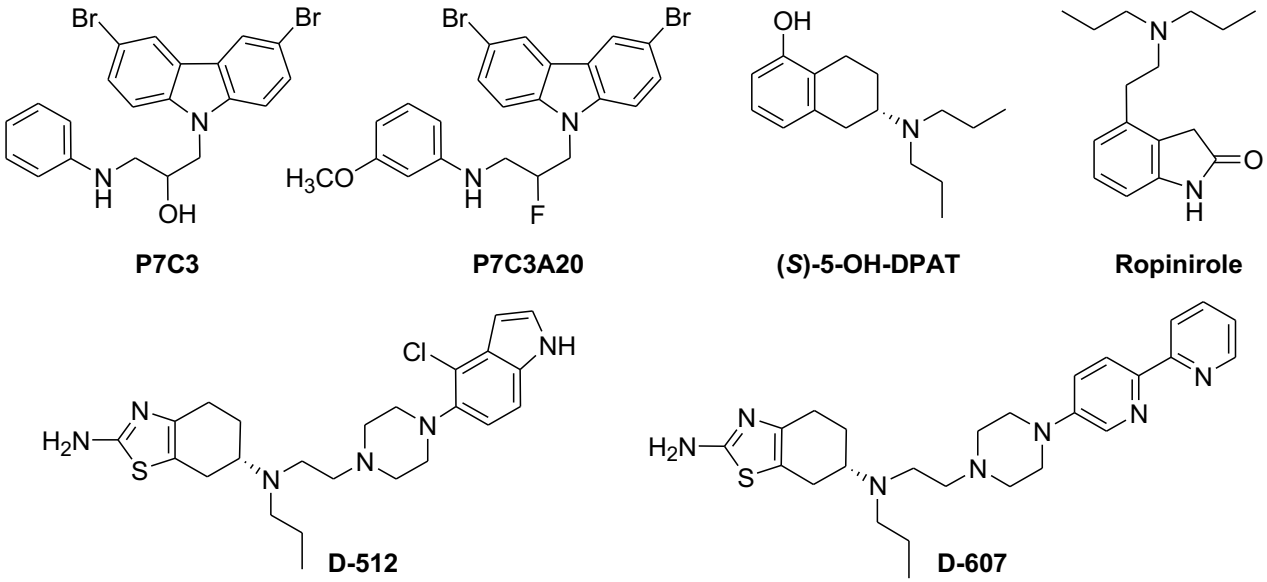


Figure 20. Molecular structures of dopamine D_2 /D_3 receptor agonists, and carbazole compounds with neuroprotective properties.

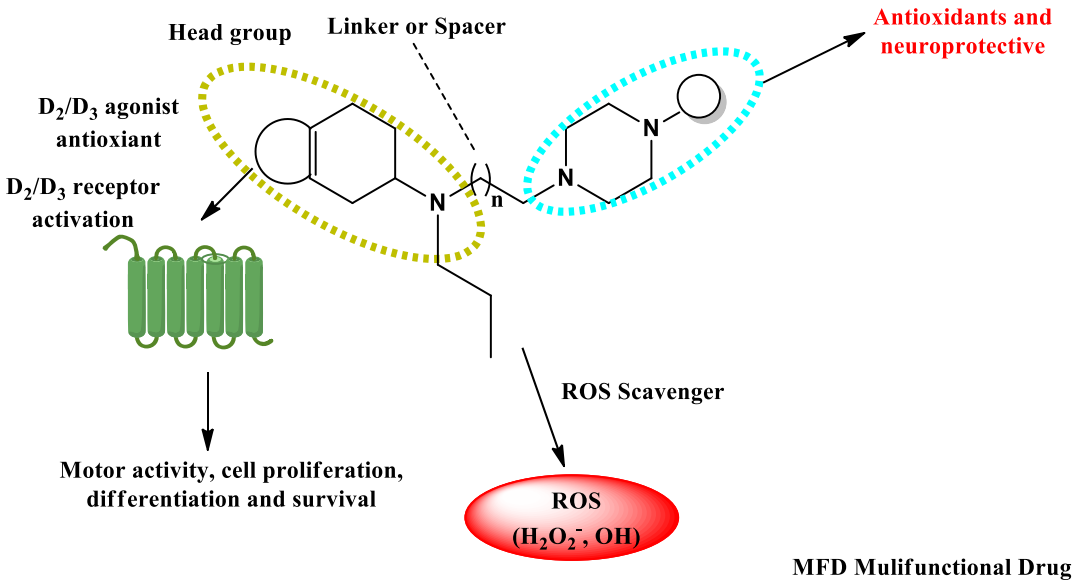
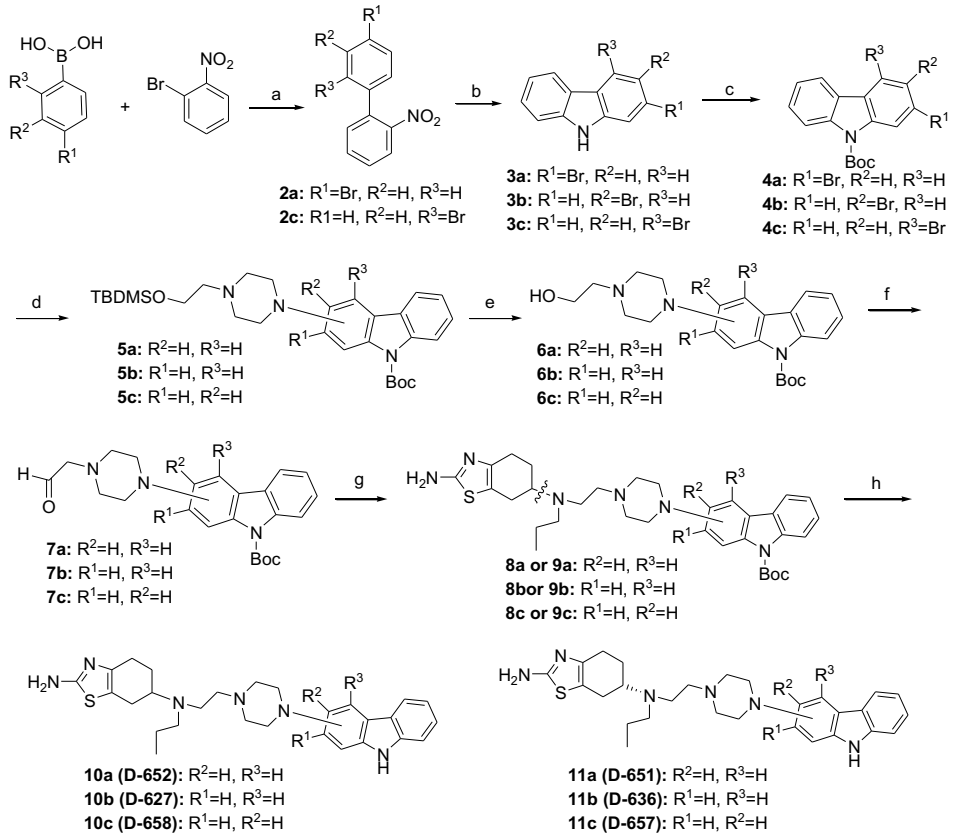


Figure 21. The hybrid molecule template for multifunctional dopamine D_2/D_3 receptor agonists.

4.2.1. Chemistry involved in the synthesis of carbazole based D2/D3 agonists.

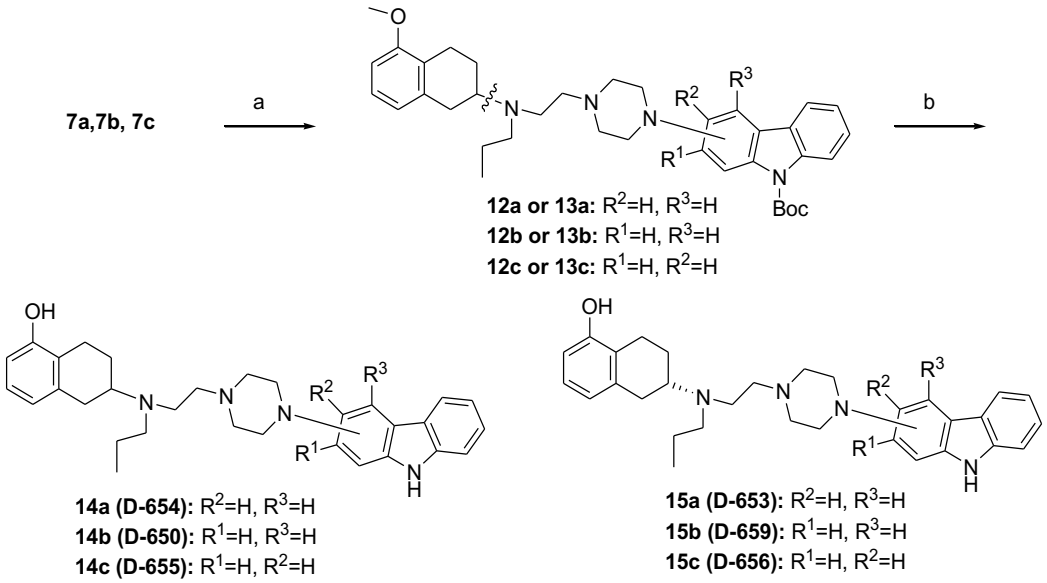
In this study, a series of compounds were synthesized by incorporating the agonist head group (aminotetralin or bioisosteric equivalent) with carbazole functionality via ethylpiperazine linker (scheme 1, 2 and 3). **Scheme 1** outlines the syntheses of final compounds (±)-**10a**, (±)-**10b**, (±)-**10c** and (-)-**11a**, (-)-**11b**, (-)-**11c**. A palladium catalyzed coupling of (4-bromophenyl)boronic acid and 1-bromo-2-nitrobenzene afforded 4'-bromo-2-nitro-1,1'-biphenyl (**2a**), which was then subjected to cyclization in the presence of PPh_3 to get 2-bromo-9*H*-carbazole (**3a**) (Kim and Lee 2013) followed by *N*-protection using di-*tert*-butyl dicarbonate in the presence of 4-dimethylaminopyridine (4-DMAP) to afford **4a**. Palladium-catalyzed cross coupling of **4a** with 1-(2-((*tert*-butyldimethylsilyl)oxy)-ethyl)piperazine (Das et al. 2015) in the presence of Cs_2CO_3 and BINAP in toluene under refluxing condition yielded intermediate **5a**. The silyl protecting group of compound **5a** was removed by treatment with *n*-Bu_4NF (TBAF) in THF to afford the alcohol **6a**, which

on subsequent oxidation in the presence of pyridine-sulfur trioxide yielded the corresponding aldehyde **7a**. Reductive amination of the aldehyde with either (±) or (-)-pramipexole in the presence of $NaBH(OAc)_3$ afforded compounds **8a** and **9a**, respectively. Finally, the amine protecting *t*-Boc groups were removed by treatment with trifluoroacetic acid to furnish the final compounds (±)-**10a** and (-)-**11a** as TFA salts. The other final compounds (±)-**10b**, (±)-**10c**, (-)-**11b** and (-)-**11c** were also prepared in a similar fashion as described above, where 3-Bromo-9*H*-carbazole and 4-Bromo-9*H*-carbazole were used as the starting materials, respectively.



Scheme 1. Synthesis of the carbazole compounds **10a, 10b, 10c, 11a, 11b,** and **11c**. Reagents and conditions: a) $Pd(PPh_3)_4$, 2M K_2CO_3, THF, 90 °C, 12 h; b) PPh_3, 1,2-dichlorobenzene, 170 °C, 12 h; c) $(Boc)_2O$, 4-DMAP, THF, rt, overnight; d) 1-[2-(tert-butyl-dimethyl-silanyloxy)-ethyl]-piperazine, $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, reflux, 24 h; e) n-Bu_4NF, THF, 0 °C to rt, 2 h; f) SO_3.py, CH_2Cl_2:DMSO (2:1), Et_3N, 0 °C to rt, 2 h; g) (±) or (-)-pramipexole, $NaBH(OAc)_3$, CH_2Cl_2, rt, 48 h; h) CF_3COOH, CH_2Cl_2, 0 °C to rt, 3 h.

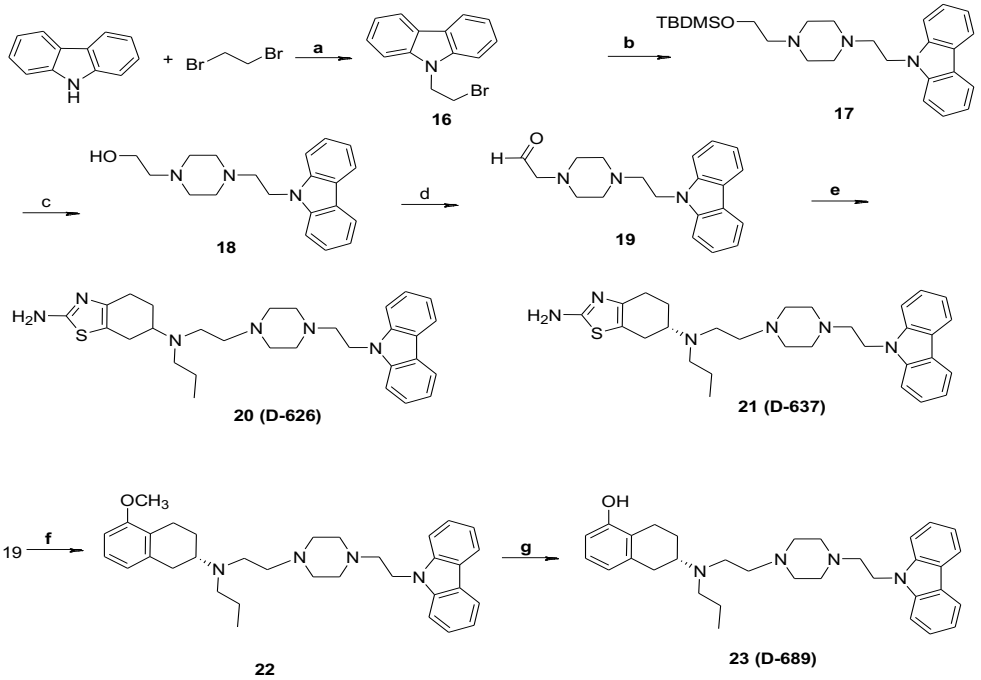
Scheme 2 depicts the syntheses of the final compounds (±)-**14a**, (±)-**14b**, (±)-**14c** and (-)-**15a**, (-)-**15b**, (-)-**15c**. To prepare these compounds, we employed (±) and (-)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine and reductively alkylated with intermediate aldehydes **7a-7c** in the presence of $NaBH(OAc)_3$ as the reducing agent to afford compounds (±)-**12a**, (±)-**12b**, (±)-**12c,** (-)-**13a**, (-)-**13b**, and (-)-**13c**. Finally, demethylation and removal of the amine protecting *t*-Boc groups were carried out in one step by refluxing with aq. HBr to give the final compounds (±)-**14a**, (±)-**14b**, (±)-**14c,** (-)-**15a**, (-)-**15b**, and (-)-**15c** as HBr salts.



Scheme 2. Synthesis of the compounds **(±)-14a** (**D-654**), **(±)-14b (D-650)**, **(±)-14c (D-655)**, **(-)-15a (D-653), (-)-15b (D-659)** and **(-)-15c (D-656)**. Reagents and conditions: a) (±) or (-)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine, $NaBH(OAc)_3$, CH_2Cl_2, rt, 48 h; b) 48% aq. HBr, reflux, 5 h.

The synthesis of three more target compounds (±)-**20**, (-)-**21,** and (-)-**23** are illustrated in **scheme 3**. *N*-alkylation was first performed by refluxing carbazole with dibromoethane in presence of a mixture of K_2CO_3, KOH and TBAB to afford 9-(2-bromoethyl)-9*H*-carbazole **16**. Base-catalyzed condensation of 1-(2-((*tert*-butyldimethylsilyl)-oxy)ethyl)piperazine with intermediate **16** yielded compound **17**, which

on TBDMS deprotection in the presence of TBAF in THF afforded alcohol **18** in excellent yield. Alcohol **18** was next oxidized in the presence of pyridine-sulfur trioxide to yield the corresponding aldehyde **19**, which was then condensed with (±) or (-)- pramipexole in the presence of $NaBH(OAc)_3$ to afford compounds (±)-**20** and (-)-**21**. These two molecules were converted to their corresponding HCl salts by treatment with ethereal HCl. To prepare compound **(-)-23** we employed (-)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine and reductively alkylated with intermediate aldehydes **19** in the presence of $NaBH(OAc)_3$ as the reducing agent to afford compound **(-)-22**. Finally, demethylation and removal of the amine protecting *t*-Boc groups were carried out in one step by refluxing with aq. HBr to give the final compounds **(-)-23** as HBr salts. All the final compounds were characterized by ^{1}H and ^{13}C NMR as well as elemental analysis.



Scheme 3. Synthesis of compounds (±)-**20 (D-626)**, (-)-**21 (D-637),** and **(-)-23 (D-689)**. reagents and conditions: a) K_2CO_3, KOH, TBAB, 50 °C, overnight;b) K_2CO_3, CH_3CN, reflux, 24 h;c) n-Bu_4NF, THF, 0 °C to rt, 3 h; d) SO_3.py, CH_2Cl_2:DMSO (2:1), Et_3N, 0 °C to rt, 2h; e) (±) or (-)-pramipexole, $NaBH(OAc)_3$, CH_2Cl_2, rt, 48 h.f) (-)-5-methoxy-1,2,3,4-

tetrahydro-naphthalen-2-yl)-propyl-amine, $NaBH(OAc)_3$, CH_2Cl_2, rt, 48 h; g) 48% aq. HBr, reflux, 5 h.

4.2.2. *In vitro* characterization of the Carbazole Based Dopamine Agonists molecules.

4.2.2.1. Potency and Agonism at DA D_2 and D_3 Receptors.

Our ongoing effort to develop multifunctional compounds for the symptomatic and disease-modifying treatment of PD is based on a hybrid drug design approach in which DA agonist head groups are covalently attached to a variety of moieties capable of producing biological effects which might be important for modulating the pathogenesis of PD (Das, Modi, and Dutta 2015; Dutta, Fei, and Reith 2002; Li et al. 2010; Shah, Rajagopalan, Xu, Voshavar, Shurubor, Beal, Andersen, et al. 2014; Yedlapudi et al. 2016; Das, Rajagopalan, et al. 2017; Das, Kandegedara, et al. 2017). Recently, we have developed compounds such as D-512 **(Figure 20)**, which have revealed superior antiparkinsonian effects *in vivo* over a clinically approved drug, ropinirole (Lindenbach et al. 2017). Selected drugs also displayed neuroprotective properties in a myriad of *in vivo* and *in vitro* assays validating our proof of concept and thus, provide a strong support for our multifunctional drug development approach (Lindenbach et al. 2017; Das, Rajagopalan, et al. 2017; Das, Kandegedara, et al. 2017; Shah, Rajagopalan, Xu, Voshavar, Shurubor, Beal, Andersen, et al. 2014; Santra et al. 2013; Yedlapudi et al. 2016; Li et al. 2010). As a part of continuing our work, we have now designed and synthesized a series of molecules where covalent modification of core carbazole moiety at different positions has been incorporated into our hybrid D_2/D_3 agonist template. The rationale of using the carbazole is based on the fact that a novel aminopropyl carbazole P7C3 and its analogue P7C3A20 **(Figure 20)** have shown proneurogenic and

neuroprotective properties in aged rats, stabilized mitochondrial membrane potential and inhibited newborn hippocampal neuron apoptosis (Pieper et al. 2010). Our current study is aimed at investigating the influence of molecular and chemical flexibility of the carbazole fragment when attached to our hybrid template as it relates to D_2/D_3 receptor binding and functional activity along with neuroprotection potential.

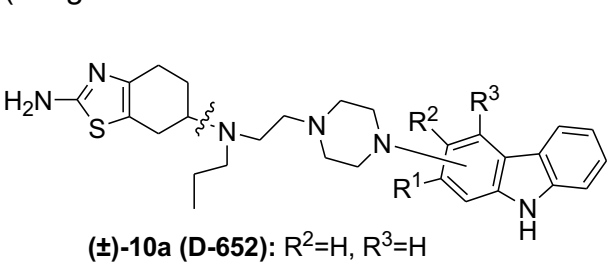
To evaluate receptor binding of the final compounds, a radioligand competition assay was conducted and the binding affinity profiles were compared with that of the reference agent (*S*)-5-OH-DPAT **(Table 1).** Binding affinity was determined by inhibition of [^{3}H] spiroperidol binding to rat DA D_2 and D_3 receptors expressed in HEK-293 cells as described by us previously (Biswas et al. 2008). **Table 1** summarizes the binding data for analogues that were synthesized. Compounds (±)-**10a-c**, which incorporate racemic 2-aminothiazole head group and a piperazine ring connected to the different positions of carbazole ring, displayed high affinity for D_3 and low to moderate affinity for D_2 receptors. When the positions of attachment are at carbon 2 and 3 of the carbazole moiety for compounds **10a** and **10b,** respectively, both the compounds displayed low affinity for D_2 and high affinity for the D_3 receptors with high selectivity (K_i, D_2 = 902 nM, D_3 = 6.18 nM, D_2/D_3 = 146 and D_2 = 612 nM, D_3 = 3.12 nM, D_2/D_3 = 196 for **10a** and **10b,** respectively). Interestingly, covalent attachment at position 4 of the carbazole ring dramatically improved the affinity for D_2 while that for D_3 receptor remained the same (K_i, D_2 = 76.9 nM, D_3 = 7.8 nM, D_2/D_3 = 9.86 for **10c**). This indicated highest tolerance of the 4-substituted carbazole derivative for interaction with the D_2 and D_3 receptors. As expected, we observed a 2-4-fold improvement in binding affinity when enantiomerically pure aminothiazole moiety was attached to the carbazole as in (-)-**11a** and (-)-**11b** compared

to their racemic counterparts (K_i, D_2 = 504 nM, D_3 = 3.94 nM, D_2/D_3 = 128 and D_2 = 135 nM, D_3 = 3.80 nM, D_2/D_3 = 35 for (-)-**11a** and (-)-**11b** respectively). However, for (-)-**11c** we did not observe much difference from its racemic version.

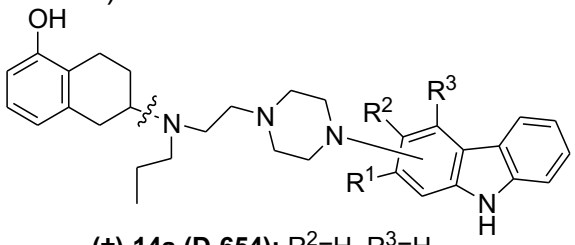
Next, we wanted to evaluate the effect of bioisosteric replacement of the aminothiazole moiety with aminotetraline functionality on the receptor binding of target compounds. In corroboration with our previous results (Ghosh et al. 2010a; Das, Kandegedara, et al. 2017). Aminotetraline substituted compounds (±)-**14a-c** and (-)-**15a-c** exhibited high affinity at both D_2 and D_3 receptors. For instance, the aminotetraline analogue **(-)-15a** has been found to have very high affinity for D_2 and subnanomolar preferential affinity for D_3 receptor compared to the corresponding thiazolidium counterpart (-)-**11a** (K_i, D_2 = 71.2 nM, D_3 = 0.40 nM, D_2/D_3 = 177 for (-)-**15a** vs D_2 = 504 nM, D_3 = 3.94 nM, D_2/D_3 = 128 for (-)-**11a**). Among the three enantiomerically pure isomers (-)-**15a-c**, which differ only in the substitution positions at the carbazole moiety, positions 2, 3 and 4, showed variable binding affinity at both D_2 and D_3 receptors (K_i, D_2 = 71.2 nM, D_3 = 0.40 nM for (-)-**15a** (; D_2 = 61.6 nM, D_3 = 1.94 nM for (-)-**15b** and D_2 = 16.9 nM, D_3 = 0.36 nM for (-)-**15c**). As discussed before, substitution at the 4-position of the carbazole aromatic ring resulted in compounds **10c**, (-)-**11c**, **14c** and (-)-**15c**) with better D_2/D_3 binding affinities in comparison to other isomeric analogues with compound (-)-**15c** exhibiting the highest affinity among all the molecules, underscoring the importance of positional attachment to the carbazole ring. Finally, the binding affinities were evaluated for another series of compounds in which the piperazine ring of the agonist fragment was appended directly to the carbazole nitrogen atom through a methylene linker. As shown in Table 1, enantiomeric compound (-)-**21** displayed relatively

higher binding affinity at D_2 and comparable affinity at D_3 receptor with moderate selectivity compared to the racemic compound (±)-**20** (K_i, D_2 = 435 nM, D_3 = 6.60 nM, D_2/D_3 = 65.9 and D_2 = 82.6 nM, D_3 = 7.18 nM, D_2/D_3 = 12 for **20** and (-)-**21,** respectively). This structural modification suggests no significant differences in DA receptor interaction between compounds where the carbazole moiety is attached either at the 2/3 positions of the aromatic ring or through the nitrogen atom; however, a prominent difference exists for compounds where the carbazole nitrogen is sterically free to probably participate in additional receptor interaction (e.g. (-)-**15c** vs (-)-**15b** and (-)-**21**).

Table 1. K_i values (nM) for inhibition of [^{3}H] spiroperidol binding (HEK - $D_{2,3}$ cells)[a] (cLogP and tPSA values are calculated using ChemDraw)



(±)-10a (D-652): R^2=H, R^3=H
(±)-10b (D-627): R^1=H, R^3=H
(±)-10c (D-658): R^1=H, R^2=H
(-)-11a (D-651): R^2=H, R^3=H
(-)-11b (D-636): R^1=H, R^3=H
(-)-11c (D-657): R^1=H, R^2=H



(±)-14a (D-654): R^2=H, R^3=H
(±)-14b (D-650): R^1=H, R^3=H
(±)-14c (D-655): R^1=H, R^2=H
(-)-15a (D-653): R^2=H, R^3=H
(-)-15b (D-659): R^1=H, R^3=H
(-)-15c (D-656): R^1=H, R^2=H

Compound	K_i (nM)				
	D_{2L}, [^{3}H]spiroperidol	D_3, [^{3}H]spiroperidol	D_{2L}/D_3	cLogP	tPSA
(-)-**5-OH-DPAT**	153 ± 32	2.07 ± 0.38	74		
(±)-**10a**	902 ± 132	6.18 ± 0.91	146	4.87	60.13
(±)-**10b**	612 ± 92	3.12 ± 0.62	196	4.87	60.13
(±)-**10c**	76.9 ± 5.2	7.8 ± 1.17	9.86	4.87	60.13
(-)-**11a**	504 ± 50	3.94 ± 0.62	128	4.87	60.13
(-)-**11b**	135 ± 12	3.8 ± 0.38	35.4	4.87	60.13

(-)-**11c**	92.4 ± 8.5	4.18 ± 0.47	22.2	4.87	60.13
(±)-**14a**	62.1 ± 7.3	2.85 ± 0.62	21.8	6.12	41.98
(±)-**14b**	37.8 ± 4.7	1.87 ± 0.41	20.2	6.12	41.98
(±)-**14c**	29.4 ± 1.3	3.61± 0.28	8.13	6.12	41.98
(-)-**15a**	71.2 ± 9.6	0.400 ± 0.038	177	6.12	41.98
(-)-**15b**	61.6 ± 3.8	1.94 ± 0.18	31.8	6.12	41.98
(-)-**15c**	16.9 ± 1.9	0.362 ± 0.032	46.9	6.12	41.98
(±)-**20**	435 ± 90	6.60 ± 1.13	65.9	5.11	
(-)-**21**	82.6 ± 13.8	7.18 ± 0.86	11.5	5.11	

[a] Results are expressed as means ± SEM for 3-6 experiments each performed in triplicate.

On the basis of the binding results, functional activities of the optically active lead compounds for human DA D_2 and D_3 receptors expressed in CHO cells were measured by monitoring stimulation of [^{35}S] GTPγS binding in comparison to stimulation by the endogenous ligand DA (Biswas et al. 2008). Comparison with the maximum stimulation (E_{max}), produced by the full agonist DA, indicates whether the compound is a full agonist, a partial agonist, or an antagonist. As shown in **Table 2**, aminothiazole containing compounds (-)-**11b** and (-)-**11c** demonstrated moderate potency at both D_2 and D_3 receptors (EC_{50} (GTPγS); D_2 = 48.7, D_3 = 0.96 nM and D_2 = 22.2, D_3 = 1.67 nM, respectively), correlating well with binding data. While (-)-**11b** showed full agonist activity at both D_2 and D_3 receptors (E_{max} = 87-93%), compound (-)-**11c** revealed partial agonist activity at D_2 and full agonism at D_3 receptor (E_{max} = 57% vs 82%, respectively for D_2 and D_3). On the other hand, aminotetraline compound (-)-**15a** displayed very high functional potency (EC_{50} (GTPγS); D_2 = 0.87 and D_3 = 0.23 nM) and full agonism (E_{max} = 85-92%)

at both the receptors. Compound (-)-**15c** was also found to be highly potent and efficacious in stimulating both receptors (EC_{50} (GTPγS); D_2 = 2.29 and D_3 = 0.22 nM; E_{max} = 74-88%). Neither compounds displayed appreciable selectivity for D_3 over D_2 **(Table 2)** and their selectivity for D_3 receptor dropped considerably when compared to the binding data **(Table 1).** We have also calculated the ClogP and tPSA values for all the compounds **(Table 1).** In general, the values indicate that these compounds should cross the blood brain barrier to produce *in vivo* CNS efficacy which we observed in case of (-)-**11b**, (-)-**15a** and (-)-**15c (Table 1)**. Therefore, our current SAR results of a series of carbazole compounds indicate that the affinity and selectivity for the D_2/D_3 receptors are governed by the nature of covalent attachment to the carbazole moiety and the structure of agonist binding head group in the hybrid molecule.

Table 2. Stimulation of [^{35}S]GTPγS binding to cloned human D_2 and D_3 receptors expressed in CHO cells[a]

	hCHO-D_2		hCHO-D_3		
	[^{35}S]GTPγS		[^{35}S]GTPγS		
Compound	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	D_2/D_3
Dopamine (DA)	146 ± 24	100	1.95 ± 0.62	100	75.0
(-)-11b	48.7 ± 6.3	87.3 ± 2.1	0.96 ± 0.25	93.4 ± 4.4	50.7
(-)-11c	22.2 ± 6.9	56.7 ± 5.1	1.67 ± 0.30	82.0 ± 7.0	13.3
(-)-15a	0.87 ± 0.098	85.2 ± 4.7	0.23 ± 0.02	92.2 ± 3.3	3.79
(-)-15c	2.29 ± 0.70	73.6 ± 10.1	0.22 ± 0.06	87.9 ± 3.8	10.3

[a]EC_{50} is the concentration producing half maximal stimulation. For each compound, maximal stimulation (E_{max}) is expressed as a percent of the E_{max} observed with 1 mM (D_2) or 100 μM (D_3) of the full agonist DA (E_{max}, %). Results are the mean ± SEM for 3–6 experiments, each performed in triplicate.

4.2.2.2. Antioxidant assay of the lead compounds.

Cellular antioxidant activity of (-)-11b, (-)-15a and (-)-15c. This experiment detection of reactive oxygen species (ROS) produced by 6-OHDA was carried out by DCFDA assay. 6-OHDA is a widely used toxin that mimics the generation of oxidative stress observed in PD and it induces neurotoxicity via its auto-oxidation and subsequent hydrogen peroxide generation (Blum et al. 2000; Soto-Otero et al. 2000). DCFDA is a non-fluorogenic dye but in the presence of ROS, it is oxidized to produce DCF which produces florescence. 6-OHDA is known to cause cell death in a dose-dependent manner via production of reactive oxygen species. From our previous and current dose-dependent experiment of 6-OHDA, we chose 75 μM 6-OHDA which can induce 40-50% cell death for our study. To examine whether our compounds (-)-**11b**, (-)-**15a** and (-)-**15c** can protect PC12 cells from the ROS produced by the exposure of 75 μM 6-OHDA, the PC12 cells were treated with 6-OHDA after pretreatment with various concentrations of drugs (5, 10, 20 μM) for 24 h, and comapred with 6-OHDA treated alone cells. As shown in Figure 3, a well over two-fold increase in ROS was observed in cells treated with 6-OHDA (75 μM) alone compared to the control untreated cells **(Figure 22)**. However, the test compounds could dose dependently decrease the production of ROS induced by 6-OHDA (75 μM) in PC12 cells. In this regard, highest dose of all the three compounds was the most efficacious in producing significant antioxidant effect. Thus at 20 μM, a reduction in 93%, 36% and 76% ROS were induced by (-)-**11b**, (-)-**15a** and (-)-**15c**, respectively. Thus, **D-653** was found to be the most potent antioxidant.

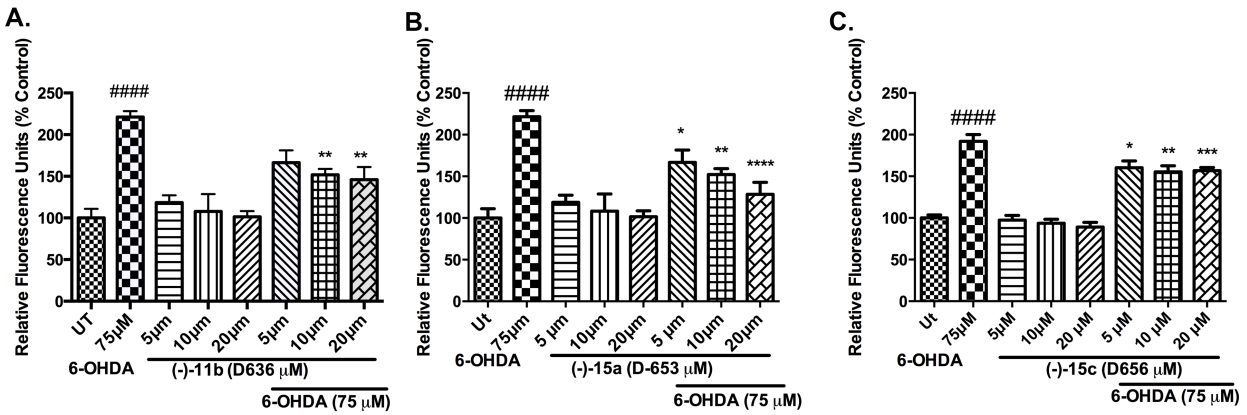


Figure 22: Detection of ROS using carboxy-H_2DCFDA on relative fluorescence of PC12 cells induced by 6-Hydroxydopamine after pretreatment with different concentrations of **D-636** (a), **D-653** (b), **D-656** (c) respectively. PC12 cells were pretreated with different doses of **D-636** (a), **D-653** (b), & **D-656** (c) for 24 h, the drug containing media was replaced with carboxy-H_2DCFDA (20 μm, 2% serum) for 30 min, the carboxy-H_2DCFDA containing media was removed, and replaced with fresh media, followed by treatment with 75 μM 6-OHDA, and incubated for 1 h. The PC12 treated with compounds were compared with non-treated cells. Data represents mean ± SDs of three independent experiments in four to six replicates. One-Way ANOVA analysis followed by Turkey's multiple comparison post doc test was performed. (*P < 0.1, **P < 0. 01, ***P < 0. 001, and ****P < 0.0001 compared to the 6-OHDA, $^{####}$P < 0.0001 compared to the control).

4.2.2.3. Neuroprotection Studies with PC12 cell line.

Neuroprotection Against 6-OHDA-Induced Toxicity. To investigate the multifunctional property of the target molecules, we next embarked on exploring the neuroprotective effect of (-)-**11b**, (-)-**15a** and (-)-**15c** in dopaminergic rat adrenal Pheochromocytoma PC12 cells against 6-OHDA-induced cytotoxicity. Treatment of PC12 cells with 6-OHDA for 24 h resulted in a significant dose-dependent neurotoxicity and the cell viability was significantly decreased to ~ 50% in cells exposed to 75 μM of 6-OHDA and this concentration was used in subsequent *in vitro* experiments (Shah, Rajagopalan, Xu, Voshavar, Shurubor, Beal, Andersen, et al. 2014). In contrast, cells treated with increasing concentrations of either (-)-**11b** or (-)-**15c** alone (0.01–30 μM) showed no cell loss at all compared to untreated controls **(Figure 23a and 23e, respectively),** indicating

the non-toxic profile of the compounds at the doses tested; however, (-)-**15a** showed some incremental toxicity starting from 20 μM dose (Figure 4c). This is an interesting finding in that a subtle change in the position of aromatic substitution from 2 to 4 in the carbazole moiety resulted in a dramatic improvement in cell survival. The potential neuroprotective effects of (-)-**11b**, (-)-**15a** and (-)-**15c** on 6-OHDA-induced toxicity were evaluated following pre-treatment with the drugs. Thus, when the cells were pre-treated with the test compounds for 24 h followed by exposure to 6-OHDA treatment for another 24 h, the compounds dose-dependently protected the cells from the neurotoxic insult and the greatest protective effect was obtained at concentration of 5 μM for (-)-**11b** and 20 μM for (-)-**15c**, both of which increased the cell survival by ~20% compared to 6-OHDA (75 μM) treated alone **(Figure 23b and 23f).** These data strongly suggest the neuroprotective effect of both the compounds on PC12 cell loss induced by 6-OHDA. Compound (-)-**15a** also revealed dose-dependent neuroprotection and the highest effect was observed at the dose of 10 μM (15%) but the effect was reversed at higher doses (Figure 4d). This could be due to the fact that the compound is little toxic to the cells at doses ≥20 μM as was seen in the drug-only toxicity experiment **(Figure 23c).**

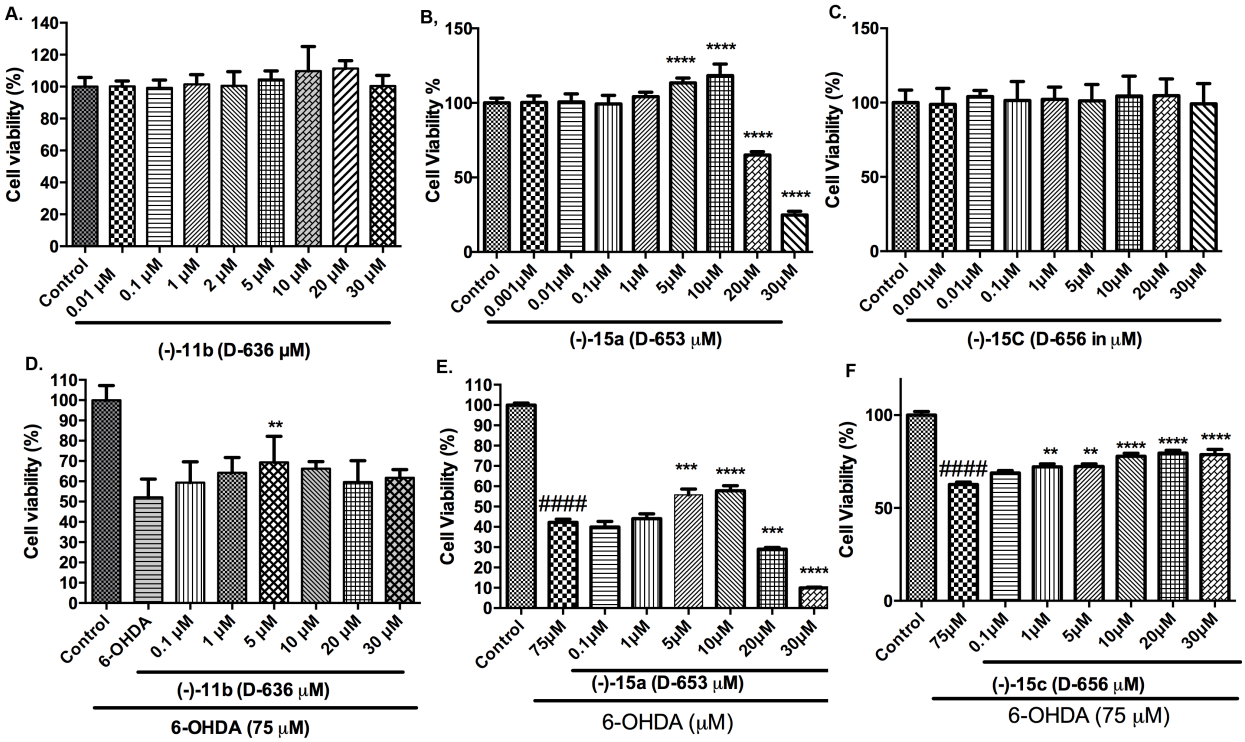


Figure 23: Dose dependent effect of D-636, D-653, and D-656 on cell viability of PC12 cells from toxicity induced by 6-Hydroxydopamine after pretreatment with different concentrations of D-636. (a,b, and c) Dose-dependent effect of D-636, D-653, and D-656 respectively. (d,e, and f) PC12 cells were pretreated with different doses of D-636, D-653, and D-656 respectively for 1 h, followed by 75 µM 6-OHDA, and incubated for 24 h. Data represents mean± SDs of three independent experiments in four to six replicates.

