SYNTHESIS OF 5-PHENOXYPRIMAQUINE DERIVATIVES AND THEIR TETRAOXANE CONJUGATES AS ANTI-MALARIAL AGENTS

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การสังเคราะห์อนุพันธ์ 5-ฟีนอกซีไพรมาควินและเตตระออกเซนคอนจูเกตเพื่อเป็นสารต้านมาลาเรีย

น.ส.สมฤดี จันทร์ส่องแสง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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สมฤดี จันทร์ส่องแสง : การสังเคราะห์อนุพันธ์ 5-ฟีนอกซีไพรมาควินและเตตระออกเซนคอนจูเกตเพื่อเป็นสารต้านมาลาเรีย. (SYNTHESIS OF 5-PHENOXYPRIMAQUINE DERIVATIVES AND THEIR TETRAOXANE CONJUGATES AS ANTI-MALARIAL AGENTS) อ.ที่ปรึกษาหลัก : ผศ. ดร.ธนธรณ์ ขอทวีวัฒนา, อ.ที่ปรึกษาร่วม : ดร.นิติพล ศรีมงคลพิทักษ์

ปัจจุบัน มีความจำเป็นต้องพัฒนายาต้านมาลาเรียชนิดใหม่เพื่อใช้รักษาเชื้อมาลาเรียสายพันธ์ดื้อยาที่มีรายงานว่าพบมากขึ้นเรื่อย ๆ งานวิจัยนี้จึงสังเคราะห์และหาฤทธิ์การต้านเชื้อมาลาเรียของสารกลุ่มไพรมาควิน ซึ่งในปัจจุบันใช้เป็นยารักษาโรคมาลาเรีย โดยเน้นการเติมหมู่แทนที่ลงบนอนุพันธ์ 5-phenoxyprimaquine ซึ่งเคยมีรายงานว่ามีค่าดัชนีการรักษามากที่สุดและมีความเป็นพิษน้อยกว่าไพรมาควิน โดยทำการศึกษาผลของหมู่แทนที่ที่ตำแหน่ง para โดยสามารถสังเคราะห์สารใหม่ทั้งหมด 3 โครงสร้าง ประกอบด้วยหมู่ CN (7e) CF_3 (7g) $CONH_2$ (7h) และเป็นสารที่มีการรายงานมาแล้วจำนวน 5 โครงสร้าง ประกอบด้วยหมู่ OMe (7b) Br (7c) Cl (7d) F (7f) และสารที่ไม่มีหมู่แทนที่ (7a) สารทั้งหมดได้พิสูจน์โครงสร้างด้วยเทคนิค ^{1}H ^{13}C ^{19}F NMR และ HRMS หลังจากนั้นนำสารทั้งหมดที่สังเคราะห์ได้ไปทดสอบฤทธิ์การต้านเชื้อมาลาเรียชนิด *P. falciparum* สายพันธุ์ 3D7 ในระยะเลือด ใช้วิธี SYBR Green I-base fluorescence (MSF) ผลปรากฏว่า 7a มีฤทธิ์ยับยั้งดีที่สุดโดยให้ค่า IC_{50} อยู่ที่ 3.65 ± 0.39 μM เปรียบเทียบกับไพรมาควินซึ่งค่า IC_{50} อยู่ที่ 11.33 ± 0.79 μM และมากกว่าอนุพันธ์อื่นๆ ซึ่งค่า IC_{50} อยู่ในช่วง 4.62 to 13.5 μM ลำดับถัดมานำสาร 7a ไปสังเคราะห์ต่อเป็นคอนจูเกตกับเตตระออกเซน (14) โดยใช้ตัวเชื่อมที่มีคาร์บอน 4 อะตอม ได้เป็น 5-ฟีนอกซีไพรมาควิน-เตตระออกเซนคอนจูเกตชนิดใหม่ (15) ซึ่งพบว่า 15 มีค่าการยับยั้งอยู่ที่ 0.38 ± 0.11 μM และมีประสิทธิภาพดีกว่าไพรมาควินเดิม 30 เท่า ยิ่งไปกว่านั้นสารประกอบใหม่ 7e และ 15 ที่มีฤทธิ์ต้านมาลาเรียสูงมีค่า selectivity index (SI) มากกว่า 25 และ 45.61 ตามลำดับ

สาขาวิชา เคมี ลายมือชื่อนิสิต .....

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Somruedee Jansongsaeng : SYNTHESIS OF 5-PHENOXYPRIMAQUINE DERIVATIVES AND THEIR TETRAOXANE CONJUGATES AS ANTI-MALARIAL AGENTS. Advisor: Asst. Prof. Dr. TANATORN KHOTAVIVATTANA Co-advisor: Dr. Nitipol Srimongkolpithak

Currently, the development of the new anti-malarial drug for the treatment of the increasing drug-resistant strains of malaria was necessary. Therefore, this research was performed by synthesis and evaluation of their antimalarial activity for the development of primaquine which currently was used for anti-malarial drug. The substituents group on 5-phenoxyprimaquine derivatives which had the highest therapeutic index and less toxicity than primaquine were emphasized. The effect of substituted group at *para* position was studied. Three new derivatives with CN (7e), CF_3 (7g), $CONH_2$ (7h) substituents, and five known derivatives with OMe (7b), Br (7c), Cl (7d), F (7f) substituents and the unsubstituted derivative (7a) of primaquine were synthesized. Their structures were characterized by ^{1}H, ^{13}C, ^{19}F NMR and HRMS techniques. In addition, all synthesized compounds were evaluated for their anti-malarial activity using Malaria SYBR Green I-base fluorescence (MSF) assay against *P. falciparum* 3D7 in the blood-stage. 7a exhibited the greatest inhibitory effects with the IC_{50} value of 3.65 ± 0.39 μM comparing with primaquine (IC_{50} = 11.33 ± 0.79 μM) and another derivative with IC_{50} values ranging from 4.62 to 13.5 μM. Subsequently, 7a conjugated with tetraoxane 14 using a linker of four carbon to afford new 5-phenoxy primaquine-tetraoxane conjugated (15). Pleasingly, 15 exhibited inhibitory effects with IC_{50} values of 0.38 ± 0.11 μM, which was 30-fold more potent than that of the primaquine. Moreover, new compound 7e and 15 with high antimalarial activity had selectivity index (SI) values of more than 25 and 45.61, respectively.

Field of Study:	Chemistry	Student's Signature .....
Academic Year:	2020	Advisor's Signature .....
		Co-advisor's Signature .....

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CHAPTER I INTRODUCTION

1. Background and significance of research

Malaria continues to be one of the world's health challenges caused by parasitic protozoan in the genus *Plasmodium*. Infection is typically transferred by female *Anopheles* mosquitoes, causing 409,000 deaths in 2019 especially children under five years of age.[1] According to the WHO, there are five different types of *Plasmodium* that can affect human, but *P. falciparum* and *P. vivax* are the two major infected pathogens. The most dangerous species is *P. falciparum*, which has developed resistance against almost all anti-malarial drugs in current use.[2] On the other hand, *P. vivax* commonly infect human and it is a cause of persistent infection.

Drug resistance and side effect are a major problem to eliminate malaria. Nowadays, the artemisinin-based combination therapies (ACTs); combinations between an artemisinin derivative and another partner drug are currently the strategy to delay malaria resistance.[3] Unfortunately, in 2007 artemisinin showed a sign of resistance in several countries, especially in Southeast Asia.[4] Because of this, new endoperoxide-based compounds with the superior on the longer haft-life are an interesting alternative to standard ACTs.[5] Recently, it was found that the clinical candidate E209 and OZ439 (**Figure 1a and 1b**), an analogue of tetraoxane and trioxolane, respectively, exhibited nanomolar efficacy against multiple strains of *P. falciparum* and *P. vivax* including the artemisinin-resistant strain.[6]

Primaquine, one of the 8-aminoquinoline a class of anti-malarial drug (PQ; **Figure 1c**), is the only drug with the radical cure ability of *P. vivax* and *P. ovale* for relapsing malaria. It is active against the temporary liver forms of all *Plasmodium* species. [7] However PQ has short half-life because of rapid metabolism especially at position 5.[8] Importantly, bulletin of the world health organization in 1981 reported that most of 5-phenoxy analogues without methyl group on the quinoline ring were substantially less toxic than primaquine.[9] The highest therapeutic index among the 8-aminoquinolines class belonged to a 5-phenoxy derivative.[10] Until 1982, Nodiff *et al*. have synthesized various 5-phenoxy derivative of primaquine and screened the anti-malarial activity in murine and monkey, the results confirmed that 5-phenoxy analogues are less toxic than PQ, for example compounds **A**, **B**, and **C** (**Figure 1d**) have no toxic lethality at the highest dose tested (640 mg/kg). The most active compounds against blood-stage parasite were the ones that have substituents R_2 = 4-F and 3-CF_3; however, the compound containing

both 4-F and 3-CF_3 substituents was found to be less active.[11] Moreover, the first successful 5-phenoxy derivative developed by the US Army programme name WR 238605 (**Figure 1e**), well-known as tafenoquine, has a longer half-life than primaquine.[12] Up to date, there is no report on the structure-activity relationship related to 5-phenoxy analogues of primaquine despite a great opportunity to enhance bioactivity and half-life further.

To combat drug resistance problems instead of using multicomponent or cocktail therapy, drugs conjugate designed for the incorporation of two biological molecules with different mode of action into a single molecule leading to several advantages was introduced. Drug conjugates can act by two different mechanisms or unique one by this PQ targeting of the liver-stage parasite while endoperoxide is fast-acting against intra-erythrocytic asexual blood-stage parasites.[13] In 2014, Miranda *et al*. synthesized drug conjugates between tetraoxane and primaquine (**Figure 1f**) and showed that these conjugates could inhibit both blood and liver stages of parasites.[14] Moreover, In 2018, Capela *et al*. reported that aryl or heteroaryl group substituted at the metabolically labile C-5 position of the primaquine after hybridization with endoperoxide moiety does not result in loss of both blood and liver stages anti-plasmodial activity together with good metabolic stability.[15] Inspired by these works, we opted to study a novel 5-phenoxy analogues of primaquine with various R groups on the phenoxy ring for anti-malarial activities against *P. falciparum* in the blood stage parasite in order to gain the structure-activity relationship (SAR). In this process, we will select the best analogue to conjugate with the tetraoxane molecule to gain more insight into the mechanism of action of primaquine and tetraoxane-based drugs. We surmise that these drug conjugates may exhibit a synergistic or additional effect, leading to increased inhibitory activity against *P. falciparum* in an asexual stage. In addition, they could be used as board activities against *P. falciparum* and *P. vivax* in the future.

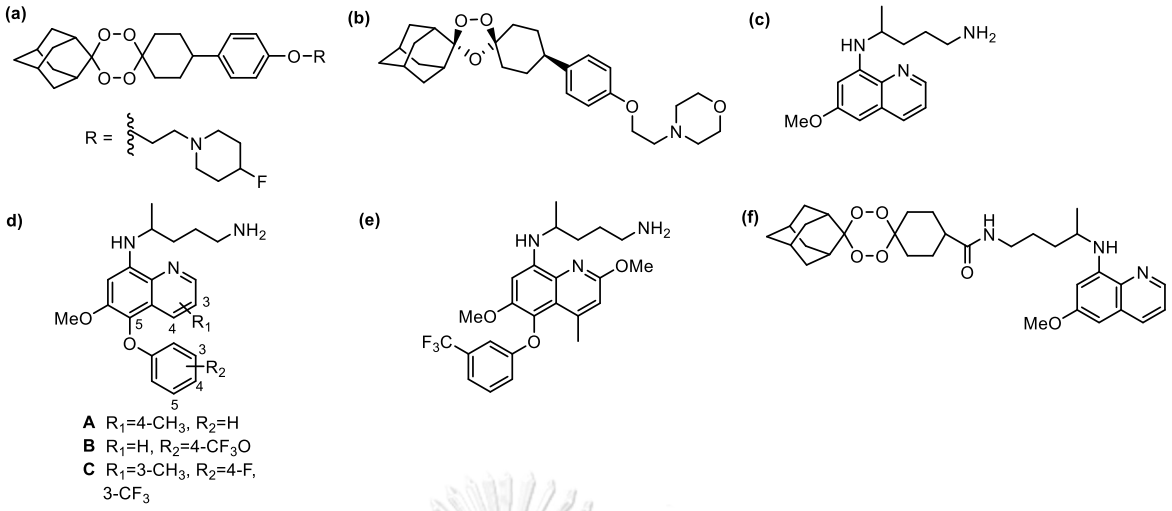


Figure 1 (a) E209 analogue of tetraoxane; **(b)** Artefenomel (OZ349); **(c)** primaquine; **(d)** 5-phenoxyprimaquine; **(e)** tafenoquine; **(f)** tetraoxane-primaquine conjugate.

2. Literature reviews

2.1 Malaria

Malaria is caused by the parasite *Plasmodium* which spread to people in 91 countries worldwide including other vertebrates. There are more than 120 species of *Plasmodium*, but human malaria is caused by only 5 species of parasite including *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax*, and *P. knowlesi*. [16] The life cycle of malaria parasite involves two hosts (**Figure 2**). The transmission of this disease is started by the infectious sporozoites from the bite of infected female *Anopheles* mosquitoes. These sporozoites travel into the human liver and mature into schizonts which are ruptured to release merozoites. In this stage, some untreated *P. vivax* and *P. ovale* persist in the liver for a long time without symptoms because asexual replication does not immediately occur, but they can be reactivated resulting in relapsed malaria. These merozoites further spread into blood circulation system for replication, and the red blood cells (erythrocytes) developed to form ring stage, trophozoites as DNA synthesis. These trophozoites further evolved into schizonts which divided into approximately 32 merozoites, [17] and it is released from the RBCs to enter the new replication cycle. In addition, hemoglobin of the host cell is ingested by the parasite, amino acids and hemozoin toxin are released, therefore, the human red blood cells broke, and as a result, the clinical symptoms are observed. [18] Not only, asexual replication but also sexual replication occurs in the human blood led to malaria transmission. Some of those trophozoites developed into female and male

gametocytes, which was the infective stage for mosquitoes, will evolve into mature gametocytes. After mosquitoes fed human blood, those mature gametocytes developed into ookinetes, oocysts, and sporozoites within the mosquito's midgut. These sporozoites were released to move into the mosquito's salivary gland, and it readily started the new cycle led to the transmission of malaria. [19]

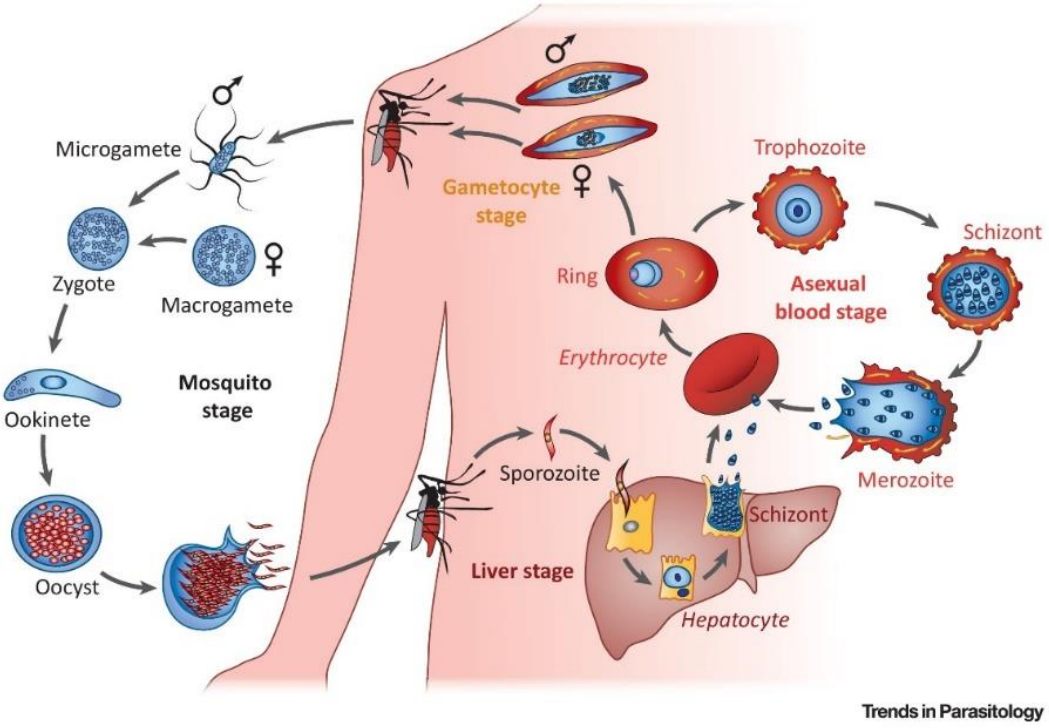


Figure 2 Life cycle of malaria parasites [16]

2.2 Anti-malarial drugs.

Currently, the WHO recommends artemisinin-based combination therapy (ACT) for the treatment of uncomplicated *P. falciparum* malaria. The combination is between a potent artemisinin derivative, which rapidly clears most parasites, and a drug from a different class, which slowly eliminates remaining parasites.[20] For the first-line treatment, chloroquine plus primaquine is generally used to cure *P. vivax* malaria in most regions. Moreover, the current drugs from the other derivatives are classified into many classes (**Figure 3**).

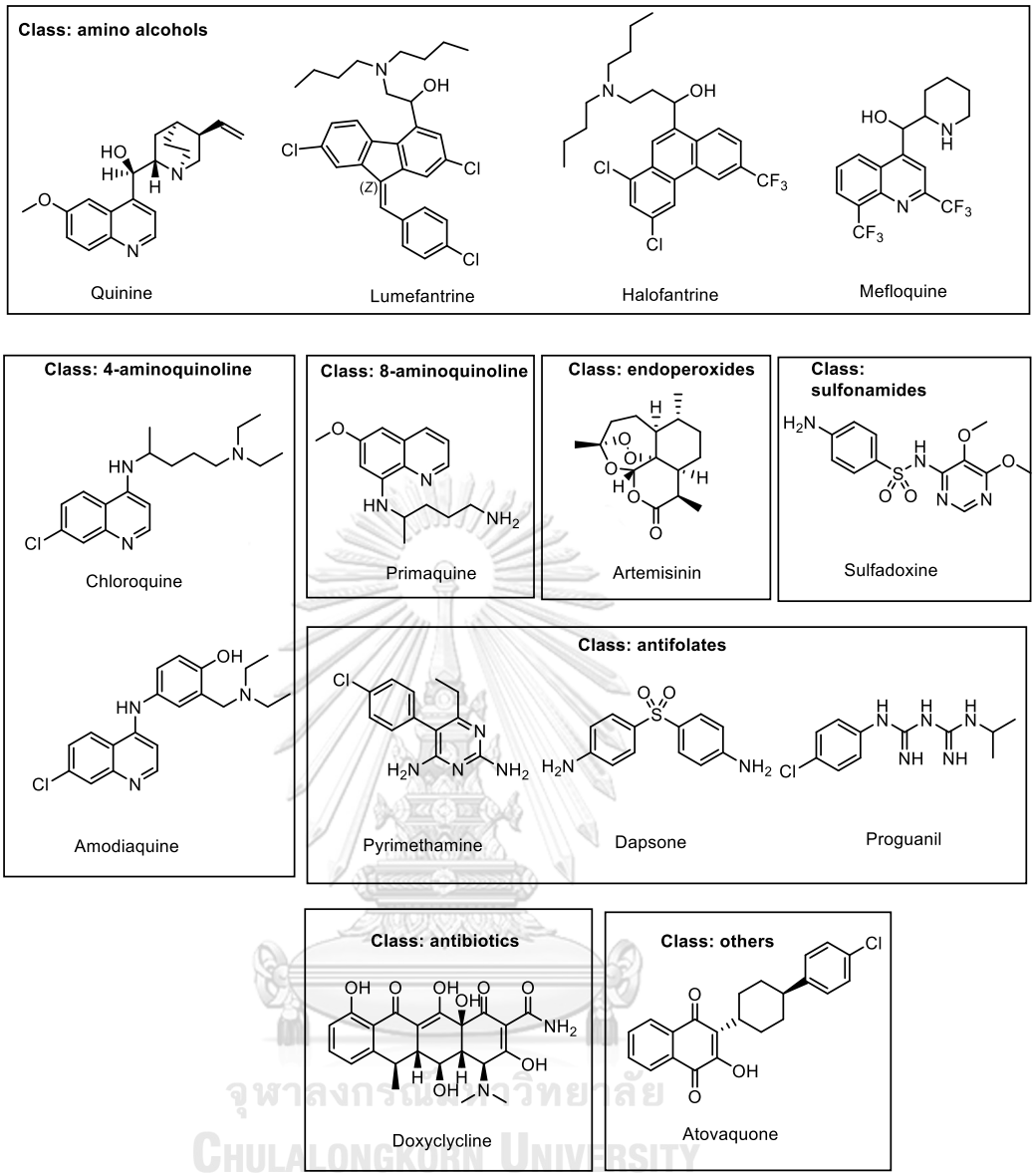


Figure 3 Chemical structure and classification of antimalarial drugs.[21]

Not only drug resistance but also the adverse effect of anti-malarial drugs is the problem to eliminate malaria. The target/mode of action, indications, and adverse effects of the drugs are clarified in **Table 1**.

Table 1 Target/mode of action, indications, and adverse effects of antimalarial drugs. [22]

Drug	Target/Mode of Action	Indications	Adverse effects
Quinine	Fast-acting erythrocytic schizonticide	IV for cerebral malaria (or quinidine); childhood or pregnancy malaria unresponsive to other agents	Cinchonism, tachycardia, sinus arrhythmias, hypoglycemia
Chloroquine*	Fast-acting erythrocytic schizontocide (mild gametocide)	Acute uncomplicated malaria; pregnancy and childhood malaria (note high resistance rates)	Pruritis, nausea, agitation (all uncommon)
Amodiaquine*	Fast-acting erythrocytic schizontocide (mild gametocide)	Acute uncomplicated malaria; prevention in travelers	Nausea, pruritus, agranulocytosis, and hepatotoxicity (long-term use)
Mefloquine	Fast-acting erythrocytic schizontocide	Acute malaria; prevention in travelers	Sleep disturbance, nightmares, nausea, rash, myalgias, psychosis, arrhythmias, pneumonitis (rare)
Halofantrine	Fast-acting erythrocytic schizontocide	Acute malaria not responding to other agents	Nausea, arrhythmias, elevated transaminases
Pyrimethamine	Fast-acting erythrocytic schizonticide (mild tissue schizontocide)	Acute malaria; pregnancy malaria (usually with sulfadoxine)	Nausea, headache, mild anemia
Sulfadoxine	Fast-acting erythrocytic schizontocide	Acute malaria; pregnancy malaria (usually with pyrimethamine)	Nausea, headache, mild anemia, hemolysis in G6PD deficiency, severe skin rashes (rare) – sulfa drug
Doxycycline	Slow-acting erythrocytic schizontocide	Acute malaria (with fast-acting agents); prevention in travelers	Nausea, photosensitivity, Esophagitis

Primaquine	Tissue schizontocide; gametocidal	Infection with *P. vivax* or *P. ovale*; relapsing infection; low risk of reinfection; prevention in travelers to high *P. vivax* areas	Nausea, methemoglobinemia (self-limiting), hemolysis in G6PD deficiency
Artemisinins	Fast-acting erythrocytic schizontocide; gametocidal	General acute treatment; IV or rectal suppository for cerebral malaria	Minimal; transient mild nystagmus or ataxia (self-limiting)
Lumefantrine	Long-acting erythrocytic schizontocide	Acute treatment (only combined with artemether) in Africa	Headache, dizziness, nausea
Dapsone	Fast-acting erythrocytic schizontocide	Acute treatment not responding to other agents	Hemolytic anemia (especially in G6PD deficiency), methemoglobinemia, jaundice, nausea, headache, insomnia, psychosis, neuropathy (rare)
Atovaquone	Fast-acting erythrocytic schizontocide	Acute uncomplicated malaria, always given in combination with proguanil	Nausea, mild elevated transaminases
Proguanil	Fast-acting erythrocytic schizonticide (mild tissue schizontocide)	Acute uncomplicated malaria, always given in combination with proguanil	Nausea, mild elevated transaminases

*Also anti-inflammatory and antipyretic. Note: use of single agents to treat acute malaria is almost never a good idea. G6PD, glucose-6-phosphate dehydrogenase; IV, intravenously

2.3 Primaquine (PQ)

Primaquine has been used for more than 60 years for radical treatment of *P. vivax* and *P. ovale* malaria. However, several cases involving severe haemolysis in malaria patients who are deficient glucose-6-phosphate dehydrogenase (G6PD) has been reported, so the use of primaquine are mainly limited, especially in South East Asia.[23] This problem affects over 350 million people globally.[24] Up to date, the mechanism of action of PQ has remained unclear. In 2016, Marcsisin *et al*. proposed that PQ is activated by CYP 2D6, a hepatic cytochrome P450 enzyme in humans, to produce 5-hydroxyprimaquine as the active species (**Figure 4**). The by-products such as H_2O_2 and some reactive oxygen species (ROS) are produced by the redox oxidation of the unstable 5-hydroxy primaquine to kill malaria parasites. [25]

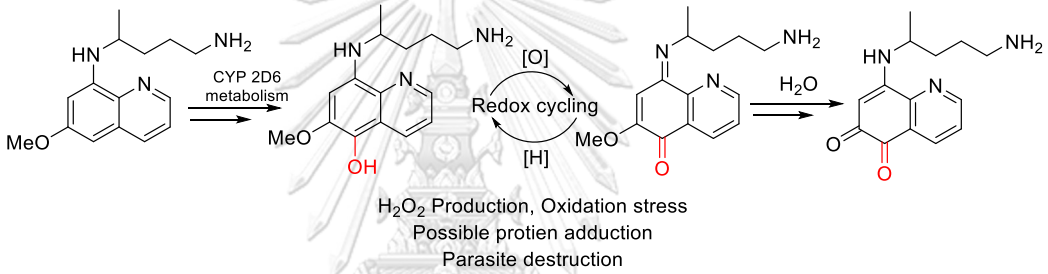
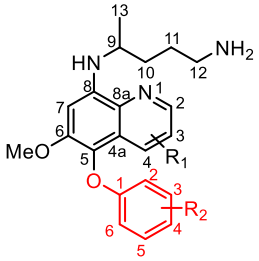


Figure 4 Proposed mechanism of primaquine CYP 2D6 metabolic activation and liver stage antimalarial activity.[25]

2.4 5-Phenoxy derivatives of primaquine

The toxicity of primaquine was decreased by the 5-phenoxy groups (**Table 2**). The result in *P. berghei* infected mice indicates that the toxic lethality was not produced at the highest dose tested (640 mg/kg) except for compounds **1a**, **1c**, and **1j**. For the demethylated derivatives on quinoline ring, the structure contains only one substituent group at R_2 position on phenoxy ring that either 3-CF_3 (**1b**) or 4-F (**1n**) exhibited the most active blood schizonticides. On the other hand, the structure contains both of those groups (**1i**) was less active than single substitution. However, the range of activity of the demethylated derivatives is not significantly different. Moreover, blood schizonticidal activity of various 5-phenoxy primaquines was dramatically increased by introduction of a methyl group on the pyridine ring especially in position 4. [11]

Table 2 Blood schizonticidal anti-malarial activity (*P. berghei*, Mouse)[11]



cures (C), [b] toxic deaths (T), [c] or MST [d]

Comp	R_1	R_2	dose	10	20	40	80	160	320	640
				mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
PQ					4.0	5.0	9.4	2T	5T	5T
4-methylprimaquine								9.0	10.0	1T
1a	4-CH_3	H		8.7	4C	3C	4C	4C	3T	2T
1b	H	3-CF_3			0.5	1.3	4.1	7.1	13.5	5C
1c	H	4-CF_3O			-0.3	-0.3	-0.3	0.5	0.7	1T
1d	H	4-CH_3O			0.4	0.4	1.6	6.0	8.6	4C
1e	H	2,4-Cl_2			0.4	0.4	0.8	2.2	4.4	10.4
1f	H	3,4-Cl_2			1.2	0.8	0.4	6.0	1C	1C
1g	H	3,5-$(CF_3)_2$			0.7	0.3	0.3	1.5	6.3	7.9
1h	4-CH_3	3,5-$(CF_3)_2$		1.7	4.3	3C	5C	4.6	5C	5C
1i	H	4-F, 3-CF_3			-0.2	0.8	3.4	5C	12.2	2C
1j	3-CH_3	4-F, 3-CF_3			5.9	9.9	4C	4.6	4C	1T
1k	4-CH_3	4-F, 3-CF_3		1C	1C	5C	5C	5C	5C	5C
1l[e]	H	H			0.3	1.5	1.7	5.1	7.5	2C
1m[e]	H	4-Cl			0.7	4.7	5.5	7.1	8.1	2C
1n[e]	H	4-F			2.1	5.7	7.5	8.9	5C	5C

Tests were carried out by the Rane Laboratory, University of Miami, FL, using blood-induced, *P. berghei* infected mice (five animals per group) [b]the number of mice surviving at 60 days postinfection. [c]Deaths prior to the 6th day. [d]Increase in mean survival time over controls; a compound is considered active if MST of the treated group is more than twice that of the control group (MST of control group, 6.1 days). [e]These data, reported earlier.[10]

2.5 Tafenoquine (TQ)

Recently, tafenoquine (**Figure 1 e**), a phenoxy analogue of primaquine, is now approved by the US FDA and Australian TGA in 2018. According to the report of medicines for malaria venture (MMV), the half-life of tafenoquine is 14-28 days which are longer than that of primaquine (4-6 hours),[26] thus allowing single-dose treatment and providing radical cure ability to prevent relapsing malaria in liver-stage malaria occurring from *P. vivax* and *P. ovale*.[27] In addition, Brueckner *et al* concluded that tafenoquine is more potent and less toxic than primaquine by the study both *in vitro* and *in vivo* using animal models. Moreover, a safe, well-tolerated, and highly effective oral chemoprophylactic agent of tafenoquine were confirmed by phase I, II, and III clinical trials studies.[28] Therefore, the presence of 5-phenoxy group may have some effect on anti-malarial activity.

