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2,4-Disubstituted Quinazolines with Antileishmanial or Antibacterial Activity

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2,4-Disubstituted Quinazolines with Antileishmanial or Antibacterial Activity

by

Megan M. Barber

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science
Department of Chemistry
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relationship

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Dedication

I would like to dedicate this thesis to my family. To my parents Joseph and Margaret Barber and my Step-Father Cecil Jones, I have truly learned what hard work, dedication, and sacrifice can do for ones future from each of them. I cannot thank you enough for the help and continued support through my years of schooling. To my sisters Ashlee, Kathleen and Shannon, your unconditional love, along with the emotional and mental support through my graduate career has allowed me to be where I am today. I would lastly like to dedicate this thesis to my significant other Chris Williams. Along with all of the above mentioned you have put up with me through it all. Thank you for understanding and always being there for me no matter what.

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List of Abbreviations

AUC area under the curve
CDC Centers for Disease Control and Prevention
DMSO dimethyl sulfoxide
EC₅₀ half maximal effective concentration
ESI electrospray ionization
ESKAPE Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species
HAI hospital acquired infection
HPLC high pressure liquid chromatography
HRMS high resolution mass spectrometry
i.p. intraperitoneal
IC₅₀ half maximal inhibitory concentration
LC-MS liquid chromatography-mass spectrometry
MeOH methanol
MIC minimum inhibitory concentration
NMR nuclear magnetic resonance
p.o. oral administration (per os)
PK pharmacokinetics
RT room temperature
SAR structure activity relationship
SI selectivity index
TLC thin layer chromatography
USF University of South Florida
WHO World Health Organization
ZOI zone of inhibition
s singlet
d doublet
t triplet
q quartet
br broad
kg kilogram
mg milligram
M moles/liter

Abstract

Herein 47 2,4-disubstituted quinazolines were synthesized and tested against *Leishmania donovani* intracellular amastigotes. A structure-activity relationship was conducted and led to the identification of quinazolines with EC₅₀s in the single digit and high nanomolar range with favorable antileishmanial selectivity indexes. Quinazoline **2.6** and **2.31** underwent *in vivo* efficacy studies in murine models of visceral leishmaniasis, reducing liver parasitemia by 12 % and 24 %, respectively, when given by the intraperitoneal route at 15 mg/kg/day x 5 days. The antileishmanial efficacy and easy of synthesis make the 2,4-disubstituted quinazoline compound series a suitable platform for the future development of antileishmanial agents.

A similar series of 50 *N*²,*N*⁴-disubstituted quinazoline-2,4-diamines has also been synthesized and tested against multi-drug resistant strains of *Acinetobacter baumannii*. Quinazolines with MICs in the single digit micromolar range were identified within the structure-activity relationship. The observed potencies of the top compounds and the easy of synthesis lead to the further investigation of *in vivo* efficacy studies and could be considered a suitable platform for the future development of anti-bacterial agents against *A. baumannii*.

Chapter 1: Introduction to Infectious Diseases and Drug Discovery

1.1 Overview

With the recent outbreak of Ebola in West Africa a stronger initiative has been called for regarding the research and development of viable drugs needed to treat neglected tropical diseases. According to the World Health Organization neglected tropical diseases are a diverse group with distinct characteristics that thrive mainly among the poorest populations.¹ The diseases are caused from four different pathogens: protozoan, bacterial, helminth, and viral. Neglected tropical diseases such as malaria, Human African trypanosomiasis, leprosy, onchocerciasis, rabies, and leishmaniasis affect over 1 billion people.² A push from the World Health Organization and others is urging countries to better fund projects researching and developing new drugs for these neglected diseases.³

Another increasing serious threat to the public health of our world is the rapid emergence of antimicrobial resistance of infections caused by parasites, viruses, fungi, and bacteria. The evolution of resistance is a natural phenomenon that can be accelerated by the use and/or the misuse of antimicrobial drugs. When first-line treatments fail, newer more expensive therapies must be used causing an increase to healthcare.⁴ In the 2014 World Health Organization's Report on global surveillance of antimicrobial resistance revealed that antibiotic resistance is no longer a prediction but that it is occurring now.⁵ This is happening worldwide and is causing once treatable common infections and minor injuries to kill once again.

The following sections will discuss the impact of the neglected tropical disease leishmaniasis and the antibiotic resistant bacteria *Acinetobacter baumannii* that plagues our

societies. *Acinetobacter* is a genus of bacteria, which can cause devastating infections in humans and has increasing antibiotic resistance. *Leishmania* is a disease caused by eukaryotic protozoa in which transmission to humans can cause several different kinds of infection ranging in severity from sores and ulcers on the body to death.⁶ Current treatments are available for *A. baumannii* and *leishmaniasis*, but due to the ability of microbes to rapidly reproduce, mutate, and evolve in quickly changing environment's, these treatments are becoming obsolete with increased resistance being developed. Investigation into novel antibiotic and antileishmanial agents is imperative in the successful treatment and possible eradication of these diseases.

1.2 Leishmaniasis

1.2.1 *Leishmania*

Alexander Russels was one of the first to clinically describe leishmaniasis, referring to it as Aleppo boil, in 1756.⁷ Leishmaniasis has been well documented since then and includes 30 species that infect mammals, 21 of these 30 species causing infection in humans, in over 90 countries world-wide.⁸ These species can be divided into two groups of old- and new-world species. The old-world species predominately occur in southern Europe, some parts of Asia and Africa, whereas the new-world species occur in the America's.⁹ Species infectious to humans include: *L. donovani* complex with 2 species (*L. donovani*, *L. infantum*), the *L. mexicana* complex with 3 main species (*L. mexicana*, *L. amazonensis*, and *L. venezuelensis*), *L. tropica*, *L. major*, *L. aethiopica*, and the subgenus *Viannia* with 4 main species (*L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) panamensis*, and *L. (V.) peruviana*).¹⁰ Table 1.1 summarizes these species showing the distribution and the main reservoirs in which the disease is contained.¹¹ The three major forms of infection for this disease are cutaneous, mucocutaneous, and visceral leishmaniasis.

Table 1.1 Leishmaniasis species, their distribution, and main reservoirs host. Information obtained from 2005 publication by Gramiccia, M. and Gradoni, L.¹¹

Disease and Associated Strains	Geographical distribution	Main reservoir host
VL ^a CL ^b <i>L. chagasi</i>	Mediterranean basin, Middle East, Central Asia, China, Central and South America	Dog
CL <i>L. major</i>	North Africa and Sub-Saharan Africa, Middle East and Central Asia	Various rodents
CL <i>L. aethiopia</i>	Ethiopia, Kenya	Rock hyraxes
CL <i>L. mexicana</i>	Central America	Various rodents
CL <i>L. amazonensis</i>	South America, north of the Amazon	Various rodents
CL <i>L. venezuelensis</i>	Venezuela	Unknown
CL, ML ^c <i>L. Viannia</i>	South America, Central America and Mexico	Numerous rain forest mammals (suspected)
CL <i>L. guyanensis</i>	Guyanas, Brazil	Sloths
CL <i>L. lainsoni</i>	Brazil, Bolivia, Peru	Rodents
CL <i>L. naiff</i>	Brazil, French Guyana, Ecuador, Peru	Armadillos
CL, ML <i>L. panamensis</i>	Central America, Colombia, Ecuador	Sloths
CL <i>L. peruviana</i>	Peruvian Andes	Dog
CL <i>L. shawi</i>	Brazil	Arboreal mammals (suspected)

^a VL visceral leishmaniasis, ^b CL cutaneous leishmaniasis, ^c ML mucocutaneous leishmaniasis

Cutaneous leishmaniasis is the most common form of infection for leishmaniasis.

Cutaneous leishmaniasis is characterized by unsightly spontaneous healing ulcers or open sores where transmission occurred. These sores can lead to disfiguring scars and disability of the infected individual.¹² Strains causing cutaneous leishmaniasis include *L. infantum*, *L. mexicana*, *L. amazonensis*, *L. venezuelensis*, *L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) panamensis*, and

L. (V.) peruviana. Mucocutaneous leishmaniasis can develop from a few species that cause cutaneous leishmaniasis and it is found primarily in Latin America. This form is the least common but can spread from the skin sores to mucosal membranes, including the nose, mouth or throat, causing destruction of the tissue leaving large holes in the area.¹³ Strains known to cause mucocutaneous leishmaniasis include *L. (V.) braziliensis* and *L. (V.) panamensis*. The most severe, common contraction of the disease is visceral leishmaniasis. This form can affect several crucial internal organs (usually the spleen, liver, or bone marrow), which could potentially result in death. Symptoms include fever, weight loss, enlarged liver and spleen, dry, thin and scaly skin, hemorrhaging of the nose, gums and skin.¹³ Strains associated with causing visceral leishmaniasis include *L. infantum* and *L. donovani*. According to the World Health Organization (WHO) it is estimated that 0.7 to 1.3 million of new cutaneous leishmaniasis cases and 200,000 to 400,000 new visceral leishmaniasis cases are reported annually, worldwide. It is also estimated that 20,000 to 30,000 deaths occur each year due to this disease.¹²

Infection in humans occurs through the bite of infected female sandflies of the genera *Phlebotomus* and *Lutzomyia* when taking a blood meal (Figure 1.1).¹⁴ Infected sandflies inject promastigotes (the flagellate stage of the *Leishmania* parasite) into the host, through the proboscis, when taking a blood meal. Macrophages and other mononuclear phagocytic cells within the host then phagocytize these promastigotes. It is within these cells that the promastigotes transform into amastigotes (does not have a visible external flagella), which quickly replicate until the host cell bursts. The amastigotes are then released into the bloodstream where the macrophages and other phagocytic cells continually phagocytize them and the replication process continues. The completion of the transmission cycle occurs when female sandflies take a blood meal from an infected individual. Once ingested the amastigotes transform

back to promastigotes in the gut of the sandflies and the cycle of transmission to another host is complete.¹³ It is estimated by the Center for Disease Control (CDC) that 350 million people in over 88 countries are at risk of contracting leishmaniasis.⁸ Although currently treatments are available, the emerging resistance to existing therapies is increasing at an alarming rate within areas at the greatest risk for infection.

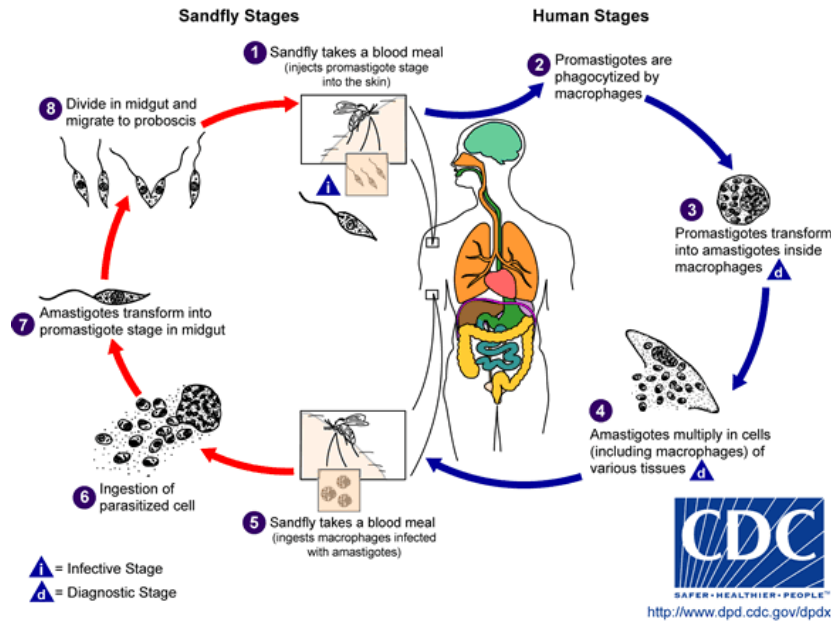


Figure 1.1 *Leishmania* life cycle¹⁴

1.2.2 Anti-leishmanial Drug Discovery

There are several approved drugs currently being used for the treatment of visceral, cutaneous, and mucocutaneous leishmaniasis. For well over a century antimonials have been the first drug of choice. Gaspar Vianna, in 1912, reported the use of the trivalent antimonial tartar emetic for the treatment of cutaneous leishmaniasis specifically caused by *L. braziliensis*.¹⁵ Trivalent and pentavalent antimonials were shown to have great success in the treatment of visceral leishmaniasis in India. This study was conducted by McCombie Young and Upendranath Brahmachari in 1982 and showed a decrease in mortality rate due to visceral

leishmaniasis in India, from 95 % to 10 % over a 10 year period.¹⁶ Two of the most frequently prescribed pentavalent antimonials, and the first line of treatment are meglumine antimoniate and sodium stibogluconate (Figure 1.2).^{7, 17} Even though pentavalent antimonials are frequently prescribed, these drugs are far from ideal. Administration is normally facilitated in a hospital and given either intravenously, or intramuscularly.¹⁸ Side effects associated with these compounds including muscle pain, fatigue, abdominal pain, nausea and vomiting, and in some cases death.^{19,}²⁰ Through the extended use of these pentavalent antimonials an increased resistance in cases of visceral leishmaniasis have been reported.²¹ Over the last 10 years (specifically in Bihar, India) antimonial resistance and therapeutic failures have become so prevalent that up to 60 % of new visceral leishmaniasis reported in this area show no response to the drugs.²²

Currently, two other drugs that are being used as a second defense against the resistant strains are amphotericin B and miltefosine (Figure 1.2). Since the 1960's amphotericin B has been in clinical use showing 90-95 % cure rate of visceral leishmaniasis strains found in India.²¹ Typical administration of this drug requires intravenous injections, dosage of 1/mg/kg, every other day for a total of 30 days. Side effects associated with amphotericin B include infusion-related fever and chills, nephrotoxicity, and hypokalemia, which require treatment to be delivered within a hospital setting.²³ New lipid formulations of amphotericin B have greatly improved upon the negative side effects. The increased cost of these new formulations make them, in general, cost prohibitive for many poor countries in need.²³ In March 2014, the FDA approved the use of miltefosine for the treatment of some cutaneous, mucosal, and visceral leishmaniasis species.¹⁸ Once used as an anticancer drug, for over a decade it has been the only orally bioavailable antileishmanial drug prescribed. Treatment with miltefosine requires a dosage of 2.5 mg/kg/day over a 28-day period and elicits only mild side effects.²⁴ In 2006, a preclinical

reproductive toxicity study in animals, showed embryo- and fetotoxicity along with teratogenic effects when dosing miltefosine 1.2 mg/kg for 10 days during gestation.²⁵ Like many of the pentavalent antimonials, decreasing efficacy rates of miltefosine are being seen in parts of India and Nepal.^{26, 27} Due to increased resistance of current antileishmanials, their unfavorable toxicity, and their cumbersome administration, the need for new antileishmanials is crucial for future successful treatment of this disease.

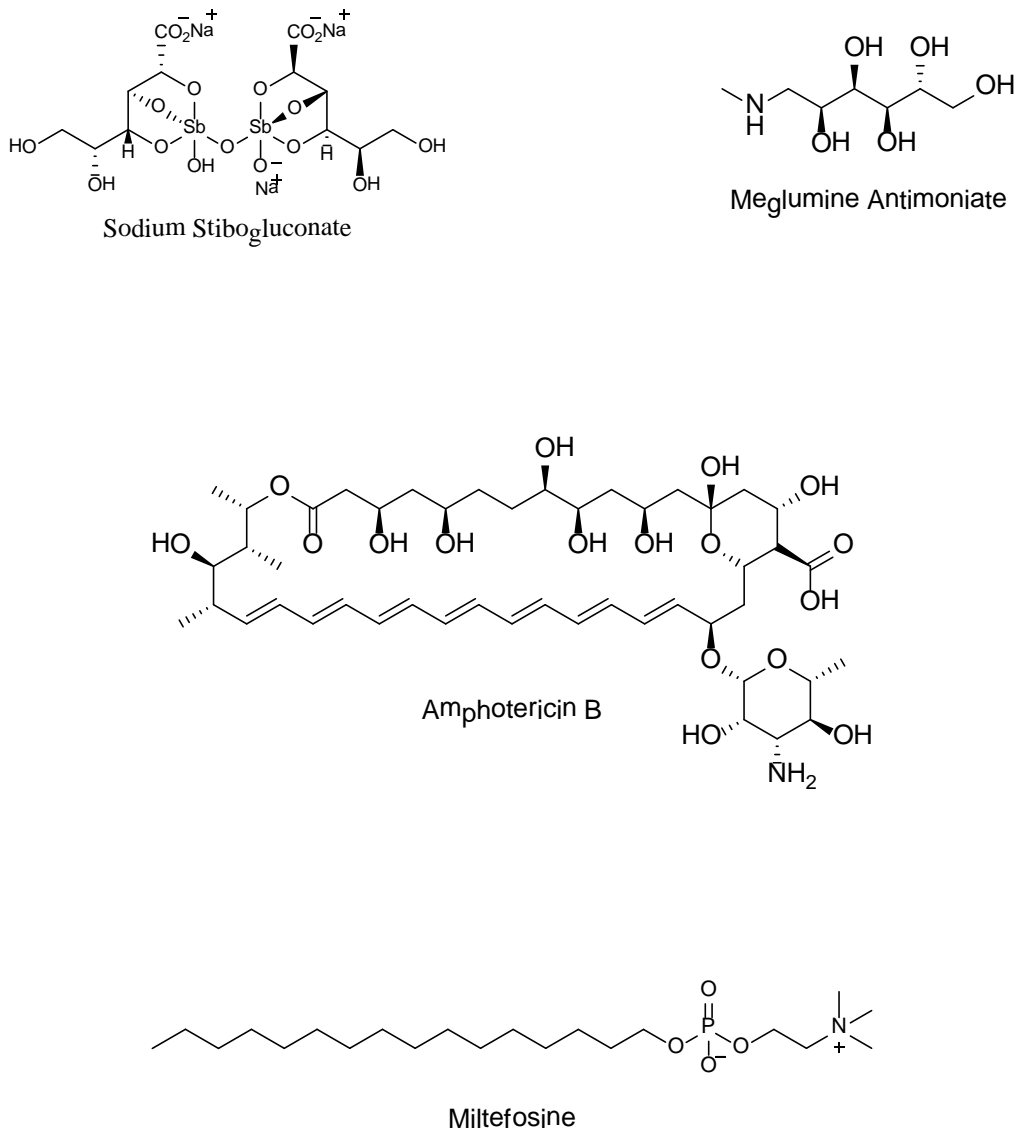


Figure 1.2 Current Antileishmanials

Recently, reports have shown that quinazoline compounds have shown favorable activity against leishmaniasis and are a promising new frontier for the development of antileishmanials. The half maximal inhibitory concentration (IC₅₀) of amastigotes of *L. donovani* with the use of quinazolines has been reported by Shaky and Gupta et al., (Figure 1.3 A and B respectively) and Bhattacharjee et al., (Figure 1.3 C) with activities below 100 ng/mL in some cases.²⁸⁻³⁰ Testing against *L. major* amastigotes, in human monocyte-derived macrophages, was conducted by Berman et al. and reported the half maximal effective concentration (EC₅₀) values as low as 0.04 nM with a series of 2,4-diaminoquinazolines (Figure 1.3 D).³¹