4.2.3. *In vivo* Efficacy of lead molecules from carbazole series compounds.

Reversal of Reserpine-induced Hypolocomotion in Rats by (-)-11b, (-)-15a, (-)-15c and Ropinirole. *In vivo* evaluation of the compounds (-)-**11b**, (-)-**15a** and (-)-**15c** in PD animal model was next performed. Reserpine induces depletion of catecholamine in the nerve terminals, resulting in a cataleptic condition in rats, which is a well-established animal model for PD (Carlsson, Lindqvist, and Magnusson 1957; Skalisz et al. 2002). Significant inhibition of locomotion of rats was observed 18 h after the administration of reserpine (5 mg/kg, sc), which indicated the development of akinesia (**Figure 24**). Compounds (-)-**11b**, (-)-**15a** and (-)-**15c** at the dose of 10 µMol/kg, ip, were not only highly

efficacious in reversing akinesia in rats, compared to reserprine treatment alone, but also demonstrated significant enhancement of locomotion for the entire duration of the study of 6 h. Among the molecules tested, (-)-**15a** was found to have the highest *in vivo* activity and this finding correlates nicely with *in vitro* functional assay where the compound exhibited subnanomolar potency for stimulation of D_2/D_3 receptors along with full agonist property. In contrast, treatment with the reference drug ropinirole at the dose of 10 μMol/kg, ip, produced a quick onset of locomotor activation compared to control but with a much shorter duration of action compared with the test compounds. The mechanism of the locomotor stimulation in this reserpine model is likely to be mediated by postsynaptic D_2/D_3 receptor activation by the compounds.

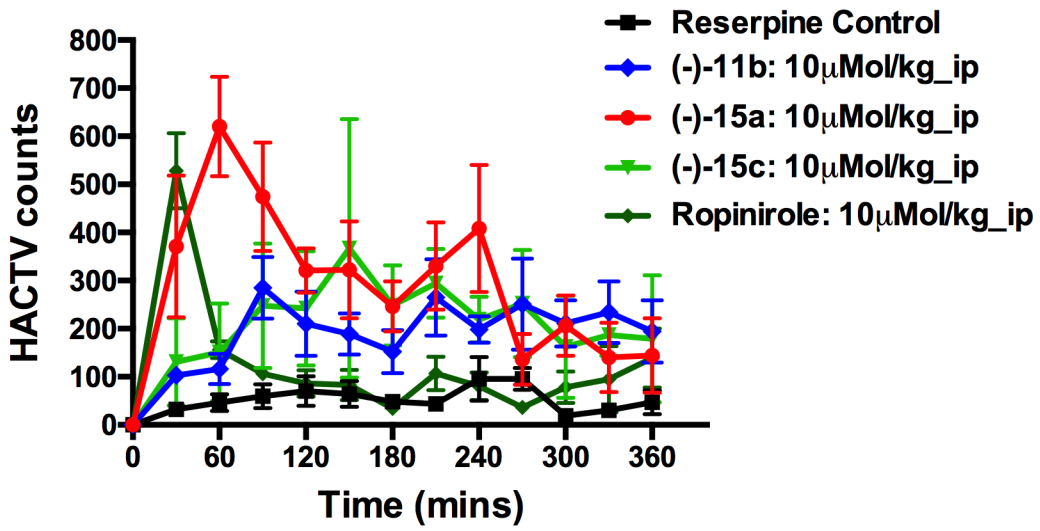


Figure 24. Effect of different drugs on reserpine (5.0 mg/kg, sc, 18h pretreatment) induced hypolocomotion in rats. Results are expressed as means + SEM for three rats. Plot represent horizontal locomotor activity at discrete 30 min interval after administration of **D-636** (10 μMol/kg, i.p), **D-656** (10 μMol/kg, i.p) and **Ropinirole** (10 μMol/kg, i.p). Significant effect was demonstrated by ANOVA analysis.

4.3. Design, and synthesis of novel multifunctional dopamine D_2 /D_3 receptors agonists containing a propargyl moiety for potential MAO-B inhibitory activity.

In this section, a series of molecules were synthesized by modifying the hybrid molecule template **(Figure 25)** as previously done for other compounds **(Figure 20).** The design of these molecules involved incorporation of the propargyl group that has been reported to show MAO-I activity, into the hybrid structure. The synthesis of these propargyl derived molecules are shown in **scheme (4, 5, and 6)**. In the current template, the piperazine linker was attached to the propargyl group through either phenyl amine or phenyl alkoxy derivatives.

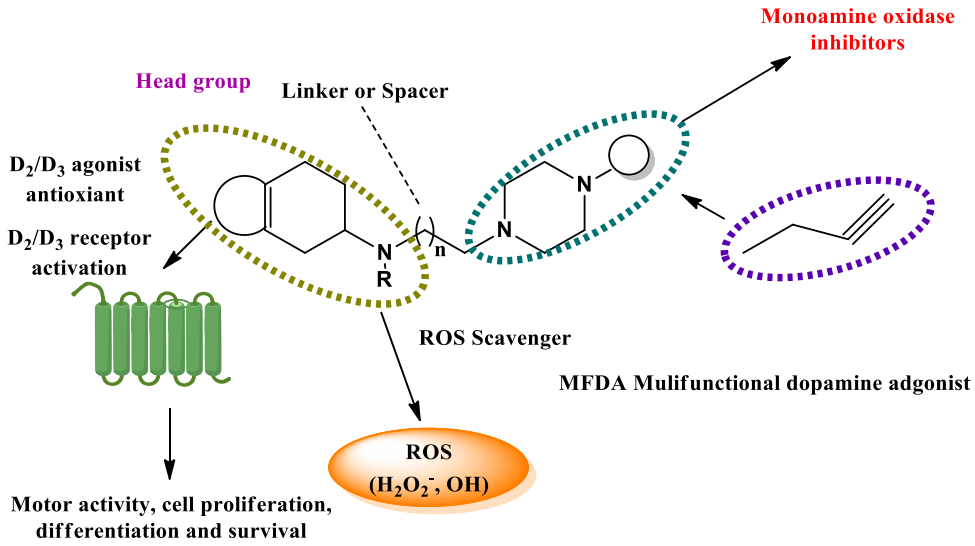
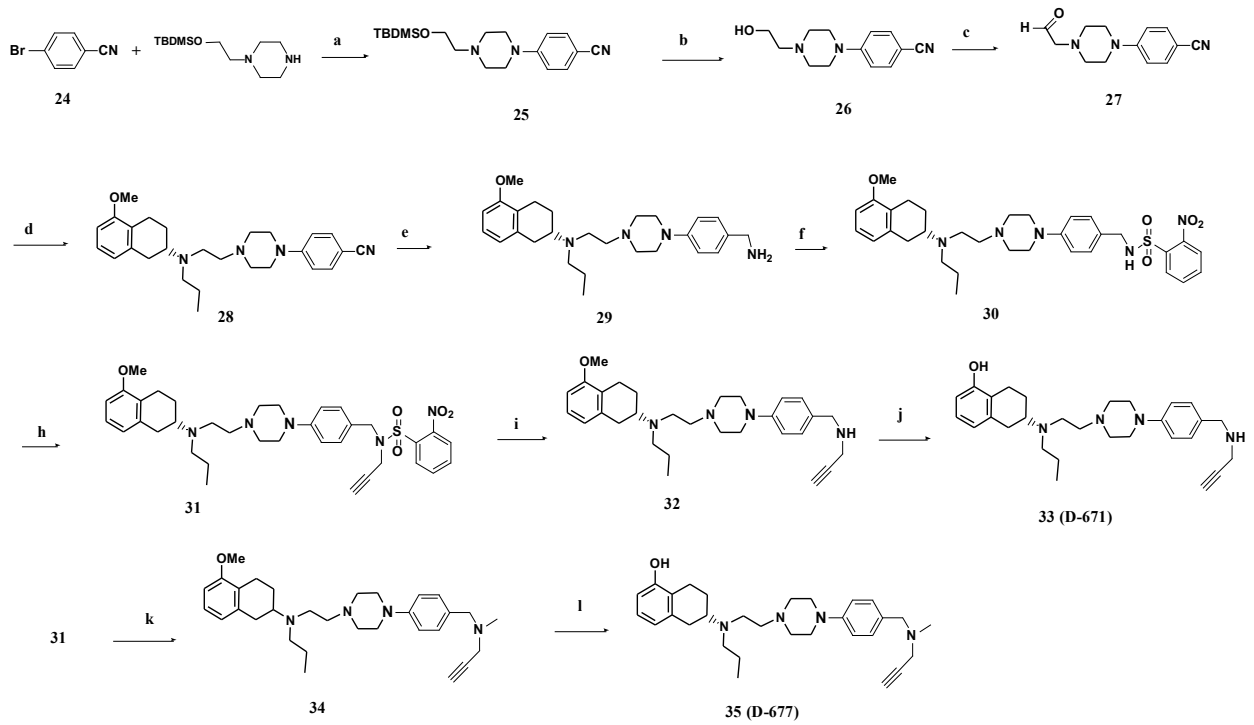


Figure 25. The hybrid molecule template for multifunctional dopamine D_2/D_3 receptor agonists that may potentially inhibit the MAO-B activity.

4.3.1. Synthesis of multifunctional dopamine D_2/D_3 receptor MAO-B inhibitors:

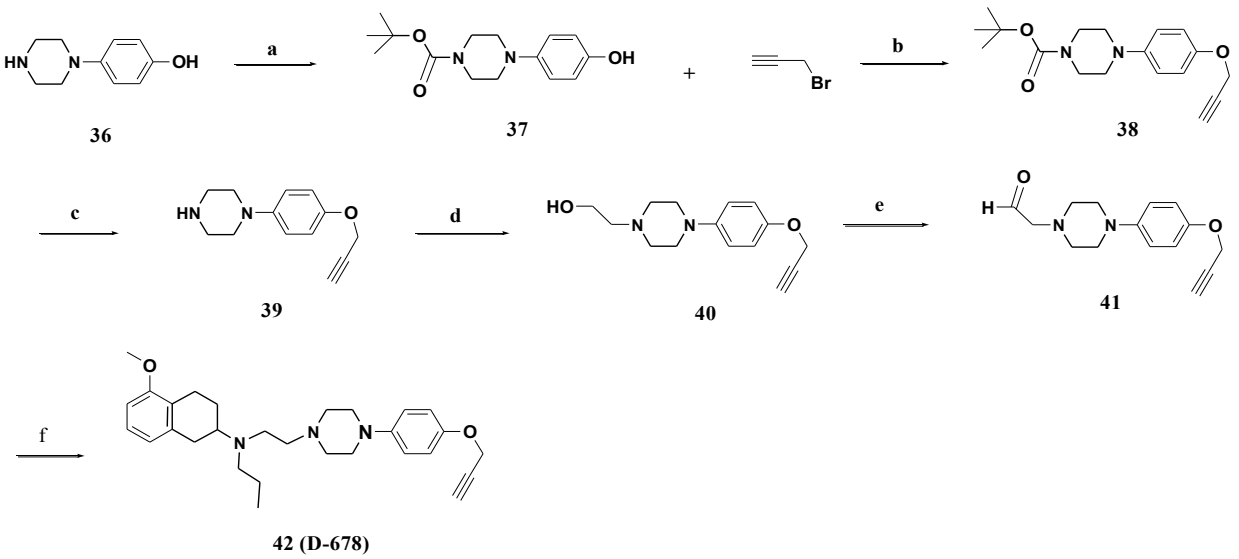
Scheme 4 outline the synthesis of two *N*-propargyl compounds **(±)-33 (D-671)**, and **(±)-35 (D-677)** in which the piperazine ring as a linker connects to the phenyl ring containing either *N*- or *O*-propargyl group. Palladium-catalyzed cross coupling of

commercially available 4-Bromo-benzonitrile **24** with 1-(2-((*tert*-butyldimethylsilyl)oxy)-ethyl)piperazine (Das et al. 2015) in the presence of Cs_2CO_3 and BINAP in toluene under refluxing condition yielded intermediate **25**. The silyl protecting group of compounds **24** was removed by treatment with *n*-Bu_4NF (TBAF) in THF to afford the alcohol **26**, which on subsequent oxidation by pyridine-sulfur trioxide yielded the corresponding aldehyde **27**. Reductive amination of the aldehyde with **(-)-5-(OH)-MPAT** in the presence of $NaBH(OAc)_3$ afforded compounds **28**. The intermediate **28** was reduced by borane:THF in1.0 M THF in presence of Conc.HCl and 25 % aq. NaOH to yield the intermediate primary amine **29**. The primary amine intermediate **29** was selectively protected by 2-nitrobenzenesulfonyl chloride to give the sulfonamide intermediate **30**. The nitrobenzenesulfonamide intermediate **30** was reacted with the commercially available propargyl bromide to get the intermediate **31**. Tertiary amine **31** was deprotected by thioglycolic acid to generate the secondary amine **32**. Finally, the compound **32** was subjected to either demethylation by refluxing with aq. HBr to give the final compound **(±)-33 (D-671)**, as HBr salt, or to *N*-methylation with 37% aqueous formaldehyde in a buffer system of NaH_2PO_4 to get the intermediate **34** which subjected to further *O*-demethylation by refluxing with 48% HBr to obtain the final compound **(±)-35 (D-677)** as HBr salt.



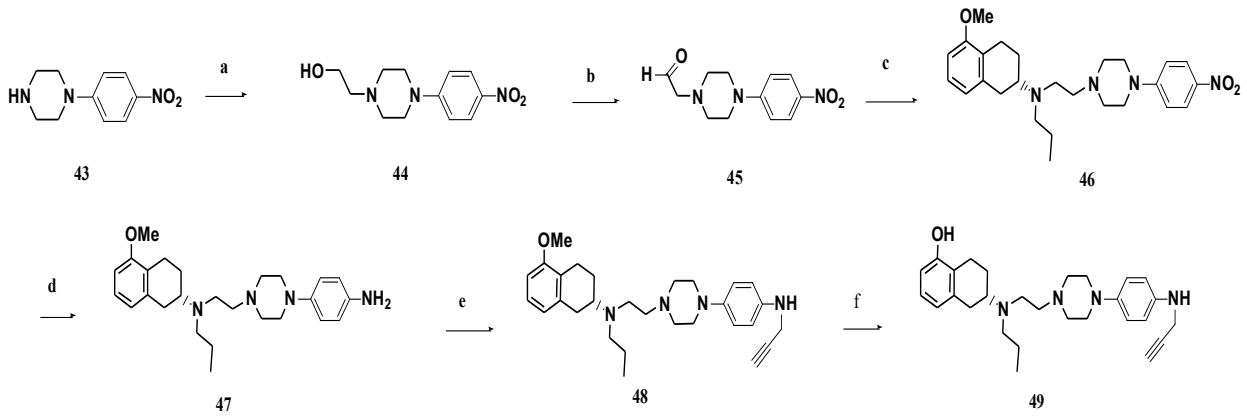
Scheme 4. Synthesis of compounds **(±)-33 (D-671)**, and **(±)-35 (D-677).** Reagents and conditions: a) $Pd(OAc)_2$, BINAP, Cs_2CO_3, Toluene, reflux, owvernight; b) TBAF, THF, 0°C to RT, 3 h; c) SO_3py, CH_2Cl_2:DMSO (2:1), Et_3N, 0°C to RT, 2h; d) (-)-DPAT, $NaBH(OAC)_3$, CH_2Cl_2, 48 h; e) dryTHF, Borne in THF, 50°C, RT, 0°C, conc. HCl, 25% aq. NaOH; f) THF, nitrobenzylsulfonylchloride, -10°C, Et_3N, RT, 1.5 h h) Propagylbromide, K_2CO_3, CH_3CN, reflux, 24 h; i) DMF, K_2CO_3 , 0°C, thioglycolic acid, 1 h, comp. 30 added; j) 48% aq. HBr, reflux, 5 h; k) 37% aq.CH_2=O, NaH_2PO_4, zinc dust, H_2O, 30°C, 48 h. l) 48% aq. HBr, reflux, 5 h.

Scheme 5 describes the synthesis of the *O*-propargyl compound **(±)-42 (D-678).** The commercially available secondary amine intermediate **36** was protected by $(Boc)_2O$ to get the tertiary amine Intermediate **37**. *O*-alkylation of compound **37** with the commercially available propargyl bromide to give compound **38**. The amine protecting group was removed by treatment with trifluoroacetic acid to get compound **39**. The intermediate **39** was refluxed with bromoethanol to produce alcohol **40,** which was subsequently oxidized by the Parikh–Doering oxidation to give the corresponding aldehyde intermediate **41.** The aldehyde intermediate was then coupled with **(±)-5-OH-MPAT** to yield the final compound **(±)-42 (D-678)**.



Scheme 5: Synthesis of compound **(±)-42 (D-678)**. **Reagents and conditions:** a) $(Boc)_2O$, DMAP, THF, 24 h; b) propagylbromide, K_2CO_3, CH_3CN, reflux, 24 h c) TFA, CH_2Cl_2, 0°C, RT, 3 h; d) 2-bromoethanol, K_2CO_3, CH_3CN, reflux, 24 h; e) SO_3py, CH_2Cl_2:DMSO (2:1), Et_3N, 0°C to RT, 2 h; f) (±)-DPAT, $NaBH(OAC)_3$, CH_2Cl_2, 48 h.

Scheme 6 describes the synthesis of compound **(-)-49.** The commercially available intermediates **1**-(4-Nitro-phenyl)-piperazine and 2-bromoethanol were refluxed in acetonitrile to get the alcohol intermediate **44**, which was subsequently oxidized by the Parikh–Doering oxidation to give the corresponding aldehyde intermediate **45**. The aldehyde intermediate **45** was then coupled with the (-)-5-OH-MPAT to get the intermediate **47**. Commercially available propargyl bromide) was used to react with the primary amine intermediate **47** to form the intermediates **48**, which was further demethylated by 48% HBr to produce the final compound **49**.



Scheme 6: Synthesis of compound **(-)-49**. reagents and conditions**:** a) 2-bromoethanol, K_2CO_3, CH_3CN, reflux, 24 h; b) SO_3py, CH_2Cl_2:DMSO (2:1), Et_3N, 0^oC to RT, 2 h; c) (±) or (-)-DPAT, $NaBH(OAC)_3$, CH_2Cl_2, 48 h; d) 10 mol.% pd/C, H_2gas, CH_3OH, 20 h; e) Propagylbromide, K_2CO_3, CH_3CN, reflux, 24 h; f) 48% aq. HBr, reflux, 5 h; g) CH_2=O, 37% aq.HCHO, NaH_2PO_4, zinc dust, H_2O, 30^oC, 48 h; h) 48% aq. HBr, reflux, 5 h.

4.3.2. *In vitro* D_2/D_3 receptor binding and functional assays with multifunctional MAOB-inhibitors.

Our effort in this work is to develop multifunctional D_2/D_3 agonists with MAO inhibitory activity to treat and modulate the progression of PD (Das, Modi, and Dutta 2015; Dutta, Fei, and Reith 2002; Li et al. 2010; Shah, Rajagopalan, Xu, Voshavar, Shurubor, Beal, Andersen, et al. 2014; Yedlapudi et al. 2016; Das, Rajagopalan, et al. 2017; Das, Kandegedara, et al. 2017). In this design DA agonist head groups are covalently attached to a propargyl moiety via a linker (Andrea Cavalli 2008; Naoi et al. 2003; Prins, Petzer, and Malan 2010). The rational of using propargyl moiety was based on the previous research work that propargyl group has monoamine oxidase inhibiton activity. The idea of including a MAO inhibiton moiety in our hybrid structure came from the previously synthesized MAO-I such as (R)-deprenyl which was shown to inhibit the MAO-B, to reduce the formation of H_2O_2 and dopaldehyde in the brain caused by MAO-B (W. 1992) (**Figure 26)**. Rasagiline (2) is a potent MAO-B inhibitor (H, Aviva, and M 2001),

considered to be useful as an adjuvant therapy to levodopa for the treatment of PD due to its ability to augment dopamine levels in the primate brain (Prins, Petzer, and Malan 2010) **(Figure 26)**.

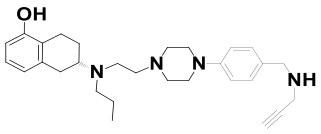
Selegiline (R)-deprenyl **Rasagiline**

Figure 26. Chemical Structures of Selegiline, and Rasagiline

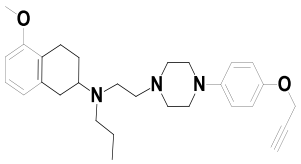
Binding affinity of the synthesized compounds was determined by inhibition of [^{3}H] spiroperidol binding to rat DA D_2 and D_3 receptors expressed in HEK-293 cells as described by us previously (Biswas et al. 2008). In general, the decrease in the D_2 binding affinity of the compound **D-677** after methylation of the secondary nitrogen atom directly attached to the propargyl group was observed. The D_2 binding affinity of compounds **35 (D-677)** decreased compared to its corresponding nonmethylated molecules **32 D-671** (K_i, D 2 = 38.8 vs. 1,079 nM, **Table 3**) as well as a reduction in D_3 binding affinity (1.30 vs. 99.0 nM, **Table 3**). The great reduction in binding affinity of the **D-677** could be explained by the importance of the hydrogen atom attached to the nitrogen. However, the bioisosteric version of **32-(D-671),** compounds **42 (D-678)** has exhibited only a slight or no effect in the D_2 binding affinity when compared to its bioisosteric **D-671** (K_i, D_2 = 41.2 vs. 38.8 nM, respectively **Table 3**) as well as slight or no effect in the D_3 binding affinity compared with the same molecule (Ki, D3=0.85 vs 1.30 nM, respectively **Table 3**) which indicates tolerance of replacement of N-atom by O-atom for the binding activity to both D_2/D_3 receptors. The similarity of the binding affinity for **D-671**, and **D-678** might be explained by the presence of the lone pair of electrons on the secondary amine and the

oxygen atom that would be necessary for the binding of these compound to the D_2/D_3 receptors.

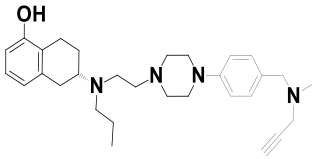
Table 3. Inhibition constants determined by Competition Experiments Assessing [^{3}H] Spiroperidol Binding to Cloned Rat D_{2L} and D_3 receptors Expressed in HEK-293 Cells[a]



(-)-33- D-671



(±)-42-D-678



(-)-34- D-677

Compound	K_i (nM)		D_{2L}/D_3	cLogP	tPSA
	D_{2L}, [^{3}H]spiroperidol	D_3, [^{3}H]spiroperidol			
(-)-5-OH-DPAT	153 ± 32	2.07 ± 0.38	74		
(-)-33- D-671	38.8 ± 5.2	1.30 ± 0.13	30	4.15	33.19
(-)-34- D-677	1,079 ± 26	99.0 ± 10	11	4.15	33.19
(±)-42-D-678	41.2 ± 5.8	0.85 ± 5.0.01	9.86	3.95	41.98

aResults are expressed as means + SEM. For 3-6 experiments each in triplicate.

Based on the binding results, compounds were selected for further *in vitro* examination by measuring the stimulation of [35^S] GTPγS binding to assess their ability to activate the human dopamine hD_2 and hD_3 expressed in CHO cells. Dopamine (DA) was used as reference because of its full agonist activity. Both **(-)-33- D-671,** and **(±)-42-D-678** showed a high potency (EC_{50} (GTPγS); D_2= 0.932, 1.64, respectively, **Table 4**), and full agonist activities for D_2 receptor with (E_{max} =97% and 91.3%, **Table 4**).

Table 4. Stimulation of [35^S] GTPγS Binding to Cloned Human D_2 and D_3 Receptors Expressed in CHO Cells[a]

Compound	hCHO-D_2		hCHO-D_3		
	[^{35}S]GTPγS EC_{50} (nM)	E_{max} (%)	[^{35}S]GTPγS EC_{50} (nM)	E_{max} (%)	D_2/D_3
Dopamine (DA)	146 ± 24	100	1.95 ± 0.62	100	75.0
(-)-33- D-671	0.932±0.212	97±17.6	0.078±0.0176	80.0±5.8	8
(±)-42-D-678	1.64±0.30	91.3±5.3	0.197±0.064	95.8±6.9	13.3

[a]EC_{50} is the concentration producing half maximal stimulation. For each compound, maximal stimulation (E_{max}%) is expressed as percent of the E_{max} observed with 1mM (D_2) or 100 μM (D_3) of the full agonist DA (E_{max} %). Results are expressed as means + SEM. For 3-6 experiments, each performed in triplicate.

4.3.3. MAO inhibition assay with multifunctional MAO-inhibitors.

A fluorescence-based enzymatic assay was carried out to evaluate the affinity of the synthesized compounds to inhibit the MAO-B activity, it was also used to evaluate the effect of changing the heteroatom group connecting to the propargyl group on the inhibition of monoamine oxidase enzyme. In this experiment, pargyline, was used as the reference compound. Pargyline is known to be a potent and selective MAO-B inhibitor. The experiment started by incubating 25 μM of the test compounds,15 μg/mL of the MAO-B enzyme with 25 μM kynuramine used as a substrate. The fluorescence of the product 4-hydroxyquinoline was measured as an assay readout. Among the three test compounds, **(-)-33- D-671**exhibited higher inhibitor effect on MAO-B than **(±)-42-D-678**, and less inhibitory effect on MAO-B. This result could be drawn from the effect of the secondary nitrogen connected directly to the propargyl group compared with the oxygen group on the compounds **(±)-42-D-678.**

These findings suggest that the hydrogen on the nitrogen atom connecting the propargyl group to the phenyl ring may potentially enhance the MAO-B enzyme inhibitory activity. Substitution of the nitrogen with the bioisosterically oxygen could be responsible for reducing the affinity to inhibit the MAO-B in compound **(±)-42-D-678** compared to compound **(-)-33- D-671**. The compound **(±)-42-D-678** has less receptor binding affinity than **(-)-33- D-671**. Moreover, compound **(-)-33- D-671**displayed better agonist potency at D_2 receptor activity in the functional receptor assays than **(±)-42-D-678**. This could suggest that there could be a relation between the D_2/D_3 receptor agonist activities and the enzyme inhibitory activity. This could also shed the point on the requirement of further modifications of the current molecular template compatibility for dopamine D_2/D_3 receptors binding, functional activity, and MAO-B enzyme interaction for the structural preference and conformational requirement.

Table 5. The IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by compounds[a]

Compound	MAO-A	MAO-B	SI[b]
	IC_{50}(μM)	IC_{50}(μM)	
Pargyline	0.28 ± 0.02	10.54 ± 0.79	37.6
(-)-33- D-671	71.90±5.805	12.38±2.282	5.80
(±)-42-D-678	57.07±0.30	24.17±0.064	2.40

[a]Results are expressed as means ± SEM for three experiments, each performed in triplicate.
[b]Selectivity index = IC_{50}(MAO-A)/IC 50 (MAO-B).

4.4. Design, development, synthesize and pharmacological studies of monoamine reuptake blockers.

We previously reported pyran based triple reuptake inhibitors (TUIs), which inhibit reuptake of all three monoamine neurotransmitters. TUIs have been implicated for production of higher efficacy in antidepressant action (Zhang et al. 2005a; Zhang et al. 2006). Our group has been working on the development of asymmetric 3,6-disubstituted and 2,4,5-trisubstituted pyran derivative targeting monoamine transporters **(Figure 4)**. These compounds exhibited affinity towards dopamine reuptake transporters and high potency for 5-HT and NE reuptake transporters (Zhang et al. 2006; Zhang et al. 2005b).

To explore the structurally novel molecular template for the monoamine transporters, derivatives of well-established 2,4,5-trisubstituted pyran were synthesized **(Figure 27)**. The goal of this project is to carry out the SAR of pyran based compounds to develop a novel multifunctional pyran molecule template to treat the motor, non-motor symptoms like depression associated with PD.

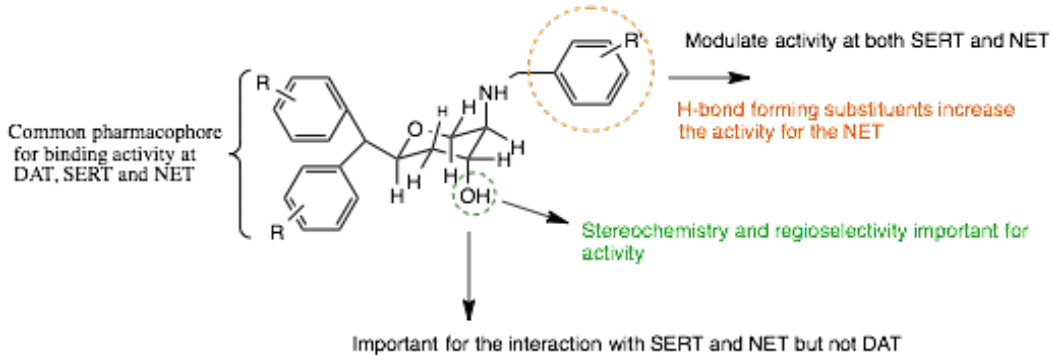


Figure 27. Pharmacophore model of pyran derivatives with monoamine transporters molecules.

In this study, the effect of connecting biphenyl substituents at position 2 on the pyran ring was explored directly without methylene bridge to the pyran ring **(Figure 28).** It was reasoned that connecting biphenyl groups directly to the pyran template could

increase the binding affinity for the dopamine transporters resulting in compounds with high selectivity and potency.

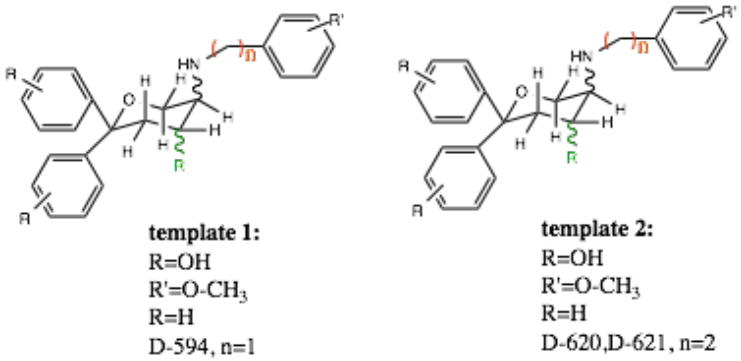
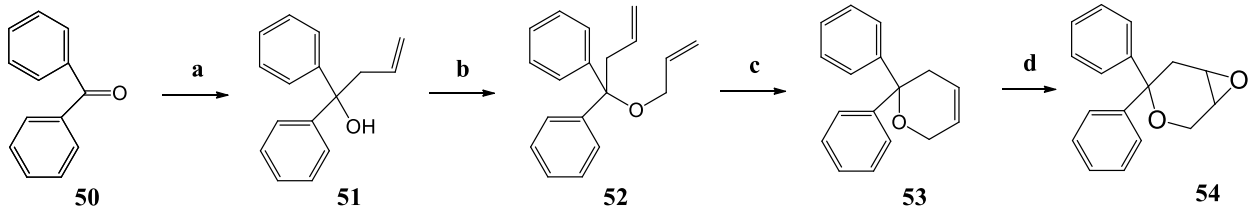


Figure 28. Initial SAR study on pyran templates (template 1: n=1, and template 2: n=2)

4.4.1. Chemistry involved in the syntheses of multifunctional D2/D3 agonist to treat depression in PD.

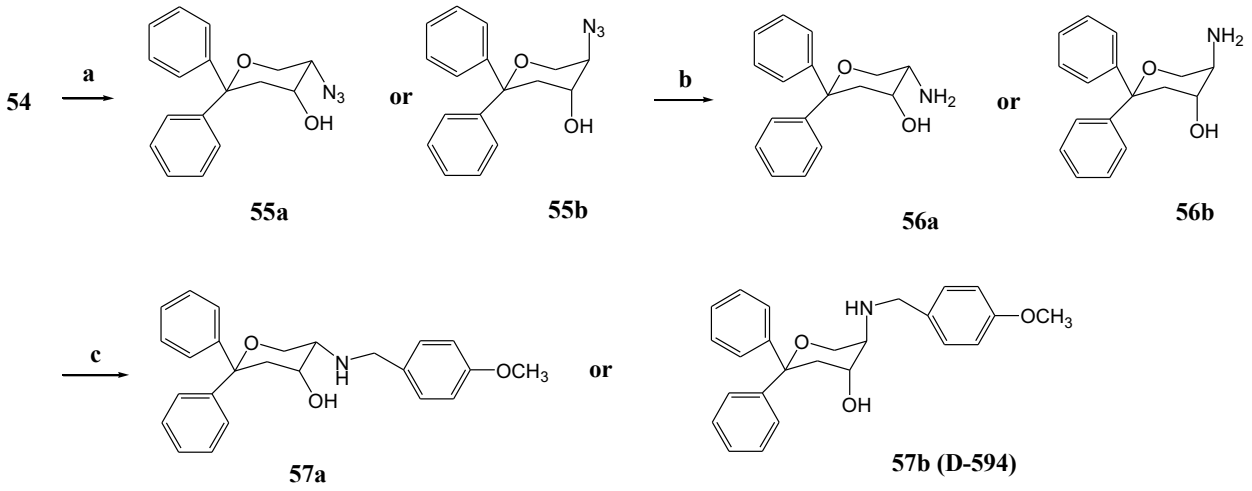
In this study, a version of the asymmetric trisubstituted pyran derivative precursor was developed as described in the following schemes.



Scheme 7; Synthesis of the intermediate racemic expoxides **54.** Reagents and conditions**:** a) allylmagnesiumbromide/CuI/THF,-78°C→RT,overnight; b) NaH/ allylbromide/DMF, 1.5h, 0°C→RT; c) Grubb'scatalyst/benzene/reflux,2h; d) mCPBA/ CH_2Cl_2,0°C→RT.

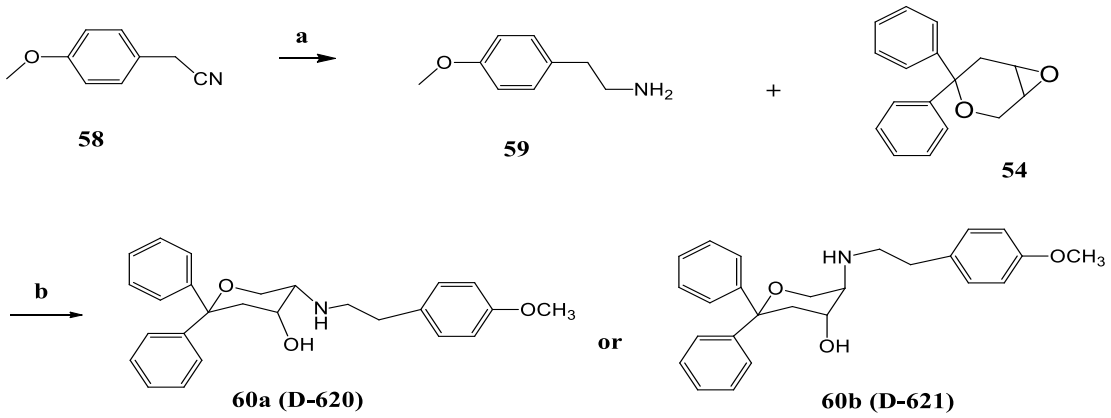
Scheme 7 outlines the synthesis of the intermediate racemic expoxides. Commercially available benzophenone **50** was treated with allymagnesium bromide in the presence of copper iodide at -78°C to obtain alcohol **51**. O-Alkylation of **51** with allybromide in the presence of NaH at 0 °C to give compound **52**, which was further converted to pyran derivative **53** via ring metastasis reaction in the presence of 1st

generation Grubbs catalyst (Sturino and Wong 1998). Direct epoxidation of olefin **53** with m-CPBA resulted in formation of low stereoselective trans-and cis-epoxide **54** (Zhang et al. 2005b).



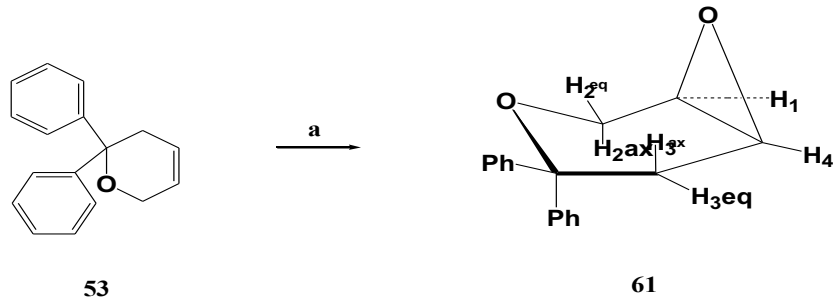
Scheme 8; Synthesis of compound **D-594**. Reagents and conditions: a) $NaN_3/CH_3OH:H_2O(8:1),NH_4Cl,80^oC,24h$; b) $CH_3OH/10\%Pd/C,30psi$; c) aldehyde $/CH_2Cl_2:CH_3OH(3:1)$, glacialaceticacid/$Na(OAC)_3BH$.

Scheme 8 describes the synthesis of the target compound **57b (D-594)** starting from epoxide **54**. The epoxide ring opening was achieved in the presence of sodium azide (NaN_3) followed by the addition of NH_4Cl at 80 ^oC to obtain the racemic azides **55**, Which was reduced in the presence of 10% Pd-C at 30psi (1 atm) to the corresponding amines **56**, which undergo further reductive amination with 4-methoxy-benzaldehyde to obtain the final compounds **57**.



Scheme 9. Synthetic scheme for **D-620** and **D-621**. Reagents and conditions: (a) BH3-Me2S added in THF at 0ºC/ 2hr RT→ 40ºC overnight (b) amine,reflux at 100ºC under N_2 overnight,ehanol. c) THF at R.T, 0ºC/2 hr, 40ºC→RT, add 2N HCl. Stirred for 1 hr at 40ºC.

