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Antimalarial Drug Discovery using Triazoles to Overcome Chloroquine Resistance

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Antimalarial Drug Discovery Using Triazoles to Overcome Chloroquine Resistance

by

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ABSTRACT

Malaria is considered as one of the most prevalent and debilitating diseases affecting humans. *Plasmodium falciparum* is the most virulent form of the parasite which developed resistance to several antimalarial drugs. Chloroquine is one of the most successful antimalarials developed that is safe, effective, and cheap. However, its use has been limited due to the emergence of drug resistance. Click chemistry, particularly, the copper(I)-catalyzed reaction between azides and alkynes has shown to have a cutting-edge advantage in medicinal chemistry by its reliability, selectivity and biocompatibility.

Triazole-based antimalarials were synthesized via copper(I)-catalyzed alkyne-azide cycloaddition reaction by modifying the aliphatic chain terminal of chloroquine. The compounds synthesized contain triazole ring directly connected to an aromatic ring or via a piperazine linker. When tested for their *in vitro* antimalarial activity against D6, Dd2 and 7G8 strains of *P. falciparum*, 12 out of 28 compounds showed better activity against chloroquine resistant strains. Particularly, PL403 and PL448 exhibited potent activity than chloroquine against CQ-resistant strains Dd2 and 7G8, with IC_{50} values of 12.8 & 14.5 nM, and 15.2 & 11 nM respectively.

The efficiency of synthesizing several triazole-based antimalarials have proven click chemistry to be fast and efficient reaction. Generally, para-substitutions and di-substitutions with electron-withdrawing groups were found to be beneficial for having better antimalarial activity for these group of click compounds. Moreover, the incorporation of piperazine linker has brought an enhanced antimalarial activity.

DEDICATION

To my mother, Zaid Araya, for her continuous love,

care and support.

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ABBREVIATIONS

WHO	World health organization
RDT	Rapid diagnostic test
PCR	Polymerase chain reaction
ELISA	Enzyme-linked immunosorbent assay
MalERA	Malaria eradication research agenda
DDT	Dichloro-diphenyl-trichloroethane
IRS	Indoor residual spraying
ITN	Insecticide treated nets
GMM	Genetically modified mosquitoes
SBET	Stand-by-emergency treatment
G6PD	Glucose-6-phosphate dehydrogenase
ACT	Artemisinin-based combination therapy
SERCA	Sarco/endoplasmic reticulum Ca^{2+}-ATPase
SP	Sulfadoxine-pyrimethamine
IV	Intravenous
RCQ	Reversed chloroquines
CQ	Chloroquine
CQR	Chloroquine resistant
CQS	Chloroquine sensitive

PfCRT	*Plasmodium falciparum* chloroquine resistant transporter
PL	Peyton lab
SC	Subcutaneous
GI	Gastro-intestinal
DV	Digestive vacuole
RA	Reversal agent
IC_{50}	Half maximal inhibitory concentration
CC	Click chemistry
CuACC	Copper-catalyzed alkyne azide cycloaddition
DBCO	Dibenzcyclooctyl
ADMET	Absorption, distribution, metabolism, excretion, and toxicity
MDR	Multidrug resistance
DFO	1,8-Diazafluoren-9-one
p-TSA	p-Toluenesulfonic acid
DIPEA	Di-isopropylethylamine

CHAPTER 1: Malaria

1.1. Introduction and Epidemiology

Malaria is a tropical infectious disease considered as one of the most prevalent and debilitating diseases affecting humans.[1] There are 3.4 billion people at risk of malaria transmission in 106 countries and territories. The WHO estimates 198 million cases of malaria and an estimated 584,000 deaths, many of them are children living in sub-Saharan Africa where a child dies every minute.[2] Malaria was eliminated from the United States in the early 1950's. However, 1500 – 2000 malaria cases are reported every year in the US from recent travelers.[3]

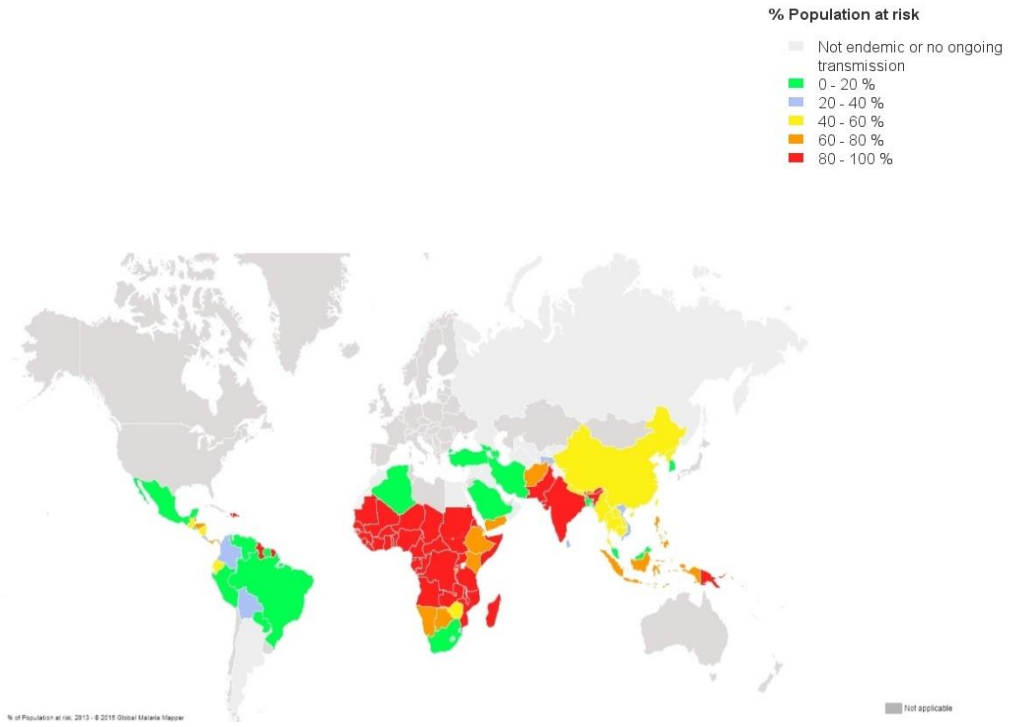


Figure 1.1. % population at risk in 2013[2]

1.2. Cause and Transmission

Malaria is caused by infection with protozoan parasites belonging to the genus *Plasmodium* transmitted by female *Anopheles* mosquitoes.[4] Although over 200 species of *Plasmodium* genus are existing, only five affect humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malarie* and *P. knowlesi*.[5]