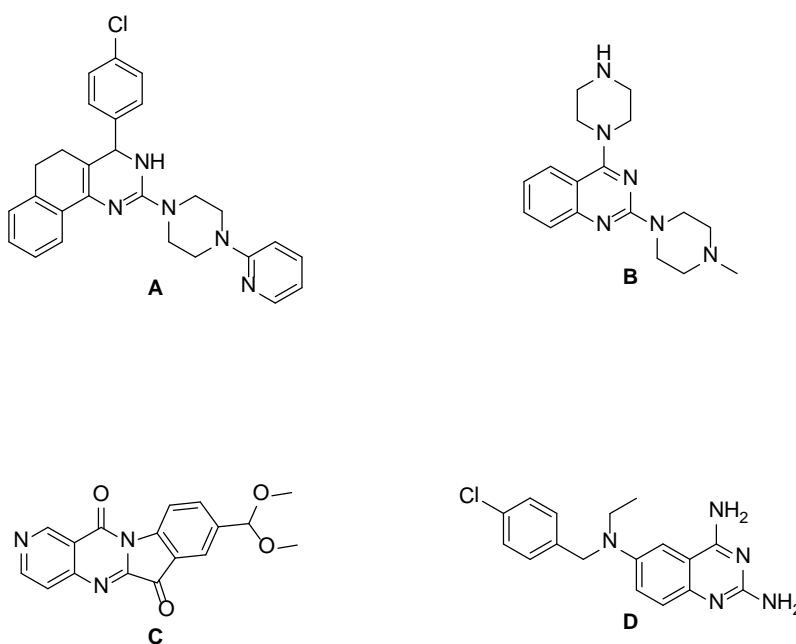


Figure 1.3 Quinazolines as Antileishmanials²⁸⁻³¹

1.2.3 *N*²,*N*⁴-disubstituted quinazoline-2,4-diamines as Antileishmanial Drug Candidates

A previous study published by the Manetsch lab in early 2014, consisted of structure-activity relationship (SAR) and structure-property relationship (SPR) studies of novel *N*²,*N*⁴-

disubstituted quinazoline-2,4-diamines involving *L. donovani* and *L. amazonensis* intracellular amastigotes.³² Within this study 29 structurally diverse quinazoline compounds were synthesized based on two initial compounds **1.1** and **1.2**. Compounds **1.1** and **1.2** were discovered from a small chemically diverse set that was tested against *L. mexicana* axenic amastigotes revealing EC₅₀ values in the single digit micromolar range (Figure 1.4).³³ The SAR conducted followed in part a Topliss operational scheme to identify new lead compounds.³² Two subseries were prepared and tested in order to optimize and validate the *N*²,*N*⁴-disubstituted quinazoline-2,4-diamines, antileishmanial activity. The first subseries focused on the optimization of the *N*²- and *N*⁴- moieties. The second subseries investigated the effect that substitution of the benzenoid ring would have on antileishmanial activity.³² Several compounds synthesized were shown to have submicromolar or single digit micromolar EC₅₀ values. In general, the aqueous solubility, the distribution coefficient (Log D), and the permeability of these quinazolines are within the acceptable ranges (solubility of >20 μM, Pe > 10 × 10⁻⁶ cm·s⁻¹, 1 < log D < 4), making them promising candidates for the development of orally bioavailable antileishmanial agents. From these results three compounds, **1.3-1.5**, were chosen for further in vivo and pharmacokinetic testing based on their EC₅₀ values, selective index SI (ratio of EC₅₀ value for J774A.1 and the value for *L. donovani*), and favorable physiochemical properties (Figure 1.4). In vivo efficacy studies revealed that compound **1.5** showed promising antileishmanial efficacy at a dosage of 5 X 15 mg/kg intraperitoneal injection (ip) with inhibition of liver parasitemia by 37 % compared to vehicle control. Compound **1.3**, dosage 5 X 30 mg/kg ip, showed no significant antileishmanial efficacy. With **1.4**, dosage 5 X 10 mg/kg ip, showed no significant difference in parasite burden when compared to the vehicle control group.³² Due to the single digit and submicromolar inhibition from in vitro studies and favorable physiochemical properties further

study into N^2, N^4 -disubstituted quinazoline-2,4-diamines as potential antileishmanial agents is warranted.

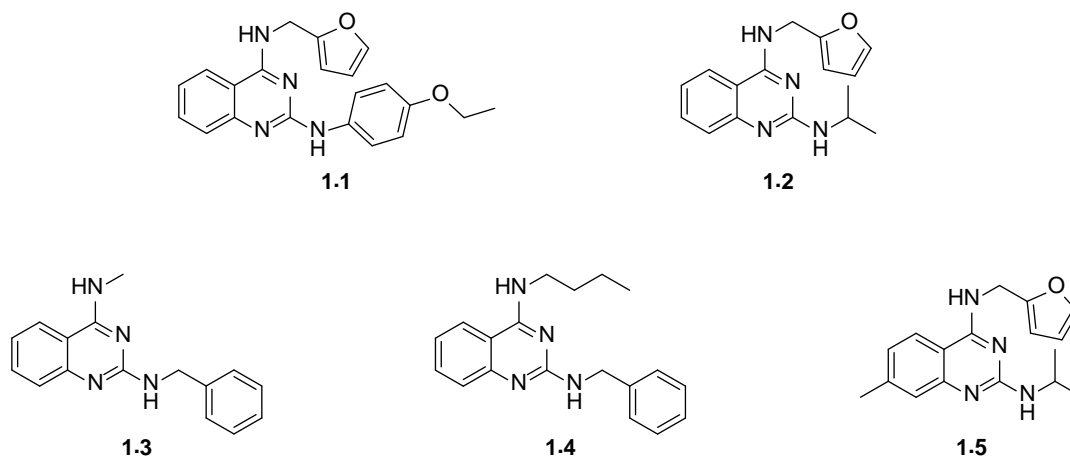


Figure 1.4 N^2, N^4 -Disubstituted quinazoline-2,4-diamines.³² Compounds 1.1 and 1.2 were initial hits discovered. Compounds 1.3-1.5 were selected for in vivo testing from developed SAR.³²

1.2.4 Research Aims

With the exuberant amount of people at risk for contracting leishmaniasis and rapid resistance documented against the newest antileishmanials, the investigation of novel antileishmanial agents is imperative. Drugs such as Amphotericin-B, Pentostam, and Miltefosine have been primarily used to treat current cases of leishmaniasis. The unfortunate and sometimes unbearable side effects, coupled with the inconvenient dosing requirements increase the need for better drugs. With the favorable antileishmanial activity shown by the initial investigation of N^2, N^4 -disubstituted quinazoline-2,4-diamines, continuation of this study is merited. The initial results made apparent three main objectives: (a) further development of an SAR based on quinazolines (b) improvement upon antileishmanial activity and key physicochemical properties (c) improve the pharmacokinetics and selective index on the entire quinazoline compound series.

1.3 Pathogenic Bacteria

1.3.1 Genus *Acinetobacter*

In 1911, reports of an aerobic, gram-negative, non-fermentative bacterium by the microbiologist Martinus Willem Beijerinck were the first known identification of the genus *Acinetobacter*.³³ The difference in the classification of bacteria being Gram-positive or Gram-negative comes from the ability to uptake crystal violet stain when using the gram staining technique in bacterial differentiation.⁴⁰ Gram-positive bacteria are able to retain the crystal violet stain during the alcohol wash due to their thick peptidoglycan layer in the cell wall. Gram-negative species have a thin peptidoglycan layer that is situated between an inner cell membrane and a bacterial outer membrane (Figure 1.5).^{41, 42} The outer bacterial membrane is a unique feature of gram-negative bacteria and is useful for the survival of these bacteria when encountering different antibiotics. The outer membrane comprised of a lipopolysaccharide acts as an endotoxin. When introduced into the circulatory system it can cause a toxic reaction resulting in fever, low blood pressure, high respiration, and even endotoxic shock, which can be fatal.^{40, 41} This outer membrane is responsible for resistance of these bacteria to lysosomes and penicillin.^{40, 41}

The CDC has identified six bacteria pathogens, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species, (collectively known as ESKAPE) as a growing concern around the world. ESKAPE pathogens encompass six different gram-positive and gram-negative bacteria that show high antibiotic resistance and are blamed for the majority of hospital-acquired infections.³⁹ There are many known strains associated with the genus *Acinetobacter* capable of causing human infection, but according to the CDC *Acinetobacter baumannii* is responsible for approximately

80 % of all clinical infections reported although the risk of infection occurring in a healthy individual is unlikely.^{34, 37} Although *A. baumannii* is typically found in the soil and water in hot, arid climates, increasing numbers of infections are being reported in climate controlled healthcare settings.^{35, 36} *A. baumannii* infections commonly occur in patients being treated in intensive care and healthcare units tending to the acutely ill.³⁶ *A. baumannii* is associated with infections of the skin and soft-tissue, urinary tract, wounds, blood stream , and surgical sites .³⁷ It has also been reported in some cases that soldiers have developed osteomyelitis from deep wound infections due to *A. baumannii* exposure.³⁷ *A. baumannii* has also been referred to as the Iraqibacter due to the multitude of cases reported in military treatment facilities during the Iraq war.³⁸

1.3.2 Antibacterials combatting *A. baumannii*

Multidrug resistance observed in *A. baumannii* clinical isolates has been attributed to the overuse of broad-spectrum antibiotics as a first line of treatment.^{36, 37, 43} Treatments for *A. baumannii* infections are limited. It is recommended that an individual's course of treatment be decided by culture-directed antimicrobial therapy.^{38, 43} *A. baumannii* has developed a plethora of resistance mechanisms due to the overuse of antibiotics and its own inherent abilities as a Gram-negative bacterium. In 2006, the Walter Reed Army Medical Center in Washington, D.C. conducted a study on the antibiotic resistance genes in multidrug resistant *A. baumannii* from 75 infected military and civilian patients. Of the isolates tested, 89 % were found to be resistant to at least three drugs classifying them as multidrug resistant.⁴⁴ Specific genes responsible for antibiotic resistance and the resistance mechanisms have been identified for *A. baumannii*. Certain β -Lactamase genes isolated from *A. baumannii* have been attributed to the resistance of a large set of β -lactam antibiotics and carbapenem.⁴³⁻⁴⁸ The identification of several

Aminoglycoside-Modifying Enzymes (AME Genes) are responsible from the resistance of a number of aminoglycoside antibiotics.^{48,49} The resistance of many aminoglycosides, quinolones, tetracyclines and trimethoprim is caused by a gene-encoding efflux pumps within the bacteria.⁵⁷ Quinolones resistance is again seen in certain point mutations in the DNA of *A. baumannii*.⁴³

Research efforts for novel antibiotics are imperative for the successful treatment of the ever-growing resistant *A. baumannii*. Recent studies conducted with quinazoline derivatives have shown promising antibiotic activity against multidrug resistant bacteria.^{50,51} Inhibition of the gram-negative species *E. aerogenes*, *K. pneumoniae* and *P. aeruginosa* was reported by Chevalier et al. in 2010 with the use of a set of quinazoline compounds affording minimal inhibitory concentrations as low as 1.25 mM.⁵⁰ Fawzy et al. reported the use of 3-substituted quinazoline derivatives (Figure 1.6 A) against *A. baumannii* with a zone of inhibition of 12 mm.⁴⁹ In 2014 the Manetsch lab reported the activity of *N*²,*N*⁴-disubstituted quinazoline-2,4-diamines against the multidrug resistant strain *Staphylococcus aureus* (MRSA) with minimum inhibitory concentrations (MICs) in the low micromolar range in addition to favorable physicochemical properties.⁵¹ Leading to testing of biological activity revealing limited potential for resistance to these agents, low toxicity, and highly effective in vivo activity, even with low dosing regimens make this compound series a suitable platform for future development of antibacterial agents. With four of the six ESKAPE pathogens showing inhibition with the use of the quinazoline class of compounds, further investigation into the potential activity against *A. baumannii* is merited.

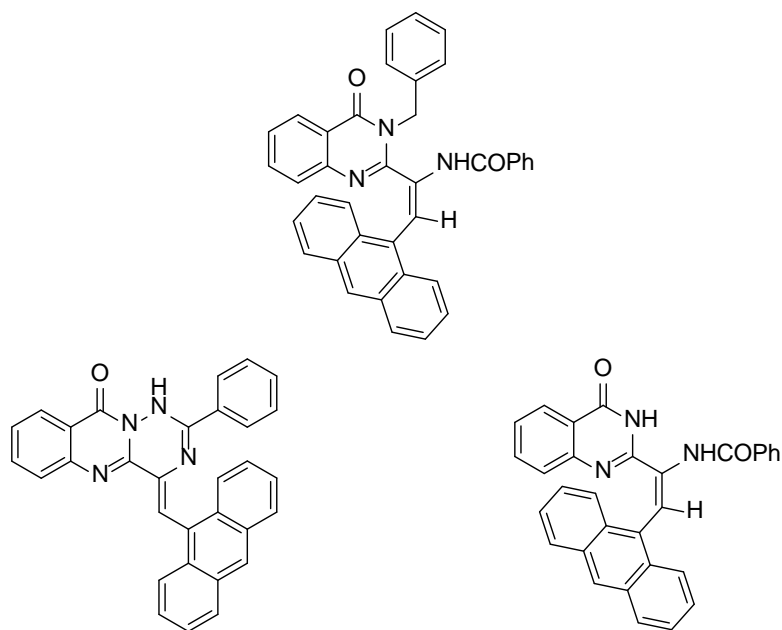


Figure 1.5 Fawzy et al. 3-substituted quinazoline derivatives⁴⁹

1.3.3 Research Aims

The deployment of a large number of soldiers to the Middle East over the past years has demonstrated the vulnerability of humans against *A. baumannii*. The quick resistance of this bacterium to commonly prescribed antibiotics increases the need for development of novel antibiotic agents. The new class of N^2, N^4 -disubstituted quinazoline-2,4-diamines, which has previously shown antibiotic activity against multidrug resistant *Staphylococcus aureus*, serves as a prospective starting point for synthetic development. From the previous study conducted by Van Horn et. Al.⁵¹ the top compounds found will be tested against *A. baumannii* to see if the indicated chemotype can invoke similar inhibition, leading to the development of a new structure activity relationship with the intention of *in vivo* testing to occur.

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Chapter 2: Quinazolines as Antileishmanials

Note to Reader

The work described in Chapter 2, section 2.2 has been reprinted from Bioorganic and Medicinal Chemistry, Xiaohua Zhu, Kurt S. Van Horn, Megan M. Barber, Siyung Yang, Michael Zhuo Wang, Roman Manetsch, Karl Werbovetz, SAR refinement of antileishmanial N^2, N^4 -disubstitutedquinazoline-2,4-diamines, available online February 18, 2015, Copyright (2015), with permission from Elsevier.

2.1 Synthetic Chemistry

The nine series of N^2, N^4 -disubstituted quinazoline-2,4-diamines were synthesized using known procedures (Figure 2.1)¹⁻³. Synthesis began with cyclization of commercially available anthranilic acids (**2.A**) and urea to afford the quinazoline-2,4-dione (**2.B**) which was then refluxed in phosphorus oxychloride to produce the 2,4-dichloroquinazoline (**2.C**). Selective substitution of the quinazoline at the 4-position occurred readily yielding the 4-amino-2-chloroquinazoline (**2.D**) and further substitution at the 2-position affords the N^2, N^4 -disubstituted quinazoline-2,4-diamines (**2.E**). Upon completion of synthetic analogues **2.C**, **2.D**, and **2.E** purification via column chromatography or recrystallization was completed to afford the pure product. Analysis of these compounds included proton and carbon spectra, as well as high resolution mass spectrometry (HRMS) to ensure products were of ≥ 95 % purity.

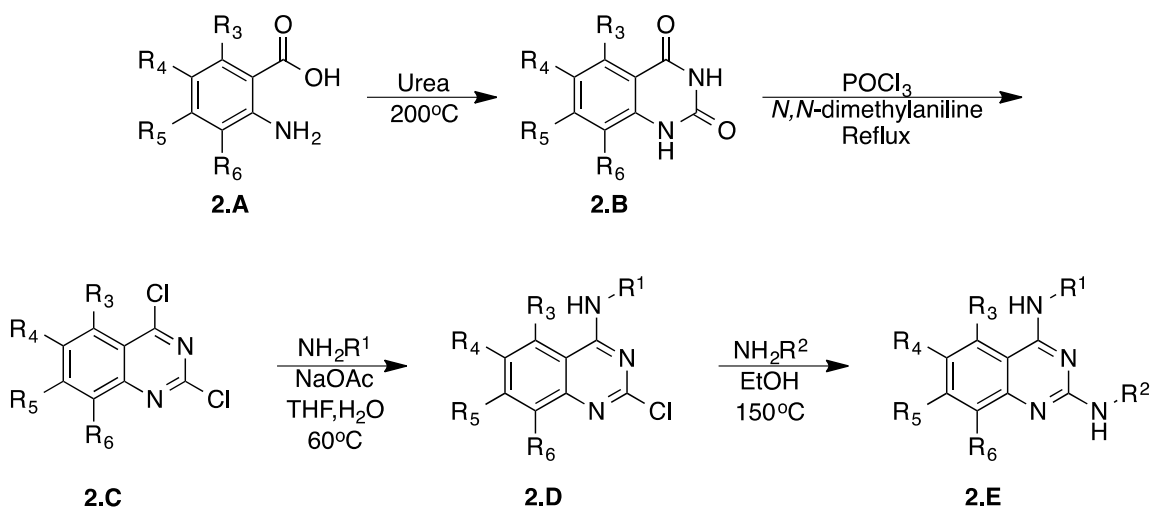


Figure 2.1 Synthesis of N^2, N^4 -disubstituted quinazoline-2,4-diamines¹⁻³

The series of *N*-benzyl-2-arylquinazoline-4-amines were synthesized using known procedures (Figure 2.2)⁴. Starting from *N*-benzyl-2-chloroquinazolin-4-amine (**2.F**), Suzuki-Miyaura Cross-Coupling conditions with tetrakis-palladium, 1M sodium carbonate, and commercially available boronic acids, under argon, to yield *N*-benzyl-2-arylquinazolin-4-amine (**2.G**). Upon completion of synthetic analogues **2.F** and **2.G** purification via column chromatography was completed to afford the pure product. Analysis of these compounds included proton and carbon spectra, as well as high resolution mass spectrometry (HRMS) to ensure products were of $\geq 95\%$ purity.

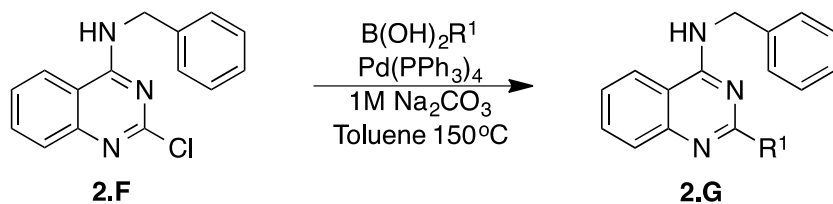


Figure 2.2 Synthesis of *N*-benzyl-2-arylquinazolin-4-amines⁴

Initially 2,4-dichloroquinazoline was reacted with phenylboronic acid at room temperature with a catalytic amount of tetrakis-palladium and a 1M solution of sodium

carbonate, under argon, in attempts to afford the desired product, 2-chloro-4-phenylquinazoline. Monitoring of the reaction by thin layer chromatography (TLC) and liquid-chromatography mass-spectrometry (LC-MS) showed the formation of three compounds with equal relative abundance: 2-chloro-4-phenylquinazoline, 4-chloro-2-phenylquinazoline, and 2,4-diphenylquinazoline. The synthesis of the series *N*-benzyl-4-arylquinazolin-2-amines (Figure 2.3) was developed to overcome selectivity issues encountered when implementing Suzuki-Miyaura Cross-Coupling conditions with 2,4-dichloroquinazoline. The hydrolysis of 2,4-dichloroquinazoline (**2.H**) to the 2-chloroquinazolin-4(1*H*)-one (**2.I**)⁵ was reacted with benzylamine to afford 2-(benzylamino)quinazolin-4(3*H*)-one (**2.J**). The chlorination of **2.J**, by refluxing in phosphorus oxychloride, yielded *N*-benzyl-4-chloroquinazolin-2-amine (**2.K**). Suzuki-Miyaura Cross-Coupling conditions with tetrakis-palladium, 1M sodium carbonate, and commercially available boronic acids, under argon, with **2.K** gave *N*-benzyl-4-arylquinazolin-2-amines as the final products. Upon completion of synthetic analogues **2.I**, **2.J**, **2.K**, and **2.L** purification via column chromatography was completed to afford the pure product. Analysis of these compounds included proton and carbon spectra, as well as high resolution mass spectrometry (HRMS) to ensure products were of $\geq 95\%$ purity.