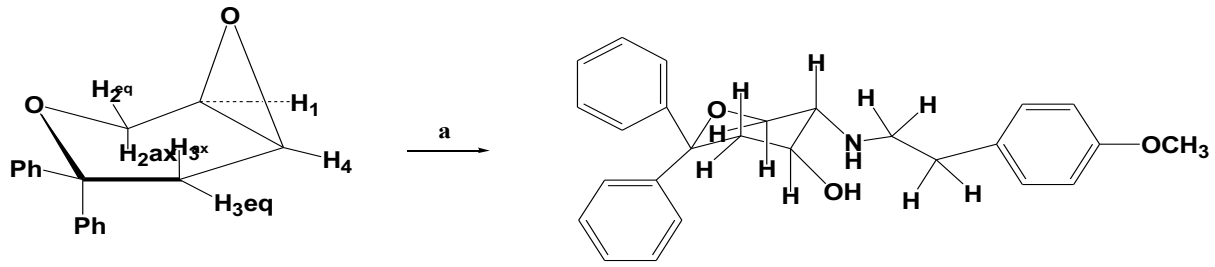
Scheme 9 outlines the synthesis of compounds **60a (D-620)** and **60b (D-621)**. 4-methoxyphenylacetonitrile **58** was treated with borane-methyl sulfide complex to obtain 2-(4-Methoxy-phenyl)-ethylamine **59** which reacted with the racemic epoxides **54** to produce compounds **60a (D-620)** and **60b (D-621).**



Scheme 10. Synthesis of a regioselective epoxide **61**. Reagents and conditions: (a) acetonitrile:DME, $Na_2B_4O_7.H_2O$, Na_2EDTA, Tertbutyl ammonium hydrogen sulfate, Epoxane, RT→-10 ºC, NaCl ice bath, oxone in aqueous Na_2EDTA 0.004 M and K_2CO_3 added.

Scheme 10 describes the synthesize of enantiomer epoxides **61** in a stereoselective manner. Alkene **53** was carried out via Shi epoxidation catalyst to give optically active cis-epoxide. The regioselectivity of shi epoxidation catalyst to give cis-epoxide was confirmed by NMR experiments. Alkene was treated with expoxone and

sodium ethylene diamine tetraacetic acid in a buffer media of potassium hydrogen monopersulfate, sodium tetraborate, tetabutylammonium hydrogen sulfate and potassium carbonate to give the cis-epoxide regioselectivity. The regioselectivity of the shi epoxidation catalyst to the give cis-epoxide was confirmed by the NMR experiments.



61 60a (D-620)

Scheme 11. Synthesis of a regioselective compound **D-620**: Reagents and conditions: (a) 2-(4-methoxyphenyl) ethan-1-amine/ ethanol was reflux 100 ^{o}C under N_2 overnight.

Scheme 11 explains the synthesis of the novel pyran compound **D-620** by introducing all the three substituents in a regiospecific manner after opening the epoxide. Cis-epoxide **61** was subjected to a regioselective ring opening in the presence of 2-(4-Methoxy-phenyl)-ethylamine to obtain compound **60a (D-620)**. The selectivity of the ring opening reaction was directed by nucleophilic attack at the site remote from the endocyclic oxygen atom where carbocationic character is better tolerated, the ring opening will involve high energy twist boat like transition state (Larin, Kochubei, and Atroshchenko 2014; Bagal et al. 2010). The relative stereochemistry of compound **60a** was confirmed by **NMR** experiments.

4.4.2. ^{1}H NMR spectrum (normal proton NMR, 2-D COSY NMR, ^{1}H homo decoupling (HMDC), and nuclear overhauser experiments (NOE)) studies for the synthesized compounds.

4.4.2.1. The study of structure of epoxide 61.

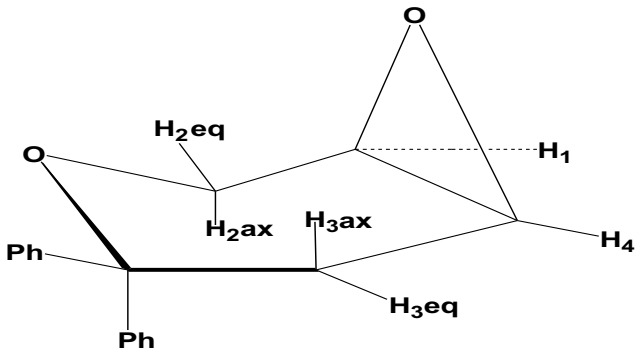


Figure 29. The assigment structure of epoxide **61.**

The NMR studies were done in C_6D_6, OCH_2 and OCH to assign the chemical shift values of the protons in $CDCl_3$. The assignment of protons was carried out by **^{1}H NMR** spectrum data (normal proton **NMR** and **2-D COSY NMR** experiments) **(Figure 29**). In compound **61** the splitting of H-4 at δ=2.95 pm (assigned from proton nmr and COSY nmr) is a triplet (*J*=4.94). Based on the MM2 minimized 3-D model of cis epoxide, the torsion angle of H1-C-C-H3ax is close to 90°. As explained above, in this cis-isomer no coupling exists (less than 1(0.66)) between H-3ax and H-4. The bt splitting of H-4 is from the coupling with H-3eq and H-1 proton, respectively, this was confirmed by the splitting pattern of H-3ax which is a doublet of doublet (J=15.8, 0.66), The coupling interactions originated from the interaction with the H-3eq and slight coupling with H-4 (J=0.66, less than 1). The splitting of H-3eq is doublet of doublet, which arises from the coupling of H-3ax and H-4 (J=15.8, 5.7), the doublet from the geminal coupling with H-3ax and another doublet from the vicinal coupling with H-4. Furthermore, **2-D COSY NMR** study demonstrated the coupling between H-4 and H-3eq and determined coupling between H-4 and H-3ax that verified H-4 is in the same phase as H-3eq.

These findings were further confirmed by ^{1}H homo decoupling **(HMDC)** and nuclear overhauser experiments **(NOE)** in **600 MHz NMR** machine in **($CDCL_3$)**: (i)

Irradiation of the proton signal at 2.25 ppm (H-1) collapsing br triplet at 2.95 ppm (H-4) into doublet and the doublet of the doublet at 3.25 ppm (H-2ax) into doublet. (ii) Irradiation of the proton signal at 2.95 ppm (H-4) resulted in collapsing the doublet of the doublet at 2.25 ppm (H1) into singlet and the doublet of doublet at 2.42 ppm (H-3eq) into doublet. (iii) Irradiation of the proton signal at 3.25 ppm (H-2ax) collapsing the doublet at 3.85 (H-2eq) and the doublet of the doublet at 2.25 (H-1). (iv) Irradiation of 3.85 ppm (H-2 eq) resulted in collapsing the signal at 3.25 ppm (H-2ax) into doublet which coupled only with H-1 proton.

Further Characterization of the compound **61**: It was though that one of the possible reasons that no such interaction was observed, may be due to the existence of a very small coupling constant between H-1 and H-2 proton. As a result, we could detect H-2ax (δ=3.25) as the doublet of the doublet pattern with a big geminal interaction (*J* = 13.6 Hz) and a small vicinal interaction with H-1 (*J* = 1.5 Hz). It was also observed that the doublet of the doublet for H1is due to a vicinal coupling interaction with H-4 (*J* =4.3 Hz) and a small vicinal interaction with H-2ax (*J* = 1.5 Hz). The coupling constant at *J* =1.5 showed that H-1 and H-2ax have interaction with H-2eq. Thus, the cis structure of epoxide was confirmed.

4.4.3. *In vitro* evaluation of the binding affinity and functional potency for the triple reuptake inhibitor compounds.

5. *In vitro* uptake inhibition studies was carried out with the three designed compounds to test their potencies for the inhibition uptake of [^{3}H]DA, [^{3}H]5-HT and [^{3}H]NE in the cell lines expressing the cloned human monoamine transporters **(Table 7)**. **D-594** was evaluated **for** its the binding affinities at the DAT, SERT, NET in the brain

by measuring its inhibitory activity for the uptake of [^{3}H] DA, [^{3}H]-5-HT, and [^{3}H]NE, and found to have weak binding affinity for the three transporters To improve the binding , a modification of the compound was done by expanding the methylene bridge connected to the nitrogen group in position 5 in the pyran template to an ethylene bridge **(Scheme 11)** to produce compound **D-620**. The reason for such structural expansion idea was to find whether the expanded designed compound fit in the binding pocket of the transporters at the receptor sites. The intermediate P-methoxybenzyl was used as a substituted group in position 5 to produce compound **D-620** that showed a high binding affinity for the dopamine transporter than **D-594** and **D-621 (Table 7)**. Moreover, compound **D-621** exhibited good binding affinity for the norepinephrine transporters. The conclusion from the binding data in table 7 that D-620 could be considered a dual reuptake transporter inhibitor as it exhibited the ability to inhibit both DAT, and NET.

Table 7. Affinity of drugs at DAT, SERT, and NET in Rat Brain.

Compound	DAT uptake, [^{3}H]DA$^{\propto}$	SERT uptake, [^{3}H]-5-HT$^{\propto}$	NET uptake, [^{3}H]NE$^{\propto}$
D-594	208±30	5,094±864	8,091±678
D-620	49.2±4.6	993±113	62.6±14.5
D-621	3,244±637	462±28	1,447±186

For uptake by DAT, SERT, and NET: [^{3}H] DA$^{\propto}$, [^{3}H]-5-HT$^{\propto}$, [^{3}H] NE$^{\propto}$ accumulation was measured. Results are the average ± SEM of three to seven independent experimental assays in triplicate.

Chapter 5- Materials and Methods

5.11. Chemistry.

Reagents and solvents were obtained from commercial suppliers and used as received unless otherwise indicated. Dry solvent was obtained according to the standard procedure. All reactions were performed under N_2 atmosphere unless otherwise indicated. Analytical silica gel 60 F254-coated TLC plates were purchased from EMD Chemicals, Inc. and were visualized with UV light or by treatment with phosphomolybdic acid (PMA), or ninhydrin. Flash column chromatographic purification was done using Whatman Purasil 60A silica gel 230−400 mesh. Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were measured on Varian 400 and 600 MHz NMR spectrometer with tetramethylsilane (TMS) as an internal standard. The NMR solvent used was either $CDCl_3$ or CD_3OD unless otherwise indicated. Autopol III automatic polarimeter (Rudolph Research Analytical) was used to record the optical rotations. MEL-TEMP II (Laboratory Devices Inc., U.S.) capillary melting point apparatus was used to record the melting points. Purity of the compounds was determined by elemental analysis and was within ±0.4% of the theoretical value (≥95% purity). Elemental analyses were performed by Atlantic Microlab, Inc., GA, USA.

Procedure A. 4'-Bromo-2-nitro-1,1'-biphenyl (2a).

To a stirring solution of 1-bromo-2-nitrobenzene (2.0 g, 9.9 mmol) and (4-bromophenyl)boronic acid (2.19 g, 10.89 mmol) in THF (25 mL) were added $Pd(PPh_3)_4$ (0.572 g, 0.50 mmol) followed by 2M K_2CO_3 (5.53 g in 20 mL water) at room temperature. The reaction mixture was stirred at 90 °C for 12 h after which it was cooled and extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO4, filtered, and

concentrated in vacuo. The crude material was purified by column chromatography over silica gel using hexane:ethyl acetate (9:1) as solvent to give compound **2a** (2.7 g, 98%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.88 (d, J = 8.4 Hz, 1H), 7.64-7.61 (m, 1H), 7.59-7.55 (m, 2H), 7.53-7.50 (m, 1H), 7.42-7.40 (m, 1H), 7.20-7.18 (m, 2H).

2-Bromo-2'-nitro-1, 1'-biphenyl (2c).

To a stirring solution of 1-bromo-2-nitrobenzene (2.50 g, 12.37 mmol) and (2-bromophenyl) boronic acid (2.73 g, 13.61 mmol) in THF (30 mL) were added $Pd(PPh_3)_4$ (0.715 g, 0.61 mmol) followed by 2M K_2CO_3 (5.53 g in 20 mL water) according to procedure A to give compound **2c** (2.18 g, 94%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.87 (d, J = 8.4 Hz, 1H), 7.44 (t, J = 7.2 Hz, 1H), 7.40 (d, J = 9 Hz, 1H), 7.30 (d, J = 7.8 Hz, 1H), 7.14 (t, J = 7.2 Hz, 1H), 7.10 (d, J = 7.2 Hz, 1H), 7.03 (d, J = 7.2 Hz, 2H).

Procedure B. 2-Bromo-9*H*-carbazole (3a).

Compound **2a** and PPh_3 were dissolved in 1,2-dichlorobenzene and the resulting solution was stirred at 170 °C for 12 h after which it was cooled and extracted with CH_2Cl_2/H_2O. The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude material was purified by column chromatography over silica gel using hexane:ethyl acetate (9:1) to yield compound **3a** (1.57 g, 89%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.04 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.62-7.58 (m, 2H), 7.52-7.47 (m, 2H), 7.39 (d, J = 8.4 Hz, 1H).

4-Bromo-9*H*-carbazole (3c).

Compound **2c** and PPh_3 were reacted in 1,2-dichlorobenzene according to procedure B to yield compound **3c** (1.25 g, 87%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.81 (d,

J = 7.8 Hz, 1H), 7.97 (s, 1H), 7.50 (t, *J* = 7.2 Hz, 1H), 7.44 (d, *J* = 7.2 Hz, 1H), 7.35 (t, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 7.8 Hz, 1H), 7.25-7.20 (m, 1H).

Procedure C. *tert*-Butyl 2-bromo-9*H*-carbazole-9-carboxylate (4a).

To a stirring solution of compound **3a** (1.5 g, 6.09 mmol) in THF (20 mL) were added $(Boc)_2O$ (1.46 g, 6.7 mmol) and DMAP (0.819 g, 6.7 mmol) in THF (20 mL) at room temperature. The reaction mixture was stirred at the same temperature for 12 h. The crude mixture was evaporated under reduced pressure, followed by extraction with EtOAc (3 × 20 mL) in water. The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 19:1) to yield compound **4a** (1.78 g, 84%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 8.48 (d, *J* = 8.4 Hz, 1H), 8.07 (d, *J* = 8.4 Hz, 1H), 7.24 (t, *J* = 7.8 Hz, 1H), 7.18 (d, *J* = 7.8 Hz, 1H), 7.11 (t, *J* = 7.2 Hz, 1H), 6.97 (t, *J* = 8.10 Hz, 1H), 1.53 (s, 9H).

***tert*-Butyl 3-bromo-9*H*-carbazole-9-carboxylate (4b).**

3-Bromo-9*H*-carbazole (**3b**) (2.0 g, 8.13 mmol) was reacted with $(Boc)_2O$ (1.95 g, 8.94 mmol) and DMAP (1.09 g, 8.94 mmol) in THF (20 mL) according to procedure C. The crude material was purified by column chromatography over silica gel using hexane:ethyl acetate (19:1) as solvent to give compound **4b** (2.8 g, ~100%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 8.27 (d, *J* = 8.4 Hz, 1H), 8.19 (d, *J* = 8.4 Hz, 1H), 8.08 (d, *J* = 2.4 Hz, 1H), 7.92 (qd, *J* = 7.8, 0.6 Hz, 1H), 7.55 (dd, *J* = 6.6, 2.4 Hz, 1H), 7.49 (td, *J* = 8.4, 1.2 Hz, 1H), 7.36 (td, *J* = 8.4, 1.2 Hz, 1H), 1.76 (s, 9H).

***tert*-Butyl 4-bromo-9*H*-carbazole-9-carboxylate (4c).**

Compound **3c** (1.5 g, 6.09 mmol) was reacted with $(Boc)_2O$ (1.46 g, 6.7 mmol) and DMAP (0.819 g, 6.7 mmol) in THF (20 mL) according to procedure C. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 19:1) to yield compound **4c** (1.78 g, 84%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.53 (s, 1H), 8.26 (d, J = 8.4 Hz, 1H), 7.94 (d, J = 7.8 Hz, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.49-7.46 (m, 2H), 7.36 (t, J = 7.2 Hz, 1H), 1.77 (s, 9H).

Procedure D. *tert*-Butyl 2-(4-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (5a).

To a mixture of compounds **4a** (0.8 g, 2.31 mmol), 1-(2-((tert-butyldimethylsilyl)oxy)-ethyl)piperazine (Das et al. 2015) (1.13 g, 4.62 mmol), BINAP (0.144 g, 0.23 mmol) and Cs_2CO_3 (2.26 g, 6.93 mmol), toluene (15 mL) was added under N_2 atmosphere. The reaction mixture was degassed by bubbling N_2 for 5 min and then $Pd(OAc)_2$ (0.039 g, 0.17 mmol) was added quickly. The system was degassed again and refluxed for 24 h under inert condition. The reaction mixture was cooled to room temperature, filtered through a pad of celite, washed with dichloromethane and concentrated in vacuum. The crude residue was purified by column chromatography (hexane:EtOAc = 4:1) to afford compound **5a** (0.97 g, 82%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.23 (d, J = 7.8 Hz, 1H), 7.92 (s, 1H), 7.84 (d, J = 7.2 Hz, 1H), 7.81 (d, J = 9.0 Hz, 1H), 7.35 (t, J = 7.2 Hz, 1H), 7.29 (t, J = 7.2 Hz, 1H), 7.00 (dd, J = 9.0, 1.8 Hz, 1H), 3.82 (t, J = 6.6 Hz, 2H), 3.32 (t, J = 4.8 Hz, 4H), 2.74 (t, J = 4.8 Hz, 4H), 2.62 (t, J = 6.6 Hz, 2H), 1.75 (s, 9H), 0.91 (s, 9H), 0.09 (s, 6H).

***tert*-Butyl 3-(4-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (5b).**

A mixture of compound **4b** (1.2 g, 3.47 mmol), 1-(2-((tert-butyldimethylsilyl)oxy)ethyl)piperazine (1.27 g, 5.20 mmol), $Pd(OAc)_2$ (0.058 g, 0.26 mmol), BINAP (0.216 g, 0.35 mmol) and Cs_2CO_3 (3.39 g, 10.4 mmol) in toluene (25 mL) was heated at 110 °C for 24 h according to procedure D. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 4:1) to give compound **5b** (1.4 g, 79%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.27 (d, J = 8.4 Hz, 1H), 8.16 (d, J = 8.4 Hz, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.48 (d, J = 2.4 Hz, 1H), 7.44 (t, J = 7.8 Hz, 1H), 7.32 (t, J = 7.8 Hz, 1H), 7.13 (dd, J = 6.6, 2.4 Hz, 1H), 3.83 (t, J = 6.6 Hz, 2H), 3.28 (t, J = 4.8 Hz, 4H), 2.77 (t, J = 4.8 Hz, 4H), 2.63 (t, J = 6.6 Hz, 2H), 1.75 (s, 9H), 0.92 (s, 9H), 0.09 (s, 6H).

***tert*-Butyl 4-(4-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (5c).**

A mixture of **4c** (2.70 g, 7.80 mmol), 1-(2-((tert-butyldimethylsilyl)oxy)-ethyl)piperazine (3.24 g, 13.25 mmol), $Pd(OAc)_2$ (0.0.13 g, 0.59 mmol), BINAP (0.49 g, 0.78 mmol) and Cs_2CO_3 (7.62 g, 23.4 mmol) in toluene (30 mL) was heated at 110°C for 24 h according to procedure D. The crude residue was purified by column chromatography (hexane:EtOAc = 4:1) to afford compound **5c** (3.24 g, 82%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.34 (dd, J = 8.1,4.8 Hz, 2H), 8.08 (d, J = 8.4 Hz, 1H), 7.45 (t, J = 7.2 Hz, 1H), 7.41-7.36 (m, 2H), 7.04 (d, J = 8.4 Hz, 1H), 3.85 (t, J = 6.6 Hz, 2H), 3.33 (d, J = 5.4 Hz, 2H), 2.99 (t, J = 5.4 Hz, 2H), 2.71 (t, J = 6.6 Hz, 4H), 1.75 (s, 9H), 0.91 (s, 9H), 0.10 (s, 6H).

Procedure E. *tert*-Butyl 2-(4-(2-hydroxyethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (6a).

Into a stirring solution of compound **5a** (0.95 g, 1.86 mmol) in THF (10 mL) was added *n*-tetrabutylammonium fluoride (2.8 mL, 2.8 mmol, 1.0 M solution in THF) at 0 °C. The reaction mixture was then stirred at room temperature for 2 h. THF was evaporated in vacuo, and the residue was diluted with CH_2Cl_2 (25 mL) and washed with a saturated solution of $NaHCO_3$. The water layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to give compound **6a** (0.595 g, 81%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.23 (d, J = 7.8 Hz, 1H), 7.94 (s, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.82 (d, J = 9.0 Hz, 1H), 7.36 (t, J = 7.2 Hz, 1H), 7.30 (t, J = 7.2 Hz, 1H), 7.00 (dd, J = 6.6, 1.8 Hz, 1H), 3.68 (t, J = 5.4 Hz, 2H), 3.34 (t, J = 4.8 Hz, 4H), 2.73 (t, J = 4.8 Hz, 4H), 2.64 (t, J = 5.4 Hz, 2H), 1.76 (s, 9H).

***tert*-Butyl 3-(4-(2-hydroxyethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (6b).**

Compound **5b** (1.2 g, 2.35 mmol) in THF (15 mL) was reacted with *n*-tetrabutylammonium fluoride (4.71 mL, 4.71 mmol, 1.0 M solution in THF) according to procedure E. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to yield compound **6b** (0.78 g, 84%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.28 (d, J = 8.4 Hz, 1H), 8.18 (d, J = 8.4 Hz, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 2.4 Hz, 1H), 7.44 (t, J = 7.8 Hz, 1H), 7.33 (t, J = 7.8 Hz, 1H), 7.13 (dd, J = 6.6, 2.4 Hz, 1H), 3.69 (t, J = 5.4 Hz, 2H), 3.29 (t, J = 4.8 Hz, 4H), 2.76 (t, J = 4.8 Hz, 4H), 2.66 (t, J = 5.4 Hz, 2H), 1.75 (s, 9H).

***tert*-Butyl 4-(4-(2-hydroxyethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (6c).**

Compound **5c** (3.30 g, 6.47 mmol) was reacted with *n*-tetrabutylammonium fluoride (9.70 mL, 9.70 mmol, 1.0 M solution in THF) in THF (30 mL) according to procedure E. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to give compound **6c** (2.01 g, 80%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.27 (d, J = 6.6 Hz, 1H), 8.17 (d, J = 7.8 Hz, 1H), 7.92 (d, J = 7.8 Hz, 1H), 7.47 (s, 1H), 7.43 (t, J = 7.2 Hz, 1H), 7.31 (t, J = 7.2 Hz, 1H), 7.11 (d, J = 9.0 Hz, 1H), 3.69 (t, J = 5.4 Hz, 2H), 3.26 (s, 4H), 2.76 (s, 4H), 2.62 (t, J = 5.4 Hz, 2H), 1.73 (s, 9H).

Procedure F. *tert*-Butyl 2-(4-(2-oxoethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (7a).

Into a stirring solution of compound **6a** (0.30 g, 0.76 mmol) in CH_2Cl_2 (6 mL) and DMSO (3 mL), was added Et_3N (0.74 mL, 5.31 mmol) at 0 °C. The reaction mixture was stirred for 5 min followed by addition of SO_3.py complex (0.604 g, 3.79 mmol) at 0 °C. Ice bath was removed, and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was quenched by addition of water and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layer was dried using Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 3:7) to give aldehyde **7a** (0.25 g, 84%). The purified aldehyde was used immediately for next step. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 9.74 (s, 1H), 8.23 (d, J = 8.4 Hz, 1H), 7.94 (s, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.36 (td, J = 7.2, 1.2 Hz, 1H), 7.29 (td, J = 7.2, 1.2 Hz, 1H), 6.97 (dd, J = 6.6, 1.8 Hz, 1H), 3.36 (t, J = 4.8 Hz, 4H), 3.24 (t, J = 1.2 Hz, 2H), 2.73 (t, J = 4.8 Hz, 4H), 1.75 (s, 9H).

***tert*-Butyl 3-(4-(2-oxoethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (7b).**

Compound **6b** (0.45 g, 1.14 mmol) in CH_2Cl_2 (10 mL) and DMSO (5 mL), was oxidized using SO_3.py complex (0.905 g, 5.69 mmol) and Et_3N (1.11 mL, 7.96 mmol) according to procedure F. The crude product was purified by silica gel column chromatography (EtOAc) to yield compound **7b** (0.35 g, 78%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 9.76 (s, 1H), 8.28 (d, *J* = 8.4 Hz, 1H), 8.18 (d, *J* = 8.4 Hz, 1H), 7.93 (d, *J* = 7.8 Hz, 1H), 7.48 (d, *J* = 2.4 Hz, 1H), 7.44 (t, *J* = 7.2 Hz, 1H), 7.32 (t, *J* = 7.2 Hz, 1H), 7.12 (dd, *J* = 6.6, 2.4 Hz, 1H), 3.31 (t, *J* = 4.2 Hz, 4H), 3.25 (t, *J* = 1.2 Hz, 2H), 2.74 (t, *J* = 4.2 Hz, 4H), 1.74 (s, 9H).

***tert*-Butyl 4-(4-(2-oxoethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (7c).**

Alcohol **6c** (0.35 g, 0.88 mmol) was oxidized using SO_3.py complex (0.704 g, 4.425 mmol), DMSO (9 mL) and Et_3N (0.86 mL, 6.19 mmol) in CH_2Cl_2 (6 mL) according to procedure F. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 3:7) to give aldehyde **7c** (0.31 g, 89%). The purified aldehyde was used immediately for next step. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 9.78 (s, 1H), 8.33 (d, *J* = 7.8 Hz, 1H), 8.27 (d, *J* = 7.8 Hz, 1H), 7.84 (d, *J* = 7.8 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.44-7.40 (m, 2H), 7.35 (t, *J* = 7.2 Hz, 1H), 7.06 (dd, *J* = 7.8, 1.8 Hz, 1H), 3.36-3.34 (m, 4H), 3.06 (t, *J* = 11.1 Hz, 2H), 2.73 (d, *J* = 11.4 Hz, 2H), 2.71 (d, *J* = 10.2 Hz, 2H), 1.75 (s, 9H).

Procedure G. *tert*-Butyl 2-(4-(2-((2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (8a).

Into a stirring solution of racemic N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine (0.058 g, 0.27 mmol) in CH_2Cl_2 (7 mL) was added aldehyde **7a** (0.12 g, 0.31 mmol). After the mixture was stirred for 1.5 h, $NaBH(OAc)_3$ (0.13 g, 0.61 mmol) was added and the mixture was stirred for another 46 h at room temperature. The reaction mixture

was quenched with a saturated solution of $NaHCO_3$ at 0 °C and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 19:1) to afford compound **8a** (0.06 g, 38%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 8.23 (d, *J* = 8.4 Hz, 1H), 7.92 (s, 1H), 7.85 (d, *J* = 7.8 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 7.35 (td, *J* = 7.2, 1.2 Hz, 1H), 7.29 (td, *J* = 7.2, 1.2 Hz, 1H), 7.00 (dd, *J* = 6.0, 2.4 Hz, 1H), 4.77 (bs, 2H), 3.33 (t, *J* = 4.8 Hz, 4H), 3.07 (m, 1H), 2.78–2.68 (m, 8H), 2.60–2.47 (m, 6H), 2.02–2.00 (m, 1H), 1.75 (s, 10H), 1.51–1.47 (m, 2H), 0.90 (t, *J* = 7.2 Hz, 3H).

***tert*-Butyl 3-(4-(2-((2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino) ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (8b).**

Aldehyde **7b** (0.15 g, 0.38 mmol) in CH_2Cl_2 (10 mL) was reacted with racemic N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine (0.073 g, 0.34 mmol) and $NaBH(OAc)_3$ (0.162 g, 0.76 mmol) according to procedure G. Crude product was purified by column chromatography (EtOAc:MeOH = 9:1) to afford compound **8b** (0.065 g, 32%). **^{1}H NMR** (400 MHz, $CDCl_3$): *δ* 8.27 (d, *J* = 8.0 Hz, 1H), 8.16 (d, *J* = 9.2 Hz, 1H), 7.93 (d, *J* = 7.6 Hz, 1H), 7.48 (d, *J* = 2.4 Hz, 1H), 7.44 (t, *J* = 7.2 Hz, 1H), 7.32 (t, *J* = 7.2 Hz, 1H), 7.12 (dd, *J* = 7.2, 2.4 Hz, 1H), 5.12 (bs, 2H), 3.28 (t, *J* = 4.8 Hz, 4H), 3.07–3.02 (m, 1H), 2.78–2.67 (m, 8H), 2.58–2.45 (m, 6H), 2.00–1.98 (m, 1H), 1.74 (s, 10H), 1.52–1.46 (m, 2H), 0.90 (t, *J* = 7.2 Hz, 3H).

***tert*-Butyl 4-(4-(2-((2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino) ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (8c).**

Aldehyde **7c** (0.15 g, 0.38 mmol) was reacted with racemic N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine (0.073 g, 0.34 mmol) and $NaBH(OAc)_3$ (0.162 g, 0.76 mmol) in CH_2Cl_2 (10 mL) according to procedure G. The crude product was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 19:1) to afford compound **8c** (0.91 g, 41%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.23 (d, *J* = 6.6 Hz, 1H), 8.28 (d, *J* = 6.6 Hz, 1H), 8.06 (d, *J* = 6.6 Hz, 1H), 7.43 (d, *J* = 5.4 Hz, 1H), 7.37 (d, *J* = 5.4 Hz, 2H), 7.02 (d, *J* = 6.0 Hz, 1H), 5.03 (s, 2H), 3.10-3.05 (m, 3H), 3.07-2.98 (m, 2H), 2.76–2.69 (m, 4H), 2.62–2.60 (m, 8H), 2.01–2.00 (m, 1H), 1.73 (s, 10H), 1.50–1.46 (m, 2H), 0.90 (t, *J* = 7.2 Hz, 3H).

(*S*)-*tert*-Butyl 2-(4-(2-((2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl) amino)ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (9a).

Aldehyde **7a** (0.125 g, 0.32 mmol) was reacted with (-)-pramipexole (0.06 g, 0.29 mmol) and $NaBH(OAc)_3$ (0.135 g, 0.64 mmol) in CH_2Cl_2 (8 mL) according to procedure G. The crude product was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 19:1) to afford compound **9a** (0.067 g, 40%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.23 (d, *J* = 8.4 Hz, 1H), 7.92 (s, 1H), 7.85 (d, *J* = 7.2 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 7.35 (td, *J* = 7.2, 1.2 Hz, 1H), 7.29 (td, *J* = 7.2, 1.2 Hz, 1H), 7.00 (dd, *J* = 6.6, 2.4 Hz, 1H), 4.76 (bs, 2H), 3.33 (t, *J* = 4.8 Hz, 4H), 3.07 (m, 1H), 2.79–2.68 (m, 8H), 2.61–2.48 (m, 6H), 2.02–2.00 (m, 1H), 1.75 (s, 10H), 1.51–1.48 (m, 2H), 0.90 (t, *J* = 7.2 Hz, 3H); $[\alpha]_D^{25}$= -44.0 (*c*=1.0 in CH_2Cl_2).

(*S*)-*tert*-Butyl 3-(4-(2-((2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl) amino)ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (9b).

Aldehyde **7b** (0.30 g, 0.76 mmol) was reacted with (-)-pramipexole (0.145 g, 0.69 mmol) and $NaBH(OAc)_3$ (0.323 g, 1.52 mmol) in CH_2Cl_2 (15 mL) according to procedure

G. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to afford compound **9b** (0.158 g, 39%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.27 (d, J = 7.2 Hz, 1H), 8.16 (d, J = 8.4 Hz, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.48 (d, J = 2.4 Hz, 1H), 7.43 (t, J = 7.2 Hz, 1H), 7.32 (t, J = 7.2 Hz, 1H), 7.12 (dd, J = 6.6, 2.4 Hz, 1H), 5.21 (bs, 2H), 3.28 (t, J = 4.8 Hz, 4H), 3.07–3.02 (m, 1H), 2.78–2.66 (m, 8H), 2.59–2.46 (m, 6H), 2.01–1.99 (m, 1H), 1.74 (s, 10H), 1.52–1.46 (m, 2H), 0.90 (t, J = 7.2 Hz, 3H).

(*S*)-*tert*-Butyl 4-(4-(2-((2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl) amino)ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (9c).

Aldehyde **7c** (0.150 g, 0.38 mmol) was reacted with (-)-pramipexole (0.73 g, 0.54 mmol) and $NaBH(OAc)_3$ (0.162 g, 0.76 mmol) in CH_2Cl_2 (10 mL) according to procedure G. The crude product was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 19:1) to afford compound **9c** (0.097 g, 43%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.32 (d, J = 7.2 Hz, 1H), 8.28 (d, J = 7.8 Hz, 1H), 8.06 (d, J = 7.8 Hz, 1H), 7.43 (d, J = 7.2 Hz, 1H), 7.40-7.35 (m, 2H), 7.30 (d, J = 7.2 Hz, 1H), 4.83 (bs, 2H), 3.33 (d, J = 8.4 Hz, 2H), 3.06 (m, 3H), 2.99 (m, 2H), 2.76–2.70 (m, 4H), 2.60 (m, 7H), 1.74 (s, 10H), 1.51–1.48 (m, 2H), 0.90 (t, J = 6.6 Hz, 3H); $[\alpha]_D^{25}$= -27.20 (c=1.0 in CH_2Cl_2).

Procedure H. N^6-(2-(4-(9*H*-carbazol-2-yl)piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo-[*d*]thiazole-2,6-diamine (10a) (D-652).

To a stirred solution of **8a** (0.055 g, 0.09 mmol) in CH_2Cl_2 (3 mL) at 0 ^{o}C, trifluoroacetic acid (3 mL) was added slowly and the reaction mixture was stirred for 3 h at room temperature. Unreacted TFA and solvent were removed under reduced pressure and the obtained TFA salt was washed with ether for several times followed by drying to yield **10a** (0.079 g, 90%). **^{1}H NMR** (600 MHz, CD_3OD): δ 7.99 (d, J = 8.4 Hz, 1H), 7.96

(d, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 1H), 7.32 (t, *J* = 7.2 Hz, 1H), 7.25 (s, 1H), 7.13 (t, *J* = 7.2 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 3.84 (m, 1H), 3.64–3.55 (m, 5H), 3.48-3.42 (m, 3H), 3.36 (s, 4H), 3.23-3.14 (m, 2H), 3.01-2.99 (m, 1H), 2.84-2.79 (m, 1H), 2.71–2.65 (m, 2H), 2.32-2.31 (m, 1H), 2.02-1.95 (m, 1H), 1.83–1.79 (m, 2H), 1.03 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): *δ* 170.28, 160.68, 145.32, 140.57, 132.69, 125.56, 124.53, 122.50, 121.20, 120.14, 119.84, 119.33, 118.79, 118.29, 117.08, 115.15, 111.37, 110.77, 109.73, 59.75, 52.49, 51.56, 50.08, 22.29, 21.88, 21.39, 18.01, 10.19; Anal. Calcd for $C_{28}H_{36}N_6S.4CF_3COOH$: C, 45.77; H, 4.27; N, 8.90. Found: C, 45.71; H, 4.64; N, 8.87.

N^6-(2-(4-(9*H*-carbazol-3-yl)piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo [*d*]thiazole-2,6-diamine (10b) (D-627).

Compound **8b** (0.05 g, 0.09 mmol) in CH_2Cl_2 (2 mL) at 0 °C, was treated with trifluoroacetic acid (2 mL) according to procedure H to obtain the TFA salt of compound **10b** (0.052 g, 84%). **^{1}H NMR** (400 MHz, CD_3OD): *δ* 8.01 (d, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 1.6 Hz, 1H), 7.41-7.36 (m, 2H), 7.32 (t, *J* = 7.2 Hz, 1H), 7.16-7.14 (m, 1H), 7.11 (t, *J* = 7.2 Hz, 1H), 3.34 (s, 1H), 3.19 (s, 4H), 2.98–2.91 (m, 2H), 2.80-2.51 (m, 12H), 2.07–2.04 (m, 1H), 1.81-1.77 (m, 1H), 1.63–1.53 (m, 2H), 0.95 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** (100 MHz, CD_3OD): *δ* 168.63, 144.25, 143.69, 143.37, 140.69, 135.84, 125.07, 123.21, 122.92, 119.50, 118.21, 118.00, 113.45, 110.87, 110.41, 107.90, 58.85, 55.41, 53.24, 53.12, 51.07, 25.37, 24.70, 23.79, 20.35, 10.43; Anal. Calcd for $C_{28}H_{36}N_6S.2CF_3COOH$: C, 53.62; H, 5.34; N, 11.73. Found: C, 53.64; H, 5.83; N, 11.34.

N^6-(2-(4-(*9H*-carbazol-4-yl)piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo [*d*]thiazole-2,6-diamine (10c) (D-658).

Compound **8c** (0.082 g, 0.14 mmol) was treated with trifluoroacetic acid (3 mL) in CH_2Cl_2 (3 mL) according to procedure H to furnish the TFA salt of **10c** (0.091g, 90%). **^{1}H NMR** (600 MHz, CD_3OD): δ 8.08 (d, J = 7.8 Hz, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.36 (t, J = 7.8 Hz, 1H), 7.31 (t, J = 7.8 Hz, 1H), 7.22 (d, J = 7.8 Hz, 1H), 7.19 (t, J = 7.2 Hz, 1H), 6.82 (d, J = 7.8 Hz, 1H), 3.94–3.89 (m, 1H), 3.83 (d, J = 7.2 Hz, 2H), 3.77-3.59 (m, 8H), 3.29 (d, J = 1.2 Hz, 1H), 3.24-3.20 (m, 3H), 3.06-3.04 (m, 1H), 2.92-2.88 (m, 1H), 2.78–2.71 (m, 2H), 2.37-2.36 (m, 1H), 2.11-2.04 (m, 1H), 1.86–1.82 (m, 2H), 1.04 (t, J = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): δ 170.32, 161.19, 146.42, 141.50, 139.78, 132.81, 125.91, 124.74, 121.78, 121.34, 118.62, 115.64, 111.66, 110.23, 107.39, 59.17, 53.36, 53.25, 52.80, 50.81, 48.60, 48.01, 44.95, 22.51, 22.03, 21.49, 18.34, 9.79; Anal. Calcd for $C_{28}H_{36}N_6S$, $3CF_3COOH$, CH_3OH, H_2O: C, 47.73; H, 5.15; N, 9.54. Found: C, 47.63; H, 4.67; N, 9.23.

(*S*)-*N*^{6}-(2-(4-(9*H*-carbazol-2-yl)piperazin-1-yl)ethyl)-*N*^{6}-propyl-4,5,6,7-tetrahydro-benzo[*d*]thiazole-2,6-diamine (11a) (D-651).

Compound **9a** (0.065 g, 0.11 mmol) was treated with trifluoroacetic acid (3 mL) in CH_2Cl_2 (3 mL) according to procedure H to furnish the TFA salt of **11a** (0.085 g, 93%). **^{1}H NMR** (600 MHz, CD_3OD): δ 7.99 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 7.8 Hz, 1H), 7.42 (d, J = 7.8 Hz, 1H), 7.33 (t, J = 7.2 Hz, 1H), 7.26 (s, 1H), 7.13 (t, J = 7.2 Hz, 1H), 7.04 (d, J = 8.4 Hz, 1H), 3.86 (m, 1H), 3.65–3.56 (m, 5H), 3.49-3.42 (m, 3H), 3.37 (s, 4H), 3.25-3.16 (m, 2H), 3.02-3.00 (m, 1H), 2.85-2.81 (m, 1H), 2.73–2.66 (m, 2H), 2.34-2.32 (m, 1H), 2.04-1.97 (m, 1H), 1.83–1.80 (m, 2H), 1.03 (t, J = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): δ 170.29, 160.41, 145.25, 140.58, 132.67, 125.55, 124.55, 122.49, 121.21, 120.15, 119.82, 119.32, 118.82, 118.24, 116.97, 115.06, 111.39, 110.76, 109.70, 59.75,

52.50, 51.55, 50.17, 22.31, 21.89, 21.40, 17.98, 10.18; $[\alpha]_D^{25}$= -24.8 (*c*=1.0 in CH_3OH); Anal. Calcd for $C_{28}H_{36}N_6S.3CF_3COOH.2H_2O$: C, 47.11; H, 5.00; N, 9.70. Found: C, 47.63; H, 4.67; N, 9.23.