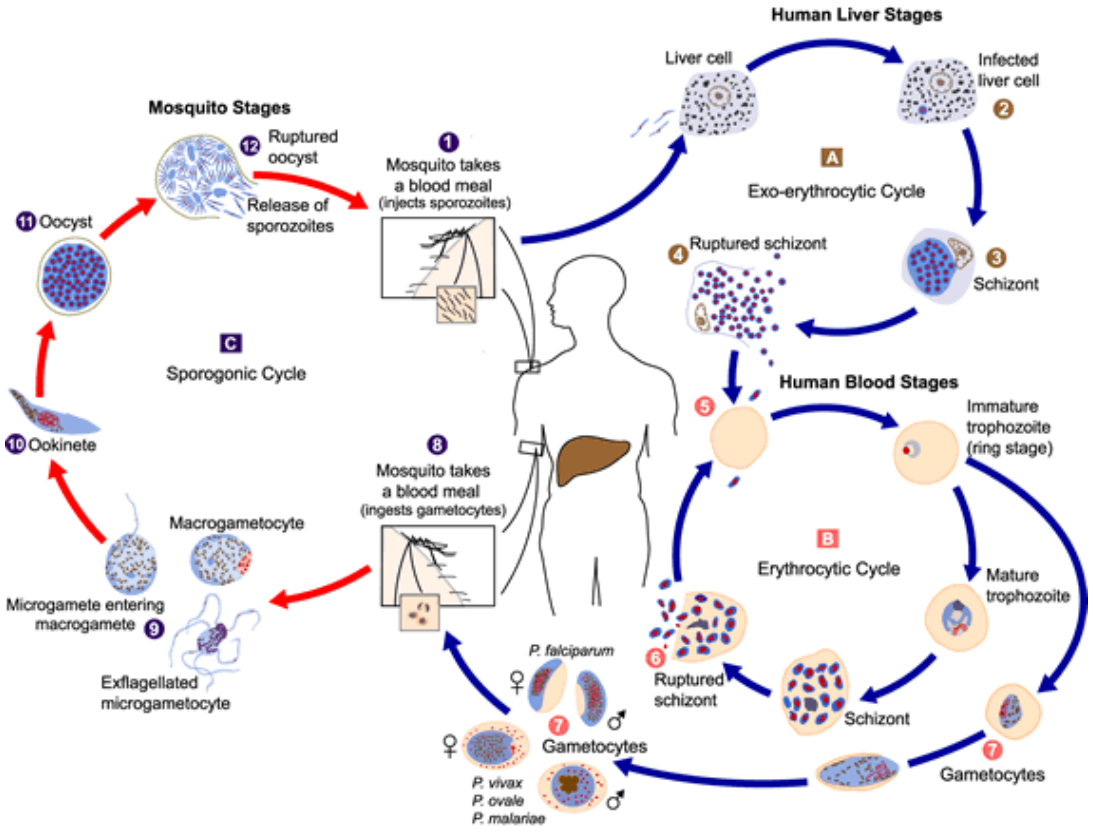


Figure 1.2. Life cycle of *Plasmodia* species[11]

Plasmodium falciparum is the most virulent form which causes potentially fatal cerebral malaria. It's characterized by high levels of parasitaemia and is responsible for about

80% of human malaria worldwide.[1, 5] Relapse is common with *P. vivax* and *ovale* due to the dormant stage (*hypnozoites*) persisting in the liver and invading the blood stream in later years. While *P. vivax* and *P. ovale* selectively infect young cells, *P. malariae* parasitizes older cells.[6] *P. knowlesi* is considered as a parasite of monkeys that can cause fatal infections in humans.[7]

Species	**Clinical features**	**Endemic areas**
Plasmodium falciparum	Tertian non-relapsing malignant malaria	Sub-Saharan Africa, Latin America, South-East Asia
Plasmodium vivax	Tertian relapsing benign malaria	South-East Asia, Latin America, Sub-Saharan Africa
Plasmodium ovale	Tertian relapsing benign malaria	South-East Asia, Pacific, East Africa
Plasmodium malariae	Quartan non-relapsing benign malaria	West Africa, Guyana, India
Plasmodium knowlesi	Quotidian, Severe malaria may occur	South-East Asia

Table 1.1. Clinical features and geographical distribution of *Plasmodia* species[8]

The malaria parasite life cycle involves two hosts: human and mosquito. During a blood meal, a *Plasmodium*-infected female *Anopheles* mosquito inoculates *sporozoites* into the human host. *Sporozoites* infect liver cells and mature into *schizonts*, which rupture and release *merozoites*. At this stage they can also remain dormant as *hypnozoites*.[9] After the initial replication in the liver, the parasites undergo asexual multiplication in the erythrocytes. *Merozoites* infect red blood cells. The ring stage *trophozoites* mature into *schizonts*, which rupture releasing *merozoites*. Some parasites differentiate into sexual erythrocytic stages (*gametocytes*). Blood stage parasites are responsible for the clinical manifestations of the disease.[10]

The *gametocytes*, male and female, are ingested by an *Anopheles* mosquito during a blood meal. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating *zygotes*. The *zygotes* in turn become motile and elongated *ookinetes* which invade mosquito gut where they develop into *oocysts*.[11] The *oocytes* give birth to *sporozoites* in the gut of a female mosquito. These migrate to the mosquito's salivary glands, and are injected into humans. Inoculation of the *sporozoites* into a new human host perpetuates the malaria life cycle.[12]

1.3. Symptoms and Diagnosis

Following an infective bite by female *Anopheles* mosquito, the infected person starts to show symptoms after 1 to 4 weeks. The most common symptoms of malaria include fever, chills, sweating, headaches, fatigue, nausea, and vomiting.[13] In *P. falciparum* malaria, untreated patients present more pernicious manifestations such as cerebral malaria, hypoglycemia, acute renal failure, metabolic acidosis, severe anemia, and pulmonary edema.[14] Severe anemia is a major cause of morbidity and mortality among children in the developing countries.

Microscopy is the basis for diagnosis of malaria providing information about the species and the density of *Plasmodium*. Polymerase chain reaction (PCR) is also used when the detection limits of parasites is below microscopy.[15] Antigen-detecting RDT detects *Plasmodium* antigen by an antibody–antigen reaction on a nitrocellulose strip.[16] Antibody could also be detected by ELISA after exposure to the parasites. Moreover, hemozoin, a

by-product of Plasmodium metabolism, has been used to detect both the disease and the species via the absorbance of laser light at specific frequencies.[17]

1.4. Treatment and Prevention

The Malaria Eradication Research Agenda (malERA) addressed several issues in malaria elimination campaigns. It focuses on integrated research agenda which is essential in making pragmatic decision for shifting from control to elimination.[18] The global malaria control strategy aims to halt the exacerbating situation of malaria by minimizing waste of resources and improving health services.[19]

Insect repellents could provide useful protection against malaria, especially in places where vector mosquitos bite early in the evening.[20] Indoor residual spraying (IRS), mainly through the use of the insecticide DDT (dichloro-diphenyl-trichloroethane) has been an important component of malaria control. Despite attempts to ban DDT completely because of issues such as effect on preterm births and lactation duration, the compound continues to be used in parts of Asia and Africa.[20, 21]

Vector control is also an essential component of prevention.[21] Insecticide-treated nets (ITNs) have consistently shown reductions in overall child mortality and in episodes of clinical malaria.[20] In areas of moderate or high transmission in Africa, deployment of pyrethroid insecticide-treated mosquito nets reduced all-cause mortality by roughly 20% in children younger than 5 years.[21] ITNs are more beneficiary than IRS as they both protect the individual user against biting and kill some of the mosquitoes that try to bite.