2.6 Artemisinin-based antimalarial drug

Artemisinin (ART), a sesquiterpene lactone from the natural product, was isolated from *Artemisia annua* which is a traditional Chinese herbal plant for malarial therapy. The very high potency of them to inhibit the parasite growth especially the chloroquine-resistant *P. falciparum* strain was reported. The endoperoxide bridge has been reported to be an important part of the antimalarial activity. Their bond was reduced by the ferrous iron resulted in cytotoxic carbon-centered radicals. The essential macromolecules of malarial parasite was alkylated by these radicals, leading to parasite's death. However, the low solubility in water or oil, poor bioavailability, and a short half-life *in vivo* (~2.5 h) are pharmacokinetically limited of the natural ART. Therefore, the semi-synthetic derivatives were synthesized to maintain the antimalarial property to overcome those problems by modifications at position C10 on their structure to improve the drug solubility (**Figure 5**) lead to the dihydroartemisinin (DHA), artemether (ATM), arteether (AE), and artesunate (ATS). Although the series of the semi-synthetic derivatives were used for the treatment of asexual blood stages of *falciparum* malaria, but the elimination half-life *in vivo* has been concerned. [29]

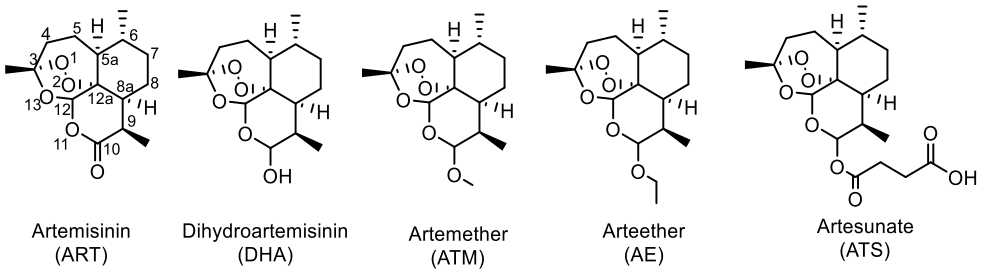


Figure 5 Chemical structure of artemisinin (ART), dihydroartemisinin (DHA), artemether (ATM), artesunate (ATS)

The 1,2,4-trioxolane derivative named arterolane (OZ277) was firstly synthesized and designed to overcome limitations associated with the artemisinin. The fast acting and comparable activity to the artemisinin were found. In addition, it was found that oral dosing was highly effective against various *P. falciparum* strains and it was then approved for combination with piperaquine in India.[30] However, the clinical study revealed that the elimination half-life was increased only threefold compare to DHA. Later, artefenomel (OZ439) was discovered (**Figure 6**). The elimination half-life in a human was increased according to the result of phase I and II clinical studies. As a result, it was used for the oral single dose to cure uncomplicated malaria by combination with another effective partner drug including piperaquine and ferroquine. These combinations are known as ART-based combination therapies (ACTs) which is currently recommended treatment for multidrug-resistant *P. falciparum* malaria by WHO. Unfortunately, the resistance to ACTs of *P. falciparum* has been reported especially in Southeast Asia and many parts of the world. [31]

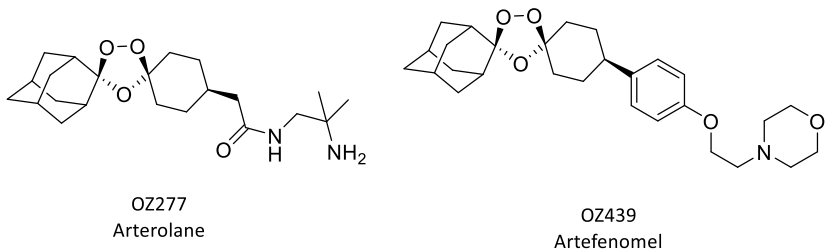


Figure 6 Chemical structure of arterolane (OZ277) and artefenomel (OZ439)

2.7 The development of E209, an analog of tetraoxane

E209, a second-generation of peroxide based drug, is fully synthetic drug containing 1,2,4,5-tetraoxacyclohexane. Achiral and the endoperoxide bond of the tetraoxane were more stable and longer *in vivo* half-lives than analog of 1,2,4-trioxolane. Symmetrical dispiro-1,2,4,5-tetroxane (**Figure 7i-7iii**) was synthesized, and their curative activities against *P. berghei in vivo* were evaluated. Especially, **7iii** had IC_{50} against *P. falciparum* comparable to artemisinin. Therefore, the potential of dispiro-1,2,4,5-tetraoxanes were confirmed by these results.[32]

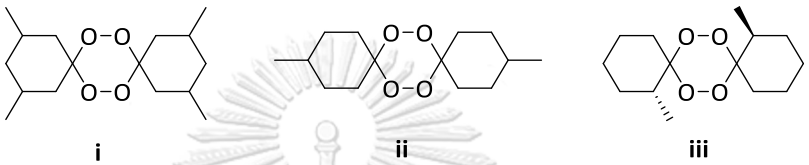


Figure 7 Dispiro-1,2,4,5-tetraoxanes

Importantly, it was found that the rapid degradation *in vivo* was prevented by the **α**-methyl substituents of this active compound. As a result, the high concentration of the drug reached to its site of action. Previously, the development of 1,2,4,5-tetraoxanes as drug candidates was studied by the O'Neill group [33]. A cyclohexyl group was replaced by a spiro adamantyl group, therefore the activity improved. Then, the RKA182 was successfully designed and synthesized as a good candidate for drug development. It was reached to the pre-clinical development. The presence of amide linkage in RKA182 caused a problem during metabolism due to its reactivity. Therefore, a series of the second-generation derivatives of RKA182 was designed to eliminate the amide linkage and enhance metabolic stability.[34, 35] (**Figure 8**)

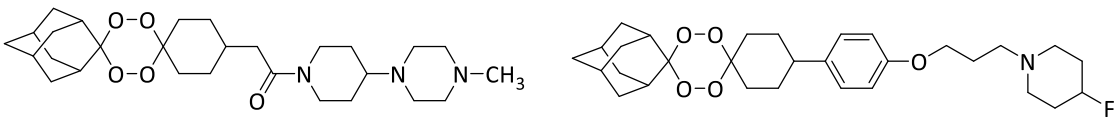


Figure 8 Structure of RKA 182 and E209

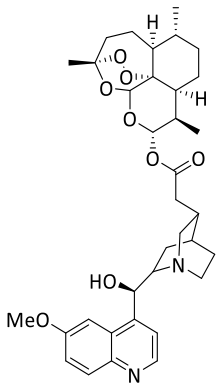
Recently, the anti-malarial property of E209, a new tetraoxane antimalarial drug candidate, was studied. The optimization of aryl-substituted side chains has been studied. An estimated half-life of E209 was 24-30 hours which higher than the artemisinin that have half-lives of 1-2 hours. Interestingly, the efficacy in vitro (mean IC_{50} range 2.9–14.0 nM) against a panel of 10 sensitive and multidrug-resistant *P. falciparum* parasite of E209 has been reported.[6]

2.8 Drug hybrid

The hybrid molecule contains the pharmacophores for each target that are separated by the metabolically stable linker, whereas the cleavage hybrid is designed to release the two drugs for interaction independently with each target. These strategies are developed for solving the limitations of drug resistance, pharmacokinetics, and pharmacokinetics that occurred from the individual components.[36]

2.8.1 artemisinin-quinine hybrid

The artemisinin-quinine was hybridized by the ester-linker (**Figure 9i**). Artemisinin, lipophilic and fast-acting but quickly eliminated drugs that are associated with high rates of recrudescence when used in monotherapy, was coupled by quinine; slow-acting and polar compound. It was found that the activity of this hybrid was superior to quinine alone, artemisinin alone, and about 3-fold to a 1:1 mixture of the two. This hybrid may be cleavage at the metabolic lability of the ester linkage to form dihydroartemisinin which was more active than artemisinin itself. Since each component has independent mechanisms of action, so the resistance of these hybrid should not occur. [37]



i

Figure 9 Artemisinin-quinine hybrid

2.8.2 cleavable and non-cleavable hybrids based on artemisinin and mefloquine.

The cleavable of diester (**Figure 10i)** and non-cleavable of C-N linkages (**Figure 10ii)** were studied. The activity of the cleavable hybrid was comparable to that of artemether and of the trifluoromethyl artemisinin derivative used in the synthesis and superior to that of mefloquine whereas the non-cleavable hybrid was ~2-4-fold less active than the cleavable one.[38]

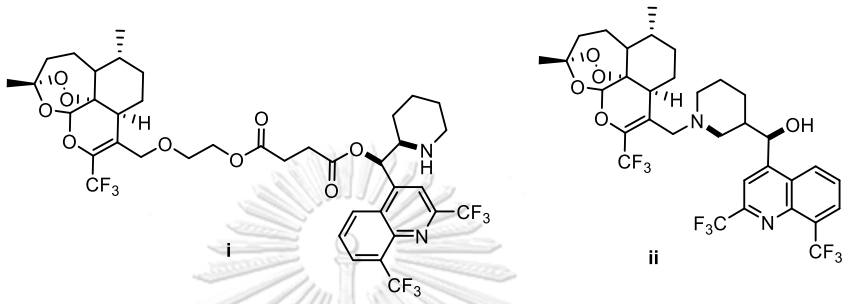
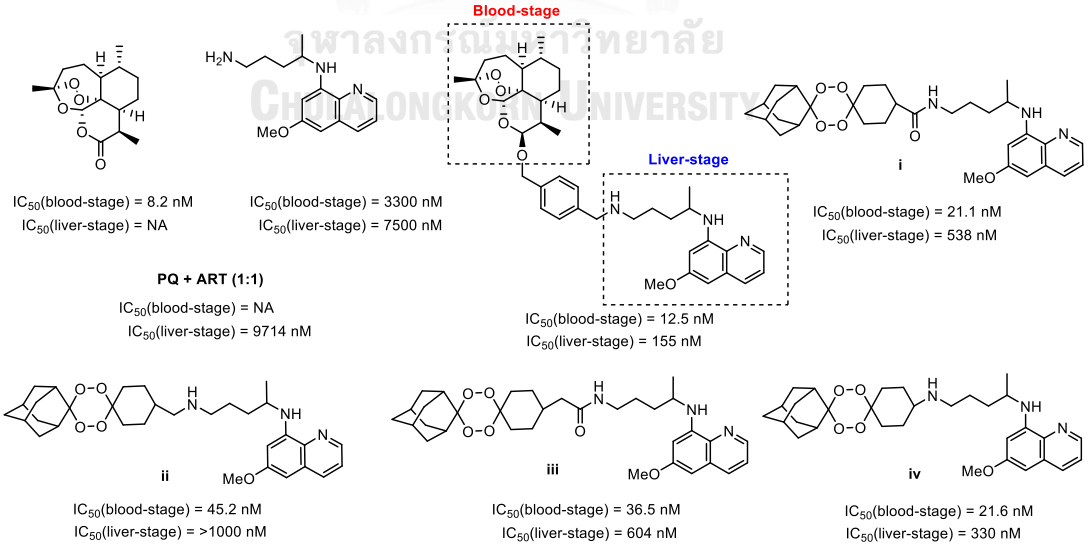


Figure 10 Cleavable and non-cleavable hybrids based on artemisinin and mefloquine **i** and **ii**.

2.8.3 Artemisinin-primaquine hybrid and tetraoxane-primaquine hybrids

All synthesized hybrids had activity against both blood and liver forms of malaria parasites. Interestingly, all hybrids had activity against the chloroquine-resistant *P. falciparum* W2 strain that was superior to primaquine alone about 100-fold. (**Figure 11i-11iv**)[14]



*Blood stage was determined against the chloroquine-resistant *P. falciparum* W2 strain whereas liver stage was determined against *P. berghei*; NA, not active (>10 μM).

Figure 11 Artemisinin-primaquine hybrid and tetraoxane-primaquine hybrids

3. Objectives

3.1 To synthesize and characterize novel 5-phenoxy analogues of primaquine by varying R group on the phenoxy ring.

3.2 To investigate the anti-malarial activities of the synthesized analogue against *P. falciparum* in order to gain the knowledge of the structure-activity relationship (SAR)

3.3 To develop the hybrid molecule based on the best 5-phenoxy analogue with a partner drug tetraoxane and investigate its anti-malarial activity.

4. Scope of research

4.1 Synthesize and characterize 5-phenoxy analogues of primaquine with various R group at *para*- position on phenoxy ring.

4.2 Investigate the bioactivity and cytotoxicity of the synthesized compound against *P. falciparum*.

4.3 Synthesize 5-phenoxyprimaquine-tetraoxane conjugates and investigate the anti-malarial activity of the conjugated drug.

5. Beneficial outcome

Three new (R = CN, $CONH_2$, CF_3) and five known (R = H, OMe, Cl, Br, F) analogs of primaquine, along with one new 5-phenoxy primaquine-tetraoxane conjugated with improved blood-stage malarial activities.

CHAPTER II EXPERIMENTS

The methodology of this project is divided into four major steps; as shown in **Figure 12**, the first, 5-phenoxy analogues with various R group on the phenoxy ring were synthesized. Second, the biological activity of the synthesized analogues against *P. falciparum* in an asexual stage was evaluated and the cytotoxicity of these analogues was also determined, then the most potent compound was further developed for conjugation with tetraoxane molecule, following by studying the anti-malarial activity of the conjugated molecule.

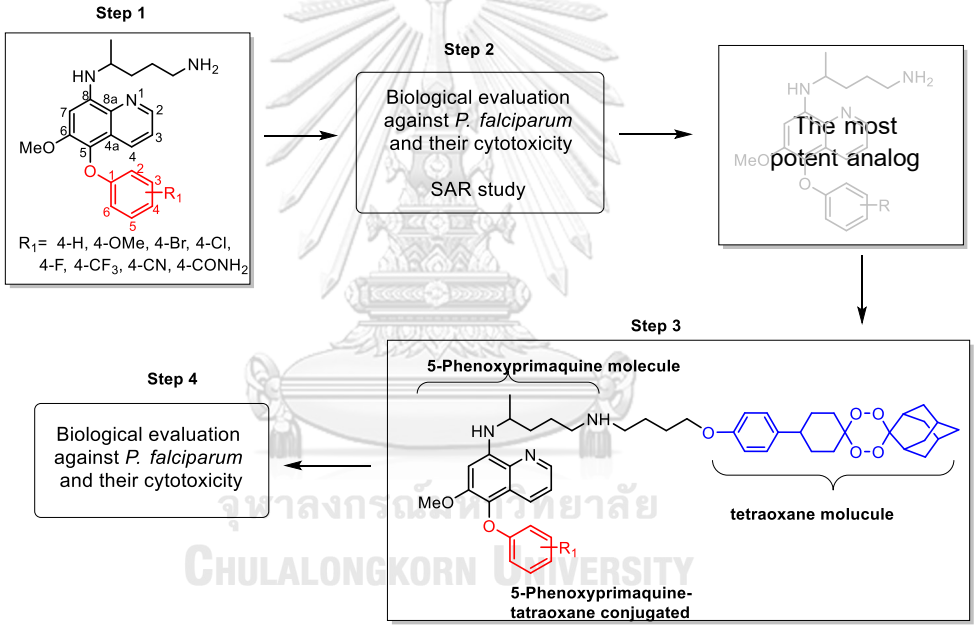


Figure 12 An overview of the research methodology.

1. Chemistry

1.1 Experimental procedure for the synthesis

All reagents and solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA), TCI chemicals (Tokyo, Japan), Fluorochem (Hadfield, Derbyshire, UK) and Merck (Darmstadt, Germany). All solvents for column chromatography from RCI Labscan (Samutsakorn, Thailand) were distilled before use. Reactions were monitored by thin-layer chromatography (TLC) using aluminium Merck TLC plates coated with silica gel 60 F_{254}. Normal phase column

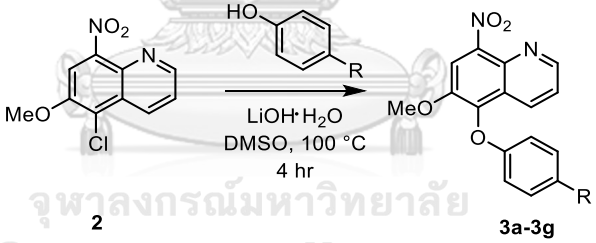
chromatography was performed using silica gel 60 (0.063-0.200 mm, 70-230 mesh ASTM, Merck, Darmstadt, Germany). Proton, carbon, and proton decoupled fluorine nuclear magnetic resonance (^{1}H, ^{13}C, and $^{19}F\{^{1}H\}$ NMR) spectra were recorded on a Bruker Advance (III) 400WB spectrometer and JEOL JNM-ECZ500/S1 (500 MHz). Chemical shifts were expressed in parts per million (ppm), *J* values were in Hertz (Hz). High-resolution mass spectra (HRMS) were obtained with a micrOTOF-Q II mass spectrometer (Bruker Daltonics) with electrospray ionization.

1.2 Synthesis and characterization analogues of primaquine in position 5

Seven derivatives of 5-phenoxy primaquine (**7a-7g**) were synthesized over 6 steps, including oxidation, nucleophilic aromatic substitution, reduction, *N*-alkylation, reductive amination, and deprotection, along with one amide derivative (**7h**) which hydrolyzed from the cyanine derivative.

1.2.1 General procedures

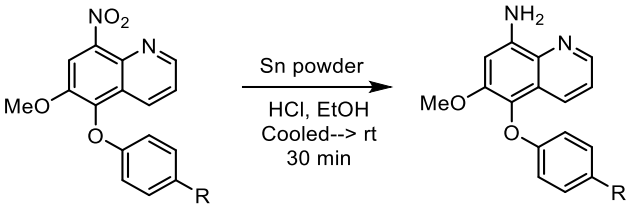
1.2.1.1 General procedure A



2 **3a-3g**

Compounds **3a-3g** were synthesized using a modified procedure.[39] A solution of **2** in DMSO in a round bottom flask was stirred at room temperature for 15 minutes. Then a solution of phenol (1.0 equiv.) and $LiOH{\cdot}H_2O$ (1.0 equiv.) in DMSO was added dropwise into a solution of starting material. After complete addition, the reaction mixture was stirred at 100 °C for 4 hours and monitored by TLC. The reaction was quenched with water, extracted with DCM 3 times and 10% NaOH 3 times. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated to give the crude product. The crude product was further purified by column chromatography (eluent: EtOAc:hexanes = 1:9 to 1:4) on silica gel to afford the product.

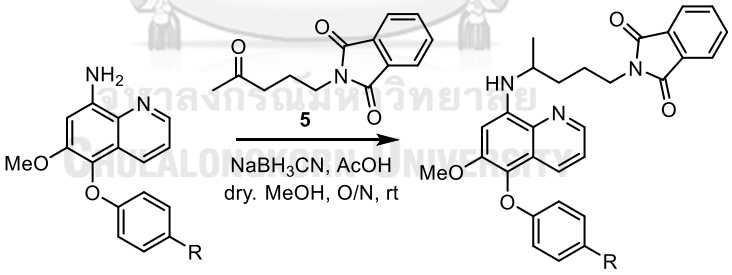
1.2.1.2 General procedure B



3a-3g **4a-4g**

Compounds **4a-4g** were synthesized using a modified procedure.[40] A solution of the appropriate 5-hydroxy-8-nitroquinoline analogue **3a-3g** (1.0 equiv.) and absolute ethanol in a round bottom flask was slowly added 12M HCl at 0 °C to prevent an exothermic reaction. Then Sn powder (10.0 equiv.) was added into the reaction. The reaction mixture was then allowed to stir at room temperature for 30 minutes and monitored by TLC. After the reaction was complete, the reaction mixture was quenched with 12M NaOH until the solution becomes neutral (pH = 7). The resulting mixture was filtered through glass Büchner filter funnel, the filtrate was then extracted with EtOAc. The combined organic layers were washed with water, dried over anhydrous $MgSO_4$, filtered, and concentrated to give the crude product. The crude product was used without further purification.

1.2.1.3 General procedure C

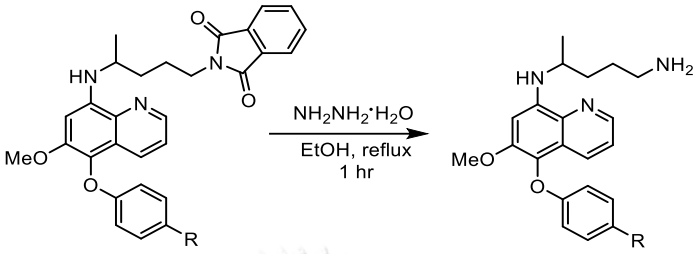


4a-4g **6a-6g**

Compounds **6a-6g** were synthesized using a modified procedure.[41] A solution of the appropriate 5-hydroxy-8-aminoquinoline analogue **4a-4g** (1.0 equiv.) and **5** (5.0 equiv.) were dissolved in anhydrous MeOH in a dry round bottom flask. Then, acetic acid was added into the reaction mixture. After stirring for 2 hours, the mixture was added $NaBH_3CN$ (2.0 equiv.). The solution was then allowed to stir at room temperature overnight and monitored by TLC. The mixture was diluted with EtOAc, washed with water and brine. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product

was further purified by column chromatography (eluent: EtOAc:hexanes = 1:9 to 1:4) on silica gel to afford the product.

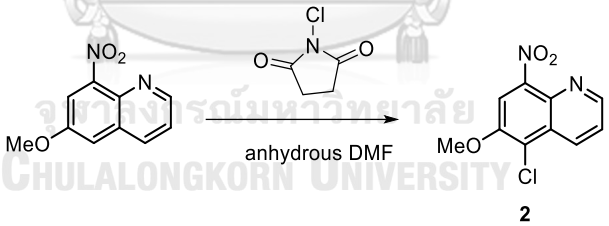
1.2.1.4 General procedure D



6a-6h **7a-7h**

Compounds **7a-7h** were synthesized using a modified procedure.[42] Compound **6a-6h** (1.0 equiv.) was dissolved EtOH in a round bottom flask. Hydrazine monohydrate (5.0 equiv.) was added into a solution and the mixture was heated at refluxed for 1 hour. A solid precipitate was observed. Then, the solution was cooled to room temperature and filtered by cotton. The filtrate was concentrated to give the crude product as a viscous oil. The crude product was purified by column chromatography (eluent: 5% to 50% MeOH:CH_2Cl_2) on silica gel.

1.2.2 Synthesis of 5-chloro-6-methoxy-8-nitroquinoline (2)

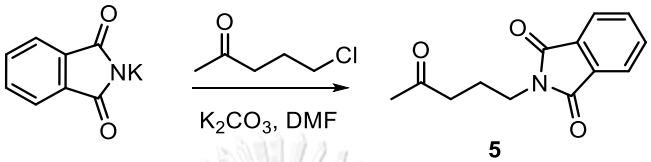


2

The title compound was synthesized using a modified procedure.[43] A solution of 6-methoxy-8-nitroquinoline (8.1 g, 40 mmol, 1.0 equiv.) in anhydrous DMF (10 mL) in a dry round bottom flask was purged with nitrogen gas, then the solution was heated at 60 °C. *N*-chlorosuccinimide (8.0 g, 60 mmol, 1.5 equiv.) was added in portionwise (3 times) into the solution. Next, the reaction mixture was stirred at 60 °C for 3 hours and monitored by TLC. After the reaction was complete, the reaction mixture was allowed cooling to room temperature, extracted with DCM, and washed with water. The combined organic layers were dried over anhydrous $MgSO_4$, concentrated to give the crude product which was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) **δ** 8.99 (dd, *J* = 4.1, 1.4 Hz, 1H, ArH), 8.62 (dd, *J* = 8.7, 1.6 Hz, 1H, ArH), 7.90 (s, 1H, ArH), 7.61 (dd, *J* = 8.7, 4.1 Hz, 1H, ArH), 4.11 (s, 3H, OMe); **^{13}C NMR** (101 MHz, $CDCl_3$) **δ** 151.7, 151.0, 147.1, 135.2, 132.2, 127.9, 123.6, 120.6, 111.4, 57.4. ^{1}H data is consistent with the literature values.[44]

1.2.3 Synthesis of 2-(4-oxopentyl)isoindoline-1,3-dione (5)



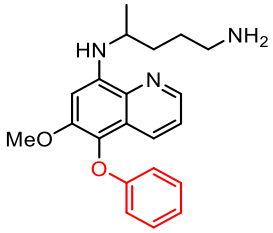
5

The title compound was synthesized using a modified procedure.[45] A solution of potassium phthalimide (4.6 g, 25 mmol, 1.0 equiv.) in anhydrous DMF (25 ml) in a dried round bottom flask was added potassium carbonate (6.9 g, 50 mmol, 2.0 equiv.). The reaction mixture was allowed to stir at room temperature for 30 minutes. Then, 5-chloro-2-pentanone (7.2 µL, 62.5 mmol, 2.5 equiv.) was added and stirring at 100 °C. After the reaction is complete, the reaction mixture was quenched with ice water. The title compound was collected by filtration.

^{1}H NMR (400 MHz, DMSO-d6) **δ** 6.97 (dq, *J* = 6.2, 4.8 Hz, 4H, ArH), 2.67 (t, *J* = 6.8 Hz, 2H, CH_2N), 1.61 (t, *J* = 7.1 Hz, 2H, CH_2CO), 1.17 (s, 3H, CH_3CO), 0.89 (q, *J* = 6.9 Hz, 2H, CH_2CH_2); **^{13}C NMR** (126 MHz, DMSO-d6) **δ** 208.2, 168.6 (2C), 134.8 (2C), 132.2 (2C), 123.5 (2C), 40.3, 37.3, 30.2, 22.6. ^{1}H and ^{13}C data are consistent with the literature values.[46]

1.2.4 Synthesis and characterization of 5-phenoxy primaquine derivatives

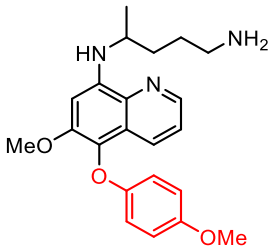
1.2.4.1 Synthesis of N^4-(6-methoxy-5-phenoxyquinolin-8-yl)pentane-1,4-diamine (7a)



The title compound was synthesized following **General procedure A** using **2** (715.9 mg, 3 mmol), phenol (282.3 mg, 3 mmol) and $LiOH{\cdot}H_2O$ (126 mg, 3 mmol) in DMSO (5 ml) to give 6-methoxy-8-nitro-5-phenoxyquinoline (**3a**) as a yellow solid (661 mg, 2.23 mmol, 74% yield). Next, **3a** (450.0 mg, 1.52 mmol) was subjected to **General procedure B** using Sn powder (1.8 g, 15.2 mmol), and 12M HCl (10 ml) in EtOH (10 ml) to give 6-methoxy-5-phenoxyquinolin-8-amine (**4a**) as a brown viscous oil (379 mg, 1.423 mmol, 94% yield). A mixture of **4a** (74 mg, 0.28 mmol) and **5** (323 mg, 1.12 mmol) were subjected to **General procedure C** using CH_3COOH (8 µL, 0.14 mmol), $NaBH_3CN$ (13.0 mg, 0.2 mmol), and anhydrous MeOH (2.5 ml) to give 2-(4-((6-methoxy-5-phenoxyquinolin-8-yl)amino)pentyl)isoindoline-1,3-dione (**6a**) as a yellow oil (75 mg, 0.16 mmol, 57% yield). Finally, **6a** (51 mg, 0.1 mmol) was subjected to **General procedure D** using hydrazine monohydrate (28 µL, 0.57 mmol), and EtOH (500 µL) to give the title product **7a** as yellow oil (33 mg, 0.09 mmol, 94% yield).