(*S*)-*N*^{6}-(2-(4-(9*H*-carbazol-3-yl)piperazin-1-yl)ethyl)-*N*^{6}-propyl-4,5,6,7-tetrahydro-benzo[*d*]thiazole-2,6-diamine (11b) (D-636).

Compound **9b** (0.15 g, 0.25 mmol) was treated with trifluoroacetic acid (8 mL) in CH_2Cl_2 (8 mL) according to procedure H to furnish the TFA salt of **11b** (0.20 g, 96%). **^{1}H NMR** (600 MHz, CD_3OD): *δ* 8.27 (s, 1H), 8.11 (d, *J* = 7.2 Hz, 1H), 7.59-7.54 (m, 2H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.44 (t, *J* = 7.2 Hz, 1H), 7.21 (t, *J* = 7.2 Hz, 1H), 3.96 (m, 1H), 3.80 (s, 4H), 3.57–3.45 (m, 2H), 3.34-3.27 (m, 2H), 3.20-3.10 (m, 7H), 2.97-2.92 (m, 1H), 2.83-2.75 (m, 2H), 2.44–2.42 (m, 1H), 2.17-2.10 (m, 1H), 1.90–1.84 (m, 2H), 1.07 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): *δ* 170.35, 160.54, 141.04, 139.17, 135.08, 132.80, 126.39, 123.44, 122.29, 119.95, 119.05, 117.60, 111.67, 111.55, 111.45, 110.90, 59.31, 54.18, 53.61, 51.21, 50.61, 22.42, 22.09, 21.50, 17.98, 9.82; $[\alpha]_D^{25}$= -17.4 (*c*=1.0 in CH_3OH); Anal. Calcd for $C_{28}H_{36}N_6S.3CF_3COOH.H_2O$: C, 48.11; H, 4.87; N, 9.90. Found: C, 48.25; H, 4.99; N, 9.58.

***(S)*-*N*^{6}-(2-(4-(9H-carbazol-4-yl)piperazin-1-yl)ethyl)-*N*^{6}-propyl-4,5,6,7-tetrahydro-benzo[*d*]thiazole-2,6-diamine (11c) (D-657).**

Compound **9c** (0.83 g, 0.14 mmol) was treated with trifluoroacetic acid (7 mL) in CH_2Cl_2 (7 mL) according to procedure H to furnish the TFA salt of **11c** (0.085 g, 93%). **^{1}H NMR** (600 MHz, CD_3OD): *δ* 8.07 (d, *J* = 7.2 Hz, 1H), 7.44 (d, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 7.8 Hz, 1H), 7.31 (t, *J* = 7.8 Hz, 1H), 7.22 (d, *J* = 7.8 Hz,, 1H), 7.19 (t, *J* = 7.2 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 3.90 (s, 1H) 3.84 (d, *J* = 6.6 Hz, 2H), 3.78–3.71 (m, 4H), 3.56-

3.43 (m, 4H), 3.29 (s, 2H), 3.26-3.21 (m, 3H), 3.06-3.04 (m, 1H), 2.92-2.88 (m, 1H), 2.78–2.71 (m, 2H), 2.36 (bs, 1H), 2.09-2.04 (m, 1H), 1.86–1.82 (m, 2H), 1.04 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): *δ* 170.32, 161.06, 146.42, 139.77, 132.80, 126.38, 124.29, 122.26, 121.30, 119.075, 118.18, 115.62, 111.61, 110.74, 109.72, 59.72, 58.72, 53.27, 52.76, 50.70, 44.91, 22.99, 22.34, 21.44, 18.30, 10.07, 9.46; $[\alpha]_D^{25}$= -25.0 (*c*=1.0 in CH_3OH); Anal. Calcd for $C_{28}H_{36}N_6S.3CF_3COOH$: C, 49.16; H, 4.73; N, 10.12. Found: C, 48.93; H, 4.84; N, 9.77.

***tert*-Butyl 2-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino) ethyl)-piperazin-1-yl)-9*H*-carbazole-9-carboxylate (12a).**

Compound **7a** (0.10 g, 0.25 mmol) was reacted with (±)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.056 g, 0.25 mmol) and $NaBH(OAc)_3$ (0.108 g, 0.51 mmol) in CH_2Cl_2 (7 mL) according to procedure G. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 1:3) to afford compound **12a** (0.115 g, 77%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 8.23 (d, *J* = 7.8 Hz, 1H), 7.91 (s,1H), 7.83 (d, *J* = 7.8 Hz, 1H), 7.79 (d, *J* = 8.4 Hz, 1H), 7.34 (t, *J* = 7.2, Hz, 1H), 7.28 (t, *J* = 7.2 Hz, 1H), 7.08 (t, *J* = 7.8 Hz, 1H), 6.98 (dd, *J* = 6.6, 1.8 Hz, 1H), 6.72 (d, *J* = 7.2, Hz, 1H), 6.64 (d, *J* = 7.8, Hz, 1H), 3.79 (s, 3H), 3.32 (t, *J* = 4.8 Hz, 4H), 3.02–2.93 (m, 2H), 2.88-2.85 (m, 1H), 2.79–2.75 (m, 3H), 2.70 (t, *J* = 4.8 Hz, 4H), 2.56–2.50 (m, 5H), 2.09–2.06 (m, 1H), 1.74 (s, 9H), 1.62–1.55 (m, 1H), 1.53-1.47 (m, 2H), 0.91 (t, *J* = 7.2 Hz, 3H).

***tert*-Butyl 3-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino) ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (12b).**

Compound **7b** (0.170 g, 0.43 mmol) was reacted with (±)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.095 g, 0.43 mmol) and $NaBH(OAc)_3$ (0.183

g, 0.86 mmol) in CH_2Cl_2 (10 mL) according to procedure G. The crude product was purified by silica gel column chromatography with EtOAc to afford compound **12b** (0.11 g, 43%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.27 (d, *J* = 7.8 Hz, 1H), 8.16 (d, *J* = 8.4 Hz, 1H), 7.92 (d, *J* = 7.8 Hz, 1H), 7.47 (d, *J* = 2.4 Hz, 1H), 7.43 (t, *J* = 7.2, Hz, 1H), 7.31 (t, *J* = 7.2 Hz, 1H), 7.12 (dd, *J* = 6.6, 2.4 Hz, 1H), 7.08 (t, *J* = 8.4 Hz, 1H), 6.72 (d, *J* = 7.2, Hz, 1H), 6.64 (d, *J* = 8.4 Hz, 1H), 3.79 (s, 3H), 3.27 (t, *J* = 4.8 Hz, 4H), 3.03–2.98 (m, 2H), 2.90-2.87 (m, 1H), 2.81–2.77 (m, 3H), 2.73 (t, *J* = 4.8 Hz, 4H), 2.59–2.51 (m, 5H), 2.11–2.08 (m, 1H), 1.74 (s, 9H), 1.63–1.57 (m, 1H), 1.55-1.49 (m, 2H), 0.91 (t, *J* = 7.2 Hz, 3H).

***tert*-Butyl 4-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino) ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (12c).**

Compound **7c** (0.10 g, 0.25 mmol) was reacted with (±)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.055 g, 0.25 mmol) and $NaBH(OAc)_3$ (0.108 g, 0.50 mmol) in CH_2Cl_2 (8 mL) according to procedure G. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 1:3) to afford compound **12c** (0.112 g, 74%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.23 (d, *J* = 8.4 Hz, 1H), 8.16 (d, *J* = 8.4, Hz, 1H), 8.09 (d, *J* = 7.2 Hz, 1H), 7.47 (d, *J* = 7.8 Hz, 1H), 7.39 (m, 2H), 7.13 (t, *J* = 7.8 Hz, 1H), 7.00 (d, *J* = 7.8 Hz, 1H), 6.70 (q, *J* = 7.8 Hz, 2H), 3.92 (bs, 2H), 3.79 (s, 3H), 3.58–3.48 (m, 4H), 2.29 (m, 2H), 3.06- 3.04 (m, 5H), 2.62, (m, 1H), 2.37 (bs, 1H), 2.09–2.06 (m, 1H), 1.74 (s, 9H), 1.62–1.55 (m, 1H), 1.53-1.47 (m, 2H), 0.92 (t, *J* = 7.2 Hz, 3H).

(*S*)-*tert*-Butyl 2-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl) amino)ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (13a).

Compound **7a** (0.10 g, 0.25 mmol) was reacted with (-)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.056 g, 0.25 mmol) and $NaBH(OAc)_3$ (0.108

g, 0.51 mmol) in CH_2Cl_2 (7 mL) according to procedure G. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 1:3) to afford compound **13a** (0.11 g, 74%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.23 (d, *J* = 8.4 Hz, 1H), 7.91 (s,1H), 7.82 (d, *J* = 7.8 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.34 (t, *J* = 7.2, Hz, 1H), 7.28 (t, *J* = 7.2 Hz, 1H), 7.08 (t, *J* = 7.8 Hz, 1H), 6.97 (dd, *J* = 6.6, 1.8 Hz, 1H), 6.71 (d, *J* = 7.2, Hz, 1H), 6.63 (d, *J* = 7.8, Hz, 1H), 3.78 (s, 3H), 3.32 (t, *J* = 4.8 Hz, 4H), 3.02–2.94 (m, 2H), 2.88-2.85 (m, 1H), 2.79–2.74 (m, 3H), 2.70 (t, *J* = 4.8 Hz, 4H), 2.56–2.50 (m, 5H), 2.08–2.06 (m, 1H), 1.74 (s, 9H), 1.61–1.54 (m, 1H), 1.52-1.47 (m, 2H), 0.91 (t, *J* = 7.2 Hz, 3H); $[\alpha]_D^{25}$= -28.6 (*c*=1.0 in CH_2Cl_2).

(*S*)- *tert*-Butyl 3-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino) ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (13b).

Compound **7b** (0.140 g, 0.456 mmol) was reacted with (-)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.070 g, 0.32 mmol) and $NaBH(OAc)_3$ (0.151 g, 0.71 mmol) in CH_2Cl_2 (10 mL) according to procedure G. The crude product was purified by silica gel column chromatography with EtOAc to afford compound 13b (0.07 g, 33%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.18 (d, *J* = 6 Hz, 1H), 8.09 (m, 1H), 7.83 (d, *J* = 7.2 Hz, 1H), 7.38-7.32 (m, 2H), 7.23 (q, *J* = 9.6, 7.5 Hz, 1H), 7.03 (d, *J* = 9 Hz, 1H), 6.99 (t, *J* = 7.8 Hz, 1H), 6.63 (d, *J* = 6.6, Hz, 1H), 6.55 (d, *J* = 7.8, Hz, 1H), 3.70 (s, 3H), 3.18 (t, *J* = 4.2 Hz, 4H), 3.27–3.26 (m, 1H), 3.12-3.10 (m, 2H), 2.93–2.90 (m, 2H), 2.81–2.78 (m, 1H), 2.69-2.64 (m, 5H), 2.48–2.43 (m, 4H), 2.03–2.00 (m, 1H), 1.65 (s, 9H), 1.54–1.47 (m, 1H), 1.45-1.37 (m, 2H), 0.82 (t, *J* = 7.2 Hz, 3H).

(*S*)-*tert*-Butyl 4-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl) amino)ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (13c).

Compound **7c** (0.10 g, 0.25 mmol) was reacted with (-)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.055 g, 0.25 mmol) and $NaBH(OAc)_3$ (0.108 g, 0.50 mmol) in CH_2Cl_2 (8 mL) according to procedure G. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 1:3) to afford compound **13c** (0.124 g, 82%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.35-8.30 (m, 2H), 7.07 (d, *J* = 7.8 Hz, 1H), 7.44-7.41 (m, 1H), 7.41-7.39 (m, 2H), 7.08 (d, *J* = 7.8 Hz, 1H), 7.04 (d, *J* = 7.8 Hz, 1H), 6.73 (d, *J* = 7.2, Hz, 1H), 6.64 (d, *J* = 8.4 Hz, 1H), 3.79 (s, 3H), 3.53-3.48 (m, 4H), 3.29 (m, 2H), 3.16-3.04 (m, 5H), 2.67-2.60 (m, 1H), 2.37 (bs, 1H), 2.03–2.01 (m, 2H), 1.85–1.84 (m, 3H), 1.75 (s, 9H), 1.26-1.24 (m, 3H), 0.92 (t, *J* = 7.2 Hz, 3H); $[\alpha]_D^{25}$= -20.3 (*c*=1.0 in CH_2Cl_2).

Procedure I. 6-((2-(4-(9*H*-carbazol-2-yl)piperazin-1-yl)ethyl)(propyl)amino)-5,6,7,8-tetrahydronaphthalen-1-ol (14a) (D-654).

A mixture of compound **12a** (0.10 g, 0.17 mmol) and 48% aqueous HBr (8 mL) was refluxed at 130 °C for 4 h. The reaction mixture was evaporated to dryness, washed with ether followed by vacuo drying to yield HBr salt of **14a** (0.095 g, 78%). **^{1}H NMR** (600 MHz, CD_3OD): δ 8.11 (d, *J* = 8.4 Hz, 1H), 8.03 (d, *J* = 7.8 Hz, 1H), 7.67-7.62 (m, 1H), 7.46 (d, *J* = 7.8 Hz, 1H), 7.38 (t, *J* = 7.2 Hz, 1H), 7.34-7.29 (m, 1H), 7.17 (t, *J* = 7.2 Hz, 1H), 6.96 (t, *J* = 7.8 Hz, 1H), 6.67 (d, *J* = 7.8, Hz, 1H), 6.62 (d, *J* = 7.8, Hz, 1H), 3.99-3.86 (m, 5H), 3.84–3.72 (m, 5H), 3.68-3.63 (m, 2H), 3.39 (m, 1H), 3.33-3.29 (m, 2H), 3.16-3.08 (m, 2H), 2.71-2.66 (m, 1H), 2.63 (m, 1H), 2.49-2.47 (m, 1H), 2.00–1.93 (m, 3H), 1.07 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): δ 154.74, 140.88, 140.12, 133.27, 129.28, 126.63, 125.85, 122.06, 121.59, 121.12, 120.99, 119.93, 119.78, 119.11, 117.61, 112.08, 110.61, 110.26, 61.26, 52.93, 51.47, 50.73, 50.47, 50.32, 45.16, 29.29, 23.55, 22.35,

18.26, 9.92, 8.31; Anal. Calcd for $C_{31}H_{38}N_4O.3HBr$: C, 51.33; H, 5.70; N, 7.72. Found: C, 51.50; H, 5.92; N, 7.46.

6-((2-(4-(9*H*-carbazol-3-yl)piperazin-1-yl)ethyl)(propyl)amino)-5,6,7,8-tetrahydro-naphthalen-1-ol (14b) (D-650).

A mixture of compound **12b** (0.08 g, 0.13 mmol) and 48% aqueous HBr (7 mL) was reflexed according to procedure I to yield HBr salt of **14b** (0.095 g, 98%). **^{1}H NMR** (600 MHz, CD_3OD): δ 8.58 (s, 1H), 8.14 (d, J = 7.8 Hz, 1H), 7.76 (d, J = 8.4 Hz, 1H), 7.61 (d, J = 9.0 Hz, 1H), 7.50 (d, J = 7.8 Hz, 1H), 7.44 (t, J = 7.2 Hz, 1H), 7.21 (t, J = 7.2 Hz, 1H), 6.94 (t, J = 7.8 Hz, 1H), 6.66 (d, J = 7.8 Hz, 1H), 6.61 (d, J = 7.8, Hz, 1H), 4.30 (s, 4H), 4.11-4.04 (m, 6H), 3.93–3.89 (m, 2H), 3.78 (s, 1H), 3.46-3.39 (m, 1H), 3.33-3.29 (m, 2H), 3.22-3.16 (m, 1H), 3.09-3.05 (m, 1H), 2.72-2.69 (m, 1H), 2.52-2.47 (m, 1H), 2.02–1.94 (m, 3H), 1.07 (t, J = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): δ 154.67, 141.05, 139.88, 133.32, 132.51, 127.22, 126.31, 123.45, 122.11, 121.60, 120.77, 120.15, 119.76, 117.91, 116.92, 112.40, 112.24, 111.96, 111.54, 111.40, 110.53, 60.95, 53.09, 50.47, 49.91, 29.34, 22.81, 22.37, 18.30, 10.26, 9.73; Anal. Calcd for $C_{31}H_{38}N_4O.3HBr.2H_2O$: C, 48.90; H, 5.96; N, 7.36. Found: C, 49.30; H, 5.86; N, 7.24.

6-((2-(4-(9*H*-carbazol-4-yl)piperazin-1-yl)ethyl)(propyl)amino)-5,6,7,8-tetrahydro-naphthalen-1-ol (14c) (D-655).

Compound **12c** (0.11 g, 0.18 mmol) was refluxed with 48% aqueous HBr (8 mL) according to procedure I to yield HBr salt of **14c** (0.097 g, 78%). **^{1}H NMR** (600 MHz, CD_3OD): δ 8.11 (d, J = 7.8 Hz, 1H), 8.07 (d, J = 7.8 Hz, 1H), 7.46-7.43 (m, 1H), 7.37-7.32 (m, 1H), 7.31-7.27 (m, 1H), 7.25-7.19 (m, 1H), 6.95 (t, J = 7.8 Hz, 1H), 6.87 (d, J = 7.8 Hz, 1H), 6.79 (d, J = 6.6, Hz, 1H), 6.61 (d, J = 7.8, Hz, 1H), 4.02 (s, 3H), 3.90–3.85 (m,

5H), 3.58-3.57 (m, 3H), 3.39 (m, 2H), 3.33-3.29 (m, 2H), 3.29-3.17 (m, 2H), 3.11-3.08 (m, 1H), 2.72-2.70 (m, 1H), 2.48 (m, 1H), 1.99–1.94 (m, 3H), 1.07 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): *δ* 154.71, 141.47, 139.70, 133.20, 126.61, 125.85, 124.74, 121.97, 121.81, 121.58, 121.25, 119.96, 118.76, 118.66, 115.57, 112.07, 110.24, 110.21,107.23 61.40, 52.89, 50.25, 48.57, 47.09, 45.57, 43.81, 29.23, 23.57, 22.30, 18.45, 9.91; Anal. Calcd for $C_{31}H_{38}N_4O.3HBr$: C, 51.33; H, 5.70; N, 7.72. Found: C, 51.07; H, 5.91; N, 8.17.

(*S*)-6-((2-(4-(9*H*-carbazol-2-yl)piperazin-1-yl)ethyl)(propyl)amino)-5,6,7,8-tetrahydronaphthalen-1-ol (15a) (D-653).

Compound **13a** (0.10 g, 0.17 mmol) was refluxed with 48% aqueous HBr (8 mL) according to procedure I to yield HBr salt of **15a** (0.10 g, 82%). **^{1}H NMR** (600 MHz, CD_3OD): *δ* 8.11 (d, *J* = 8.4 Hz, 1H), 8.03 (d, *J* = 7.8 Hz, 1H), 7.62 (m, 1H), 7.46 (d, *J* = 7.8 Hz, 1H), 7.38 (t, *J* = 7.2 Hz, 1H), 7.34-7.28 (m, 1H), 7.17 (t, *J* = 7.2 Hz, 1H), 6.96 (t, *J* = 7.8 Hz, 1H), 6.68 (d, *J* = 7.8, Hz, 1H), 6.62 (d, *J* = 7.8, Hz, 1H), 3.99 (s, 3H), 3.93-3.85 (m, 2H), 3.82–3.63 (m, 7H), 3.39 (m, 1H), 3.33-3.29 (m, 2H), 3.17-3.08 (m, 2H), 2.70 (m, 1H), 2.63-2.62 (m, 1H), 2.49-2.47 (m, 1H), 2.01–1.94 (m, 3H), 1.07 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): *δ* 154.73, 140.86, 140.14, 133.28, 129.27, 126.62, 125.82, 122.08, 121.60, 121.09, 120.97, 119.93, 119.76, 119.10, 117.58, 112.08, 110.60, 110.26, 72.29, 61.27, 52.94, 51.83, 51.45, 50.78, 50.49, 41.61, 29.31, 23.48, 22.35, 18.26, 9.92, 8.31; $[\alpha]_D^{25}$= -21.5 (*c*=1.0 in CH_3OH); Anal. Calcd for $C_{31}H_{38}N_4O.3HBr$: C, 51.33; H, 5.70; N, 7.72. Found: C, 51.44; H, 5.93; N, 7.47.

(*S*)-6-((2-(4-(9*H*-carbazol-3-yl)piperazin-1-yl)ethyl)(propyl)amino)-5,6,7,8-tetra-hydronaphthalen-1-ol (15b) (D-659).

Compound **13b** (0.07 g, 0.12 mmol) was refluxed with 48% aqueous HBr (7 mL) according to procedure I to yield HBr salt of **15b** (0.085 g, 97%). **^{1}H NMR** (600 MHz, CD_3OD): δ 8.58 (s, 1H), 8.15 (q, *J* = 7.8 Hz, 2H), 7.76 (d, *J* = 6.6 Hz, 1H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.43 (q, *J* = 7.8 Hz, 1H), 7.21 (d, *J* = 7.8 Hz, 1H), 6.94 (t, *J* = 7.2 Hz, 1H), 6.66 (d, *J* = 7.2 Hz, 1H), 6.61 (d, *J* = 7.8, Hz, 1H), 4.17 (s, 4H), 3.91-3.85 (m, 6H), 3.87–3.85 (m, 2H), 3.57 (s, 1H), 3.50-3.49 (m, 1H), 3.32-3.28 (m, 2H), 3.17-3.14 (m, 1H), 3.08-3.05 (m, 1H), 2.69-2.67 (m, 1H), 2.51-2.46 (m, 1H), 1.96–1.93 (m, 3H), 1.48 (t, *J* = 6.0 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): δ 157.19, 151.08, 147.936, 147.513.32, 138.93, 137.85, 133.55, 132.78, 129.08,127.16, 126.92, 122.87, 122.74, 121.64, 119.43, 118.42, 117.40, 107.78, 106.90, 106.54, 57.16, 55.21, 51.85, 50.41, 48.10, 40.131, 29.71, 25.60, 23.87, 22.08, 11.92; $[\alpha]_D^{25}$= -22.2 (*c*=1.0 in CH_3OH); Anal. Calcd for $C_{31}H_{38}N_4O.4HBr$: C, 46.18; H, 5.25; N, 6.95. Found: C, 46.13; H, 5.66; N, 7.59.

(*S*)-6-((2-(4-(9*H*-carbazol-4-yl)piperazin-1-yl)ethyl)(propyl)amino)-5,6,7,8-tetra-hydronaphthalen-1-ol (15c) (D-656).

Compound **13c** (0.112 g, 0.18 mmol) was refluxed with 48% aqueous HBr (9 mL) according to procedure I to yield HBr salt of **15c** (0.10 g, 82%). **^{1}H NMR** (600 MHz, CD_3OD): δ 8.09 (d, *J* = 7.2 Hz, 1H), 7.34 (d, *J* = 8.4 Hz, 1H), 7.33 (t, *J* = 7.2 Hz, 1H), 7.26 (dd, *J* = 4.8, 2.4 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 2H), 6.92 (t, *J* = 7.8 Hz, 1H), 6.72 (d, *J* = 7.2 Hz, 1H), 6.65 (d, *J* = 7.2 Hz, 1H), 6.62 (d, *J* = 7.8, Hz, 1H), 4.02 (s, 3H), 3.90-3.84 (m, 6H), 3.59–3.56 (m, 2H), 3.40 (m, 2H), 3.30-3.28 (m, 2H), 3.19-3.14 (m, 1H), 3.06-3.05 (m, 1H), 2.71-2.68 (m, 1H), 2.47 (bs, 1H), 1.96–1.92 (m, 3H), 1.05 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): δ 154.69, 141.41, 139.73, 133.24, 127.24, 125.30, 124.28, 122.56, 121.57, 121.21,120.28, 119.63, 119.28, 118.33, 115.52, 112.43, 111.75, 110.77,

109.71, 107.68, 61.27, 52.94, 51.83, 60.95, 52.82, 50.31, 44.47, 43.68, 22.53, 18.97, 9.92, ; $[\alpha]_D^{25}$= -17.8 (*c*=1.0 in CH_3OH); Anal. Calcd for **)** $C_{31}H_{38}N_4O.2HBr.\ CH_2Cl_2$: C, 52.69; H, 5.80; N, 7.68. Found: C, 52.23; H, 6.18; N, 7.96.

9-(2-bromoethyl)-9*H*-carbazole (16).

A suspension of carbazole (1.0 g, 5.98 mmol), K_2CO_3 (1.82 g, 13.16 mmol), tetrabutylammonium bromide (0.039 g, 0.12 mmol) and KOH (2.25 g, 40.07 mmol) in dibromoethane (10 mL, 119.61 mmol) was stirred at 50 °C under N_2 overnight. The reaction mixture was filtered off and diluted with CH_2Cl_2. The organic layer was washed with water, dried over sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography with petroleum ether to afford compound **16** (0.52 g, 32%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.10 (d, *J* = 7.6 Hz, 2H), 7.51-7.42 (m, 4H), 7.29-7.25 (m, 2H), 4.71 (t, *J* = 7.2 Hz, 2H), 3.68 (t, *J* = 7.2 Hz, 2H).

9-(2-(4-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)ethyl)-9*H*-carbazole (17).

A mixture of compound **16** (0.7 g, 2.55 mmol), 1-(2-((tert-butyldimethylsilyl)-oxy)ethyl)piperazine (0.75 g, 3.06 mmol), and K_2CO_3 (1.06 g, 7.66 mmol) in acetonitrile (20 mL) was refluxed for 24 h under inert condition. The reaction mixture was cooled to room temperature, filtered, washed with EtOAc and concentrated in vacuo. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 3:2) to give compound **17** (0.81 g, 73%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.09 (d, *J* = 7.2 Hz, 2H), 7.49-7.41 (m, 4H), 7.25-7.21 (m, 2H), 4.45 (t, *J* = 7.2 Hz, 2H), 3.76 (t, *J* = 6.8 Hz, 2H), 2.76 (t, *J* = 7.2 Hz, 2H), 2.68-2.57 (m, 6H), 2.54 (t, *J* = 6.4 Hz, 4H), 0.90 (s, 9H), 0.06 (s, 6H).

2-(4-(2-(9*H*-carbazol-9-yl)ethyl)piperazin-1-yl)ethanol (**18**).

Compound **17** (0.875 g, 2.0 mmol) was treated with *n*-tetrabutylammonium fluoride (4.0 mL, 4.0 mmol, 1.0 M solution in THF) in THF (12 mL) according to procedure E. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 5:1) to give compound **18** (0.525 g, 81%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.09 (d, J = 7.2 Hz, 2H), 7.48-7.41 (m, 4H), 7.25-7.21 (m, 2H), 4.44 (t, J = 7.2 Hz, 2H), 3.62 (t, J = 5.4 Hz, 2H), 2.82-2.76 (m, 4H), 2.61-2.55 (m, 8H).

2-(4-(2-(9*H*-carbazol-9-yl)ethyl)piperazin-1-yl)acetaldehyde (19).

Alcohol **18** (0.30 g, 0.93 mmol) was oxidized using SO_3.py complex (0.74 g, 4.64 mmol), DMSO (3 mL) and Et_3N (0.90 mL, 6.49 mmol) in CH_2Cl_2 (6 mL) according to procedure F. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to give aldehyde **19** (0.23 g, 77%). The purified aldehyde was used immediately for next step. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 9.67 (s, 1H), 8.07 (d, J = 7.8 Hz, 2H), 7.47-7.41 (m, 4H), 7.23-7.21 (m, 2H), 4.44-4.42 (m, 2H), 3.38-3.36 (m, 2H), 2.78-2.74 (m, 4H), 2.60-2.56 (m, 6H).

***N*^{6}-(2-(4-(2-(9*H*-carbazol-9-yl)ethyl)piperazin-1-yl)ethyl)-*N*^{6}-propyl-4,5,6,7-tetra-hydrobenzo[*d*]thiazole-2,6-diamine (20) (D-626).**

Compound **19** (0.14 g, 0.44 mmol) was reacted with (±)-pramipexole (0.083 g, 0.39 mmol) and $NaBH(OAc)_3$ (0.185 g, 0.87 mmol) in CH_2Cl_2 (10 mL) according to procedure G. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 4:1) to afford compound **20** (0.115 g, 57%). The compound was converted to HCl salt. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.07 (d, J = 8.4 Hz, 2H), 7.47-7.39 (m, 4H), 7.23-7.20 (m, 2H), 5.20 (bs, 2H), 4.41 (t, J = 7.2 Hz, 2H), 3.04–2.97 (m, 1H), 2.75 (t, J = 7.2 Hz, 2H), 2.69–2.48 (m, 14H), 2.45 (t, J = 7.2 Hz, 4H), 1.97–1.94 (m, 1H), 1.73-1.63 (m, 1H), 1.49–1.40

(m, 2H), 0.87 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$): *δ* 166.01, 144.77, 140.29, 125.70, 122.90, 120.39, 118.96, 116.88, 108.54, 58.52, 58.08, 56.12, 53.59, 53.48, 48.28, 40.94, 26.46, 25.82, 25.09, 22.33, 11.85; Anal. Calcd for $C_{30}H_{40}N_6S.5HCl.CH_3OH$: C, 50.25; H, 6.61; N, 11.72. Found: C, 50.81; H, 7.00; N, 11.33.

(*S*)-*N*^{6}-(2-(4-(2-(9*H*-carbazol-9-yl)ethyl)piperazin-1-yl)ethyl)-*N*^{6}-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine (21) (D-637).

Compound **19** (0.19 g, 0.59 mmol) was reacted with (-)-pramipexole (0.112 g, 0.53 mmol) and $NaBH(OAc)_3$ (0.25 g, 1.18 mmol) in CH_2Cl_2 (12 mL) according to procedure G. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 4:1) to afford compound **21** (0.13 g, 47%). The compound was converted to HCl salt. **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 8.07 (d, *J* = 7.8 Hz, 2H), 7.45 (t, *J* = 7.8 Hz, 2H), 7.40 (d, *J* = 7.8 Hz, 2H), 7.22 (t, *J* = 7.8 Hz, 2H), 5.11 (bs, 2H), 4.41 (t, *J* = 7.2 Hz, 2H), 3.03–2.98 (m, 1H), 2.74 (t, *J* = 7.2 Hz, 2H), 2.69–2.48 (m, 14H), 2.45 (t, *J* = 7.2 Hz, 4H), 1.96–1.94 (m, 1H), 1.71-1.64 (m, 1H), 1.48–1.41 (m, 2H), 0.87 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, $CDCl_3$): *δ* 165.85, 144.91, 140.28, 125.68, 122.89, 120.39, 118.95, 117.00, 108.53, 58.58, 58.08, 56.13, 53.63, 53.52, 48.33, 40.95, 26.52, 25.84, 25.10, 22.35, 11.85; $[\alpha]_D^{25}$= -19.8 (*c*=1.0 in CH_2Cl_2); Anal. Calcd for $C_{30}H_{40}N_6S.4HCl.2CH_3OH$: C, 52.89; H, 7.21; N, 11.57. Found: C, 53.16; H, 7.14; N, 11.68.

{2-[4-(2-Carbazol-9-yl-ethyl)-piperazin-1-yl]-ethyl}-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (22).

Compound **19** (0.140.0g, 0.435 mmol) was reacted with (-)-DPAT (0.0.095 g, 0.435 mmol) and $NaBH(OAc)_3$ (0.184 g, 0.87 mmol) in CH_2Cl_2 (12 mL) according to procedure G. The crude product was purified by silica gel column chromatography (EtOAc:MeOH =

4:1) to afford compound **22** (0.09 g, 32.3%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.03 (d, *J* = 7.8 Hz, 2H), 7.42-7.39 (m, 2H), 7.17 (d, *J* = 7.2 Hz, 2H), 7.05-7.01 (m, 2H), 6.64 (t, J= 7.8 Hz, 2H), 6.59 (t, J= 8.4 Hz, 2H), 4.37 (t, *J* = 7.8 Hz, 2H), 3.74 (s, 1H), 3.61 (t, J= 6 Hz, 2H), 3.54 (t, J= 5.4 Hz, 2H), 3.46 (t, J= 4.8 Hz, 2H), 3.34 (t, J= 5.4 Hz, 2H), 3.24 (t, J= 5.4 Hz, 2H), 3.03 (d, *J* = 4.2 Hz, 1H), 3.00 (d, *J* = 4.2 Hz, 1H), 2.95 (dd, J= 3.6, 1.8 Hz, 2H), 2.92 (d, *J* = 3.6 Hz, 2H), 2.89 (dd, J= 2.4, 2.4 Hz, 2H), 2.88-2.85 (m, 4H), 2.80 (m, 2H), 2.77 (d, *J* = 3.0 Hz, 1H), 2.76-2.69 (m, 3H), 2.66-2.64 (m, 2H), 2.02-2.00 (m, 2H), 1.61 (dd, J=7.8 Hz, 7.2 Hz, 1H), 1.5 (qd, J= 6.6, 5.5 Hz, 2H), 1.42 (dd, J= 6.0, 5.4 Hz, 1H), 0.91 (t, J= 7.8 Hz, 3H), 0.87 (t, *J* = 7.2 Hz, 3H).

6-({2-[4-(2-Carbazol-9-yl-ethyl)-piperazin-1-yl]-ethyl}-propyl-amino)-5,6,7,8-tetrahydro-naphthalen-1-ol (23) (D-689).

Compound **22** (0.09 g, 0.172 mmol) was dissolved in dryCH_2Cl_2 and BBr_3 was added at -40 °C for 2 h, the reaction mixture was stirred overnight at room temp. The reaction was quenched with an ice water, and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (EtOAC:MeOH = 8:2) to yield compound **23** (0.025 g, 60%). **^{1}H NMR** (600 MHz, CD_3OD): δ 8.18 (s, 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.46 (d, *J* = 7.8 Hz, 2H), 6.94 (t, *J* = 7.2 Hz, 3H), 6.61 (d, *J* = 6.6, Hz, 5H), 4.43 (s, 1H), 3.44-3.37 (m, 4H), 3.20-3.18 (m, 4H), 2.99-2.96 (m, 4H), 2.87-2.83 (m, 4H), 2.75-2.74 (m, 2H), 2.64-2.63 (m, 3H), 2.45-2.42 (m, 3H), 2.33–2.32 (m, 4H), 1.79-1.75 (m, 4H), 1.04 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): δ 154.74, 154.71, 154.66, 138.77, 132.97, 129.77, 128.78, 126.62, 123.83, 123.55, 122.51, 121.65, 120.70, 119.70, 118.80, 112.99, 112.60, 112.01, 110.56,

111.43, 111.11, 55.00, 54.67, 54.20, 53.87, 32.19, 31.78, 31.37, 25.94, 25.42, 25.31, 24.81, 22.81, 22.22, 21.44, 21.08, 20.37, 20.20, 19.57, 18.94, 10.75, 10.41, 10.09, 9.79, 9.14; $[\alpha]_D^{25}$= -27.2 (*c*=1.0 in CH_3OH); Anal. Calcd for $C_{33}H_{45}Cl_3N_4O.3HCl$: C, 63.92; H, 7.31; N, 9.04.

4-{4-[2-(tert-Butyl-dimethyl-silanyloxy)-ethyl]-piperazin-1-yl}-benzonitrile (25).

A mixture of 4-Bromo-benzonitrile **24** (1.0g, 5.49 mmol), 1-(2-((tert-butyldimethylsilyl)oxy)ethyl) piperazine (2.015 g, 8.24 mmol), $Pd(OAc)_2$ (0.0925 g, 0.412 mmol), BINAP (0.342 g, 0.55 mmol) and Cs_2CO_3 (5.37 g, 16.49 mmol) in toluene (25 mL) was heated at 110 °C for 24 h according to procedure D. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 4:1) to give compound **25** (1.71 g, 90%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 7.37 (t, J=4.8 Hz, 2H), 6.76 (d, *J* = 8.4 Hz, 2H), 3.70 (t, *J* = 6.0 Hz, 2H), 3.23 (d, J=7.2, 1H), 2.57 (t, J=4.2, 4H), 2.49 (t, J=5.4 Hz, 2H), 1.13 (td, J=6, 1.2 Hz, 2H), 0.89 (s, 9H), 0.08 (s, 6H).

Procedure J. 4-[4-(2-Hydroxy-ethyl)-piperazin-1-yl]-benzonitrile (26).

Into a stirring solution of compound **25** (1.71 g, 4.95 mmol) in THF (50 mL) was added *n*-tetrabutylammonium fluoride (7.42 mL, 7.42 mmol, 1.0 M solution in THF) at 0 °C. The reaction mixture was then stirred at room temperature for 5 h. THF was evaporated in vacuo, and the residue was diluted with CH_2Cl_2 (25 mL) and washed with a saturated solution of $NaHCO_3$. The water layer was extracted with EtOAc (3 × 50 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to give compound **26** as brown powder (0.880 g, 60.1%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 7.48 (dd, *J* = 5.4, 1.8 Hz, 2H), 6.84 (d, *J* = 5.4, 1.8

Hz, 2H), 3.67 (t, *J* = 5.4 Hz, 2H), 3.37(t, *J* = 2.4 Hz, 4H), 3.33 (t, *J* = 4.8 Hz, 4H), 2.61 (t, *J* = 5.4 Hz, 2H).

4-[4-(2-Oxo-ethyl)-piperazin-1-yl]-benzonitrile (27).

Compound **26** (0.880 g, 3.81 mmol) in CH_2Cl_2 (30 mL) and DMSO (15 mL), was oxidized using SO_3.py complex (3.028 g, 19.025 mmol) and Et_3N (3.71 mL, 26.635 mmol) according to procedure F. The crude product was purified by silica gel column chromatography (EtOAc) to yield compound **27** (0.133 g, 62%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 9.60 (t, J=1.2 Hz, 1H), 7.37 (d, *J* = 7.8 Hz, 2H), 7.76 (d, *J* = 8.4 Hz, 2H), 3.39 (d, J=7.2, 1H), 3.27 (t, J=7.8, 3H), 2.56 (t, J=7.2 Hz, 4H), 1.13 (td, J=6, 1.2 Hz, 2H).

***(s)*-4-(4-{2-[(5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-benzonitrile (28).**

Compound **27** (0.133 g, 0.542 mmol) was reacted with (-)-5-OMe-MPAT (0.107g, 0.488 mmol) and $NaBH(OAc)_3$ (0.229 g, 1.08 mmol) in CH_2Cl_2 (15 mL) according to procedure G. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 4:1) to afford compound **28** (0.454 g, 32.3%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 7.44 (dd, *J* = 5.4, 1.8 Hz, 2H), 7.05 (t, *J* = 7.8 Hz, 1H), 6.81 (dd, *J* = 5.4, 1.8 Hz, 2H), 6.68 (dd, J= 4.2, 3 Hz, 1H), 6.62 (dd, J= 4.8, 3 Hz, 1H), 3.77 (s, 3H), 3.29 (t, J= 5.4 Hz, 4H), 2.98 (d, *J* = 2.4 Hz, 1H), 2.95 (d, *J* = 6.0 Hz, 1H), 2.80 (dd, J= 4.2, 3 Hz, 1H), 2.71–2.68 (m, 2H), 2.66 (t, *J* = 7.8 Hz, 1H), 2.52–2.48 (m, 5H), 1.55-1.53 (m, 2H), 1.45 (dd, J= 7.8, 7.2 Hz, 2H), 1.23(t, J= 7.2 Hz, 2H), 0.91 (td, J= 5.4, 2.4 Hz, 2H), 0.87 (t, *J* = 7.2 Hz, 3H).

Procedure K. {2-[4-(4-Aminomethyl-phenyl)-piperazin-1-yl]-ethyl}-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (29).