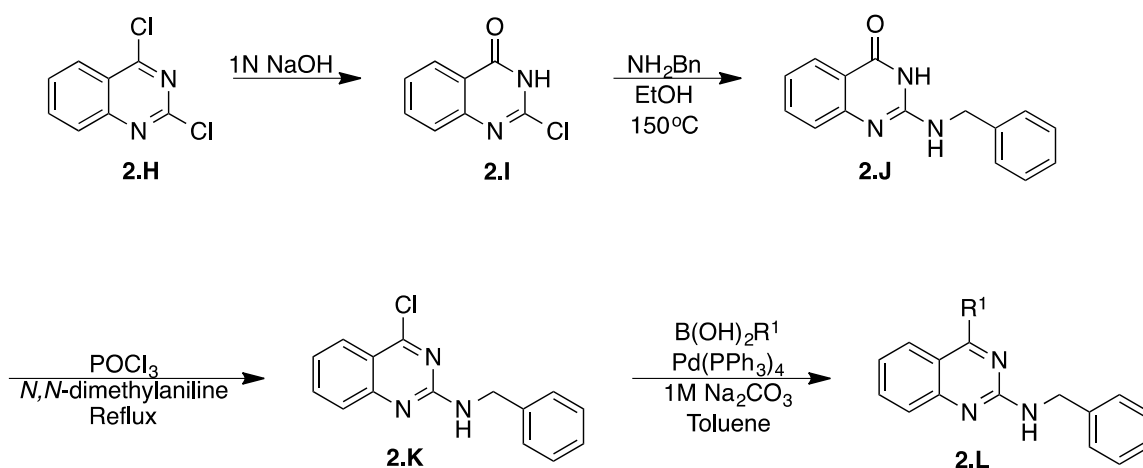


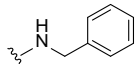
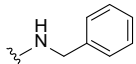
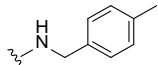
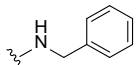
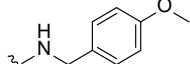
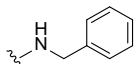
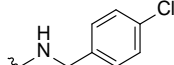
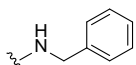
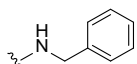
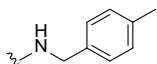
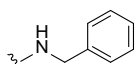
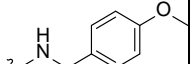
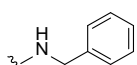
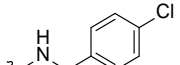
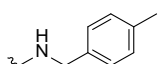
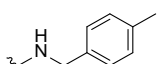
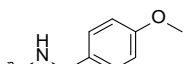
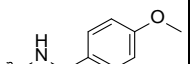
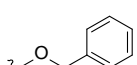
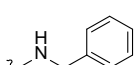
Figure 2.3 Synthesis of *N*-benzyl-4-arylquinazolin-2-amines⁵

2.2 Anti-Leishmanial Activity

2.2.1 Structure Activity Relationship

In an earlier study conducted by the Manetsch group in 2014, showed the in vitro potency of N^2, N^4 -dibenzylquinazoline-2,4-diamine **1** against intracellular *L. donovani*.⁶ The antileishmanial potency and selectivity of analogs of this compound are given in Table 2.1. All but two of these compounds displayed EC_{50} values against intracellular *L. donovani* ranging from 0.39 to 1.1 μM . No clear trend for in vitro antileishmanial activity is observed for compounds substituted with methyl, methoxy, and chloro- substituents at the *para* position of the benzyl groups at N^2 and N^4 , although a methoxy group appears to decrease antileishmanial activity if it is placed at the *para* position of the N^4 benzyl group as in **2.2** ($EC_{50} = 2.5 \mu\text{M}$) and **2.8** ($EC_{50} = 6.0 \mu\text{M}$). Replacing a nitrogen atom with an oxygen atom at position 4 does not appear to affect antileishmanial activity (compare **1** with **2.9**). The antileishmanial selectivity indexes (SI) of these compounds was modest; seven displayed SI values of 5.1–8.2 for intracellular *L. donovani* compared to murine J774.A1 macrophages while three of these molecules exhibited SI values <3 . The effect of an aryl substitution at position 4 when paired with a benzyl substitution at the N^2 atom was also examined, as was an aryl substitution at position 2 when paired with a benzyl substitution at the N^4 atom (Table 2.2). These compounds (**2.10–2.12**) were not active against *L. donovani* up to a concentration of 10 μM .

Table 2.1: Probing the *para*-position of benzyl substituents of N^2,N^4 -disubstituted quinazolin-2,4-diamines⁹

Compound	R ¹	R ²	<i>L. donovani</i> EC ₅₀ ^a μM	J774A.1 EC ₅₀ ^b μM	SI
Reference 1			0.67 ± 0.27 ^c	5.5 ± 1.4 ^c	8.2
2.1			0.39 ± 0.16	2.1 ± 0.2	5.4
2.2			2.5 ± 0.2	5.2 ± 2.0	2.1
2.3			0.82 ± 0.23	4.6 ± 2.4	5.6
2.4			0.089 ± 0.10	4.5 ± 2.1	5.1
2.5			0.72 ± 0.10	4.1 ± 2.5	5.7
2.6			0.61 ± 0.13	3.2 ± 0.1	5.2
2.7			1.1 ± 0.5	2.9 ± 1.1	2.6
2.8			6.0 ± 2.9	2.2 ± 1.4	0.37
2.9			0.65 ± 0.13	3.3 ± 0.1	5.1


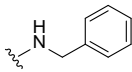

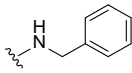
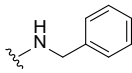

^a EC₅₀ value is the mean ± standard deviation of at least three independent experiments. The control drug for the in vitro intracellular antileishmanial assay is amphotericin B, which displays an EC₅₀ = 41 ± 8 nM against *L. donovani* (n = 20).

^b EC₅₀ value is the mean ± standard deviation of at least three independent experiments.

Podophyllotoxin is the control compound for the in vitro cytotoxicity assay, exhibiting an EC₅₀ = 21 ± 5 nM against the J774.A1 macrophages (n = 11).

^c From Van Horn et al.⁶

Table 2.2: Probing with 2-phenyl or 4-phenyl-substituted quinazolines⁹

Compound	R ¹	R ²	<i>L. donovani</i> ^a EC ₅₀ μM	J774A.1 EC ₅₀ μM	SI
2.10			>10	ND ^b	ND
2.11			>10	ND	ND
2.12			>10	ND	ND

^a The reported value is based on two determinations. The control drug for the in vitro intracellular antileishmanial assay is amphotericin B, which displays an EC₅₀ = 41 ± 8 nM against *L. donovani* (n = 20).

^b Not determined

The most potent quinazoline-2,4-diamine against intracellular *L. donovani* in vitro from the previous study⁶ was *N*²-benzyl-*N*⁴-methylquinazoline-2,4-diamine **2**, which displayed an EC₅₀ of 0.15 μM against intracellular *L. donovani* and a selectivity index of 100. Table 2.3 shows a series of *N*⁴-methylated analogs of this compound. A dramatic decrease in activity is observed as smaller substituents were placed at *N*². Aside from the reduction in activity compared to **2.2**, there is no clear SAR trend in this series, as antileishmanial potency fluctuated when going from the *N*²-cyclopentyl derivative **2.14** (EC₅₀ = 4.4 μM) to the *N*²-isopropyl and *N*²-ethyl derivatives **2.15** and **2.16** (EC₅₀ values >25 μM) to the *N*²-methyl derivative **2.17** (EC₅₀ = 7.6 μM). Those *N*⁴-methyl derivatives that were tested displayed less toxicity to J774 macrophages compared to the dibenzylated quinazolines shown in Table 2.1, however.

Table 2.3: Probing *N*²-substituents of *N*⁴-methyl-quinazolin-2,4-diamines⁹


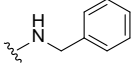
Compound	R ¹	R ²	<i>L. donovani</i> EC ₅₀ ^a μM	J774A.1 EC ₅₀ ^b μM	SI
Reference 2			0.15 ± 0.02 ^c	15 ± 1 ^c	100

Table 2.3 (Continued)

2.13			20 ± 5	ND	ND
2.14			4.4 ± 1.2	39 ± 10	8.9
2.15			>25	ND	ND
2.16			>25	ND	ND
2.17			7.6 ± 1.3	>133	>17.5
2.18			>25	ND	ND

^a EC₅₀ value is the mean ± standard deviation of at least three independent experiments. The control drug for the in vitro intracellular antileishmanial assay is amphotericin B, which displays an EC₅₀ = 41 ± 8 nM against *L. donovani* (n = 20).

^b EC₅₀ value is the mean ± standard deviation of at least three independent experiments. Podophyllotoxin is the control compound for the in vitro cytotoxicity assay, exhibiting an EC₅₀ = 21 ± 5 nM against the J774.A1 macrophages (n = 11).

^c From Van Horn et al.⁶

Earlier work showed that *N*⁴-(furan-2-ylmethyl)-*N*²-isopropyl-7-methylquinazoline-2,4-diamine exhibited promising activity in *L. donovani*-infected mice when given at a dose of 15 mg/kg/day for five days by the ip route.⁶ We thus synthesized analogs where either the furan-2-ylmethyl substituent at *N*⁴ or the isopropyl substituent at *N*² were held constant and substituents at the other exocyclic nitrogen atom were varied (Tables 2.4 and 2.5) in an effort to identify compounds with improved in vitro activity to take forward to in vivo evaluation. Of the compounds possessing an *N*⁴-(furan-2-ylmethyl) substituent (Table 2.4), only the derivative **2.22** bearing a cyclohexyl group at *N*² displayed in vitro potency similar to reference compound **3**, but **2.22** showed negligible selectivity for intracellular *L. donovani* compared to J774 macrophages.

Table 2.4: Probing N^4 -substituents of N^2 -furfuryl-quinazolin-2,4-diamines⁹

Compound	R ¹	R ²	<i>L. donovani</i> EC ₅₀ ^a μM	J774A.1 EC ₅₀ ^b μM	SI
Reference 3			2.5 ± 0.4 ^c	17 ± 6 ^c	6.8
2.19			>25	ND	ND
2.20			>25	ND	ND
2.21			>25	ND	ND
2.22			4.0 ± 2.5	5.3 ± 0.8	1.3

^a EC₅₀ value is the mean ± standard deviation of at least three independent experiments. The control drug for the in vitro intracellular antileishmanial assay is amphotericin B, which displays an EC₅₀ = 41 ± 8 nM against *L. donovani* (n = 20).

^b EC₅₀ value is the mean ± standard deviation of at least three independent experiments.

Podophyllotoxin is the control compound for the in vitro cytotoxicity assay, exhibiting an EC₅₀ = 21 ± 5 nM against the J774.A1 macrophages (n = 11).

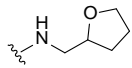










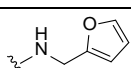

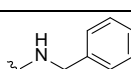
^c From Van Horn et al.⁶

When the N^2 -isopropyl group was held constant (Table 2.5, compounds **2.23–2.31**), promising in vitro antileishmanial activity was observed with the N^4 -benzyl (**2.23**, EC₅₀ = 2.0 μM), N^4 -phenyl (**2.24**, EC₅₀ = 1.9 μM), and N^4 -isopropyl (**2.26**, EC₅₀ = 2.3 μM) substituted analogs. Of these three compounds, the antileishmanial selectivity is best with the diisopropyl derivative **2.26** (SI >13). In the series of N^4 -isopropyl derivatives (Table 2.5, compounds **2.29–2.31**), the N^2 -benzyl derivative **2.31** displayed outstanding in vitro potency against *L. donovani* and good selectivity (SI = 19).

Table 2.5: Probing of 4- and 2-substitutions of N^2 -isopropyl or N^4 -isopropyl mono-substituted quinazolin-2,4-diamines⁹

Compound	R ¹	R ²	<i>L. donovani</i> EC ₅₀ ^a μM	J774A.1 EC ₅₀ ^b μM	SI
2.23			2.0 ± 0.1	6.7 ± 1.2	3.4
2.24			1.9 ± 0.2	12 ± 0	6.3

Table 2.5 (Continued)

2.25			8.9 ± 0.1	46 ± 4	5.2
2.26			2.3 ± 0.5	>30	>13
2.27			6.1 ± 2.8	31 ± 5	5.1
2.28			6.3 ± 1.9	>30	>4.8
2.29			8.0 ± 2.0	>40	>5
2.30			11.0 ± 3.0	8.3 ± 5.8	0.75
2.31			0.38 ± 0.09	7.2 ± 0.2	19

^a EC₅₀ value is the mean ± standard deviation of at least three independent experiments. The control drug for the in vitro intracellular antileishmanial assay is amphotericin B, which displays an EC₅₀ = 41 ± 8 nM against *L. donovani* (n = 20).

^b EC₅₀ value is the mean ± standard deviation of at least three independent experiments. Podophyllotoxin is the control compound for the in vitro cytotoxicity assay, exhibiting an EC₅₀ = 21 ± 5 nM against the J774.A1 macrophages (n = 11).

2.2.2 *In vivo* Efficacy Studies and Pharmacokinetic Studies

Compounds **2.6** and **2.31** were selected for the *in vivo* evaluation of PK properties and for efficacy in a murine model of visceral leishmaniasis on the basis of their sub-micromolar in vitro antileishmanial potency (EC₅₀ values of 0.61 and 0.38 μM, respectively) and fair to good selectivity (SI values of 5.2 and 19, respectively). The mean plasma and tissue concentration–time profiles of **2.6** and **2.31** after ip administration (10 mg/kg) are shown in Figure 2.4 and the relevant pharmacokinetic parameters are listed in Table 2.6. Compared with **2.31**, **2.6** showed a much slower decrease of plasma concentration with a longer terminal *t*_{1/2} (9.3 h vs 2.1 h). Both compounds accumulated in the target tissues (Table 2.6) with tissue-to-plasma partition coefficients ranging from 3.2 to 16 for **2.31** and 20 to 21 for **2.6**. After adjusting for dose, **2.6** exhibited 2.8-fold greater plasma AUC than **2.31**. Longer plasma half-life and greater AUC of

2.6 are consistent with **2.6** being more metabolically stable (Table 2.6) and exhibiting greater tissue partitioning. Both **2.6** and **2.31** were then selected for *in vivo* antileishmanial evaluation. First, these compounds were examined for their toxicity to BALB/c mice. While **2.6** and **2.31** were toxic to the mice at a dose of 30 mg/kg/day x 5 by the ip route, the compounds had no apparent adverse effects on the mice when given by this route at 10 mg/kg/day x 5. This dose was thus chosen for evaluation in our murine model of visceral leishmaniasis.⁷ Quinazoline **2.31** reduced liver parasitemia by only 23.8 ± 7.5 % and compound **2.6** decreased liver parasitemia by 12.0 ± 3.2 %, both given ip, compared to infected control groups. The same dose of the control drug miltefosine exhibited 93.6 ± 1.4 % inhibition of liver parasitemia when given ip, consistent with our previously reported results.^{6,8}

Table 2.6: Pharmacokinetics of 2.6 and 2.31 after ip administration in mice⁹

Parameter ^a	Compound 2.31			Compound 2.6		
	Plasma	Liver	Spleen	Plasma	Liver	Spleen
AUC ($\mu\text{M} \cdot \text{h}$)	7.14			15.7		
$t_{1/2}$ (h)	2.1			9.3		
C_{max} (μM) ^b	2.15	20.2 (0.54)	16.5 (0.18)	1.4	25.3 (8.51)	26.6 (11.4)
T_{max} (h)	0.25			0.5		
V_z/F (L/kg)	1.15			22.3		
Mic $t_{1/2}$ (min) ^c		42			88	

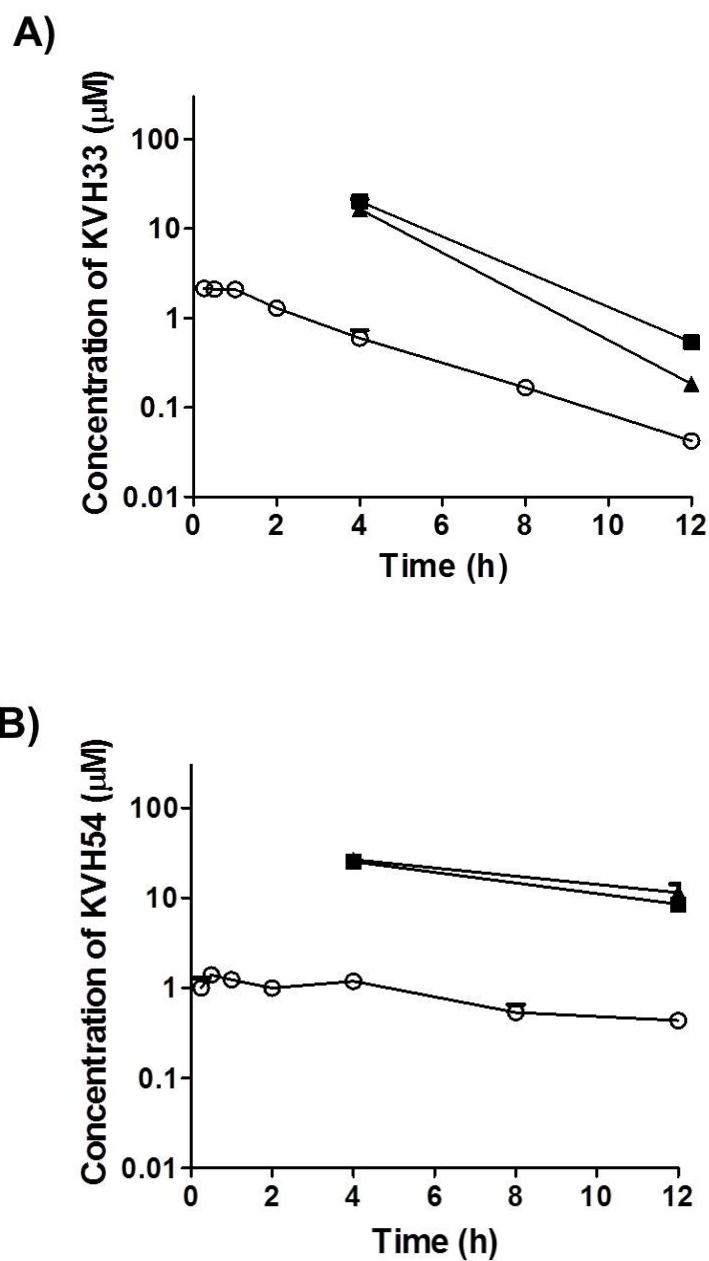


Figure 2.4 Pharmacokinetic Evaluation (See Page 30). Mean plasma (○), liver (■), and spleen (▲) concentration-time profiles of **2.31** (A) and **2.6** (B) after i.p. administration at a dose of 10 mg/kg to mice. Symbols and error bars represent the mean and standard error of triplicate determinations.⁹

2.2.3 Summary of Initial Structure Activity Relationship Study

As illustrated by the *in vivo* studies reported here with **2.6** and **2.31**, the toxicity of the 2,4-quinazoline diamines in mice continues to limit the dose of these compounds that can be administered, restricting the ability to conduct efficacy studies in the murine model of visceral leishmaniasis. The synthesis and evaluation of the 2,4-quinazoline diamines reported here provides further information regarding the antileishmanial SAR of these molecules, however, and lends clues for the synthesis of new analogs with lower toxicity. Future efforts will involve the preparation and testing of 2,4-quinazoline diamines containing isopropyl or related small alkyl substitutions at the exocyclic nitrogen atoms to maximize efficacy and exposure and minimize toxicity *in vivo*.