^{1}H NMR (500 MHz, $CDCl_3$) **δ** 8.56 (dd, *J* = 4.1, 1.0 Hz, 1H, ArH), 8.07 (dd, *J* = 8.4, 1.2 Hz, 1H, ArH), 7.31 – 7.24 (m, 3H, ArH), 6.99 (td, *J* = 7.4, 0.7 Hz, 1H, ArH), 6.90 (d, *J* = 8.6 Hz, 2H, ArH), 6.49 (s, 1H, ArH), 6.10 (s, 1H, NH), 3.93 (s, 3H, OCH_3), 3.72 (s, 1H, CH), 2.85 (t, *J* = 6.7 Hz, 2H, CH_2), 1.84 – 1.65 (m, 4H, CH_2), 1.37 (d, *J* = 6.3 Hz, 3H, CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) **δ**159.5, 150.5, 144.9, 143.1, 133.8, 129.9, 129.6, 124.7, 124.5, 122.1, 121.5, 115.0, 93.7, 57.1, 48.2, 41.6, 34.2, 28.9, 20.7; **HRMS** (ESI^+): *m/z* calcd for $C_{21}H_{26}N_3O_2^+$ $[M+H]^+$ 352.2020, found 352.2052.

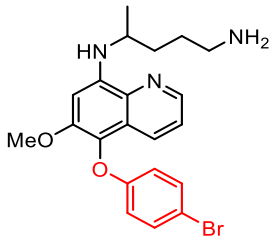
1.2.4.2 Synthesis of N^4-(6-methoxy-5-(4-methoxyphenoxy)quinolin-8-yl)pentane-1,4-diamine (7b)



The title compound was synthesized following **General procedure A** using **2** (715.9 mg, 3 mmol), 4-methoxyphenol (372.4 mg, 3 mmol) and $LiOH{\cdot}H_2O$ (125.8 mg, 3 mmol) in DMSO (5 ml) to give 6-methoxy-5-(4-methoxyphenoxy)-8-nitroquinoline (**3b**) as a brown solid (809 mg, 2.48 mmol, 83% yield). Next, **3b** (620.0 mg, 1.90 mmol) was subjected to **General procedure B** using Sn powder (2.2 g, 19.0 mmol), and 12M HCl (15 ml) in EtOH (15 ml) to give 6-methoxy-5-(4 methoxyphenoxy)-8-aminoquinoline (**4b**) as a green solid (248 mg, 0.84 mmol, 44% yield). A mixture of **4b** (88 mg, 0.30 mmol) and **5** (346.8 mg, 1.50 mmol) were subjected to **General procedure C** using CH_3COOH (8 µL, 0.15 mmol), $NaBH_3CN$ (16.0 mg, 0.3 mmol), and anhydrous MeOH (2.5 ml) to give 2-(4-((6-methoxy-5-(4-methoxyphenoxy)quinolin-8yl)amino) pentyl) isoindoline-1,3-dione (**6b**) as a yellow oil (53 mg, 0.10 mmol, 35% yield). Finally, **6b** (53 mg, 0.1 mmol) was subjected to **General procedure D** using hydrazine monohydrate (26 µL, 0.52 mmol), and EtOH (500 µL) to give the title product **7b** as yellow oil (41 mg, 0.10 mmol, 100% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (dd, *J* = 4.1, 1.6 Hz, 1H, ArH), 8.05 (dd, *J* = 8.4, 1.5 Hz, 1H, ArH), 7.25 (dd, *J* = 8.5, 4.1 Hz, 1H, ArH), 6.78 (q, *J* = 9.3 Hz, 4H, ArH), 6.44 (s, 1H, ArH), 6.05 (s, 1H, NH), 3.90 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.68 (dd, *J* = 11.9, 5.9 Hz, 1H, CH), 2.79 (t, *J* = 6.8 Hz, 2H, NH_2), 1.82 – 1.61 (m, 4H, NH_2), 1.34 (d, *J* = 6.3 Hz, 3H, CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ154.3, 153.6, 150.6, 144.9, 143.0, 133.9, 129.9, 125.1, 124.8, 122.0, 115.6 (2C), 114.7 (2C), 93.8, 57.2, 55.8, 48.2, 41.7, 34.2, 29.1, 20.7; **HRMS** (ESI^+): *m/z* calcd for $C_{22}H_{28}N_3O_3^+$ $[M+H]^+$ 382.2125, found 382.2161.

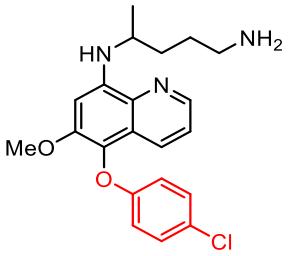
1.2.4.3 Synthesis of N^4-(5-(4-bromophenoxy)-6-methoxyquinolin-8-yl) pentane-1,4-diamine(7c)



The title compound was synthesized following **General procedure A** using **2** (715.9 mg, 3 mmol), 4-bromophenol (519.0 mg, 3 mmol) and $LiOH{\cdot}H_2O$ (126.0 mg, 3 mmol) in DMSO (5 ml) to give 6-methoxy-5-(4-bromophenoxy)-8-nitroquinoline (**3c**) as a brown solid (1.1 g, 2.93 mmol, 98% yield). Subsequently, NO_2 group was reduced to NH_2 using a modified procedure from *Bioorg. Med. Chem*. **2016**, *24*, 1790. [47] A solution of **3c** (638.0 mg, 1.7 mmol, 1.0 equiv.) was dissolved EtOH (20 mL) in a round bottom flask. Next, 10% Pd on carbon (63 mg, 0.6 mmol, 1.0 equiv.) was added into the solution. The reaction mixture was then allowed to stir at room temperature under H_2 and monitored by TLC. After the reaction is complete, The Pd was filtered out through glass Büchner filter funnel, the filtrate was then extracted with EtOAc. The combined organic layers were washed with water, dried over anhydrous $MgSO_4$, filtered, and concentrated to give the crude product. The crude product was used without further purification to give 5-(4-bromophenoxy)-6-methoxyquinolin-8-amine (**4c**) as a yellow soild (117 mg, 0.34 mmol, 20% yield). A mixture of **4c** (69 mg, 0.20 mmol) and **5** (231 mg, 1.00 mmol) were subjected to **General procedure C** using CH_3COOH (5 µL, 0.10 mmol), $NaBH_3CN$ (10.0 mg, 0.2 mmol), and anhydrous MeOH (1.5 ml) to give 2-(4-((5-(4-bromophenoxy)-6-methoxyquinolin-8yl)amino)pentyl) hexahydro-1*H*-isoindole-1,3(2*H*)-dione (**6c**) as a yellow oil (88 mg, 0.16 mmol, 80% yield). Finally, **6c** (67 mg, 0.12 mmol) was subjected to **General procedure D** using hydrazine monohydrate (30 µL, 0.62 mmol), and EtOH (600 µL) to give the title product **7c** as yellow oil (28 mg, 0.07 mmol, 54% yield).

^{1}H NMR (500 MHz, $CDCl_3$) **δ** 8.54 (s, 1H, ArH), 7.98 (d, *J* = 8.0 Hz, 1H, ArH), 7.37 – 7.22 (m, 3H, ArH), 6.75 (d, *J* = 8.7 Hz, 2H, ArH), 6.43 (s, 1H, ArH), 6.11 (s, 1H, ArH), 3.89 (s, 3H, OCH_3), 3.68 (s, 1H, CH), 2.78 (s, 2H, CH_2), 1.86 – 1.52 (m, 4H, CH), 1.35 (d, *J* = 5.9 Hz, 3H, CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) **δ** 158.7, 150.4, 144.9, 143.4, 133.7, 132.4 (2C), 129.5, 124.4, 124.0, 122.2, 116.8 (2C), 113.7, 93.2, 56.9, 48.2, 42.1, 34.2, 30.0, 20.7; **HRMS** (ESI^+): *m/z* calcd for $C_{21}H_{25}BrN_3O_2^+$ $[M+H]^+$ 430.1125, found 430.1148.

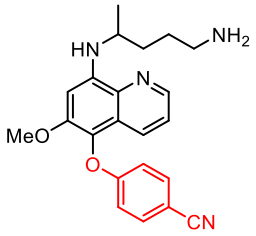
1.2.4.4 Synthesis of N^4-(6-methoxy-5-(4-chlorophenoxy)quinolin-8-yl)pentane-1,4-diamine (7d)



The title compound was synthesized following **General procedure A** using **2** (715.9 mg, 3 mmol), 4-chlorophenol (386.0 mg, 3 mmol) and $LiOH{\cdot}H_2O$ (125.8 mg, 3 mmol) in DMSO (5 ml) to give 6-methoxy-5-(4-chlorophenoxy)-8-nitroquinoline (**3d**) as a yellow solid (509 mg, 1.54 mmol, 51% yield). Next, **3d** (463.0 mg, 1.40 mmol) was subjected to **General procedure B** using Sn powder (1.6 g, 14.0 mmol), and 12M HCl (11 ml) in EtOH (11 ml) to give 6-methoxy-5-(4-chlorophenoxy)-8-aminoquinoline (**4d**) as a green viscous oil (420 mg, 1.40 mmol, 100% yield). A mixture of **4d** (75 mg, 0.25 mmol) and **5** (289.0 mg, 1.25 mmol) were subjected to **General procedure C** using CH_3COOH (7 µL, 0.125 mmol), $NaBH_3CN$ (16.0 mg, 0.25 mmol), and anhydrous MeOH (1.5 ml) to give 2-(4-((6-methoxy-5-(4-chlorophenoxy)quinolin-8yl)amino) pentyl)isoindoline-1,3-dione (**6d**) as a yellow viscous oil (57 mg, 0.11 mmol, 44% yield). Finally, **6d** (56 mg, 0.11 mmol) was subjected to **General procedure D** using hydrazine monohydrate (29 µL, 0.57 mmol), and EtOH (700 µL) to give the title product **7d** as yellow oil (29 mg, 0.075 mmol, 68% yield).

^{1}H NMR (500 MHz, $CDCl_3$) **δ** 8.54 (dd, *J* = 4.2, 1.6 Hz, 1H, ArH), 7.99 (dd, *J* = 8.5, 1.6 Hz, 1H, ArH), 7.30 – 7.25 (m, 1H, ArH), 7.17 (d, *J* = 9.0 Hz, 2H, ArH), 6.80 (d, *J* = 9.0 Hz, 2H, ArH), 6.43 (s, 1H, ArH), 3.89 (s, 3H, OCH_3), 3.69 (dd, *J* = 11.8, 5.8 Hz, 1H, CH), 2.79 (t, *J* = 6.1 Hz, 2H, CH_2), 1.80 – 1.56 (m, 4H, CH_2), 1.35 (d, *J* = 6.3 Hz, 3H, CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) **δ** 158.1, 150.4, 144.9, 143.3, 133.7, 129.6, 129.4 (2C), 126.4, 124.5, 124.2, 122.2, 116.3 (2C), 93.3, 57.0, 48.1, 41.4, 34.1, 20.7; **HRMS** (ESI^+): *m/z* calcd for $C_{21}H_{25}ClN_3O_2^+$ $[M+H]^+$ 386.1630, found 386.1666.

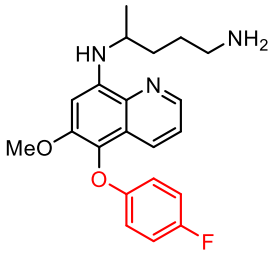
1.2.4.5 Synthesis of 4-((8-((5-aminopentan-2-yl)amino)-6-methoxyquinolin-5-yl)oxy)benzonitrile (7e)



The title compound was synthesized following **General procedure A** using **2** (1.4 g, 6 mmol), 4-hydroxybenzonitrile (714.0 mg, 6 mmol) and $LiOH{\cdot}H_2O$ (252.0 mg, 6 mmol) in DMSO (10 ml) to give 4-((6-methoxy-8-nitroquinolin-5-yl)oxy)benzonitrile (**3e**) as a yellow solid (1.9 g, 5.91 mmol, 98% yield). Next, **3e** (358.0 mg, 1.1 mmol) was subjected to **General procedure B** using Sn powder (1.3 g, 11.0 mmol), and 12M HCl (8 ml) in EtOH (8 ml) to give 4-((8-amino-6-methoxyquinolin-5-yl)oxy)benzonitrile (**4e**) as a black viscous oil (367 mg, 1.26 mmol, 100% yield). A mixture of **4e** (70 mg, 0.24 mmol) and **5** (56.0 mg, 0.24 mmol) were subjected to **General procedure C** using CH_3COOH (40 µL, 0.72 mmol), $NaBH_3CN$ (15.0 mg, 0.24 mmol), and anhydrous MeOH (2.0 ml) to give 4-((8-((5-(1,3-dioxoisoindolin-2-yl)pentan-2-yl)amino)-6-methoxyquinolin-5-yl)oxy)benzonitrile (**6e**) as a yellow viscous oil (54 mg, 0.10 mmol, 44% yield). Finally, **6e** (30 mg, 0.06 mmol) was subjected to **General procedure D** using hydrazine monohydrate (30 µL, 0.6 mmol), and EtOH (500 µL) to give the title product **7e** as yellow oil (26 mg, 0.06 mmol, 100% yield).

^{1}H NMR (500 MHz, $CDCl_3$) **δ** 8.56 (dd, *J* = 4.2, 1.4 Hz, 1H, ArH), 7.93 (dd, *J* = 8.5, 1.5 Hz, 1H, ArH), 7.53 (d, *J* = 8.7 Hz, 2H, ArH), 7.30 (dd, *J* = 8.5, 4.1 Hz, 1H, ArH), 6.93 (d, *J* = 8.7 Hz, 2H, ArH), 6.42 (s, 1H, ArH), 6.17 (brs, 1H, NH), 3.88 (s, 3H, OCH_3), 3.69 (s, 1H, CH), 2.79 (t, *J* = 6.8 Hz, 2H, CH_2NH), 1.82 – 1.57 (m, 4H, CH_2CH_2), 1.35 (d, *J* = 6.3 Hz, 3H, CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) **δ** 162.9, 150.3, 145.0, 143.8, 134.2 (2C), 133.5, 129.1, 124.1, 123.0, 122.5, 119.2, 115.9 (2C), 105.0, 92.5, 56.8, 48.2, 42.1, 34.2, 29.9, 20.7; **HRMS** (ESI^+): *m/z* calcd for $C_{22}H_{25}N_4O_2^+$ $[M+H]^+$ 377.1972, found 377.1978.

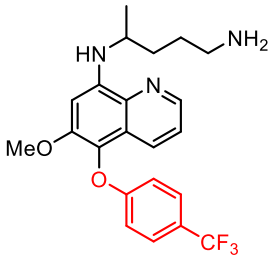
1.2.4.6 Synthesis of N^4-(5-(4-fluorophenoxy)-6-methoxyquinolin-8-yl) pentane-1,4-diamine (7f)



The title compound was synthesized following **General procedure A** using **2** (716.0 mg, 3 mmol), 4-fluorophenol (336.0 mg, 3 mmol) and $LiOH{\cdot}H_2O$ (126.0 mg, 3 mmol) in DMSO (5 ml) to give 5-(4-fluorophenoxy)-6-methoxy-8-nitroquinoline (**3f**) as a yellow solid (1.0 g, 3.18 mmol, 100% yield). Next, **3f** (628.5 mg, 2.0 mmol) was subjected to **General procedure B** using Sn powder (2.4 g, 20.0 mmol), and 12M HCl (14 ml) in EtOH (14 ml) to give 5-(4-fluorophenoxy)-6-methoxyquinolin-8-amine (**4f**) as a black viscous oil (400 mg, 1.41 mmol, 70% yield). A mixture of **4f** (79.5 mg, 0.28 mmol) and **5** (64.7 mg, 0.28 mmol) were subjected to **General procedure C** using CH_3COOH (32 µL, 0.56 mmol), $NaBH_3CN$ (52.5 mg, 0.84 mmol), and anhydrous MeOH (2.0 ml) to give 2-(4-((5-(4-fluorophenoxy)-6-methoxyquinolin-8-yl)amino) pentyl)isoindoline-1,3-dione (**6f**) as a yellow viscous oil (47 mg, 0.094 mmol, 34% yield). Finally, **6f** (35 mg, 0.07 mmol) was subjected to **General procedure D** using hydrazine monohydrate (20 µL, 0.42 mmol), and EtOH (1ml) to give the title product **7f** as yellow oil (27 mg, 0.07 mmol, 100% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (d, *J* = 4.0 Hz, 1H, ArH), 8.01 (d, *J* = 8.4 Hz, 1H, ArH), 7.25 (dd, *J* = 7.7, 3.2 Hz, 1H, ArH), 6.90 (t, *J* = 8.6 Hz, 2H, ArH), 6.79 (dd, *J* = 9.0, 4.2 Hz, 2H, ArH), 6.43 (s, 1H, ArH), 6.03 (brs, 1H, NH), 3.88 (s, 3H, OCH_3), 3.67 (s, 1H, CH), 2.89 (s, 2H, CH_2NH), 1.89 – 1.62 (m, 4H, CH_2CH_2), 1.30 (d, *J* = 6.0 Hz, 3H, CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 158.7, 156.1 (d, $^1J_{CF}$ = 177.0 Hz), 150.6, 144.7, 142.7, 125.0, 124.7, 122.2, 116.3 (d, $^3J_{CF}$ = 7.9 Hz), 115.9 (d, $^2J_{CF}$ = 15.8 Hz) (4C), 115.8, 94.3, 57.1, 48.1, 40.0, 33.8, 24.6, 20.5; **^{19}F{^{1}H} NMR** (471 MHz, $CDCl_3$) δ -123.31; **HRMS** (ESI^+): *m/z* calcd for $C_{21}H_{25}FN_3O_2^+$ $[M+H]^+$ 370.1925, found 370.1925.

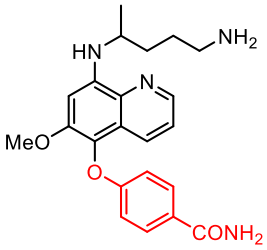
1.2.4.7 Synthesis of N^4-(5-(4-fluorophenoxy)-6-methoxyquinolin-8-yl) pentane-1,4-diamine (7g)



The title compound was synthesized following **General procedure A** using **2** (716.0 mg, 3 mmol), 4-hydroxybenzotrifluoride (486.0 mg, 3 mmol) and $LiOH{\cdot}H_2O$ (126.0 mg, 3 mmol) in DMSO (5 ml) to give 6-methoxy-8-nitro-5-(4-(trifluoromethyl)phenoxy)quinoline (**3g**) as a pale yellow solid (149 mg, 0.41 mmol, 14% yield). Next, **3g** (149 mg, 0.41 mmol) was subjected to **General procedure B** using Sn powder (485 mg, 4.09 mmol), and 12M HCl (3.5 ml) in EtOH (3.5 ml) to give 6-methoxy-5-(4-(trifluoromethyl)phenoxy)quinolin-8-amine (**4g**) as an orange viscous oil (95 mg, 0.33 mmol, 82% yield). A mixture of **4g** (91 mg, 0.32 mmol) and **5** (148 mg, 0.64 mmol) were subjected to **General procedure C** using CH_3COOH (38 µL, 0.64 mmol), $NaBH_3CN$ (20 mg, 0.32 mmol), and anhydrous MeOH (3.0 ml) to give 2-(4-((6-methoxy-5-(4 (trifluoromethyl)phenoxy) quinoline-yl)amino)pentyl) isoindoline-1,3-dione (**6g**) as an orange viscous oil (22 mg, 0.04 mmol, 13% yield). Finally, **6g** (22 mg, 0.04 mmol) was subjected to **General procedure D** using hydrazine monohydrate (20 µL, 0.40 mmol), and EtOH (1 ml) to give the title product **7g** as yellow viscous oil (20 mg, 0.04 mmol, 100% yield).

^{1}H NMR (500 MHz, acetone-d_6) δ 8.72 – 8.48 (m, 1H, ArH), 8.00 (ddd, *J* = 8.5, 3.5, 1.7 Hz, 1H, ArH), 7.61 (d, *J* = 8.0 Hz, 2H, ArH), 7.41 (ddd, *J* = 8.3, 4.1, 1.5 Hz, 1H, ArH), 7.01 (d, *J* = 8.3 Hz, 2H, ArH), 6.71 (dd, *J* = 16.1, 14.9 Hz, 1H, ArH), 3.91 (dd, *J* = 4.4, 1.7 Hz, 3H, OCH_3), 3.40 3.17 (m, 2H, CH_2NH), 2.87 (t, *J* = 7.0 Hz, 2H, CH), 1.94 – 1.68 (m, 4H, CH_2CH_2), 1.35 (d, *J* = 6.3 Hz, 3H, CH_3); **^{13}C NMR** (126 MHz, acetone-d_6) δ 150.7, 144.8, 144.6, 144.0, 133.4, 131.0, 128.7 (q, $^3J_{CF}$ = 4.1 Hz), 126.9 (q, $^2J_{CF}$ = 65.1 Hz), 124.0, 122.9, 122.7 (q, $^1J_{CF}$ = 265.0 Hz), 122.5, 115.3 (3C), 92.7, 56.1, 50.5, 47.9, 41.2, 34.6, 20.1; **^{19}F NMR** (471 MHz, acetone-d_6) δ -61.81; **HRMS** (ESI^+): *m/z* calcd for $C_{22}H_{25}F_3N_3O_2^+$ $[M+H]^+$ 420.1893, 420.1939.

1.2.4.8 Synthesis of 4-((8-((5-aminopentan-2-yl)amino)-6-methoxyquinolin-5-yl)oxy)benzamide (7h)



The title compound was synthesized following **General procedure A** using **2** (1.5 g, 6.29 mmol), 4-hydroxybenzonitrile (1.8 g, 15.7 mmol) and $LiOH{\cdot}H_2O$ (660 mg, 15.7 mmol) in DMSO (10 ml) to give 4-((6-methoxy-8-nitroquinolin-5-yl) oxy)benzonitrile (**3e**) as a yellow solid (801 mg, 2.50 mmol, 40% yield). Next, **3e** (777 mg, 2.42 mmol) was subjected to **General procedure B** using Sn powder (2.8 g, 24.2 mmol), and 12M HCl (15 ml) in EtOH (15 ml) to give 4-((8-amino-6-methoxy quinolin-5-yl)oxy)benzonitrile (**4e**) as a black viscous oil (521 mg, 1.79 mmol, 74% yield). A mixture of **4e** (501 mg, 1.72 mmol) and **5** (1.1 g, 4.73 mmol) were subjected to **General procedure C** using CH_3COOH (100 µL, 1.72 mmol), $NaBH_3CN$ (108 mg, 1.72 mmol), and anhydrous MeOH (4.0 ml) to give 4-((8-((5-(1,3-dioxoisoindolin-2-yl)pentan-2-yl)amino)-6-methoxyquinolin-5-yl)oxy)benzonitrile (**6e**) as a yellow viscous oil (890 mg, 1.75 mmol, 100% yield). Subsequently, CN was hydrolysed to COOH using a procedure from *J. Mater. Chem. C.* **2016**, *4,* 5335. [48] A solution of **6e** (890 mg, 1.75 mmol, 1.0 equiv.) was dissolved by 50% H_2SO_4 (5 mL) in a round bottom flask. The reaction mixture was heated at 80 ºC for 6 hours. After the reaction is complete, the mixture was neutralized with sat. $NaHCO_3$ and extracted with EtOAc. The combined organic layers were washed with water, dried over anhydrous $MgSO_4$, filtered, and concentrated to give the crude product. The crude product was further purified by column chromatography (eluent: 1%-2% MeOH in DCM) on silica gel to afford 4-((8-((5-(1,3-dioxoisoindolin-2-yl)pentan-2-yl)amino)-6-methoxyquinolin-5-yl)oxy) benzamide (**6h**) as yellow viscous oil (541 mg, 1.03 mmol, 58% yield). Finally, **6h** (461 mg, 0.88 mmol) was subjected to **General procedure D** using hydrazine monohydrate (435 µL, 8.8 mmol), and EtOH (5 mL) to give the title product **7h** as yellow oil (204 mg, 0.52 mmol, 59% yield).

