2010

Synthesis of chrysosplenol B, chrysosplenol D, and their analogs and synthesis of 3-(carboxymethylthio)-picolinic acid and its analog

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Synthesis of chrysosplenol B, chrysosplenol D, and their analogs and synthesis of 3-(carboxymethylthio)-picolinic acid and its analog

by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

Major: Organic Chemistry

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2010

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GENERAL INTRODUCTION

Synthesis of natural products or their analogs is interesting and challenging work. During the course of the investigation, chemists can not only prepare various kinds of bioactive compounds, but also discover new synthetic methodologies. The first chapter of this thesis deals with the development of modified methods to synthesize chrysosplenol B, chrysosplenol D, and their analogs. Both chrysosplenol B and chrysosplenol D have the flavone skeleton with 3-methoxyl and 5-hydroxyl groups. They have ability to potentiate the potent anti-malarial activity of artemisinin and have ability to potentiate the activity of norfloxacin. The second chapter deals with the synthetic effort towards 3-(carboxymethylthio)-picolinic acid and a functionalized analog. They were synthesized in order to determine the minimum structural requirements for activity. This may enable researchers to study the mechanisms of molecular recognition of phosphoenolpyruvate (PEP) and oxaloacetate (OAA) by cytosolic phosphoenolpyruvate carboxykinase (cPEPCK).

Explanation of Thesis Format

This thesis is divided into two chapters preceded by introductions. All the chapters are treated as separate sections. The numbering of the compounds, schemes and references are, therefore, listed independently in each section. The first chapter deals with the synthesis of chrysosplenol B, chrysosplenol D and their analogs. The second chapter deals with the synthesis of 3-(carboxymethylthio)-picolinic acid and its analog. A general summary of both papers follows the second chapter.

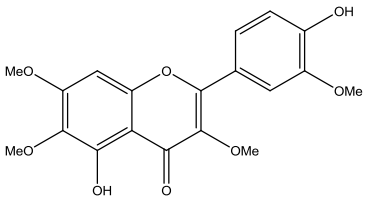
CHAPTER 1

Synthesis of chrysosplenol B and chrysosplenol D and their analogs

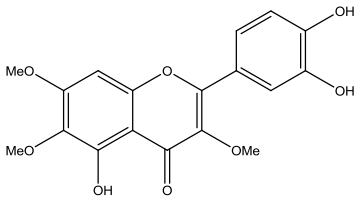
Introduction

Flavonoids are a class of phenolic compounds widely distributed in plants. Over one thousand individual types are known, and the list is constantly expanding. It has been found that components of this kind have many medical functions, such as diuretic, laxative, antispasmodic, antihypertensive and anti-inflammatory actions.[1]

Artemisia annua L. (*Asteraceae*) is an annual herb endemic to the northern parts of Chahar and Suiyuan provinces in China where it is known as 'quinghao' and it has been used to treat chills and fever for more than 2000 years.[2, 3] It had been reported that some flavones from *A. annua* potentiated the antimicrobial activity of artemisinin against *Plasmodium falciparum* and other malarial causing parasites.[4,5] It has been proposed that this activity is due to its inhibition of the multidrug resistant (MDR) pumps in *Plasmodium falciparum* and in *Staphylococcus aureus*.[6] Flavonols which bear a free hydroxyl group at C-3 were ineffective as inhibitors of the *S. aureus* MDR pump.[4] Chrysosplenol B **(1)**, also known as chrysosplenetin, and chrysosplenol D **(2)** are isolated from *A. annua* [3-6] and showed potentiated artemisinin activity against *Plasmodium falciparum*. Both flavonols bear methoxy groups at C-3 as shown in Figure 1.[3]



Chrysosplenol B **(1)**



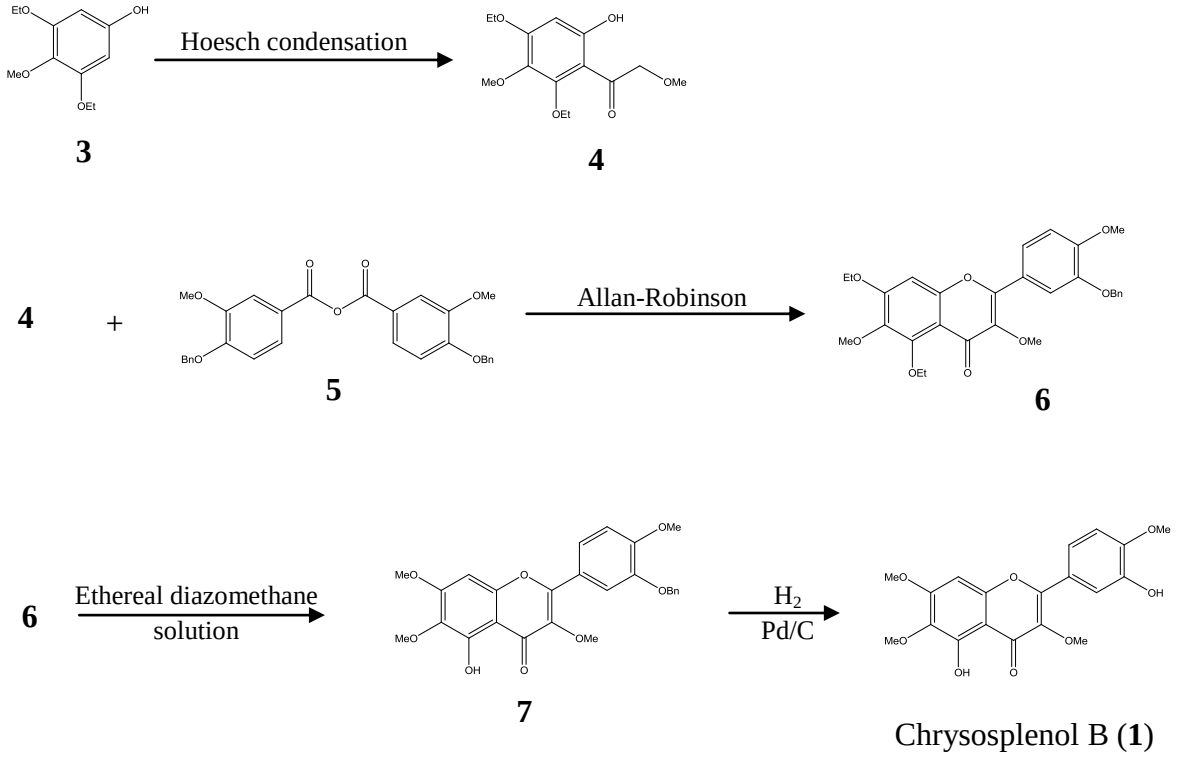
Chrysosplenol D **(2)**

Figure 1

Chrysosplenol B **(1)** and chrysosplenol D **(2)** are also isolated from *Chrysosplenium* plants[7-9], a medicinal herb for the treatment of the common cold, which is considered to be mainly due to rhinovirus infection. Tsuchiya and coworkers reported that 3-methoxyl and 5-hydroxyl groups on the flavone skeleton conferred potent specific antiviral activity against rhinovirus.[7] Both chrysosplenol B **(1)** and chrysosplenol D **(2)** bear 3-methoxyl and 5-hydroxyl groups.

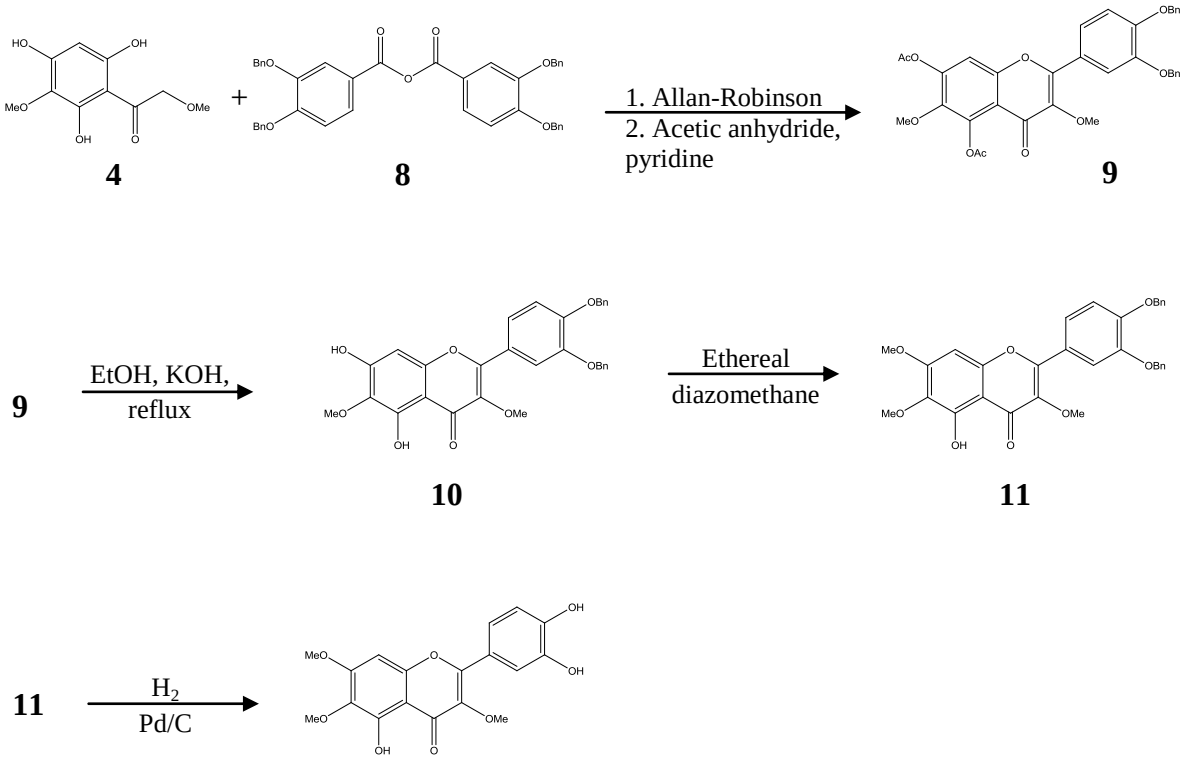
The 3-methoxylated flavones are rare in nature. Therefore, total synthesis of chrysosplenol B **(1)** and chrysosplenol D **(2)** has been studied.[6, 10, 11] Fukui and coworkers started the synthesis of chrysosplenol B with conversion of iretol **(3)** to 2, 4, 6-trihydroxy-3, ω-dimethoxyacetophenone **(4)** by a Hoesch condensation with methoxyacetonitrile. The ketone was subjected to the Allan-Robinson flavone synthesis using *o*-benzylvanillic anhydride **(5)** to give 4'-benzyloxy-5, 7-dihydroxy-3, 3', 6-trimethoxyflavone **(6)**. The partial methylation of hydorxyflavone **(6)** with diazomethane gave 4'-benzyloxy-5-hydroxy-3, 3', 6, 7-tetramethoxyflavone **(7)**, which was then debenzylated to give 4', 5-dihydroxy-3, 3', 6, 7-tetramethoxyflavone, chrysosplenol B **(1)**.[10]