Into a stirred solution of compound **28** (454 g, 1.05 mmol) in 30 ml dry THF, borane:THF complex in1.0 M THF (2.102 mL, 2.102 mmol) was added dropwise at R. T. The reaction mixtures was stirred at 50 °C for 1.5 h, cooled to R.T. Water and conc.HCl (2 mL) were added slowly at 0 °C. The solvent was evaporated and 10 ml of 25 % aq. NaOH was added at 0°C. The water layer was extracted with EtOAC (3 × 50 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (DCM:MeOH = 9:1) to give compound **29** (0.880 g, 60.1%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 7.47 (d, *J* = 9.0, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 7.09-7.06 (m, 1H), 6.88 (d, J= 8.4, 1H), 6.83 (d, J= 9.0 Hz, 1H), 6.69 (d, J= 7.8 Hz, 1H), 6.63 (d, J= 8.4 Hz, 1H), 3.79 (s, 3H), 3.31 (t, J= 5.4 Hz, 2H), 3.19 (t, *J* = 5.4 Hz, 2H), 2.99 (d, *J* = 4.8 Hz, 1H), 2.96 (d, J= 3.6 Hz, 1H), 2.93-2.89 (m, 1H), 2.88-2.82 (m, 2H), 2.73-2.70 (m, 3H), 2.64-2.61 (m, 3H), 2.52-2.48 (m, 4H), 2.04 (d, J= 12 Hz, 1H), 1.55 (dd, J= 6.6, 5.4 Hz, 2H), 1.48 (dd, J= 7.8, 7.2 Hz, 2H), 0.88 (t, J= 7.2 Hz, 3H).

Procedure L. N-[4-(4-{2-[(5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-benzyl]-2-nitro-benzenesulfonamide (30).

Into a stirring solution of 2-nitrobenzenesulfonyl chloride (0.4614 g, 0.208 mmol) in THF (5 mL), Et 3 N (0.131 mL, 0.937 mmol) was added (S)-4,5,6,7tetrahydrobenzo[d]thiazole-2,6-diamine **29** (0.10 g, 0.229 mmol) at −10 °C, and the resulting suspension was then stirred at room temperature for 1.5 h. The suspension was first filtered to remove precipitated triethylammonium chloride, and the filtrate was concentrated in vacuo (Brown et al. 2009). Water was added, and CH_2Cl_2 (3 × 15 mL) was used to extract the product. The combined organic layer was dried over Na_2SO_4, and

the solvent was removed in vacuo to obtain the intermediate **30**. The crude mixture was was purified by a silica gel column chromatography (0.134 g, 94%). **^{1}H NMR** (600 MHz, 123CD 3 OD): *δ* 7.24 (d, *J* = 0.6, 1H), 7.18 (d, *J* = 8.4 Hz, 1H), 7.07 (t, *J* = 7.8 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 2H), 6.70 (d, *J* = 7.2 Hz, 1H), 6.64 (d, *J* = 7.2 Hz, 1H), 3.79 (s, 3H), 3.18-3.16 (m, 3H), 3.02-2.96 (m, 1H), 2.93-2.91 (m, 1H), 2.87 (t, J= 5.4 Hz, 1H), 2.85-2.83 (m, 1H), 2.74-2.71 (m, 2H), 2.68 (t, J=5.4 Hz, 1H), 2.65-2.63 (m, 3H), 2.58-2.55 (m, 1H), 2.52-2.51 (m, 3H), 2.34 (d, J=1.8 Hz, 1H), 2.10-2.04 (m, 1H), 1.98-1.89(m,2H), 1.61-1.51 (m, 2H), 1.51-1.45 (m, 1H), 0.94-0.91 (m, 2H), 0.89 (s, 3H), 0.07-0.05 (m, 2H).

Procedure M. N-[4-(4-{2-[(5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl amino]-ethyl}-piperazin-1-yl)-benzyl]-2-nitro-N-prop-2-ynyl-benzenesulfonamide (31).

Compound **30** (0.134 g, 0.217 mmol), potassium carbonate (0.90 g, 0.651 mmol), and propargyl bromide (0.31 mL, 0.261 mmol) were suspended in acetonitrile (7 mL) and the reaction mixture was heated to approximately 50 °C for 5 h. After cooling to room temperature, the reaction mixture was filtered, and the filtrate was condensed in vacuo. Water was added, and the compound was extracted with EtOAC (3 × 15 mL). The combined organic layer was dried over Na_2SO_4, and the solvent was removed in vacuo to give the crude product, which was purified by column chromatography using (1:9) MeOH:EtOAC to give intermediate **31** (0.345 g, 60%). **^{1}H NMR** (600 MHz, 123CD 3 OD): *δ* 7.97 (d, *J* = 7.8, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 7.69-7.59 (m, 2H), 7.24 (dd, J= 3.6, 1.2 Hz, 1H), 7.19 (d, J=7.8 Hz, 1H), 7.06 (t, =8.4, 2H), 6.83 (d, J=7.8 Hz, 1H), 6.71 (dd, J= 7.8, 7.8, 2H), 6.64 (d, J= 7.8, 1H), 4.48 (s, 1H), 4.20 (s, 1H), 3.98 (s, 1H), 3.79 (s, 3H), 3.34 (d, J=1.2 Hz, 1H), 3.17 (d, J= 4.2 Hz, 2H), 3.11 (d, J= 4.8 Hz, 2H), 2.99 (d, J= 5.4

Hz, 1H), 2.96 (d, J=3.6 Hz, 1H), 2.88-2.85 (m, 1H), 2.87-2.77 (m, 3H), 2.62-2.48 (m, 8H), 2.13-2.11 (m, 1H), 2.080-2.07 (m, 1H), 1.58 (dd, J= 5.4, 4.8 Hz, 2H), 1.51-1.50 (m, 2H), 0.89 (t, J= 7.2 Hz, 3H).

Procedure N. (5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-{2-[4-(4-prop-2-ynylaminomethyl-phenyl)-piperazin-1-yl]-ethyl}-amine (32).

Potassium carbonate (0.118 g, 1.364 mmol) was suspended in DMF (6 mL) and the suspension was cooled to 0 °C followed by slow addition of thioglycolic acid (0.070 mL, 0.758 mmol) and propargyl bromide (0.194 ml, 1.63 mmol). The mixture was stirred for 1 h at room temperature. A solution of the intermediate **31** (0.345 g, 0.522 mmol) in DMF (15 mL) was added, the reaction mixture was heated to 50 °C and stirred overnight. The reaction mixture was quenched carefully by adding 1N NaOH at room temperature, and CH_2Cl_2 (4 × 30 mL) was used to extract the product. The combined organic layer was dried over Na_2SO_4, and the solvent was removed in vacuo to give the crude, which was purified by a silica gel column chromatography using EtOAC:MeOH (9:1) to give compound **32** (0.30 g, 64%). **^{1}H NMR** (600 MHz, CDCl 3): δ ppm 7.46 (dd, *J* = 5.4, 1.8 Hz, 1H), 7.22 (d, *J* = 1.8 Hz, 1H), 7.07 (d, *J* = 7.8 Hz, 1H), 6.85 (d, J= 9.0 Hz, 2H), 6.84-6.82 (m, 2H), 6.70 (d, J=7.8 Hz, 1H), 6.64 (d, J=7.8 Hz, 1H), 3.79 (s, 3H), 3.38 (d, J=2.4 Hz, 2H), 3.34-3.30 (m, 3H), 3.19 (d, J= 5.4 Hz, 2H), 2.99 (dd, J= 4.8 Hz, 2H), 2.87-2.84 (m, 2H), 2.76-2.74 (m, 3H), 2.66 (d, J= 4.2 Hz, 2H), 2.62(d, J= 3.6 Hz, 2H), 2.55-2.53 (m, 4H), 2.24 (d, J= 1.8 Hz, 1H), 1.58-1.56 (m, 2H), 1.50-1.49 (m, 2H), 0.89 (t, J=7.2 Hz, 3H).

Procedure O. 6-(Propyl-{2-[4-(4-prop-2-ynylaminomethyl-phenyl)-piperazin-1-yl]-ethyl}-amino)-5,6,7,8-tetrahydro-naphthalen-1-ol (33).

Compound **32** (0.050 g, 0.105 mmol) was dissolved in dry CH_2Cl_2 and BBr_3 was added at -40 °C for 2 h. The reaction mixture was stirred overnight at room temp. The reaction was quenched by adding ice water, and the aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (EtOAC:MeOH = 8:2) to yield the final compound **33 (D-671)** (0.025 g, 60%). **^{1}H NMR** (600 MHz, CD_3OD): δ ppm 7.48 (d, *J* = 8.4 Hz, 1H), 7.25 (d, *J* = 0.6 Hz, 1H), 7.07 (d, *J* = 8.4 Hz, 1H), 6.84 (d, J= 9.0 Hz, 2H), 6.70 (d, J=7.8 Hz, 1H), 6.64 (d, J=7.8 Hz, 1H), 3.31 (d, J= 5.4 Hz, 4H), 3.00-2.99 (m, 1H), 2.99-2.96 (m, 144H), 2.86-2.86 (m,1H), 2.7-2.70 (m, 3H), 2.64-2.61 (m, 3H), 2.52-2.50 (m, 3H), 1.57-1-54 (m, 2H), 1.49-1.46 (m, 2H), 0.89 (t, J=7.2 Hz, 3H). **^{13}C NMR** (150 MHz, CD_3OD): *δ* 233.87, 229.67, 223.82, 218.35, 210.53, 204.88, 199.54, 196.80, 190.59, 156.53, 156.20, 121.25, 118.65, 108.23, 101. 50, 66.22, 55.28, 51.11, 46.77, 42.15, 31.26, 30.02, 29.69, 25.49, 22.68, 14.12, 8.71; $[\alpha]_D^{25}$= -22 (*c*=1.0 in CH_3OH); Anal. Calcd for $C_{28}H_{40}N_4O_3.3HBr$, $2H_2O$: C, 54.21; H, 7.53; N, 8.72. Found: C 54.31; H, 7.29; N, 8.54.

Procedure P. (5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-[2-(4-{4-[(methyl-prop-2-ynyl-amino)-methyl]-phenyl}-piperazin-1-yl)-ethyl]-propyl-amine (34).

A mixture of compound **32** (0.7 g, 0.147 mmol), 37% aqueous formaldehyde (6.13 ml, 0.221 mmol), and NaH_2PO_4 (0.176 g, 0.147 mmol) were suspended in (10 mL) water and stirred for 5 min at room temperature. The mixture was refluxed at 30 °C for 48 h. The reaction was quenched by adding water and the crude was extracted with CH_2Cl_2 (3 * 10 mL). The crude material was purified by a silica gel column chromatography (EtOAC: MeOH= 9:1) to give compound **34** (0.51 g, 71%). **^{1}H NMR** (600 MHz, $CDCl_3$): 7.13-7.10

(m, 2H), 6.86-6.84 (m, 2H), 6.71-6.65 (m, 3H), 3.79 (s, 3H), 3.65-6.63 (m, 1H), 3.53-3.50 (m, 1H), 3.37 (s, 2H), 3.19 (s, 3H), 3.11-3.07 (m, 2H), 3.01-2.98 (m, 4H), 2.76-2.72 (m, 3H), 2.60-2.56 (m, 4H), 2.66 (d, J= 4.2 Hz, 2H), 2.62 (d, J= 3.6 Hz, 2H), 2.54-2.49 (m, 5H), 2.24-2.24 (m, 1H), 1.99-1.95 (m, 3H), 1.69-1.63 (m, 2H), 1.54-150 (m, 2H), 1.01 (d, J= 7.2 Hz, 3H), 0.89 (t, J=11.4 Hz, 3H).

6-{[2-(4-{4-[(Methyl-prop-2-ynyl-amino)-methyl]-phenyl}-piperazin-1-yl)-ethyl]-propyl-amino}-5,6,7,8-tetrahydro-naphthalen-1-ol (35).

Compound **34** (0.050 g, 0.105 mmol) was dissolved in dry CH_2Cl_2 and BBr_3 was slowly added at -40 °C for over a period of 2 h. The reaction mixture was stirred overnight at room temp according to procedure O. The crude product was purified by silica gel column chromatography (EtOAC:MeOH = 8:2) to yield compound **35 (D-677)** (0.020 g, 55%). **^{1}H NMR** (600 MHz, CD_3OD): 7.26-7.22 (m, 1H), 7.09-7.06 (m, 1H), 6.86-6.82 6 (m, 2H), 6.70 (d, J=7.2 Hz, 1H), 6.64 (d, J=7.8 Hz, 1H), 3.39-6.38 (m, 1H), 3.34-3.30 (m, 3H), 63.19 (d, J=4.2 Hz, 2H), 3.01-2.94 (m, 2H), 2.87-2.84 (m, 3H), 2.75-2.74 (m, 3H), 2.66 (d, J= 4.2 Hz, 2H), 2.62 (d, J= 3.6 Hz, 2H), 2.54-2.49 (m, 5H), 2.24-2.24 (m, 1H), 2.07-2.02 (m, 1H), 1.58-1.54 (m, 1H), 1.49 (t, J=6.6 Hz, 2H), 0.89 (t, J=11.4 Hz, 3H). **^{13}C NMR** (150 MHz, CD_3OD): δ 232.81, 229.21, 222.31, 217.46, 211.65, 202.40, 197.89, 194.32, 190.12, 154.96, 153.01, 150,89, 120.76, 117.30, 106.80, 101. 02, 64.57, 53.79, 50.70, 45.77, 41.58, 30.79, 29.92, 27.45, 24.56, 20.94, 12.83, 8.06; $[\alpha]_D^{25}$= -25 (*c*=1.0 in CH_3OH); Mp. 205-210 °C, Anal. Calcd for $C_{30}H_{42}N_4O.3HCl$, H_2O: C, 54.33; H, 7.69; N, 7.20. Found: C 54.05; H, 7.22; N, 7.12.

4-(4-Hydroxy-phenyl)-piperazine-1-carboxylic acid tert-butyl ester (37).

A mixture of 4-Piperazin-1-yl-phenol **36** (1.0g, 5.49 mmol), 1-(2-((tert-butyldimethylsilyl)oxy)ethyl) piperazine (2.015 g, 8.24 mmol), $Pd(OAc)_2$ (0.0925 g, 0.412 mmol), BINAP (0.342 g, 0.55 mmol) and Cs_2CO_3 (5.37 g, 16.49 mmol) in toluene (25 mL) was heated at 110 °C for 24 h according to procedure D. The crude material was purified by a silica gel column chromatography (hexane:EtOAc = 4:1) to give compound **25** (1.71 g, 90%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.24-6.87 (m,4H), 3.55 (t, J= 1.8 Hz, 4H), 2.94 (s, 2H), 2.86 (s, 2H), 1.46 (s, 9H).

4-(4-Prop-2-ynyloxy-phenyl)-piperazine-1-carboxylic acid tert-butyl ester (38).

Compound **37** (0.134 g, 0.217 mmol), potassium carbonate (0.90 g, 0.651 mmol), and propargyl bromide (0.31 ml, 0.261 mmol) were suspended in acetonitrile (7 mL). The stirring mixture was heated to approximately 50 °C for 5 h according to procedure M. The crude, Procedure I which was purified by column chromatography using (1:9) MeOH:EtOAC to give intermediate 31 (0.345 g, 60%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.24-6.87 (m,4H), 4.62 (d, J= 1.8 Hz, 2H), 3.55 (t, J= 1.8 Hz, 4H), 2.94 (s, 2H), 2.86 (s, 2H), 2.49-2.48 (m,1H), 1.46 (s, 9H).

1-(4-Prop-2-ynyloxy-phenyl)-piperazine (39).

Into a stirring solution of compound **38** (0.110 g, 0.293 mmol) in THF (10 mL) was added *n*-tetrabutylammonium fluoride (0.127 ml, 0.44 mmol, 1.0 M solution in THF) at 0 °C by following procedure E. The crude product was purified by silica gel column chromatography (EtOAC:MeOH = 9:1) to give compound **39** (0.50 g, 78.7%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 6.89-6.88 (m, 4H), 4.61 (d, *J* = 2.4 Hz, 2H), 3.04-3.02 (m, 8H), 2.50 (t, J= 2.4 Hz, 1H).

Procedure Q. 2-[4-(4-Prop-2-ynyloxy-phenyl)-piperazin-1-yl]-ethanol (40).

A suspension of 1-(4-Prop-2-ynyloxy-phenyl)-piperazine **39** (0.130 g, 0.601 mmol), potassium carbonate (48.13 g, 348.27 mmol), and 2-Bromo-ethanol (0.017 mL, 0.24 mmol) in acetonitrile (5 mL) was refluxed under N_2 overnight. The reaction mixture was filtered off, and the filtered was evaporated under reduced pressure. The residue was then diluted with ether, washed with water, dried over Na_2SO_4, filtered, and concentrated to give alcohol **40** (0.085g, 54.32%). **^{1}H NMR** (600MHZ, CDCL3): δ 6.92-6.87 (m, 4H), 4.61 (d, *J* = 2.4 Hz, 2H), 3.84-3.82 (m, 2H), 3.34-3.31 (m, 2H), 3.05-3.04 (m, 4H), 3.00 (m, 4H), 2.50 (t, J= 2.4 Hz, 1H).

[4-(4-Prop-2-ynyloxy-phenyl)-piperazin-1-yl]-acetaldehyde (41).

Compound **40** (0.200 g, 0.768 mmol) in CH_2Cl_2 (10 mL) and DMSO (5 mL), was oxidized using SO_3.py complex (3.028 g, 19.025 mmol) and Et_3N (0.611 mL, 3.84 mmol) according to procedure F. The crude product was purified by silica gel column chromatography (EtOAC) to yield compound **41** (0.170 g, 85.6%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.84 (s, 1H), 7.00 (dd, J= 6.0, 1.8 Hz, 1H), 6.61-6.59 (m, 1H), 6.17-6.13 (m, 2H), 4.77 (d, J= 0.6 Hz, 1H), 3.92-3.90 (m, 4H), 3.84-3.82 (m, 2H), 3.33-3.31 (m, 4H), 3.26-3.23 (m, 4H).

(5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-{2-[4-(4-prop-2-ynyloxy-phenyl)-piperazin-1-yl]-ethyl}-amine (42, D-678).

Compound **41** (0.170 g, 0.658 mmol) was reacted with (±)-5-OMe-MPAT (0.129 g, 0.592 mmol) and $NaBH(OAc)_3$ (0.278 g, 1.316 mmol) in CH_2Cl_2 (15 mL) according to procedure G. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 4:1) to obtain compound **42** (**D-678)** (0.060 g, 32.3%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 7.24-6.92 (m, 1H), 6.90-6.87 (m, 4H), 6.69 (d, J= 7.8 Hz, 1H), 6.63 (d, J=

7.8 Hz, 1H), 4.63-4.61 (m, 2H), 3.79 (s, 3H), 3.69-3.68 (m, 1H), 3.52-3.50 (m, 1H), 3.34-3.33 (m, 1H), 3.12-3.05 (m, 4H), 3.03-3.01 (m, 1H), 2.99-2.98 (m, 1H), 2.96-2.95 (m, 1H), 2.85-2.83 (m, 1H), 2.76-2.71 (m, 2H), 2.66-2.63 (m, 3H).2.53-2.50 (m, 1H), 2.49-2.47 (m, 2H), 0.87 (t, J= 7.8 Hz, 3H). **^{13}C NMR** (150 MHz, CD_3OD): *δ* 235.34, 231.04, 224.94, 219.39, 214.29, 207.74, 199.97, 197.85, 195.48, 159.34, 157.36, 153,32, 126.06, 122.76, 110.21, 107. 43, 69.93, 59.20, 55.60, 49.35, 44.67, 34.23, 32.79, 29.98, 28.45, 25.53, 16.93, 14. 67, 10.65; $[\alpha]_D^{25}$= -24 (*c*=1.0 in CH_3OH); Mp = 195 °C, Anal. Calcd for $C_{29}H_{42}N_3O_2.3HCl$, H_2O, C, H, N.

2-[4-(4-Nitro-phenyl)-piperazin-1-yl]-ethanol (44).

A mixture of 2-bromoethanol (0.274 mL, 3.86 mmol), **1**-(4-Nitro-phenyl)-piperazine **43** (2.0 g, 9.65 mmol), and K_2CO_3 (4.0 g, 28.95 mmol) in acetonitrile (25 mL) was refluxed for 24 h under inert condition according to procedure Q. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 3:2) to give compound **44** (1.20 g, 49.5%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 6.82-6.80 (m, 2H), 6.50-7.44 (m, 2H), 3.76 (t, *J* = 6.8 Hz, 2H), 2.76 (t, *J* = 7.2 Hz, 2H), 2.68-2.57 (m, 4H), 2.54 (t, *J* = 6.4 Hz, 4H).

[4-(4-Nitro-phenyl)-piperazin-1-yl]-acetaldehyde (45).

Compound **44** (1.20 g, 4.77 mmol) in CH_2Cl_2 (20 mL) and DMSO (10 mL), was oxidized using SO_3.py complex (3.80 g, 23.87 mmol) and Et_3N (4.65 mL, 33.43 mmol) according to procedure F. The crude product was purified by silica gel column chromatography (EtOAc) to yield compound **45** (0.80 g, 67.2%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 6.82-6.79 (d, *J* = 9.6, 2.4 Hz, 2H), 6.69-6.64 (m, 2H), 4.10 (t, *J* = 7.2 Hz, 2H), 3.25 (t, *J* = 1.2 Hz, 2H), 2.74 (t, *J* = 4.2 Hz, 4H), 2.25 (m, 2H).

(5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-{2-[4-(4-nitro-phenyl)-piperazin-1-yl]-ethyl}-propyl-amine (46).

Compound **45** (0.251 g, 1.007 mmol) was reacted with (-)-5-OMe-MPAT (0.197g, 0.906 mmol) and $NaBH(OAc)_3$ (0.427 g, 2.015 mmol) in CH_2Cl_2 (15 mL) according to procedure G. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 4:1) to afford compound **46** (0.30 g, 65.7%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 8.10 (dd, *J* = 4.8, 2.4 Hz, 2H), 7.08 (t, *J* = 7.8 Hz, 1H), 6.79 (dd, *J* = 7.8, 1.8 Hz, 2H), 6.69 (d, *J* = 7.8 Hz, 1H), 6.64 (d, *J* = 8.4 Hz, 1H), 3.79 (s, 3H), 3.40 (t, *J* = 5.4 Hz, 4H), 3.00 (d, *J* = 4.2 Hz, 1H), 2.79 (d, *J* = 3.6 Hz, 1H), 2.95-2.93 (m, 1H), 2.85-2.82 (m, 1H), 2.76–2.70 (m, 3H), 2.65–2.60 (m, 4H), 2.53–2.48 (m, 5H), 2.03 (dd, *J* = 4.2, 3.0 Hz, 4H), 1.59-1.52 (m, 2H), 1.50–1.44 (m, 1H), 0.87 (t, *J* = 7.2 Hz, 3H).

Procedure R. {2-[4-(4-Amino-phenyl)-piperazin-1-yl]-ethyl}-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (47).

Compound **46** (0.30, 0.661) was dissolved in methanol (15 mL) and the mixture was hydrogenated on a parr apparatus (1 atm) in the presence of 10% Pd-C (.070 g, 10 wt %) at 30 psi for overnight. Then, the reaction mixture was filtered through a short bed of Celite, and the filtrate was concentrated under reduced pressure on a rotary evaporator. The crude was purified via column chromatography using MeOH:EtOAc (1:10) as the eluent to afford the amine **48** (0.20 g, 71.4%). %). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 7.08 (t, *J* = 8.4 Hz, 1H), 6.80-6.78 (m, 2H), 6.70 (d, *J* = 7.2 Hz, 1H), 6.64-6.62 (m, 3H), 3.79 (s, 3H), 3.41 (s, 2H), 3.05 (t, J= 4.8 Hz, 4H), 2.99 (dq, J= 3.6, 1.8 Hz, 1H), 2.92 (tq, J= 2.4,1.8 Hz, 1H), 2.86 (d, J= 2.4 Hz, 1H), 2.83 (d, J= 3.0 Hz, 1H), 2.75-2.71(m,

2H), 2.67-2.63 (m, 4H), 2.55-2.48 (m, 5H), 2.07-2.03 (m, 1H), 1.60-1.55 (m, 2H), 1.51-1.44 (m, 1H), 0.89 (t, *J* = 7.2 Hz, 3H).

Procedure S. (5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-{2-[4-(4-prop-2-ynylamino-phenyl)-piperazin-1-yl]-ethyl}-amine (48).

Compound **47** (0.109 g, 0.332 mmol), potassium carbonate (0.137 g, 0.995 mmol), and propargyl bromide (0.047 mL, 0.398 mmol) were suspended in acetonitrile (10 mL). according to procedure N. The crude compound was purified by column chromatography using (1:9) MeOH:EtOAC to give compound **48** (0.456 g, 40%). **^{1}H NMR** (600 MHz, CDCl 3): δ ppm 7.08-7.06 (m, 1H), 6.85 (td, J= 3.6, 1.8 Hz, 2H), 6.69 (d, J= 7.8 Hz, 1H), 6.67-6.63 (m, 3H), 3.89 (dd, J = 4.2, 2.4 Hz, 2H), 3.79 (s, 3H), 3.60 (d, J = 6.6. Hz, 1H), 3.98 (dd, J= 13.8, 4.2 Hz, 2H), 2.94-2.88 (m, 1H), 2.85 (d, J= 2.4 Hz, 2H), 2.82 (d, J= 2.4 Hz, 2H), 2.76-2.82 (m, 3H), 2.64-2.63 (m, 4H), 2.53-2.47 (m, 5H), 2.19 (s, 1H), 2.06-2.03 (m, 1H), 1.59 (m, 2H), 1.50-1.44 (m, 2H), 0.88 (t, J= 7.8, 3H).

6-(Propyl-{2-[4-(4-prop-2-ynylamino-phenyl)-piperazin-1-yl]-ethyl}-amino)-5,6,7,8-tetrahydro-naphthalen-1-ol (49).

Compound **48** (0.0456 g, 0.140 mmol) was dissolved in dryCH_2Cl_2 (5 mL) and BBr_3 (0.110 ml, 0.705 mmol) was added at -40 °C for 2 h, the reaction mixture was stirred overnight at room temp according to procedure O. The crude product was purified by silica gel column chromatography (EtOAC:MeOH = 8:2) to yield HBr salt of **49** (0.025 g, 60%). **^{1}H NMR** (600 MHz, CD_3OD): *δ* 7.12 (d, *J* = 7.2 Hz, 1H), 6.97 (d, *J* = 7.8 Hz, 1H), 6.90-6.82 (m, 2H), 6.71 (d, *J* = 4.8 Hz, 1H), 6.68-6.67 (m, 1H), 4.10 (q, J= 7.2 Hz, 3H), 3.79 (s, 2H), 3.65-3.63 (m, 2H), 3.33 (s, 3H), 3.22-3.00 (m,7H), 2.79 (s, 1H), 2.59-2.53 (m, 4H), 2.32 (t, *J* = 7.2 Hz, 1H); 2.27 (t, *J* = 7.2 Hz, 1H); 2.1-2.03 (m, 3H), 1.61 (t, *J* =

8.4 Hz, 3H), 1.07 (t, *J* = 6.0 Hz, 3H). **^{13}C NMR** (150 MHz, CD_3OD): *δ* 155.43, 142.45, 142.83, 135.09, 128.67, 128.48, 128.49, 124.59, 123.49, 122.48, 121.32, 120.56, 120.34, 120.05, 119.59, 115.69, 113.34, 113.01, 75.30, 65.29, 56.39, 54.89, 54.21, 53.98, 53.30, 46.34, 34.09, 26.40, 24.39, 22.23, 13.41, 11.98; $[\alpha]_D^{25}$= -26.08 (*c*=1.0 in CH_3OH); Anal. Calcd for $C_{28}H_{38}N_4O.3HBr$: C, H, N.

Procedure T. 1,1-Diphenyl-but-3-en-1-ol (51).

Into a mixture of benzophenone (**50)** (5.0 g, 27.40 mmol) in anhydrous THF (50.0 mL), cupper iodide (CuI) (0.52 g, 2.74 mmol) at -78 ºC under nitrogen was added allyl magnesium chloride in THF (3.39 g, 2 mmol) slowly under stirring condition. The reaction mixture was warmed slowly to room temperature and stirred overnight. The mixture was cooled to 0 ºC and sat. NH_4Cl and water were added. The organic layer was separated, and the aqueous layer was extracted with EtOAC (3 * 100 ml). The organic layer was combined, dried over Na_2SO_4 and concentrated in rotary evaporator. The crude product was mostly pure compound **51** (6.14 g, 99%) and used for the next step without further purification. **^{1}H NMR** (500MHz, $CDCl_3$) δ, 7.42–7.45 (m,4H), 7.27–7.23 (m,4H), 7.18–7.12 (m,2H), 5.68–7.62 (m,1H), 5.20–5.12 (m,2H), 2.58 (S, 1H).

Procedure U. **1,1-Diphenyl-but-3-en-1- (prop-3-en)ol ether (52).**

The alcohol **51** (6.14 g, 27.37 mmol) was taken in an oven-dried RB flask equipped with a magnetic stir-bar. Anhydrous DMF (50ml) was then added to the flask via syringe. The solution was cooled to 0 ºC, and NaH 60% (13.45 g, 560.42 mmol) was added portionwise . The reaction mixture was stirred for 15-20 min. Next the allyl bromide (18.42, 152.26 mmol) was added dropwise, after 5 min the water bath was removed, and the reaction mixture was stirred at room temperature for 1.5 h. Reaction mixture was cooled

again to 0 °C and the reaction was quenched with ethyl acetate followed by addition of water. The crude was purified by gradient column chromatography using hexan:EtOAC (10:1) to yield compound **52** (7.0 g, 96%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.24-7.30 (m,4H), 7.21-7.19 (m, 4H), 7.13-7.11 (m,2H), 5.86-5.80 (m, 1H), 5.57-5.50 (m,1H), 5.29-5.26 (m,1H), 5.06-5.03(m, 1H), 4.96-4.94 (m, 1H), 4.91-4.88 (m, 2H), 3.07 (dd, J= 5.4, 1.2Hz), 2.91-2.79 (m, 2H).

Procedure V. 2,2-Diphenyl-3,6-dihydro-2H-pyran (53).

Into a stirred solution of vinyl ether **(52)** dissolved in anhydrous benzene under continuous flow of nitrogen at room temperature, 1st generation Grubb's catalyst (0.44 g, 0.53 mmol) was added. The solution mixture was slowly heated to reflux at 90 °C for 2 h. After the reaction mixture was cooled to room temperature, the solvent was removed under reduced pressure on a rotary evaporator. The crude residue was purified by gradient column chromatography using hexane:EtOAC (9.5:0.5) to yield the cyclic olefin **53** (3.25 g, 51.1%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.36 (dd, J=7.8, 0.6 Hz, 4H), 7.31-7.29 (m, 4H), 7.24-7.21 (m, 2H), 6.0-5.96 (m, 1H), 5.64 (dt, J=2.4,1.8, 1H), 4.06-4.04 (m, 2H), 2.82-2.80 (m, 2H). kimkk

Procedure W. 2,2-Diphenyl-7-oxa-bicyclo[4.1.0]heptane (54).

m-CPBA (1.64 g, 9.35 mmol, 50% wt/wt in water), was added portion wise to a solution of alkene **53** (1.50 g, 6.35 mmol) in DCM (15 ml) at 0 °C. The ice bath was removed, and the reaction mixture was stirred at room temperature for 24 h. Next, the reaction mixture was cooled again to 0 °C and quenched with saturated $NaHCO_3$ (100 mL). The organic layer was separated, and the aqueous layer was extracted with additional DCM (2 × 50 ml). The organic layers were combined and washed with brine

100 mL. The organic layer was separated, dried over Na_2SO_4, and concentrated over rotary evaporator. The crude product was purified via gradient silica gel column chromatography using hexane:EtOAC, 8:1 to obtain pure racemic epoxide **54 (wrong structure in the scheme)** (0.53 g, 50%). **^{1}H NMR** (600 MHz, $CDCl_3$): minor portion; δ 7.44-7.38 (m, 4H), 7.30-7.23 (m, 5H), 7.18-7.15 (m, 1H), 4.22-4.16 (m, 1H), 4.07 (dd, J= 6.11, 5.49 Hz, 1H), 3.99-3.94 (m, 1H), 3.37-3.30 (m, 2H), 2.50-2.46 (m, 1H), 2.29 (s, 1H), major portion: δ 7.46-7.44 (m, 2H), 7.38 (t, J= 7.63 Hz), 7.30- 7.22 (m, 5H), 7.17- 7.14 (m, 1H), 4.11 (J=7.02, 5.88 Hz, 1Hz), 4.04- 3.99 (m, 1Hz), 3.94-3.90 (m, 1H), 3.58 (t, J= 11.60 Hz, 1H), 3.20 (dd, J= 9.77, 4.27, Hz, 1 H), 2.42 (d, J= 2.13 Hz, 1H), 2.03-1.98 (m, 1H).

Procedure X. 2-Azido-5,5-diphenyl-cyclohexanol (55 a, and b).

Epoxide 54 (0.43 g, 1.70 mmol) was dissolved in MeOH:H_2O (8:1). Sodium azide (0.55 g, 8.52 mmol) and NH_4Cl (0.18 g, 3.40 mmol) were added at once. The mixture was then stirred for 48 h at 80 °C under a continuous flow of N_2, cooled to room temperature, and quenched with water (50 mL). The solution was extracted with EtOAC (3 × 50 mL), the organic layers were combined and washed with brine (25 mL). The organic layer was separated, dried over Na_2SO_4, and concentrated under reduced pressure on a rotary evaporator. The crude product was purified by gradient silica gel column chromatography using Hexan:EtOAC to obtain the racemic azide **(55 a and b)** (0.23 g, 46 %).**1H NMR** (600 MHz, $CDCl_3$): δ 7.44-7.43 (m, 4H), 7.30-7.23 (t, 5H), 7.26-7.19 (m, 1H), 4.15-4.12 (m, 1H), 3.56 (dd, J= 8.4, 6.0 Hz, 1H), 3.37-3.30 (m, 2H), 2.97-2.91 (m, 1H), 2.50-2.46 (m, 1H), 2.30-2.26 (m, 1H).

Procedure Y. 2-Amino-5-(1-methylene-but-2-enyl)-5-phenyl-cyclohexanol (56 a, and b).

The azides **(55 a, and b)** (0.32 g, 0.78 mmol) was dissolved in methanol (5 mL) and the mixture was hydrogenated on a parr apparatus (1 atm) in the presence of 10% Pd-C (0.026 g, 10 wt %) at 30 psi for overnight according to procedure R. The crude was purified via column chromatography using MeOH:DCM (1:10) as the eluent to afford the amine **(56a and b)** (0.050 g, 50%). **1H NMR** (600 MHz, $CDCl_3$): δ 7.44-7.43 (m, 4H), 7.30-7.23 (t, 5H), 7.26-7.19 (m, 1H), 4.15-4.12 (m, 1H), 3.56 (dd, J= 8.4, 6.0 Hz, 1H), 2.97-2.91 (m, 1H), 1.80–1.48 (m, 2 H), 1.28–1.72 (m, 1 H).

Procedure R. 2-(4-Methoxy-benzylamino)-5,5-diphenyl-cyclohexanol (57a and b).

4-methoxy-benzaldehyde (0.035 g, 0.26 mmol) was dissolved in a mixture of 1,2-dichloromethane (3 mL)/methanol (1 mL) and glacial acetic acid (0.015 µL, 0.26 mmol) was then added. Then, amine **(56a and b)** (0.107 g, 0.38 mmol) was added and the solution stirred at room temperature for 2 h following which $Na(OAc)_3BH$ (0.0.05 g, 0.24 mmol) was added. The resulting mixture was then stirred at room temperature for 24 hours, cooled to 0 °C, diluted with DCM (12 mL) and quenched by the addition of water (18 mL). The organic layer was separated, and the aqueous layer was extracted with additional DCM (3 × 18 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure on a rotary evaporator. The crude residue was purified by gradient silica gel column chromatography using DCM:MeOH (10:1) to obtain compounds **(57a (D-594) and 57 b)** (68 mg, 46%). (c = 1, MeOH). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.46-7.44 (m, 2 H), 7.33 (t, J = 7.93 Hz, 2 H), 7.32-7.27 (m, 2 H), 7.23-7.22 (m,

2 H), 7.20-7.18 (m, 2 H), 7.13 (t, J = 7.32 Hz, 2 H), 6.84–6.82 (m, 2 H), 4.1-4.07 (m, 1H), 3.78 (s, 3H), 3.59-3.53 (m, 1H), 3.12-3.07 (m, 2H), 2.76-2.71 (m, 1H), 1.93-1.88 (m, 2H), **^{13}C NMR** (100 MHz, $CDCl_3$): δ 158.7, 147.61, 132.1, 129.2, 128.7, 127.1, 126.63, 113.8, 81.2, 69.3, 69.6, 61.2, 55.2, 55.24, 50.86, 42.4. The free base was converted into corresponding hydrochloride salt. Mp = 250–255 °C. Anal. ($C_{25}H_{28}NO_2$. HCl) C, H, N.

Procedure Z. 2-(4-Methoxy-phenyl)-ethylamine (59).

4-methoxyphenylacetonitrile (2.0 g, 13.59 mmol) was dissolved in THF (11 mL) at room temperature and the reaction mixture was cooled to 0 ^oC followed by addition of borone-methyl sulfide complex (5.74 g, 47.56 mmol). The reaction mixture was stirred for 2 h, then gradually warmed to 40 ^oC and stirred overnight. The reaction mixture was slowly quenched by adding 2N HCl solution (16 ml), the quenched mixture was stirred for 1 h at 40 ^oC followed by the addition of conc.NH_4OH (5 ml) then EtOAC, and dibasic sodium phosphate. The organic layer was separated, dried over Na_2SO_4, and concentrated over rotary evaporator to get compound **(59)**. The crude was mostly pure for use in the next step without further purification (0.1 g, 0.396 mmol) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.06–7.05 (m, 2 H), 6.84-6.82 (m, 2 H), 4.21-4.17 (m, 2 H), 3.71 (s, 3 H), 3.54-3.53 (s, 2H).

Procedure Z'. 2-[2-(4-Methoxy-phenyl)-ethylamino]-5,5-diphenyl-cyclohexanol (60a and b).

A mixture of epoxide (0.1 g, 0.396 mmol) and 2-(4-methoxyphenyl) ethan-1-amine (1.198g, 7.92 mmol) in ethanol was refluxed at 100 ^oC under N_2 overnight. The reaction was quenched with saturated $NaHCO_3$, and DCM was added. The organic layer was separated, dried over Na_2SO_4, and concentrated in rotary evaporator. The crude product

was purified via gradient silica gel column chromatography using MeOH:DCM (1:10) to get compounds **(60a (D-620), and b (D-621))** (0.096 and 0.025 g, 64% and 17%). Major yield: **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.45 (d, J= 7.2 Hz, 2H), 7.33 (t, J= 7.5Hz, 2H), 7.28 (d, J= 7.8 Hz, 2H), 7.23 (t, J= 7.8 Hz, 1H), 7.22 (t, J= 7.8 Hz,2H), 7.14 (t, J= 7.5 Hz, 1H), 7.07(d, J=8.4 Hz, 2H), 6.8 (d, J= 8.4 Hz, 2H), 4.07 (dd, J= 11.50, 4.8 Hz, 1H), 3.77 (s, 3H), 3.50 (td, J= 11.60, 4.1 Hz, 1H), 3.09 (dd, J= 11.60, 4.3 Hz, 1H), 3.05 (t, J= 11.0 Hz, 1H), 2.95 (dt, J= 14.7 Hz, 1H), 2.78 (m, 1H), 2.69 (btw d&t, J= 10.6, 4.8 Hz, 1H), 2.68 (m, 1H), 1.91 (dd, J= 13.8, 4.3 Hz, 1H). **^{1}H NMR** (600 MHz, CDCl 3): Minor yield: δ 7.67–7.63 (m, 2 H), 7.54-7.52 (m, 2H), 7.46-7.43 (m, 2H), 7.28-7.20 (m, 2H), 7-08-7.01 (m, 2H), 6.83 (d, J= 9.0 Hz, 2H), 6.82-6.78 (m, 2H), 4.06 (dd, J = 7.2, 4.2 Hz, 1 H), 3.78 (s, 3 H), 3.53 (dd, J = 6.6, 6.0 Hz, 2 H),3.42 (dd, J = 6.6, 6.0 Hz, 1 H), 3.09 (dd, J = 9.0, 4.2 Hz, 1 H), 2.78-2.73 (m, 2 H), 2.71-2.69 (m, 2 H), 2.67–2.63 (m, 2 H), 1.91 (dd, J=11.4, 2.4 HZ, 1H), 0.88–0.82 (m, 3 H). **^{13}C NMR** (150 MHz, $CDCL_3$): δ 158.053, 147.646, 142.104, 131.537, 129.550, 128.737, 128.077, 127.219, 127.101, 126.647, 124.858, 113.92, 81.287, 69.264, 64.701, 61.974, 55.226, 48.787, 42.451, 36.038. **(60a (D-620), and b (D-621))**; Mp = 195 °C. Anal. ($C_{26}H_{30}NO_3$. HCl) C, H, N and Mp = 200 °C. Anal. ($C_{26}H_{30}NO_3$. HCl) C, H, N respectively.