^{1}H NMR (500 MHz, Methanol-d4) δ 8.51 (dd, *J* = 4.1, 1.4 Hz, 1H, ArH), 8.16 (dd, *J* = 5.7, 3.4 Hz, 1H, ArH), 7.96 (dd, *J* = 11.1, 4.1 Hz, 1H, ArH), 7.77 (s, 1H, ArH), 7.76 (s, 2H, ArH), 7.32 (dd,

J = 8.4, 4.1 Hz, 1H, ArH), 6.81 (dd, *J* = 8.3, 1.4 Hz, 2H, ArH), 6.58 (d, *J* = 4.1 Hz, 1H, ArH), 3.84 (s, 3H, OCH_3), 3.82-3.77 (m, 1H, CH), 2.95 (t, *J* = 7.2 Hz, 2H, CH_2NH), 1.76 (t, *J* = 10.6 Hz, 4H, CH_2CH_2), 1.31 (t, *J* = 5.5 Hz, 3H, CH_3); **^{13}C NMR** (126 MHz, Methanol-d4) **δ** 170.5, 162.5, 150.4, 144.8, 143.5, 133.4, 131.7 (2C), 129.3, 129.0, 126.7, 125.6, 124.2, 123.8, 122.1 (2C), 114.4, 93.3, 55.8, 39.6, 33.3, 19.5; HRMS (ESI+): m/z calcd for $C_{22}H_{27}N_4O_3^+$ $[M+H]^+$ 395.2078, 395.2114

1.3 Synthesis and characterization of tetraoxane unit

The E209 analog of tetraoxane (**11**) was synthesized over 4 steps, including acetylation, gem dihydroperoxide intermediate formation, cyclisation, and deprotection by using a reported procedure from P. M. O'Neill *et al*. 2017. [6] Then, **11** was further synthesized to be aldehyde **13** by ozonolysis of alkene **12**. The alkylated product **14**, a precursor for conjugation with 5-phenoxy primaquine, was synthesized using 1,4-dibromobutane. (**Figure 13**)

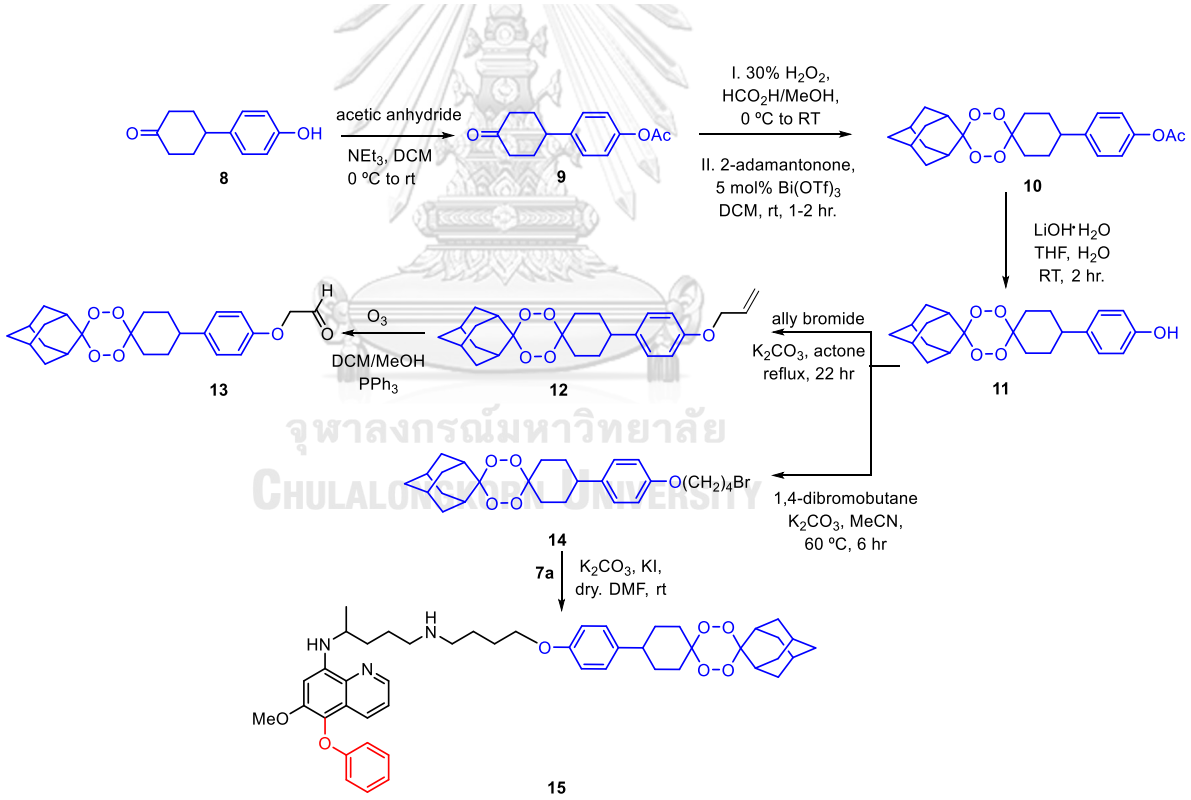
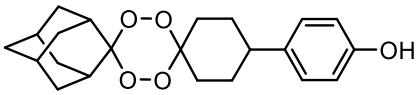


Figure 13 Synthesis of tetraoxane unit

1.3.1 Synthesis of 4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo [3.3.1.13,7] decan]-4-yl)phenol (11)



The title compound was synthesized using a reported procedure.[6] A solution of 4-(4-hydroxyphenyl)cyclohexanone (**8**) (9.0 g, 47.3 mmol, 1.0 equiv.) and NEt_3 (13 mL, 94.6 mmol, 2.0 equiv.) in DCM (90 mL) was added acetic anhydride (141 mL, 141.9 mmol, 3.0 equiv.) dropwise at 0 °C via syringe. After complete addition, the solution was allowed to stir at room temperature for 3 hours and monitored by TLC. The reaction mixture was extracted with water, sat. $NaHCO_3$, and brine, respectively. Then, the combined organic layers were dried over anhydrous Na_2SO_4, concentrated to give the crude product of 4-(4-oxocyclohexyl)phenyl acetate (**9**) as a white solid (13.6 g, 58.42 mmol, quantitative yield).

^{1}**H NMR** (500 MHz, $CDCl_3$) **δ** 7.24 (d, *J* = 8.3 Hz, 2H, ArH), 7.03 (d, *J* = 8.4 Hz, 2H, ArH), 3.02 (tt, *J* = 15.0, 5.0 Hz, 1H, CH), 2.50 (dd, *J* = 10.7, 5.0 Hz, 4H, CH_2), 2.29 (s, 3H, $COCH_3$), 2.21 (d, *J* = 13.7 Hz, 2H, CH_2), 1.91 (dd, *J* = 11.3, 7.5 Hz, 2H, CH_2).

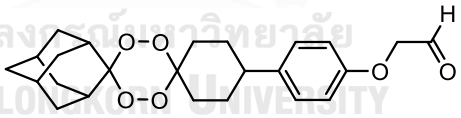
Next, **9** (10.0 g, 43.0 mmol, 1.0 equiv.) was dissolved in 1:1 HCO_2H/MeCN (50 mL: 50 mL) in a tree-neck round bottom flask. Next, 30% H_2O_2 (42 mL) was slowly added into a solution at 0 °C. After complete addition, the reaction mixture was stirred at room temperature for 2 hours. Then, the reaction mixture was extracted with DCM, water, sat. $NaHCO_3$, and brine, respectively. The combined organic layers were dried over anhydrous Na_2SO_4 and filtered to give the filtrate as an intermediate **I** (concentrated to 50 mL). The filtrate **I** was used for next step, a solution of **I** and 2-adamantanone (6.5 g, 43.0 mmol, 1.0 equiv.) were added $Bi(OTf)_3$ (1.4 g, 2.2 mmol, 5 mol%). The reaction mixture was allowed to stir at room temperature for 1 hour and monitored by TLC. Then, the mixture was filtered through a plug of silica and concentrated to give the crude product. The title compound was collected by flash column chromatography (eluent: EtOAc:hexanes = 0.5:10 to 1:10) to give 4-(dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo [3.3.1.1^{3,7}]decan]-4-yl)phenyl acetate (**10**) as a white solid (413 mg, 0.99 mmol, 22%).

^{1}H NMR (500 MHz, $CDCl_3$) **δ** 7.22 (d, *J* = 8.5 Hz, 2H, ArH), 7.00 (d, *J* = 8.5 Hz, 2H, ArH), 3.24 (d, *J* = 43.5 Hz, 2H, CH), 2.61 (tt, *J* = 15.0, 5.0 Hz, 1H, CH), 2.29 (s, 3H, $COCH_3$), 2.13 – 1.58 (m, 20H, CH/CH_2)

Finally, a solution of **10** (410 mg, 0.96 mmol, 1.0 equiv.) was dissolved in THF (6 mL) and water (2 mL) in a round bottom flask. Next, $LiOH{\cdot}H_2O$ (120 mg, 2.87 mmol, 3.0 equiv.) was added into the solution. The reaction mixture was then stirred at room temperature for 3 hours and monitored by TLC. After the reaction is completed, the mixture was neutralized with dilute HCl. Then, most of THF was evaporated out under reduced pressure. The residue was extracted 2 times with DCM. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated to give the crude product which was used without further purification to give the title product **11** as a white solid (326 mg, 0.875 mmol, 92% yield).

^{1}H NMR (500 MHz, $CDCl_3$) **δ** 7.05 (d, *J* = 8.5 Hz, 2H, ArH), 6.74 (d, *J* = 8.1 Hz, 2H, ArH), 3.21 (d, *J* = 38.1 Hz, 1H, CH), 2.52 (tt, *J* = 11.8, 3.6 Hz, 1H, CH), 2.11 – 1.56 (m, 21H, CH/CH_2); **^{13}C NMR** (126 MHz, $CDCl_3$) **δ** 154.03, 138.20, 127.98, 115.31, 110.65, 107.73, 42.83, 37.04, 33.25, 27.15. ^{1}H NMR data and ^{13}C NMR data are consistent with the literature values (*Nat. Commun.* **2017**, *8*, 15159)

1.3.2 2-(4-((1*r*,3*r*,5*r*,7*r*)-dispiro[adamantane-2,3'-[1,2,4,5]tetraoxane-6',1''-cyclohexan]-4''-yl) phenoxy)acetaldehyde (13)

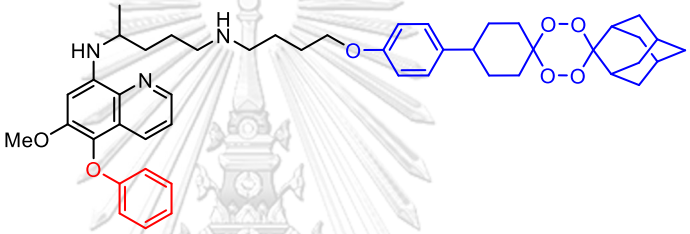


A solution of **11** (326 mg, 0.875 mmol, 1.0 equiv.) and K_2CO_3 (484 mg, 3.50 mmol, 4.0 equiv.) in acetone 8 mL was added allyl bromide (302 µL, 3.50 mmol, 4.0 equiv.). After complete addition, the solution was allowed to stir at reflux for 24 hours and monitored by TLC. The reaction mixture was cooled down to room temperature. The solid of K_2CO_3 was filtered out to give the filtrate which was concentrated to give the crude product. The crude product was further purified by flash column chromatography (eluent: EtOAc:hexanes = 0:100 to 1:9) to give (1*r*,3*r*,5*r*,7*r*)-4''-(4-(allyloxy)phenyl)dispiro[adamantane-2,3'-[1,2,4,5]tetraoxane-6',1'' cyclohexane] (**12**) as a white solid (249 mg, 0.603 mmol, 70% yield). Next, **12** (517 mg, 1.253 mmol, 1.0 equiv.) in MeOH (1 mL) and CH_2Cl_2 (8 mL) at -78 ºC was bubbled by ozone until the

colorless solution is saturated and appeared blue solution. The solution was then bubbled by nitrogen gas for 20 mins. After that, PPh_3 (1.6 g, 6.26 mmol, 5.0 equiv.) was added portion-wise into the solution. The reaction mixture was stirred at -78 ºC for 1 hour and then at room temperature for 1 hour. The reaction mixture was concentrated to give crude product The crude product was further purified by column chromatography (eluent: EtOAc:hexanes = 1:9 to 1:4) on silica gel to afford the title product as pale brown oil (88 mg, 0.21 mmol, 17% yield).

1.4 Synthesis of 5-phenoxy primaquine-tetraoxane conjugate

1.4.1 N^1-(4-(4-((1*r*,3*r*,5*r*,7*r*)-dispiro[adamantane-2,3'-[1,2,4,5]tetraoxane-6',1"-cyclohexan]-4"-yl) phenoxy)butyl)-N^4-(6-methoxy-5-phenoxyquinolin-8-yl)pentane-1,4-diamine (15)



The title compound was synthesized using a modified procedure [49] To a solution of **14** (15 mg, 0.03 mmol, 1 equiv.), K_2CO_3 (4 mg, 0.03 mmol, 1 equiv.), and KI (1 mg, 0.006 mmol, 0.2 equiv.) in anhydrous DMF (500 µL). A solution of N^4-(6-methoxy-5-phenoxyquinolin-8-yl)pentane-1,4-diamine (**7a**) (10 mg, 0.03 mmol, 1.0 equiv.) in anhydrous DMF (500 µL) was added. After complete addition, the solution was allowed to stir at room temperature overnight and monitored by TLC. The reaction mixture was quenched with water and extracted with DCM. Then, the combined organic layers were dried over anhydrous Na_2SO_4, concentrated to give the crude product. The crude product was further purified by column chromatography (eluent: 1%-3% MeOH:CH_2Cl_2) on silica gel to afford the title product as yellow viscous oil (12 mg, 0.015 mmol, 51% yield).

^{1}H NMR (500 MHz, $CDCl_3$) **δ** 8.52 (ddd, *J* = 4.1, 1.6, 0.5 Hz, 1H, ArH), 8.04 (ddd, *J* = 4.1, 1.6, 0.5 Hz, 1H, ArH), 7.24 – 7.19 (m, 3H, ArH), 7.06 (d, *J* = 8.7 Hz, 2H, ArH), 6.95 (td, *J* = 7.3, 0.5 Hz, 1H, ArH), 6.85 (d, *J* = 7.8 Hz, 2H, ArH), 6.72 (d, *J* = 8.4 Hz, 2H, ArH), 6.54 (s, 1H, ArH), 3.90 (s, 3H, OCH_3), 3.86 – 3.77 (m, 2H, OCH_2), 3.72 (dd, *J* = 12.5, 6.3 Hz, 1H, CH), 3.22 (d, *J* = 38.2 Hz, 1H, CH), 2.98 – 2.81 (m, 4H, NCH_2), 2.51 (tt, *J* = 11.6, 3.5 Hz, 1H, NH), 2.08 – 1.59 (m, 23H, CH/CH_2), 1.30 (d, *J* = 6.3 Hz, 3H, CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) **δ** 159.4, 157.1, 150.5, 145.1,

142.7, 138.4, 134.0, 130.0, 129.6, 127.8, 125.1, 124.7, 122.1, 121.6, 114.9, 114.4, 110.6, 107.6, 94.8, 67.2, 57.2, 48.5, 48.3, 47.9, 42.8, 37.0, 34.4, 33.2, 29.8, 27.2, 26.7, 24.1, 20.7; HRMS (ESI+): m/z calcd for $C_{47}H_{59}N_3NaO_7^+$ $[M+Na]^+$ 800.4245, found 800.4242.

2. Biology

2.1 Parasite culture and antimalarial testing *in vitro*.

P. falciparum strains, 3D7 (wild-type drug sensitive strain) was used in this study. This parasite was maintained continuously in human erythrocytes at 37°C under 3% CO_2 in RPMI 1640 culture media (Gibco, USA) supplemented with 25 mM HEPES (Sigma), pH 7.4, 0.2% $NaHCO_3$, 40 mg/mL gentamicin, and 10% human serum. *In vitro* antimalarial activity was determined by using the malaria SYBR green I-based fluorescence (MSF) method. Briefly, 0.09 ml of cultured 1% ring-stage synchronized parasites were transferred to individual wells of a standard 96-well microtiter plate and *in vitro* culture continued for 48 hours, with 0.01 ml of compound at different concentration in each well. The compounds were already dissolved in DMSO and the final concentration of DMSO in each well was 0.1% which causes no effect on the parasite viability. Following 48 hr, SYBR Green I was then added to each well and fluorescence signals were measured by spectrofluorometer at ex485/em535 nm. The results were read as concentration of each compound that exhibit 50% growth inhibition (IC_{50}) from the dose-response curve established from the fluorescence signals at each concentration of compounds. [50]

2.2 Cytotoxicity testing by sulforhodamine B (SRB) colorimetric assay.

Cytotoxicity test of selected analogues against African green monkey kidney fibroblast (Vero cells) was obtained from Bioassay laboratory, BIOTEC, NSTDA, Thailand. They were maintained continuously in MEM/EBSS medium (Hyclone Laboratories Inc., South Logan, Utah) supplemented with 10% heated fetal bovine serum (GE Healthcare, PAA Laboratories GmbH, Pasching, Aurstria), 2.2 g/l Sodium bicarbonate (Emsure, ACS, Reag. Ph Eur, Germany), and 1% sodium pyruvate (Sigma). Cytotoxicity was determined by using the sulforhodamine B (SRB) assay. 1.9×10^4 Vero cells were incubated at 37 °C, 5% CO_2 for 72 hours. Then, the cells were fixed with 10% trichloroacetic acid (Sigma) at 4 °C for 45 minutes, washed and dried at room

temperature overnight. Then the plate was stained with 0.057% (W/V) sulforhodamine B (SRB) (Sigma), washed with 1% (V/V) acetic acid, washed, and left to dry at room temperature overnight. Finally, 10X Tris-base was added to each well to dissolve protein-bound dye. The OD was determined at wavelength 510 nm. The IC_{50} value of each compound was determined from the dose-response curve. [51, 52]

CHAPTER III RESULTS & DISCUSSIONS

1. Chemistry

1.1 Synthesis of 5-phenoxy primaquine derivatives

Seven derivatives of 5-phenoxy primaquine (**7a-7g**) were synthesized over 6 steps, including oxidation, nucleophilic aromatic substitution, reduction, *N*-alkylation, reductive amination, and deprotection, along with one amide derivative (**7h**) which was hydrolyzed from the cyanine derivative.

In the first step, the commercially available 6-methoxy-8-nitroquinoline was first chlorinated with *N*-chlorosuccinimide (NCS) by the basic reaction in organic chemistry (**Figure 14**). The position 5 and 7 at quinoline ring are more favored for the reaction due to the mesomeric electron-donating effect of 6-OMe group, but the position 7 is sterically hindered from neighboring group. Therefore, the chlorinated product has occurred as a major product at only position 5 in good yield.

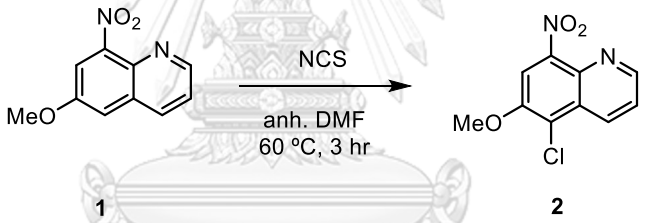
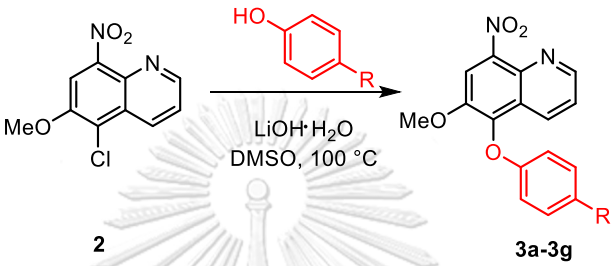


Figure 14 Oxidation of **1** to create chlorinated product **2** by NCS.

According to tafenoquine (**Figure 1 e**), a well-known anti-malarial drug with improved blood stage activity and drug haft-life compared to primaquine. Its structure contained a trifluoromethyl group at *-meta* position of the 5-phenoxy ring. However, their substituent group-activity relationship remained unclear. The electronic effect at the *-para* position on the 5-phenoxy ring was studied. The phenol contained small substituents group either electron withdrawing group or electron donating group (-OMe, -Br, -Cl, -F, $-CF_3$, - CN, $-CONH_2$) to compare the anti-malarial activity in blood stage with unsubstituted phenol. Therefore, the chlorinated product **2** was further used for synthesis of 5-phenoxy analog of primaquine *via* nucleophilic aromatic substitution reaction (S_NAr) with various phenol under basic condition. This method is commonly used in organic synthesis to functionalize aromatic molecules. The first attempt using anhydrous DMF as a solvent was unsuccessful. The chlorinated product

was not reacted with the phenol, due to the insolubility of the base, $LiOH{\cdot}H_2O$. Therefore, the solvent was changed to dimethyl sulfoxide. At 100 ºC, both the starting material and LiOH was well dissolved, and the reaction proceeded to give the intermediate **3a-3g** in **Table 3** using **General procedure A.**

Table 3 Synthesis of **3a-3g** by nucleophilic aromatic substitution (S_NAr) reaction.



2 **3a-3g**

Compound	R	Yield (%)
3a	H	74
3b	OMe	83
3c	Br	98
3d	Cl	51
3e	CN	40
3f	F	100
3g	CF_3	25

This S_NAr reaction takes place by a two-step mechanism. In the first and rate-determining step, C5 at quinoline ring where is the electron deficiency because it bonded to the chlorine atom, is attacked by phenoxide ion ($C_6H_5O^-$; a nucleophile). This step is accelerated and stabilized because of the resonance effect of the *p*-NO_2 group. A tetrahedral intermediate was formed by rehybridization of sp^2 to sp^3 carbon. In the second step, the aromaticity of quinoline ring was restored by loss of a leaving group. (**Figure 15**)

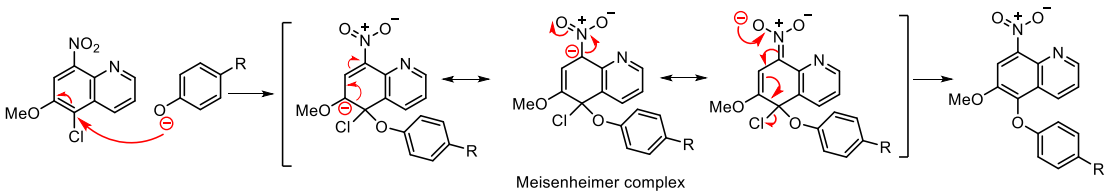
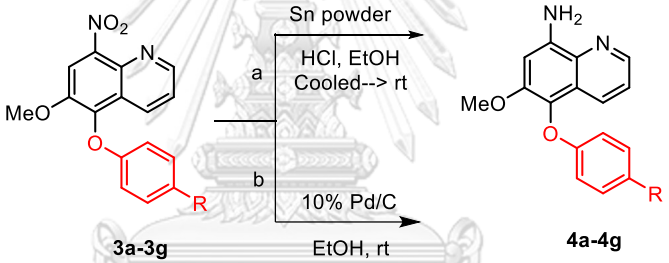


Figure 15 Proposed mechanism of S_NAr reaction.

Subsequently, the nitro group of **3a-3g** was reduced using either Sn in concentrated HCl or 10% Pd/C to generate amine intermediate **4a-4g** in **table 4** using **General procedure B.** All amine derivatives (**4a-4g**) were synthesized using route a, but the exception was **4c** (R = Br) using route b.

Table 4 Synthesis of **4a-4g** by reduction reaction via Sn/HCl or 10% Pd/C



Compound	R	Method	% yield
4a	H	a	94
4b	OMe	a	44
4c	Br	b	20
4d	Cl	a	100
4e	CN	a	100
4f	F	a	70
4g	CF_3	a	100

The initial experiment began with **4a** (R = H) and **4c** (R = Br). These two compounds were reduced by Pd/H_2. However, the reaction proceeded very slowly at room temperature over two nights. Alternatively, Sn and concentrated HCl were used instead, and the reaction and the

desired products were achieved in good yields. The proposed mechanism is shown in **Figure 16**.

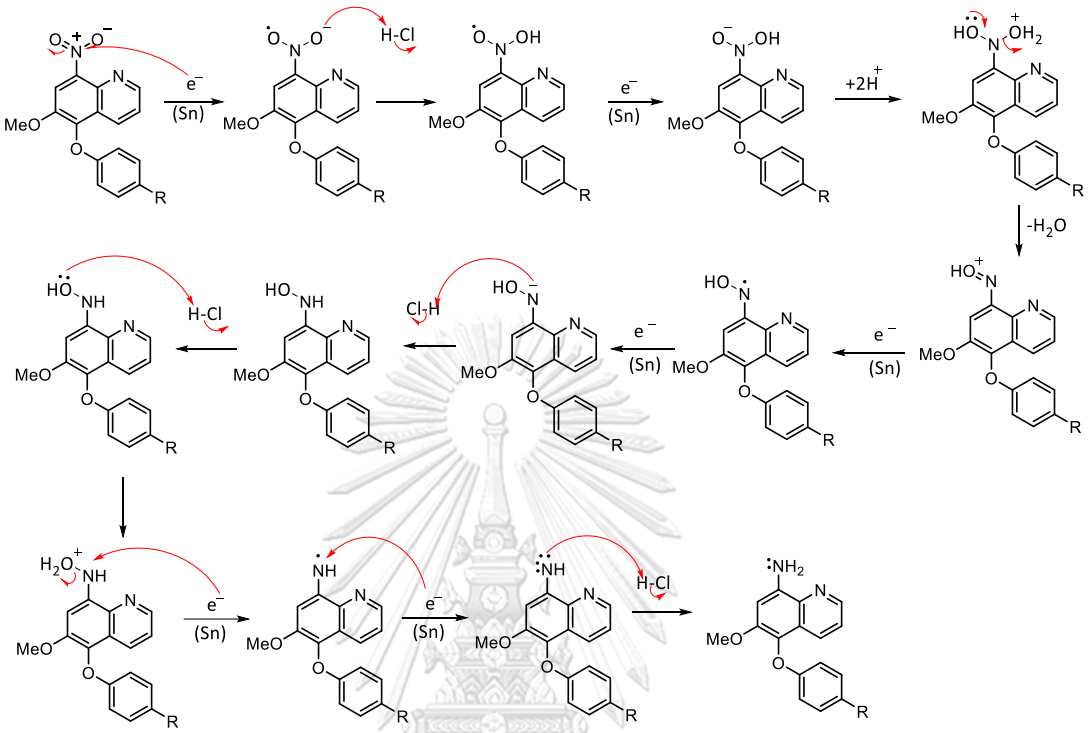


Figure 16 Proposed mechanism of Sn/HCl reduction

In addition, *N*-alkylation of potassium phthalimide with 1,4-dibromopentane was easily synthesized to afford precursor **5** in good yield. (**Figure 17**)

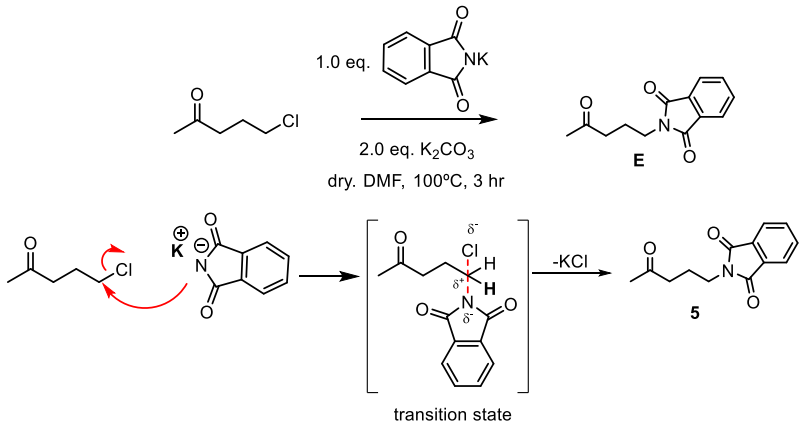


Figure 17 Synthesis of 5 via S_N2 reaction

Next, incorporation of the alkyl side chain to the 8-amino group **4a-4g** was attempted *via* reductive amination reaction. The amine compound **4a** was initially alkylated by 2-(4-bromopentyl)isoindoline-1,3-dione in small scale reaction using a reaction tube under neat condition at 120 ºC to give the phthalimide protected terminal amino **i** in good yield (80%) (**Figure 18**)[6]. However, the later scale-up reaction was unsuccessful because the mixture was unable to stirred efficiently under neat conditions. Therefore, reductive amination was attempted as an alternative pathway. This reaction occurred by the nucleophilic addition reaction between nucleophilic nitrogen **4a-4g** and the electrophilic carbonyl **5** to generate an imine intermediate. However, for this step, the rate of the reaction at room temperature was too slow leading to low yield, so the temperature was increased to 50 ºC to accelerate the imine formation. The iminium ion produced was then reduced by sodium cyanoborohydride ($NaBH_3CN$) as a mild reducing agent to produce **6a-6g** in moderate yield. The mechanism of action of these is shown in **figure 19**. In addition, CN derivative (**6e**) was further hydrolyzed to amide derivative (**6h**) in moderate yield (**Table 5**).

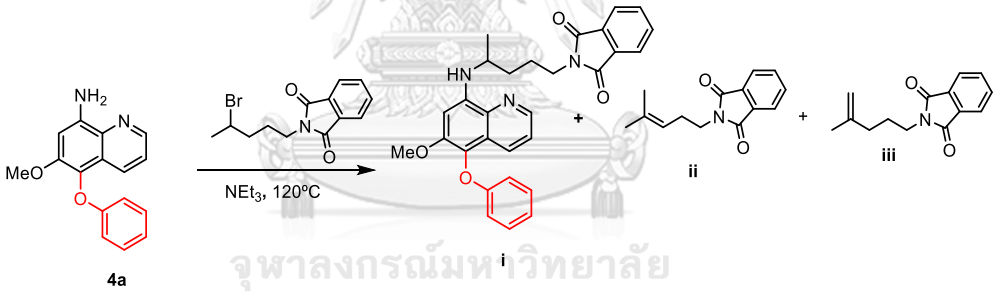
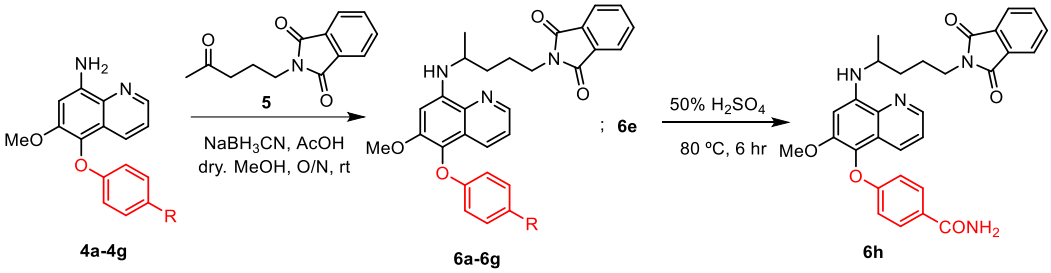


Figure 18 *N*-Alkylation of **4a** and 2-(4-bromopentyl)isoindoline-1,3-dione.