Deltamethrin and Permethrin are the most commonly used insecticides constituting about 60 and 22% of the global usage respectively.[22]

The genome of *Anopheles gambiae*, one of the most efficient vectors of malaria, has now been sequenced providing candidate genes in generating Genetically Modified Mosquitoes (GMM).[24] This 20-year old program uses site-specific gene recombination technologies to insert the antipathogen effector genes in the integration sites of the genome to develop *A. gambie* that are fully refractory to *plasmodium*.[22]

Drug chemoprophylaxis has also proved to be one of the most effective preventive strategy especially for travellers to malaria endemic areas. It is essential to emphasize that no chemoprophylactic regimen gives complete protection and must always be accompanied by primary protection such as ITN and insect repellants. The prophylaxis drug should be continued to be taken for at least 4 weeks after the last possible exposure to take into account for the pre-erythrocytic phase of the plasmodial cycle.[8]

Common antimalarials used as chemoprophylactic agents include chloroquine, proguanil, mefloquine, doxycycline, primaquine and tefenoquine. Proguanil in combination with chloroquine and atovaquone (Malorone) is also administered. Some antimalarial drugs that have well established therapeutic roles have no rational use as malaria chemotherapeutic agents (Table 2). Although chloroquine is less useful as chemoprophylaxis in most places, it is still used in Central America and Hispaniola. In the US, mefloquine (1989), doxycycline (1992) and atovaquone/proguanil (2001) are the three currently approved drugs for malaria prevention.[8, 25]

Antimalarial drug	Severe adverse event or contraindication observed
Amodiaquine	agranulocytosis and hepatotoxicity
Halofantrine	cardiac dysrhythmias
Pyrimethamine-dapsone	Agranulocytosis
Pyrimethamine-sulfadoxine	severe skin reactions (e.g. Stevens-Johnson syndrome)
Quinine	long-term use predisposes to blackwater fever
qinghaosu derivatives (i.e. artesunate)	severe CNS lesions in animal studies in long-term use of these short-acting drugs

Table 1.2. Medications that should not be used for malaria chemoprophylaxis

The WHO recommends occupational and long-term travellers to carry Stand-By Emergency Treatment (SBET) drugs for self treatment when medical care is not accessible within 24 hours from onset of exposure and symptoms. Combination drugs used for uncomplicated *P. falciparum* malaria such as artemether/lumefantrine, atovaquone-proguanil, quinine plus doxycycline or clindamycin can be used as SBET drugs.[8]

Almost two decades have been spent on development of malaria vaccines. So far, most efforts have been put in developing pre-erythrocytic stage vaccines designed to destroy infected hepatocytes or prevent their invasion by sporozoites.[20] RTS,S/AS01 is a hybrid molecule in which the circumsporozoite protein of *P. falciparum* is expressed with hepatitis-B surface antigen (HBsAg) in yeast and boosted with the potent ASO adjuvant.[21]

In coincidence with World Malaria Day 2015, the RTS,S Clinical Trials Partnership reported final results of a Phase 3 trial of the RTS,S/AS01 vaccine which were conducted in seven countries in Africa. After 38 and 48 months, the results indicated 25.9 and

36.3% protection in children at 6-12 weeks and 5-7 months respectively when 3 doses were given at 0, 1, 2 with a booster at 20 months or a control vaccine.[26]

Moreover, various medicinal plants are reported to possess insect-repellent activity against mosquitoes including *Azadirachta indica, Lantana camara, Vitex negundo, Mentha piperita, Tagetes minuta, Eucalyptus* and *Cymbopogon species*.[23] In some definite epidemiological situations, environmental management activies comprising drainage of mosquitoes breeding sites, house design improvements and utilising larvivorous fish have proved to be effective.[20]

1.5. Antimalarial Chemotherapy

When malaria prevention fails and a person is infected by the parasite, several treatment options are available. The treatment depends on the type the *Plasmodium* species, the stage of the parasite, the mechanism of action and the half life of the drug.

Quinine was the first natural antimalarial extracted from the bark of cinchona tree in Peru around the 17th century. It was the pioneer drug and has been used for more than two centuries until its resistance emerged.[27] In addition, the common side effect symptoms of cinchonism associated with it decreased its use. Because of resistance, quinine is taken in combination with secondary agents such as doxycycline, clindamycin or SP. Although its sterioisomer, quinidine, could be given IV for *P. falciparum* malaria, it is basically indicated as anti-arrhythmic.[29]

The 4-aminoquinolines are an important class of antimalarials which comprises of chloroquine, amodiaquine and piperaquine.[27] Chloroquine is one of the classic drugs

which became a mainstay for malaria treatment in the mid-20th century. Chloroquine and amodiaquine have blood schizontocidal effects against asexual stages of the parasite in blood.[28] Despite the reported cases of hepatotoxicity and agranulocytosis for amodiaquine, the lack of new drugs for drug-resistant malaria made it attractive.[29]

Another important class are the 4-methanoquinolines including mefloquine, halofantrine and lumifantrine.[27] In addition to neuropsychiatric side effects, drug resistance in southeast asia has limited the use of mefloquine. Halofantrine is another aminoalcohol effective for treating chloroquine-resistant malaria, but cardiotoxicity is associated with its use. Lumefantrine is a racemic fluorene derivative which is only available in coformulation with artemether.[29]

Primaquine, pamaquine and bulaquine belong to a class of 8-aminoquinolines.[27] Primaquine is one of the few drugs effective against both *gametocytes* of *P. falciparum* and *hypnozites* of *P. vivax* and *P. ovale*. However, its short half-life (6 hrs) makes it contraindicated in patients with G6PD deficiency, because it has to be taken for two weeks to overcome relapse in *P. ovale* and *vivax* infections.[28]

Artemisinin belongs to a class of sesquiterterpene lactones, extracted from a sweet woodworm *Artemisia annua*.[29] Artemisinin derivatives such as artemether, artesunate and arteether have been used recently in China and SE Asia for the management of malaria. Their high gametocidal effect and low resistance profile made them the mainstay combination therapy.[28] Unfortunately artemisinin can not be used alone due to its short half-life, and hence the WHO highly recommends administration of ACTs. It has been

suggested that these endoperoxides act by damaging Sarco-Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA).[29]