Scheme 1. Synthesis of chrysosplenol B (1)



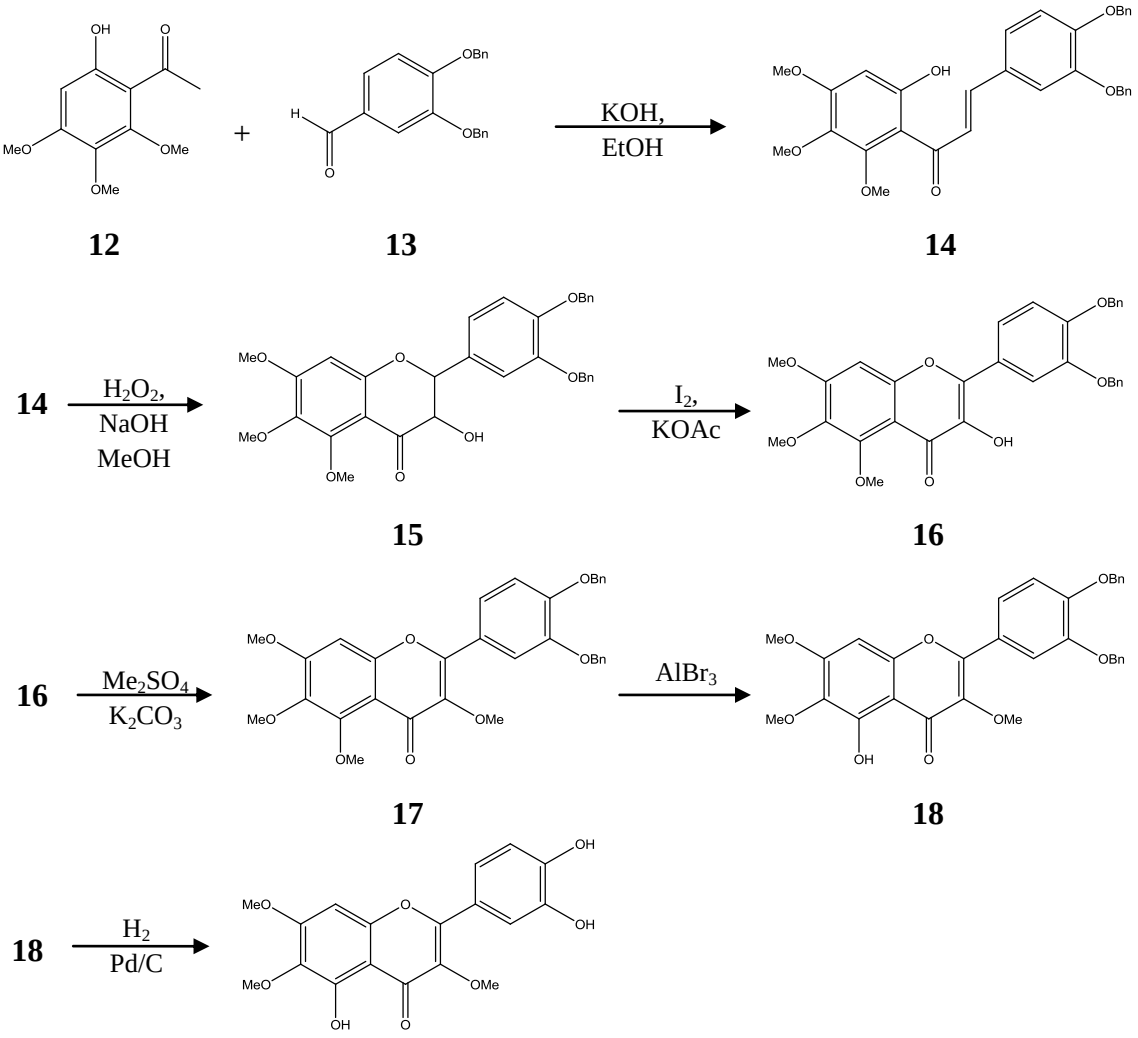
About a year later, Fukui and Nakayama synthesized chrysosplenol D **(2)** in similar manner. By using the method of Allan-Robinson's flavone synthesis, the reaction of the ketone **4** with 3, 4-dibenzyloxybenzoic anhydride **(8)** and potassium 3, 4-dibenzyloxybenzoate, followed by treatment with alcoholic potassium hydroxide gave a mixture of flavones. The mixture was acetylated without further purification to yield 5, 7-diacetoxy-3', 4'-dibenzyloxy-3, 6-dimethoxyflavone **(9)**. Treatment of flavone **9** with dilute alkali gave 3', 4'-dibenzyloxy-5, 7-dihydroxy-3, 6-dimethoxyflavone **(10)**. The partial methylation of flavone **10** gave 3', 4'-dibenzyloxy-5-hydroxy-3, 6, 7-trimethoxyflavone **(11)**. The debenzylation of flavone **11** yielded chrysosplenol D **(2)**. This synthesis gave chrysosplenol D (**2**); however, it only gave 26% yield of flavone **9** from ketone **4.**[11]

Scheme 2. Synthesis of chrysosplenol D (2) by Fukui and Nakayama



Chrysosplenol D **(2)** was also synthesized by Kraus and Roy using a different route as shown in Scheme 3. Chalcone **14** was synthesized via an aldol condensation of ketone **12** with aldehyde **13**. Dihydroflavonol **15** was formed from Algar-Flynn-Olyamada (AFO) reaction of chalcone **14.** Iodine oxidation of **15** produced flavonol **16** in 78% yield. Compound **16** was methylated to afford 3-methoxyflavone **17**. Aluminum tribromide mediated selective demethylation of 3-methoxyflavone **17** and debenzylation of **18** using a catalytic amount of 10% Pd/C afforded chrysosplenol D **(2)**.[6]

Scheme 3. Synthesis of chrysosplenol D (2) by Kraus and Roy



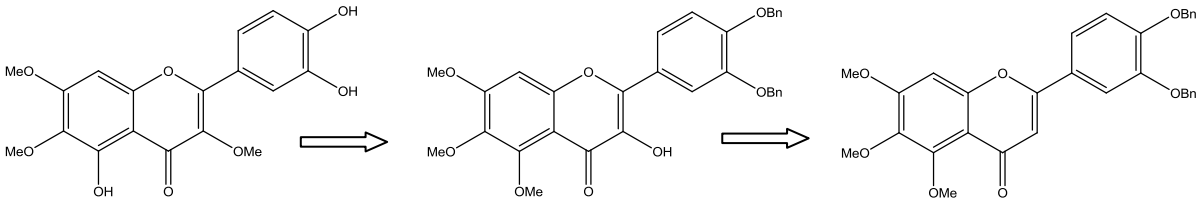
When we tried to reproduce this procedure, the AFO reaction did not proceed very well. This reaction gave mostly starting material.

Therefore, we report herein an alternate synthesis of chrysosplenol D **(2)**. The synthesis of chrysosplenol B **(1)** was carried out by a modification of this route.

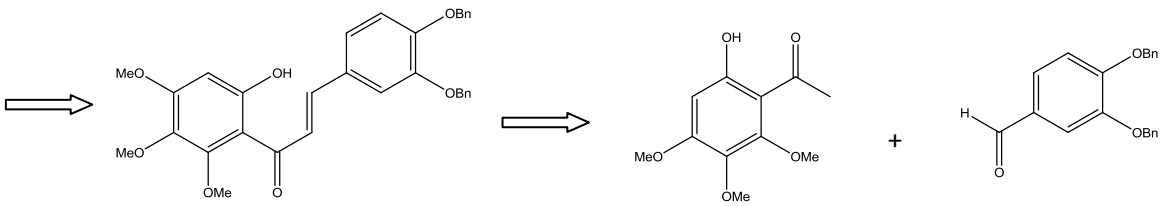
Results and discussion

Our synthesis of chrysosplenol D **(2)** involved the synthesis of a chalcone intermediate from aldol condensation of ketone **12** with aldehyde **13** which would be subjected to oxidation to a flavone then to flavonol, methylation, and deprotection.