Table 5 Synthesis of **6a-6g** by reductive amination between **4a-4g** and **5**



Compound	R	% yield
6a	H	57
6b	OMe	35
6c	Br	80
6d	Cl	44
6e	CN	44
6f	F	34
6g	CF_3	25
6h	$CONH_2$	58

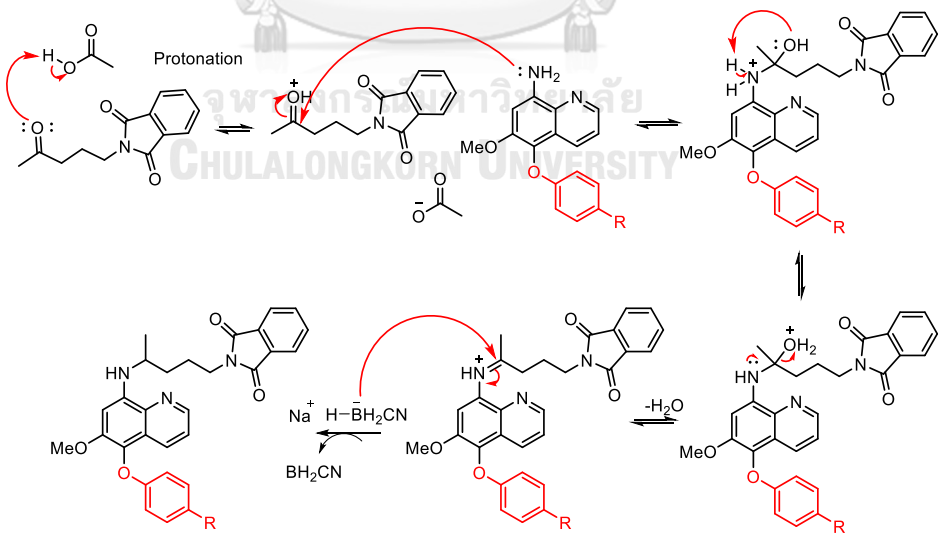
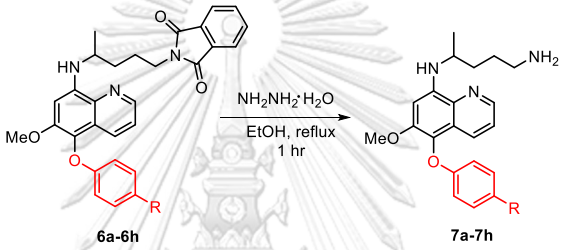


Figure 19 Mechanism of action of reductive amination reaction

Finally, the phthalimide deprotection of **6a-6h** was achieved using hydrazine monohydrate to give the desired 5-phenoxy primaquine **7a-7h** in good yield (**Table 6**). The mechanism of action of phthalimide deprotection by hydrazine is shown in **figure 20**. In the first step, carbonyl carbon was substituted by a lone pair of nitrogen of hydrazine. Subsequently, the ring opened. Proton was then transferred by an intramolecular nucleophilic acyl substitution reaction. Finally, the tetrahedral intermediate broke to provide the desired primary amine and the by-product of the phthalhydrazide.

Table 6 Synthesis of **7a-7h** by phthalimide deprotection of **6a-6h**.



Compound	R	% yield
7a	H	94
7b	OMe	100
7c	Br	54
7d	Cl	68
7e	CN	100
7f	F	100
7g	CF_3	85
7h	$CONH_2$	100

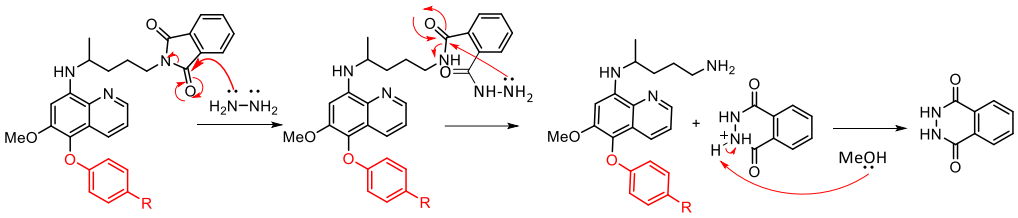


Figure 20 Mechanism of action of phthalimide deprotection by hydrazine

1.2 Synthesis of tetraoxane unit and novel 5-phenoxy primaquine-tetraoxane conjugated compound

The E209 analog of tetraoxane (**11**) was successfully synthesized. In the first step, the commercially available phenol **8** was acetylated by acetic anhydride in the presence of triethylamine to give **9** in quantitative yield. Next, this acetyl protected compound was peroxidised under acid-catalytic conditions to form the gem dihydroperoxide intermediate which then reacted with adamantan-2-one to provide a protected tetraoxane **10** in low yield.[6] After that, **10** was deprotected under the basic condition to afford the desired tetraoxane **11** in excellent yield. In addition, **11** was further converted to aldehyde **13** in low yield by ozonolysis of alkene **12** which was prepared by *O*-alkylation of **11** with allyl bromide. On the other hand, **11** was alkylated by 1,4-dibromobutane to give the alkylated product **14** in good yield. This precursor was further alkylated by primary amine of **7a** to give the novel 5-phenoxy primaquine-tetraoxane conjugated compound **15** in moderate yield without disubstituted product (**Figure 21**).

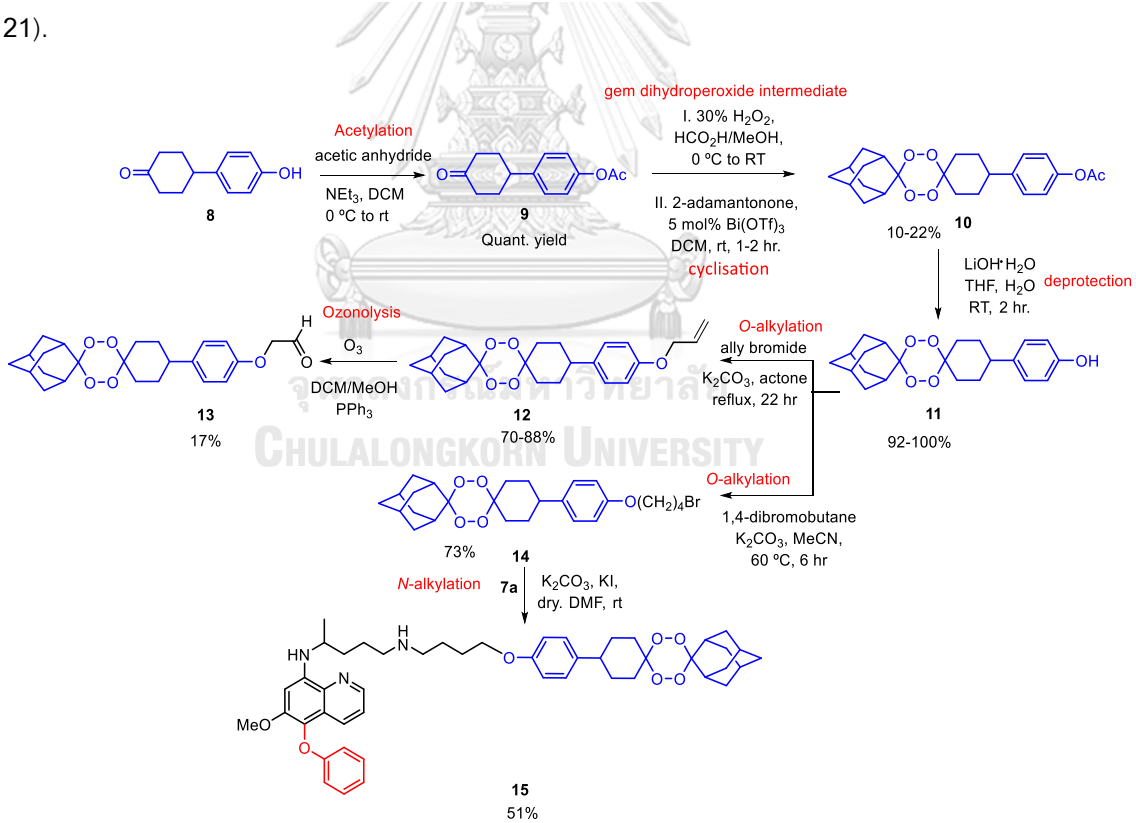


Figure 21 The result from synthesis of tetraoxane unit

1.3 Unsuccessful synthesis of 5-phenoxy primaquine-tetraoxane conjugated compounds.

1.3.1 Attempted synthesis of the novel conjugate by substitution of tetraoxane molecule into position 5 of primaquine.

The S_NAr reaction of compound **2** with a phenoxide ion under basic condition gave the product in good yield, but the reduction of nitro group into amine was unsuccessful because the endoperoxide bond was easily decomposed to ketone in the presence of acid. Alternatively, Pd/C under H_2 was used instead (**Figure 22**); however, many spots of by-products were observed on TLC plate. Therefore, we did not pursue this synthetic route because there are many remaining steps to afford the desired conjugate.

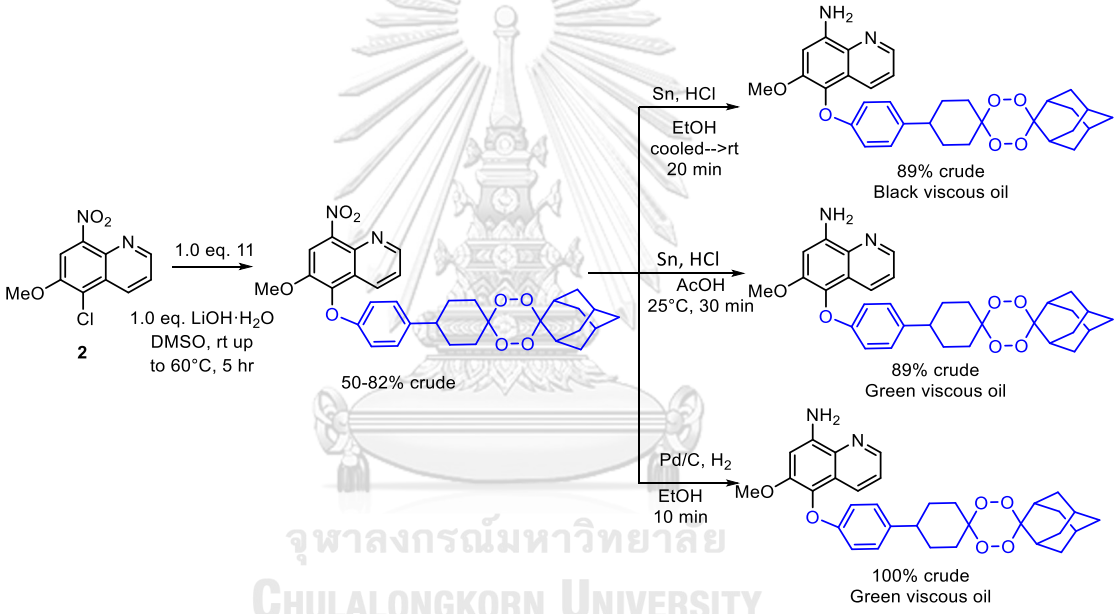


Figure 22 Reduction of nitro group of compound contains endoperoxide bond to amine using Sn/HCl and Pd/C under H_2

1.3.2 Attempted synthesis of the novel conjugate of ozonide phenol OZ288

The synthesis of this conjugate was not successful. Alkylation of OZ288 which was provided by BIOTEC with 1,4-dibromobutane in the first step did not provide the desired product when the reaction was performed at 55-60 ºC. The reaction temperature was then increased to 70 ºC; however, this led to the decomposition of the compounds (**Figure 23**).

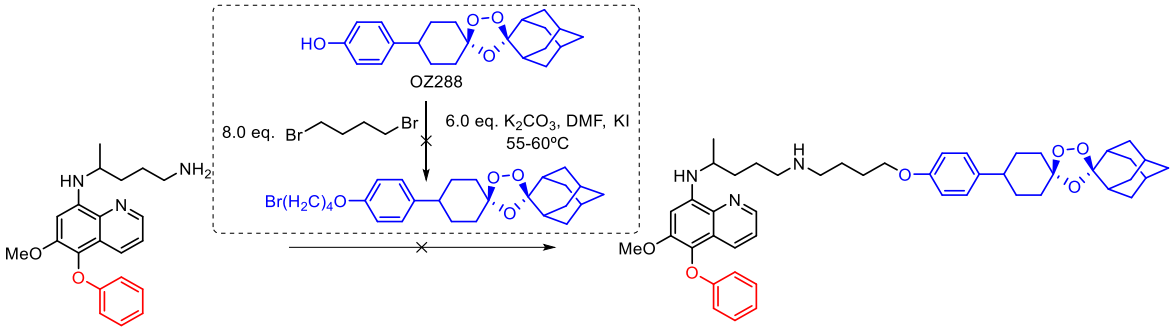


Figure 23 *O*-alkylation of ozonide phenol (OZ288) with 1,4-dibromobutane

1.2.3 Attempted synthesis of the novel conjugate of dispiro 1,2,4,5-tetraoxane derivative

The bromoacetic acid was initially alkylated by **11** to form an amide linker of this conjugate. Phenolates of **11** are formed when phenolic hydroxy groups are exposed to basic conditions. This phenolate anion can be reacted with the bromoacetic acid using a catalytic amount of KI to promote this reaction. The iodide will act as an external nucleophile to generate a highly reactive iodide intermediate. Anhydrous DMF as a polar aprotic solvent was used to prevent *C*-alkylated by-product; however, the target product was not observed. They did not react with each other to form the product even at 60ºC. (**Figure 24**)

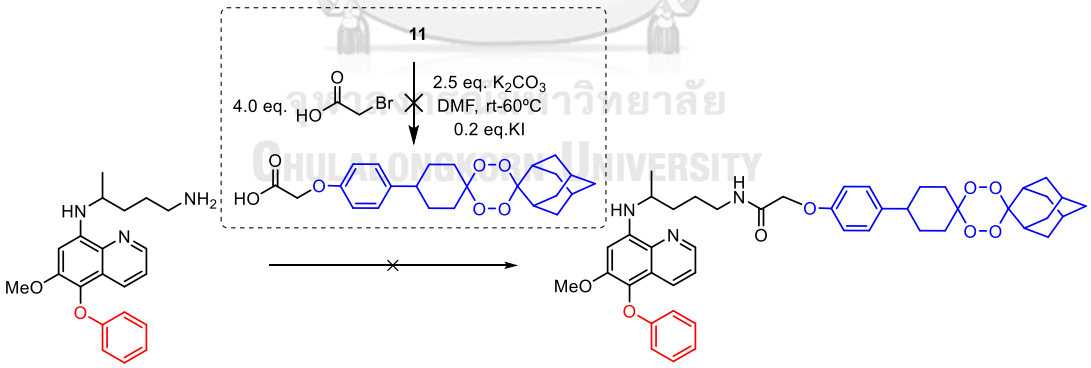


Figure 24 *O*-alkylation of tetraoxane (**11**) with bromoacetic acid using KI.

2. Biology

2.1 Antimalarial activity

Table 7 *In vitro* antimalarial activities (IC_{50}) against *P. falciparum* 3D7 and cytotoxicity (CC_{50}) against African green monkey kidney fibroblast (Vero cells)

Compd.	R	IC_{50} μM	CC_{50} μM	Selectivity Index (SI)
		P. falciparum 3D7 (blood stage)	Vero Cells	
Primaquine biphosphate	-	11.33 ± 0.79	>100	>9
7a	H	3.65 ± 0.39	37.49 ± 5.24	10.27
7b	OCH_3	7.89 ± 0.50	55.62 ± 4.34	7.05
7c	Br	7.03 ± 1.31	42.16 ± 3.25	6.00
7d	Cl	8.20 ± 0.83	46.38 ± 5.68	5.66
7e	CN	4.62 ± 0.56	>100	>25
7f	F	4.97 ± 0.40	47.47 ± 3.23	9.55
7g	CF_3	4.63 ± 0.44	22.66 ± 3.74	4.89
7h	$CONH_2$	13.5 ± 1.57	>100	>7
Conjugate 15	H	0.38 ± 0.11	17.33 ± 0.36	45.61
PYR	-	0.05 ± 0.005	ND	-
Elipticine	-	ND	5.97± 0.14	-

SI = CC_{50} (μM) on monkey Vero cells/IC_{50} (μM) in blood stage; Each IC_{50} and CC_{50} value represent the mean ± SD (n = 3), ND = not determined, PYR = pyrimethamine which targets dihydrofolate reductase of *P. vivax* (*PvDHFR*).

Since primaquine is the only tissue schizonticide drug available for radical treatment of *P. vivax* or *P. ovale* infections. It had weak blood schizonticide activity as a result of oxidative stress which was generated from its hydroxylated metabolites at position 5 **(Figure 4)**, but it also produced hemolytic side effect. The effects of the various R group of 5-phenoxy derivatives of primaquine were studied. The anti-malarial activity in **Table 7** revealed that most of them had a better inhibitory activity with the IC_{50} value of 3.65 – 7.89 μM against asexual forms of *P. falciparum* 3D7 chloroquine-sensitive strain than the standard primaquine with the IC_{50} value of 11.33 ± 0.79 μM. In contrast, **7h** with the IC_{50} of 13.5 ± 1.57 μM had less potency than the primaquine. According to the proposed mechanism of action of chloroquine, a well-known anti-malarial drug. In blood stage infection, hemoglobin is digested to amino acids and heme by parasites in its acidic digestive vacuole. This heme is toxic to parasites. Then, this heme is

transformed to hemozoin by parasites through the heme polymerization process.[53] Therefore, the presence of the phenoxy group at position 5 of primaquine may have a significance about this process with the result of increasing inhibitory activity superior to that of the primaquine.[54] However, this data assessed that the correlation between the various R group on the structure and their blood stage activity were not significantly different. As expected, **15** exhibited inhibitory activity with IC_{50} values of 0.38 ± 0.11 μM, which was 30-fold more potent than that of the primaquine alone. Since the metabolic instability of amide derivative of lead candidate RKA182 (**Figure 8**) was reported.[34] Therefore, **15** with different mode of action between the 5-phenoxyprimaquine molecule and the tetraoxane molecule was conjugated by an amine linker which was designed to eliminate the metabolic liability. However, this conjugate had less anti-malarial activity in blood stage than that of **11** which had IC_{50} = 29 nM (unpublished result). Once 5-phenoxyprimaquine part inhibited heme polymerization similar to tafenoquine.[55] As a result, there is a lot of free heme which contains ferrous iron to reduce the endoperoxide bond of the tetraoxane part result in cytotoxic carbon-centered radicals. The essential macromolecules of malarial parasite could be alkylated by these radicals, leading to parasite's death. Therefore, these drug conjugates may exhibit a synergistic effect, leading to increased inhibitory activity against *P. falciparum* in an asexual stage. However, the anti-malarial activity of **11** and 1:1 of 5-phenoyprimaquine: tetraoxane were not evaluated for comparison, so the actual source of activity could not conclude for this work. In addition, a heme polymerization assay will be tested to confirm this proposed mechanism of our conjugate in the future.[56] In addition, all synthesized compounds will be also studied with a chloroquine-resistant strain of *Plasmodium falciparum*. The cytotoxicity of eight 5-phenoxyprimaquine derivatives (**7a-7h**) including one 5-phenoxyprimaquine-tetraoxane conjugated (**15**) was then assessed using the African green monkey kidney fibroblast (Vero cells) with sulforhodamine B (SRB) colorimetric assay. Then, the selectivity index (SI) was calculated as the ratio of the 50% cytotoxic concentration (CC_{50}) to the 50% inhibitory concentration (IC_{50}). The results in **Table 7** show that those compounds had cytotoxic concentration (CC_{50}) values and selectivity indexes from 17.33 to more than 100 μM and from 4.89 to more than 25, respectively. All synthesized compounds are less toxic comparing with ellipticine which is an anti-cancer drug. Interestingly, **7e** and **15** with high antimalarial activity had SI values of more than 25 and 45.61, respectively.

CHAPTER IV CONCLUSION

In conclusion, three new (R = CN, $CONH_2$, CF_3) and five known (R = H, OMe, Cl, Br, F) derivatives of 5-phenoxy primaquine were successfully synthesized over six steps with moderate overall yields. Moreover, one new 5-phenoxy primaquine-tetraoxane conjugate was also synthesized over eleven steps. The anti-malarial activity of all synthesized compounds was investigated using Malaria SYBR Green I-base fluorescence (MSF) assay against *P. falciparum* 3D7 in the blood-stage. Comparing with the primaquine as a reference compound (IC_{50} = 11.33 ± 0.79 μM), most of the 5-phenoxy primaquine derivatives exhibited greater inhibition toward *P. falciparum* 3D7 with IC_{50} values ranging from 4.62 to 8.2 μM, but the exception was **7h** (R = $CONH_2$, IC_{50} = 13.5 ± 1.57 μM); however, there was no significant difference in correlation between the effects of the substituted group at the para position on the phenoxy ring and their malarial activity. Therefore, the presence of the phenoxy group in position 5 can enhance the inhibition of malarial parasite. Subsequently, the most potent derivative (**7a**) was conjugated with tetraoxane **14** using a linker of four carbon to afford new 5-phenoxy primaquine-tetraoxane conjugated (**15**). This conjugate was again evaluated for their anti-malarial activity using the same previous method. The synthesized conjugate (**15**) exhibited inhibitory effects with IC_{50} values of 0.38 ± 0.11 μM, which was 30-fold more potent than that of the primaquine. Then, all synthesized compounds were assessed using the African green monkey kidney fibroblast (Vero cells) with sulforhodamine B (SRB) colorimetric assay. The selectivity index (SI) was then calculated. Compound **7e** and **15** with high antimalarial activity had SI values of more than 25 and 45.61, respectively. Therefore, this CN (**7e**) and new 5-phenoxyprimaquine-tetraoxane conjugated **15** could be used for further drug discovery study in the future.

APPENDIX

Appendix A NMR spectra of 2 and 5

5-chloro-6-methoxy-8-nitroquinoline (2)

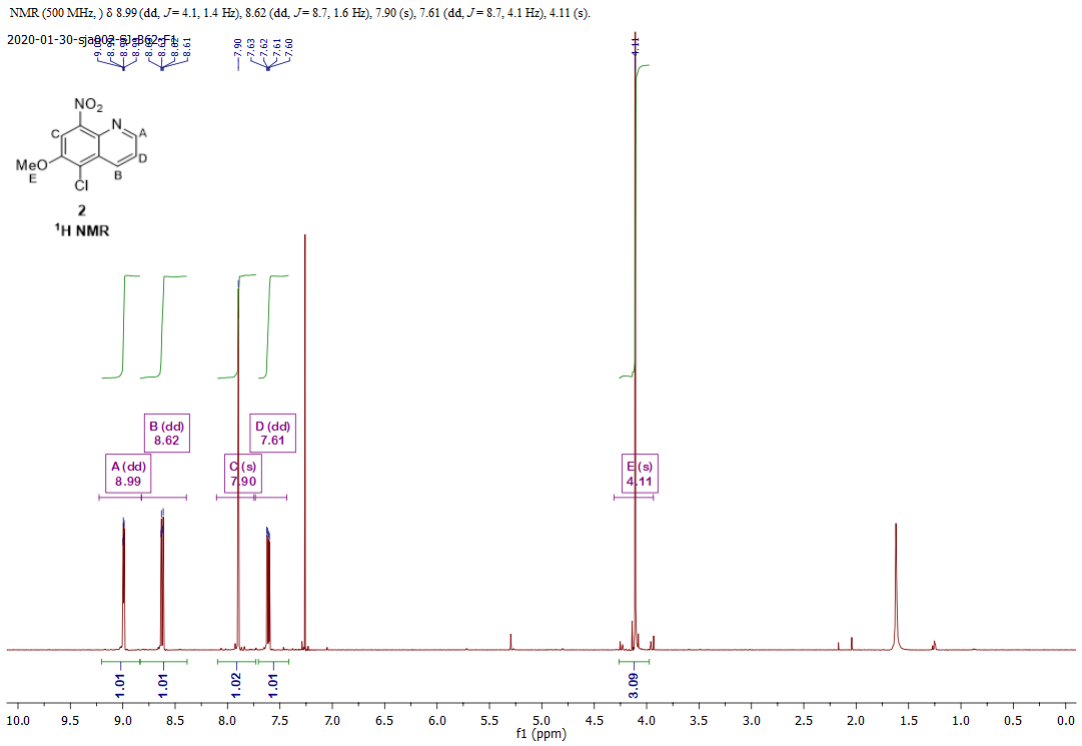


Figure 25 ^{1}H NMR of spectrum of 2

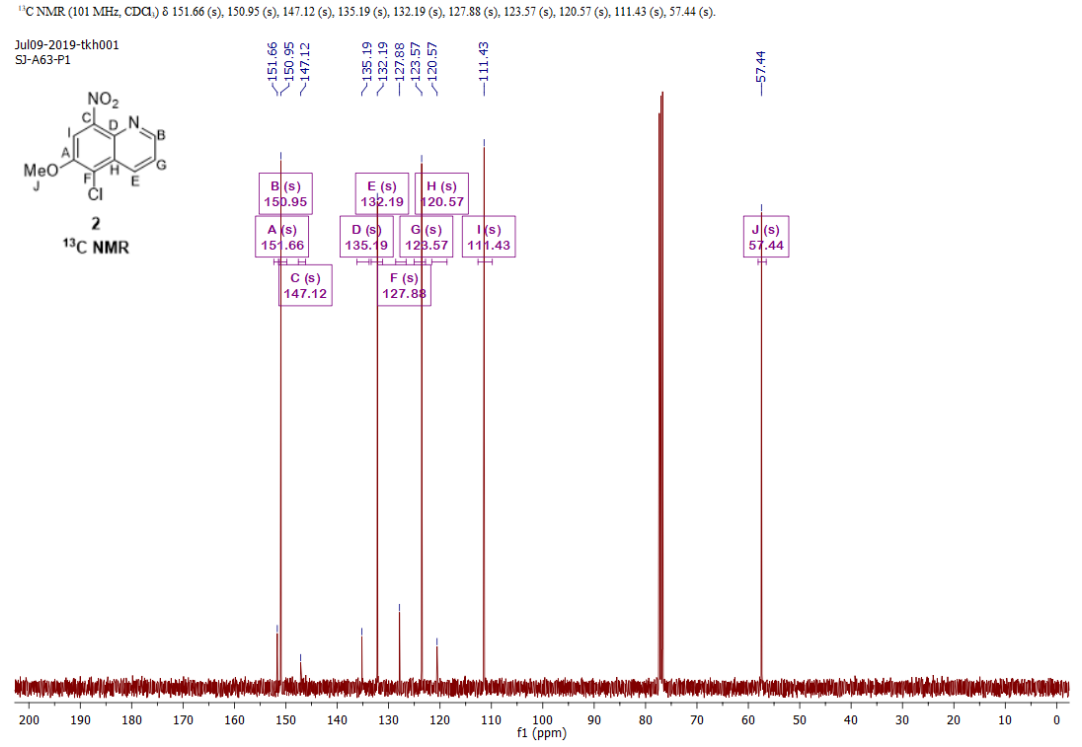


Figure 26 ^{13}C NMR of spectrum of 2

2-(4-oxopentyl)isoindoline-1,3-dione (5)