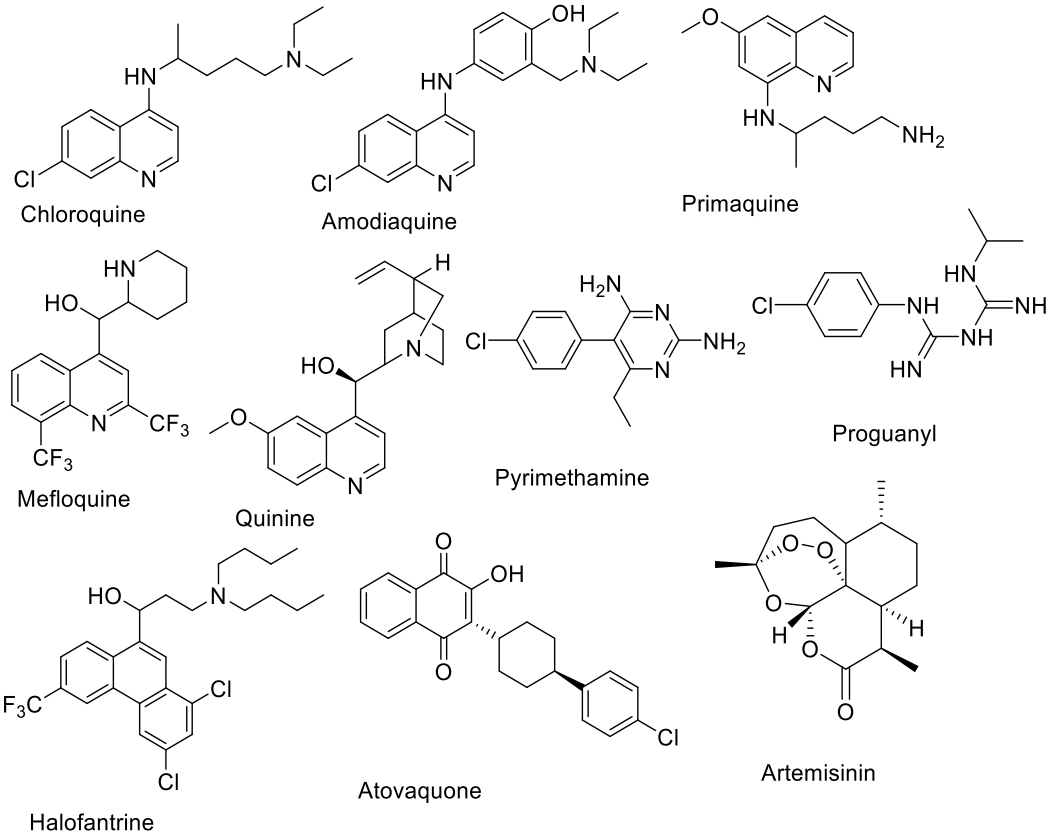


Figure 1.3. Currently available antimalarial drugs

Atovaquone is a hydroxy-naphthoquinone which acts by collapsing mitochondrial membrane potential of the parasite.[27] It's usually used for treatment and prevention of chloroquine-resistant *P. falciparum* in combination with proguanil (Malarone). Proguanil is a biguanide antifolate that synergistically acts by reducing the concentration of atovaquone needed.[29]

Sulfadoxime and pyrimethamine are antifolate inhibitors which inhibit DNA synthesis of the parasite by specifically targeting *dihydropteroate synthase* and *dihydrofolate*

reductase enzymes. SP (Fansidar) is very effective when administered in combination with amodiaquine.[29] Dapsone is another sulfone drug used with chlorproguanil (lapdap), although withdrawn due to its hemolytic toxicity. Antibiotics are also recommended for use in quinoline-resistant strains. Tetracyclines (tetracycline & doxycycline) and macrolides (clindamycin & azithromycin) act by inhibiting RNA synthesis. They are very beneficial in combination therapy with quinine.[31]

Research and development of new and recycled antimalarial drugs is still in progress, and many drugs are in their preclinical and clinical phases. 4-aminoquinoline drugs such as AQ-13 and N-tert-butyl-isoquine (GSK369796) are analogues of chloroquine and amodiaquine which are in Phase II clinical and Preclinical trials respectively. AQ-13 is being studied for uncomplicated *P. falciparum* malaria but there is a concern of cross resistance with chloroquine.[33] N-tert-butyl-isoquine is highly effective and possesses better overall preclinical safety profile than chloroquine or amodiaquine.[34]

Tefenoquine and NPC-1161B are 8-aminoquinolines under inverstigation for the treatment of the hypnozoite stages of *P. vivax* and *P. ovale* that are responsible for relapse of these malaria species. Tefenoquine has an advantage over primaquine in that it is long acting (2-3 weeks) for treating liver stages of *P. vivax*. Tafenoquine is currently in late stage development as an anti-malarial prophylactic agent, and NPC-1161B is in late preclinical development.[35]

The disadvantages of semisynthetic artemisinin derivatives are their short half-lives, dependence on plant source for starting material, and alarming reports of ACT treatment failures which call for a need for new analogues to this class of antimalarials. OZ277

(Arterolane) was the first synthetic ozonide to be evaluated clinically and is now launched in India in combination with piperaquine, and has obtained regulatory approval to introduce in seven African countries.

OZ439 is another improved synthetic ozonide candidate designed to provide a single-dose oral cure in humans. It has successfully completed Phase I clinical trials and is currently undergoing Phase IIb trials in malaria patients in combination with piperaquine. When compared to OZ277 and other former endoperoxides, OZ439 is active against all asexual erythrocytic stages of *P. falciparum* and exhibits a substantial increase in the pharmacokinetic half-life.[36]

Mutations on *dihydrofolate reductase* enzyme compromised the efficacy of antifolate drugs. Research in developing new antifolates such as P65, P218, and WR99210 is in-advance. P218 has proven to potently inhibit both wild-type and clinically relevant mutated forms of *Plasmodium falciparum*. Its suitable pharmacologic and safety profile made it to be an oral antimalarial candidate among this class.[37] KAE609 (Cipargamin) is a first-in-class agent with a new mechanism of action by inhibiting pfATP4 (Ca^{2+}-ATPase). This spiroindolone showed a rapid antimalarial activity in phase II clinical trial for the treatment of uncomplicated *P. falciparum* and *vivax* malaria.[38]