Procedure Z". 2,2-Diphenyl-7-oxa-bicyclo[4.1.0]heptane (61).

The alkene (0.20 g, 0.85 mmol) **(53)** was dissolved in a mixture of acetonitrile:DME (1:2) (15 mL), A buffer $Na_2B_4O_7.H_2O$ (0.01 g, 0.05 mmol) in 0.0004 M aqueous Na_2EDTA (?) was added. Tertbutyl ammonium hydrogen sulfate (0.0114 g, 0.034 mmol), and epoxane (ketone) (0.065 g, 0.25 mmol) were then added with stirring. The mixture was cooled to about -10 ^{o}C bath temperature by using an NaCl ice bath. A solution of oxone

in aqueous Na_2EDTA 0.004 M and a solution of K_2CO_3 (0.68 g, 4.91 mmol) in water were added dropwise separately over a period of 2 h via droplets to give the chiral epoxide **(61)** (0.074 g, 35%). **^{1}H NMR** (600 MHz, CDCl3): δ 7.31-6.92 (m, 10H), 3.85 (d, *J*= 13.6 Hz, 1H), 3.25 (dd, *J*= 13.6, 1.5 Hz, 1H), 2.95 (bt, *J*= 4.9 Hz, 1H), 2.42 (dd, *J*= 15.8, 5.8 Hz, 1H), 2.25 (dd, *J*= 4.3, 1.5 Hz, 1H), 2.20 (dd, *J*= 15.8, 0.66 Hz, 1H).

2-[2-(4-Methoxy-phenyl)-ethylamino]-5,5-diphenyl-cyclohexanol (60a).

A mixture of chiral epoxide **(61)** (0.1 g, 0.396 mmol) and 2-(4-methoxyphenyl) ethan-1-amine (1.19 g, 7.92 mmol) in ethanol was refluxed at 100 °C under N_2 overnight. The reaction was followed according to procedure Z'. The crude product was purified via silica gel column chromatography using MeOH:DCM (1:10) to get compounds **60a (D-620)** (0.096 g, 64%). **^{1}H NMR** (600 MHz, CDCl 3): δ 7.45 (d, J= 7.2 Hz, 2H), 7.33 (t, J= 7.5Hz, 2H), 7.28 (d, J= 7.8 Hz, 2H), 7.23 (t, J= 7.8 Hz, 1H), 7.22 (t, J= 7.8 Hz,2H), 7.14 (t, J= 7.5 Hz, 1H), 7.07(d, J=8.4 Hz, 2H), 6.8 (d, J= 8.4 Hz, 2H), 4.07 (dd, J= 11.50, 4.8 Hz, 1H), 3.77 (s, 3H), 3.50 (td, J= 11.60, 4.1 Hz, 1H), 3.09 (dd, J= 11.60, 4.3 Hz, 1H), 3.05 (t, J= 11.0 Hz, 1H), 2.95 (dt, J= 14.7 Hz, 1H), 2.78 (m, 1H), 2.69 (btw d&t, J= 10.6, 4.8 Hz, 1H), 2.68 (m, 1H), 1.91 (dd, J= 13.8, 4.3 Hz, 1H). **^{13}C NMR** (150 MHz, $CDCL_3$): δ 158.053, 147.646, 142.104, 131.537, 129.550, 128.737, 128.077, 127.219, 127.101, 126.647, 124.858, 113.92, 81.287, 69.264, 64.701, 61.974, 55.226, 48.787, 42.451, 36.038. Mp = 195 °C. Anal. ($C_{26}H_{30}NO_3$. HCl) C, H, N.

5.2. Evaluation of binding affinity and functional potencies at dopamine D_2 and D_3 receptors.

Binding affinity was evaluated by inhibition of [^{3}H] spiroperidol (15.0 Ci/mmol, Perkin-Elmer) binding to DA rD_2 and rD_3 receptors expressed in HEK-293 cells in a buffer

containing 0.9% NaCl to determine the inhibition constants (K_i) of the synthesized compounds (Zhen et al. 2010; Ghosh et al. 2010b). The Cheng–Prusoff equation was used to convert the observed IC_{50} into inhibition constants (K_i) (Zhen et al. 2010). Functional activity of test compounds in activating dopamine hD_2 and hD_3 receptors expressed in CHO cells was measured by stimulation of [^{35}S] GTPγS (1250 Ci/mmol, Perkin-Elmer) binding in comparison to stimulation by ymthe full agonist DA. All these procedures were described by us previously (Biswas et al. 2008; Zhen et al. 2010; Ghosh et al. 2010b).

5.3. Animal Experiments: *(In vivo* study of D-636, D-653 and D-656 in Parkinsonian rats).

5.3.1. Drugs and chemicals.

The following commercially available drug was used in the experiment: reserpine hydrochloride (Alfa Aesar). The TFA salt of (-)-**11b** (**D-636)** and HBr salts of (-)-**15a** (**D-653**) and (-)-**15c** (**D-656**) were dissolved in water. Reserpine was dissolved in 20 µL of glacial acetic acid and further diluted with 5.5% glucose solution. The compounds for this study were administered in a volume of 0.1−0.2 mL for subcutaneous administration and 0.5-0.7 ml for interaperitoneal adminstration into each rat.

5.3.2. Animals.

In rodent studies, animals were male and female Sprague-Dawley rats from Harlan (Indianapolis, IN) weighing 220-225 g unless otherwise specified. Animals were maintained in sawdust-lined cages in a temperature and humidity-controlled environment at 22 ± 1 °C and 60 ± 5% humidity, respectively. A 12 h light/dark cycle was maintained, with lights on from 6:00 a.m. to 6:00 p.m. They were group-housed with unrestricted

access to food and water. All experiments were performed during the light component. All animal use procedures were in compliance with the Wayne State University Animal Investigation Committee, consistent with AALAC guidelines.

5.3.3. Reversal of reserpine-induced hypolocomotion in rats.

The ability of compounds **D-636**, **D-653** and **D-656** to reverse reserpine-induced hypolocomotion was investigated according to a reported procedure (McCall et al. 2005). Reserpine (5.0 mg/kg, sc) was administered 18 h before the injection of drug or vehicle. The rats were placed individually in the chambers for 1 h for acclimatization before administration of the test drugs or vehicle. Immediately after administration of drug or vehicle, animals were individually placed in Opto-Varimex 4 animal activity monitor chamber (Columbus Instruments, Ohio, USA) to start measuring locomotor activity. Locomotion was monitored for 6 h. Consecutive interruption of two infrared beams, situated 50 cm apart and 4 cm above the cage floor, in the monitor chamber recorded movement. The data were presented as horizontal activity (HACTV). The effect of individual doses of drugs on locomotor activity was compared with respect to saline treated controls (mean ± SEM). The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. The effect was considered significant if the difference from control group was observed at $p < 0.05$.

5.4. In vitro study of D-636, D-653 and D-656 using PC12 cells.

5.4.1. Cell Cultures and Treatments.

PC12 cells (ATCC CRL1721.1, Manassas, VA, USA), a rat adrenal pheochromocytoma cell line, were cultured in T-75 flasks (Greiner Bio One, Frickenhausen, Germany) and maintained in RPMI 1640 medium supplemented with

10% heat inactivated horse sérum, 5% fetal bovine sérum, 100 U/ ml penicillin, and 100 μg/mL streptomycin at 37 °C in 95% air/5% CO_2. Stock solutions of **D-636**, **D-653**, and **D-656** were prepared in dimethylsulfoxide (DMSO) and stored at -20 °C, a stock solution of 6-Hydroxydopamine (6-OHDA) was stored at -80 °C, a solution of 6-carboxy-2',7'-dichlorodihydroflourescence diacetate (carboxy-DCFDA) was prepared fresh in DMSO before addition. All stock solution were stored for the period of the experiments.

5.4.2. Antioxidant activity studies: Measurement of antioxidant activity:

To determine the effects of **D-636, D-653** and **D-656** in decreasing reactive oxygen species (ROS) in PC12 cells produced by the neurotoxin 6-OHDA, a quantitative fluorometric ROS assay was performed. PC12 cells were plated at 30,000 cells/well density in 100 μL media in 96-well black plates and incubated at 37 °C under 5% CO_2 atmosphere for 24 h. The cells were treated for 24 h with various concentrations of compounds **D-636, D-653** and **D-656**. Then the drugs containing media were removed and replaced with DCFDA 20 μm for 30 min in an incubator (37 °C, 5% CO_2). The DCFDA containing media was then removed and the cells were washed with PBS buffer to remove the traces of the dye. Fresh culture media was added followed by treatment with 75 μM of 6-OHDA alone for an additional 1 h under the same conditions. After incubation for 1 h, the fluorescence was measured using spectrophotometer fluorescence generated microplate reader (Biotek Epoch, Winooski, VT, USA) at excitation 497 nm and emission at 527nm. Data from at least three experiments were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test using GraphPad software (version 6, San Diego, CA, USA). The specific fluorescence emission was calculated after subtraction of the DCFDA untreated control cells from the DCFDA

treated cells for both the background and 1h treatment conditions. This was followed by the division of the 1h treatment data by the background activity which was determined from vials before treatment with 6-OHDA, to derive the final data point.

5.4.3. Neuroprotection Studies.

5.4.3.1. Assessment of Cell Viability:

PC12 cells (ATCC CRL1721.1, Manassas, VA, USA), a rat adrenal pheochromocytoma cell line, were cultured in T-75 flasks (Greiner Bio One, Frickenhausen, Germany) and maintained in RPMI 1640 medium supplemented with 10% heat inactivated horse sérum, 5% fetal bovine sérum, 100 U/ml penicillin, and 100 μg/mL streptomycin at 37 °C in 95% air/5% CO_2. Stock solutions of **D-636**, **D-653**, **D-656**, and 6-hydroxydopamine (6-OHDA) were prepared in dimethylsulfoxide (DMSO) and aliquots were stored at -20 °C and -80 °C, respectively. For all experiments assessing neuroprotective effects of the test compounds, PC12 cells were pretreated with indicated concentrations of **D-636**, **D-653** and **D-656** for 24 h and then treated with 75 μM 6-OHDA for another 24 h. The control cells were treated with above media containing 0.01% DMSO only.

To determine the neuroprotective effects of **D-636**, **D-653** and **D-656** in the presence of neurotoxin 6-OHDA, a quantitative colorimetric MTT assay was performed. PC12 cells were plated at 17000 cells/well density in 100 μL of media in 96-well plates and incubated at 37 °C under 5% CO_2 atmosphere for 24 h. Cells were treated with varying concentrations of the test compounds to determine their direct effect on cell viability. Neuroprotection experiments were conducted by treating cells for 24 h with varying concentrations of **D-636**, **D-653** and **D-656**. Then the drug containing media was

replaced with fresh culture media followed by treatment with 75 μM of 6-OHDA alone for an additional 24 h under the same condition. After incubation for 24 h, 5 mg/mL MTT solution (prepared in Dulbecco's phosphate-buffered saline) was added to the cells (to a final concentration of 0.5 mg/mL) and the plates were further incubated at 37 ^{o}C in 95% air/5% CO_2 atmosphere for 3-4 h to produce dark-blue formazan crystals. Afterward, the plates were centrifuged at 450 *g* for 10 min and the supernatants were carefully removed. Formazan crystals were dissolved by adding 100 μL of methanol:DMSO (1:1) mixture to each well and shaking gently at 400 rpm for 30 min at room temperature on a Thermomix R shaker (Eppendorf, Hamburg, Germany). The absorbance was measured on a microplate reader (Biotek Epoch, Winooski, VT, USA) at 570 nm with background correction performed at 690 nm. Data from at least three experiments were analyzed using GraphPad software (version 6, San Diego, CA, USA). Cell viability was defined as percentage reduction in absorbance compared to untreated controls. The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test.

5.5. Human monoamine oxidase inhibition (hMAO) studies.

This study was done to determine the ability of selected compounds in inhibiting MAO enzymes. A fluorometric screening was carried out according to the protocols with some modifications (Zhengyin et al. 2004; Novaroli et al. 2005; Chimenti et al. 2013). The enzyme activity was determined by measuring the fluorescence generated from the oxidation of kynuramine to 4-hydroxyquinoline (4-HQ) catalyzed by monoamine oxidase enzyme. In this experiment the MAO enzyme source was used containing recombinant hMAO-A and hMAO-B microsomes from insect cells as commercially available in Sigma-

Aldrich. They were stored at −80 °C as pre-aliquoted to avoid repeated freeze-thaw cycles. During experiment preparation, they were liquefied rapidly in a 37 °C water bath and kept on ice until use. In this study, the substrate for both MAO-A and MAO-B was kynuramine, and buffer assay was potassium phosphate (0.1 M, pH 7.4, made isotonic with KCl 20.2 mM). The final volume of the 146 reactions was set to 200 µL. The reference compound for this assay was a pargyline, a known potent MAO-B inhibitor.

5.5.1. Initial hMAO-B inhibition screening

Selected compounds were first examined at a dose of 25 µM for their inhibitory activity against hMAO-B. The final concentration of enzyme and substrate were set at 15 µg/mL and 25 µM, respectively. Stocks of compounds (50 µM) were prepared in DMSO, whose percentage was kept at 0.05% in the final assay reaction mixture. Substrate (50 µL/well) and compound solutions (100 µL/well) were added into a black 96-well plate and pre-incubated at 37 °C for 10 min. The control wells received assay buffer instead of compound solutions. The enzymatic reaction was initiated by the addition of hMAO-B solution (50 µL/well). The reaction mixture was then incubated at 37 °C for 20 min, and subsequently 2N NaOH aqueous solution (75 µL/well) was added to terminate the reaction. The fluorescence of 4-HQ was measured in triplicates using the Synergy Hybrid H1 fluorescence microplate reader (BioTek) at the wavelength pair of 310/400 nm (excitation/emission), and the readings were averaged and normalized with respect to the control. Assays were carried out in three independent experiments.

5.5.2. IC 50 values determination

Compounds, which reduced the hMAO-B activity to near half in the initial screening at 25 µM were considered hit molecules and were tested further for the IC 50 values for

both hMAO-A and hMAO-B to determine their selectivity ratio. Seven doses of test compounds (0-250 µM) were used, and the final concentration of the enzyme was set to 15 µg/mL. The final concentrations of substrate for measuring either hMAO-A or hMAO-B activity were set to 40 µM and 25 µM, respectively. The fluorescence was measured in either duplicates or triplicates, and the IC 50 values were determined from non-linear regression of dose-response curves using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). Assays were carried out in three independent experiments.

Monoamine reuptake inhibitors studies.

5.5.3. Inhibition of monoamine uptake by cloned human biogenic amine transporters in heterologous cells.

Inhibition of substrate uptake by cloned human transporters was measured with stably transfected human embryonic kidney (HEK) 293 cells as in our previous work (E A Reith et al. 2012). The cell lines were obtained and used in uptake assays as described in the same paper (E A Reith et al. 2012). [^{3}H]DA ([ring 2,5,6- 3 H]dopamine (45.0 Ci/mmol, PerkinElmer, Boston, MA, U.S.A) was used for monitoring DAT and NET, DA was used as a reference because of its excellent substrate for NET (Santra, Gogoi, Gopishetty, Antonio, Zhen, E A Reith, et al. 2012), [^{3}H]5-HT ([1,2- ^{3}H] serotonin (27.9 Ci/mmol, Perkin-Elmer) was the radioligand for monitoring SERT.

Drug stocks contained an additional 0.01% (w/v) bovine serum albumin in order to reduce absorption of drug to the walls of the assay plates. At least five triplicate concentrations of each test compound were studied, spaced evenly around the IC_{50} value which was converted to K_i with the Cheng-Prusoff equation (Soumava et al. 2012).

5.6. Statistical analysis

Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). For all the *in vitro* assays, the data were analyzed by one-way analysis of variance (ANOVA) analysis followed by Tukey's multiple comparison post hoc test unless otherwise specified. And for the *in vivo* assays, one-way ANOVA analysis followed by Dunnett's analysis was used. The effect was considered significant if the difference from control group was observed at $p < 0.05$.

CHAPTER 6

CONCLUSION

Parkinson's disease (PD) is a progressive age-related neurodegenerative disorder that is characterized by the loss of dopamine and the degeneration of the DA neurons in the SNpc of the central nervous system (CNS). The pathogenesis of PD has been identified as multifactorial in nature. Therefore, drugs that target a single biological target, have been found to be insufficient to treat PD (Nagal and Singla 2012). Therefore, it is hypothesized that designing and development of multifunctional molecules having multiple pharmacological properties targeting multiple pathogenic pathways associated with the progression and development of PD should be beneficial as disease-modifying agent for the treatment of PD.

The first main objective represents the development of a novel series of multifunctional dopamine D_2/D_3 agonists based on carbazole derivatives that can potentially protect the neurons from degeneration in PD. The dopamine D_2/D_3 receptor agonists pharmacophore were attached to the various carbazole moieties via va linkers to build various hybrid molecules. A series of compounds were synthesized, and characterized *in vitro* assays which was followed by in vivo assay for selected lead molecules.. Compounds (-)-**11b**, (-)-**15a** and (-)-**15c** exhibited high affinity and full agonist activity at both D_2 and D_3 receptors. In PD animal model, the lead molecules exhibited potent activity and high efficay in augmenting the locomotor activity with a long duration of action by reversing hypolocomotion in reserpinized rats, which indicated their potential as anti-PD drugs. To glean insight into their possible multifunctional property, the data presented here also shows that both (-)-**11b** and (-)-**15c** are neuroprotective in an *in vitro*

model of dopaminergic PC12 cells treated with the neurotoxin 6-OHDA to demonstrate a significant dose-dependent reduction of toxicity induced by treatment with the neurotoxin 6-hydroxydopamine. Therefore, supports the notion that multifunctional drugs like (-)-**11b** and (-)-**15c** have the potential not only to ameliorate motor dysfunction in PD patients but also to modify disease progression by protecting DA neurons from neurotoxic insults in addition to restoring their function. This study will, therefore, shed additional light on the importance of carbazole moiety (Głuszyńska 2015) as a potential molecular component in hybrid drug design approach for developing disease-modifying therapeutics for PD. Further mechanistic studies to ascertain the disease-modifying effects of the compounds are currently underway and will be published in due course.

The second objective was to design and synthesize a series of novel dopamine D_2/D_3 receptor agonists that should selectively inhibit MAO-B activity also. Such molecules were designed by combining the D_2/D_3 receptor agonist fragments with the propargyl group. The designed compounds were evaluated for the binding activity for D_2/D_3 receptors first. In this SAR study, a noticeable reduction in the D_2 binding affinity of the compound **(-)-35-(D-677)** was observed when the compound underwent methylation of the secondary nitrogen atom directly attached to the propargyl group. A reduction in the D_3 binding affinity was also observed. Conversely, the O-analog version **(±)-42 D-678),** exhibited similar D_2 binding affinity when compared to **(-)-33 D-671** The similarity of the binding affinity for **D-(-)-33 D-671**, and **(±)-42 D-678** might be explained by the presence of the lone pair of electrons on the secondary amine and the oxygen atom that would be necessary for the binding of these compound to the D_2/D_3 receptors possibly via H-bonding interaction. In the case of functional activities, both **(-)-33 D-671 and (±)-**

42 D-678 produce full agonist activity. It was interesting that the compound **(±)-42 D-678** remained full agonist at D_2 receptor even after the substitution of the nitrogen atom and also exerted a slight improvement in the potency at the D_3 receptor. Compounds were further tested in the *in vitro* enzymatic assays and compound **(-)-33 D-671** was shown to be more active than **(±)-42 D-678**, demonstrating potent inhibition of the MAO-B.

The determination of IC_{50} values suggested that both compounds **(-)-33 D-671 and (±)-42 D-678** are slightly more selective for MAO-B than MAO-A. Further structural modification and characterization studies are required to improve the selectivity of the current lead compound to inhibit the MAO-B.

Our final goal was to develop novel multifunctional triple reuptake inhibitors (TUIs) to treat the motor, and the non-motor symptoms like depression associated with PD. Our drug development study was based on the modification of the pyran template using different substituted groups. To develop suitable TUIs and to further understand the effect of the structural modifications on the activity profile for the three monoamine transporters, we further expanded our previous SAR studies with 2,3,5-trisubstituted pyran compounds. Compound **D-594** was synthesized by introducing biphenyl groups directly to the pyran moiety to evaluate their effect on the profile of the uptake inhibition activity. Compound **D-594** exhibited moderate potency at DAT and weak potency at both SERT and NET. To follow up on the SAR studies to improve the binding activity of this compound, further modifications have been made by substituting the methylene bridge with the ethylene bridge to develop compounds **D-620**, and **D-621** which were subjected to *in vitro* inhibition assays. Compound **D-621** exhibited low potency at both DAT and NET transporters with moderate potency at SERT transporter while compound **D-620**

exhibited balanced potency at both DAT and NET transporters with moderate potency at SERT transporter. Introduction of methylene atom to build the ethylene bridge in general showed increasing the activity for the SERT. Compound **D-620**, was identified as the lead compound from this SAR study and it exhibited dual reuptake inhibitor activity (DNRI-type) profile. This outcome suggests further study and structural modifications are necessary to develop TUIs with potent activity at three monoamine transporters.

REFERENCES

Biswas, S., S. Hazeldine, B. Ghosh, I. Parrington, E. Kuzhikandathil, M. E. Reith, and A. K. Dutta. 2008. 'Bioisosteric heterocyclic versions of 7-{[2-(4-phenyl-piperazin-1-yl)ethyl]propylamino}-5,6,7,8-tetrahydronaphthalen-2- ol: identification of highly potent and selective agonists for dopamine D3 receptor with potent in vivo activity', *J Med Chem*, 51: 3005-19.

Brown, Dennis A., Manoj Mishra, Suhong Zhang, Swati Biswas, Ingrid Parrington, Tamara Antonio, Maarten E. A. Reith, and Aloke K. Dutta. 2009. 'Investigation of various N-heterocyclic substituted piperazine versions of 5/7-{[2-(4-aryl-piperazin-1-yl)-ethyl]-propyl-amino}-5,6,7,8-tetrahydro-naphthalen-2-ol: Effect on affinity and selectivity for dopamine D3 receptor', *Bioorganic & Medicinal Chemistry*, 17: 3923-33.

Chimenti, Paola, Anél Petzer, Simone Carradori, Melissa D'Ascenzio, Romano Silvestri, Stefano Alcaro, Francesco Ortuso, Jacobus P. Petzer, and Daniela Secci. 2013. 'Exploring 4-substituted-2-thiazolylhydrazones from 2-, 3-, and 4-acetylpyridine as selective and reversible hMAO-B inhibitors', *European Journal of Medicinal Chemistry*, 66: 221-27.

Das, B., S. Vedachalam, D. Luo, T. Antonio, M. E. Reith, and A. K. Dutta. 2015. 'Development of a Highly Potent D2/D3 Agonist and a Partial Agonist from Structure-Activity Relationship Study of N(6)-(2-(4-(1H-Indol-5-yl)piperazin-1-yl)ethyl)-N(6)-propyl-4,5,6,7-tetrahydroben zo[d]thiazole-2,6-diamine Analogues: Implication in the Treatment of Parkinson's Disease', *J Med Chem*, 58: 9179-95.

E A Reith, Maarten, Solav Ali, Audrey Hashim, Imran Sheikh, Naresh Theddu, Narendra V Gaddiraju, Suneet Mehrotra, Kyle Schmitt, Thomas Murray, Henry Sershen, Ellen Unterwald, and Franklin Davis. 2012. *Novel C-1 Substituted Cocaine Analogs Unlike Cocaine or Benztropine*.

Ghosh, Balaram, Tamara Antonio, Bhaskar Gopishetty, Maarten Reith, and Aloke Dutta. 2010. 'Further delineation of hydrophobic binding sites in dopamine D2/D3 receptors for N-4 substituents on the piperazine ring of the hybrid template 5/7-{[2-(4-aryl-piperazin-1-yl)-ethyl]-propyl-amino}-5,6,7,8-tetrahydro-naphthalen-2-ol', *Bioorganic & Medicinal Chemistry*, 18: 5661-74.

McCall, R. B., K. J. Lookingland, P. J. Bedard, and R. M. Huff. 2005. 'Sumanirole, a highly dopamine D2-selective receptor agonist: in vitro and in vivo pharmacological characterization and efficacy in animal models of Parkinson's disease', *J Pharmacol Exp Ther*, 314: 1248-56.

Novaroli, L., M. Reist, E. Favre, A. Carotti, M. Catto, and P. A. Carrupt. 2005. 'Human recombinant monoamine oxidase B as reliable and efficient enzyme source for inhibitor screening', *Bioorg Med Chem*, 13: 6212-7.

Santra, Soumava, Sanjib Gogoi, Bhaskar Gopishetty, Tamara Antonio, Juan Zhen, Maarten E A Reith, and Aloke Dutta. 2012. *Structural Exploration of (3 S ,6 S)-6-Benzhydryl- N -benzyltetrahydro-2 H -pyran-3-amine Analogues: Identification of Potent Triple Monoamine Reuptake Inhibitors as Potential Antidepressants*.

Soumava, Santra, Gogoi Sanjib, Gopishetty Bhaskar, Antonio Tamara, Zhen Juan, Reith Maarten E. A., and Dutta Aloke K. 2012. 'Structural Exploration of (3S,6S)-6-Benzhydryl-N-benzyltetrahydro-2H-pyran-3-amine Analogues: Identification of

Potent Triple Monoamine Reuptake Inhibitors as Potential Antidepressants', *ChemMedChem*, 7: 2093-100.

Zhen, Juan, Tamara Antonio, Aloke K. Dutta, and Maarten E. A. Reith. 2010. 'Concentration of receptor and ligand revisited in a modified receptor binding protocol for high-affinity radioligands: [3H]Spiperone binding to D2 and D3 dopamine receptors', *Journal of Neuroscience Methods*, 188: 32-38.

Zhengyin, Yan, Caldwell Gary W., Zhao Boyu, and Reitz Allen B. 2004. 'A high-throughput monoamine oxidase inhibition assay using liquid chromatography with tandem mass spectrometry', *Rapid Communications in Mass Spectrometry*, 18: 834-40.

Andrea Cavalli, * Maria Laura Bolognesi, * Anna Minarini, Michela Rosini, Vincenzo Tumiatti, Maurizio Recanatini, andCarlo Melchiorre. 2008. 'Multi-target-Directed Ligands To Combat Neurodegenerative Diseases', *Journal of Medicinal Chemistry*, 51: 347-72.

Bagal, S. K., S. G. Davies, J. A. Lee, P. M. Roberts, A. J. Russell, P. M. Scott, and J. E. Thomson. 2010. 'An oxidation and ring contraction approach to the synthesis of (+/-)-1-deoxynojirimycin and (+/-)-1-deoxyaltronojirimycin', *Org Lett*, 12: 136-9.

Biswas, S., S. Hazeldine, B. Ghosh, I. Parrington, E. Kuzhikandathil, M. E. Reith, and A. K. Dutta. 2008. 'Bioisosteric heterocyclic versions of 7-{[2-(4-phenyl-piperazin-1-yl)ethyl]propylamino}-5,6,7,8-tetrahydronaphthalen-2- ol: identification of highly potent and selective agonists for dopamine D3 receptor with potent in vivo activity', *J Med Chem*, 51: 3005-19.

Blum, D., S. Torch, M. F. Nissou, A. L. Benabid, and J. M. Verna. 2000. 'Extracellular toxicity of 6-hydroxydopamine on PC12 cells', *Neurosci Lett*, 283: 193-6.

Carlsson, A., M. Lindqvist, and T. Magnusson. 1957. '3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists', *Nature*, 180: 1200.

Das, B., A. Kandegedara, L. Xu, T. Antonio, T. Stemmler, M. E. A. Reith, and A. K. Dutta. 2017. 'A Novel Iron(II) Preferring Dopamine Agonist Chelator as Potential Symptomatic and Neuroprotective Therapeutic Agent for Parkinson's Disease', *ACS Chem Neurosci*, 8: 723-30.

Das, B., G. Modi, and A. Dutta. 2015. 'Dopamine D3 agonists in the treatment of Parkinson's disease', *Curr Top Med Chem*, 15: 908-26.

Das, B., S. Rajagopalan, G. S. Joshi, L. Xu, D. Luo, J. K. Andersen, S. V. Todi, and A. K. Dutta. 2017. 'A novel iron (II) preferring dopamine agonist chelator D-607 significantly suppresses alpha-syn- and MPTP-induced toxicities in vivo', *Neuropharmacology*, 123: 88-99.

Das, B., S. Vedachalam, D. Luo, T. Antonio, M. E. Reith, and A. K. Dutta. 2015. 'Development of a Highly Potent D2/D3 Agonist and a Partial Agonist from Structure-Activity Relationship Study of N(6)-(2-(4-(1H-Indol-5-yl)piperazin-1-yl)ethyl)-N(6)-propyl-4,5,6,7-tetrahydroben zo[d]thiazole-2,6-diamine Analogues: Implication in the Treatment of Parkinson's Disease', *J Med Chem*, 58: 9179-95.

Dutta, A. K., X. S. Fei, and M. E. Reith. 2002. 'A novel series of hybrid compounds derived by combining 2-aminotetralin and piperazine fragments: binding activity at D2 and D3 receptors', *Bioorg Med Chem Lett*, 12: 619-22.

Ghosh, B., T. Antonio, B. Gopishetty, M. Reith, and A. Dutta. 2010. 'Further delineation of hydrophobic binding sites in dopamine D(2)/D(3) receptors for N-4 substituents on the piperazine ring of the hybrid template 5/7-{[2-(4-aryl-piperazin-1-yl)-ethyl]-

propyl-amino}-5,6,7,8-tetrahydro-naphthale n-2-ol', *Bioorg Med Chem*, 18: 5661-74.

Gluszynska, A. 2015. 'Biological potential of carbazole derivatives', *Eur J Med Chem*, 94: 405-26.

H, Youdim Moussa B, Gross Aviva, and Finberg John P M. 2001. 'Rasagiline [N-propargyl-1R(+)-aminoindan], a selective and potent inhibitor of mitochondrial monoamine oxidase B', *British Journal of Pharmacology*, 132: 500-06.

Johnson, M., T. Antonio, M. E. Reith, and A. K. Dutta. 2012. 'Structure-activity relationship study of N(6)-(2-(4-(1H-Indol-5-yl)piperazin-1-yl)ethyl)-N(6)-propyl-4,5,6,7-tetrahydroben zo[d]thiazole-2,6-diamine analogues: development of highly selective D3 dopamine receptor agonists along with a highly potent D2/D3 agonist and their pharmacological characterization', *J Med Chem*, 55: 5826-40.

Kim, Mounggon, and Jun Yeob Lee. 2013. 'Synthesis of 2- and 4-substituted carbazole derivatives and correlation of substitution position with photophysical properties and device performances of host materials', *Organic Electronics*, 14: 67-73.

Larin, E. A., V. S. Kochubei, and Y. M. Atroshchenko. 2014. 'Regio- and stereoselective synthesis of new diaminocyclopentanols', *Beilstein J Org Chem*, 10: 2513-20.

Li, C., S. Biswas, X. Li, A. K. Dutta, and W. Le. 2010. 'Novel D3 dopamine receptor-preferring agonist D-264: Evidence of neuroprotective property in Parkinson's disease animal models induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and lactacystin', *J Neurosci Res*, 88: 2513-23.

Lindenbach, D., B. Das, M. M. Conti, S. M. Meadows, A. K. Dutta, and C. Bishop. 2017. 'D-512, a novel dopamine D2/3 receptor agonist, demonstrates greater anti-

Parkinsonian efficacy than ropinirole in Parkinsonian rats', *Br J Pharmacol*, 174: 3058-71.

Loris, Z. B., A. A. Pieper, and W. D. Dietrich. 2017. 'The neuroprotective compound P7C3-A20 promotes neurogenesis and improves cognitive function after ischemic stroke', *Exp Neurol*, 290: 63-73.

Luo, D., H. Sharma, D. Yedlapudi, T. Antonio, M. E. Reith, and A. K. Dutta. 2016. 'Novel multifunctional dopamine D2/D3 receptors agonists with potential neuroprotection and anti-alpha synuclein protein aggregation properties', *Bioorg Med Chem*, 24: 5088-102.

MacMillan, K. S., J. Naidoo, J. Liang, L. Melito, N. S. Williams, L. Morlock, P. J. Huntington, S. J. Estill, J. Longgood, G. L. Becker, S. L. McKnight, A. A. Pieper, J. K. De Brabander, and J. M. Ready. 2011. 'Development of proneurogenic, neuroprotective small molecules', *J Am Chem Soc*, 133: 1428-37.

Modi, G., T. Antonio, M. Reith, and A. Dutta. 2014. 'Structural modifications of neuroprotective anti-Parkinsonian (-)-N6-(2-(4-(biphenyl-4-yl)piperazin-1-yl)-ethyl)-N6-propyl-4,5,6,7-tetrahydrobe nzo[d]thiazole-2,6-diamine (D-264): an effort toward the improvement of in vivo efficacy of the parent molecule', *J Med Chem*, 57: 1557-72.

Naoi, M., W. Maruyama, M. B. Youdim, P. Yu, and A. A. Boulton. 2003. 'Anti-apoptotic function of propargylamine inhibitors of type-B monoamine oxidase', *Inflammopharmacology*, 11: 175-81.

Pieper, A. A., S. Xie, E. Capota, S. J. Estill, J. Zhong, J. M. Long, G. L. Becker, P. Huntington, S. E. Goldman, C. H. Shen, M. Capota, J. K. Britt, T. Kotti, K. Ure, D.

J. Brat, N. S. Williams, K. S. MacMillan, J. Naidoo, L. Melito, J. Hsieh, J. De Brabander, J. M. Ready, and S. L. McKnight. 2010. 'Discovery of a proneurogenic, neuroprotective chemical', *Cell*, 142: 39-51.

Prins, Louis H. A., Jacobus P. Petzer, and Sarel F. Malan. 2010. 'Inhibition of monoamine oxidase by indole and benzofuran derivatives', *European Journal of Medicinal Chemistry*, 45: 4458-66.

Santra, S., L. Xu, M. Shah, M. Johnson, and A. Dutta. 2013. 'D-512 and D-440 as novel multifunctional dopamine agonists: characterization of neuroprotection properties and evaluation of in vivo efficacy in a Parkinson's disease animal model', *ACS Chem Neurosci*, 4: 1382-92.

Shah, M., S. Rajagopalan, L. Xu, C. Voshavar, Y. Shurubor, F. Beal, J. K. Andersen, and A. K. Dutta. 2014. 'The high-affinity D2/D3 agonist D512 protects PC12 cells from 6-OHDA-induced apoptotic cell death and rescues dopaminergic neurons in the MPTP mouse model of Parkinson's disease', *J Neurochem*, 131: 74-85.

Skalisz, L. L., V. Beijamini, S. L. Joca, M. A. Vital, C. Da Cunha, and R. Andreatini. 2002. 'Evaluation of the face validity of reserpine administration as an animal model of depression--Parkinson's disease association', *Prog Neuropsychopharmacol Biol Psychiatry*, 26: 879-83.

Soto-Otero, R., E. Mendez-Alvarez, A. Hermida-Ameijeiras, A. M. Munoz-Patino, and J. L. Labandeira-Garcia. 2000. 'Autoxidation and neurotoxicity of 6-hydroxydopamine in the presence of some antioxidants: potential implication in relation to the pathogenesis of Parkinson's disease', *J Neurochem*, 74: 1605-12.

Sturino, Claudio F., and Jerome C. Y. Wong. 1998. 'The ring-closing metathesis of vinyl ethers with Grubbs' catalyst for the synthesis of dihydropyrans', *Tetrahedron Letters*, 39: 9623-26.

W., Olanow C. 1992. 'An introduction to the free radical hypothesis in Parkinson's disease', *Annals of Neurology*, 32: S2-S9.

Wang, S. N., T. Y. Xu, X. Wang, Y. F. Guan, S. L. Zhang, P. Wang, and C. Y. Miao. 2016. 'Neuroprotective Efficacy of an Aminopropyl Carbazole Derivative P7C3-A20 in Ischemic Stroke', *CNS Neurosci Ther*, 22: 782-8.

Wu, X., J. Kosaraju, W. Zhou, and K. Y. Tam. 2017. 'Neuroprotective Effect of SLM, a Novel Carbazole-Based Fluorophore, on SH-SY5Y Cell Model and 3xTg-AD Mouse Model of Alzheimer's Disease', *ACS Chem Neurosci*, 8: 676-85.

Yedlapudi, D., G. S. Joshi, D. Luo, S. V. Todi, and A. K. Dutta. 2016. 'Inhibition of alpha-synuclein aggregation by multifunctional dopamine agonists assessed by a novel in vitro assay and an in vivo Drosophila synucleinopathy model', *Sci Rep*, 6: 38510.

Yoon, H. J., S. Y. Kong, M. H. Park, Y. Cho, S. E. Kim, J. Y. Shin, S. Jung, J. Lee, Farhanullah, H. J. Kim, and J. Lee. 2013. 'Aminopropyl carbazole analogues as potent enhancers of neurogenesis', *Bioorg Med Chem*, 21: 7165-74.

Zhang, S., F. Fernandez, S. Hazeldine, J. Deschamps, J. Zhen, M. E. Reith, and A. K. Dutta. 2006. 'Further structural exploration of trisubstituted asymmetric pyran derivatives (2S,4R,5R)-2-benzhydryl-5-benzylamino-tetrahydropyran-4-ol and their corresponding disubstituted (3S,6S) pyran derivatives: a proposed pharmacophore model for high-affinity interaction with the dopamine, serotonin, and norepinephrine transporters', *J Med Chem*, 49: 4239-47.

Zhang, S., J. Zhen, M. E. Reith, and A. K. Dutta. 2005a. 'Discovery of novel trisubstituted asymmetric derivatives of (2S,4R,5R)-2-benzhydryl-5-benzylaminotetrahydropyran-4-ol, exhibiting high affinity for serotonin and norepinephrine transporters in a stereospecific manner', *J Med Chem*, 48: 4962-71.

Zhang, Shijun, Juan Zhen, Maarten E. A. Reith, and Aloke K. Dutta. 2005b. 'Discovery of Novel Trisubstituted Asymmetric Derivatives of (2S,4R,5R)-2-benzhydryl-5-benzylaminotetrahydropyran-4-ol, Exhibiting High Affinity for Serotonin and Norepinephrine Transporters in a Stereospecific Manner', *Journal of Medicinal Chemistry*, 48: 4962-71.