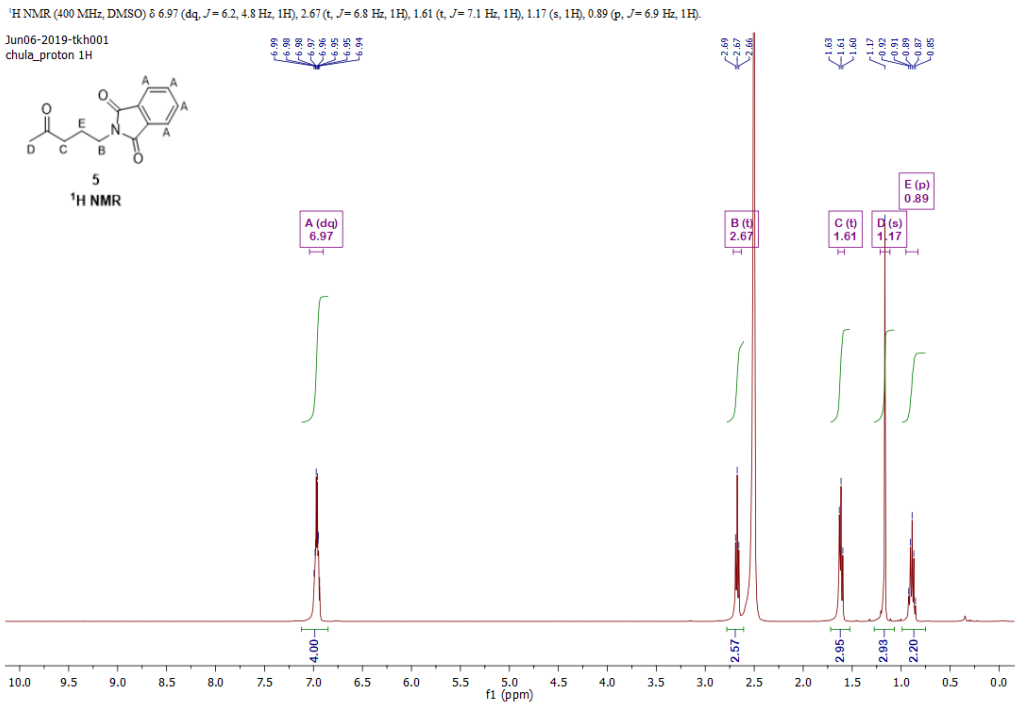


Figure 27 ^{1}H NMR of spectrum of 5

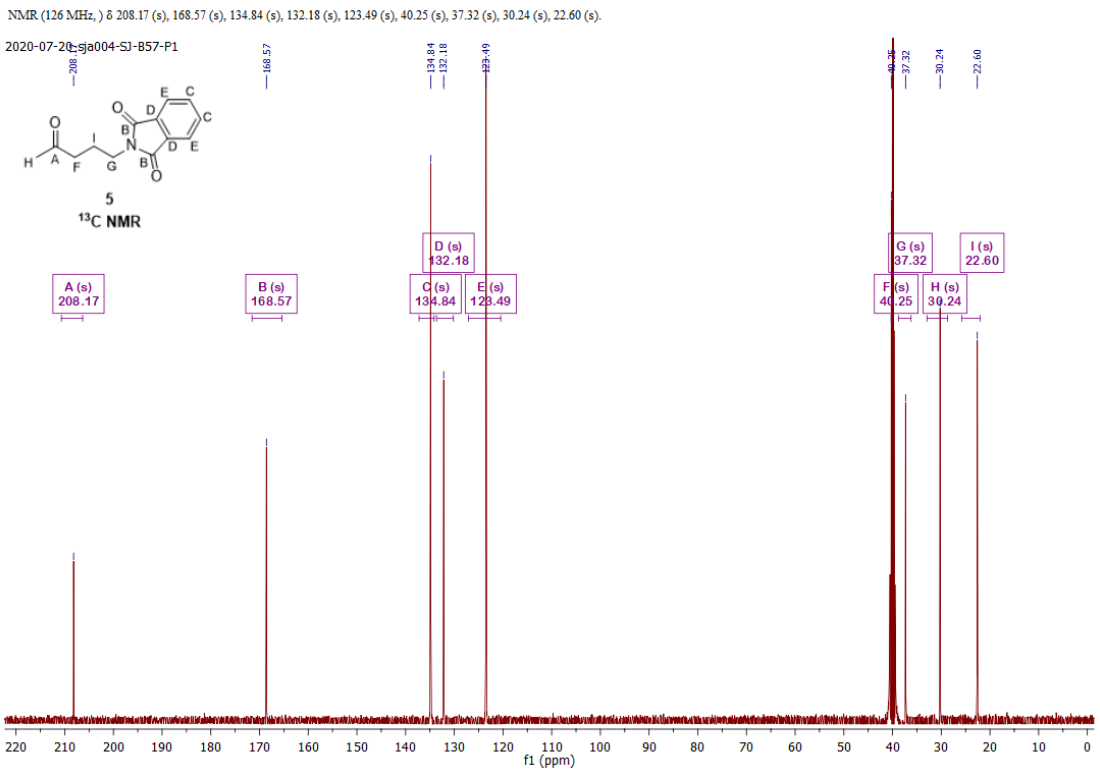


Figure 28 ^{13}C NMR of spectrum of 5

Appendix B NMR and HRMS spectra of 7a-7h

N^4-(6-methoxy-5-phenoxyquinolin-8-yl)pentane-1,4-diamine (7a)

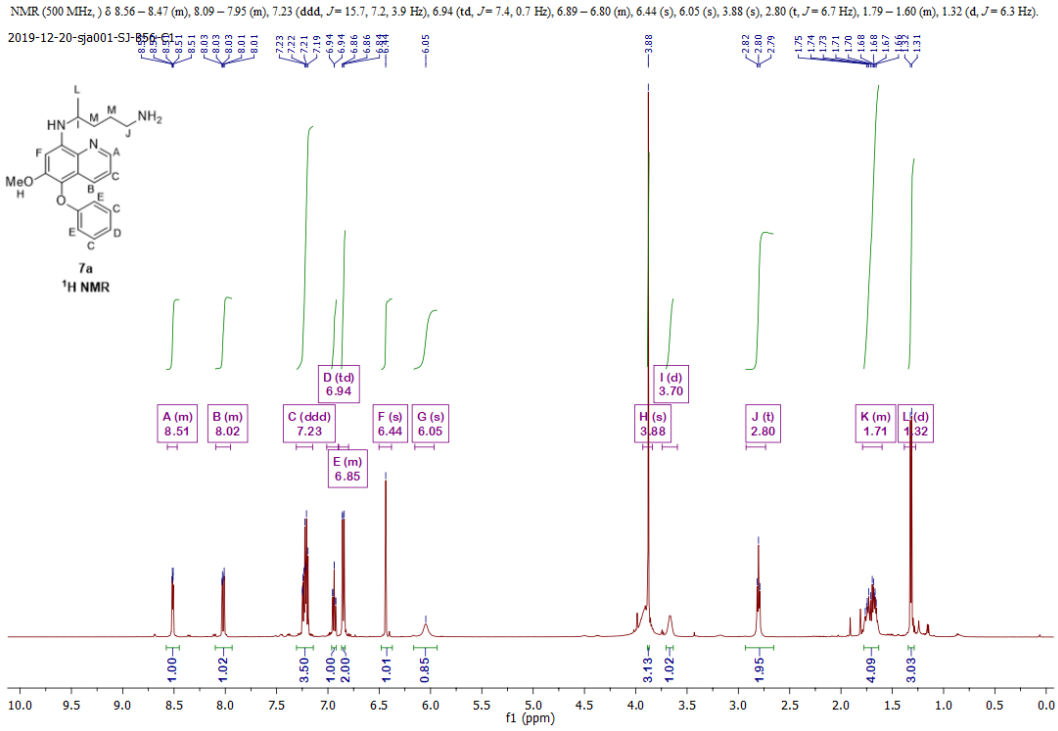


Figure 29 ^{1}H NMR of spectrum of **7a**

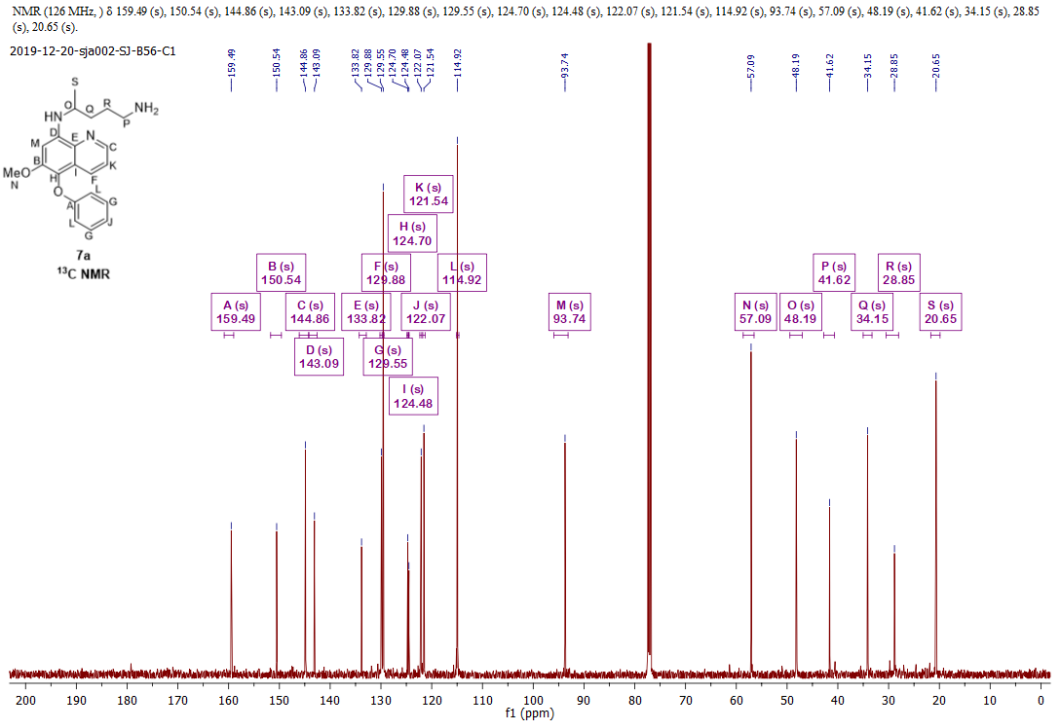


Figure 30 ^{13}C NMR of spectrum of **7a**

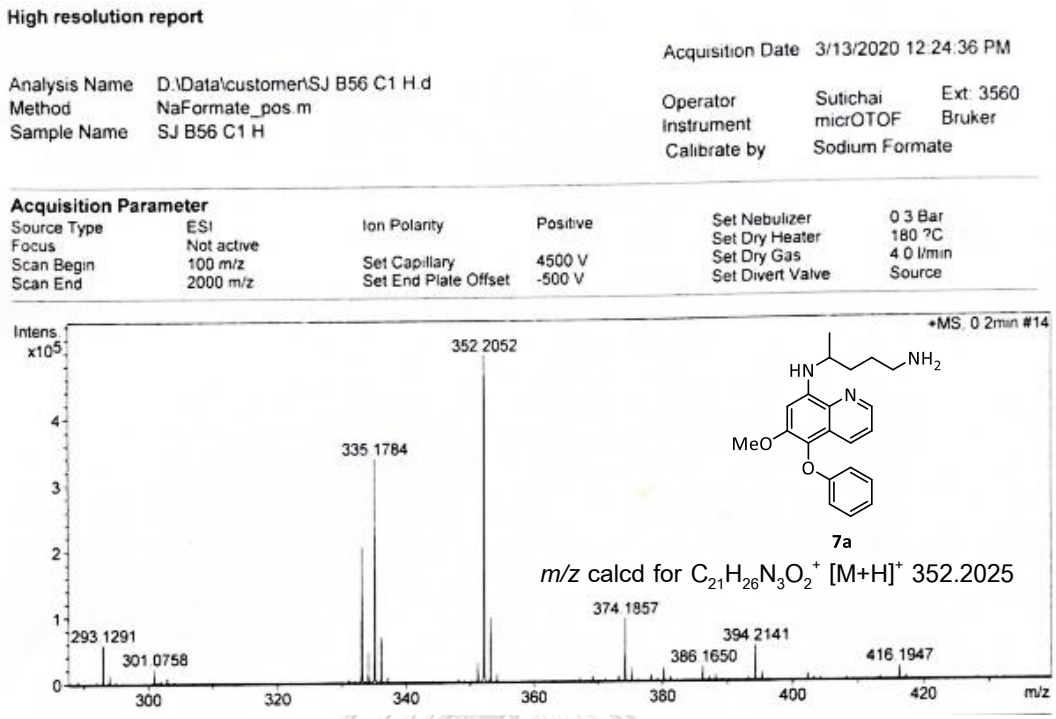


Figure 31 HRMS spectrum of 7a

N^4-(6-methoxy-5-(4-methoxyphenoxy)quinolin-8-yl)pentane-1,4-diamine (7b)

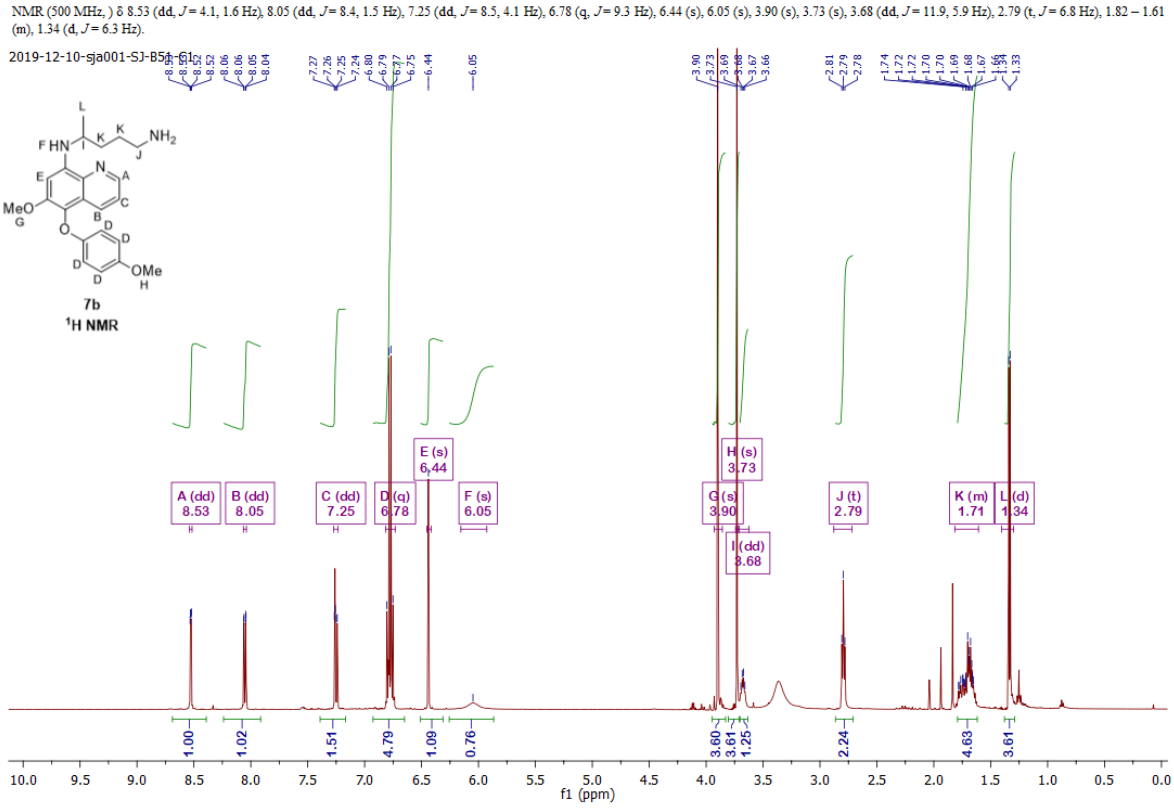


Figure 32 ^{1}H NMR of spectrum of 7b

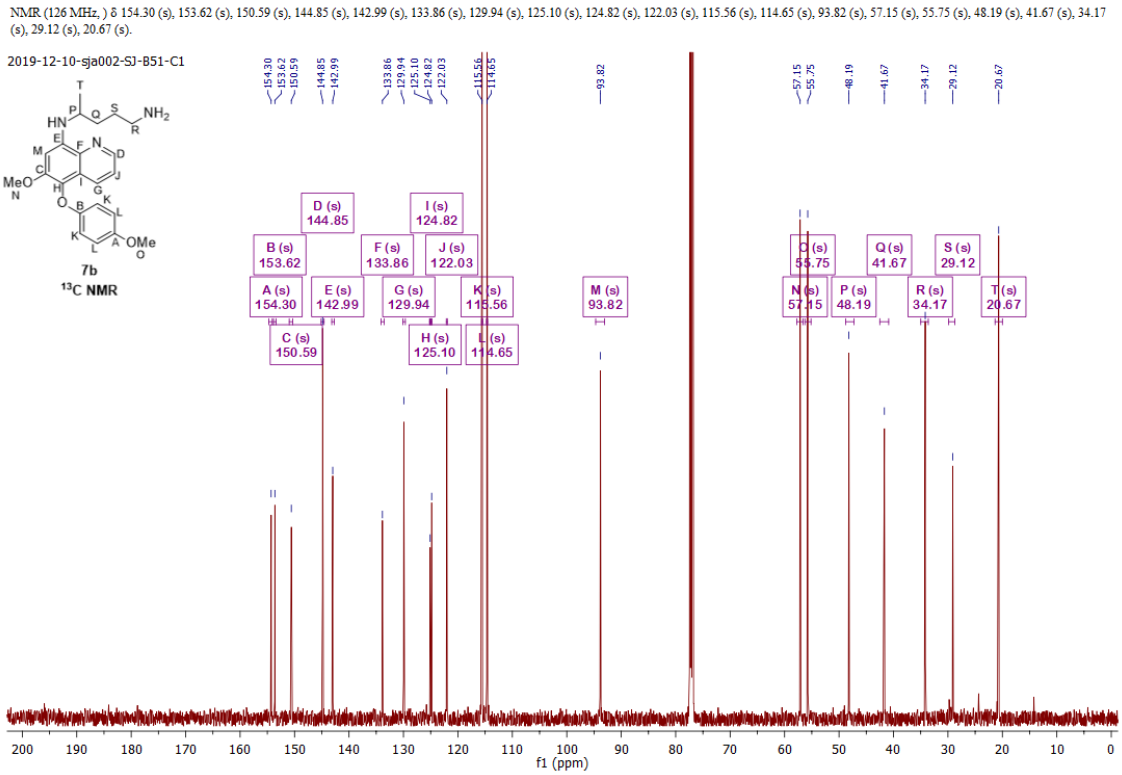


Figure 33 ^{13}C NMR of spectrum of **7b**

High resolution report

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		Calibrate by	Sodium Formate

Acquisition Parameter

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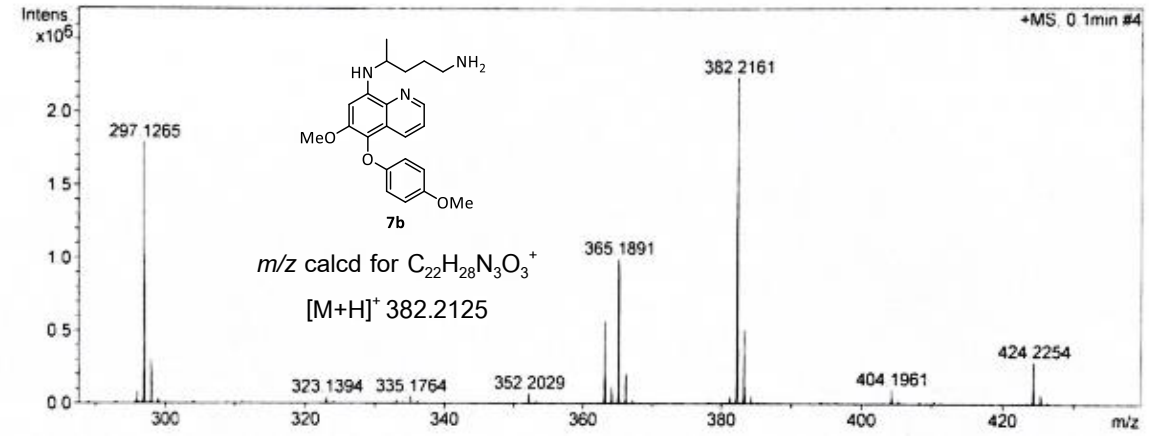

Figure 34 HRMS spectrum of **7b**

N^4-(5-(4-bromophenoxy)-6-methoxyquinolin-8-yl)pentane-1,4-diamine (7c)

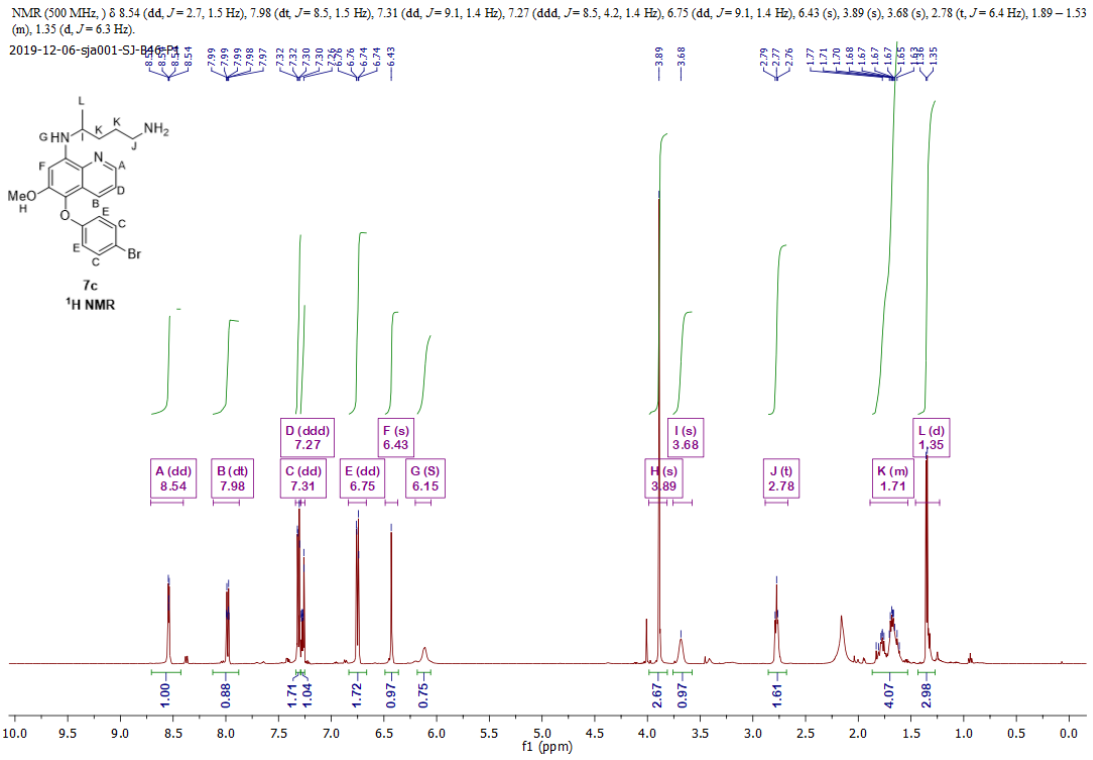

Figure 35 ^{1}H NMR of spectrum of **7c**

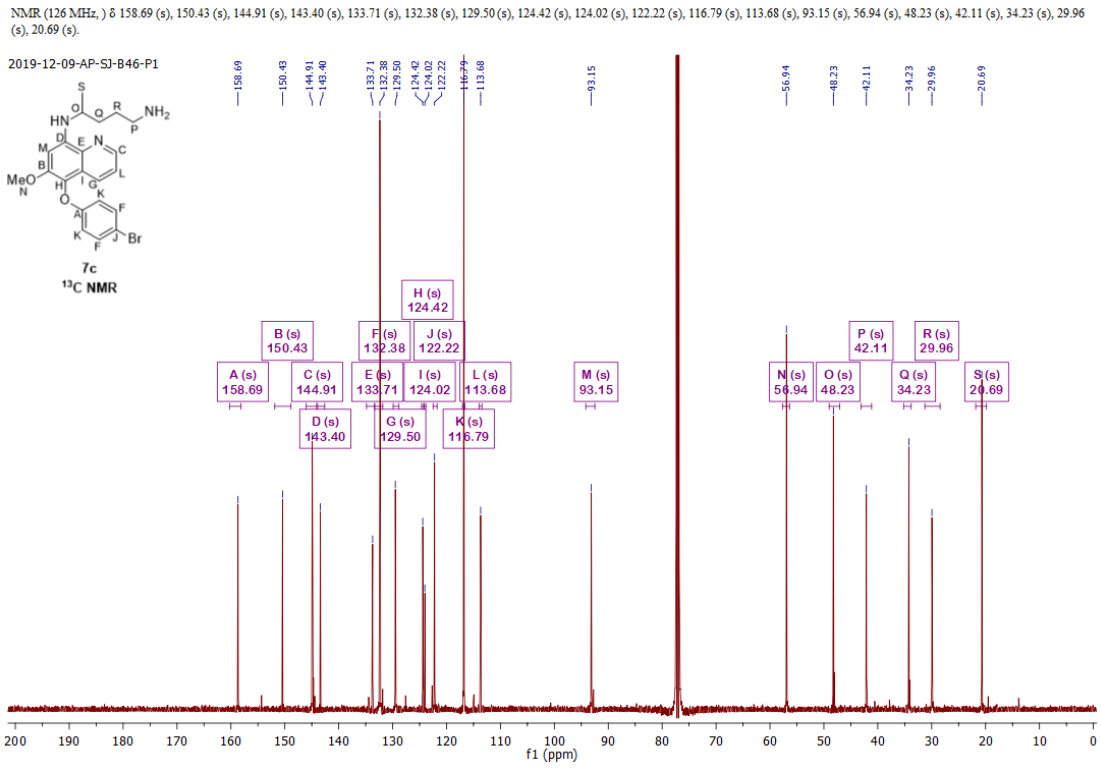

Figure 36 ^{13}C NMR of spectrum of **7c**

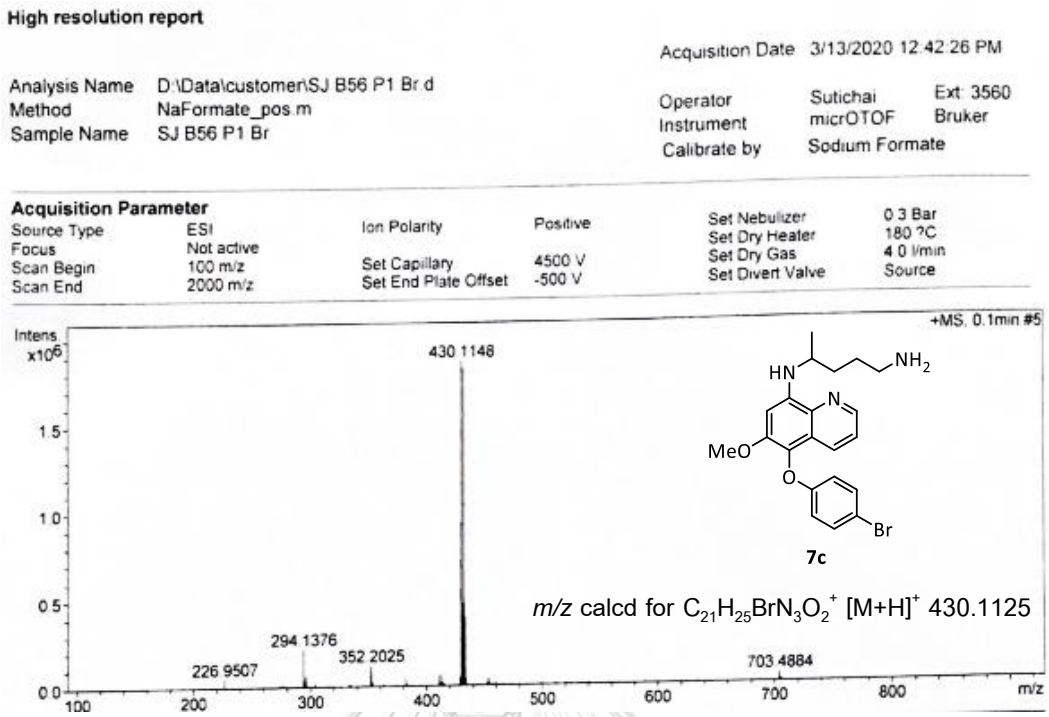

Figure 37 HRMS spectrum of **7c**

N^4-(6-methoxy-5-(4-chlorophenoxy)quinolin-8-yl)pentane-1,4-diamine (7d)