Scheme 4. Retrosynthesis of chrysosplenol D



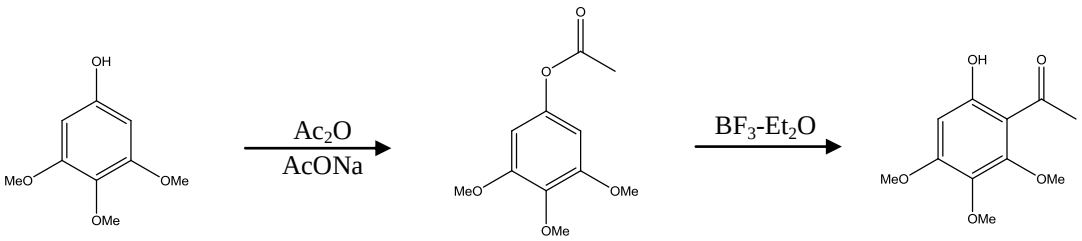
Chrysosplenol D (**2**) **16** **19**



14 **12** **13**

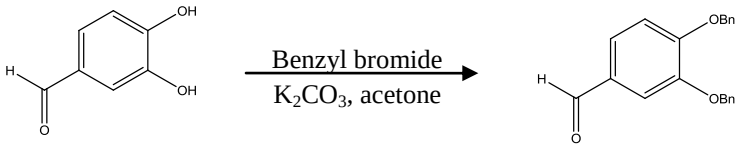
Ketone **12** was synthesized by *O*-acetylation of commercially available 3, 4, 5-trimethoxyphenol **(20)**, followed by boron trifluoride-catalyzed Fries rearrangement of **21** to afforded ketone **12** in high yield (96 %) (Scheme 5).[12] Aldehyde **13** was obtained with good yield (67 %) by protecting commercially available 3, 4-dihydroxybenzaldehyde **(22)** with benzyl bromide (Scheme 6).[13]

Scheme 5



20 **21** **12**

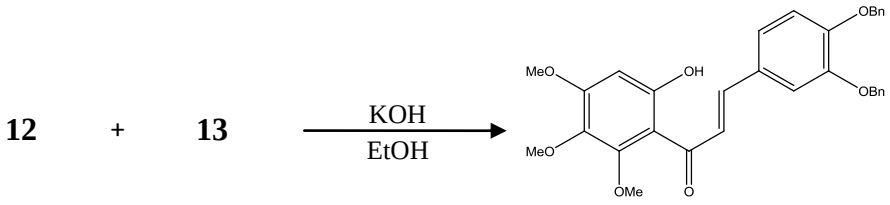
Scheme 6



22 **13**

An aldol condensation between ketone **12** and aldehyde **13** gave the chalcone **14** in 84 % yield (Scheme 7).[14] Ketone **12** and aldehyde **13** had similar R_f values. To avoid purification problems, excess ketone was used to complete the consumption of the aldehyde.

Scheme 7

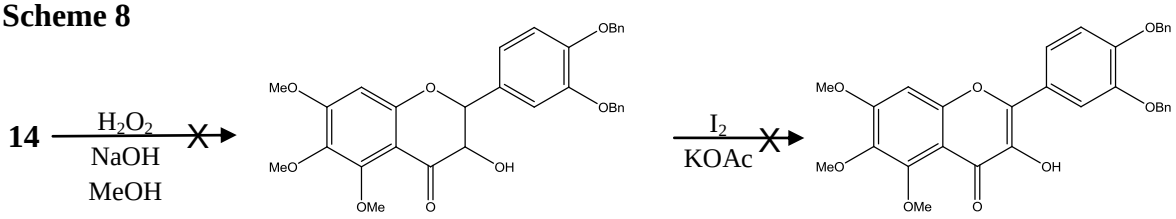


14

In the Kraus and Roy synthesis of chrysosplenol D **(2)**, they obtained a flavonol as the product in the AFO oxidation. They obtained dihydroflavonol **15** and then formed

flavonol **16** by iodine oxidation.[6] However, the AFO reaction did not work well for the synthesis of *o*-methoxyflavones as shown in Scheme 8. This reaction gave mostly starting material.

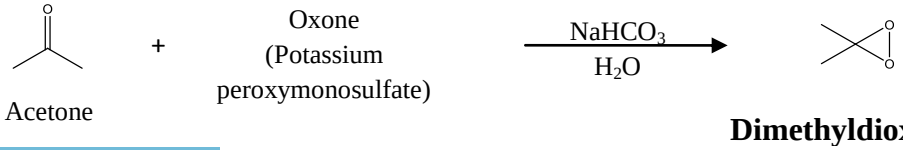
Scheme 8



15 **16**

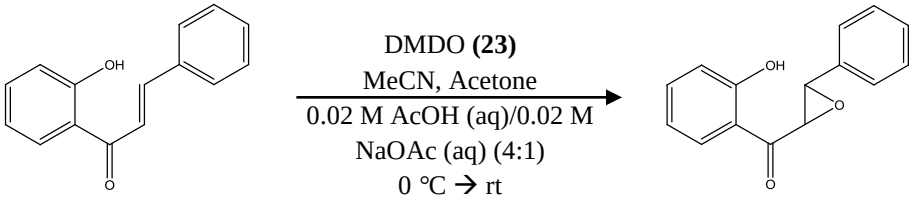
Therefore, we tried different routes. Dimethyldioxirane (DMDO) **(23)** has proven to be a very useful epoxidising agent, forming epoxide in good yields. Burke and O'Sullivan showed the successful epoxidation of unsubstituted (E)-2'-hydroxychalcone **(24)** to (E)-2'-hydroxychalcone epoxide **(25)** and also observed cyclisation of (E)-2'-hydroxychalcone **(24)** to *trans*-2,3-dihydroflavonol **(26)** as shown in Scheme 10.[15] We have synthesized DMDO **(23)** using simplified procedure employed by Murray and Singh as shown in Scheme 9.[16] With our hydroxychalcone **14**, we cyclized the chalcone using DMDO **(23)**[15] and then formed flavonol **16** by iodine oxidation[6] as shown in Scheme 11. However, the reaction gave low yield (< 5 %) due to low concentration of DMDO **(23)**. This reaction gave mostly starting material.

Scheme 9. Synthesis of dimethyldioxirane (DMDO) (23)

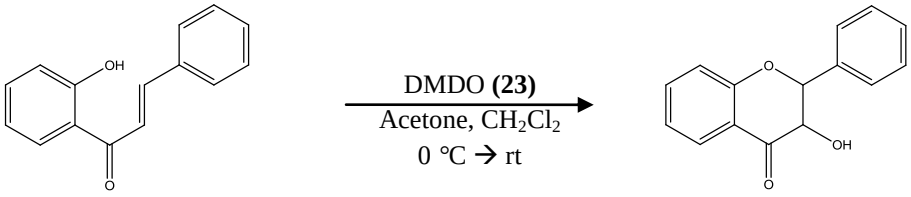


Dimethyldioxirane (23)

Scheme 10

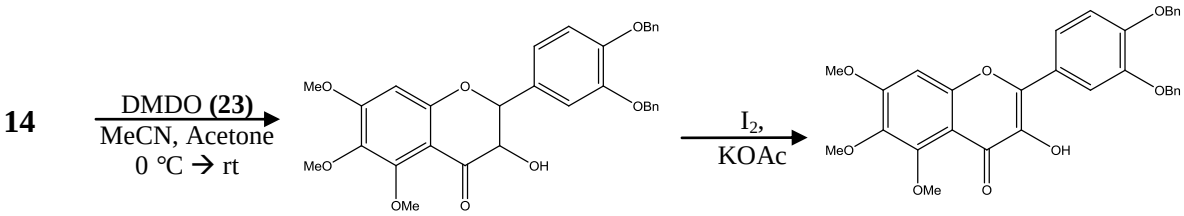


24 **25**



24 **26**

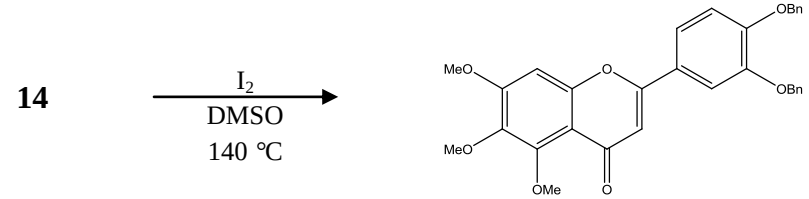
Scheme 11



15 **16**

We tried oxidizing the chalcone **14** to flavone **19.**[17] The reaction with iodine gave a low yield (< 10 %) and intractable mixture of compounds.

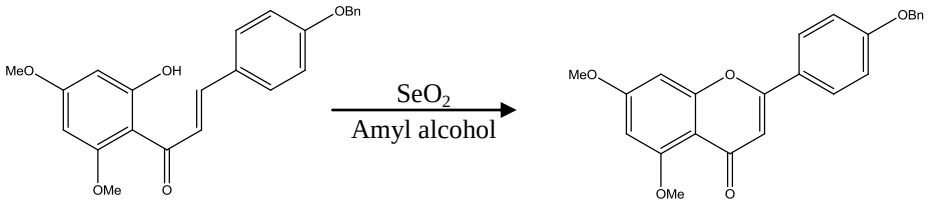
Scheme 12



19

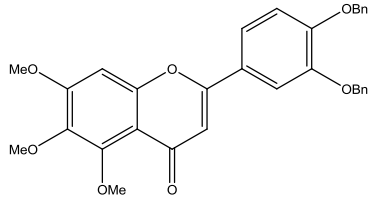
Mahal and coworkers oxidized dimethoxymonobenzyloxy chalcones by means of selenium dioxide oxidation in amyl alcohol (Scheme 13).[18] Since we had a similar system, we tried an oxidation using selenium dioxide to yield flavone **19** in 75 % yield (Scheme 14).