2.3 Expanded Structure Activity Relationship Study

Following the success of the previous study a second SAR was developed in the hopes of lowering toxicity of the 2,4-quinazoline diamines. Beginning with Table 2.7 the table consists of compounds with substitution of various piperazine moieties at the 2-position while keeping a *N*-benzylamine constant in the 4-position. When tested against *L. donovani* compounds **2.32-2.36**, although retaining single digit micromolar inhibition concentrations, decreased potency compared with the reference compound. However compound **2.36** did show an increase in antileishmanial selectivity (SI=12.5). Compound **2.32** had a complete loss of activity when compared to the reference compound.

Table 2.7: Probing 2-substitutions of *N*⁴-benzyl-2-piperazinyl-substituted quinazolin-2,4-diamines

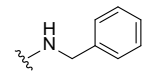
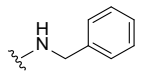
Compound	R ¹	R ²	<i>L. donovani</i> EC ₅₀ ^a μM	J774A.1 EC ₅₀ ^b μM	SI
Reference 1			0.67 ± 0.27 ^c	5.5 ± 1.4 ^c	8.2

Table 2.7 (Continued)

2.32			>10	ND	ND
2.33			5.2	13	2.5
2.34			3.5	15	4.3
2.35			3.4	7.5	2.2
2.36			1.6	20	12.5

^a

EC₅₀ value is the mean ± standard deviation of at least three independent experiments. The control drug for the in vitro intracellular antileishmanial assay is amphotericin B, which displays an EC₅₀ = 41 ± 8 nM against *L. donovani* (n = 20).

^b EC₅₀ value is the mean ± standard deviation of at least three independent experiments.

Podophyllotoxin is the control compound for the in vitro cytotoxicity assay, exhibiting an EC₅₀ = 21 ± 5 nM against the J774.A1 macrophages (n = 11).

^c From Van Horn et al.⁶

Further investigation of the effects of piperazines on the activity against *L. donovani* were studied with the synthetic series in Table 2.8, where 4-benzylpiperazine-1-yl is constant in the 4-position and the 2-position is substituted with different piperazino- or a morpholino- substituent. Compound **2.39** showed an increase in inhibition activity compared to **2.35** against *L. donovani*. Compounds **2.37**, although not as potent as **2.35**, still exhibited single digit micromolar inhibition concentration and a six fold increase against the J774A.1 mammalian cell line and a high antileishmanial selectivity (SI =30.7). Compounds **2.38**, **2.40**, and **2.41** lost inhibition activity against *L. donovani*.

Table 2.8: Probing 2-substitutions of 4-(4-benzylpiperazin-1-yl)-2-quinazoline

Compound	R ¹	R ²	<i>L. donovani</i> EC ₅₀ ^a μM	J774A.1 EC ₅₀ ^b μM	SI
2.37			5.2	46	8.8
2.38			>10	ND	ND
2.39			2.8	86	30.7
2.40			>10	44	ND
2.41			>10	ND	ND

^a EC₅₀ value is the mean ± standard deviation of at least three independent experiments. The control drug for the in vitro intracellular antileishmanial assay is amphotericin B, which displays an EC₅₀ = 41 ± 8 nM against *L. donovani* (n = 20).











^b EC₅₀ value is the mean ± standard deviation of at least three independent experiments. Podophyllotoxin is the control compound for the in vitro cytotoxicity assay, exhibiting an EC₅₀ = 21 ± 5 nM against the J774.A1 macrophages (n = 11).

The final series of compounds was designed to investigate the effects of substitution on the benzoid ring with a new core-scaffold of either *N*²-methyl-*N*⁴-benzyl-quinazolin-2,4-diamine, *N*²,*N*⁴-bis(4-chlorobenzyl)quinazoline-2,4-diamine, or *N*⁴-(4-chlorobenzyl)-*N*²-(4-methoxybenzyl)quinazoline-2,4-diamine. With EC₅₀ values against intracellular *L. donovani* at or below 1.2 μM and favorable antileishmanial selectivity, especially **2.45** SI=37.8, introduction of substitution along the benzoid ring has shown compounds with improved in vitro activity to possibly take forward to *in vivo* evaluation.

Table 2.9: Probing benzenoid ring of the quinazoline scaffold

Compound	R ¹	R ²	R	<i>L. donovani</i> EC ₅₀ ^a μM	J774A.1 EC ₅₀ ^b μM	SI
2.42			6-Cl	0.35	4.8	13.7

Table 2.9 (Continued)

2.43			7-Cl	1.2	7.3	6.1
2.44			6-OMe	2.5	ND	ND
2.45			7-Cl	0.37	14	37.8
2.46			7-Cl	0.48	5.1	10.6
2.47			7-Cl	1.2	ND	ND

^a EC₅₀ value is the mean \pm standard deviation of at least three independent experiments. The control drug for the in vitro intracellular antileishmanial assay is amphotericin B, which displays an EC₅₀ = 41 \pm 8 nM against *L. donovani* (n = 20).

^b EC₅₀ value is the mean \pm standard deviation of at least three independent experiments. Podophyllotoxin is the control compound for the in vitro cytotoxicity assay, exhibiting an EC₅₀ = 21 \pm 5 nM against the J774.A1 macrophages (n = 11).

2.4 Summary

Following the publication reported from the Manetsch lab earlier this year, an additional 22 molecules were synthesized and tested. Compounds which exhibited the most potent activity, submicromolar range, against *L. donovani* incorporated the quinazoline-2,4-diamine scaffolds with *N*-benzyl substituents in the 2- and/ or 4- position and an *N*²-benzyl-6-chloro-*N*⁴-methyl substituent combinations. Enhancement of cytotoxicity values for compounds was achieved with either a mono or disubstitution of piperazino- substituents. With this enhancement, retention of single digit micromolar EC₅₀s against *L. donovani* was still observed making this a possible platform for the future development of anti-leishmanial agents. *In vitro* efficacy of the piperazino- substituted compounds could potentially be improved with substitution along the benzoid ring of the scaffold, as this has shown to generally improve activity and antileishmanial selectivity. Preliminary data from current *in vivo* hamster studies indicates promising potential

for the class of N^2-N^4 -quinazolin-diamines to become useful bioavailable anti-leishmanial agents.

2.5 Experimental Section

2.5.1 General Information

All commercially available chemical reagents, except for the boronic acids used, and anhydrous solvents were purchased from either Sigma Aldrich, Oakwood Products, Inc. or TCI America and used without any further purification. Boronic acids used were purchased through Frontier Scientific. NMR spectra were recorded at ambient temperature on a 500 MHz Varian NMR spectrometer in the solvent indicated. All ^1H NMR experiments are reported in δ units, parts per million (ppm) downfield of TMS and were measured relative to the signals for chloroform (7.26 ppm), methanol (3.31 ppm) and dimethyl sulfoxide (2.50 ppm). All ^{13}C NMR spectra were reported in ppm relative to the signals for chloroform (77 ppm), methanol (49 ppm) and dimethyl sulfoxide (39.5 ppm) with ^1H decoupled observation. Data for ^1H NMR are reported as follows: chemical shift (δ), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, sext = sextet, sept = septet, oct = octet m = multiplet), integration and coupling constant (Hz), whereas ^{13}C NMR analyses were reported in terms of chemical shift. NMR data was analyzed by using MestReNova Software ver. 5.3.2-4936. High resolution mass spectra (HRMS) were performed on an Agilent LC/MSD TOF system G3250AA. Analytical thin layer chromatography (TLC) was performed on silica gel 60 F254 pre-coated plates (0.25 mm) from EMD Chemical Inc. and components were visualized by ultraviolet light (254 nm). Silicycle silica gel 230-400 (particle size 40-63 μm) mesh was used for all flash column chromatography.

2.5.2 General Procedures

Procedure A: Cyclization of Anthranilic Acids to the Corresponding Quinazoline-2,4-diones

One equivalent of the commercially available anthranilic acid and three equivalents of urea were combined in a mortar and pestle until a homogenous mixture was obtained. This powder was then transferred to a round bottom flask and heated to 200°C uncovered. After 3 hours the mixture was cooled, 10 mL of water was added, the solid filtered and subsequently washed with 40 mL of water. Crude product was dried and no further purification was completed.

Procedure B: Chlorination of Quinazoline-2,4-diones to the Corresponding 2,4-Dichloroquinazoline

One equivalent of quinazoline-2,4-dione and one equivalent of *N,N*-dimethylaniline were combined in a round bottom flask, 12 equivalents of phosphorus oxychloride was then added. The mixture was refluxed under argon until the presence of starting material was no longer seen by TLC or by LC-MS (6-24 hours). Upon completion the reaction mixture was cooled and slowly added over ice, amount of ice equaled to ten times that of the reaction volume. Upon precipitation the reaction was filtered and washed with water to afford the crude 2,4-dichloroquinazoline which was purified by column chromatography using hexane and ethyl acetate (hexane/ethyl acetate = 5:1).

Procedure C: Amine Substitution of 2,4-Dichloroquinazolines to Yield 4-Amino-substituted-2-chloroquinazoline

One equivalent of the crude 2,4-dichloroquinazoline, 1.1 equivalents of sodium acetate, and 1.1 equivalents of selected primary amine were combined in a round bottom flask and mixed with a three to one solution of tetrahydrofuran and water to afford a 0.1 M solution. The reaction

was heated to 65°C and monitored until no starting material was seen by TLC or LC-MS. The reaction was diluted with ethyl acetate and the organic layer separated. This organic layer was washed three times with equal amounts of water and then dried over sodium sulfate. The crude 4-amino-substituted-2-chloroquinazoline was then purified by column chromatography using hexane and ethyl acetate (hexane/ethyl acetate = 5:1).

Procedure D: Amine Substitution of 4-Aminosubstituted-2-chloroquinazolines to Yield 2,4-Diamino-substituted Quinazolines

One equivalent of 4-aminosubstituted-2-chloroquinazoline and 1.5 equivalents of amine were combined with ethanol to create a 0.2 M solution which was heated to 150°C in a sealed tube. The reaction was monitored by TLC and LC-MS for the absence of starting material (8-18 hours). Solvent was evaporated and crude product was purified via column chromatography (dichloromethane/methanol = 10:1).

Procedure E: Arylation of Chloro-Substituted Quinazolines to Yield Aryl-Substituted Quinazolines

Mono-chloro-substituted quinazoline (0.37 mmol), boronic acid (0.37 mmol), and 5 mol % of tetrakis-palladium catalyst, were mixed with toluene (1.25 mL) and 1 M solution of disodium carbonate (0.25 mL) in a round bottom flask and kept at room temperature under argon. The progression of the reaction was monitored by TLC and LCMS until no starting material was observed. The reaction mixture was then diluted with dichloromethane (2.0 mL) and the organic layer was separated and washed with an equal volume of water three times and subsequently dried over sodium sulfate. Purification of the final product was completed by column chromatography (hexane/ethyl acetate = 5:1).

2.5.3 Compound Characterization

Compounds **2.1-2.31**, **2.45-2.47**, *N*⁴-benzyl-2-chloroquinazolin-4-amine, 2,4,6-trichloroquinazolin-4-amine, 2,4,7-trichloroquinazolin-4-amine, and 2,4-dichloro-6-methoxyquinazolin-4-amine have been previously reported.^{9,10}

***N*-benzyl-2-(4-methylpiperazin-1-yl)quinazolin-4-amine (2.32):** *N*²-benzyl-2-chloroquinazolin-4-amine 0.10 g (0.37 mmol) was reacted with 4-methylpiperazine and purified according to general procedure D to furnish 0.08 g of the title compound as a beige crystalline solid in 53 % yield. ¹H NMR (500 MHz, CD₃OD) δ 7.69 (dd, *J* = 8.3, 1.3 Hz, 1H), 7.50 (ddd, *J* = 8.1, 6.6, 1.3 Hz, 1H), 7.46 (dd, *J* = 8.5, 1.3 Hz, 1H), 7.33 – 7.28 (m, 4H), 7.26 – 7.22 (m, 1H), 7.08 (ddd, *J* = 8.2, 6.6, 1.4 Hz, 1H), 4.89 (s, 3H), 3.89 – 3.82 (m, 4H), 3.64 (dd, *J* = 6.2, 3.8 Hz, 4H), 3.52 (s, 2H). ¹³C NMR (126 MHz, CD₃OD) δ 152.0, 150.6, 143.1, 131.5, 124.2, 119.8, 118.8, 118.3, 116.0, 113.8, 113.0, 102.7, 46.3, 36.6, 36.1, 35.2. HRMS: *m/z* calculated for C₂₀H₂₃N₅ [M+H]⁺ 334.2026; found 334.2039. R_f = 0.26 (DCM/MeOH 10:1)

***N*-benzyl-2-(4-cyclohexylpiperazin-1-yl)quinazolin-4-amine (2.33):** *N*²-benzyl-2-chloroquinazolin-4-amine 90.0 mg (0.33 mmol) was reacted with 4-cyclohexylpiperazine and purified according to general procedure D to furnish 27.0 mg of the title compound as a beige crystalline solid in 20 % yield. ¹H NMR (500 MHz, CD₃OD) δ 7.91 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.53 (ddd, *J* = 8.4, 7.0, 1.5 Hz, 1H), 7.40 (dd, *J* = 8.5, 1.1 Hz, 1H), 7.38 – 7.34 (m, 2H), 7.28 (dd, *J* = 8.4, 6.8 Hz, 2H), 7.22 – 7.18 (m, 1H), 7.13 (ddd, *J* = 8.2, 6.9, 1.2 Hz, 1H), 4.76 (s, 2H), 3.82 (dd, *J* = 6.2, 4.0 Hz, 4H), 2.60 (t, *J* = 5.1 Hz, 4H), 2.32 (ddd, *J* = 11.1, 7.5, 3.3 Hz, 1H), 1.92 – 1.86 (m, 2H), 1.80 (dt, *J* = 12.2, 3.0 Hz, 2H), 1.30 – 1.09 (m, 6H). ¹³C NMR (126 MHz, CD₃OD) δ 152.1, 150.4, 142.8, 131.5, 124.4, 119.9, 118.9, 118.4, 115.8, 113.9, 113.21, 102.7, 55.8, 40.5, 36.13, 35.5, 20.0, 17.7, 17.3. HRMS: *m/z* calculated for C₂₅H₃₁N₅ [M+H]⁺ 402.2652; found 402.2660. R_f = 0.26 (DCM/MeOH 10:1)

***N*-benzyl-2-(phenylpiperazin-1-yl)quinazolin-4-amine (2.34):** *N*²-benzyl-2-chloroquinazolin-4-amine 90 mg (0.33 mmol) was reacted with phenylpiperazine and purified according to general procedure D to furnish 21.0 mg of the title compound as a beige crystalline solid in 21 % yield. ¹H NMR (500 MHz, CD₃OD) δ 7.91 – 7.88 (m, 1H), 7.55 – 7.51 (m, 1H), 7.42 – 7.36 (m, 3H), 7.30 – 7.27 (m, 2H), 7.24 – 7.18 (m, 3H), 7.13 – 7.09 (m, 1H), 6.97 – 6.93 (m, 2H), 6.85 – 6.81 (m, 1H), 4.77 (s, 2H), 3.95 – 3.91 (m, 4H), 3.09 (dd, *J* = 10.3, 5.1 Hz, 4H). ¹³C NMR (126 MHz, CD₃OD) δ 160.2, 158.8, 151.6, 151.2, 139.6, 132.4, 128.6, 128.0, 126.9, 126.5, 124.0, 121.9, 121.2, 119.9, 116.4, 110.8, 49.5, 44.2, 43.9. HRMS: *m/z* calculated for C₂₅H₂₅N₅ [M+H]⁺ 396.2183; found 396.2178. R_f = 0.41 (DCM/MeOH 10:1)

***N*-benzyl-2-(4-benzylpiperazin-1-yl)quinazolin-4-amine (2.35):** *N*²-benzyl-2-chloroquinazolin-4-amine 0.10 g (0.37 mmol) was reacted with 4-benzylpiperazine and purified according to general procedure D to furnish 38.0 mg of the title compound as a beige crystalline solid in 23 % yield. ¹H NMR (500 MHz, CD₃OD) δ 8.09 (dd, *J* = 8.2, 0.9 Hz, 1H), 7.71 (ddd, *J* = 8.4, 7.0, 1.4 Hz, 1H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.55 – 7.53 (m, 2H), 7.46 (dd, *J* = 10.4, 4.8 Hz, 2H), 7.38 (t, *J* = 7.3 Hz, 1H), 7.30 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 1H), 4.93 (s, 2H), 4.03 – 3.97 (m, 4H), 3.52 – 3.48 (m, 1H), 2.80 – 2.75 (m, 4H), 2.07 (d, *J* = 10.8 Hz, 2H), 1.97 (d, *J* = 12.3 Hz, 2H), 1.41 (d, *J* = 7.7 Hz, 2H). ¹³C NMR (126 MHz, CD₃OD) δ 160.2, 158.8, 151.6, 139.6, 132.4,

128.6, 128.0, 126.9, 126.5, 124.0, 122.0, 121.2, 119.9, 116.4, 110.8, 49.5, 44.2, 43.9. HRMS: m/z calculated for $C_{26}H_{27}N_5$ $[M+H]^+$ 410.2339; found 410.2344. $R_f = 0.28$ (DCM/MeOH 10:1)

***N*-benzyl-2-(4-(2,4-difluorophenyl)piperazin-1-yl)quinazolin-4-amine (2.36):** N^2 -benzyl-2-chloroquinazolin-4-amine 90.0 mg (0.33 mmol) was reacted with 2,4-difluorophenylpiperazine and purified according to general procedure D to furnish 87.0 mg of the title compound as a yellow crystalline solid in 50 % yield. 1H NMR (500 MHz, CD_3OD) δ 7.87 (dd, $J = 8.2, 1.4$ Hz, 1H), 7.47 (ddd, $J = 8.3, 6.8, 1.5$ Hz, 1H), 7.40 (dd, $J = 8.5, 1.2$ Hz, 1H), 7.35 – 7.31 (m, 2H), 7.23 (dd, $J = 8.4, 6.8$ Hz, 2H), 7.18 – 7.13 (m, 1H), 7.06 (ddd, $J = 8.1, 6.8, 1.3$ Hz, 1H), 6.87 – 6.79 (m, 2H), 6.73 (dddd, $J = 9.0, 7.9, 2.9, 1.3$ Hz, 1H), 4.73 (s, 2H), 3.92 – 3.87 (m, 4H), 2.90 – 2.84 (m, 4H). ^{13}C NMR (126 MHz, CD_3OD) δ 161.0, 159.9, 159.8, 158.0, 157.9, 157.6, 157.5, 155.6, 155.5, 152.4, 140.6, 137.7, 137.7, 137.6, 137.6, 133.2, 128.9, 127.9, 127.4, 125.2, 122.8, 122.0, 120.7, 120.7, 120.6, 120.6, 111.8, 111.3, 111.3, 111.2, 111.1, 105.1, 104.9, 104.9, 104.7, 51.7, 51.7, 45.2, 44.9. HRMS: m/z calculated for $C_{25}H_{23}F_2N_5$ $[M+H]^+$ 432.1994; found 432.2007. $R_f = 0.74$ (DCM/MeOH 10:1)