Alves, Guido, Elin Forsaa, Kenn Freddy Pedersen, Michaela Gjerstad, and Jan Petter Larsen. 2008. *Epidemiology of Parkinson's disease*.

Amos D. Korczyn, MD, MSc. 2004. 'Drug treatment of Parkinson's disease', *Dialogues Clin Neurosci*, 6: 315–22.

Bashir, Maryam, Afifa Bano, Abdul Ijaz, and Bashir Chaudhary. 2015. 'Recent Developments and Biological Activities of N-Substituted Carbazole Derivatives: A Review', *Molecules*, 20: 13496.

Collier, Timothy J., Nicholas M. Kanaan, and Jeffrey H. Kordower. 2011. 'Ageing as a primary risk factor for Parkinson's disease: evidence from studies of non-human primates', *Nature reviews. Neuroscience*, 12: 359-66.

De Jesús-Cortés, Héctor, Pin Xu, Jordan Drawbridge, Sandi Jo Estill, Paula Huntington, Stephanie Tran, Jeremiah Britt, Rachel Tesla, Lorraine Morlock, Jacinth Naidoo, Lisa M. Melito, Gelin Wang, Noelle S. Williams, Joseph M. Ready, Steven L.

McKnight, and Andrew A. Pieper. 2012. 'Neuroprotective efficacy of aminopropyl carbazoles in a mouse model of Parkinson disease', *Proceedings of the National Academy of Sciences of the United States of America*, 109: 17010-15.

Dushanova, Juliana. 2012. *Diagnostics, rehabilitation and models of Parkinson's disease*.

Facecchia, Katie, Lee-Anne Fochesato, Sidhartha D. Ray, Sidney J. Stohs, and Siyaram Pandey. 2011. 'Oxidative Toxicity in Neurodegenerative Diseases: Role of Mitochondrial Dysfunction and Therapeutic Strategies', *Journal of Toxicology*, 2011: 12.

Głuszyńska, Agata. 2015. 'Biological potential of carbazole derivatives', *European Journal of Medicinal Chemistry*, 94: 405-26.

Gopishetty, Bhaskar, Stuart Hazeldine, Soumava Santra, Mark Johnson, Gyan Modi, Solav Ali, Juan Zhen, Maarten Reith, and Aloke Dutta. 2011. *Further Structure−Activity Relationship Studies on 4-((((3 S ,6 S)-6-Benzhydryltetrahydro-2 H -pyran-3-yl)amino)methyl)phenol: Identification of Compounds with Triple Uptake Inhibitory Activity as Potential Antidepressant Agents*.

Hindle, John V. 2010. 'Ageing, neurodegeneration and Parkinson's disease', *Age and Ageing*, 39: 156-61.

Loris, Zachary B., Andrew A. Pieper, and W. Dalton Dietrich. 2017. 'The neuroprotective compound P7C3-A20 promotes neurogenesis and improves cognitive function after ischemic stroke', *Experimental Neurology*, 290: 63-73.

Sheikh, Saba, Safia, Ejazul Haque, and Snober Mir. 2012. *Neurodegenerative Diseases: Multifactorial Conformational Diseases and Their Therapeutic Interventions*.

Visanji, Naomi, and Connie Marras. 2015. 'The relevance of pre-motor symptoms in Parkinson's disease', *Expert Review of Neurotherapeutics*, 15: 1205-17.

Visanji, Naomi P., Patricia L. Brooks, Lili-Naz Hazrati, and Anthony E. Lang. 2013. 'The prion hypothesis in Parkinson's disease: Braak to the future', *Acta Neuropathologica Communications*, 1: 2.

Abramov, Sonia Gandhi and Andrey Y. 2012. 'Mechanism of Oxidative Stress in Neurodegeneration', *Oxidative Medicine and Cellular Longevity*, 2012: 11.

Ahlawat, Abhilasha, Ajay Rana, Nidhi Goyal, and Dr Saurabh Sharma. 2014. *Potential role of nitric oxide synthase isoforms in pathophysiology of neuropathic pain*.

Ahmadinejad, Fereshteh, Simon Geir Møller, Morteza Hashemzadeh-Chaleshtori, Gholamreza Bidkhori, and Mohammad-Saeid Jami. 2017. 'Molecular Mechanisms behind Free Radical Scavengers Function against Oxidative Stress', *Antioxidants*, 6: 51.

Alves, Guido, Elin Forsaa, Kenn Freddy Pedersen, Michaela Gjerstad, and Jan Petter Larsen. 2008. *Epidemiology of Parkinson's disease*.

Amos D. Korczyn, MD, MSc. 2004. 'Drug treatment of Parkinson's disease', *Dialogues Clin Neurosci*, 6: 315–22.

Andrea Cavalli, * Maria Laura Bolognesi, * Anna Minarini, Michela Rosini, Vincenzo Tumiatti, Maurizio Recanatini, andCarlo Melchiorre. 2008. 'Multi-target-Directed Ligands To Combat Neurodegenerative Diseases', *Journal of Medicinal Chemistry*, 51: 347-72.

'Antidepressants.' in., *Manual of Clinical Psychopharmacology*.

Aquilano, Katia, Sara Baldelli, Giuseppe Rotilio, and Maria Rosa Ciriolo. 2008. 'Role of Nitric Oxide Synthases in Parkinson's Disease: A Review on the Antioxidant and Anti-inflammatory Activity of Polyphenols', *Neurochemical Research*, 33: 2416-26.

Arora, A., and P. Fletcher. 2013. 'Problem based review: a patient with Parkinson's disease', *Acute Med*, 12: 246-50.

Bagal, S. K., S. G. Davies, J. A. Lee, P. M. Roberts, A. J. Russell, P. M. Scott, and J. E. Thomson. 2010. 'An oxidation and ring contraction approach to the synthesis of (+/-)-1-deoxynojirimycin and (+/-)-1-deoxyaltronojirimycin', *Org Lett*, 12: 136-9.

Bansal, Yogita, and Om Silakari. 2014. 'Multifunctional compounds: Smart molecules for multifactorial diseases', *European Journal of Medicinal Chemistry*, 76: 31-42.

Bashir, Maryam, Afifa Bano, Abdul Ijaz, and Bashir Chaudhary. 2015. 'Recent Developments and Biological Activities of N-Substituted Carbazole Derivatives: A Review', *Molecules*, 20: 13496.

Bédard, Catherine, Marie-Josée Wallman, Emmanuelle Pourcher, Peter V. Gould, André Parent, and Martin Parent. 2011. 'Serotonin and dopamine striatal innervation in Parkinson's disease and Huntington's chorea', *Parkinsonism Relat Disord*, 17: 593-98.

Benjamin C.L. Lai, MD, MSc , Joseph K.C. Tsui, MD, FRCP(UK), FRCPC. 2001. 'Epidemiology of Parkinson's disease', *BCMJ*, 43: 133-37.

Biswas, S., S. Hazeldine, B. Ghosh, I. Parrington, E. Kuzhikandathil, M. E. Reith, and A. K. Dutta. 2008. 'Bioisosteric heterocyclic versions of 7-{[2-(4-phenyl-piperazin-1-yl)ethyl]propylamino}-5,6,7,8-tetrahydronaphthalen-2- ol: identification of highly

potent and selective agonists for dopamine D3 receptor with potent in vivo activity', *J Med Chem*, 51: 3005-19.

Blair, Hannah A., and Sohita Dhillon. 2017. 'Safinamide: A Review in Parkinson's Disease', *CNS Drugs*, 31: 169-76.

Blanpied, Thomas A., Richard J. Clarke, and Jon W. Johnson. 2005. 'Amantadine Inhibits NMDA Receptors by Accelerating Channel Closure during Channel Block', *The Journal of Neuroscience*, 25: 3312-22.

Blaya, Meghan O., Helen M. Bramlett, Jacinth Naidoo, Andrew A. Pieper, and W. Dalton Dietrich. 2014. 'Neuroprotective Efficacy of a Proneurogenic Compound after Traumatic Brain Injury', *Journal of Neurotrauma*, 31: 476-86.

Blesa, Javier, Ines Trigo-Damas, Anna Quiroga-Varela, and Vernice R. Jackson-Lewis. 2015. 'Oxidative stress and Parkinson's disease', *Frontiers in Neuroanatomy*, 9.

Blum, D., S. Torch, M. F. Nissou, A. L. Benabid, and J. M. Verna. 2000. 'Extracellular toxicity of 6-hydroxydopamine on PC12 cells', *Neurosci Lett*, 283: 193-6.

Borut Poljsak, Dušan Šuput, and Irina Milisav. 2013. 'Achieving the Balance between ROS and Antioxidants: When to Use the Synthetic Antioxidants', *Oxidative Medicine and Cellular Longevity*, 2013: 11.

Brown, Dennis A., Manoj Mishra, Suhong Zhang, Swati Biswas, Ingrid Parrington, Tamara Antonio, Maarten E. A. Reith, and Aloke K. Dutta. 2009. 'Investigation of various N-heterocyclic substituted piperazine versions of 5/7-{[2-(4-aryl-piperazin-1-yl)-ethyl]-propyl-amino}-5,6,7,8-tetrahydro-naphthalen-2-ol: Effect on affinity and selectivity for dopamine D3 receptor', *Bioorganic & Medicinal Chemistry*, 17: 3923-33.

Bruno, Dubois, Tolosa Eduardo, Katzenschlager Regina, Emre Murat, Lees Andrew J., Schumann Günther, Pourcher Emmanuelle, Gray Julian, Thomas Gail, Swartz Jina, Hsu Timothy, and Moline Margaret L. 2012. 'Donepezil in Parkinson's disease dementia: A randomized, double-blind efficacy and safety study', *Movement Disorders*, 27: 1230-38.

Bruno, P. Guiard, Mansari Mostafa El, and Blier Pierre. 2009. 'Prospect of a Dopamine Contribution in the Next Generation of Antidepressant Drugs: The Triple Reuptake Inhibitors', *Current Drug Targets*, 10: 1069-84.

Butini, S., K. Nikolic, S. Kassel, H. Brückmann, S. Filipic, D. Agbaba, S. Gemma, S. Brogi, M. Brindisi, G. Campiani, and H. Stark. 2016. 'Polypharmacology of dopamine receptor ligands', *Progress in Neurobiology*, 142: 68-103.

Calabresi, Paolo, Veronica Ghiglieri, Petra Mazzocchetti, Ilenia Corbelli, and Barbara Picconi. 2015. 'Levodopa-induced plasticity: a double-edged sword in Parkinson's disease?', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370.

Carlsson, A., M. Lindqvist, and T. Magnusson. 1957. '3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists', *Nature*, 180: 1200.

Céu Mateus, Joana Coloma. 2013. 'Health Economics and Cost of Illness in Parkinson's Disease', *European Neurological Review*, 8: 6-9.

Chan, Hiu-Fai, Prashanth L. Kukkle, Marcelo Merello, Shen-Yang Lim, Yu-Yan Poon, and Elena Moro. 2013. 'Amantadine improves gait in PD patients with STN stimulation', *Parkinsonism Relat Disord*, 19: 316-19.

Chan, Hiu-Fai, Lk Prashanth, Marcelo Merello, Shen-Yang Lim, Yu-Yan Poon, and Elena Moro. 2012. *Amantadine improves gait in PD patients with STN stimulation*.

Chen, Shengdi, Piu Chan, Shenggang Sun, Haibo Chen, Baorong Zhang, Weidong Le, Chunfeng Liu, Guoguang Peng, Beisha Tang, Lijuan Wang, Yan Cheng, Ming Shao, Zhenguo Liu, Zhenfu Wang, Xiaochun Chen, Mingwei Wang, Xinhua Wan, Huifang Shang, Yiming Liu, Pingyi Xu, Jian Wang, Tao Feng, Xianwen Chen, Xingyue Hu, Anmu Xie, and Qin Xiao. 2016. 'The recommendations of Chinese Parkinson's disease and movement disorder society consensus on therapeutic management of Parkinson's disease', *Translational Neurodegeneration*, 5: 12.

Chimenti, Paola, Anél Petzer, Simone Carradori, Melissa D'Ascenzio, Romano Silvestri, Stefano Alcaro, Francesco Ortuso, Jacobus P. Petzer, and Daniela Secci. 2013. 'Exploring 4-substituted-2-thiazolylhydrazones from 2-, 3-, and 4-acetylpyridine as selective and reversible hMAO-B inhibitors', *European Journal of Medicinal Chemistry*, 66: 221-27.

Chinta, Shankar J., and Julie K. Andersen. 2008. 'Redox imbalance in Parkinson's disease', *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1780: 1362-67.

Cohen, R. M., I. C. Campbell, Michelle Dau'phin, J. F. Tallman, and D. L. Murphy. 1982. 'Changes in α- and β-receptor densities in rat brain as a result of treatment with monoamine oxidase inhibiting antidepressants', *Neuropharmacology*, 21: 293-98.

Collier, Timothy J., Nicholas M. Kanaan, and Jeffrey H. Kordower. 2011. 'Ageing as a primary risk factor for Parkinson's disease: evidence from studies of non-human primates', *Nature reviews. Neuroscience*, 12: 359-66.

Cui, Shi-Shuang, Juan-Juan Du, Rao Fu, Yi-Qi Lin, Pei Huang, Ya-Chao He, Chao Gao, Hua-Long Wang, and Sheng-Di Chen. 2017. 'Prevalence and risk factors for depression and anxiety in Chinese patients with Parkinson disease', *BMC Geriatrics*, 17: 270.

Cummings, J. L. 1992. *Depression and Parkinson's Disease: A Review*.

Das, B., A. Kandegedara, L. Xu, T. Antonio, T. Stemmler, M. E. A. Reith, and A. K. Dutta. 2017. 'A Novel Iron(II) Preferring Dopamine Agonist Chelator as Potential Symptomatic and Neuroprotective Therapeutic Agent for Parkinson's Disease', *ACS Chem Neurosci*, 8: 723-30.

Das, B., G. Modi, and A. Dutta. 2015. 'Dopamine D3 agonists in the treatment of Parkinson's disease', *Curr Top Med Chem*, 15: 908-26.

Das, B., S. Rajagopalan, G. S. Joshi, L. Xu, D. Luo, J. K. Andersen, S. V. Todi, and A. K. Dutta. 2017. 'A novel iron (II) preferring dopamine agonist chelator D-607 significantly suppresses alpha-syn- and MPTP-induced toxicities in vivo', *Neuropharmacology*, 123: 88-99.

Das, B., S. Vedachalam, D. Luo, T. Antonio, M. E. Reith, and A. K. Dutta. 2015. 'Development of a Highly Potent D2/D3 Agonist and a Partial Agonist from Structure-Activity Relationship Study of N(6)-(2-(4-(1H-Indol-5-yl)piperazin-1-yl)ethyl)-N(6)-propyl-4,5,6,7-tetrahydroben zo[d]thiazole-2,6-diamine Analogues: Implication in the Treatment of Parkinson's Disease', *J Med Chem*, 58: 9179-95.

Dauer, W., and S. Przedborski. 2003. 'Parkinson's disease: mechanisms and models', *Neuron*, 39: 889-909.

De Jesús-Cortés, Héctor, Pin Xu, Jordan Drawbridge, Sandi Jo Estill, Paula Huntington, Stephanie Tran, Jeremiah Britt, Rachel Tesla, Lorraine Morlock, Jacinth Naidoo, Lisa M. Melito, Gelin Wang, Noelle S. Williams, Joseph M. Ready, Steven L. McKnight, and Andrew A. Pieper. 2012. 'Neuroprotective efficacy of aminopropyl carbazoles in a mouse model of Parkinson disease', *Proceedings of the National Academy of Sciences of the United States of America*, 109: 17010-15.

'Depression and Parkinson's disease: a review'. 1992. *American Journal of Psychiatry*, 149: 443-54.

Dézsi, Livia, and László Vécsei. 2014. 'Safinamide for the treatment of Parkinson's disease', *Expert Opinion on Investigational Drugs*, 23: 729-42.

Dias, Vera, Eunsung Junn, and M. Maral Mouradian. 2013. *The Role of Oxidative Stress in Parkinson's Disease*.

Dichter, Gabriel. 2010. *Anhedonia in Unipolar Major Depressive Disorder: A Review~!2009-10-26~!2010-02-18~!2010-04-08~!*

Dick, F. D., G. De Palma, A. Ahmadi, N. W. Scott, G. J. Prescott, J. Bennett, S. Semple, S. Dick, C. Counsell, P. Mozzoni, N. Haites, S. Bezzina Wettinger, A. Mutti, M. Otelea, A. Seaton, P. Söderkvist, and A. Felice. 2007. 'Environmental risk factors for Parkinson's disease and parkinsonism: the Geoparkinson study', *Occupational and Environmental Medicine*, 64: 666-72.

Dinesh, Dhingra, Joshi Parul, Gupta Arun, and Chhillar Ritu. 2012. 'Possible Involvement of Monoaminergic Neurotransmission in Antidepressant-like activity of Emblica officinalis Fruits in Mice', *CNS Neuroscience & Therapeutics*, 18: 419-25.

Drummond, Nicola J., Nick O. Davies, Janet E. Lovett, Mark R. Miller, Graeme Cook, Thomas Becker, Catherina G. Becker, Donald B. McPhail, and Tilo Kunath. 2017. 'A synthetic cell permeable antioxidant protects neurons against acute oxidative stress', *Scientific Reports*, 7: 11857.

Dushanova, Juliana. 2012. *Diagnostics, rehabilitation and models of Parkinson's disease*.

Dutta, A. K., X. S. Fei, and M. E. Reith. 2002. 'A novel series of hybrid compounds derived by combining 2-aminotetralin and piperazine fragments: binding activity at D2 and D3 receptors', *Bioorg Med Chem Lett*, 12: 619-22.

Dutta, A. K., S. Santra, H. Sharma, C. Voshavar, L. Xu, O. Mabrouk, T. Antonio, and M. E. Reith. 2014. 'Pharmacological and behavioral characterization of D-473, an orally active triple reuptake inhibitor targeting dopamine, serotonin and norepinephrine transporters', *PLoS One*, 9: e113420.

Dutta, Aloke K., Bhaskar Gopishetty, Sanjib Gogoi, Solav Ali, Juan Zhen, and Maarten Reith. 2011. 'The novel trisubstituted pyran derivative D-142 has triple monoamine reuptake inhibitory activity and exerts potent antidepressant-like activity in rodents', *European Journal of Pharmacology*, 671: 39-44.

E A Reith, Maarten, Solav Ali, Audrey Hashim, Imran Sheikh, Naresh Theddu, Narendra V Gaddiraju, Suneet Mehrotra, Kyle Schmitt, Thomas Murray, Henry Sershen, Ellen Unterwald, and Franklin Davis. 2012. *Novel C-1 Substituted Cocaine Analogs Unlike Cocaine or Benztropine*.

E. Burke, Robert, Patrizia Debetto, Alessandro Negro, Diego Guidolin, Stephen Skaper, and Pietro Giusti. 1999. *α-Synuclein and Parkinson's disease*.

Edmondson, D. E., and C. Binda. 2018. 'Monoamine Oxidases', *Subcell Biochem*, 87: 117-39.

Eisenhofer, Graeme, Irwin J. Kopin, and David S. Goldstein. 2004. 'Catecholamine Metabolism: A Contemporary View with Implications for Physiology and Medicine', *Pharmacological Reviews*, 56: 331-49.

Entzeroth, Michael, and Anil K. Ratty. 2017. 'Monoamine Oxidase Inhibitors°™Revisiting a Therapeutic Principle', *Open Journal of Depression*, Vol.06No.02: 39.

Exner, Nicole, Anne Kathrin Lutz, Christian Haass, and Konstanze F Winklhofer. 2012. 'Mitochondrial dysfunction in Parkinson's disease: molecular mechanisms and pathophysiological consequences', *The EMBO Journal*, 31: 3038-62.

Facecchia, Katie, Lee-Anne Fochesato, Sidhartha D. Ray, Sidney J. Stohs, and Siyaram Pandey. 2011. 'Oxidative Toxicity in Neurodegenerative Diseases: Role of Mitochondrial Dysfunction and Therapeutic Strategies', *Journal of Toxicology*, 2011: 12.

Fernandez-Espejo, Emilio. 2004. 'Pathogenesis of parkinson’s disease', *Molecular Neurobiology*, 29: 15-30.

Finberg, John P. M. 2014. 'Update on the pharmacology of selective inhibitors of MAO-A and MAO-B: Focus on modulation of CNS monoamine neurotransmitter release', *Pharmacology & Therapeutics*, 143: 133-52.

Foley, P., M. Gerlach, M. B. H. Youdim, and P. Riederer. 2000. 'MAO-B inhibitors: multiple roles in the therapy of neurodegenerative disorders?', *Parkinsonism Relat Disord*, 6: 25-47.

Garbis, Hanneke, and Patricia R. McElhatton. 2007. '2.11 - Psychotropic drugs.' in, *Drugs During Pregnancy and Lactation (Second Edition)* (Academic Press: Oxford).

Gartlehner G, Hansen RA, Morgan LC, et al. 'Second-Generation Antidepressants in the Pharmacologic Treatment of Adult Depression: An Update of the 2007 Comparative Effectiveness Review', *Comparative Effectiveness Reviews*, 46.

Gasparini, Fabrizio, Th Di Paolo, #xe9, #xe8, se, and Baltazar Gomez-Mancilla. 2013. 'Metabotropic Glutamate Receptors for Parkinson's Disease Therapy', *Parkinson's Disease*, 2013: 11.

Ghosh, B., T. Antonio, B. Gopishetty, M. Reith, and A. Dutta. 2010a. 'Further delineation of hydrophobic binding sites in dopamine D(2)/D(3) receptors for N-4 substituents on the piperazine ring of the hybrid template 5/7-{[2-(4-aryl-piperazin-1-yl)-ethyl]-propyl-amino}-5,6,7,8-tetrahydro-naphthale n-2-ol', *Bioorg Med Chem*, 18: 5661-74.

Ghosh, Balaram, Tamara Antonio, Bhaskar Gopishetty, Maarten Reith, and Aloke Dutta. 2010b. 'Further delineation of hydrophobic binding sites in dopamine D2/D3 receptors for N-4 substituents on the piperazine ring of the hybrid template 5/7-{[2-(4-aryl-piperazin-1-yl)-ethyl]-propyl-amino}-5,6,7,8-tetrahydro-naphthalen-2-ol', *Bioorganic & Medicinal Chemistry*, 18: 5661-74.

Gluszynska, A. 2015. 'Biological potential of carbazole derivatives', *Eur J Med Chem*, 94: 405-26.

Głuszyńska, Agata. 2015. 'Biological potential of carbazole derivatives', *European Journal of Medicinal Chemistry*, 94: 405-26.

Goetz, Christopher G. 2011. 'The History of Parkinson's Disease: Early Clinical Descriptions and Neurological Therapies', *Cold Spring Harb Perspect Med*, 1.

Gogoi, S., T. Antonio, S. Rajagopalan, M. Reith, J. Andersen, and A. K. Dutta. 2011. 'Dopamine D(2)/D(3) agonists with potent iron chelation, antioxidant and neuroprotective properties: potential implication in symptomatic and neuroprotective treatment of Parkinson's disease', *ChemMedChem*, 6: 991-5.

Goldenberg, Marvin M. 2008. 'Medical Management of Parkinson's Disease', *Pharmacy and Therapeutics*, 33: 590-606.

Goldman, Samuel M. 2014. 'Environmental Toxins and Parkinson's Disease', *Annual Review of Pharmacology and Toxicology*, 54: 141-64.

Gopishetty, B., S. Hazeldine, S. Santra, M. Johnson, G. Modi, S. Ali, J. Zhen, M. Reith, and A. Dutta. 2011a. 'Further structure-activity relationship studies on 4-((((3S,6S)-6-benzhydryltetrahydro-2H-pyran-3-yl)amino)methyl)phenol: identification of compounds with triple uptake inhibitory activity as potential antidepressant agents', *J Med Chem*, 54: 2924-32.

Gopishetty, Bhaskar, Stuart Hazeldine, Soumava Santra, Mark Johnson, Gyan Modi, Solav Ali, Juan Zhen, Maarten Reith, and Aloke Dutta. 2011b. *Further Structure−Activity Relationship Studies on 4-((((3 S ,6 S)-6-Benzhydryltetrahydro-2 H -pyran-3-yl)amino)methyl)phenol: Identification of Compounds with Triple Uptake Inhibitory Activity as Potential Antidepressant Agents*.

———. 2011c. 'Further Structure−Activity Relationship Studies on 4-((((3S,6S)-6-Benzhydryltetrahydro-2H-pyran-3-yl)amino)methyl)phenol: Identification of

Compounds with Triple Uptake Inhibitory Activity as Potential Antidepressant Agents', *Journal of Medicinal Chemistry*, 54: 2924-32.

group, Parikson's study. 1989. 'Datatop: A multicenter controlled clinical trial in early parkinson's disease: parkinson study group', *Archives of Neurology*, 46: 1052-60.

H, Youdim Moussa B, Gross Aviva, and Finberg John P M. 2001. 'Rasagiline [N-propargyl-1R(+)-aminoindan], a selective and potent inhibitor of mitochondrial monoamine oxidase B', *British Journal of Pharmacology*, 132: 500-06.

H, Youdim Moussa B, and Bakhle Y S. 2006. 'Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness', *British Journal of Pharmacology*, 147: S287-S96.

Haddad, Dominik, and Ken Nakamura. 2015. 'Understanding the susceptibility of dopamine neurons to mitochondrial stressors in Parkinson's disease', *FEBS letters*, 589: 3702-13.

Hague, S M, S Klaffke, and O Bandmann. 2005. 'Neurodegenerative disorders: Parkinson's disease and Huntington's disease', *Journal of Neurology, Neurosurgery & Psychiatry*, 76: 1058-63.

Hall, Edward D., Paula K. Andrus, Jo A. Oostveen, John S. Althaus, and Philip F. Von Voigtlander. 1996. 'Neuroprotective effects of the dopamine D2/D3 agonist pramipexole against postischemic or methamphetamine-induced degeneration of nigrostriatal neurons', *Brain Research*, 742: 80-88.

Hancock, Dana B., Eden R. Martin, Gregory M. Mayhew, Jeffrey M. Stajich, Rita Jewett, Mark A. Stacy, Burton L. Scott, Jeffery M. Vance, and William K. Scott. 2008.

'Pesticide exposure and risk of Parkinson's disease: A family-based case-control study', *BMC Neurology*, 8: 6-6.

Hansen, R. A., G. Gartlehner, K. N. Lohr, B. N. Gaynes, and T. S. Carey. 2005. 'EFficacy and safety of second-generation antidepressants in the treatment of major depressive disorder', *Annals of Internal Medicine*, 143: 415-26.

Happe, Kevin. 2007. 'Monoamine Oxidase Inhibitors.' in, *xPharm: The Comprehensive Pharmacology Reference* (Elsevier: New York).

Hassan, Mohammad Q., Sidney J. Stohs, and Wallace J. Murray. 1985. 'Effects of vitamins E and A on 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced lipid peroxidation and other biochemical changes in the rat', *Archives of Environmental Contamination and Toxicology*, 14: 437-42.

Hastings, Teresa G. 2009. 'The role of dopamine oxidation in mitochondrial dysfunction: implications for Parkinson's disease', *Journal of Bioenergetics and Biomembranes*, 41: 469-72.

Heman-Ackah, Sabrina, Martina Hallegger, Mahendra Rao, and Matthew Wood. 2013. 'RISC in PD: the impact of microRNAs in Parkinson's disease cellular and molecular pathogenesis', *Frontiers in Molecular Neuroscience*, 6.

Hemmerle, Ann M., James P. Herman, and Kim B. Seroogy. 2012. 'Stress, depression and Parkinson's disease', *Experimental Neurology*, 233: 79-86.

Hindle, John V. 2010. 'Ageing, neurodegeneration and Parkinson's disease', *Age and Ageing*, 39: 156-61.

Hisahara, Shin, and Shun Shimohama. 2011. 'Dopamine Receptors and Parkinson's Disease', *International Journal of Medicinal Chemistry*, 2011.

Hurwitz, B. 2014. 'Urban observation and sentiment in James Parkinson's Essay on the Shaking Palsy (1817)', *Lit Med*, 32: 74-104.

Hussain, Al-Shimali M., Waleed M. Renno, Hanaa L. Sadek, Noura M. Kayali, Aseel Al-Salem, Muddanna S. Rao, and Khalid M. Khan. 2018. 'Monoamine oxidase-B inhibitor protects degenerating spinal neurons, enhances nerve regeneration and functional recovery in sciatic nerve crush injury model', *Neuropharmacology*, 128: 231-43.

Hwang, Onyou. 2013. 'Role of Oxidative Stress in Parkinson's Disease', *Experimental Neurobiology*, 22: 11-17.

Irwin, David J., Virginia M. Y. Lee, and John Q. Trojanowski. 2013. 'Parkinson's disease dementia: convergence of α-synuclein, tau and amyloid-β pathologies', *Nature Reviews Neuroscience*, 14: 626.

J. Rojas, Rafael, Dale Edmondson, Terri Almos, Roderick Scott, and Mark E. Massari. 2015. *Reversible and irreversible small molecule inhibitors of monoamine oxidase B (MAO-B) investigated by biophysical techniques*.

J. Z Igmond, Michael, and Robert E. B Urke. 2018. *PATHOPHYSIOLOGY O F PARKINSON'S D ISEASE*.

Jaimes, Subhashini Bolisetty and Edgar A. 2013. 'Mitochondria and Reactive Oxygen Species: Physiology and Pathophysiology', *International Journal of Molecular Sciences*, 14: 6306-44.

Jankovic, J. 2007. 'Parkinson’s disease: clinical features and diagnosis', *Neurol Neurosurg Psychiatry*, ;79: 368–76.

Jankovic, Joseph, and L. Giselle Aguilar. 2008. 'Current approaches to the treatment of Parkinson's disease', *Neuropsychiatric Disease and Treatment*, 4: 743-57.

Jayaraj, Richard L., Kuppusamy Tamilselvam, Thamilarasan Manivasagam, and Namasivayam Elangovan. 2013. 'Neuroprotective Effect of CNB-001, a Novel Pyrazole Derivative of Curcumin on Biochemical and Apoptotic Markers Against Rotenone-Induced SK-N-SH Cellular Model of Parkinson's Disease', *Journal of Molecular Neuroscience*, 51: 863-70.

Jellinger, K. A. 2000. *Cell death mechanism in Parkinson's disease*.

Jenner, Peter. 2003. 'Oxidative stress in Parkinson's disease', *Annals of Neurology*, 53: S26-S38.

Jenner, Peter, and C. Warren Olanow. 2006. 'The pathogenesis of cell death in Parkinson's disease', *Neurology*, 66: S24-S36.

Jha, Saurabh Kumar, Niraj Kumar Jha, Dhiraj Kumar, Rashmi K. Ambasta, and Pravir Kumar. 2017. 'Linking mitochondrial dysfunction, metabolic syndrome and stress signaling in Neurodegeneration', *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1863: 1132-46.

Jinling Liu , Jiangchuan Dong , Lei Wang, Ying Su , Peng Yan, Shenggang Sun. 2013. 'Comparative Efficacy and Acceptability of Antidepressants in Parkinson's Disease: A Network Meta-Analysis', *journal.pone*, 8.

Johnson, M., T. Antonio, M. E. Reith, and A. K. Dutta. 2012. 'Structure-activity relationship study of N(6)-(2-(4-(1H-Indol-5-yl)piperazin-1-yl)ethyl)-N(6)-propyl-4,5,6,7-tetrahydroben zo[d]thiazole-2,6-diamine analogues: development of highly

selective D3 dopamine receptor agonists along with a highly potent D2/D3 agonist and their pharmacological characterization', *J Med Chem*, 55: 5826-40.

JP, Hughes, Rees S, Kalindjian SB, and Philpott KL. 2011. 'Principles of early drug discovery', *British Journal of Pharmacology*, 162: 1239-49.

Jr., James David Adams. 2012. 'Parkinson's Disease°™Apoptosis and Dopamine Oxidation', *Open Journal of Apoptosis*, Vol.01No.01: 8.

Kalia, Lorraine V., and Anthony E. Lang. 2015. 'Parkinson's disease', *The Lancet*, 386: 896-912.

Kim, Mounggon, and Jun Yeob Lee. 2013. 'Synthesis of 2- and 4-substituted carbazole derivatives and correlation of substitution position with photophysical properties and device performances of host materials', *Organic Electronics*, 14: 67-73.

Klein, Christine, and Ana Westenberger. 2012. 'Genetics of Parkinson's Disease', *Cold Spring Harb Perspect Med*, 2.

Larin, E. A., V. S. Kochubei, and Y. M. Atroshchenko. 2014. 'Regio- and stereoselective synthesis of new diaminocyclopentanols', *Beilstein J Org Chem*, 10: 2513-20.

Levant, Beth. 1997. 'The D3 Dopamine Receptor: Neurobiology and Potential Clinical Relevance', *Pharmacological Reviews*, 49: 231-52.

Li, C., S. Biswas, X. Li, A. K. Dutta, and W. Le. 2010. 'Novel D3 dopamine receptor-preferring agonist D-264: Evidence of neuroprotective property in Parkinson's disease animal models induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and lactacystin', *J Neurosci Res*, 88: 2513-23.

Liang, Y., and Elliott Richelson. 2008. *Triple reuptake inhibitors: Next-generation antidepressants*.

Lindenbach, D., B. Das, M. M. Conti, S. M. Meadows, A. K. Dutta, and C. Bishop. 2017. 'D-512, a novel dopamine D2/3 receptor agonist, demonstrates greater anti-Parkinsonian efficacy than ropinirole in Parkinsonian rats', *Br J Pharmacol*, 174: 3058-71.

Liu, Zewen, Tingyang Zhou, Alexander C. Ziegler, Peter Dimitrion, and Li Zuo. 2017. 'Oxidative Stress in Neurodegenerative Diseases: From Molecular Mechanisms to Clinical Applications', *Oxidative Medicine and Cellular Longevity*, 2017: 11.

Livia, Dezsi, and Vecsei Laszlo. 2017. 'Monoamine Oxidase B Inhibitors in Parkinson's Disease', *CNS & Neurological Disorders - Drug Targets*, 16: 425-39.

Löhle, Matthias, and Heinz Reichmann. 2011. 'Controversies in Neurology: why monoamine oxidase B inhibitors could be a good choice for the initial treatment of Parkinson's disease', *BMC Neurology*, 11: 112.

Lopez-Munoz, F., C. Alamo, G. Juckel, and H. J. Assion. 2007. 'Half a century of antidepressant drugs: on the clinical introduction of monoamine oxidase inhibitors, tricyclics, and tetracyclics. Part I: monoamine oxidase inhibitors', *J Clin Psychopharmacol*, 27: 555-9.

Loris, Z. B., A. A. Pieper, and W. D. Dietrich. 2017a. 'The neuroprotective compound P7C3-A20 promotes neurogenesis and improves cognitive function after ischemic stroke', *Exp Neurol*, 290: 63-73.

Loris, Zachary B., Andrew A. Pieper, and W. Dalton Dietrich. 2017b. 'The neuroprotective compound P7C3-A20 promotes neurogenesis and improves cognitive function after ischemic stroke', *Experimental Neurology*, 290: 63-73.

Luo, D., H. Sharma, D. Yedlapudi, T. Antonio, M. E. Reith, and A. K. Dutta. 2016. 'Novel multifunctional dopamine D2/D3 receptors agonists with potential neuroprotection and anti-alpha synuclein protein aggregation properties', *Bioorg Med Chem*, 24: 5088-102.

M Gardner, D., K. I Shulman, S. E Walker, and S. A. N. Tailor. 1996. *The making of a user friendly MAOI diet*.

M Goldenberg, Marvin. 2008. *Medical Management of Parkinson's Disease*.

MacMillan, K. S., J. Naidoo, J. Liang, L. Melito, N. S. Williams, L. Morlock, P. J. Huntington, S. J. Estill, J. Longgood, G. L. Becker, S. L. McKnight, A. A. Pieper, J. K. De Brabander, and J. M. Ready. 2011. 'Development of proneurogenic, neuroprotective small molecules', *J Am Chem Soc*, 133: 1428-37.

Malco, Rossi, Bruno Verónica, Arena Julieta, Cammarota Ángel, and Merello Marcelo. 'Challenges in PD Patient Management After DBS: A Pragmatic Review', *Movement Disorders Clinical Practice*, 0.

Marsh, Laura. 2013. 'Depression and Parkinson's Disease: Current Knowledge', *Current Neurology and Neuroscience Reports*, 13: 409.

Martins Branco, Diogo, Daniela Arduino, A. Raquel Esteves, Diana Silva, Sandra Cardoso, and Catarina Oliveira. 2010. 'Cross-talk between mitochondria and proteasome in Parkinson's disease pathogenesis', *Frontiers in Aging Neuroscience*, 2.

Massano, João, and Kailash P. Bhatia. 2012. 'Clinical Approach to Parkinson's Disease: Features, Diagnosis, and Principles of Management', *Cold Spring Harb Perspect Med*, 2: a008870.

Matsunaga, Shinji, Taro Kishi, and Nakao Iwata. 2015. 'Combination Therapy with Cholinesterase Inhibitors and Memantine for Alzheimer's Disease: A Systematic Review and Meta-Analysis', *International Journal of Neuropsychopharmacology*, 18: pyu115.

McCall, R. B., K. J. Lookingland, P. J. Bedard, and R. M. Huff. 2005. 'Sumanirole, a highly dopamine D2-selective receptor agonist: in vitro and in vivo pharmacological characterization and efficacy in animal models of Parkinson's disease', *J Pharmacol Exp Ther*, 314: 1248-56.

McDowell, F. H., and J. E. Lee. 1970. 'L-DOPA in Parkinson's Disease', *California Medicine*, 113: 44-46.

McKee, Ann C., Robert C. Cantu, Christopher J. Nowinski, E. Tessa Hedley-Whyte, Brandon E. Gavett, Andrew E. Budson, Veronica E. Santini, Hyo-Soon Lee, Caroline A. Kubilus, and Robert A. Stern. 2009. 'Chronic Traumatic Encephalopathy in Athletes: Progressive Tauopathy following Repetitive Head Injury', *Journal of neuropathology and experimental neurology*, 68: 709-35.

MD, Stacey L. Kowal MSc Timothy M. Dall MS Ritashree Chakrabarti PhD Michael V. Storm BS Anjali Jain. 2013. 'The current and projected economic burden of Parkinson's disease in the United States', *Movement Disorders*, 28.

Meiser, Johannes, Daniel Weindl, and Karsten Hiller. 2013. 'Complexity of dopamine metabolism', *Cell Communication and Signaling*, 11: 34.

Mercuri, Nicola Biagio, and Giorgio Bernardi. 2005. 'The 'magic' of l-dopa: why is it the gold standard Parkinson's disease therapy?', *Trends in Pharmacological Sciences*, 26: 341-44.

Michael-Titus, Adina, Patricia Revest, and Peter Shortland. 2010. '16 - DEPRESSION AND ANXIETY.' in, *The Nervous System (SECOND EDITION)* (Churchill Livingstone).

Michel, P. P., E. C. Hirsch, and S. Hunot. 2016. 'Understanding Dopaminergic Cell Death Pathways in Parkinson Disease', *Neuron*, 90: 675-91.

Modi, G., T. Antonio, M. Reith, and A. Dutta. 2014. 'Structural modifications of neuroprotective anti-Parkinsonian (-)-N6-(2-(4-(biphenyl-4-yl)piperazin-1-yl)-ethyl)-N6-propyl-4,5,6,7-tetrahydrobe nzo[d]thiazole-2,6-diamine (D-264): an effort toward the improvement of in vivo efficacy of the parent molecule', *J Med Chem*, 57: 1557-72.