Scheme 13



27 **28**

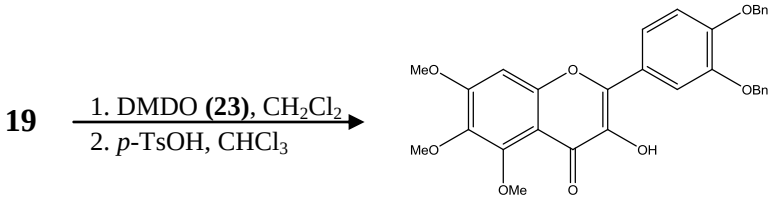
Scheme 14



19

With flavone **19** in hand, flavonol **16** was produced using DMDO **(23)** as shown in Scheme 15.[17] However, the reaction went in low yield (17 % maximum) and also gave demethylated compound. Flavonol **16** and the demethylated compound had similar R_f values and created purification problems.

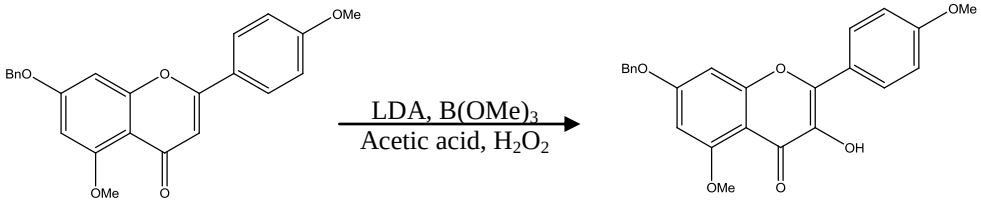
Scheme 15



16

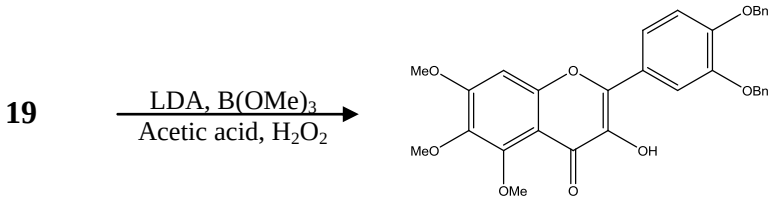
Adams and coworkers proposed an alternative oxidation procedure involving deprotonation, oxidation, and hydrolysis as shown in Scheme 16.[19] The reaction proceeded via an anion at C-3 position from deprotonation of **19** with lithium diisopropylamide (LDA) which was trapped by trimethylborate. The resulting borate was oxidized using hydrogen peroxide to give flavonol **16** in 62 % yield.

Scheme 16



29 **30**

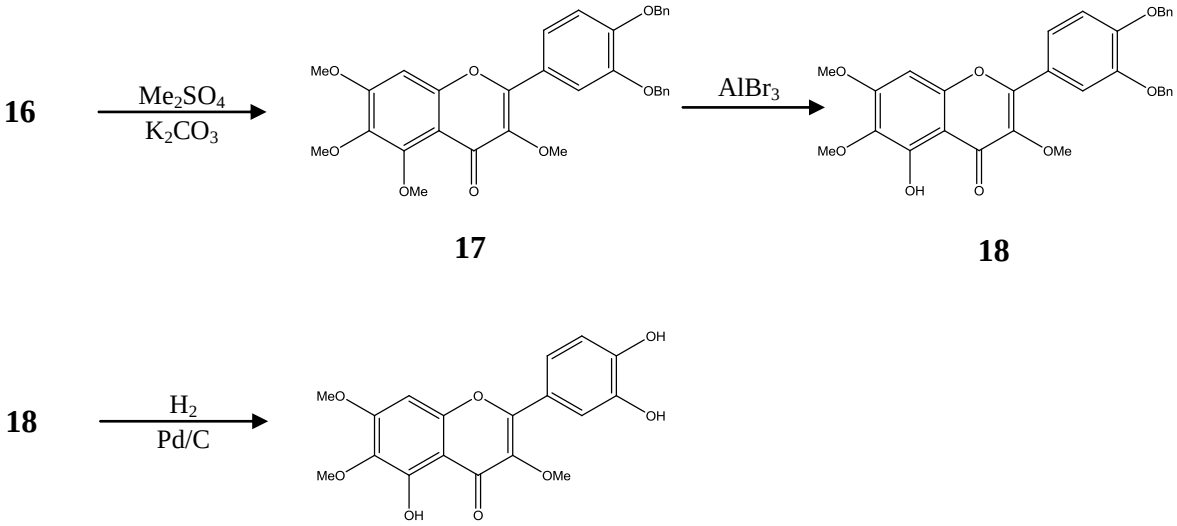
Scheme 17



16

Flavonol **16** was methylated to afford 3-methoxyflavone **17**. Aluminum tribromide mediated selective demethylation[6] of **17** and debenzylation[6] of **18** using a catalytic amount of 10 % Pd/C afforded chrysosplenol D **(2)** in 60 % yield. The overall yield from **12** is 15 %.

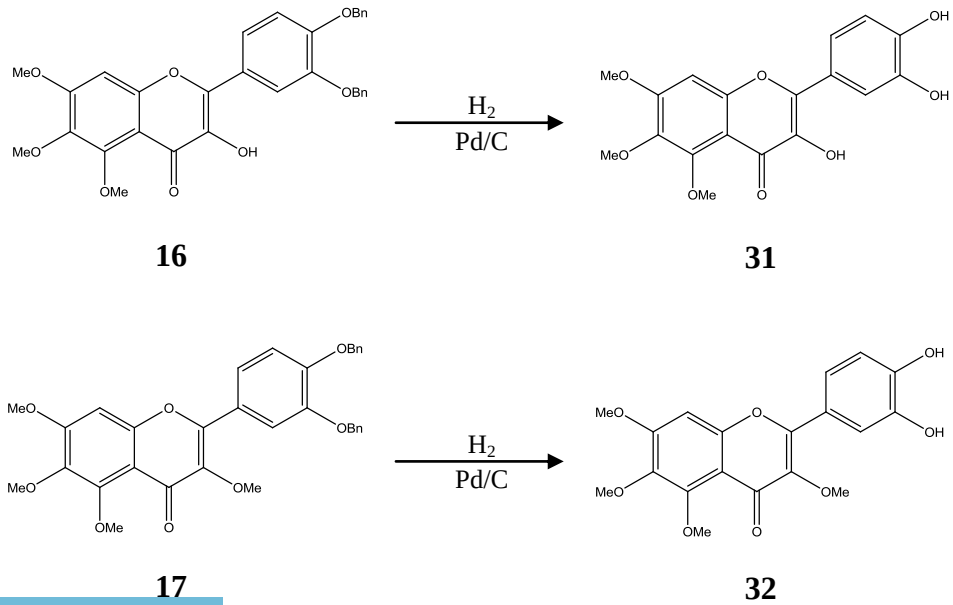
Scheme 18



Chrysosplenol D **(2)**

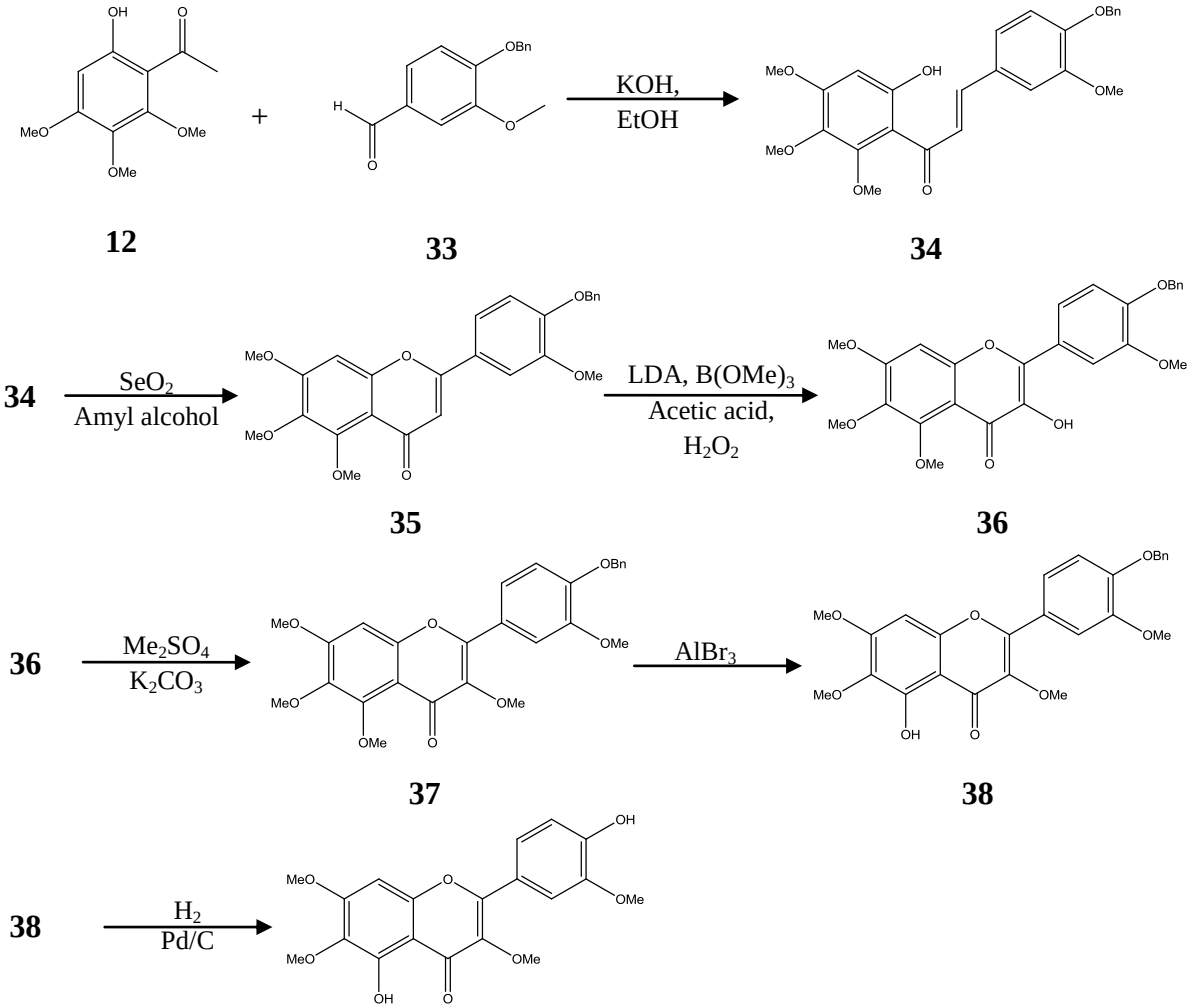
To investigate the activities of the functional groups, two more derivatives of chrysosplenol D **(2)** were synthesized. Compounds 3', 4'-dibenzyloxy-3-hydroxy-5, 6, 7-trimethoxyflavone **(16)**, and 3', 4'-dibenzyloxy-3, 5, 6, 7-tetramethoxyflavone **(17)** were debenzylated to form analogs **31** and **32**, respectively.