4-(4-benzylpiperazin-1-yl)-2-chloroquinazoline: Commercially available 2,4-dichloroquinazoline 0.31 g (1.76 mmol) was reacted with 4-benzylpiperazine and purified according to general procedure C to furnish 0.44 g of the title compound in 67 % yield. 1H NMR (500 MHz, CD_3OD) δ 8.02 (dd, $J = 8.4, 0.9$ Hz, 1H), 7.80 (ddd, $J = 8.4, 7.0, 1.3$ Hz, 1H), 7.69 (dd, $J = 8.4, 0.8$ Hz, 1H), 7.52 (ddd, $J = 8.3, 7.0, 1.3$ Hz, 1H), 7.40 – 7.32 (m, 4H), 7.30 – 7.26 (m, 1H), 3.98 – 3.94 (m, 4H), 3.61 (s, 2H), 2.69 – 2.64 (m, 4H). ^{13}C NMR (126 MHz, CD_3OD) δ 164.9, 156.0, 152.7, 137.0, 133.5, 129.2, 128.0, 127.1, 126.2, 125.6, 125.4, 114.1, 62.4, 52.5, 49.0. $R_f = 0.32$ (DCM/MeOH 10:1)

***N*-benzyl-4-(benzylpiperazin-1-yl)quinazolin-2-amine (2.37):** 4-(4-benzylpiperazin-1-yl)-2-chloroquinazoline 90.0 mg (0.27 mmol) was reacted with benzylamine and purified according to general procedure D to furnish 25.0 mg of the title compound as a beige crystalline solid in 32 % yield. 1H NMR (500 MHz, CD_3OD) δ 7.70 (dd, $J = 8.3, 0.9$ Hz, 1H), 7.51 (ddd, $J = 8.4, 6.9, 1.4$ Hz, 1H), 7.41 (d, $J = 8.3$ Hz, 1H), 7.34 – 7.29 (m, 6H), 7.25 (ddd, $J = 8.6, 6.7, 3.5$ Hz, 3H), 7.20 – 7.16 (m, 1H), 7.07 (ddd, $J = 8.2, 7.0, 1.2$ Hz, 1H), 4.61 (s, 2H), 3.67 – 3.62 (m, 4H), 3.49 (s, 2H), 2.51 (s, 4H). ^{13}C NMR (126 MHz, CD_3OD) δ 165.7, 158.7, 153.2, 136.9, 132.5, 129.3, 128.0, 128.0, 127.1, 126.8, 126.4, 125.3, 120.5, 62.5, 52.5, 49.1, 44.6. HRMS: m/z calculated for $C_{26}H_{27}N_5$ $[M+H]^+$ 410.2339; found 410.2353. $R_f = 0.13$ (DCM/MeOH 10:1)

2,4-bis(4-benzylpiperazin-1-yl)quinazoline (2.38): 4-(4-benzylpiperazin-1-yl)-2-chloroquinazoline 60.0 mg (0.18 mmol) was reacted with 4-benzylpiperazine and purified according to general procedure D to furnish 73.0 mg of the title compound as a beige crystalline solid in 74 % yield. 1H NMR (500 MHz, CD_3OD) δ 7.79 (d, $J = 8.1$ Hz, 1H), 7.57 (t, $J = 7.6$ Hz, 1H), 7.49 (d, $J = 8.3$ Hz, 1H), 7.39 (d, $J = 7.1$ Hz, 2H), 7.35 (t, $J = 7.5$ Hz, 2H), 7.30 – 7.23 (m, 3H), 7.15 (t, $J = 7.5$ Hz, 1H), 7.04 (d, $J = 8.1$ Hz, 2H), 6.87 (t, $J = 7.3$ Hz, 1H), 4.05 – 4.0 (m, 4H), 3.76 (s, 4H), 3.63 (s, 2H), 3.25 – 3.22 (m, 4H), 2.73 – 2.66 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.7, 158.2, 154.2, 151.5, 137.8, 132.5, 129.2, 128.4, 127.3, 126.1, 125.2, 120.7, 120.0, 116.5, 112.1, 63.1, 52.9, 49.8, 49.5, 44.1, 29.8. HRMS: m/z calculated for $C_{30}H_{34}N_6$ $[M+H]^+$ 479.6312; found 479.6330. $R_f = 0.56$ (DCM/MeOH 10:1)

4-(4-benzylpiperazin-1-yl)-2-(4-cyclohexylpiperazin-1-yl)quinazoline (2.39): 4-(4-benzylpiperazin-1-yl)-2-chloroquinazoline 60.0 mg (0.18 mmol) was reacted with 4-cyclohexylpiperazine and purified according to general procedure D to furnish 65.0 mg of the title compound as a beige crystalline solid in 65 % yield. ¹H NMR (500 MHz, CD₃OD) δ 7.88 (dd, *J* = 8.3, 1.0 Hz, 1H), 7.68 (ddd, *J* = 8.3, 6.8, 1.4 Hz, 1H), 7.63 (dd, *J* = 8.5, 1.1 Hz, 1H), 7.52 – 7.46 (m, 4H), 7.42 (ddd, *J* = 9.4, 4.0, 2.0 Hz, 1H), 7.26 (ddd, *J* = 8.2, 6.8, 1.3 Hz, 1H), 4.07 – 4.0 (m, 4H), 3.86 – 3.79 (m, 4H), 3.71 (s, 2H), 3.48 (dt, *J* = 3.3, 1.6 Hz, 1H), 2.83 – 2.74 (m, 8H), 2.07 (d, *J* = 10.5 Hz, 2H), 1.96 (d, *J* = 12.2 Hz, 2H), 1.48 – 1.35 (m, 5H). ¹³C NMR (126 MHz, CD₃OD) δ 165.5, 161.5, 157.9, 153.7, 137.0, 132.4, 129.2, 128.0, 127.1, 125.1, 120.7, 111.7, 63.8, 62.6, 52.5, 49.2, 48.7, 43.6, 28.1, 25.8, 25.5. HRMS: *m/z* calculated for C₂₉H₃₈N₆ [M+H]⁺ 471.3231; found 471.3232. R_f = 0.32 (DCM/MeOH 10:1)

4-(4-benzylpiperazin-1-yl)-2-(4-methylpiperazin-1-yl)quinazoline (2.40): 4-(4-benzylpiperazin-1-yl)-2-chloroquinazoline 60.0 mg (0.18 mmol) was reacted with 4-methylpiperazine and purified according to general procedure D to furnish 50.0 mg of the title compound as a beige crystalline solid in 59 % yield. ¹H NMR (500 MHz, CD₃OD) δ 7.70 (d, *J* = 8.2 Hz, 1H), 7.53 – 7.49 (m, 1H), 7.47 – 7.44 (m, 1H), 7.35 – 7.29 (m, 4H), 7.25 (dt, *J* = 8.3, 1.8 Hz, 1H), 7.08 (dd, *J* = 11.3, 3.8 Hz, 1H), 3.87 (s, 4H), 3.69 – 3.61 (m, 4H), 3.54 (s, 2H), 2.61 – 2.55 (m, 4H), 2.52 – 2.45 (m, 4H), 2.31 (s, 3H). ¹³C NMR (126 MHz, CD₃OD) δ 166.8, 159.2, 155.0, 138.3, 133.7, 130.5, 129.3, 128.4, 126.4, 126.4, 122.1, 112.9, 63.8, 55.8, 53.7, 50.5, 46.1, 44.6. HRMS: *m/z* calculated for C₂₄H₃₀N₆ [M+H]⁺ 403.2605; found 403.2610. R_f = 0.35 (DCM/MeOH 10:1)

4-(4-(4-benzylpiperazin-1-yl)quinazolin-2-yl)morpholine (2.41): 4-(4-benzylpiperazin-1-yl)-2-chloroquinazoline 60.0 mg (0.18 mmol) was reacted with morpholine and purified according to general procedure D to furnish 37.0 mg of the title compound as a beige crystalline solid in 54 % yield. ¹H NMR (500 MHz, DMSO) δ 7.73 (d, *J* = 8.2 Hz, 1H), 7.56 – 7.52 (m, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.33 (d, *J* = 4.3 Hz, 4H), 7.25 (dd, *J* = 8.5, 4.3 Hz, 1H), 7.10 (dd, *J* = 11.1, 4.0 Hz, 1H), 3.74 – 3.70 (m, 4H), 3.66 – 3.62 (m, 4H), 3.54 (s, 2H), 2.58 – 2.52 (m, 4H), 2.48 (dd, *J* = 5.0, 3.3 Hz, 4H). ¹³C NMR (126 MHz, DMSO) δ 165.4, 158.1, 154.1, 138.4, 133.0, 129.4, 128.7, 127.5, 126.2, 125.8, 121.2, 111.9, 66.6, 62.5, 52.8, 49.8, 44.6. HRMS: *m/z* calculated for C₂₃H₂₇N₅O [M+H]⁺ 390.2288; found 390.2295.

2,6-dichloro-*N*-methylquinazolin-4-amine: 0.13 g (0.54 mmol) of 2,4,6-trichloroquinazoline was reacted with methylamine and purified according to general procedure C to furnish 0.11 g of the title compound in 92 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.69 (d, *J* = 9.1 Hz, 1H), 7.37 (dd, *J* = 9.1, 2.6 Hz, 1H), 6.97 (d, *J* = 2.7 Hz, 1H), 3.86 (s, 3H), 3.22 (d, *J* = 4.8 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 161.2, 158.9, 151.5, 139.6, 126.9, 123.5, 122.2, 111.7, 28.6. R_f = 0.82 (DCM/MeOH 10:1)

***N*²-benzyl-6-chloro-*N*⁴-methylquinazoline-2,4-diamine (2.42):** 0.10 g (0.44 mmol) of 2,6-dichloro-*N*-methylquinazolin-4-amine was reacted with benzylamine and purified according to general procedure D to furnish 87.0 mg of the title compound as a white crystalline solid in 45 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.48 – 7.44 (m, 2H), 7.44 – 7.37 (m, 3H), 7.33 (t, *J* = 7.6 Hz, 2H), 7.27 (q, *J* = 3.4, 2.4 Hz, 1H), 4.73 (d, *J* = 5.9 Hz, 2H), 3.09 (d, *J* = 4.8 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 160.0, 159.6, 150.5, 139.8, 133.0, 128.5, 127.7, 127.3, 127.1, 125.8,

120.2, 111.9, 45.6, 28.1. HRMS: m/z calculated for C₁₆H₁₅ClN₄ [M+H]⁺ 299.7701; found 299.7703. R_f = 0.33 (DCM/MeOH 10:1)

2,7-dichloro-N-methylquinazolin-4-amine: 0.20 g (0.86 mmol) of 2,4,7-trichloroquinazoline was reacted with methylamine and purified according to general procedure C to furnish 0.17 g of the title compound in 72 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.72 (d, J = 2.0 Hz, 1H), 7.62 (d, J = 8.8 Hz, 1H), 7.39 (dd, J = 8.7, 2.0 Hz, 1H), 3.22 (d, J = 4.9 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 161.2, 158.9, 151.5, 139.6, 126.9, 122.2, 123.5, 111.7, 28.6. R_f = 0.80 (DCM/MeOH 10:1)

N²-benzyl-7-chloro-N⁴-methylquinazoline-2,4-diamine (2.43): 0.15 g (0.66 mmol) of 2,7-dichloro-N-methylquinazolin-4-amine was reacted with benzylamine and purified according to general procedure D to furnish 66.0 mg of the title compound as a white crystalline solid in 68 % yield. ¹H NMR (500 MHz, DMSO) δ 8.21 – 8.11 (m, 1H), 8.08 (d, J = 8.5 Hz, 1H), 7.51 (d, J = 7.8 Hz, 2H), 7.44 (t, J = 7.8 Hz, 2H), 7.35 (q, J = 7.5, 6.3 Hz, 2H), 7.18 (d, J = 8.6 Hz, 1H), 4.70 (d, J = 6.4 Hz, 2H), 3.53 (s, 1H), 3.09 (d, J = 4.5 Hz, 3H). ¹³C NMR (126 MHz, DMSO) δ 160.9, 153.5, 141.7, 137.4, 128.7, 128.0, 127.0, 125.4, 124.2, 120.7, 111.0, 110.8, 44.6, 28.2. HRMS: m/z calculated for C₁₆H₁₅ClN₄ [M+H]⁺ 299.7701; found 299.7698. R_f = 0.38 (DCM/MeOH 10:1)

2-chloro-6-methoxy-N-methylquinazolin-4-amine: 0.10 g (0.44 mmol) of 2,4-dichloro-6-methoxyquinazoline was reacted with methylamine and purified according to general procedure C to furnish 81.0 mg of the title compound in 83 % yield. ¹H NMR (500 MHz, CDCl₃) δ 8.12 (d, J = 7.5 Hz, 1H), 7.56 (dd, J = 7.5, 1.5 Hz, 1H), 6.78 (d, J = 1.4 Hz, 1H), 3.87 (s, 2H), 2.91 (s, 2H), 2.30 (s, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 160.7, 158.5, 154.2, 148.7, 127.3, 121.8, 110.5, 109.0, 55.8, 28.3. R_f = 0.48 (DCM/MeOH 10:1)

N²-benzyl-6-methoxy-N⁴-methylquinazoline-2,4-diamine (2.44): 70.0 mg (0.24 mmol) of 2-chloro-6-methoxy-N-methylquinazolin-4-amine was reacted with benzylamine and purified according to general procedure D to furnish 30.0 mg of the title compound as a white crystalline solid in 45 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.45 (d, J = 9.1 Hz, 1H), 7.41 (d, J = 7.1 Hz, 2H), 7.32 (t, J = 7.6 Hz, 2H), 7.28 – 7.21 (m, 2H), 6.87 (d, J = 2.7 Hz, 1H), 4.73 (d, J = 5.7 Hz, 2H), 3.83 (s, 3H), 3.11 (d, J = 4.7 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 160.3, 158.5, 154.2, 146.7, 140.2, 128.4, 127.7, 127.0, 126.9, 123.1, 111.0, 101.4, 55.7, 45.7, 28.1. HRMS: m/z calculated for C₁₇H₁₈N₄ [M+H]⁺ 295.3510; found 295.3509. R_f = 0.26 (DCM/MeOH 10:1)

2.6 References

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Chapter 3: Quinazolines as Potential Antibacterial Agents

3.1 Synthetic Chemistry

For the general synthesis of the series N^2, N^4 -disubstituted quinazoline-2,4-diamines please refer to section 2.1.

Synthesis of N^2 -benzyl-6-bromo- N^4 -methylquinazoline-2,4-diamine followed procedures outlined in Figure 2.1. The series of N^2 -benzyl- N^4 -methyl-6-alken-yl-quinazoline-2,4-diamine, N^2 -benzyl- N^4 -methyl-6-aryl-quinazoline-2,4-diamine, and N^2 -benzyl- N^4 -methyl-6-alkan-yl-quinazoline-2,4-diamine were synthesized following reported procedures (Figure 3.1).¹ Suzuki-Miyaura Cross-Coupling conditions with tetrakis-palladium, saturated sodium bicarbonate, and commercially available boronic acids, under argon, with **3.A** afforded both the N^2 -benzyl- N^4 -methyl-6-alken-yl-quinazoline-2,4-diamine and N^2 -benzyl- N^4 -methyl-6-aryl-quinazoline-2,4-diamine, which were purified by column chromatography and had proton spectra, carbon spectra, and HRMS characterization completed. The subsequent hydrogenation of **3.B** with palladium on carbon in methanol under 1 atm at room temperature gave the products of N^2 -benzyl- N^4 -methyl-6-alkan-yl-quinazoline-2,4-diamine with which purification by column chromatography and characterization as described above was conducted.

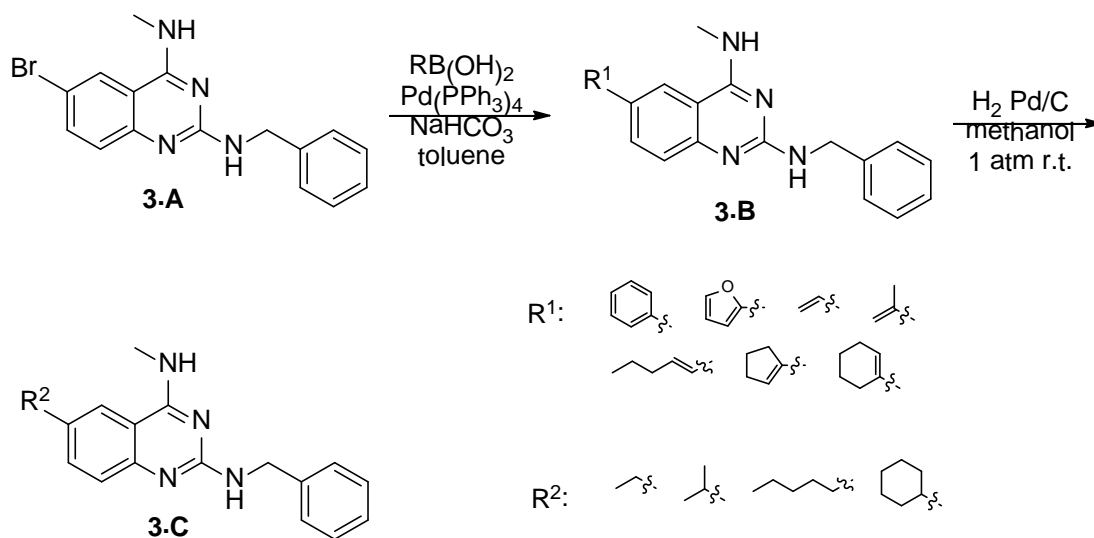
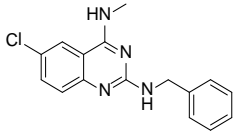
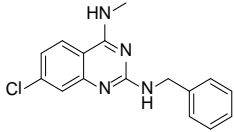
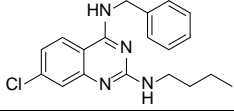
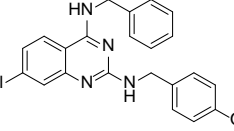
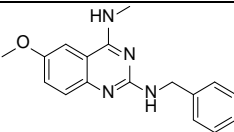
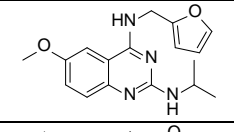
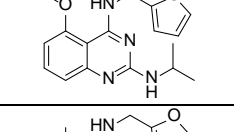
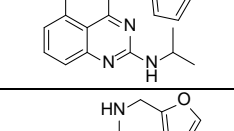
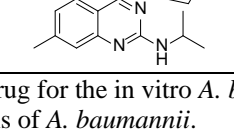


Figure 3.1 Synthesis of N^2 -benzyl- N^4 -methyl-6-alken-yl-quinazoline-2,4-diamine, N^2 -benzyl- N^4 -methyl-6-aryl-quinazoline-2,4-diamine, and N^2 -benzyl- N^4 -methyl-6-alkan-yl-quinazoline-2,4-diamine

3.2 Initial Anti-Bacterial Activity

The initial success of the N^2, N^4 -disubstituted quinazoline-2,4-diamine compound class as antibacterial agents against MRSA motivated us to question the potential of these compounds to target other multi-drug resistant bacteria.² This initiated the testing of top frontrunner compounds from previously reported, antibacterial and antileishmanial N^2, N^4 -disubstituted quinazoline-2,4-diamines to be tested against six different clinical isolates of multi-drug resistant *A. baumannii* strains leading to the construction of tables 3.1 and 3.2.²⁻⁴ Compounds **3.1**, **3.2**, and **3.5** have not been published but were previously discussed in the follow-up SAR conducted for chapter 2. In both tables little to no activity was reported for most compounds against the *A. baumannii* strain 1403. However compounds **3.1**, **3.2**, and **3.5** displayed modest antibacterial activity at single digit micromolar MIC concentrations against less resistant strains. These results indicate the potential of quinazoline series to develop a new SAR focusing *A. baumannii*.