Mongeau, R., P. Blier, and C. de Montigny. 1997. 'The serotonergic and noradrenergic systems of the hippocampus: their interactions and the effects of antidepressant treatments', *Brain Research Reviews*, 23: 145-95.

Moore, Darren J., Andrew B. West, Valina L. Dawson, and Ted M. Dawson. 2005. 'MOLECULAR PATHOPHYSIOLOGY OF PARKINSON'S DISEASE', *Annual Review of Neuroscience*, 28: 57-87.

Morphy, Richard, Corinne Kay, and Zoran Rankovic. 2004. 'From magic bullets to designed multiple ligands', *Drug Discovery Today*, 9: 641-51.

Morphy, Richard, and Zoran Rankovic. 2005. 'Designed Multiple Ligands. An Emerging Drug Discovery Paradigm', *Journal of Medicinal Chemistry*, 48: 6523-43.

Müller, Walter E., Anne Eckert, Christopher Kurz, Gunter Peter Eckert, and Kristina Leuner. 2010. 'Mitochondrial Dysfunction: Common Final Pathway in Brain Aging

and Alzheimer's Disease—Therapeutic Aspects', *Molecular Neurobiology*, 41: 159-71.

Murata, Miho. 2009. 'Levodopa in the early treatment of Parkinson's disease', *Parkinsonism Relat Disord*, 15: S17-S20.

Mythri, Rajeswara Babu, C. Venkateshappa, G. Harish, Anita Mahadevan, Uday B. Muthane, T. C. Yasha, M. M. Srinivas Bharath, and S. K. Shankar. 2011. 'Evaluation of Markers of Oxidative Stress, Antioxidant Function and Astrocytic Proliferation in the Striatum and Frontal Cortex of Parkinson's Disease Brains', *Neurochemical Research*, 36: 1452-63.

Nagal, Anubhav, and Rajeev K. Singla. 2012. *Parkinson's Disease: Diagnosis, Therapeutics & Management*.

Naoi, M., W. Maruyama, M. B. Youdim, P. Yu, and A. A. Boulton. 2003. 'Anti-apoptotic function of propargylamine inhibitors of type-B monoamine oxidase', *Inflammopharmacology*, 11: 175-81.

'Neurocognitive Disorders.' in., *Diagnostic and Statistical Manual of Mental Disorders*.

Nissinen, Erkki, and Pekka T. Männistö. 2010. 'Biochemistry and Pharmacology of Catechol-O-Methyltransferase Inhibitors.' in Erkki Nissinen (ed.), *International Review of Neurobiology* (Academic Press).

Noreddine, Benkerroum. 2016. 'Biogenic Amines in Dairy Products: Origin, Incidence, and Control Means', *Comprehensive Reviews in Food Science and Food Safety*, 15: 801-26.

Novaroli, L., M. Reist, E. Favre, A. Carotti, M. Catto, and P. A. Carrupt. 2005. 'Human recombinant monoamine oxidase B as reliable and efficient enzyme source for inhibitor screening', *Bioorg Med Chem*, 13: 6212-7.

Oestergaard, S., and C. Møldrup. 2011. 'Improving outcomes for patients with depression by enhancing antidepressant therapy with non-pharmacological interventions: A systematic review of reviews', *Public Health*, 125: 357-67.

Olanow, Tatton. 1999. 'ETIOLOGY AND PATHOGENESIS OF PARKINSON'S DISEASE', *Annual Review of Neuroscience*, 22: 123-44.

Oscar Bernal-Pacheco, Natlada Limotai, Criscely L. and Hubert H. Fernandez. 2012. 'Nonmotor Manifestations in Parkinson Disease', *The neurologist*, 18: 1-16.

Ossig, C., and H. Reichmann. 2013. 'Treatment of Parkinson's disease in the advanced stage', *Journal of Neural Transmission*, 120: 523-29.

Papp, M., V. Klimek, and P. Willner. 1994. 'Parallel changes in dopamine D2 receptor binding in limbic forebrain associated with chronic mild stress-induced anhedonia and its reversal by imipramine', *Psychopharmacology (Berl)*, 115: 441-6.

'Parkinson Disease: Neurologic Pathways & Drug Targets'. 2015. TUSOM | Pharmwiki.

Perier, Celine, and Miquel Vila. 2011. 'Mitochondrial Biology and Parkinson's Disease', *Cold Spring Harb Perspect Med*.

Pichler, Irene, Fabiola Del Greco M, Martin Gögele, Christina M. Lill, Lars Bertram, Chuong B. Do, Nicholas Eriksson, Tatiana Foroud, Richard H. Myers, Pd Gwas Consortium, Michael Nalls, Margaux F. Keller, Consortium International Parkinson's Disease Genomics, Consortium Wellcome Trust Case Control, Beben Benyamin, John B. Whitfield, Consortium Genetics of Iron Status, Peter P.

Pramstaller, Andrew A. Hicks, John R. Thompson, and Cosetta Minelli. 2013. 'Serum Iron Levels and the Risk of Parkinson Disease: A Mendelian Randomization Study', *PLOS Medicine*, 10: e1001462.

Pieper, A. A., S. Xie, E. Capota, S. J. Estill, J. Zhong, J. M. Long, G. L. Becker, P. Huntington, S. E. Goldman, C. H. Shen, M. Capota, J. K. Britt, T. Kotti, K. Ure, D. J. Brat, N. S. Williams, K. S. MacMillan, J. Naidoo, L. Melito, J. Hsieh, J. De Brabander, J. M. Ready, and S. L. McKnight. 2010. 'Discovery of a proneurogenic, neuroprotective chemical', *Cell*, 142: 39-51.

Poewe, W., K. Seppi, C. M. Tanner, G. M. Halliday, P. Brundin, J. Volkmann, A. E. Schrag, and A. E. Lang. 2017. 'Parkinson disease', *Nat Rev Dis Primers*, 3: 17013.

Pringsheim, Tamara, Nathalie Jette, Alexandra Frolkis, and Thomas D.L. Steeves. 2014. 'The prevalence of Parkinson's disease: A systematic review and meta-analysis', *Movement Disorders*, 29: 1583-90.

Prins, Louis H. A., Jacobus P. Petzer, and Sarel F. Malan. 2010. 'Inhibition of monoamine oxidase by indole and benzofuran derivatives', *European Journal of Medicinal Chemistry*, 45: 4458-66.

Przedborski, S. 2005. 'Pathogenesis of nigral cell death in Parkinson's disease', *Parkinsonism Relat Disord*, 11 Suppl 1: S3-7.

Rahal, Anu, Amit Kumar, Vivek Singh, Brijesh Yadav, Ruchi Tiwari, Sandip Chakraborty, and Kuldeep Dhama. 2014. 'Oxidative Stress, Prooxidants, and Antioxidants: The Interplay', *BioMed Research International*, 2014: 19.

Ratner, Marcia, and Robert G. Feldman. 2004. *Environmental Toxins and Parkinson's Disease*.

Rezende Figueira, Tiago, Mario Barros, Anamaria A Camargo, Roger F Castilho, Julio Ferreira, Alicia Kowaltowski, Francis E Sluse, Nadja Souza-Pinto, and Anibal Eugênio Vercesi. 2012. *Mitochondria as a Source of Reactive Oxygen and Nitrogen Species: From Molecular Mechanisms to Human Health*.

Riederer, P., and M. B. Youdim. 1986. 'Monoamine oxidase activity and monoamine metabolism in brains of parkinsonian patients treated with l-deprenyl', *J Neurochem*, 46: 1359-65.

Riederer, Peter, and Gerd Laux. 2011. *MAO-inhibitors in Parkinson's Disease*.

Rimessi, Alessandro, Maurizio Previati, Federica Nigro, Mariusz R. Wieckowski, and Paolo Pinton. 2016. 'Mitochondrial reactive oxygen species and inflammation: Molecular mechanisms, diseases and promising therapies', *The International Journal of Biochemistry & Cell Biology*, 81: 281-93.

Rinne, Juha O., Ismo Ulmanen, and Myung-Sik Lee. 2003. 'Catechol-O-Methyl Transferase (COMT) Inhibitors in Patients with Parkinson's Disease', *American Journal of Pharmacogenomics*, 3: 11-15.

Samluk, Lukasz, Piotr Chroscicki, and Agnieszka Chacinska. 2018. 'Mitochondrial protein import stress and signaling', *Current Opinion in Physiology*, 3: 41-48.

Santos, M. H. Silla. 1996. 'Biogenic amines: their importance in foods', *International Journal of Food Microbiology*, 29: 213-31.

Santra, S., S. Gogoi, B. Gopishetty, T. Antonio, J. Zhen, M. E. Reith, and A. K. Dutta. 2012. 'Structural exploration of (3S,6S)-6-benzhydryl-N-benzyltetrahydro-2H-pyran-3-amine analogues: identification of potent triple monoamine reuptake inhibitors as potential antidepressants', *ChemMedChem*, 7: 2093-100.

Santra, S., L. Xu, M. Shah, M. Johnson, and A. Dutta. 2013. 'D-512 and D-440 as novel multifunctional dopamine agonists: characterization of neuroprotection properties and evaluation of in vivo efficacy in a Parkinson's disease animal model', *ACS Chem Neurosci*, 4: 1382-92.

Santra, Soumava, Sanjib Gogoi, Bhaskar Gopishetty, Tamara Antonio, Juan Zhen, Maarten E A Reith, and Aloke Dutta. 2012. *Structural Exploration of (3 S ,6 S)-6-Benzhydryl- N -benzyltetrahydro-2 H -pyran-3-amine Analogues: Identification of Potent Triple Monoamine Reuptake Inhibitors as Potential Antidepressants*.

Sarkar, Sumit, James Raymick, and Syed Imam. 2016. 'Neuroprotective and Therapeutic Strategies against Parkinson's Disease: Recent Perspectives', *International Journal of Molecular Sciences*, 17: 904.

Sarker, Subhodeep, Rene Weissensteiner, Ilka Steiner, Harald H Sitte, Gerhard F Ecker, Michael Freissmuth, and Sonja Sucic. 2010. 'THE HIGH-AFFINITY BINDING SITE FOR TRICYCLIC ANTIDEPRESSANTS RESIDES IN THE OUTER VESTIBULE OF THE SEROTONIN TRANSPORTER', *Molecular Pharmacology*.

Saxena, Smita, and Pico Caroni. 2011. 'Selective Neuronal Vulnerability in Neurodegenerative Diseases: from Stressor Thresholds to Degeneration', *Neuron*, 71: 35-48.

Schafer, Freya Q., and Garry R. Buettner. 2001. 'Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple', *Free Radical Biology and Medicine*, 30: 1191-212.

Schapira, Anthony H. 2009a. 'Etiology and Pathogenesis of Parkinson Disease', *Neurologic Clinics*, 27: 583-603.

Schapira, Anthony H. V. 'Neurobiology and treatment of Parkinson's disease', *Trends in Pharmacological Sciences*, 30: 41-47.

———. 2009b. 'Neurobiology and treatment of Parkinson's disease', *Trends in Pharmacological Sciences*, 30: 41-47.

———. 2011. 'Monoamine Oxidase B Inhibitors for the Treatment of Parkinson's Disease', *CNS Drugs*, 25: 1061-71.

Shah, M., S. Rajagopalan, L. Xu, C. Voshavar, Y. Shurubor, F. Beal, J. K. Andersen, and A. K. Dutta. 2014. 'The high-affinity D2/D3 agonist D512 protects PC12 cells from 6-OHDA-induced apoptotic cell death and rescues dopaminergic neurons in the MPTP mouse model of Parkinson's disease', *J Neurochem*, 131: 74-85.

Shah, Mrudang, Subramanian Rajagopalan, Liping Xu, Chandrashekhar Voshavar, Yevgeniya Shurubor, Flint Beal, K. Andersen Julie, and K. Dutta Aloke. 2014. 'The high-affinity D2/D3 agonist D512 protects PC12 cells from 6-OHDA-induced apoptotic cell death and rescues dopaminergic neurons in the MPTP mouse model of Parkinson's disease', *Journal of Neurochemistry*, 131: 74-85.

Shahul Hameed, Ging-Yuek Robin Hsiung. 2011. 'The role of mitochondria in aging, neurodegenerative disease, and future therapeutic options', *BCMJ*, 53: 188-92.

Shankar J. chinta, Julie K. Andersen. 2008. 'Redox imbalance in Parkinson's disease', *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1780.

Sharma, Horrick, Soumava Santra, and Aloke Dutta. 2015. 'Triple reuptake inhibitors as potential next-generation antidepressants: a new hope?', *Future Medicinal Chemistry*, 7: 2385-406.

Sheikh, Saba, Safia, Ejazul Haque, and Snober Mir. 2012. *Neurodegenerative Diseases: Multifactorial Conformational Diseases and Their Therapeutic Interventions*.

Shoichet MS, Tate CC, Baumann MD, et al. . 2008. 'Strategies for Regeneration and Repair in the Injured Central Nervous System'.

Siegfried Kösel, Götz Hofhaus, Alexander Maassen, Peter Vieregge and Manuel B. Graeber. 1999. 'Role of Mitochondria in Parkinson Disease', *Biol. Chem.*, 380: 865 – 70.

Skalisz, L. L., V. Beijamini, S. L. Joca, M. A. Vital, C. Da Cunha, and R. Andreatini. 2002. 'Evaluation of the face validity of reserpine administration as an animal model of depression--Parkinson's disease association', *Prog Neuropsychopharmacol Biol Psychiatry*, 26: 879-83.

Slattery, D. A., and J. F. Cryan. 2012. 'Using the rat forced swim test to assess antidepressant-like activity in rodents', *Nat Protoc*, 7: 1009-14.

Soto, Claudio. 2003. 'Unfolding the role of protein misfolding in neurodegenerative diseases', *Nature Reviews Neuroscience*, 4: 49.

Soto-Otero, R., E. Mendez-Alvarez, A. Hermida-Ameijeiras, A. M. Munoz-Patino, and J. L. Labandeira-Garcia. 2000. 'Autoxidation and neurotoxicity of 6-hydroxydopamine in the presence of some antioxidants: potential implication in relation to the pathogenesis of Parkinson's disease', *J Neurochem*, 74: 1605-12.

Soumava, Santra, Gogoi Sanjib, Gopishetty Bhaskar, Antonio Tamara, Zhen Juan, Reith Maarten E. A., and Dutta Aloke K. 2012. 'Structural Exploration of (3S,6S)-6-Benzhydryl-N-benzyltetrahydro-2H-pyran-3-amine Analogues: Identification of

Potent Triple Monoamine Reuptake Inhibitors as Potential Antidepressants', *ChemMedChem*, 7: 2093-100.

Spinelli, Kateri J., Jonathan K. Taylor, Valerie R. Osterberg, Madeline J. Churchill, Eden Pollock, Cynthia Moore, Charles K. Meshul, and Vivek K. Unni. 2014. 'Presynaptic Alpha-Synuclein Aggregation in a Mouse Model of Parkinson's Disease', *The Journal of Neuroscience*, 34: 2037-50.

Stahl, Stephen M., James F. Pradko, Barbara R. Haight, Jack G. Modell, Carol B. Rockett, and Susan Learned-Coughlin. 2004. 'A Review of the Neuropharmacology of Bupropion, a Dual Norepinephrine and Dopamine Reuptake Inhibitor', *Primary Care Companion to The Journal of Clinical Psychiatry*, 6: 159-66.

Stanley, Fahn, and Cohen Gerald. 1992. 'The oxidant stress hypothesis in Parkinson's disease: Evidence supporting it', *Annals of Neurology*, 32: 804-12.

Strydom, Belinda, Sarel F. Malan, Neal Castagnoli, Jacobus J. Bergh, and Jacobus P. Petzer. 2010. 'Inhibition of monoamine oxidase by 8-benzyloxycaffeine analogues', *Bioorganic & Medicinal Chemistry*, 18: 1018-28.

Sturino, Claudio F., and Jerome C. Y. Wong. 1998. 'The ring-closing metathesis of vinyl ethers with Grubbs' catalyst for the synthesis of dihydropyrans', *Tetrahedron Letters*, 39: 9623-26.

Sulzer, David. 2007. 'Multiple hit hypotheses for dopamine neuron loss in Parkinson's disease', *Trends in Neurosciences*, 30: 244-50.

Takalo, Mari, Antero Salminen, Hilkka Soininen, Mikko Hiltunen, and Annakaisa Haapasalo. 2013. 'Protein aggregation and degradation mechanisms in

neurodegenerative diseases', *American Journal of Neurodegenerative Disease*, 2: 1-14.

Tanzi, Lars Bertram and Rudolph E. 2005. 'The genetic epidemiology of neurodegenerative disease', *The Juournal of Clinical Investigation*, 115: 1449 - 57.

Taylor, Chirisse, Ashwana D. Fricker, Lakshmi A. Devi, and Ivone Gomes. 2005. 'Mechanisms of action of antidepressants: from neurotransmitter systems to signaling pathways', *Cellular signalling*, 17: 549-57.

Teixeira, Fábio G., Miguel F. Gago, Paulo Marques, Pedro Silva Moreira, Ricardo Magalhães, Nuno Sousa, and António J. Salgado. 2018. 'Safinamide: a new hope for Parkinson's disease?', *Drug Discovery Today*, 23: 736-44.

Teo, Kay Cheong, and Shu-Leong Ho. 2013. 'Monoamine oxidase-B (MAO-B) inhibitors: implications for disease-modification in Parkinson's disease', *Translational Neurodegeneration*, 2: 19.

Titova, Nataliya, and K. Ray Chaudhuri. 2017. 'Chapter Forty-Five - Personalized Medicine and Nonmotor Symptoms in Parkinson's Disease.' in K. Ray Chaudhuri and Nataliya Titova (eds.), *International Review of Neurobiology* (Academic Press).

Turrens, Julio F. 2003. 'Mitochondrial formation of reactive oxygen species', *The Journal of Physiology*, 552: 335-44.

Van der Schyf, C. J. 2011. 'The use of multi-target drugs in the treatment of neurodegenerative diseases', *Expert Rev Clin Pharmacol*, 4: 293-8.

Van der Schyf, Cornelis, Werner Geldenhuys, and Moussa Youdim. 2006. *Multifunctional drugs with different CNS targets for neuropsychiatric disorders*.

Van der Schyf, Cornelis J., and Werner J. Geldenhuys. 2011. 'Multimodal drugs and their future for Alzheimer's and Parkinson's disease.' in Moussa B. H. Youdim and Peter Douce (eds.), *International Review of Neurobiology* (Academic Press).

Vila, Miquel, David Ramonet, and Celine Perier. 2008. 'Mitochondrial alterations in Parkinson's disease: new clues', *Journal of Neurochemistry*, 107: 317-28.

Visanji, Naomi, and Connie Marras. 2015. 'The relevance of pre-motor symptoms in Parkinson's disease', *Expert Review of Neurotherapeutics*, 15: 1205-17.

Visanji, Naomi P., Patricia L. Brooks, Lili-Naz Hazrati, and Anthony E. Lang. 2013. 'The prion hypothesis in Parkinson's disease: Braak to the future', *Acta Neuropathologica Communications*, 1: 2.

W., Olanow C. 1992. 'An introduction to the free radical hypothesis in Parkinson's disease', *Annals of Neurology*, 32: S2-S9.

Wang, Gelin, Ting Han, Deepak Nijhawan, Pano Theodoropoulos, Jacinth Naidoo, Sivaramakrishnan Yadavalli, Hamid Mirzaei, Andrew A Pieper, Joseph M Ready, and Steven L McKnight. 2014. 'P7C3 Neuroprotective Chemicals Function by Activating the Rate-Limiting Enzyme in NAD Salvage', *Cell*, 158: 1324-34.

Wang, S. N., T. Y. Xu, X. Wang, Y. F. Guan, S. L. Zhang, P. Wang, and C. Y. Miao. 2016. 'Neuroprotective Efficacy of an Aminopropyl Carbazole Derivative P7C3-A20 in Ischemic Stroke', *CNS Neurosci Ther*, 22: 782-8.

Warner, Thomas T., and Anthony H. V. Schapira. 2003. 'Genetic and environmental factors in the cause of Parkinson's disease', *Annals of Neurology*, 53: S16-S25.

Willis, Gregory L., and Reuven Sandyk. 1992. 'Sensitivity of Dopamine Receptors in the Lateral Hypothalamus is Altered in 6-Hydroxydopamine Treated Rats', *International Journal of Neuroscience*, 65: 199-207.

Wim Mandemakers, Vanessa A. Morais and Bart De Strooper. 2007. 'A cell biological perspective on mitochondrial dysfunction in Parkinson disease and other neurodegenerative diseases', *Journal of Cell Science*, 120: 1707- 16.

Wimalasena, Kandatege. 2011. 'Vesicular Monoamine Transporters: Structure-Function, Pharmacology, and Medicinal Chemistry', *Medicinal research reviews*, 31: 483-519.

Wu, X., J. Kosaraju, W. Zhou, and K. Y. Tam. 2017. 'Neuroprotective Effect of SLM, a Novel Carbazole-Based Fluorophore, on SH-SY5Y Cell Model and 3xTg-AD Mouse Model of Alzheimer's Disease', *ACS Chem Neurosci*, 8: 676-85.

Xu, Shengli, and Piu Chan. 2015. 'Interaction between Neuromelanin and Alpha-Synuclein in Parkinson's Disease', *Biomolecules*, 5: 1122.

Yedlapudi, D., G. S. Joshi, D. Luo, S. V. Todi, and A. K. Dutta. 2016. 'Inhibition of alpha-synuclein aggregation by multifunctional dopamine agonists assessed by a novel in vitro assay and an in vivo Drosophila synucleinopathy model', *Sci Rep*, 6: 38510.

Yin, T. C., J. K. Britt, H. De Jesus-Cortes, Y. Lu, R. M. Genova, M. Z. Khan, J. R. Voorhees, J. Shao, A. C. Katzman, P. J. Huntington, C. Wassink, L. McDaniel, E. A. Newell, L. M. Dutca, J. Naidoo, H. Cui, A. G. Bassuk, M. M. Harper, S. L. McKnight, J. M. Ready, and A. A. Pieper. 2014. 'P7C3 neuroprotective chemicals block axonal degeneration and preserve function after traumatic brain injury', *Cell Rep*, 8: 1731-40.

Yoon, H. J., S. Y. Kong, M. H. Park, Y. Cho, S. E. Kim, J. Y. Shin, S. Jung, J. Lee, Farhanullah, H. J. Kim, and J. Lee. 2013. 'Aminopropyl carbazole analogues as potent enhancers of neurogenesis', *Bioorg Med Chem*, 21: 7165-74.

Youdim, Moussa B. H. 2010. 'Why do we need multifunctional neuroprotective and neurorestorative drugs for Parkinson's and Alzheimer's diseases as disease modifying agents', *Experimental Neurobiology*, 19: 1-14.

Youdim, Moussa B. H., and Jerry J. Buccafusco. 2005. 'Multi-functional drugs for various CNS targets in the treatment of neurodegenerative disorders', *Trends in Pharmacological Sciences*, 26: 27-35.

Zhang, Li, Valina L. Dawson, and Ted M. Dawson. 2006. 'Role of nitric oxide in Parkinson's disease', *Pharmacology & Therapeutics*, 109: 33-41.

Zhang, Pei-Lan, Yan Chen, Chen-Hao Zhang, Yu-Xin Wang, and Pedro Fernandez-Funez. 2018. 'Genetics of Parkinson’s disease and related disorders', *Journal of Medical Genetics*, 55: 73-80.

Zhang, S., F. Fernandez, S. Hazeldine, J. Deschamps, J. Zhen, M. E. Reith, and A. K. Dutta. 2006. 'Further structural exploration of trisubstituted asymmetric pyran derivatives (2S,4R,5R)-2-benzhydryl-5-benzylamino-tetrahydropyran-4-ol and their corresponding disubstituted (3S,6S) pyran derivatives: a proposed pharmacophore model for high-affinity interaction with the dopamine, serotonin, and norepinephrine transporters', *J Med Chem*, 49: 4239-47.

Zhang, S., J. Zhen, M. E. Reith, and A. K. Dutta. 2005a. 'Discovery of novel trisubstituted asymmetric derivatives of (2S,4R,5R)-2-benzhydryl-5-benzylaminotetrahydropyran-4-ol, exhibiting high affinity for serotonin and

norepinephrine transporters in a stereospecific manner', *J Med Chem*, 48: 4962-71.

Zhang, Shijun, Juan Zhen, Maarten E. A. Reith, and Aloke K. Dutta. 2005b. 'Discovery of Novel Trisubstituted Asymmetric Derivatives of (2S,4R,5R)-2-benzhydryl-5-benzylaminotetrahydropyran-4-ol, Exhibiting High Affinity for Serotonin and Norepinephrine Transporters in a Stereospecific Manner', *Journal of Medicinal Chemistry*, 48: 4962-71.

Zhen, Juan, Tamara Antonio, Aloke K. Dutta, and Maarten E. A. Reith. 2010. 'Concentration of receptor and ligand revisited in a modified receptor binding protocol for high-affinity radioligands: [3H]Spiperone binding to D2 and D3 dopamine receptors', *Journal of Neuroscience Methods*, 188: 32-38.

Zhengyin, Yan, Caldwell Gary W., Zhao Boyu, and Reitz Allen B. 2004. 'A high-throughput monoamine oxidase inhibition assay using liquid chromatography with tandem mass spectrometry', *Rapid Communications in Mass Spectrometry*, 18: 834-40.

Zhou, Chun, Yong Huang, and Serge Przedborski. 2008. 'Oxidative Stress in Parkinson's Disease: A Mechanism of Pathogenic and Therapeutic Significance', *Annals of the New York Academy of Sciences*, 1147: 93-104.

Zhu, Kangdi, Jacobus J. van Hilten, and Johan Marinus. 2016. 'Associated and predictive factors of depressive symptoms in patients with Parkinson's disease', *J Neurol*, 263: 1215-25.

Alexander, G. E. 2004. 'Biology of Parkinson's disease: pathogenesis and pathophysiology of a multisystem neurodegenerative disorder', *Dialogues Clin Neurosci*, 6: 259-80.

Biswas, S., S. Hazeldine, B. Ghosh, I. Parrington, E. Kuzhikandathil, M. E. Reith, and A. K. Dutta. 2008. 'Bioisosteric heterocyclic versions of 7-{[2-(4-phenyl-piperazin-1-yl)ethyl]propylamino}-5,6,7,8-tetrahydronaphthalen-2- ol: identification of highly potent and selective agonists for dopamine D3 receptor with potent in vivo activity', *J Med Chem*, 51: 3005-19.

Cavalli, A., M. L. Bolognesi, A. Minarini, M. Rosini, V. Tumiatti, M. Recanatini, and C. Melchiorre. 2008. 'Multi-target-directed ligands to combat neurodegenerative diseases', *J Med Chem*, 51: 347-72.

Cenci, M. A., K. E. Ohlin, and P. Odin. 2011. 'Current options and future possibilities for the treatment of dyskinesia and motor fluctuations in Parkinson's disease', *CNS Neurol Disord Drug Targets*, 10: 670-84.

Das, B., A. Kandegedara, L. Xu, T. Antonio, T. Stemmler, M. E. A. Reith, and A. K. Dutta. 2017. 'A Novel Iron(II) Preferring Dopamine Agonist Chelator as Potential Symptomatic and Neuroprotective Therapeutic Agent for Parkinson's Disease', *ACS Chem Neurosci*, 8: 723-30.

Das, B., G. Modi, and A. Dutta. 2015. 'Dopamine D3 agonists in the treatment of Parkinson's disease', *Curr Top Med Chem*, 15: 908-26.

Das, B., S. Rajagopalan, G. S. Joshi, L. Xu, D. Luo, J. K. Andersen, S. V. Todi, and A. K. Dutta. 2017. 'A novel iron (II) preferring dopamine agonist chelator D-607 significantly suppresses alpha-syn- and MPTP-induced toxicities in vivo', *Neuropharmacology*, 123: 88-99.

Das, B., S. Vedachalam, D. Luo, T. Antonio, M. E. Reith, and A. K. Dutta. 2015. 'Development of a Highly Potent D2/D3 Agonist and a Partial Agonist from

Structure-Activity Relationship Study of N(6)-(2-(4-(1H-Indol-5-yl)piperazin-1-yl)ethyl)-N(6)-propyl-4,5,6,7-tetrahydroben zo[d]thiazole-2,6-diamine Analogues: Implication in the Treatment of Parkinson's Disease', *J Med Chem*, 58: 9179-95.

Gluszynska, A. 2015. 'Biological potential of carbazole derivatives', *Eur J Med Chem*, 94: 405-26.

Hearn, M. G., Y. Ren, E. W. McBride, I. Reveillaud, M. Beinborn, and A. S. Kopin. 2002. 'A Drosophila dopamine 2-like receptor: Molecular characterization and identification of multiple alternatively spliced variants', *Proc Natl Acad Sci U S A*, 99: 14554-9.

Johnson, M., T. Antonio, M. E. Reith, and A. K. Dutta. 2012. 'Structure-activity relationship study of N(6)-(2-(4-(1H-Indol-5-yl)piperazin-1-yl)ethyl)-N(6)-propyl-4,5,6,7-tetrahydroben zo[d]thiazole-2,6-diamine analogues: development of highly selective D3 dopamine receptor agonists along with a highly potent D2/D3 agonist and their pharmacological characterization', *J Med Chem*, 55: 5826-40.

Klein, C., and A. Westenberger. 2012. 'Genetics of Parkinson's disease', *Cold Spring Harb Perspect Med*, 2: a008888.

Loris, Z. B., A. A. Pieper, and W. D. Dietrich. 2017. 'The neuroprotective compound P7C3-A20 promotes neurogenesis and improves cognitive function after ischemic stroke', *Exp Neurol*, 290: 63-73.

Luo, D., H. Sharma, D. Yedlapudi, T. Antonio, M. E. Reith, and A. K. Dutta. 2016. 'Novel multifunctional dopamine D2/D3 receptors agonists with potential neuroprotection and anti-alpha synuclein protein aggregation properties', *Bioorg Med Chem*, 24: 5088-102.

MacMillan, K. S., J. Naidoo, J. Liang, L. Melito, N. S. Williams, L. Morlock, P. J. Huntington, S. J. Estill, J. Longgood, G. L. Becker, S. L. McKnight, A. A. Pieper, J. K. De Brabander, and J. M. Ready. 2011. 'Development of proneurogenic, neuroprotective small molecules', *J Am Chem Soc*, 133: 1428-37.

Mandel, S., E. Grunblatt, P. Riederer, M. Gerlach, Y. Levites, and M. B. Youdim. 2003. 'Neuroprotective strategies in Parkinson's disease : an update on progress', *CNS Drugs*, 17: 729-62.

Modi, G., T. Antonio, M. Reith, and A. Dutta. 2014. 'Structural modifications of neuroprotective anti-Parkinsonian (-)-N6-(2-(4-(biphenyl-4-yl)piperazin-1-yl)-ethyl)-N6-propyl-4,5,6,7-tetrahydrobe nzo[d]thiazole-2,6-diamine (D-264): an effort toward the improvement of in vivo efficacy of the parent molecule', *J Med Chem*, 57: 1557-72.

Pieper, A. A., S. Xie, E. Capota, S. J. Estill, J. Zhong, J. M. Long, G. L. Becker, P. Huntington, S. E. Goldman, C. H. Shen, M. Capota, J. K. Britt, T. Kotti, K. Ure, D. J. Brat, N. S. Williams, K. S. MacMillan, J. Naidoo, L. Melito, J. Hsieh, J. De Brabander, J. M. Ready, and S. L. McKnight. 2010. 'Discovery of a proneurogenic, neuroprotective chemical', *Cell*, 142: 39-51.

Poewe, W., K. Seppi, C. M. Tanner, G. M. Halliday, P. Brundin, J. Volkmann, A. E. Schrag, and A. E. Lang. 2017. 'Parkinson disease', *Nat Rev Dis Primers*, 3: 17013.

Przedborski, S. 2017. 'The two-century journey of Parkinson disease research', *Nat Rev Neurosci*, 18: 251-59.

Salat, D., and E. Tolosa. 2013. 'Levodopa in the treatment of Parkinson's disease: current status and new developments', *J Parkinsons Dis*, 3: 255-69.

Schapira, A. H., and C. W. Olanow. 2004. 'Neuroprotection in Parkinson disease: mysteries, myths, and misconceptions', *Jama*, 291: 358-64.

Shah, M., S. Rajagopalan, L. Xu, C. Voshavar, Y. Shurubor, F. Beal, J. K. Andersen, and A. K. Dutta. 2014. 'The high-affinity D2/D3 agonist D512 protects PC12 cells from 6-OHDA-induced apoptotic cell death and rescues dopaminergic neurons in the MPTP mouse model of Parkinson's disease', *J Neurochem*, 131: 74-85.

Surmeier, D. J., J. A. Obeso, and G. M. Halliday. 2017a. 'Selective neuronal vulnerability in Parkinson disease', *Nat Rev Neurosci*, 18: 101-13.

Surmeier, D. James, José A. Obeso, and Glenda M. Halliday. 2017b. 'Selective neuronal vulnerability in Parkinson disease', *Nature Reviews Neuroscience*, 18: 101.

Wang, S. N., T. Y. Xu, X. Wang, Y. F. Guan, S. L. Zhang, P. Wang, and C. Y. Miao. 2016. 'Neuroprotective Efficacy of an Aminopropyl Carbazole Derivative P7C3-A20 in Ischemic Stroke', *CNS Neurosci Ther*, 22: 782-8.

Wu, X., J. Kosaraju, W. Zhou, and K. Y. Tam. 2017. 'Neuroprotective Effect of SLM, a Novel Carbazole-Based Fluorophore, on SH-SY5Y Cell Model and 3xTg-AD Mouse Model of Alzheimer's Disease', *ACS Chem Neurosci*, 8: 676-85.

Yoon, H. J., S. Y. Kong, M. H. Park, Y. Cho, S. E. Kim, J. Y. Shin, S. Jung, J. Lee, Farhanullah, H. J. Kim, and J. Lee. 2013. 'Aminopropyl carbazole analogues as potent enhancers of neurogenesis', *Bioorg Med Chem*, 21: 7165-74.

Youdim, M. B., and Y. J. Oh. 2013. 'Promise of neurorestoration and mitochondrial biogenesis in Parkinson's disease with multi target drugs: an alternative to stem cell therapy', *Exp Neurobiol*, 22: 167-72.

ABSTRACT

CARBAZOLE BASED MULTIFUNCTIONAL DOPAMINE AGONISTS AND RELATED MOLECULES AS POTENTIAL SYMPTOMATIC AND DISEASE MODIFYING THERAPEUTIC AGENTS FOR PARKINSON'S

by

ASMA ELMABRUK

August 2018

Advisor: Dr. Aloke Dutta

Major: Pharmaceutical Sciences

Degree: Doctor of Philosophy

Parkinson's disease (PD) is a progressive neurodegenerative disease that develops from gradual depletion of dopamine (DA) and dopaminergic neurons in the substantia nigra pars compacta (SNc) with the accumulation of intraneuronal proteinaceous matter named as Lewy bodies. The four cardinal symptoms associated with PD are tremor, rigidity, bradykinesia, and postural instability. Although the exact mechanism and etiology of PD are not fully understood, several factors have been implicated in the pathogenesis and progression of PD including protein aggregation, oxidative stress, mitochondrial dysfunction, environmental, and genetic factors.

The current therapy of Parkinson's disease is categorized into four classes: levodopa, DA agonists, monoamine oxidase inhibitors (MAO-Is), catechol-o-methyl transferase inhibitors (COMT-Is) and Dopamine agonist (DA). Even though these medications are available to treat PD, they only reduce the symptoms and do not slow or stop the disease progression; in addition to developing the severe side effects such as dyskinesia and motor fluctuation with long-term therapy. To overcome the concerns associated with

current PD medications, a new strategy has been adopted by developing multifunctional molecules to target multiple factors implicated in the pathogenesis of the disease that could be beneficial to treat the patients.

The hypothesis of this dissertation is to develop novel multifunctional dopamine D_2/D_3 agonist molecules with neuroprotective, antioxidants properties to modulate the pathogenic pathway while addressing the symptomatic deficits. Specifically, our hybrid structure strategy which combines D_2/D_3 agonist head groups to the other moieties that are suitable to modulate the pathogenic pathway of PD, led to development of molecules to validate our proof of concept.

In this project, the structure activity relationship (SAR) study was carried out based on our hybrid structure strategy template that was previously established. Three main objectives were set forward in this project: the first is to design and develop multifunctional molecules by covalently attaching D_2/D_3 agonist head groups such as pramipexole and 5-OH-DPAT to various carbazole moieties through a piperazine linker. The lead molecules (-)-**11b**, (-)-**15a** and (-)-**15c** exhibited high affinity for both D_2 and D_3 receptors whereas in GTPγS functional assay, the compounds showed potent agonist activity at both D_2 and D_3 receptors (EC_{50} (GTPγS); D_2 = 48.7 nM, D_3 = 0.96 nM for **11b**, D_2 = 0.87 nM, D_3 = 0.23 nM for **15a** and D_2 = 2.29 nM, D_3 = 0.22 nM for **15c**). In PD animal model study, the test compounds exhibited potent *in vivo* activity by reversing hypolocomotion in reserpinized rats with a long duration of action compared to the reference drug. In a cellular antioxidant assay, compounds **(-)-11b**, **(-)-15a** and **(-)-15c** exhibited potent activity in reducing oxidative stress induced by neurotoxin 6-hydroxydopamine (6-OHDA). Also, in a cell-based PD neuroprotection model, these lead compounds significantly increased

cell survival from toxicity of 6-OHDA, thereby, producing neuroprotection effect. These observations suggest that the lead carbazole-based dopamine agonists are promising multifunctional molecules for a viable symptomatic and disease modifying therapy of PD and should be further investigated. The second objective is to combine D_2/D_3 agonist head groups with monoamine oxidase inhibiton property. Based on the results from in vitro receptor assays and enzymatic inhibition assay of the generated compounds led to the identification of compounds **(−)-33 (D-671)** and **(±)-42 (D-678)** as the lead compounds that demand further modification. The third main objective is to develop novel multifunctional triple reuptake inhibitor based on the modification of the pyran template that was previously established by us to treat the motor, non-motor symptoms like depression associated with PD. The designed compounds were evaluated for their binding affinities for the DAT, SERT, NET in the brain tissue. Based on the results of the affinity data of the initial compounds for the **DAT, SERT, NET**, cis-isomer compound 60a **(D-620)** exhibited high affinity for both **DAT** and **NET** that could be considered as a dual inhibitor for the monoamine reuptake transporters. According to this finding **60a (D-621)** was identified as the lead compound that requires further modification.

AUTOBIOGRAPHICAL STATEMENT

Asma Elmabruk

Education

Bachelor of Pharmacy (1st Pharmacy degree in Libya). 2002
Garyounis University, School of Pharmacy
Graduate Non-degree certificate 2011

Awards and Honors

Wayne State University
Thomas C. Rumble University Graduate Fellowship 2018
Graduate Research Assistantship 2015-2017
Garyounis University, School of Pharmacy
Libyan of Higher Education, international Scholarship 2009-2015
B.S. award with high honor 1996-2002

Publication

Elmabruk A., Das B., Yedlapudi D., Xu L.,Antonio A., Reith M., Dutta A. Design, Synthesis and Pharmacological Characterization of Carbazole Based Dopamine Agonists as Potential Symptomatic and Neuroprotective Therapeutic Agents for Parkinson's Disease. Under review.

License and Certification

Pharmacy Teaching Certificate 2018
Medical Therapy Management Certificate 2018
Pharmacy -Based immunization Delivery Certificate of Achievement 2018
FPGEE Pharmacy Certificate 2017
Libyan Pharmacy Practice License 2003-present
Professional Arabic and Religion Teaching Certificate. 2003