Scheme 19



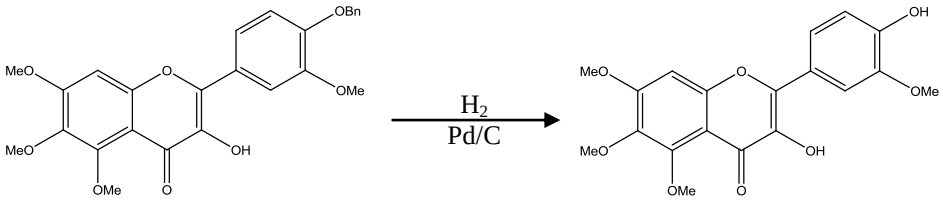
Chrysosplenol B **(1)** has a similar structure as chrysosplenol D **(2)** and was synthesized in similar manner. The synthesis started with an aldol condensation between ketone **12** and aldehyde **33** which formed chalcone **34**, which was oxidized by selenium dioxide to form flavone **35**. Then flavone **35** was further oxidized by LDA and H_2O_2 which was methylated to afford 3-methoxyflavone **37**. Aluminum tribromide mediated selective demethylation of **37** and debenzylation of **38** using a catalytic amount of 10 % Pd/C afforded chrysosplenol B **(1)** in 67% yield. The overall yield from **12** is 16 %.

Scheme 20. Synthesis of chrysosplenol B (1)

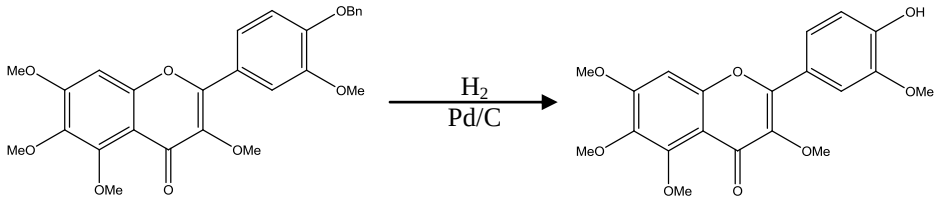


Similar to chrysosplenol D **(2)**, two more derivatives of chrysosplenol B **(1)** were synthesized. Both 4'-benzyloxy-3-hydroxy-5, 3', 6, 7-tetramethoxyflavone **(36)** and 4'-benzyloxy-3, 5, 3', 6, 7-pentaoxyflavone **(37)** were debenzylated to form **39** and **40**, respectively.

Scheme 21



36 **39**



37 **40**

In conclusion, we have synthesized chrysosplenol B **(1)**, chrysosplenol D **(2)** and their analogs. Both chrysosplenol B **(1)** and chrysosplenol D **(2)** have the flavone skeleton with 3-methoxyl and 5-hydroxyl groups which have ability to potentiate the anti-malarial activity of artemisinin and the activity of norfloxacin. The synthesis started from 1-(6-hydroxy-2, 3, 4-trimethoxyphenyl)ethanone **(12)** for both natural products. Chrysosplenol B **(1)** was synthesized in six steps with 16 % overall yield. Chrysosplenol D **(2)** was also synthesized in six steps with 15 % overall yield. Our synthesis has led to an alternative method of forming flavones from chalcones using selenium dioxide.

Experimental section

General.

All ^{1}H NMR spectra were recorded at 300 MHz or 400 MHz unless otherwise noted. Unless otherwise noted, reactions were carried out under an argon atmosphere. Thin-layer chromatography was performed using commercially prepared 60-mesh silica gel plates (Whatman K6F), and visualization was effected using short wavelength UV light (254 nm). All reagents were used directly as obtained commercially unless otherwise noted.

3, 4, 5-Trimethoxyphenyl acetate (21).

To a stirred solution of 3, 4, 5-trimethoxyphenol **(20)** (5.0 g, 27 mmol) in acetic anhydride (25 mL, 0.27 mol) at room temperature was added sodium acetate (5.0 g, 61 mmol). The mixture was heated for 2 hours at 110 ℃. The mixture was concentrated under vacuum, diluted with water, and extracted with dichloromethane (3 x 25 mL), and dried over anhydrous $MgSO_4$. The solvent was evaporated to yield 3, 4, 5-trimethoxyphenyl acetate **21** (5.92 g, 96 % yield) as a light brown oil.[12]

1-(6-Hydroxy-2, 3, 4-trimethoxyphenyl)ethanone (12).

To a stirred solution of acetate **21** (3.0 g, 13 mmol) in glacial acetic acid (3 mL) at room temperature was added dropwise boron trifluoride-diethyl ether (6.0 mL, 47 mmol). The mixture was heated for 2 hours at 70 ℃. It was cooled and then poured into 10 % aqueous NaOH (125 mL). The mixture was washed with ether, the aqueous layer was cooled, acidified with concentrated HCl, then extracted with dichloromethane. The combined organic

layers were dried over anhydrous $MgSO_4$. The solvent was evaporated, and the crude residue was purified by silica gel column chromatography (hexanes/EtOAc, 3:1) to yield compound **12** (2.56 g, 85 % yield) as a brown oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.23 (s, 1H), 3.98 (s, 3H), 3.88 (s, 3H), 3.77 (s, 3H), 2.65 (s, 3 H).[12]

3, 4-Dibenzyloxybenzaldehyde (13).

To a stirred solution of 3, 4-dihydroxybenzaldehyde **(22)** (2.0 g, 14.5 mmol) in acetone (40 mL) at room temperature was added benzyl bromide (5.2 mL, 44 mmol), and potassium carbonate (9.0 g, 66 mmol). The mixture was heated to reflux for 4 hours. After completion of the reaction, the solid material was filtered off, and the solvent was evaporated. The residue was purified by silica gel column chromatography (hexanes/EtOAc, 95:5) to yield compound **13** (3.07 g, 67 % yield) as white solid: mp 84-87 ℃: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.81 (s, 1H), 7.38-7.32 (m, 12H), 7.02 (d, 1H), 5.27 (s, 2H), 5.22 (s, 2H).[13]

3', 4'Dibenzyloxy-6-hydroxy-2, 3, 4-trimethoxychalcone (14).

To a stirred solution of ketone **12** (1.3 g, 5.7 mmol) and aldehyde **13** (1.5 g, 4.7 mmol) in EtOH (50 mL) at 0 ℃ was added freshly powdered KOH (89.2%, 1.5 g, 24 mmol). The mixture was boiled for 24 hours. The solvent was evaporated to approximately one-fifth the original volume. Ice-cold H_2O (~ 3mL) was added, and the mixture was neutralized with 2 N HCl. The mixture was extracted with ethyl acetate (3 x 50 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated, and the crude residue was purified by silica gel column chromatography (hexanes/EtOAc, 3:1) to yield compound **14** (2.08 g, 84 % yield) as a

yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 2H), 7.49-7.25 (m, 11H), 7.21 (s, 1H), 6.94 (d, 1H, *J* = 8.4 Hz), 6.29 (s, 1H), 5.23 (s, 4H), 3.90 (s. 3H), 3.84 (s, 6H).[6]

3', 4'-Dibenzyloxy-5, 6, 7-methoxyflavone (19).

To a stirred solution of chalcone **14** (1.0 g, 1.9 mmol) in amyl alcohol (25 mL) at room temperature was added selenium dioxide (0.74 g, 6.6 mmol). The mixture was heated at 136 ℃ for 12 hours. After completion of the reaction, the selenium was filtered and washed with ether. The combined solutions were extracted with 20 % aqueous NaOH and dried over anhydrous $MgSO_4$. The solvent was evaporated, and the crude residue was purified by silica gel column chromatography (hexanes/EtOAc, 1:1) to yield compound **19** (0.75 g, 75 % yield) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49-7.35 (m, 12H), 7.00 (d, 1H), 6.74 (s, 1H), 6.52 (s, 1H), 5.25 (s, 4H), 3.98 (s, 6H), 3.92 (s, 3H).[21]

3', 4'-Dibenzyloxy-3-hydroxy-5, 6, 7-trimethoxyflavone (16).