Table 3.1: Study Focusing on the Benzoid Ring Substitution of quinazoline-2,4-diamines²⁻⁴

Compound	R	1403 MIC μM ^a	1646 MIC μM	1649 MIC μM	1650 MIC μM	1651 MIC μM	1652 MIC μM	IC ₅₀ THP-1 (μM) ^b
3.1		50	2	25	15	6	20	ND ^c
3.2		>50	6	30	>50	10	50	ND
3.3		>25	ND	ND	ND	ND	ND	ND
3.4		>25	ND	ND	ND	ND	ND	ND
3.5		>50	2	25	50	10	30	ND
3.6		>25	ND	ND	ND	ND	ND	ND
3.7		>25	ND	ND	ND	ND	ND	ND
3.8		>25	ND	ND	ND	ND	ND	ND
3.9		>25	ND	ND	ND	ND	ND	ND

^a The control drug for the in vitro *A. baumannii* assay is Tigecycline, which displays an MIC= 0.85 μM against all tested strains of *A. baumannii*.

^b Tetracycline is the control compound for the in vitro cytotoxicity assay exhibiting an IC₅₀ = 148.5 μM against the THP-1 cell-line.

^c Not determined.

Table 3.2: Study Focusing on the 2,4-position of quinazoline-2,4-diamines²⁻⁴

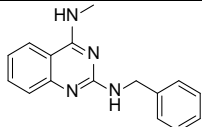
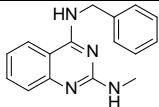
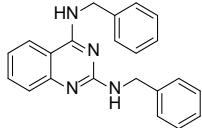
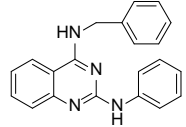
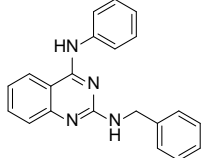
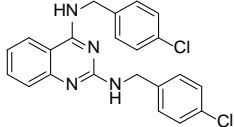
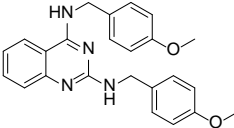
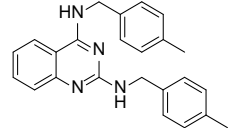
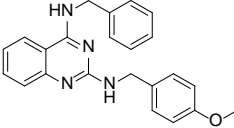
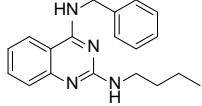
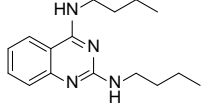
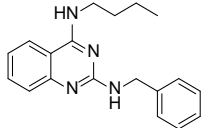
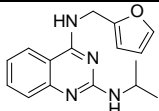
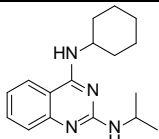
Compound	R	1403 MIC μM ^a	1646 MIC μM	1649 MIC μM	1650 MIC μM	1651 MIC μM	1652 MIC μM	IC ₅₀ THP-1 (μM) ^b
3.10		>50	50	>50	>50	>50	>50	>50
3.11		>50	50	>50	>50	>50	>50	>50
3.12		>25	ND ^c	ND	ND	ND	ND	ND
3.13		>25	ND	ND	ND	ND	ND	ND
3.14		>25	ND	ND	ND	ND	ND	ND
3.15		>25	ND	ND	ND	ND	ND	ND
3.16		>25	ND	ND	ND	ND	ND	ND
3.17		>25	ND	ND	ND	ND	ND	ND
3.18		>25	ND	ND	ND	ND	ND	ND
3.19		>25	ND	ND	ND	ND	ND	ND
3.20		>25	ND	ND	ND	ND	ND	ND

Table 3.2 (Continued)

3.21		>25	ND	ND	ND	ND	ND	ND
3.22		>25	ND	ND	ND	ND	ND	ND
3.23		>25	ND	ND	ND	ND	ND	ND

^aThe control drug for the in vitro *A. baumannii* assay is Tigecycline, which displays an MIC= 0.85 μ M against all tested strains of *A. baumannii*.

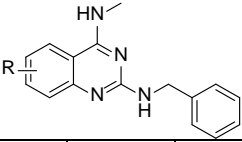
^bTetracycline is the control compound for the in vitro cytotoxicity assay, exhibiting an IC₅₀= 148.5 μ M against the THP-1 cell-line.

^c Not determined.

3.3 Structure-Activity Relationship

Table 3.3 was developed with the intention of varying the benzoid ring substitution at the 6 or 7-position for continuation of compounds **3.1**, **3.2**, and **3.5** series. Compounds **3.24** and **3.27** kept similar activity against all strains as compared with the reference compounds **3.1**, **3.2**, and **3.5**. Compound **3.26** displayed decreased activity in all tested strains when compared to other reported compounds in this table. There was however compound **3.25** with increased potency against the 1403 strain, when compared to the reference compounds. Until this point, the most active compound against the 1403 strain had an MIC of 50 μ M, whereas **3.25** was at least five times more potent while also having an EC₅₀ concentration of 12.9 μ M for the THP-1 cell line. THP-1 is a human monocyte cell line derived from an acute monocytic leukemia patient.⁵ Cytotoxicity was also determined for compound **3.24** (EC₅₀ = 6.1 μ M) but was found to be almost twice as toxic in comparison to **3.25**.

Table 3.3: Probing Benzoid Ring Substitution of *N*²-benzyl-*N*⁴-methyl-quinazolines-2,4-diamines

								
Compound	R	1403 MIC μM ^a	1646 MIC μM	1649 MIC μM	1650 MIC μM	1651 MIC μM	1652 MIC μM	IC ₅₀ THP-1 (μM) ^b
3.24	6-Br	50	2	12	15	8	20	6.1
3.25	6-Me	10	2	25	50	10	20	12.9
3.26	7-Br	>50	6	25	>50	10	>50	ND ^c
3.27	7-Me	50	12	25	50	12	35	ND

^a The control drug for the in vitro *A. baumannii* assay is Tigecycline, which displays an MIC= 0.85 μM against all tested strains of *A. baumannii*.

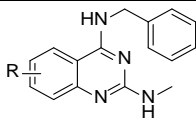
^b Tetracycline is the control compound for the in vitro cytotoxicity assay, exhibiting an IC₅₀= 148.5 μM against the THP-1 cell-line.

^c Not determined.

Tables 3.4 and 3.5 were developed to determine the effects on activity when variation of the amine in the 2 or 4-position occurred. Table 3.4 scaffold now contains the benzylamine at the 4-position and the methylamine at the 2-position. With this different 2,4-substitution, as compared with Table 3.3, almost all activity in every tested strain, except 1646 and 1651, was lost. This dramatic loss in activity across the multiple strains indicates that the 2-benzylamine and 4-methylamine substitution could possibly be a key to the compounds activity against *A. baumannii*. The synthesized compounds in Table 3.5 studied the extension of the aromatic ring substituent from a 2-benzylamine to a 2-phenethylamine and its effects on the activity against the six strains. This substitution caused a loss of activity in all strains, except 1646 and 1652, further

indicating that the *N*²-methyl-*N*⁴-benzyl-quinazolines-2,4-diamine scaffold is key to antibacterial activity with these strains.

Table 3.4: Probing Benzoid Ring Substitution of *N*²-methyl- *N*⁴-benzyl-quinazolines-2,4-diamines

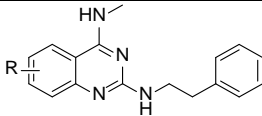
								
Compound	R	1403 MIC μM ^a	1646 MIC μM	1649 MIC μM	1650 MIC μM	1651 MIC μM	1652 MIC μM	IC ₅₀ THP-1 (μM) ^b
3.28	6-Cl	>50	4	50	50	6	>50	ND ^c
3.29	6-Br	>50	2	50	>50	>50	>50	ND
3.30	6-Me	>50	6	>50	>50	12	>50	ND
3.31	6-OMe	>50	6	>50	>50	8	>50	ND
3.32	7-Cl	>50	4	>50	>50	>50	>50	ND
3.33	7-Br	>50	4	>50	>50	>50	>50	ND
3.34	7-Me	>50	12	>50	>50	>50	>50	ND

^a The control drug for the in vitro *A. baumannii* assay is Tigecycline, which displays an MIC= 0.85 μM against all tested strains of *A. baumannii*.

^b Tetracycline is the control compound for the in vitro cytotoxicity assay, exhibiting an IC₅₀ = 148.5 μM against the THP-1 cell-line.

^c Not determined.

Table 3.5: Probing the benzoid ring of *N*⁴-methyl- *N*²-phenethyl-quinazolin-2,4-diamines

								
Compound	R	1403 MIC μM ^a	1646 MIC μM	1649 MIC μM	1650 MIC μM	1651 MIC μM	1652 MIC μM	IC ₅₀ THP-1 (μM) ^b
3.35	6-H	>50	12	>50	>50	>50	>50	ND ^c
3.36	6-Cl	>50	2	>50	>50	>50	>50	ND
3.37	6-Ome	>50	4	>50	>50	>50	>50	ND
3.38	6-Br	>50	2	>50	>50	10	>50	ND
3.39	6-Me	>50	2	>50	>50	10	>50	ND

^a The control drug for the in vitro *A. baumannii* assay is Tigecycline, which displays an MIC= 0.85 μM against all tested strains of *A. baumannii*.

^b Tetracycline is the control compound for the in vitro cytotoxicity assay, exhibiting an IC₅₀ = 148.5 μM against the THP-1 cell-line.

^c Not determined.

So far, compound **3.25** has been the compound with the best *in vitro* activity against strain 1403 and it was therefore selected for further development leading to the synthetic development of Table 3.6, which aims to explore the extension of substituents at the 6-position of the benzoid ring. Due to synthetic restrictions discussed in section 3.1, the generation of the alkenyl substituted quinazolines as synthetic intermediates was first needed, which were hydrogenated in the subsequent step to yield alkyl-substituted quinazolines. A noticeable trend was observed with compounds **3.44-3.50** with large and non-planar side chains being more potent. Compound **3.44** with a 6-pentenyl (MIC = 10 μM, strain 1403) compared to 6-pentyl-substituted analogue **3.45**, (MIC = 2 μM, strain 1403) had a five fold increase in activity.

Similarly, an increase in potency was observed starting from the aromatic 6-phenyl-quinazoline **3.49**, to quinazolines with the 6-cyclohexenyl **3.47** and the 6-cyclohexyl **3.48** substitution against all strains. The identification of the top compounds **3.44**, **3.45**, **3.47**, and **3.48** were selected for cytotoxicity testing. IC₅₀ concentrations from **3.44** and **3.45** remained relatively the same (5.6 μM and 5.4 μM respectively). However there was an increase in IC₅₀ concentrations from 2.3 μM for compound **3.47** to 11.1 μM for compound **3.48** proving better selectivity with the most potent compound generated by this SAR.

Table 3.6: Probing of the 6-position of N²-benzyl-N⁴-methyl-quinazolines-2,4-diamines

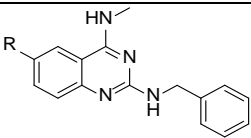











								
Compound	R	1403 MIC μM ^a	1646 MIC μM	1649 MIC μM	1650 MIC μM	1651 MIC μM	1652 MIC μM	IC ₅₀ THP-1 (μM) ^b
Reference 3.24	6-Me	10	2	25	50	10	20	12.9
3.40		25	2	10	20	25	25	ND ^c
3.41		30	2	10	30	2	30	ND
3.42		> 50	2	25	> 50	10	> 50	ND
3.43		>50	25	35	>50	35	>50	ND
3.44		10	2	10	25	2	30	5.6
3.45		2	2	2	2	2	10	5.4

Table 3.6 (Continued)

3.46		> 50	2	50	> 50	30	> 50	ND
3.47		2	2	10	10	2	30	2.3
3.48		2	2	2	2	2	10	11.1
3.49		30	10	20	20	2	50	ND
3.50		40	25	> 50	> 50	15	> 50	ND

^a The control drug for the in vitro *A. baumannii* assay is Tigecycline, which displays an MIC= 0.85 μ M against all tested strains of *A. baumannii*.

^b Tetracycline is the control compound for the in vitro cytotoxicity assay, exhibiting an IC₅₀ = 148.5 μ M against the THP-1 cell-line.

^c Not determined.

3.4 Summary

From the 23 previously reported compounds **3.1-3.23**, three *N*²,*N*⁴-disubstituted quinazoline-2,4-diamines **3.1**, **3.2**, and **3.5** stood out as a good initial hits for the development of the *A. baumannii* antibacterial structure-activity relationship study. Exploring the different substitutions at the 6- or 7-position of the benzoid ring led to the identification of compound **3.25** with an activity in the low micromolar range for the majority of the multi-drug resistant *A. baumannii* strains tested. Further variation of the 6-position side chain developed four new compounds **3.44**, **3.45**, **3.47**, and **3.48**, with single digit micromolar MIC concentrations for at least half of the tested strains. The identification of *N*²,*N*⁴-disubstituted quinazoline-2,4-diamines with single digit micromolar MIC concentrations from the initial SAR shows promise for the further development these compounds as antibacterial agents against *A. baumannii*.

3.5 Experimental Section

3.5.1 General Information

All commercially available chemical reagents, except for the boronic acids used, and anhydrous solvents were purchased from either Sigma Aldrich, Oakwood Products, Inc. or TCI America and used without any further purification. Boronic acids used were purchased through Frontier Scientific. NMR spectra were recorded at ambient temperature on a 500 MHz Varian NMR spectrometer in the solvent indicated. All ^1H NMR experiments are reported in δ units, parts per million (ppm) downfield of TMS and were measured relative to the signals for chloroform (7.26 ppm), methanol (3.31 ppm) and dimethyl sulfoxide (2.50 ppm). All ^{13}C NMR spectra were reported in ppm relative to the signals for chloroform (77 ppm), methanol (49 ppm) and dimethyl sulfoxide (39.5 ppm) with ^1H decoupled observation. Data for ^1H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, sext = sextet, sept = septet, oct = octet m = multiplet), integration and coupling constant (Hz), whereas ^{13}C NMR analyses were reported in terms of chemical shift. NMR data was analyzed by using MestReNova Software ver. 5.3.2-4936. High resolution mass spectra (HRMS) were performed on an Agilent LC/MSD TOF system G3250AA. Analytical thin layer chromatography (TLC) was performed on silica gel 60 F254 pre-coated plates (0.25 mm) from EMD Chemical Inc. and components were visualized by ultraviolet light (254 nm). Silicycle silica gel 230-400 (particle size 40-63 μm) mesh was used for all flash column chromatography.

3.5.2 General Procedures

Procedure A: Suzuki-Miyaura Cross Coupling with *N*²-benzyl-*N*⁴-methyl-6-bromo-quinazolin-2,4-diamine to yield *N*²-benzyl-*N*⁴-methyl-6-alken-yl-quinazolin-2,4-diamine and *N*²-benzyl-*N*⁴-methyl-6-aryl-quinazolin-2,4-diamine

One equivalent of *N*²-benzyl-*N*⁴-methyl-6-bromo-quinazolin-2,4-diamine, 1.5 equivalents of the boronic acid, 5 mol-% of tetrakis-palladium, a saturated solution of sodium bicarbonate, and anhydrous dimethoxyethane were combined in a sealed microwave tube, under argon, and heated in the microwave to 150°C. The reaction was monitored by TLC and LCMS until no starting material was observed. The reaction was cooled to room temperature, diluted with dichloromethane. The organic layer was separated and washed with equal volume of water three times, then dried over sodium sulfate. Purification of final product was completed by column chromatography using dichloromethane and methanol (dichloromethane/methanol = 10:1).

Procedure B: Hydrogenation of *N*²-benzyl-*N*⁴-methyl-6-alken-yl-quinazolin-2,4-diamine to yield *N*²-benzyl-*N*⁴-methyl-6-alkan-yl-quinazolin-2,4-diamine

One equivalent of *N*²-benzyl-*N*⁴-methyl-6-alken-yl-quinazolin-2,4-diamine was combined with one equivalent of palladium on carbon and hydrogen gas in methanol to afford a 2mg/mL solution. The reaction was monitored by LC-MS until no starting material was present. The reaction was filtered over celite and rinsed with three equal volumes of methanol.

Procedure C: Cyclization of Anthranilic Acids to the Corresponding Quinazolin-2,4-diones

One equivalent of the commercially available anthranilic acid and three equivalents of urea were combined in a mortar and pestle until a homogenous mixture was obtained. This powder was then transferred to a round bottom flask and heated to 200°C uncovered. After 3 hours the mixture was cooled, 10 mL of water was added, the solid filtered and subsequently

washed with 40 mL of water. Crude product was dried and no further purification was completed.

Procedure D: Chlorination of Quinazoline-2,4-diones to the Corresponding 2,4-Dichloroquinazoline

One equivalent of quinazoline-2,4-dione and one equivalent of *N,N*-dimethylaniline were combined in a round bottom flask, 12 equivalents of phosphorus oxychloride was then added. The mixture was refluxed under argon until the presence of starting material was no longer seen by TLC or by LC-MS (6-24 hours). Upon completion the reaction mixture was cooled and slowly added over ice, amount of ice equaled to ten times that of the reaction volume. Upon precipitation the reaction was filtered and washed with water to afford the crude 2,4-dichloroquinazoline which was purified by column chromatography using hexane and ethyl acetate (hexane/ethyl acetate = 5:1).

Procedure E: Amine Substitution of 2,4-Dichloroquinazolines to Yield 4-Amino-substituted-2-chloroquinazoline

One equivalent of the crude 2,4-dichloroquinazoline, 1.1 equivalents of sodium acetate, and 1.1 equivalents of selected primary amine were combined in a round bottom flask and mixed with a three to one solution of tetrahydrofuran and water to afford a 0.1 M solution. The reaction was heated to 65°C and monitored until no starting material was seen by TLC or LC-MS. The reaction was diluted with ethyl acetate and the organic layer separated. This organic layer was washed three times with equal amounts of water and then dried over sodium sulfate. The crude 4-amino-substituted-2-chloroquinazoline was then purified by column chromatography using hexane and ethyl acetate (hexane/ethyl acetate = 5:1).

Procedure F: Amine Substitution of 4-Aminosubstituted-2-chloroquinazolines to Yield 2,4-Diamino-substituted Quinazolines

One equivalent of 4-aminosubstituted-2-chloroquinazoline and 1.5 equivalents of amine were combined with ethanol to create a 0.2 M solution which was heated to 150°C in a sealed tube. The reaction was monitored by TLC and LC-MS for the absence of starting material (8-18 hours). Solvent was evaporated and crude product was purified via column chromatography (dichloromethane/methanol 10:1).