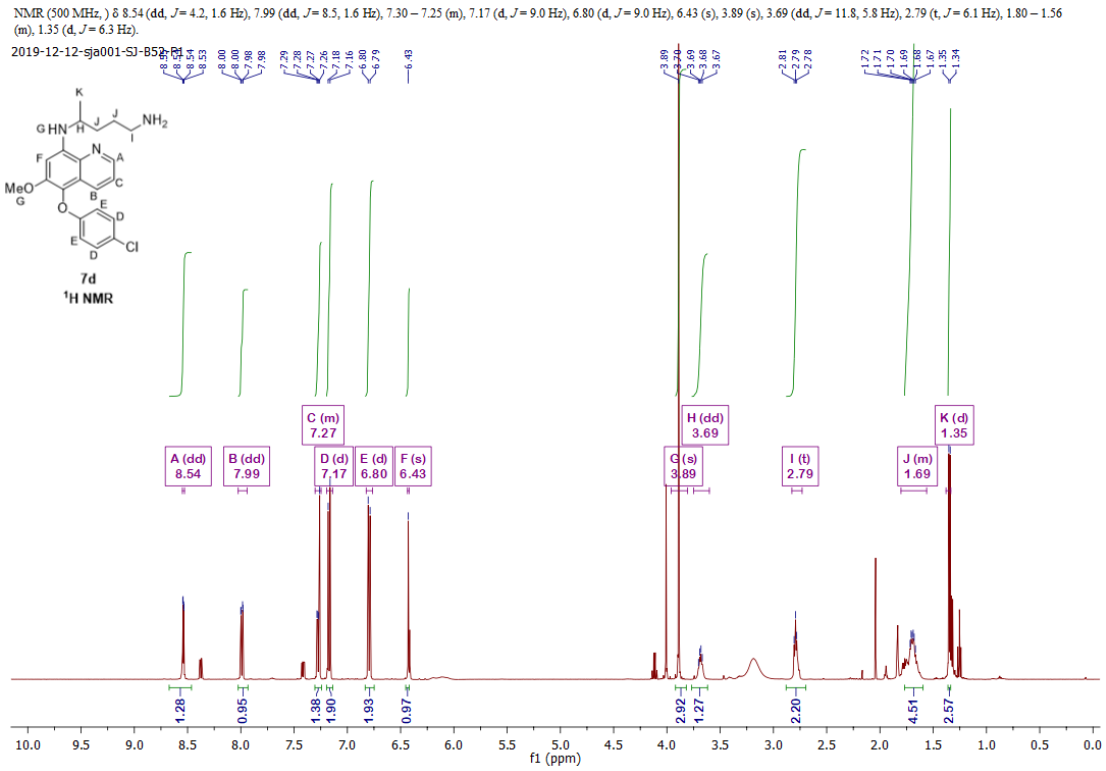

Figure 38 ^{1}H NMR of spectrum of **7d**

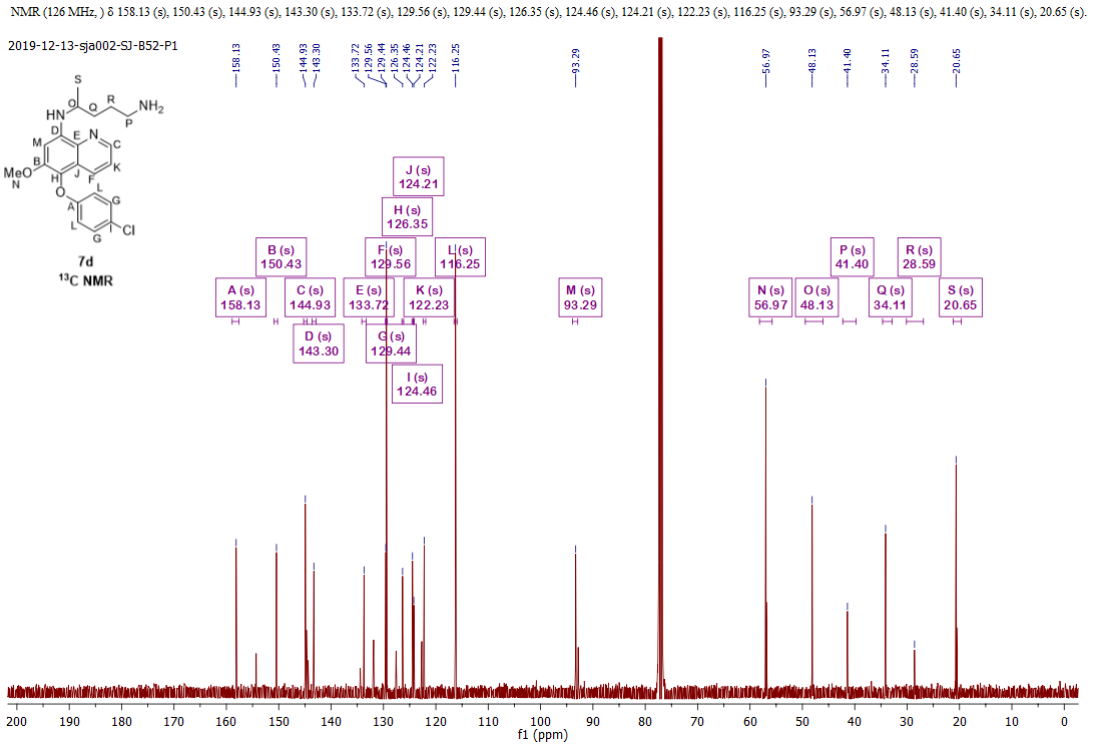

Figure 39 ^{13}C NMR of spectrum of **7d**

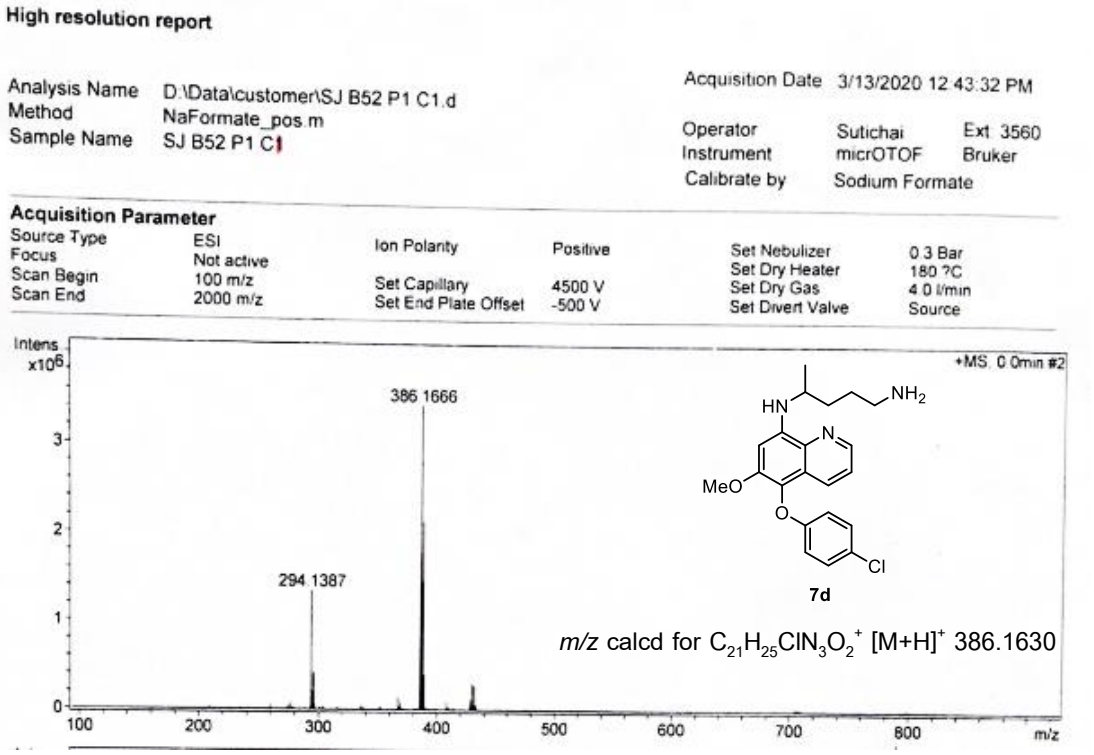

Figure 40 HRMS spectrum of **7d**

4-((8-((5-aminopentan-2-yl)amino)-6-methoxyquinolin-5-yl)oxy)benzonitrile (7e)

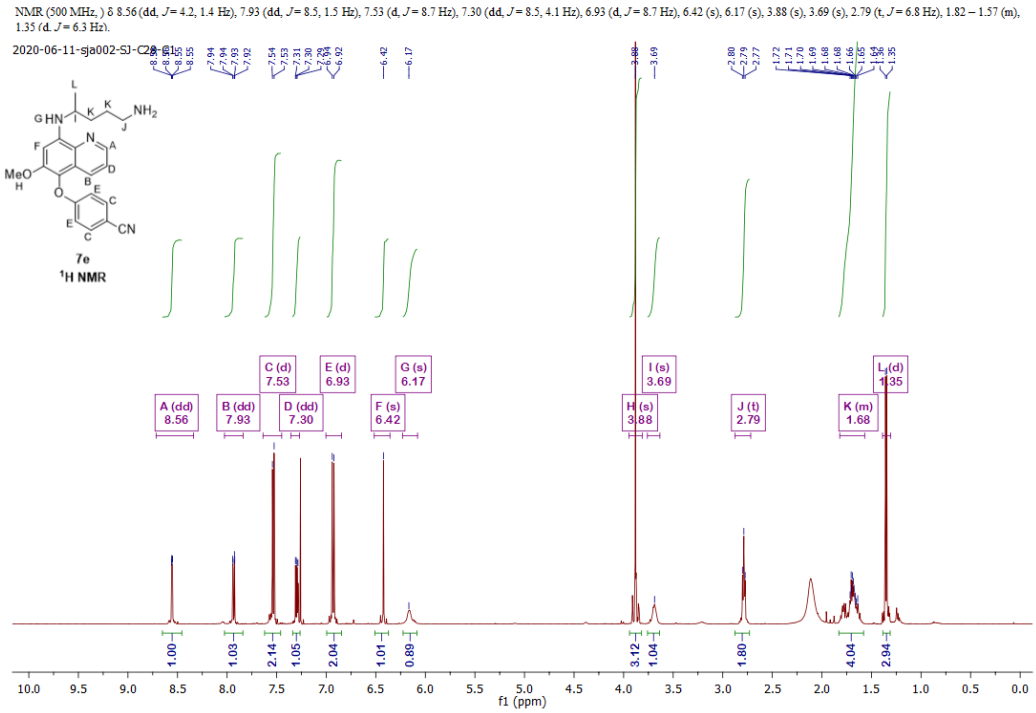

Figure 41 ^{1}H NMR of spectrum of **7e**

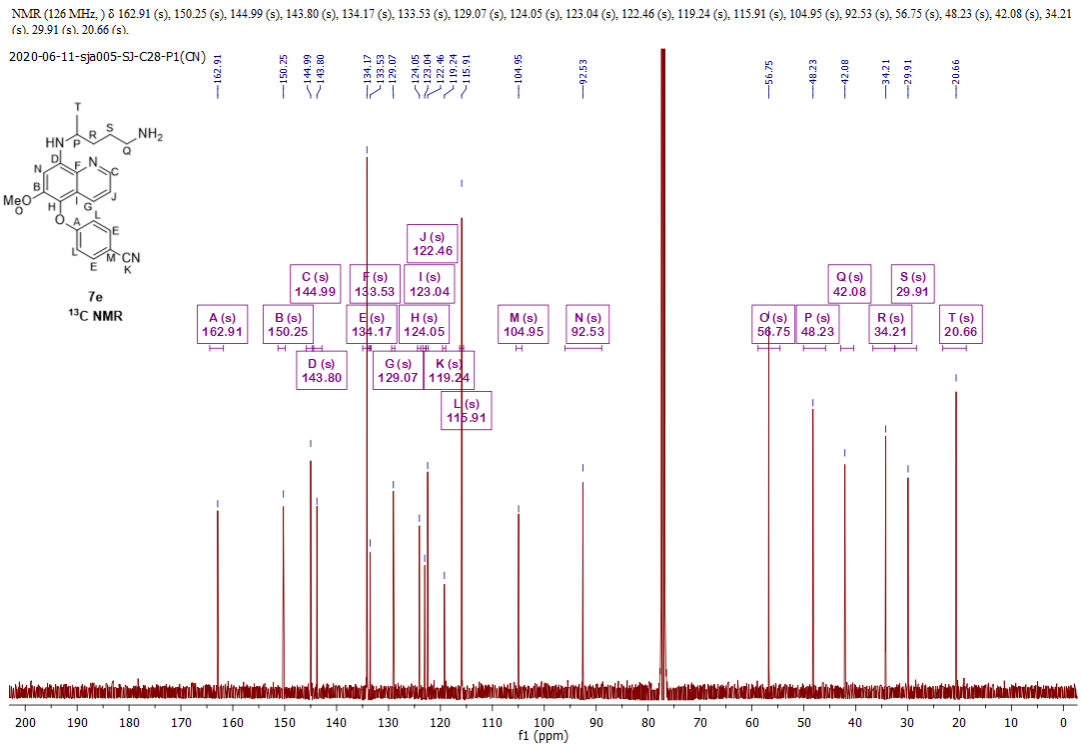

Figure 42 ^{13}C NMR of spectrum of **7e**

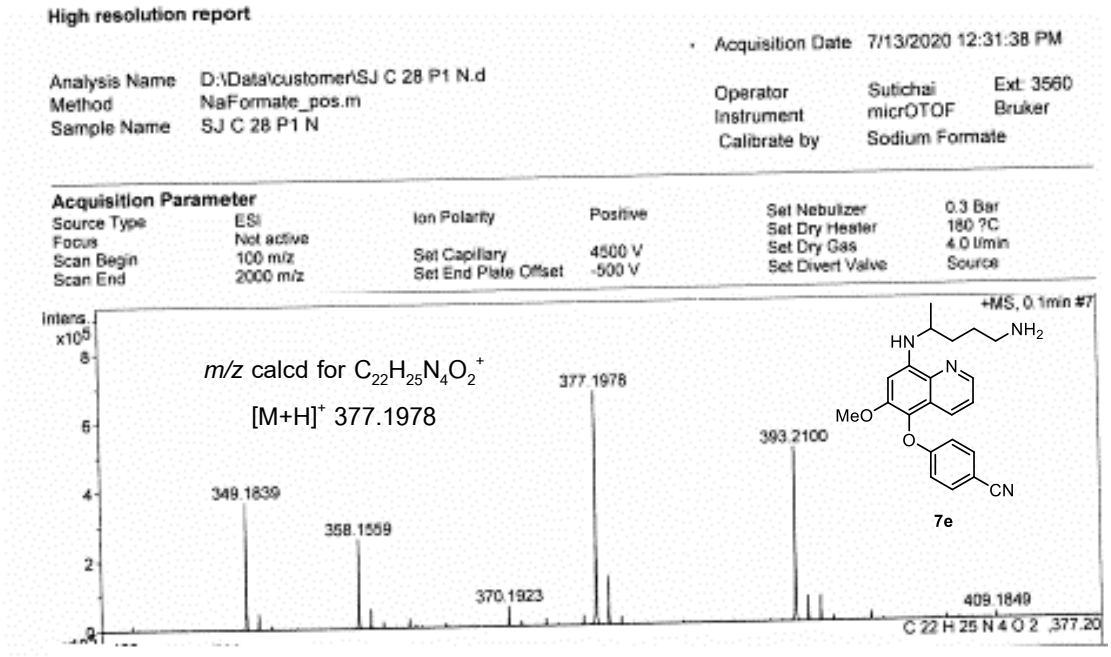

Figure 43 HRMS spectrum of **7e**

N[4]-(5-(4-fluorophenoxy)-6-methoxyquinolin-8-yl)pentane-1,4-diamine (**7f**)

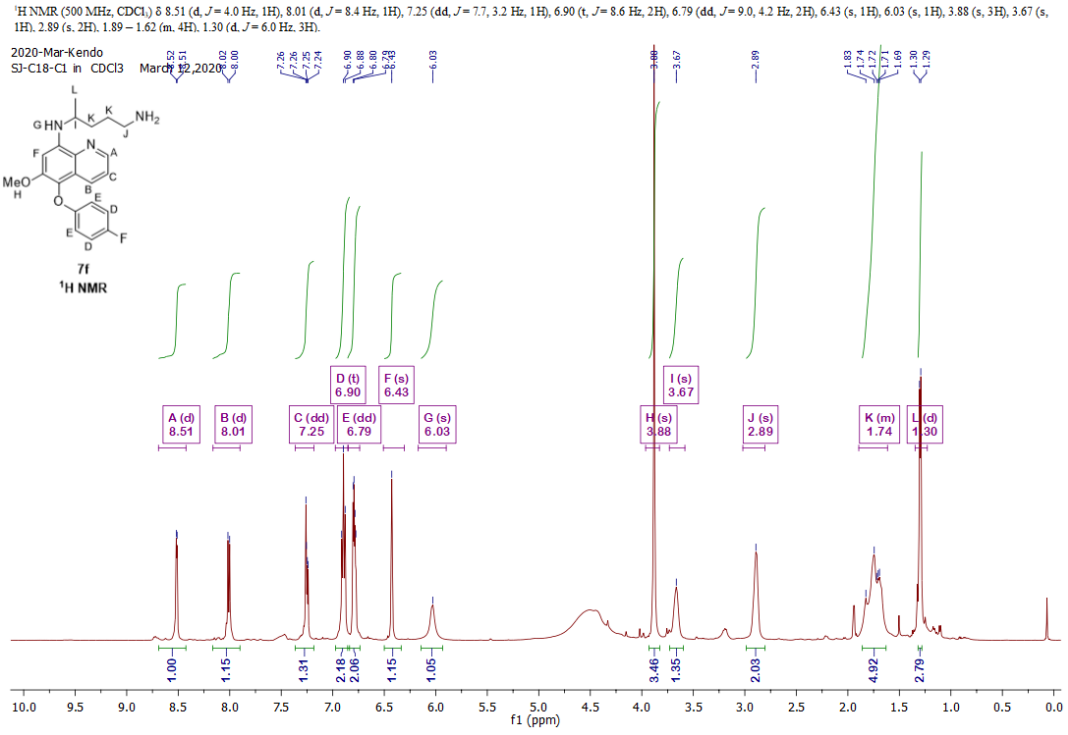

Figure 44 ^{1}H NMR of spectrum of **7f**

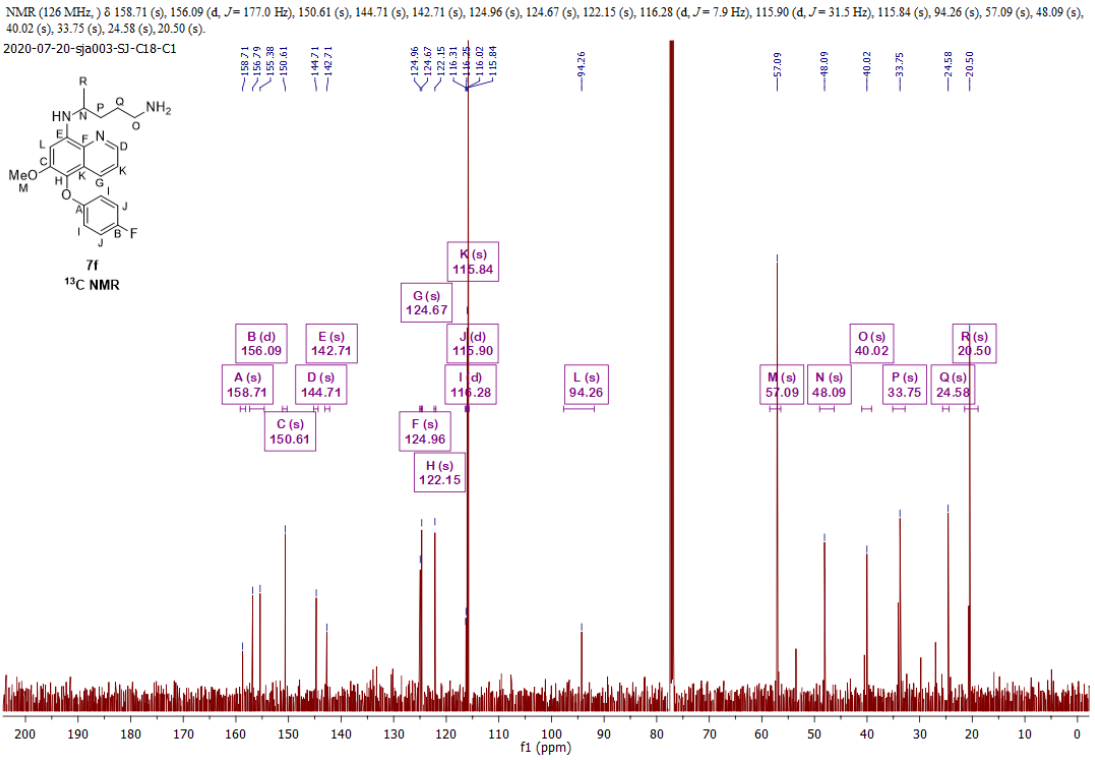

Figure 45 ^{13}C NMR of spectrum of **7f**

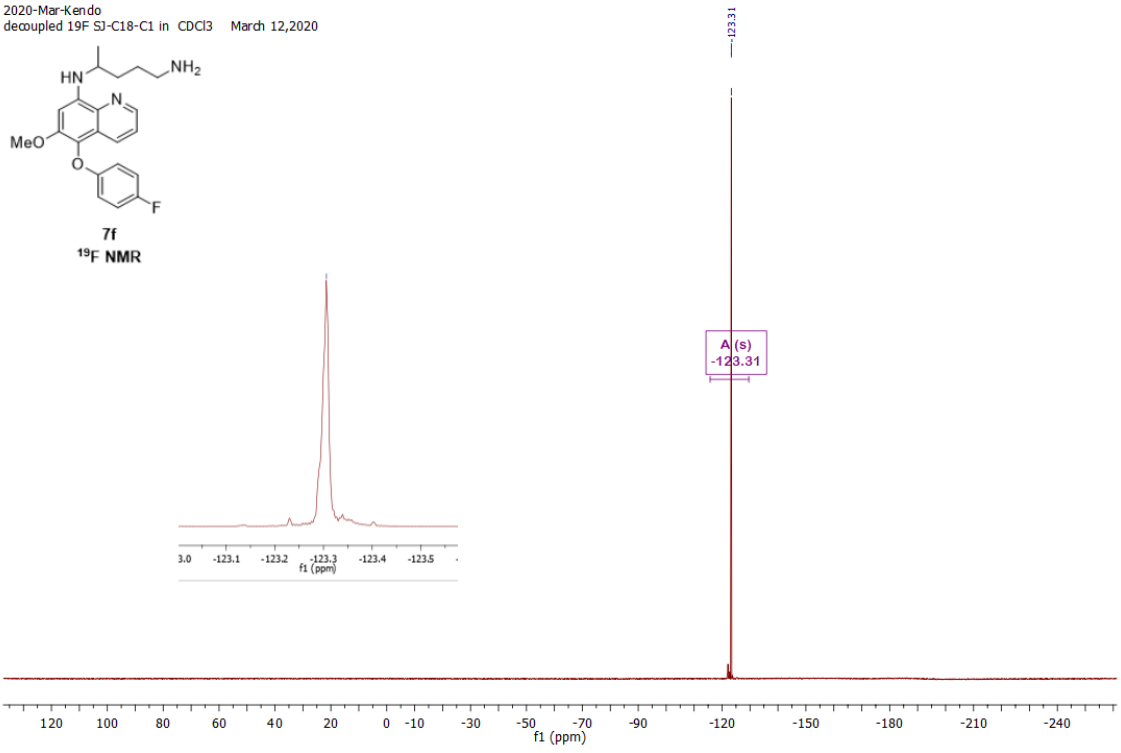

Figure 46 ^{19}F{^{1}H} NMR of spectrum of **7f**

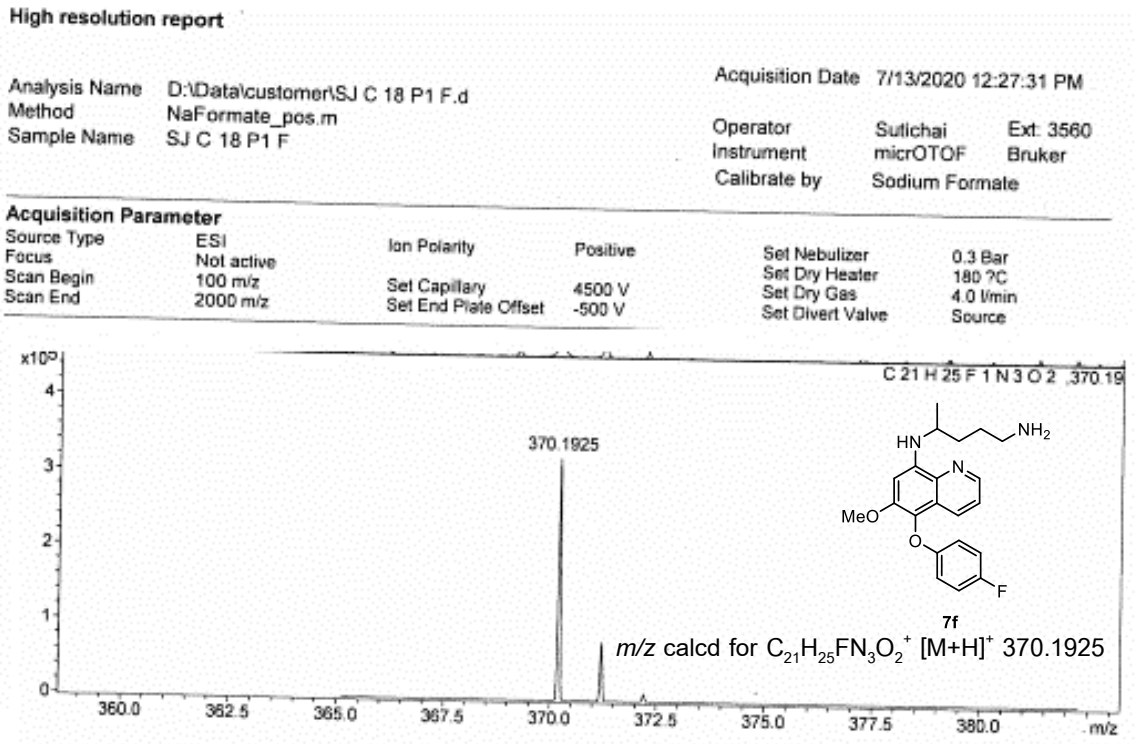

Figure 47 HRMS spectrum of **7f**

N^4-(5-(4-fluorophenoxy)-6-methoxyquinolin-8-yl)pentane-1,4-diamine (7g)