Using dimethyldioxirane:

To a stirred solution of chalcone **19** (0.1 g, 0.19 mmol) in dichloromethane (4 mL) at 0 ℃ was added dimethyldioxirane (8 mL). The mixture was stirred at 4 ℃ under Argon gas overnight. The solvent was evaporated and the residue was dissolved in chloroform (5 mL) and then catalytic amount of *p*-toluenesulfonic acid was added. The mixture was stirred at 0 ℃ for 30 minutes. The solvent was evaporated, and the crude residue was purified by silica gel column chromatography (hexanes/EtOAc, 5:2) to yield compound **16** (18 mg, 17 % yield)

as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52-7.29 (m, 12H), 7.02 (d, 1H), 6.71 (s, 1H), 6.49 (s, 1H), 5.22 (s, 4H), 3.97 (s, 6H), 3.92 (s, 3H).[6]

Using lithium diisopropylamide:

To a stirred solution of diisopropylamine (0.06 mL, 0.46 mmol) in THF (2 mL) at -78 ℃ was added butylithium. The mixture was stirred for 30 minutes at -78 ℃. A solution of flavone **19** (0.20 g, 0.38 mmol) in THF (5 mL) was added dropwise at -78 ℃. After 5 minutes, a solution of freshly distilled trimethyl borate in THF (1 mL) was added and the mixture was stirred for 40 minutes at -78 °C. Glacial acetic acid (30 µL, 0.06 mmol) was added followed by 30 % hydrogen peroxide (45 µL). The reaction was allowed to warm to room temperature for 1 hour and quenched with saturated $NaHCO_3$. The mixture was extracted with EtOAc and dried over anhydrous $MgSO_4$. The solvent was evaporated, and the crude residue was purified by silica gel column chromatography (hexanes/EtOAc, 1:1) to yield compound **16** (0.13 g, 62 % yield) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.89 (d, 1H), 7.80-7.27(m, 10H), 7.04 (d, 1H), 6.73 (s, 1H), 5.28 (s, 4H), 4.03 (s, 3H), 4.00 (s, 3H), 3.93 (s, 3H).[6]

3', 4'- Dibenzyloxy-3, 5, 6, 7-tetramethoxyflavone (17).

To a stirred solution of flavonol **16** (100 mg, 0.19 mmol) and anhydrous K_2CO_3 (230 mg, 1.66 mmol) in dry acetone (5 mL) at room temperature, Me_2SO_4 (210 mg, 1.66 mmol) was added dropwise. The mixture was heated for 9 hours. The solvent was removed, and the crude residue was purified by silica gel column chromatography (hexanes/EtOAc, 1:1) to

yield compound **17** (84 mg, 82 % yield) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.65, (d, 1H), 7.50-7.32 (m, 10H), 7.01 (s, 1H), 6.67 (s, 1H), 5.27 (s, 4H), 4.00 (s, 3H), 3.96 (s, 3H), 3.91 (s, 3H), 3.72 (s, 3H).[6]

3', 4'-Dibenzyloxy-5-hydroxy-3, 6, 7-trimethoxyflavone (18).

To a stirred solution of 3-methoxyflavone **17** (70 mg, 0.13 mmol) in MeCN (1 mL) at 0 ℃, was added dropwise a solution of $AlBr_3$ (236 mg, 0.88 mmol) in MeCN (3.5 mL). The mixture was stirred at 0 ℃, for 20 minutes, and then 2 % aqueous HCl solution (7.5 mL) was added. The solution was heated at 75 ℃ for 25 minutes and then cooled to room temperature. The solvent was evaporated, extracted with CH_2Cl_2 (2x25 mL), and dried over Na_2SO_4. The solvent was evaporated and the crude residue was purified by silica gel column chromatography (hexanes/EtOAc, 3:2) to yield compound **18** (53 mg, 78 % yield) as a yellow oil : ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.65, (s, 1H), 7.48-7.33 (m, 10H), 7.05 (d, 1H, J = 9.0 Hz), 6.44 (s, 1H), 5.27 (s, 4H), 4.15 (s, 3H), 3.95 (s, 3H), 3.71 (s, 3H).[6]

Chrysosplenol D (2).

A mixture of 3-methoxyflavone **18** (40 mg, 0.074 mmol) and a catalytic amount of 10 % Pd/C in MeOH/EtOAc (20 mL, 1:1) was stirred in H_2 atmosphere at room temperature for 1 hour. The mixture was purified by silica gel column chromatography (hexanes/EtOAc, 1:1) to yield the natural product **2** (16 mg, 60 % yield) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, 1H), 7.56 (d, 1H), 7.16 (s, 1H), 6.42 (s, 1H), 4.09 (s, 3H), 3.93 (s, 3H), 3.75 (s, 3H).[6]

3, 3', 4'-Trihydroxy-5, 6, 7-trimethoxyflavone (31).

A mixture of flavonol **16** (30 mg, 0.055 mmol) and a catalytic amount of 10 % Pd/C in MeOH/EtOAc (15 mL, 1:1) was stirred in H_2 atmosphere at room temperature for 1 hour. The mixture was purified by silica gel column chromatography (hexanes/EtOAc, 1:1) to yield compound **31** (13 mg, 65 % yield) as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.62 (d, 1H), 7.46 (d, 1H), 6.70 (s, 1H), 3.96 (s, 3H), 3.90 (s, 3H), 3.85 (s, 3H).

3', 4'-Dihydroxy-3, 5, 6, 7-tetramethoxyflavone (32).

A mixture of 3-methoxyflavone **17** (60 mg, 0.108 mmol) and a catalytic amount of 10 % Pd/C in MeOH/EtOAc (30 mL, 1:1) was stirred in H_2 atmosphere at room temperature for 1 hour. The mixture was purified by silica gel column chromatography (hexanes/EtOAc, 1:1) to yield compound **32** (28mg , 69 % yield) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.66, (d, 1H), 7.00 (s, 1H), 6.64 (s, 1H), 3.98 (s, 3H), 3.95 (s, 3H), 3.89 (s, 3H), 3.75 (s, 3H).[22]

4-Benzyloxy-3-methoxybenzaldehyde (33).

To a stirred solution of 4-hydroxy-3-methoxybenzaldehyde **(12)** (2.0 g, 13mmol) in acetone (40 mL) at room temperature was added benzyl bromide (2.3 mL, 20 mmol), and potassium carbonate (4.5 g, 33 mmol). The mixture was boiled for 4 hours. After completion of the reaction, the solid material was filtered off, and the solvent was evaporated. The residue was purified by silica gel column chromatography (hexanes/EtOAc, 95:5) to yield

compound **33** (2.7 g, 84 % yield) as white solid: mp 87-90 ℃: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.81 (s, 1H), 7.44-7.31 (m, 7H), 6.98 (d, 1H), 5.21 (s, 2H), 3.91 (s, 3H).[20]

4'-Benzyloxy-6-hydroxy-2, 3, 4, 3'-tetramethoxychalcone (34).

To a stirred solution of ketone **12** (1.1 g, 5.0 mmol) and aldehyde **33** (1.0 g, 4.1 mmol) in EtOH (40 mL) at 0 ℃ was added freshly powdered KOH (89.2%, 1.3 g, 21 mmol). The mixture was boiled for 24 hours. The solvent was evaporated to approximately one-fifth the original volume. Ice-cold H_2O (~ 3mL) was added, and the mixture was neutralized with 2 N HCl. The mixture was extracted with ethyl acetate (3 x 50 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated, and the crude residue was purified by silica gel column chromatography (hexanes/EtOAc, 3:1) to yield compound **34** (1.4 g, 75 % yield) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.46-7.28 (m, 7H), 7.22 (s, 1H), 6.94 (d, 1H), 6.27 (s, 1H), 5.20 (s, 2H), 3.97 (s, 6H), 3.96 (s, 3H), 3.91 (s, 3H).[14]

4'-Benzyloxy-5, 6, 7, 3'-tetramethoxyflavone (35).

To a stirred solution of chalcone **34** (1.0 g, 2.2 mmol) in amyl alcohol (25 mL) at room temperature was added selenium dioxide (0.86 g, 7.8 mmol). The mixture was boiled at 136 ℃ for 12 hours. After completion of the reaction, the selenium was filtered off and washed with ether. The combined solutions were extracted with 20 % aqueous NaOH and dried over anhydrous $MgSO_4$. The solvent was evaporated, and the cr,ude residue was purified by silica gel column chromatography (hexanes/EtOAc, 1:1) to yield compound **35**

(0.76 g, 76 % yield) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43-7.29 (m, 7H), 6.95 (d, 1H), 6.75 (s, 1H), 6.54 (s, 1H), 5.19 (s, 2H), 3.96 (s, 6H), 3.94 (s, 3H), 3.89 (s, 3H).[21]

4'-Benzyloxy-3-hydroxy-5, 6, 7, 3'-tetramethoxyflavone (36).

To a stirred solution of diisopropylamine (0.12 mL, 1.6 mmol) in THF (1 mL) at -78 ℃ was added butylithium. The mixture was stirred for 30 minutes at -78 ℃. A solution of flavone **33** (600 mg, 1.3 mmol) in THF (10 mL) was added dropwise at -78 ℃. After 5 minutes, a solution of freshly distilled trimethyl borate in THF (2 mL) was added and the mixture was stirred for 40 minutes at -78 ℃. Glacial acetic acid (60 μL, 0.12 mmol) was added followed by 30 % hydrogen peroxide (90 μL). The reaction was allowed to warm to room temperature for 1 hour and quenched with saturated $NaHCO_3$. The mixture was extracted with EtOAc and dried over anhydrous $MgSO_4$. The solvent was evaporated, and the crude residue was purified by silica gel column chromatography (hexanes/EtOAc, 1:1) to yield compound **36** (0.38 g, 61 % yield) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.86 (d, 1H), 7.79-7.28 (m, 5H), 7.02 (d, 1H), 6.72 (s, 1H), 5.24 (s, 2H), 3.98 (s, 6 H), 3.96 (s, 3H), 3.90 (s, 3H).[8]

4'-Benzyloxy-3, 5, 6, 7, 3'-pentamethoxyflavone (37).