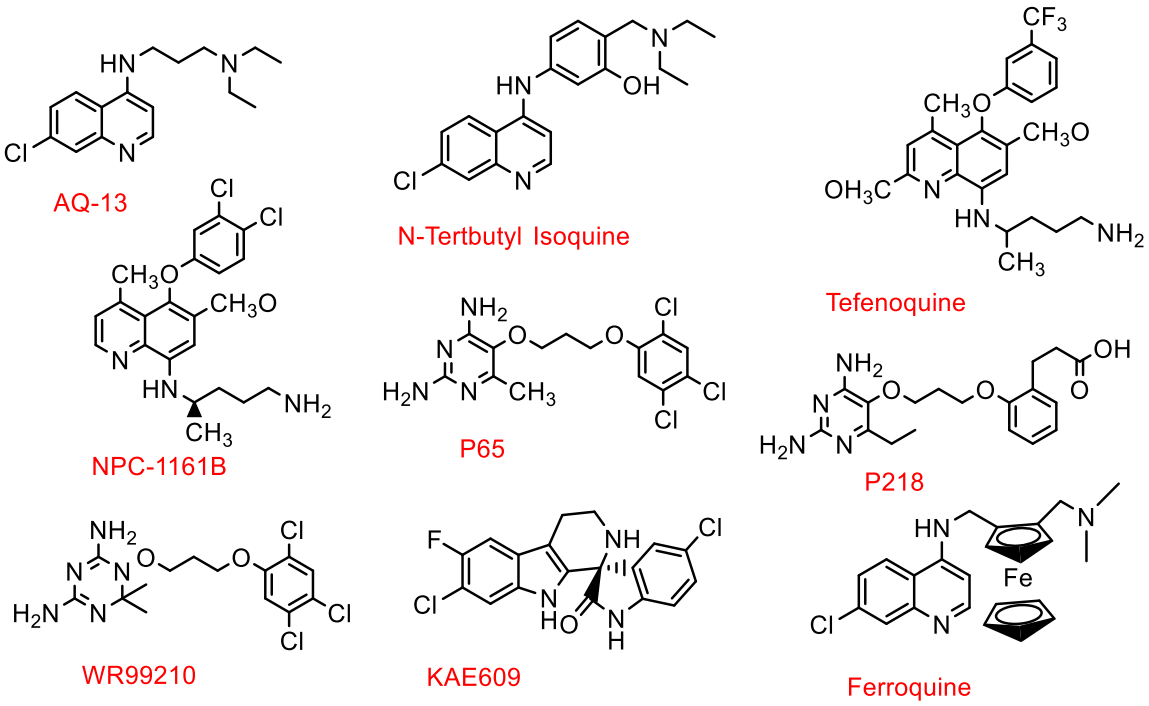


Figure 1.4. Antimalarial drug candidates in clinical development

1.6. Resistance to Antimalarial Drugs

The *plasmodium* parasite developed acquired resistance to antimalarial drugs. The type of the resistance varies with the species, the strain, and the drug used. Many factors account for the development and spread of resistance including drug-use patterns, characteristics of the drug, host factors, parasite characteristics, vector, and environmental factors.[39]

Drug resistance to several novel antimalarial drugs has been reported when used as monotherapy and combination drugs. *Plasmodium falciparum* is the main concern and cause of resistance among the five species. Chloroquine resistance to *P. vivax* is largely concentrated in Indonesia and Papua New Guinea. Chloroquine retained adequate efficacy against *P. ovale* and *P. malariae*, but delayed parasite clearance times is

observed.[40] There are recent reports of treatment failure and clinical resistance to artemisinin-based therapy in western Cambodia.[41]

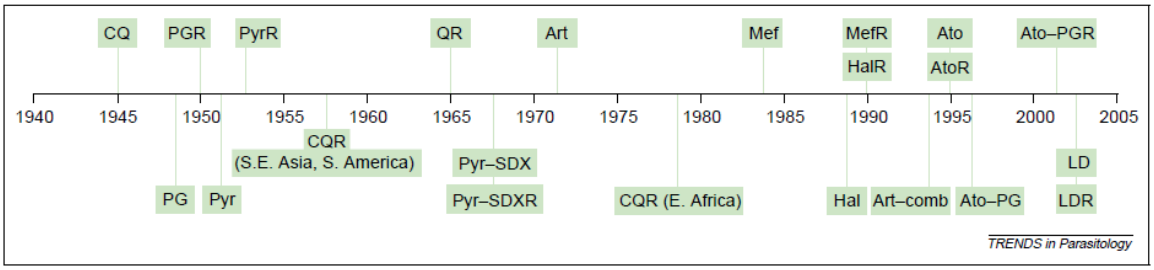


Figure 1.5. Approximate dates of introduction and treatment failure of antimalarial drugs [Abbreviations: Chloroquine (CQ), Atovaquine-Proguanil (PG), Pyrimethamine (Pyr), Quinine (Q), Sulfadoxine (SDX), Artimisinin and derivatives (Art), Mefloquine (Mef), Halofantrine (Hal), Atovaquone (Ato) , LapDap: Chlorproguanil–Dapsone (LD), Resistance (R), Combinations (Comb)]. [42]

Genetic mutations in *P. falciparum* strains have been linked with phenotypes of clinical resistance and putative markers have emerged and reported.[39]

Gene	**Product**	**Genetic Determinant**	**Drug**
pfcrt	Transporter	Thr76	Chloroquine
pfmdr 1	Transporter	Tyr86	Chloroquine, mefloquine, quinine, dihydroartemisinin, artesunate
dhps	Dihydropteroate Synthetase	Gly437, Glu540, Gly581	Sulfadoxine
dhfr	Dihydrofolate Reductase	Asn108, Arg59, Ile51, Leu164	Pyrimethamine
cytb	Cytochrome b	Ser268	Atovaquone

Table 1.3. Mutations in *P. falciparum* associated with resistance to antimalarial drugs[39]

In order to counter drug resistance and delay the emergence of more resistance, many combination therapies have been adapted. The drugs to be used in combined therapies might require to have separate mechanisms of action against same stage of the parasite. Artemisinin derivatives along with other antimalarial classes present effort towards

achieving such therapeutic level.[39] Current combination regimens recommended by the WHO include artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine, and artesunate-SP.[31]

1.7.Reversed Chloroquines

Chloroquine (Resochin) was first synthesized in 1934 by a German chemist, Hans Andersag, by modifying mepacrine, replacing the acridine ring with a quinoline ring.[43] Chloroquine is a weak base containing a side-chain diethylamine nitrogen (pKa = 10.2) and a quinoline ring nitrogen (pKa = 8.1). It is administered as a racemic mixture of two enantiomers of which the (+)-CQ enantiomer is more potent against CQ-resistant strains of *P. falciparum* than the (-)-chloroquine enantiomer.[45] Chloroquine is indicated for treatment and prevention of acute uncomplicated malaria.[47]

Chloroquine could be administered in oral, subcutaneous, intramuscular and rectal routes. When taken orally, CQ is rapidly absorbed from GI tract with bioavailability of 75-80 %.[45] It has an onset of action of 1-2 hrs and half-life of 3-6 days.[45] CQ is metabolized in liver by CYP3A, CYP2C8, and CYP2D6 enzymes to mono-desethyl CQ (30-40%) and bisdesethyl CQ (5-10%).[45] The CQ side chain is further dealkylated to form 7-chloro-4-aminoquinoline. About 70% of the ingested drug is excreted unchanged in urine.[45]

CQ targets the biochemical pathway in the acidic digestive vacoule (DV) of *P. falciparum*. Heme released during metabolism of hemoglobin biocrystallizes to inert hemozoin by the *Plasmodium* parasite. CQ accumulates in the DV and blocks heme

biocrystallization to hemozoin by forming cytotoxic CQ–heme complexes and other mechanisms that kill the parasites by making the cell-membrane permeable.[46]