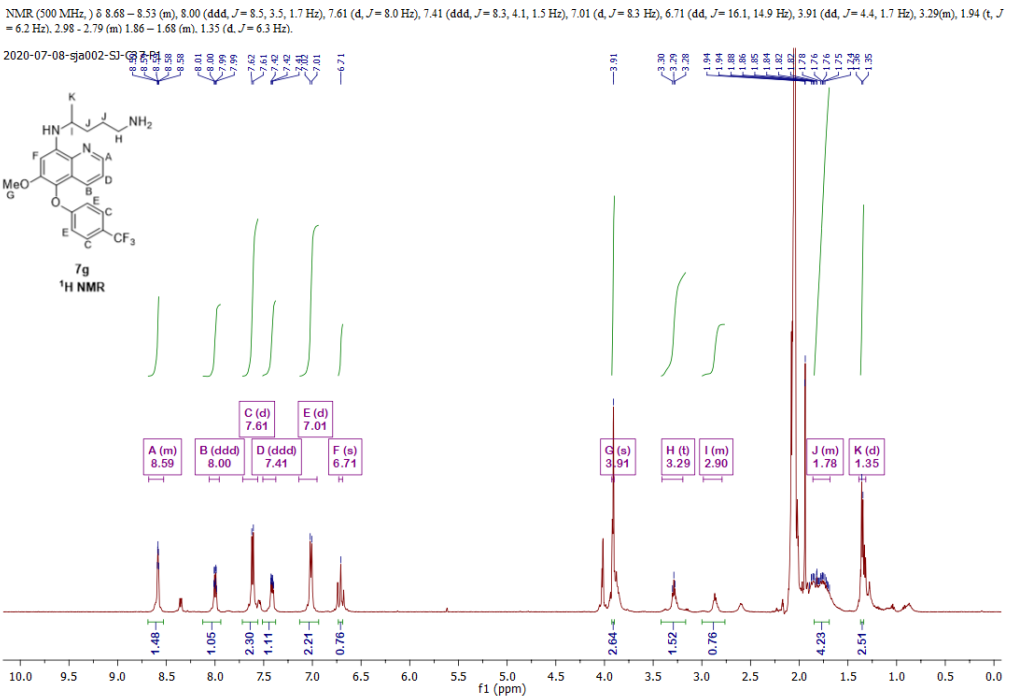

Figure 48 ^{1}H NMR of spectrum of **7g**

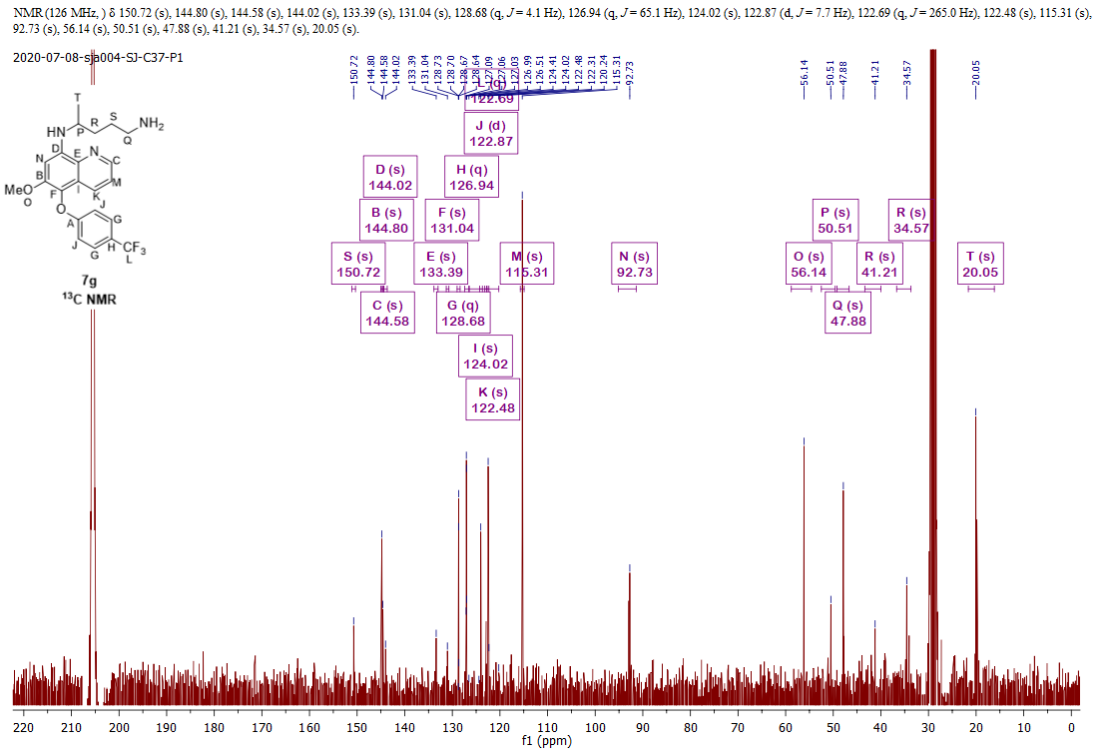

Figure 49 ^{13}C NMR of spectrum of **7g**

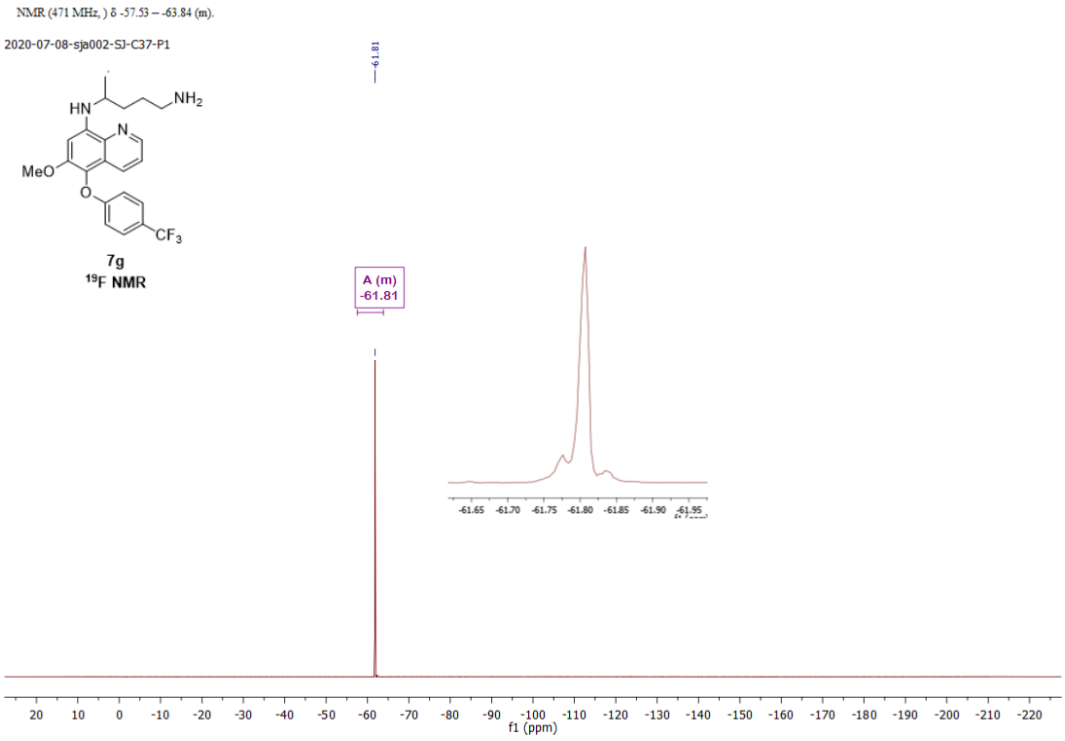

Figure 50 ^{19}F NMR of spectrum of **7g**

High resolution report

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		Calibrate by	Sodium Formate

Acquisition Parameter

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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

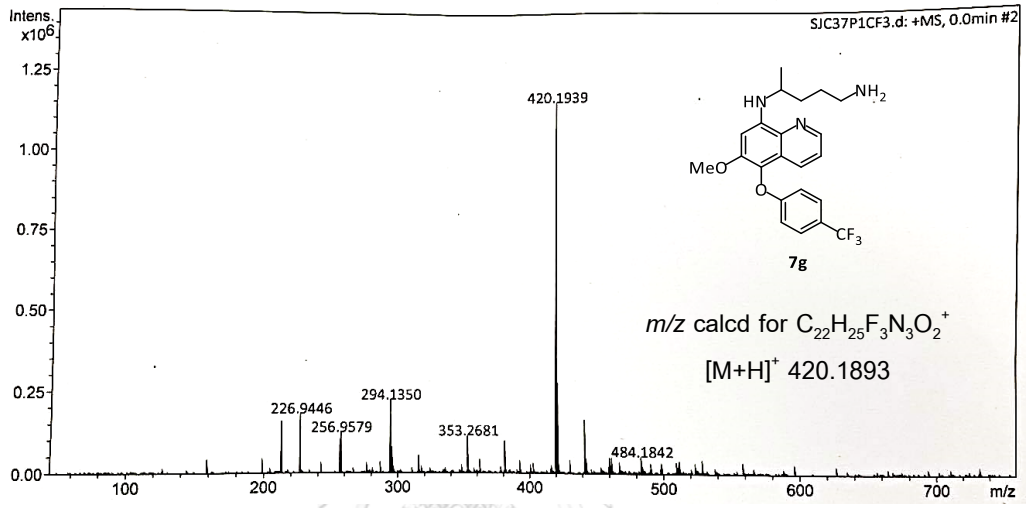

Figure 51 HRMS spectrum of 7g

4-((8-((5-aminopentan-2-yl)amino)-6-methoxyquinolin-5-yl)oxy)benzamide (7h)

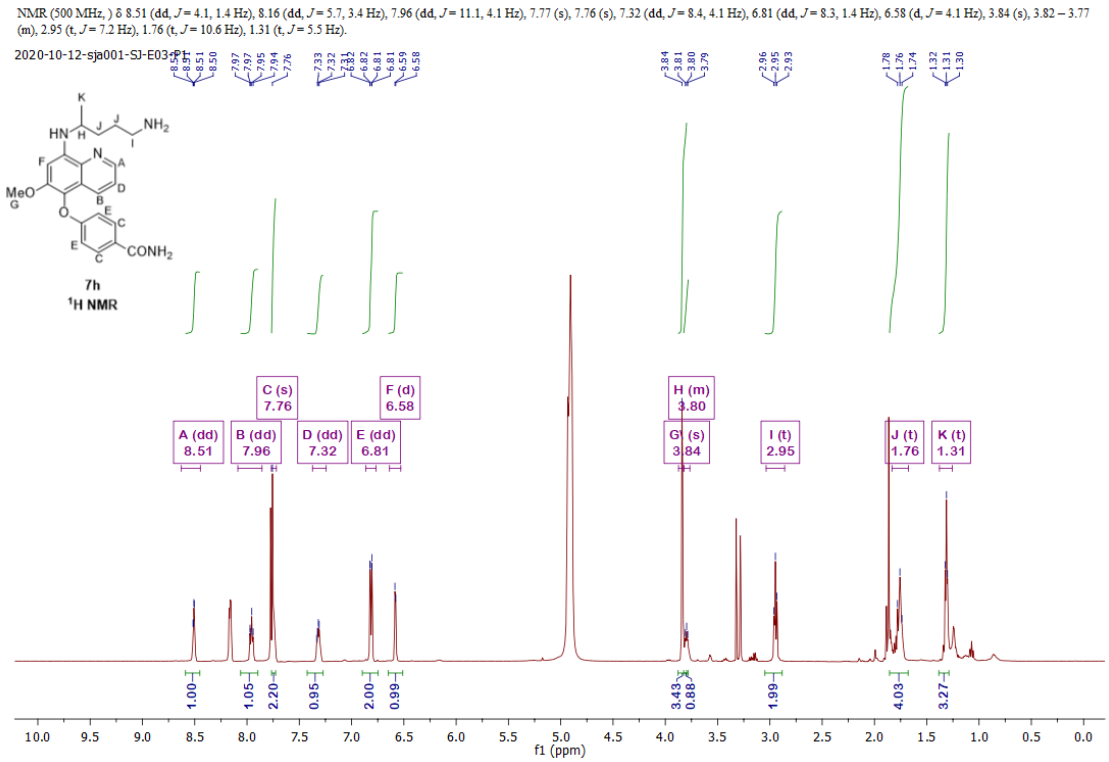

Figure 52 ^{1}H NMR of spectrum of **7h**

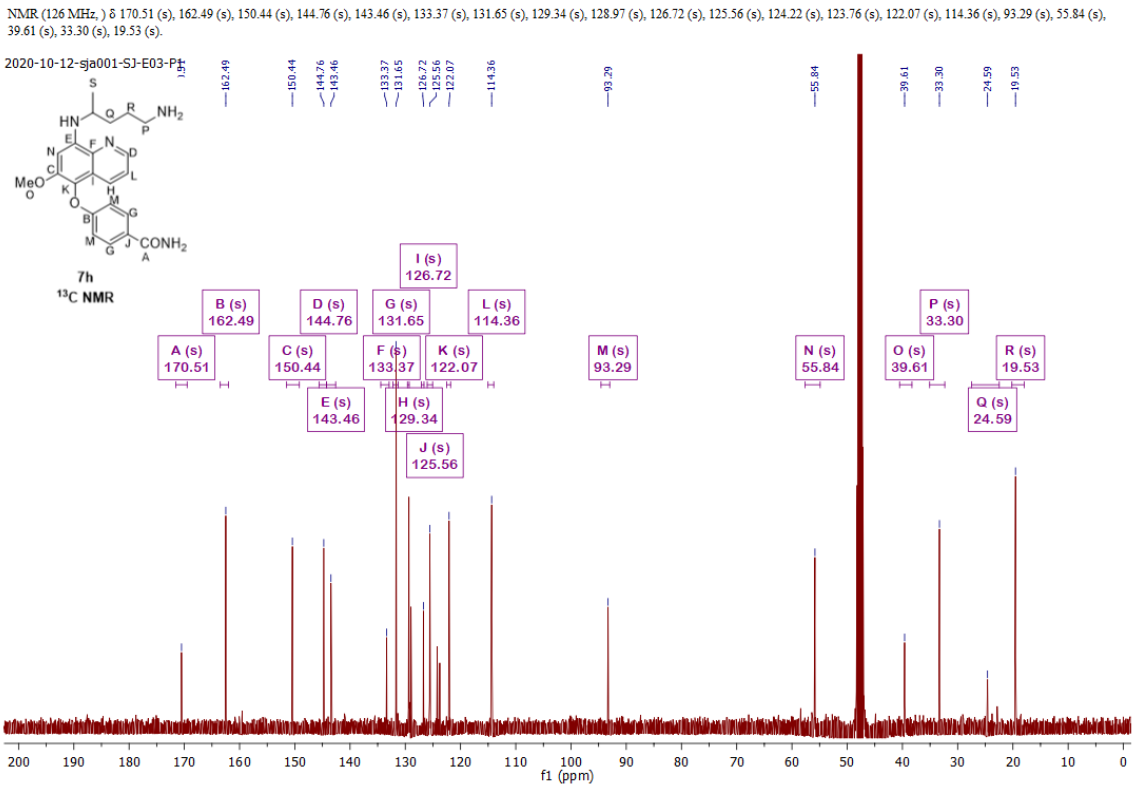

Figure 53 ^{13}C NMR of spectrum of **7h**

Mass Spectrum List Report

Analysis Info

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Sample Name	SJ-E03-P1	Instrument / Ser#	micrOTOF-Q II 10335
Comment			

Acquisition Parameter

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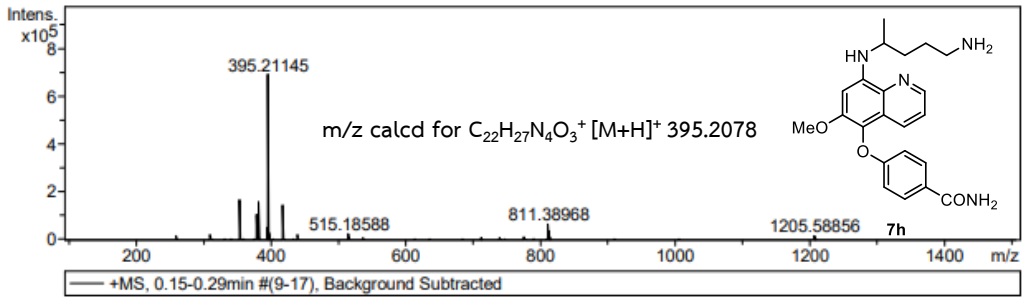

Figure 54 HRMS spectrum of **7h**

Appendix C NMR of 11 and 15

4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)phenol (11)

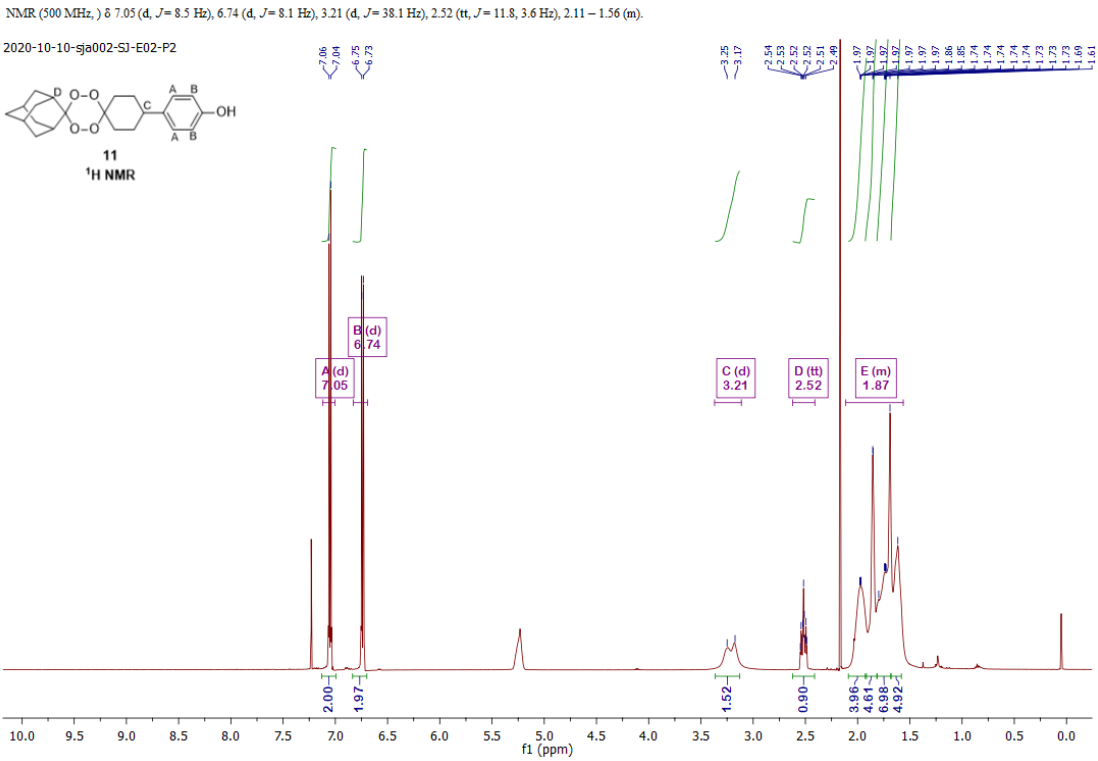

Figure 55 ^{1}H NMR of **11**

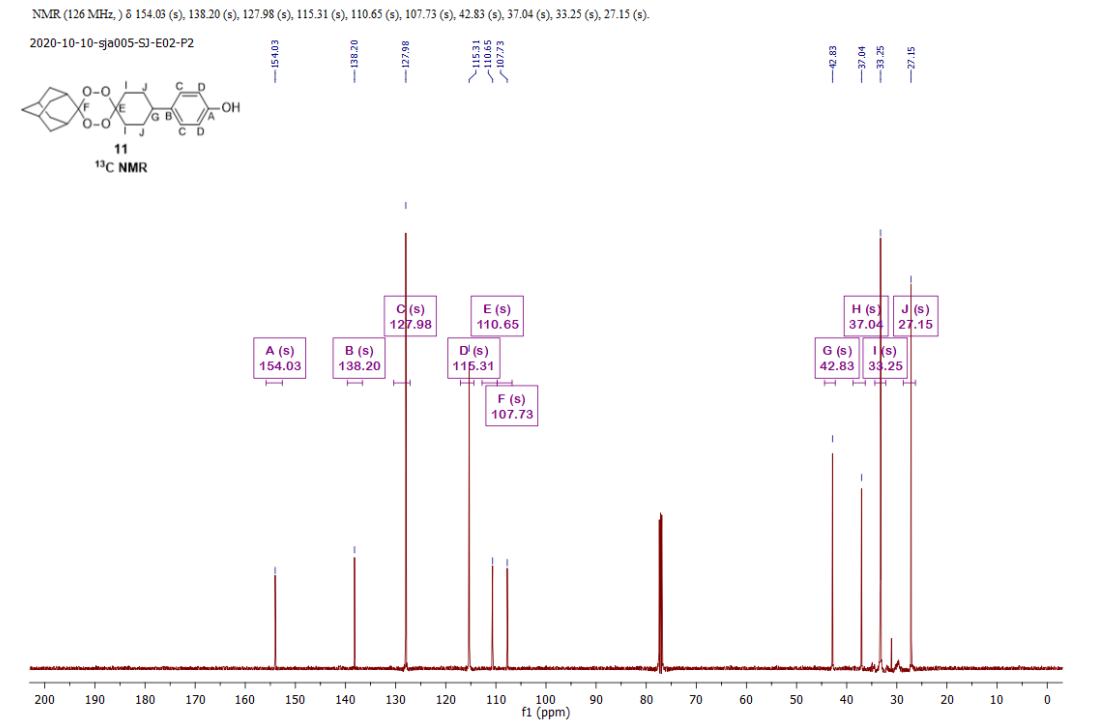

Figure 56 ^{13}C NMR of **11**

N^1-(4-(4-((1*r*,3*r*,5*r*,7*r*)-dispiro[adamantane-2,3'-[1,2,4,5]tetraoxane-6',1''-cyclohexan]-4''-yl) phenoxy)butyl)-N^4-(6-methoxy-5-phenoxyquinolin-8-yl)pentane-1,4-diamine (15)

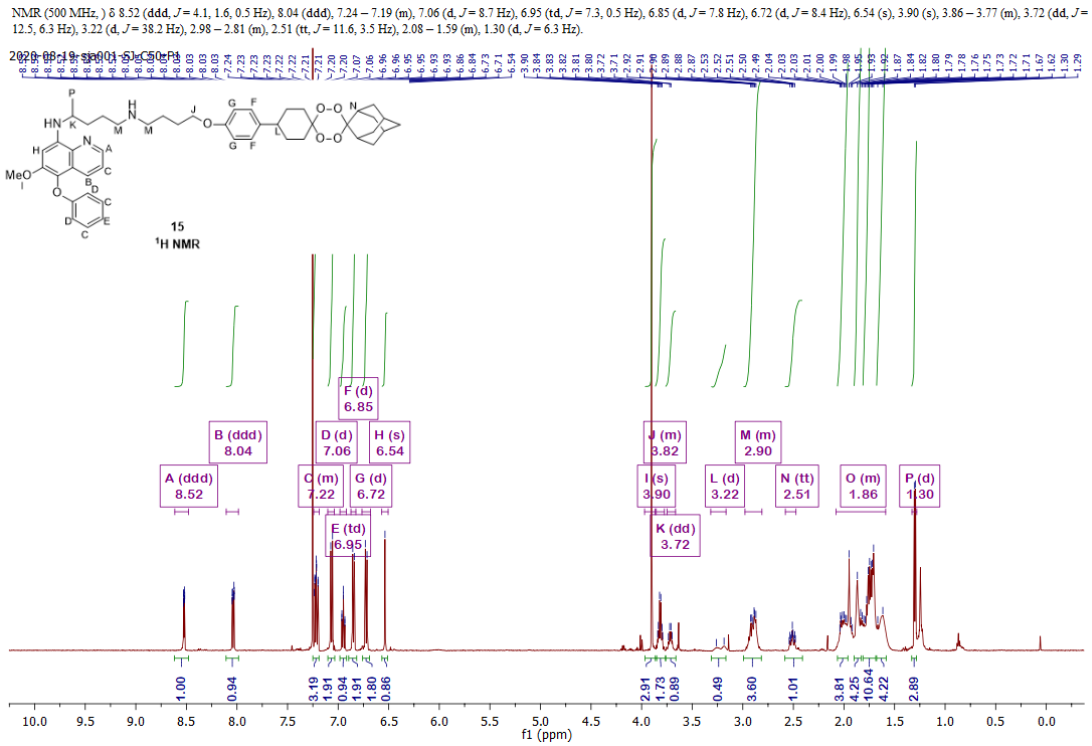

Figure 57 ^{1}H NMR of **15**

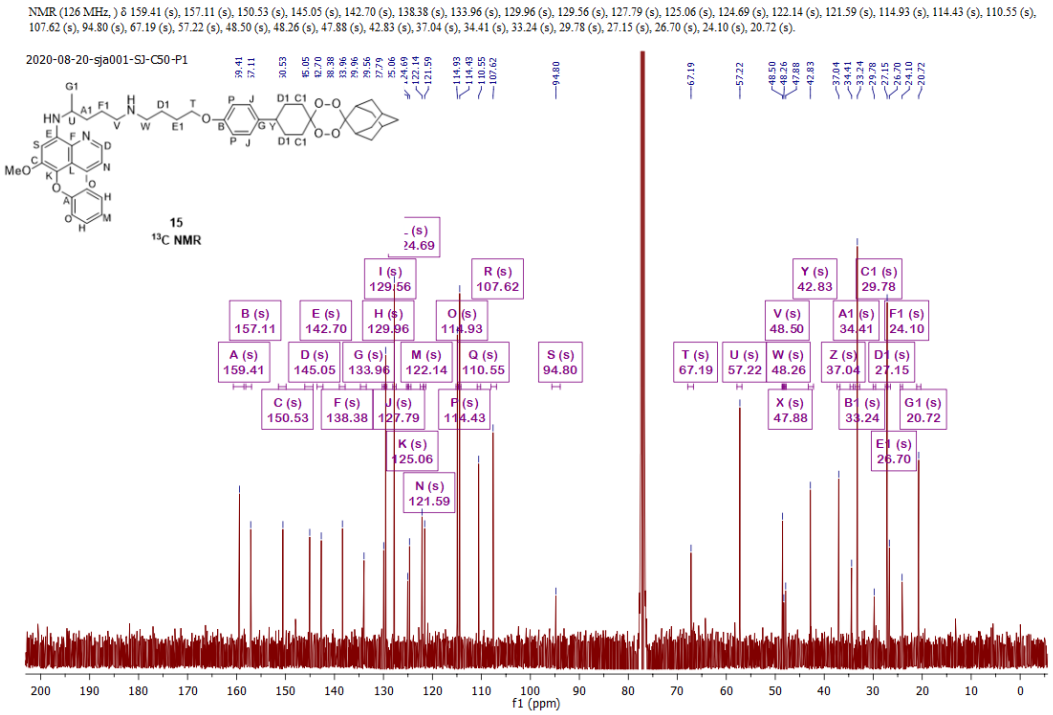

Figure 58 ^{13}C NMR of **15**

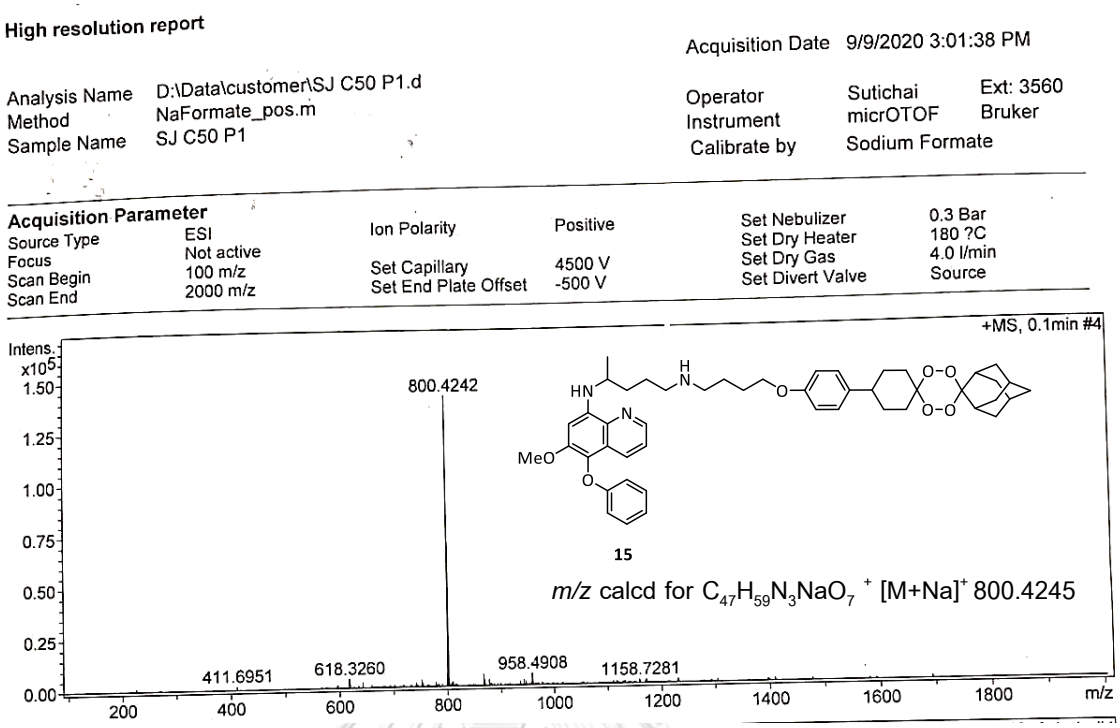

Figure 59 HRMS spectrum of 15

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