To a stirred solution of flavonol **36** (200 mg, 0.43 mmol) and anhydrous K_2CO_3 (534 mg, 3.9 mmol) in dry acetone (10 mL) at room temperature, Me_2SO_4 (488 mg, 3.87 mmol) was added dropwise. The mixture was boiled for 9 hours. The solvent was removed, and the crude residue was purified by silica gel column chromatography (hexanes/EtOAc, 1:1) to

yield compound **37** (174 mg, 84 % yield) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.61 (d, 1H), 7.54-7.28 (m, 5H), 6.98 (s, 1H), 6.63 (s, 1H), 5.22 (s, 2H), 3.99 (s, 6H), 3.97 (s, 3H), 3.89 (s, 3H), 3.74 (s, 3H).[22]

4'-Benzyloxy-5-hydroxy-3, 6, 7, 3'-tetramethoxyflavone (38).

To a stirred solution of 3-methoxyflavone **37** (100 mg, 0.21 mmol) in MeCN (2 mL) at 0 ℃, was added dropwise a solution of $AlBr_3$ (390 mg, 1.46 mmol) in MeCN (5 mL). The mixture was stirred at 0 ℃, for 20 minutes, and then 2 % aqueous HCl solution (10 mL) was added. The solution was boiled at 75 ℃ for 25 minutes and then cooled to room temperature. The solvent was evaporated, extracted with CH_2Cl_2 (2x25 mL), and dried over Na_2SO_4. The solvent was evaporated and the crude residue was purified by silica gel column chromatography (hexanes/EtOAc, 3:2) to yield compound **38** (79 mg, 81 % yield) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.59, (s, 1H), 7.56-7.29 (m, 5H), 7.01 (d, 1H), 6.41 (s, 1H), 5.24 (s, 2H), 3.97 (s, 6H), 3.89 (s, 3H), 3.71 (s, 3H).[22]

Chrysosplenol B (1).

A mixture of 3-methoxyflavone **38** (60 mg, 0.13 mmol) and a catalytic amount of 10% Pd/C in MeOH/EtOAc (25 mL, 1:1) was stirred in H_2 atmosphere at room temperature for 1 hour. The mixture was purified by silica gel column chromatography (hexanes/EtOAc, 1:1) to yield the natural product **1** (32 mg, 67 % yield) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (s, 1H), 7.63 (d, 1H), 7.19 (s, 1H), 6.57 (s, 1H), 4.00 (s, 6H), 3.92 (s, 3H), 3.72 (s, 3H).[10]

3, 4'-Dihydroxy-5, 6, 7, 3'-tetramethoxyflavone (39).

A mixture of flavonol **36** (40 mg, 0.086 mmol) and a catalytic amount of 10 % Pd/C in MeOH/EtOAc (20 mL, 1:1) was stirred in H_2 atmosphere at room temperature for 1 hour. The mixture was purified by silica gel column chromatography (hexanes/EtOAc, 1:1) to yield compound **39** (19 mg, 59 % yield) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.67 (d, 1H), 7.43 (d, 1H), 6.68 (s, 1H), 3.98 (s, 6H), 3.91 (s, 3H), 3.87 (s, 3H).[8]

4'-Hydroxy-3, 5, 6, 7, 3'-pentamethoxyflavone (40).

A mixture of 3-methoxyflavone **37** (40 mg, 0.084 mmol) and a catalytic amount of 10 % Pd/C in MeOH/EtOAc (40 mL, 1:1) was stirred in H_2 atmosphere at room temperature for 1 hour. The mixture was purified by silica gel column chromatography (hexanes/EtOAc, 1:1) to yield compound **40** (20 mg, 63 % yield) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.67 (d, 1H), 7.13 (d, 1H), 6.68 (s, 1H), 3.98 (s, 6H), 3.91 (s, 3H), 3.87 (s, 3H), 3.71 (s, 3H).[7]

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CHAPTER 2

Synthesis of 3-(carboxymethylthio)-picolinic acid and its analog

Introduction

Phosphenolpyruvate carboxykinase (PEPCK) is an enzyme used in the natural process of gluconeogenesis.[1] It converts oxaloacetate (OAA) to phosphoenolpyruvate (PEP), using GTP or ITP as the phosphoryl donor. In vertebrates, PEPCK occurs as two distinct isozymes, cytosolic and mitochondrial, which are products of different genes.[2-5] Most reactions of gluconeogenesis can use the glycolysis enzymes in the opposite direction; however, the pyruvate kinase enzyme is irreversible. Therefore, the enzymes pyruvate carboxylase and phosphoenolpyruvate carboxykinase are used to provide an alternate path for effectively reversing its actions.

Transcription of the PEPCK gene is stimulated by glucagon, glucocorticoids, retinoic acid, and adenosine 3', 5'-monophosphate (cAMP), while it is inhibited by insulin. Insulin inhibits the transcription of many of the stimulatory elements and, therefore, considered dominant of these factors. PEPCK activity is also inhibited by hydrazine sulfate, and the inhibition decreases the rate of gluconeogenesis. Also, the use of siRNA to inhibit PEPCK in a diabetic mouse model shows elimination of the hyperglycemia upon PEPCK inhibition. Therefore, it would be advantageous to find novel inhibitors of PEPCK and/or an inhibitor scaffold for preparing analogs and derivatives for novel inhibitors of PEPCK.[1,2]

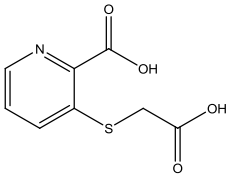
In efforts to characterize the active sites of the isozymes of PEPCK, studies have been performed using analogs of PEP or OAA, either as reversible inhibitors or as alternative

substrates. Derivatives of PEP with alkyl or halo substitutions have been used to demonstrate the stereospecificity of the reaction catalyzed by mitochondrial PEPCK (mPEPCK) from avian liver.[6-9] Studies have shown that α-hydroxyl and α-sulfhydryl carboxylic acids are poor substrates for that enzyme's phosphoryl transfer reaction.[1, 2, 6]

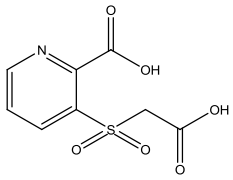
The molecular structure configuration for interacting with a binding site of PEPCK was investigated to find novel inhibitors of PEPCK. The PEPCK inhibitors are characterized by having a size capable of fitting into and interaction with the PEPCK binding site and at least one of the following: (a) a first terminal substituent have co-planar atoms acting as metal ligands to the active site metal ion PEPCK; (b) at least one of an atom or substituent at positions 2 or 3 from the first terminal substituent includes a neutral carbon center or include an oxygen, sulfur, selenium, or other atom with similar physiochemical properties; (c) at least one of an atom or substituent at positions 2 or 3 from the first terminal substituent is devoid of an electropositive atom or substituent; or (d) a second terminal substituent opposite of the first terminal substituent, having an atom that is a hydrogen bonding acceptor and/or is negatively charged.[1]

Holyoak and others also concluded the ability of substrate analogs to inhibit PEPCK depended upon their overall size and the orientation, electronic properties and charge of their functional groups. His study systematically evaluated substrate analogs of PEP and OAA as reversible inhibitors of PEPCK when tested against PEP. With the exception of three compounds, all were bifunctional, being predominantly bicarboxylic acids, biphosphonic acids, or bisulfonic acids. Some of the bifunctional compounds were also phosphoryl or sulfonyl monocarboxylic acids. The structure-function analysis illustrated the mechanism of

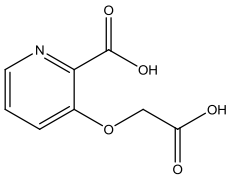
molecular recognition used by PEPCK, which could be exploited to develop novel and selective inhibitors of the enzyme. [1, 2]



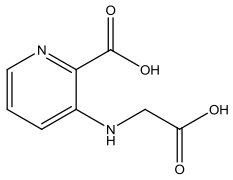
3-(Carboxymethylthio)-picolinic acid **(1)**



3-(Carboxymethylsulfonyl)-picolinic acid **(2)**



3-(Carboxymethoxy)-picolinic acid **(3)**



3-(Carboxymethylamino)-picolinic acid **(4)**

Figure 1. Four different picolinic acid compounds

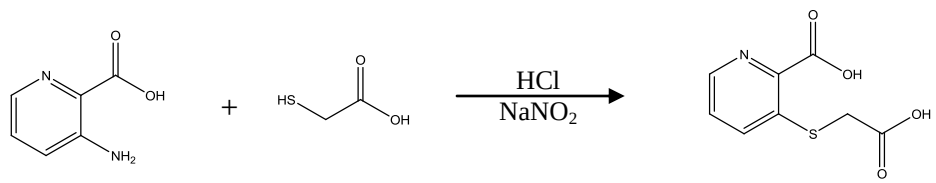
Knowing some characteristics of the inhibitors, development of novel inhibitors of the enzyme has been studied. One of the most important criteria in choosing compounds for the inhibitors was that they possess functional groups similar to those of the substrates. Therefore, we focused on synthesizing compounds which were bicarboxylate and bicarboxylate with sulfonyl group, 3-(carboxymethylthio)-picolinic acid **(1)** and its analog,

3-(carboxymethylsulfonyl)-picolinic acid **(2)** as shown in Figure 1. In the past, 3-(carboxymethoxy)-2-picolinic acid **(3)** and 3-(carboxymethylamino)-picolinic acid **(4)** were known and studied.[10- 12] However, 3-(carboxymethylthio)-picolinic acid **(1)** is not a very well known compound. Only one synthesis of 3-(carboxymethylthio)-picolinic acid **(1)** has been reported in 1926 by Plazek and Sucharda.[12] The synthesis of 3-(carboxymethylthio)-picolinic acid **(1)** was carried by the same route. The analog of 3-(carboxymethylthio)-picolinic acid **(1)**, 3-(carboxymethylsulfonyl)-picolinic acid **(2)** was synthesized by oxidation with dimethyldioxirane (DMDO).[15]

Results and discussion

When the synthesis of 3-(carboxymethylthio)-picolinic acid **(1)** was started, there was only one direct synthesis reported for this compound. Our synthesis of 3-(carboxymethylthio)-picolinic acid **(1)** was started by reproducing method employed by Plazek and Sucharda as shown in Scheme 1.[12] The reaction started with commercially available β-aminopicolinic acid. At first trial, reaction gave very low yield (< 5%) after silica gel column chromatography with intractable mixture of compounds that was not investigated any further.