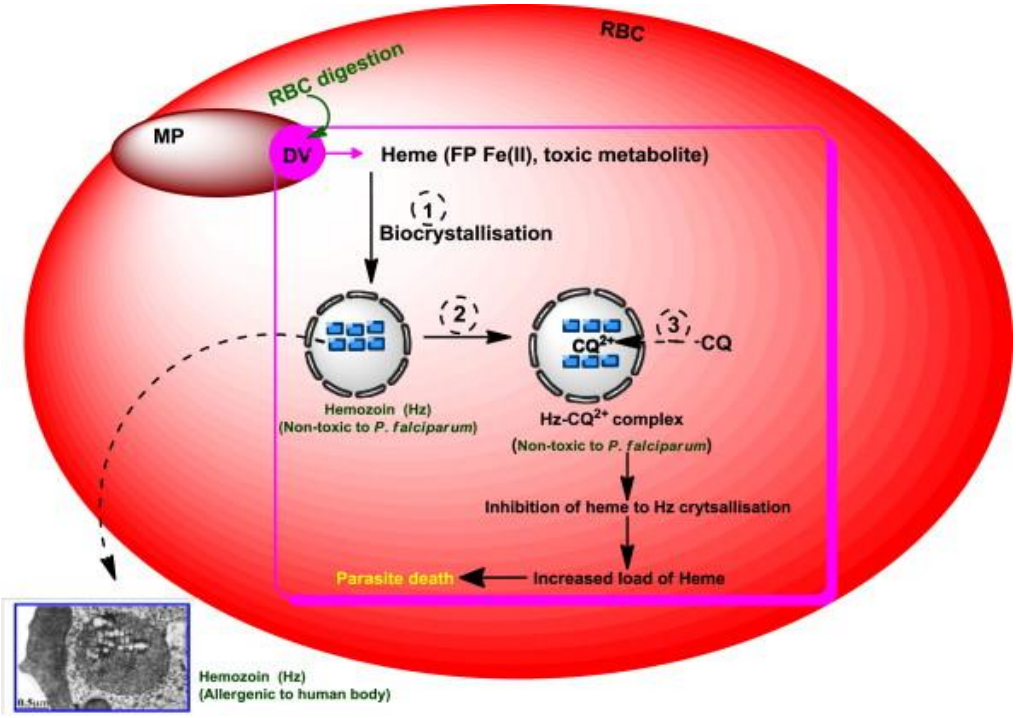


Figure 1.6. Mechanism of action of CQ and electron micrograph of the DV[48]

In the DV of *Plasmodium falciparum* (pH = 5.5), CQ is trapped at high concentration in its doubly protonated and membrane-impermeable form.[47] The CQ-resistant strain accumulates 4-10 times less CQ than CQ-sensitive strains of *P. falciparum*, thus the intracellular CQ concentration will be less toxic to the parasite.[46]

Chloroquine is effective, cheap, and safe to children and pregnant women. It has been one of the most successful antimalarials ever developed and used as the most reliable firstline prophylaxis for 40 years. The first cases of resistance were reported from South America in 1961. The resistance to CQ is linked to mutation in the DV membrane protein

of *Plasmodium falciparum* chloroquine resistant transporter (PfCRT).[44] The change from lysine (K) to threonine (T) in the transmembrane domain of PfCRT appears to be required for the resistance.[49]

Parasite type and origin	**PfCRT position and encoded aminoacid**									
	72	**74**	**75**	**76**	**97**	**220**	**271**	**326**	**356**	**371**
Chloroquine sensitive										
Wild type	C	M	N	K	H	A	Q	N	I	R
106/1 (reverant)	C	I	E	K	H	S	E	S	I	I
Chloroquine resistant										
Southeast Asia & Africa, type E1a	C	I	E	T	H	S	E	S	T	I
Southeast Asia & Africa, type E1b	C	I	E	T	H	S	E	S	I	I
Papua New Guinea, type P1	S	M	N	T	H	S	Q	D	L	R
South America, type W1a	S	M	N	T	H	S	Q	D	L	R
South America, type W1b	C	M	N	T	H	S	Q	D	L	R
South America, type W2	C	M	E	T	Q	S	Q	N	I	T

Table 1.4. Mutant forms of PfCRT and association of the K76T marker with chloroquine resistant *P. falciparum* from different geographic regions[50]

Several reversal agents or chemosentisezers that reverse the resistance by inhibiting the efflux of chloroquine via membrane transporters of the DV of *Plasmodium falciparum* resistant strain have been reported. Verapamil and two other calcium channel blockers, vinblastine and daunomycin, are among the first drugs reported that slowed the release and increased the accumulation of chloroquine by resistant *P. falciparum*.[52] Subsequently the reversal effect of tricyclic antidepressant, desipramine, and selective serotonin re-uptake inhibitor (SSRI), fluoxetine, were reported.[53, 54] Fluoxetine showed an increased reversal activity than verapamil when tested against CQ-resistant W2 clone of *P. falciparum*.[54]

Antihistamines such as chlorpheniramine (racemic) and promethazine also showed reversal of chloroquine resistance *in vitro* against CQ-resistant *P. falciparum*.[55,56]

Combination of chemosentisizers had also displayed an additive effect in potentiating CQ accumulation in the DV of the resistant parasite.[57] The antimalarial drug, primaquine, also has a synergic activity with chloroquine and it blocks PfCRT.[58] It was implicated by recent study that the mutant PfCRT uses reversal agents such as verapamil, chlorpheniramine, desipramine, prometazine, fluoxetine and primaquine as substrates.[59]

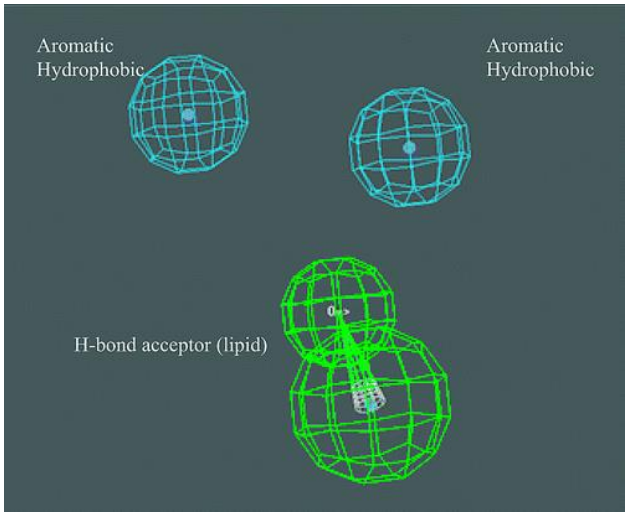


Figure 1.7. Pharmacophore of chloroquine reversal resistance[60]

Bhattacharjee et al. have developed a pharmacophore for reversal agents.[60] The pharmacophore has a general structure of two aromatic hydrophobic groups and a hydrogen bond acceptor, preferably at a side chain nitrogen atom. The class of drugs which qualify for this pharmacophore include calcium channel blockers, antihistamines, tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRI's).