3.5.3 Compound Characterization

Compounds **3.1**, **3.2**, and **3.5** were reported as **2.48**, **2.49**, **2.50** and 2,4-dichloro-6-methylquinazoline, 2,4-dichloro-7-methylquinazoline, 2,4,6-trichloroquinazoline, 2,4,7-trichloroquinazoline, and 2,4-dichloro-6-methoxyquinazoline were reported in the previous chapter. Compounds **3.3**, **3.4**, **3.6-3.23**, *N*-benzyl-2,6-dichloroquinazolin-4-amine, and 2-chloro-*N*-methylquinazolin-4-amine were previously reported.^{2,3}

6-bromo-2,4-dichloroquinazoline: 3.0 g (13.9 mmol) of commercially available 2-amino-5-bromobenzoic acid was reacted according to general procedure C to give crude 6-bromoquinazoline-2,4(1*H*,3*H*)-dione. Without further purification, 3.40 g (14.12 mmol) of crude 6-bromoquinazoline-2,4(1*H*,3*H*)-dione was reacted and purified according to general procedure D to give 1.18 g of the title compound as a beige solid in 30 % yield. ¹H NMR (500 MHz, CDCl₃) δ 8.41 (d, *J* = 2.1 Hz, 1H), 8.05 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.88 (d, *J* = 8.9 Hz, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 162.8, 155.4, 151.0, 139.7, 129.6, 128.2, 123.4, 123.2. R_f = 0.88 (DCM/MeOH 10:1)

6-bromo-2-chloro-*N*-methylquinazolin-4-amine: 0.10 g (0.36 mmol) of 6-bromo-2,4-dichloroquinazoline was reacted with methylamine and purified according to general procedure E to furnish 89.0 mg of the title compound in 91 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.82 (d, *J* = 2.0 Hz, 1H), 7.79 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.64 (d, *J* = 8.8 Hz, 1H), 5.98 (s, br, 1H), 3.22 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 160.5, 158.1, 149.4, 136.8, 129.6, 123.4, 119.3, 114.6, 28.7. R_f = 0.54 (DCM/MeOH 10:1)

***N*²-benzyl-6-bromo-*N*⁴-methylquinazoline-2,4-diamine (3.24):** 80.0 mg (0.29 mmol) of 6-bromo-2-chloro-*N*-methylquinazolin-4-amine was reacted with benzylamine and purified according to general procedure F to furnish 78.0 mg of the title compound as a white crystalline solid in 78 % yield. ¹H NMR (500 MHz, DMSO) δ 8.34 (d, *J* = 2.5 Hz, 1H), 8.16 (s, 1H), 7.71 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.50 (d, *J* = 7.7 Hz, 2H), 7.46 – 7.40 (m, 2H), 7.36 – 7.29 (m, 2H), 4.70 (s, 2H), 3.08 (s, 3H). HRMS: *m/z* calculated for C₁₆H₁₅BrN₄ [M+H]⁺ 343.0553; found 343.0544. R_f = 0.51 (DCM/MeOH 10:1)

2-chloro-*N*,6-dimethylquinazolin-4-amine: 0.15 g (0.70 mmol) of 2,4-dichloro-6-methylquinazoline was reacted with methylamine and purified according to general procedure E to furnish 95.0 mg of the title compound in 66 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.63 (d, *J* = 8.5 Hz, 1H), 7.53 (d, *J* = 8.5 Hz, 1H), 7.45 (s, 1H), 6.13 (s, br, 1H), 3.20 (d, *J* = 4.9 Hz, 3H), 2.45 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 161.2, 157.0, 148.9, 136.3, 135.2, 127.4, 120.0, 113.2, 28.5, 21.6. R_f = 0.44 (DCM/MeOH 10:1)

***N*²-benzyl-*N*⁴,6-dimethylquinazoline-2,4-diamine (3.25):** 80.0 mg (0.38 mmol) of 2-chloro-*N*,6-dimethylquinazolin-4-amine was reacted with benzylamine and purified according to general procedure F to furnish 47.0 mg of the title compound as a beige crystalline solid in 57 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.42 – 7.38 (m, 3H), 7.36 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.34 – 7.23 (m, 4H), 4.74 (s, 2H), 3.09 (d, *J* = 4.8 Hz, 3H), 2.38 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 160.5, 158.9, 149.4, 140.1, 134.4, 130.6, 128.4, 127.7, 126.9, 125.1, 120.3, 111.0, 45.6, 28.0, 21.2. HRMS: *m/z* calculated for C₁₇H₁₈N₄ [M+H]⁺ 279.1604; found 279.1607. R_f = 0.55 (DCM/MeOH 10:1)

7-bromo-2,4-dichloroquinazoline: 5.0 g (23.2 mmol) of commercially available 2-amino-6-bromobenzoic acid was reacted according to general procedure C to give crude 7-bromoquinazoline-2,4(1*H*,3*H*)-dione. Without further purification, 5.20 g (21.6 mmol) of crude 7-bromoquinazoline-2,4(1*H*,3*H*)-dione was reacted and purified according to general procedure D to give 2.82 g of the title compound as a beige solid in 47 % yield. ¹H NMR (500 MHz, CDCl₃) δ 8.19 (d, *J* = 1.7 Hz, 1H), 8.12 (d, *J* = 8.9 Hz, 1H), 7.83 (dd, *J* = 8.9, 1.7 Hz, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 164.0, 156.2, 152.8, 133.0, 131.6, 130.5, 127.2, 121.1. R_f = 0.90 (DCM/MeOH 10:1)

7-bromo-2-chloro-*N*-methylquinazolin-4-amine: 0.10 g (0.36 mmol) of 7-bromo-2,4-dichloroquinazoline was reacted with methylamine and purified according to general procedure E to furnish 95.0 mg of the title compound in 97 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.94 (d, *J* = 1.8 Hz, 1H), 7.56 (dd, *J* = 8.7, 1.9 Hz, 1H), 7.52 (d, *J* = 8.7 Hz, 1H), 5.98 (s, 1H), 3.24 (d, *J* = 4.9 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 161.3, 158.8, 151.6, 130.4, 129.6, 128.0, 122.0, 112.0, 28.6. R_f = 0.35 (DCM/MeOH 10:1)

***N*²-benzyl-7-bromo-*N*⁴-methylquinazoline-2,4-diamine (3.26):** 90.0 mg (0.33 mmol) of 7-bromo-2-chloro-*N*-methylquinazolin-4-amine was reacted with benzylamine and purified according to general procedure F to furnish 58.0 mg of the title compound as a white crystalline solid in 51 % yield. ¹H NMR (500 MHz, CD₃OD) δ 7.69 (d, *J* = 8.7 Hz, 1H), 7.50 (s, 1H), 7.38 (d, *J* = 7.9 Hz, 2H), 7.30 (t, *J* = 7.7 Hz, 2H), 7.22 (d, *J* = 7.4 Hz, 1H), 7.19 (dd, *J* = 8.7, 2.0 Hz, 1H), 4.67 (s, 2H), 3.03 (s, 3H). ¹³C NMR (126 MHz, DMSO) δ 161.7, 160.8, 153.8, 141.9, 129.0, 128.2, 127.5, 127.3, 126.6, 125.6, 123.6, 111.4, 44.8, 28.5. HRMS: *m/z* calculated for C₁₆H₁₅BrN₄ [M+H]⁺ 343.0553; found 343.0548. R_f = 0.59 (DCM/MeOH 10:1)

2-chloro-*N*⁴,7-dimethylquinazolin-4-amine: 0.15 g (0.70 mmol) of 2,4-dichloro-7-methylquinazoline was reacted with methylamine and purified according to general procedure E to furnish 0.13 g of the title compound in 86 % yield. ¹H NMR (500 MHz, CDCl₃) δ 8.12 (d, *J* = 8.6 Hz, 1H), 7.75 (s, 1H), 7.55 (dd, *J* = 8.6, 1.3 Hz, 1H), 3.21 (s, 3H), 2.61 (s, 3H). ¹³C NMR

(126 MHz, CDCl₃) δ 161.5, 157.8, 150.8, 144.3, 128.0, 127.0, 120.6, 111.2, 28.5, 21.9. R_f = 0.38 (DCM/MeOH 10:1)

N²-benzyl-N⁴,7-dimethylquinazoline-2,4-diamine (3.27): 0.11 g (0.53 mmol) of 2-chloro-N,7-dimethylquinazolin-4-amine was reacted with benzylamine and purified according to general procedure F to furnish 0.13 g of the title compound as a beige crystalline solid in 88 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.60 (d, *J* = 8.4 Hz, 1H), 7.51 (s, 1H), 7.25 (dd, *J* = 8.4, 1.7 Hz, 1H), 3.20 (d, *J* = 4.8 Hz, 3H), 4.80 (s, 2H), 2.48 (s, 3H). HRMS: *m/z* calculated for C₁₇H₁₈N₄ [M+H]⁺ 279.1604; found 279.1598. R_f = 0.49 (DCM/MeOH 10:1)

N⁴-benzyl-6-chloro-N²-methylquinazoline-2,4-diamine (3.28): 80.0 mg (0.26 mmol) of *N*-benzyl-2,6-dichloroquinazolin-4-amine was reacted with methylamine and purified according to general procedure F to furnish 72.0 mg of the title compound as a beige crystalline solid in 67 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.49 – 7.36 (m, 7H), 7.34 (dd, *J* = 5.8, 2.7 Hz, 1H), 4.79 (s, 2H), 3.06 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 160.3, 159.0, 150.8, 138.3, 133.1, 129.0, 128.8, 128.0, 127.7, 127.3, 125.7, 120.2, 45.2, 28.4. HRMS: *m/z* calculated for C₁₆H₁₅ClN₄ [M+H]⁺ 299.1058; found 299.1058. R_f = 0.28 (DCM/MeOH 10:1)

N-benzyl-6-bromo-2-chloroquinazolin-4-amine: 0.10 g (0.36 mmol) of 6-bromo-2,4-dichloroquinazoline was reacted with benzylamine and purified according to general procedure E to furnish 0.13 g of the title compound in 99 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.84 – 7.79 (m, 2H), 7.67 (d, *J* = 8.8 Hz, 1H), 7.44 – 7.36 (m, 5H), 6.01 (s, br, 1H), 4.86 (d, *J* = 5.3 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 159.6, 158.0, 149.7, 138.3, 136.9, 129.7, 129.0, 128.4, 128.3, 123.4, 119.4, 114.4, 46.0. R_f = 0.60 (DCM/MeOH 10:1)

N⁴-benzyl-6-bromo-N²-methylquinazoline-2,4-diamine (3.29): 0.12 g (0.34 mmol) *N*-benzyl-6-bromo-2-chloroquinazolin-4-amine was reacted with methylamine and purified according to general procedure F to furnish 92.0 mg of the title compound as a beige crystalline solid in 88 % yield. ¹H NMR (500 MHz, DMSO) δ 8.65 (s, br, 1H), 8.48 (s, 1H), 7.75 (d, *J* = 8.9, 1H), 7.55 – 7.40 (m, 5H), 6.81 (s, br, 1H), 4.88 (d, *J* = 5.8 Hz, 2H), 2.98 (t, *J* = 3.6 Hz, 3H). ¹³C NMR (126 MHz, DMSO) δ 160.8, 159.6, 151.9, 140.4, 135.7, 129.0, 128.3, 127.7, 127.5, 125.9, 113.3, 112.0, 44.2, 28.6. HRMS: *m/z* calculated for C₁₆H₁₅BrN₄ [M+H]⁺ 343.0553; found 343.0549. R_f = 0.52 (DCM/MeOH 10:1)

N-benzyl-2-chloro-6-methylquinazolin-4-amine: 0.10 g (0.47 mmol) of 2,4-dichloro-6-methylquinazoline was reacted with benzylamine and purified according to general procedure E to furnish 0.13 g of the title compound in 86 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.68 (s, 1H), 7.56 (d, *J* = 8.4 Hz, 1H), 7.45 – 7.32 (m, 6H), 6.05 (s, br, 1H), 4.86 (s, 2H), 2.47 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 160.3, 156.9, 149.2, 137.4, 136.39, 135.4, 131.8, 128.9, 128.4, 127.6, 119.9, 113.0, 45.7, 21.6. R_f = 0.61 (DCM/MeOH 10:1)

N⁴-benzyl-N²,6-dimethylquinazoline-2,4-diamine (3.30): 0.11 g (0.41 mmol) *N*-benzyl-2-chloro-6-methylquinazolin-4-amine was reacted with methylamine and purified according to general procedure F to furnish 92.0 mg of the title compound as a beige crystalline solid in 81 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.39 – 7.28 (m, 8H), 4.81 (d, *J* = 5.4 Hz, 2H), 3.05 (d, *J* = 5.1 Hz, 3H), 2.37 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 159.8, 159.6, 150.1, 138.8, 134.4,

130.5, 128.7, 128.0, 127.5, 125.4, 120.1, 110.6, 45.0, 28.5, 21.2. HRMS: m/z calculated for $C_{17}H_{18}N_4$ [M+H]⁺ 279.1604; found 279.1595. R_f = 0.55 (DCM/MeOH 10:1)

***N*-benzyl-2-chloro-6-methoxyquinazolin-4-amine:** 0.10 g (0.51 mmol) of 2,4-dichloro-6-methoxyquinazoline was reacted with benzylamine and purified according to general procedure E to furnish 0.12 g of the title compound in 93 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.69 (d, J = 9.1 Hz, 1H), 7.38 (m, 6H), 6.95 (d, J 2.4 Hz, 1H), 6.16 (s, br, 1H), 4.86 (d, J = 5.2 Hz, 2H), 3.86 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 160.1, 157.7, 155.5, 146.1, 137.5, 129.3, 128.9, 128.4, 128.0, 124.4, 113.6, 100.5, 55.8, 45.8. R_f = 0.63 (DCM/MeOH 10:1)

***N*⁴-benzyl-6-methoxy-*N*²-methylquinazoline-2,4-diamine (3.31):** 0.10 g (0.33 mmol) *N*-benzyl-2-chloro-6-methoxyquinazolin-4-amine was reacted with methylamine and purified according to general procedure F to furnish 85.0 mg of the title compound as a beige crystalline solid in 78 % yield. ¹H NMR (500 MHz, DMSO) δ 8.74 (s, br, 1H), 7.82 (s, 1H), 7.61 (d, J = 6.7 Hz, 2H), 7.57 – 7.38 (m, 5H), 6.66 (s, 1H), 4.96 (s, 2H), 4.02 (s, 3H), 3.02 (s, 3H). ¹³C NMR (126 MHz, DMSO) δ 160.2, 159.2, 154.3, 146.5, 140.8, 129.1, 128.4, 127.6, 126.2, 123.9, 111.6, 104.2, 56.5, 44.3, 28.9. HRMS: m/z calculated for $C_{17}H_{18}N_4O$ [M+H]⁺ 295.1553; found 295.1543. R_f = 0.29 (DCM/MeOH 10:1)

***N*-benzyl-2,7-dichloroquinazolin-4-amine:** 0.20 g (0.86 mmol) of 2,4,7-trichloroquinazoline was reacted with benzylamine and purified according to general procedure E to furnish 0.26 g of the title compound in 98 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.74 (s, 1H), 7.62 (d, J = 8.8 Hz, 1H), 7.42 – 7.32 (m, 6H), 6.18 (s, br, 1H), 4.85 (d, J = 5.2 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 160.4, 158.8, 151.7, 139.8, 137.0, 129.0, 128.4, 128.2, 127.1, 127.0, 122.2, 111.5, 45.9. R_f = 0.66 (DCM/MeOH 10:1)

***N*⁴-benzyl-7-chloro-*N*²-methylquinazoline-2,4-diamine (3.32):** 0.25 g (0.82 mmol) *N*-benzyl-2,7-dichloroquinazolin-4-amine was reacted with methylamine and purified according to general procedure F to furnish 77.0 mg of the title compound as a beige crystalline solid in 48/ % yield. ¹H NMR (500 MHz, CD₃OD) δ 7.85 (d, J = 8.7 Hz, 1H), 7.42 – 7.28 (m, 5H), 7.21 (d, J = 10.6, 1H), 7.03 (d, J = 8.7, 1H), 4.78 (s, 2H), 2.93 (s, 3H). ¹³C NMR (126 MHz, CD₃OD) δ 160.7, 160.0, 152.2, 139.3, 138.2, 128.0, 127.3, 126.6, 123.8, 122.2, 120.8, 109.5, 43.9, 27.1. HRMS: m/z calculated for $C_{16}H_{15}ClN_4$ [M+H]⁺ 299.1058; found 299.1046. R_f = 0.38 (DCM/MeOH 10:1)

***N*-benzyl-7-bromo-2-chloroquinazolin-4-amine:** 0.10 g (0.36 mmol) of 7-bromo-2,4-dichloroquinazoline was reacted with benzylamine and purified according to general procedure E to furnish 0.12 g of the title compound in 97 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.94 (s, 1H), 7.53 (s, 2H), 7.43 – 7.33 (m, 5H), 6.1 (s, br, 1H) 4.85 (d, J = 5.3 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 159.6, 158.0, 149.7, 138.3, 136.9, 129.7, 129.0, 128.4, 128.3, 123.4, 119.4, 114.4, 45.9. R_f = 0.71 (DCM/MeOH 10:1)

***N*⁴-benzyl-7-bromo-*N*²-methylquinazoline-2,4-diamine (3.33):** 0.11 g (0.33 mmol) *N*-benzyl-7-bromo-2-chloroquinazolin-4-amine was reacted with methylamine and purified according to general procedure F to furnish 90.0 mg of the title compound as a beige crystalline solid in 79 % yield. ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.70 (s, 1H), 8.20 (d, J = 9.0 Hz, 1H), 7.56 (dd, J =

27.3, 7.7 Hz, 5H), 7.41 (dd, $J = 29.9, 7.6$ Hz, 2H), 6.91 (s, 1H), 4.92 (s, 2H), 3.71 (s, 1H), 3.01 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 161.1, 160.4, 154.2, 140.5, 129.1, 128.3, 127.6, 126.7, 125.8, 123.4, 120.6, 111.1, 44.2, 28.7. HRMS: m/z calculated for $\text{C}_{16}\text{H}_{15}\text{BrN}_4$ $[\text{M}+\text{H}]^+$ 343.0553; found 343.0543. $R_f = 0.58$ (DCM/MeOH 10:1)

***N*-benzyl-2-chloro-7-methylquinazolin:** 0.10 g (0.47 mmol) of 2,4-dichloro-7-methylquinazoline was reacted with benzylamine and purified according to general procedure E to furnish 0.12 g of the title compound in 96 % yield. ^1H NMR (500 MHz, CDCl_3) δ 7.57 (d, $J = 8.4$ Hz, 1H), 7.54 (s, 1H), 7.42 – 7.31 (m, 5H), 7.27 – 7.24 (m, 1H), 4.85 (d, $J = 5.3$ Hz, 2H), 2.49 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 160.6, 157.7, 151.1, 144.5, 137.4, 128.9, 128.3, 128.1, 127.2, 120.6, 111.0, 45.7, 28.4, 21.9. $R_f = 0.68$ (DCM/MeOH 10:1)

***N*⁴-benzyl-*N*²,7-dimethylquinazoline-2,4-diamine (3.34):** 0.10 g (0.35 mmol) *N*-benzyl-2-chloro-7-methylquinazolin was reacted with methylamine and purified according to general procedure F to furnish 77.0 mg of the title compound as a beige crystalline solid in 76 % yield. ^1H NMR (500 MHz, CDCl_3) δ 7.41 – 7.34 (m, 5H), 7.31 (d, $J = 6.9$ Hz, 2H), 6.89 (d, $J = 8.2$ Hz, 1H), 4.80 (d, $J = 5.4$ Hz, 2H), 3.06 (d, $J = 5.0$ Hz, 3H), 2.41 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 160.3, 159.8, 152.2, 143.2, 138.8, 128.7, 128.0, 127.5, 125.1, 122.8, 120.6, 108.8, 45.0, 28.4, 21.8. HRMS: m/z calculated for $\text{C}_{17}\text{H}_{18}\text{N}_4$ $[\text{M}+\text{H}]^+$ 279.1604; found 279.1597. $R_f = 0.47$ (DCM/MeOH 10:1)

***N*⁴-methyl-*N*²-phenethylquinazoline-2,4-diamine (3.35):** 0.10 g (0.52 mmol) of 2-chloro-*N*-methylquinazolin-4-amine was reacted with phenethylamine and purified according to general procedure F to furnish 55.0 mg of the title compound as a beige crystalline solid in 55 % yield. ^1H NMR (500 MHz, CDCl_3) δ 8.08 – 8.02 (m, 2H), 7.78 (td, $J = 7.5, 1.5$ Hz, 1H), 7.52 (td, $J = 7.5, 1.5$ Hz, 1H), 7.31 – 7.23 (m, 2H), 7.26 (s, 3H), 7.27 – 7.16 (m, 1H), 3.58 (s, 1H), 3.51 (t, $J = 5.2$ Hz, 2H), 2.91 (s, 2H), 2.79 – 2.73 (m, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 160.9, 159.4, 151.1, 139.6, 132.7, 128.9, 128.6, 126.2, 124.7, 121.4, 121.1, 111.3, 42.9, 36.3, 28.0. HRMS: m/z calculated for $\text{C}_{17}\text{H}_{18}\text{N}_4$ $[\text{M}+\text{H}]^+$ 278.1531; found 278.1529. $R_f = 0.40$ (DCM/MeOH 10:1)