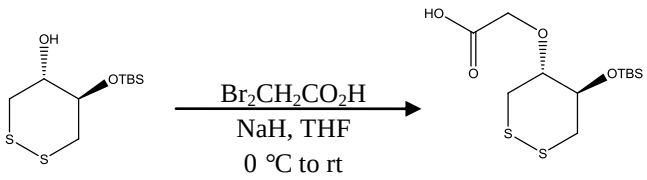
Scheme 1



5 **6** **1**

With low yield, we searched for different route. Calandra and coworkers developed a route toward internally disulfide protected β-mercaptoalcohol **8** as shown in Scheme 2.[13]

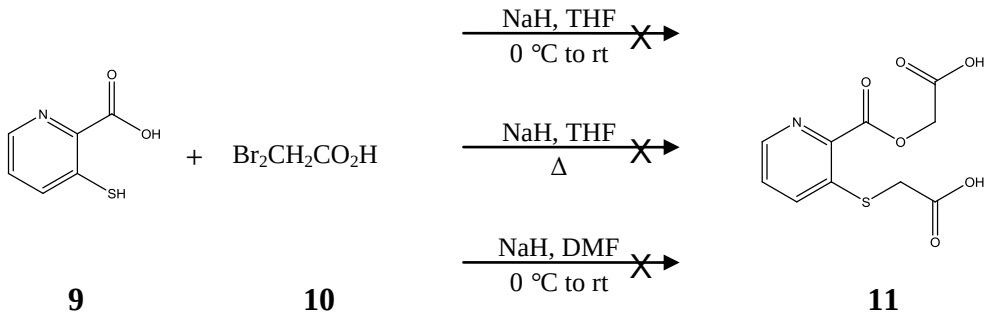
Scheme 2



7 **8**

With similar connectivity, we incorporated this route to our synthesis toward 3-(carboxymethylthio)-picolinic acid. Our synthesis began using commercially available 3-mercaptopicolinic acid as shown in Scheme 3. However this reaction only gave starting material. Therefore, we tried heating the reaction; however, again it only returned starting material. Then, we tried using a different solvent. Instead of tetrahydrofuran (THF), we used dimethylformamide (DMF) for the solvent. With DMF, we got some starting material and intractable mixture of compounds, but no desired product.

Scheme 3

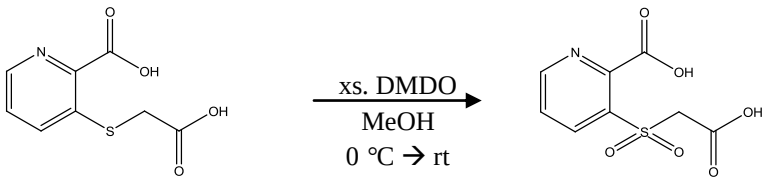


9 **10** **11**

While searching for another route, we found a paper which also followed the method by Plazek and Sucharda and was able to obtain 3-(carboxymethylthio)-picolinic acid.[14] With this proof, we have reproduced the method by Plazek and Sucharda again. This trial, again, gave very low yield (< 5%) after silica gel column chromatography with intractable mixture of compounds. However, we were able to increase yield by recrystallization. Somehow ^{1}H NMR spectra of crude product showed more pure product than recrystallized product. Therefore this compound was sent to Dr. Holyoak without purification to test for its activity.

Since we had some experience with dimethyldioxirane (DMDO), we have used DMDO to oxidize 3-(carboxymethylthio)-picolinic acid **(1)** to synthesize 3-(carboxymethylsulfonyl)-picolinic acid **(2)**. We have had problems with the concentration of DMDO, therefore we used excess DMDO to react with 3-(carboxymethylthio)-picolinic acid **(1)** as shown in Scheme 4[15]. This reaction was carried smoothly with yield of 32 %.

Scheme 4



1 **2**

In conclusion, we have successfully synthesized 3-(carboxymethylthio)-picolinic acid **(1)** and 3-(carboxymethylsulfonyl)-picolinic acid **(2)**. Both of these compounds are bicarboxylates, 3-(carboxymethylsulfonyl)-picolinic acid **(2)** with sulfonyl group which

meets criteria in choosing compounds that they possess functional groups similar to those of the substrates, OAA and PEP. These compounds can help researchers to study the mechanisms of molecular recognition of phosphoenolpyruvate and oxaloacetate by cytosolic phosphoenolpyruvate carboxykinase.

Experimental section

General.

All ^{1}H NMR spectra were recorded at 300 MHz or 400 MHz unless otherwise noted. Unless otherwise noted, reactions were carried out under an argon atmosphere. Thin-layer chromatography was performed using commercially prepared 60-mesh silica gel plates (Whatman K6F), and visualization was effected using short wavelength UV light (254 nm). All reagents were used directly as obtained commercially unless otherwise noted.

3-(Carboxymethylthio)-picolinic acid (1).

To hot concentrated hydrochloric acid (2.2 mL), β-aminopicolinic acid **(5)** (1.5 g, 10.6 mmol) was dissolved and then quickly cooled. Separated crystals were added in concentrated sodium nitrite solution (0.8 g). Clear, brownish liquid is poured into 0 ℃ of thioglycolic acid **(6)** (1.0 g, 10.9 mmol) in water (~2 mL). After 2 hours of stirring 1.5 mL of concentrated hydrochloric acid is added. The reaction was let stand overnight to obtain compound **1** (384 mg, 17 % yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.44 (d, 1H), 8.22 (d, 1H), 7.73 (m, 1H), 3.90 (s, 2H); LRMS (EI): *m/z* 215 (M+2), 78, 45 (100%)

3-(Carboxymethylsulfonyl)-picolinic acid (2).

To a solution of 3-(carboxymethylthio)-picolinic acid **(1)** (100 mg, 0.469 mmol) in methanol (5 mL) was added dropwise DMDO in acetone at 0 ℃. The solution was stirred to room temperature for 2 hours. After evaporation of the solvent, the residue was dried under vacuum to yield compound **2** (37 mg, 32 % yield) as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.63 (d, 1H), 8.53 (d, 1H), 8.15 (m, 1H), 4.32 (s, 2H).

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GENERAL CONCLUSION

In the first chapter, we investigated the synthesis of chrysosplenol B, chrysosplenol D, and their analogs. We have modified the Kraus and Roy synthesis of chrysosplenol D. From Kraus and Roy synthesis, we were unable to obtain flavonols using Algar-Flynn-Olyamada reaction. However, this research has led to an alternative method of forming flavonols from chalcones. We have oxidized chalcones to flavones using selenium dioxide and further oxidized flavones to flavonols using lithium diisopropylamide, trimethylborate and hydrogen peroxide. With our modified method, we were able to synthesized chrysosplenol D in six steps from 1-(6-hydroxy-2, 3, 4-trimethoxyphenyl)ethanone in 15 % yield. Chrysosplenol B was also synthesized in six steps from 1-(6-hydroxy-2, 3, 4-trimethoxyphenyl)ethanone in 16 % yield. Both chrysosplenol B and chrysosplenol D have the flavone skeleton with 3-methoxyl and 5-hydroxyl groups, which have ability to potentiate the potent anti-malarial activity of artemisinin and have ability to potentiate the activity of norfloxacin.

In the second chapter, we have synthesized 3-(carboxymethylthio)-picolinic acid and its analog, 3-(carboxymethylsulfonyl)-picolinic acid. Both of these compounds are bicarboxylates, 3-(carboxymethylsulfonyl)-picolinic acid with sulfonyl group which meets criteria in choosing compounds that they possess functional groups similar to those of the substrates, oxaloacetate, and phosphoenolpyruvate. These compounds can help researchers to study the mechanisms of molecular recognition of phosphoenolpyruvate and oxaloacetate by cytosolic phosphoenolpyruvate carboxykinase.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Dr. George A. Kraus, my major professor and mentor, for his continuous support and guidance throughout my coursework and research. I would also like to thank Dr. Malika Jeffries-El and Dr. Gordon J. Miller for being on my program of study committee.

I would like to thank Dr. Hakwon Kim for his motivation and guidance. I would also like to thank past and present Kraus group members for their helpful suggestions and exciting discussions. They made the work place really lively and enjoyable. I am also grateful to all of my friends and colleagues at Iowa State University.

Finally, I would like to thank my parents and brother for their endless supports and encouragements throughout my whole educational experience. I would also like to thank all of my other family members for their thoughts and prayers.