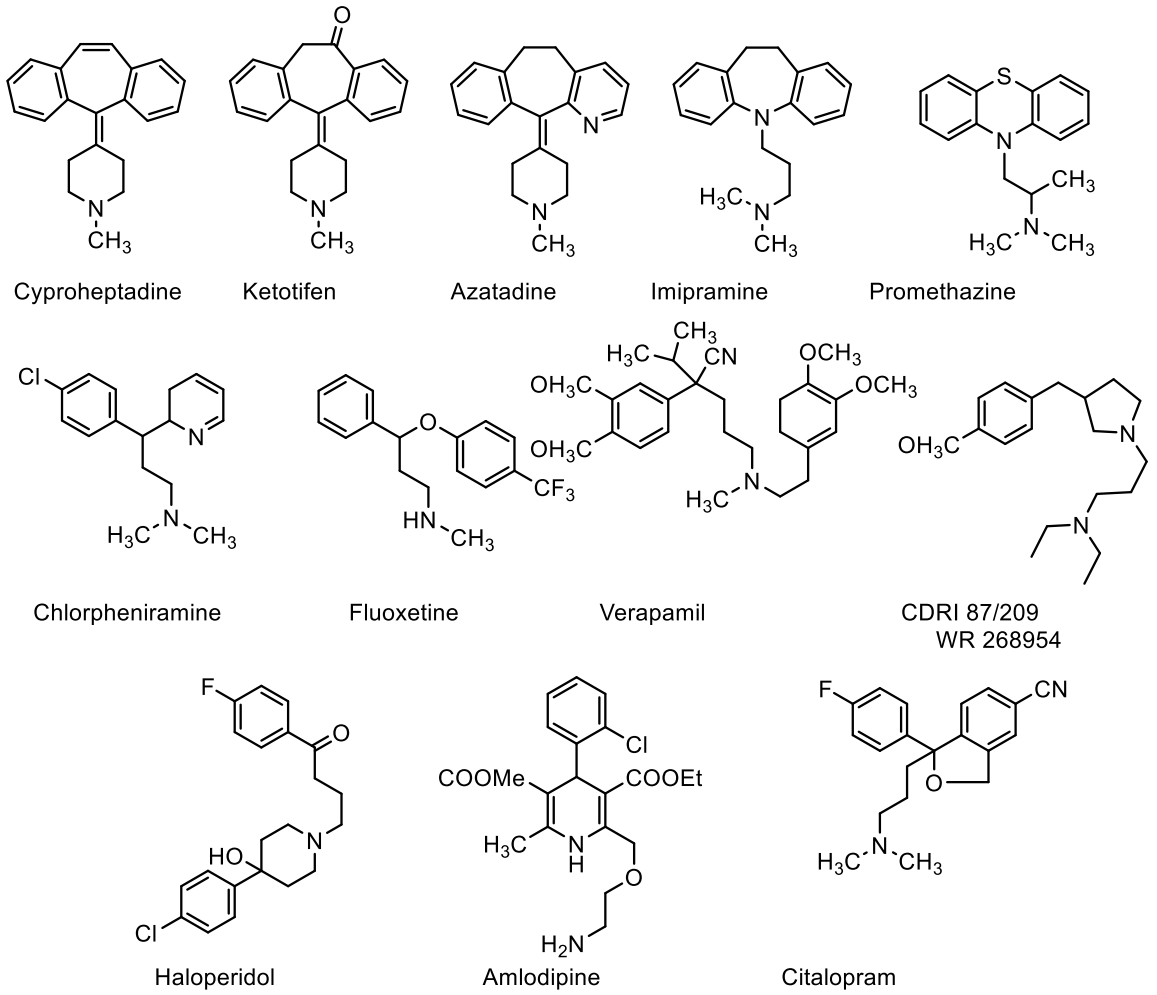
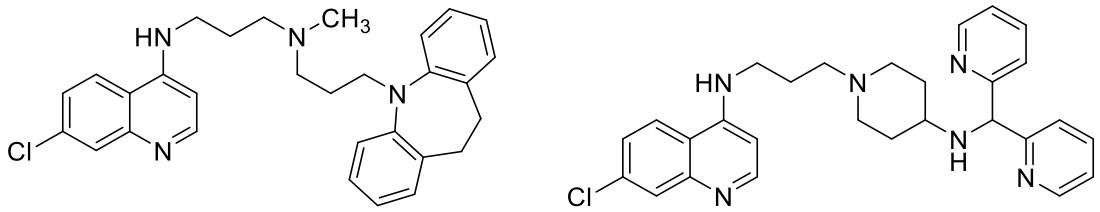


Figure 1.8. Structures of known CQ-resistance reversal agents used as pharmacophores

To circumvent chloroquine resistance, reversed chloroquines were designed in Peyton lab. These are hybrid drugs comprising quinoline nucleus and reversal agent moiety. In earlier studies, the tricyclic antidrepressant drug, imipramine, showed the highest potency when tested for its CQ resistance reversal effect against W2 clone of *P. falciparum*. [61] The first hybrid molecule, PL01, was synthesized by linking chloroquine moiety and imipramine as a RA. The hybrid analogue was found to be very effective against both CQ-sensitive and CQ-resistant strains of *P. falciparum* when compared to chloroquine.[62]

Modifications were made to PL01 to increase the oral availability. As a result a lead antimalarial drug candidate, PL69, was developed. It was found to be effective for CQ-resistant malaria *in vitro.* The IC_{50} values of PL69 against D6, Dd2, and 7G8 strains of *P. falciparum* were 0.9, 1.6, and 1.8 nM, when compared to CQ values of 6.9, 102, and 106 nM. PL69 was also orally available in a mouse model of malaria. *In vivo*, it cured mice infected with *P. berghei* at <64 mg/kg/day, which is equimolar dose with of chloroquine.[63]



PL01 PL69

Figure 1.9. The hit (PL01) and the lead (PL69) RCQ's synthesized in Peyton lab

Moreover, the mechanism of action was indicated to be similar to CQ, by inhibiting the formation of hemozoin. PL69 (DM1157) is now in late-stage preclinical studies by DesignMedix. This lead RCQ candidate has physico-chemical features that makes it a favourable partner drug for antimalarial combination therapy. The drug discovery research is still on progress to furnish more back-up drugs for the pipeline.[64]

CHAPTER 2: Click Chemistry

2.1. Introduction

Nature's giant molecules are constructed from a small building blocks by few types of reactions for connecting them together. Biological macromolecules such as polysaccharides, proteins and nucleic acids are all condensation polymers of small subunits formed by carbon-heteroatom bonds. Following Mother Nature's strategy, Sharpless, Kolb, and Finn identified a group of reactions and developed an approach called 'Click Chemistry' in 2001.[65] The main objective of click chemistry was to develop an array of selective, powerful and modular blocks which are reliable and have wide application.[66]

Sharpless and coworkers have set criteria that a process must meet to fulfill these reactions: "*The reaction must be modular, wide in scope, give very high yields, generate only inoffensive by-products that can be removed by non-cromatographic methods, and be stereospecific (but not necessarily enantioselective). The required process characteristics include simple reaction conditions, readily available starting materials and reagents, the use of no solvents or a solvent that is benign (such as water) or easily removed, and simple product isolation*." [66,67]

Finn and Fokin have developed common attributes of click reactions that many click compounds are derived from alkenes and alkynes, and that the bond is formed between carbon and heteroatom (mostly N, O & S). In addtion click reactions are strongly exothermic and are usually either fusion or condensation processes. Moreover, many

click reactions are highly tolerant of water. In point of fact, they are often accelerated by the presence of water.[68]