6-chloro-*N*⁴-methyl-*N*²-phenethylquinazoline-2,4-diamine (3.36): 90.0 mg (0.39 mmol) 2,6-dichloro-*N*-methylquinazolin-4-amine was reacted with phenethylamine and purified according to general procedure F to furnish 55.0 mg of the title compound as a beige crystalline solid in 48 % yield. ^1H NMR (400 MHz, CDCl_3) δ 7.44 – 7.40 (m, 2H), 7.35 (d, $J = 9.4$ Hz, 1H), 7.31 – 7.24 (m, 4H), 7.23 – 7.17 (m, 1H), 3.08 (d, $J = 4.8$ Hz, 3H), 2.93 (t, $J = 7.2$ Hz, 2H), 1.22 (t, $J = 7.0$ Hz, 2H). ^{13}C NMR (126 MHz, CDCl_3) δ 160.0, 159.7, 150.5, 139.6, 133.0, 128.9, 128.5, 127.1, 126.3, 125.6, 120.3, 111.8, 42.8, 36.2, 28.1. HRMS: m/z calculated for $\text{C}_{17}\text{H}_{17}\text{ClN}_4$ $[\text{M}+\text{H}]^+$ 312.8073; found 312.7996. $R_f = 0.38$ (DCM/MeOH 10:1)

6-methoxy-*N*⁴-methyl-*N*²-phenethylquinazoline-2,4-diamine (3.37): 60.0 mg (0.20 mmol) 2,4-dichloro-6-methoxyquinazoline was reacted with phenethylamine and purified according to general procedure F to furnish 17.0 mg of the title compound as a beige crystalline solid in 23 % yield. ^1H NMR (500 MHz, CDCl_3) δ 7.43 (d, $J = 9.1$ Hz, 1H), 7.31 (t, $J = 7.4$ Hz, 2H), 7.29 – 7.26 (m, 2H), 7.22 (dt, $J = 9.1, 2.6$ Hz, 2H), 6.88 (d, $J = 2.7$ Hz, 1H), 3.80 – 3.75 (m, 2H), 3.14 (d, $J = 4.7$ Hz, 3H), 2.96 (t, $J = 7.2$ Hz, 2H). ^{13}C NMR (126 MHz, CDCl_3) δ 160.3, 158.2, 154.3,

145.8, 139.6, 128.9, 128.6, 128.5, 126.2, 123.2, 110.8, 101.6, 55.7, 42.9, 36.3, 28.2. HRMS: m/z calculated for $C_{18}H_{20}N_4$ $[M+H]^+$ 232.1868; found 232.1869. $R_f = 0.29$ (DCM/MeOH 10:1)

6-bromo- N^4 -methyl- N^2 -phenethylquinazoline-2,4-diamine (3.38): 65.0 mg (0.24 mmol) 6-bromo-2-chloro- N -methylquinazolin-4-amine was reacted with phenethylamine and purified according to general procedure F to furnish 31.0 mg of the title compound as a beige crystalline solid in 37 % yield. 1H NMR (500 MHz, $CDCl_3$) δ 7.64 (d, $J = 2.2$ Hz, 1H), 7.57 (dd, $J = 8.9, 2.2$ Hz, 1H), 7.32 (t, $J = 7.5$ Hz, 3H), 7.29 – 7.25 (m, 2H), 7.25 – 7.21 (m, 1H), 3.81 – 3.73 (m, 2H), 3.11 (d, $J = 4.6$ Hz, 3H), 2.96 (t, $J = 7.2$ Hz, 2H). HRMS: m/z calculated for $C_{17}H_{17}BrN_4$ $[M+H]^+$ 357.0714; found 357.0714. $R_f = 0.59$ (DCM/MeOH 10:1)

$N^4,6$ -dimethyl- N^2 -phenethylquinazoline-2,4-diamine (3.39): 75.0 mg (0.36 mmol) 2-chloro- $N,6$ -dimethylquinazolin-4-amine was reacted with phenethylamine and purified according to general procedure F to furnish 49.0 mg of the title compound as a beige crystalline solid in 46 % yield. 1H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, $J = 8.3$ Hz, 3H), 7.35 (d, $J = 8.5$ Hz, 5H), 7.33 – 7.28 (m, 9H), 7.26 (d, $J = 7.4$ Hz, 6H), 7.21 (d, $J = 6.5$ Hz, 7H), 3.78 (s, 6H), 3.09 (s, 9H), 2.96 (dd, $J = 13.7, 6.5$ Hz, 10H), 2.34 (s, 9H). HRMS: m/z calculated for $C_{18}H_{20}N_4O$ $[M+H]^+$ 293.1764; found 293.1761. $R_f = 0.34$ (DCM/MeOH 10:1)

N^2 -benzyl- N^4 -methyl-6-vinylquinazoline-2,4-diamine (3.40): 50.0 mg (0.14 mmol) of N^2 -benzyl-6-bromo- N^4 -methylquinazoline-2,4-diamine was reacted with commercially available vinyl boronic acid dibutyl ester and purified according to general procedure A to furnish 13.0 mg of the title compound as a yellow solid in 31 % yield. 1H NMR (500 MHz, CD_3OD) δ 7.85 (d, $J = 1.9$ Hz, 1H), 7.69 (dd, $J = 8.7, 1.9$ Hz, 1H), 7.40 – 7.36 (m, 2H), 7.32 – 7.28 (m, 3H), 7.24 – 7.20 (m, 1H), 6.75 (dd, $J = 17.6, 11.0$ Hz, 1H), 5.82 – 5.76 (m, 1H), 5.22 (d, $J = 11.0$ Hz, 1H), 4.68 (s, 2H), 3.05 (s, 3H). HRMS: m/z calculated for $C_{18}H_{18}N_4$ $[M+H]^+$ 291.1636; found 291.1633. $R_f = 0.33$ (DCM/MeOH 10:1)

N^2 -benzyl-6-ethyl- N^4 -methylquinazoline-2,4-diamine (3.41): 10.0 mg (0.03 mmol) of N^2 -benzyl- N^4 -methyl-6-vinylquinazoline-2,4-diamine was reacted with palladium on carbon and purified according to general procedure B to furnish 7.0 mg of the title compound as a yellow solid in 70 % yield. 1H NMR (500 MHz, $CDCl_3$) δ 7.45 – 7.38 (m, 4H), 7.34 – 7.29 (m, 3H), 7.25 (d, $J = 7.0$ Hz, 1H), 4.73 (d, $J = 4.7$ Hz, 2H), 3.10 (d, $J = 4.7$ Hz, 3H), 2.68 (d, $J = 7.6$ Hz, 2H), 1.27 – 1.25 (m, 3H). HRMS: m/z calculated for $C_{18}H_{20}N_4$ $[M+H]^+$ 293.1765; found 293.1768.

N^2 -benzyl- N^4 -methyl-6-(prop-1-en-2-yl)quinazoline-2,4-diamine (3.42): 80.0 mg (0.23 mmol) of N^2 -benzyl-6-bromo- N^4 -methylquinazoline-2,4-diamine was reacted with commercially available isopentyl pinacol boronic ester and purified according to general procedure A to furnish 22.0 mg of the title compound as a yellow solid in 31 % yield. 1H NMR (500 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.60 (d, $J = 8.8$ Hz, 1H), 7.32 (d, $J = 7.7$ Hz, 2H), 7.28 – 7.23 (m, 3H), 7.21 (d, $J = 7.2$ Hz, 1H), 5.46 (s, 1H), 5.06 (s, 1H), 4.64 (s, 2H), 3.08 (s, 3H), 2.16 (s, 3H). HRMS: m/z calculated for $C_{19}H_{20}N_4$ $[M+H]^+$ 305.1765; found 305.1766.

N^2 -benzyl-6-isopropyl- N^4 -methylquinazoline-2,4-diamine (3.43): 10.0 mg (0.03 mmol) of N^2 -benzyl- N^4 -methyl-6-(prop-1-en-2-yl)quinazoline-2,4-diamine was reacted with palladium on

carbon and purified according to general procedure B to furnish 5.0 mg of the title compound as a yellow solid in 50 % yield. ¹H NMR (500 MHz, CD₃OD) δ 7.83 (d, J = 1.9 Hz, 1H), 7.59 (dd, J = 8.5, 1.9 Hz, 1H), 7.40 (d, J = 7.2 Hz, 2H), 7.33 (t, J = 7.6 Hz, 3H), 7.27 – 7.23 (m, 1H), 4.72 (s, 2H), 3.0 (p, J = 6.9 Hz, 1H), 1.31 (s, 3H), 1.30 (s, 3H). HRMS: m/z calculated for C₁₉H₂₂N₄ [M+H]⁺ 307.1643; found 307.1643.

(E)-N²-benzyl-N⁴-methyl-6-(pent-1-en-1-yl)quinazoline-2,4-diamine (3.44): 80.0 mg (0.23 mmol) of N²-benzyl-6-bromo-N⁴-methylquinazoline-2,4-diamine was reacted with commercially available (E)-1-Pentenyl pinacol boronic ester and purified according to general procedure A to furnish 24.0 mg of the title compound as a yellow solid in 31 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.62 (s, 1H), 7.53 (t, J = 7.1 Hz, 1H), 7.36 (d, J = 8.0 Hz, 3H), 7.31 – 7.26 (m, 2H), 7.23 (t, J = 5.0 Hz, 1H), 6.34 (dd, J = 16.0, 3.8 Hz, 1H), 6.21 (dt, J = 14.2, 6.7 Hz, 1H), 4.68 (s, 2H), 3.08 (d, J = 4.0 Hz, 3H), 2.15 (t, J = 6.5 Hz, 2H), 1.51 – 1.43 (m, 2H), 1.27 (s, 1H), 0.93 (td, J = 7.4, 3.4 Hz, 3H). HRMS: m/z calculated for C₂₁H₂₄N₄ [M+H]⁺ 333.2064; found 333.2064.

N²-benzyl-N⁴-methyl-6-pentylquinazoline-2,4-diamine (3.45): 11.0 mg (0.03 mmol) of (E)-N²-benzyl-N⁴-methyl-6-(pent-1-en-1-yl)quinazoline-2,4-diamine was reacted with palladium on carbon and purified according to general procedure B to furnish 7.0 mg of the title compound as a yellow solid in 70 % yield. ¹H NMR (500 MHz, CD₃OD) δ 7.67 (d, J = 1.8 Hz, 1H), 7.43 (dd, J = 8.5, 1.9 Hz, 1H), 7.38 (d, J = 7.2 Hz, 2H), 7.30 (td, J = 8.5, 8.0, 2.5 Hz, 3H), 7.24 – 7.20 (m, 1H), 4.68 (s, 2H), 3.05 (s, 3H), 2.67 (t, J = 7.7 Hz, 2H), 1.70 – 1.62 (m, 3H), 1.38 – 1.32 (m, 4H), 0.91 (t, J = 6.9 Hz, 3H). HRMS: m/z calculated for C₂₁H₂₆N₄ [M+H]⁺ 335.2083; found 335.2083.

N²-benzyl-6-(cyclopent-1-en-1-yl)-N⁴-methylquinazoline-2,4-diamine (3.46): 80.0 mg (0.23 mmol) of N²-benzyl-6-bromo-N⁴-methylquinazoline-2,4-diamine was reacted with commercially available 1-Cyclopentenylboronic acid pinacol ester and purified according to general procedure A to furnish 8.0 mg of the title compound as a yellow solid in 10 % yield. ¹H NMR (500 MHz, CDCl₃) δ 7.74 (dd, J = 8.7, 2.0 Hz, 1H), 7.41 (dd, J = 8.0, 3.8 Hz, 4H), 7.32 (t, J = 7.4 Hz, 2H), 7.27 (d, J = 2.9 Hz, 1H), 6.18 (t, J = 2.3 Hz, 1H), 4.74 (d, J = 4.9 Hz, 2H), 3.12 (d, J = 4.6 Hz, 3H), 2.76 – 2.70 (m, 2H), 2.56 (ddt, J = 7.7, 5.1, 2.5 Hz, 2H), 2.08 – 2.02 (m, 2H). HRMS: m/z calculated for C₂₁H₂₂N₄ [M+H]⁺ 331.1999; found 331.1995.

N²-benzyl-6-(cyclohex-1-en-1-yl)-N⁴-methylquinazoline-2,4-diamine (3.47): 80.0 mg (0.14 mmol) of N²-benzyl-6-bromo-N⁴-methylquinazoline-2,4-diamine was reacted with commercially available cyclohexenyl boronic acid and purified according to general procedure A to furnish 11.0 mg of the title compound as a yellow solid in 14 % yield. ¹H NMR (500 MHz, CD₃OD) δ 7.85 (d, J = 2.0 Hz, 1H), 7.68 (dd, J = 8.7, 2.1 Hz, 1H), 7.39 (d, J = 7.2 Hz, 2H), 7.30 (dt, J = 9.9, 7.7 Hz, 3H), 7.25 – 7.21 (m, 1H), 6.19 (dt, J = 4.1, 2.2 Hz, 1H), 4.69 (s, 2H), 3.07 (s, 3H), 2.46 (ddt, J = 6.3, 4.2, 2.1 Hz, 2H), 2.27 – 2.21 (m, 2H), 1.85 – 1.79 (m, 2H), 1.72 – 1.66 (m, 3H). HRMS: m/z calculated for C₂₂H₂₄N₄ [M+H]⁺ 345.2078; found 345.2077.

N²-benzyl-6-cyclohexyl-N⁴-methylquinazoline-2,4-diamine (3.48): 10.0 mg (0.03 mmol) of N²-benzyl-6-(cyclohex-1-en-1-yl)-N⁴-methylquinazoline-2,4-diamine was reacted with palladium on carbon and purified according to general procedure B to furnish 8.0 mg of the title compound as a yellow solid in 80 % yield. ¹H NMR (500 MHz, CD₃OD) δ 7.70 (d, J = 1.9 Hz, 1H), 7.46 (dd, J = 8.6, 2.0 Hz, 1H), 7.39 (d, J = 7.2 Hz, 2H), 7.30 (dd, J = 8.5, 6.7 Hz, 3H), 7.24 – 7.20 (m,

1H), 4.68 (s, 2H), 3.05 (s, 3H), 2.57 (ddd, J = 11.5, 8.3, 3.2 Hz, 1H), 1.88 (ddt, J = 8.9, 6.2, 3.0 Hz, 4H), 1.48 (dddd, J = 19.2, 12.8, 10.4, 3.3 Hz, 4H), 1.35 – 1.30 (m, 2H). HRMS: m/z calculated for C₂₂H₂₆N₄ [M+H]⁺ 347.2235; found 347.2231.

N²-benzyl-N⁴-methyl-6-phenylquinazoline-2,4-diamine (3.49): 50.0 mg (0.14 mmol) of N²-benzyl-6-bromo-N⁴-methylquinazoline-2,4-diamine was reacted with commercially available phenyl boronic acid and purified according to general procedure A to furnish 11.0 mg of the title compound as a yellow solid in 22 % yield. ¹H NMR (500 MHz, CD₃OD) δ 8.19 – 8.16 (m, 1H), 7.91 – 7.87 (m, 1H), 7.68 (d, J = 7.7 Hz, 2H), 7.48 – 7.40 (m, 5H), 7.34 (q, J = 7.6 Hz, 3H), 7.25 (d, J = 7.3 Hz, 1H), 4.72 (s, 2H), 3.09 (s, 3H). HRMS: m/z calculated for C₂₂H₂₀N₄ [M+H]⁺ 341.1763; found 341.1763. R_f = 0.36 (DCM/MeOH 10:1)

N²-benzyl-6-(furan-2-yl)-N⁴-methylquinazoline-2,4-diamine (3.50): 50.0 mg (0.14 mmol) of N²-benzyl-6-bromo-N⁴-methylquinazoline-2,4-diamine was reacted with commercially available 2-furyl boronic acid and purified according to general procedure A to furnish 6.0 mg of the title compound as a yellow solid in 13 % yield. ¹H NMR (500 MHz, CD₃OD) δ 8.20 (d, J = 1.9 Hz, 1H), 7.88 (dd, J = 8.8, 1.9 Hz, 1H), 7.56 (d, J = 1.8 Hz, 1H), 7.40 (d, J = 7.2 Hz, 2H), 7.36 (d, J = 8.8 Hz, 1H), 7.31 (t, J = 7.7 Hz, 2H), 7.23 (t, J = 7.4 Hz, 1H), 6.76 (d, J = 3.3 Hz, 1H), 6.53 (dd, J = 3.4, 1.8 Hz, 1H), 4.69 (s, 2H), 3.07 (s, 3H). HRMS: m/z calculated for C₂₀H₁₈N₄O [M+H]⁺ 331.1551; found 331.1549. R_f = 0.43 (DCM/MeOH 10:1)

3.6 References

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Chapter 4: Future Plans

4.1 2,4-disubstituted Quinazolines as Antileishmanials

After the initial SAR conducted by the Manetsch lab in 2014 a secondary SAR was completed.¹ Quinazoline-2,4-diamines with aromatic substituents at both N^2 and N^4 exhibited potent *in vitro* antileishmanial activity but relatively low selectivity, while compounds substituted with small alkyl groups at either N^2 or N^4 generally showed lower antileishmanial potency but were less toxic to a murine macrophage cell line.² Based on their *in vitro* antileishmanial potency, N^4 -benzyl- N^2 -(4-chlorobenzyl)quinazoline-2,4-diamine (**2.6**) and N^2 -benzyl- N^4 -isopropylquinazoline-2,4-diamine (**2.31**) were selected for *in vivo* evaluation of their pharmacokinetic and antileishmanial properties. While **2.6** displayed a longer plasma half-life and a greater area under the curve than **2.31**, both compounds showed low efficacy in an acute murine visceral leishmaniasis model.² Although there is still some hindrance in dosing caused by the toxicity of the compounds in *in vivo* murine models, preliminary data from hamster studies shows a promising lead with decreased toxicity, for the animal, at higher concentrations for the quinazoline class. The expanded SAR conducted found that the introduction of a piperazine moiety can help increase the antileishmanial selectivity index but does decrease the EC_{50} activity within the *L. donovani in vitro* testing. Further investigation of these molecules to increase their efficacy while retaining their selectivity could yield promising analogues with more favorable properties, which would be useful for further biological testing of this series.

4.2 2,4-Disubstituted Quinazolines as Antibacterials

Top compounds from previously reported 2,4-disubstituted-quinazolin-2,4-amines with the Manetsch lab were tested against *A. baumannii* to determine the initial series with which an SAR can be constructed.¹⁻³ With the development of an initial SAR dedicated to antibacterial activity against multi-drug resistant *A. baumannii* identification of six compounds, **3.24**, **3.25**, **3.44**, **3.45**, **3.47**, and **3.48**, with low to single digit micromolar MIC concentrations against all tested strains were identified. Substitution changes varying from the N^2 -methyl- N^4 -benzyl-quinazolines-2,4-diamine scaffold decreased efficacy of the compounds. Whereas the addition of a large, non-planar side chains to the 6-position helped to increase both efficacy and antibacterial selectivity as seen in **3.48**. Cytotoxicity data for the top six compounds indicated little selectivity over the THP-1 cell line. Improvement of this antibacterial selectivity while retaining and/or improvement of the efficacy are required for the further biological study of these compounds. Compound **3.25** and **3.48** are potential candidates for further biological testing and *in vivo* testing due to their low single digit MIC concentrations along with the highest IC_{50} values. Further expansion of this SAR with substitutions in the 5- or 8-position could be studied as well as introducing larger, non-planer substituents in the 6-position to determine if this could aid in the selectivity problem or improve the efficacy.

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Appendices

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