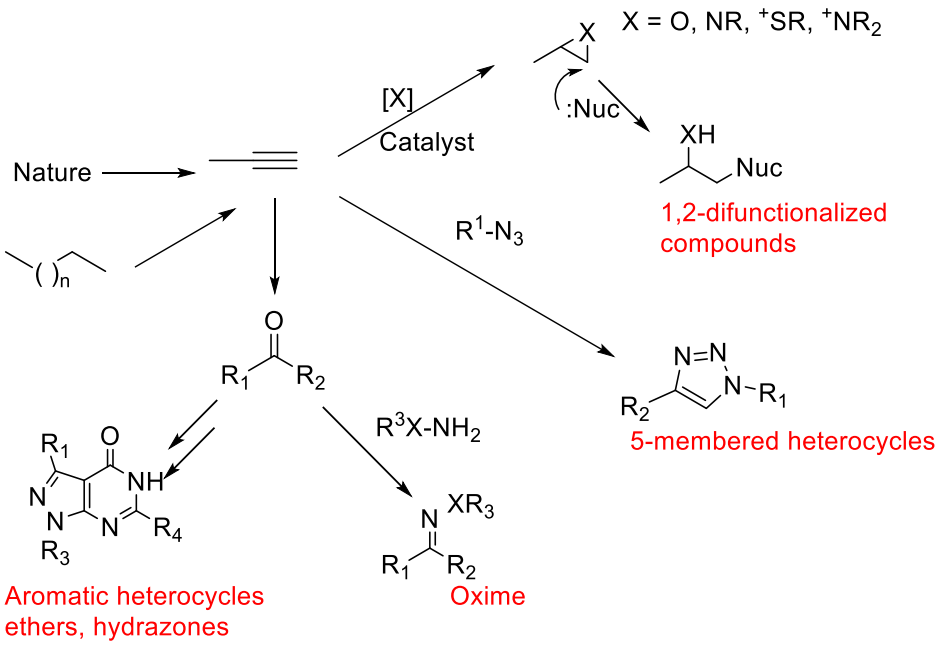
2.2. Types of click reactions

There are mainly four types of click reactions that use olefins and acetylenes as their carbon framework for forming carbon-heteroatom bond. These reactions include:

- Cycloaddition reactions: for example hetero-Diels-Alder and 1,3-dipolar cycloadditions
- Nucleophilic ring-opening on strained heterocyclic electrophiles including epoxides, aziridines, cyclic sulfates & sulfamidates and aziridinium & episulfonium ions[69]
- Non-aldol carbonyl chemistry including formation of oxime ethers, hydrazones and aromatic heterocycles
- Addition to carbon–carbon multiple bonds such as epoxidation, dihydroxylation, aziridination, and nitrosyl and sulfenyl halide additions[70]

2.3. Huigsen 1,3-Cycloaddition reactions

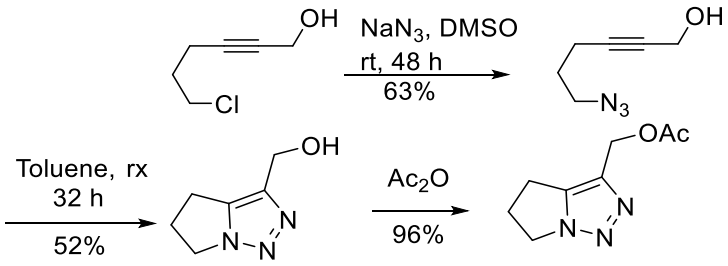
The potential and the mechanism of 1,3-dipolar cycloaddition reaction between alkynes and azides to form 1,2,3-triazoles was unveilled by Huisgen et al. in the 1960s.[71] However, the reaction was not utilized to its full extent until the copper(I)-catalyzed Azide-Alkyne cycloaddition (CuAAC) version reaction is discovered.[72]



Scheme 2.1. Click Chemistry – energetically highly favorable linking reactions

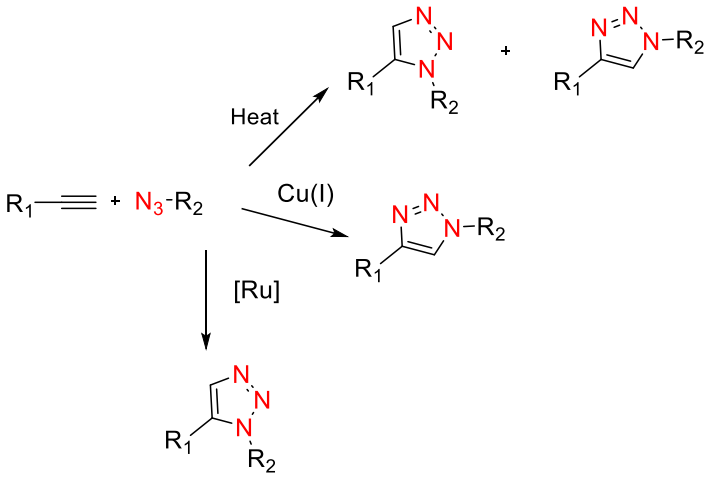
The potential of this reaction is very high due to the wide range of substituents involving alkynes and azides. Several triazoles have been made by using the Huigsen 1,3-cycloaddition reaction before the advent of catalysed reactions. Pearson et al. described the synthesis of monosubstituted dihydropyrrolo-1,2,3-triazole analogue of *antitumor dehydropyrrolizidine alkaloid* using an intramolecular azide–alkyne cycloaddition, by refluxing the azide in toluene in 55% yield.[73]

Nevertheless, this reaction has suffered a lack of selectivity for more than 40 years because of the weak directing effect of the substituents yielding a mixture of the 1,4- and the 1,5-regioisomers. Another disadvantage of this reaction is that the formation of products require long heating and the two regioisomers are challenging to separate using classical chromatographic procedures.[74]



Scheme 2.2. Synthesis of antitumor dehydropyrrolizidine analogue

When the reactants are heated without a catalyst, mixture of 1,4 and 1,5-triazoles are formed. When the reaction is catalysed by ruthenium, only the 1,5-regioisomer is formed. This was shown by Imperio et al. on the synthesis of Steganacin and Podophyllotoxun analogues to replace the lactone ring by 1,5-disubstituted triazole selectively.[75] However, the copper catalysed azide-alkyne cycloaddition (CuAAC) reaction produces only the 1,4-regioisomer.



Scheme 2.3. The 1,3-dipolar cycloaddition between azides and alkynes

2.4. Copper catalysed Azide-Alkyne cycloaddition

The copper(I)-catalyzed cycloaddition reaction between azides and terminal alkynes to give 1,4-disubstituted 1,2,3-triazoles has become the benchmark of 'click chemistry'. This reaction is very reliable, selective, biocompatible and exhibits a broad scope.[76] It was reported that the copper (I) salts were able to accelerate the reaction by up to 10 million times. [77,80]

One of the starting materials, azide, has a unique advantage of stability towards H_2O, O_2 and other organic synthetic conditions. Azide and alkyne functional groups are not reactive to other functionalities because of their high kinetic barrier.[81] Usually the reaction proceeds to completion in 6-36 hours at ambient temperature (room temperature to 50^0C). The catalyst is prepared *in situ* by reducing Cu(II) to Cu(I) salts using sodium ascorbate/ascorbic acid to get a cheaper and purer source. Sodium Ascorbate has proven to be an excellent reducing agent at 0.25-2% catalyst concentration.

The common solvents used include mixture of *tert*-butanol/ethanol and water without using any additional organic solvent. However, when reducing agent is not used, copper(I) salts including CuI and CuOTf : C_6H_6 can be used directly by utilizing one equivalent of nitrogen base (triethyl amine, DIPEA) in a cosolvent (acetonitrile, tetrahydrofuran, dichloromethane and toluene).[77]

The CuAAC is a very versatile reaction in that it proceeds best in aqueous systems including serum and whole blood, over a broad temperature range (0-160°C). Its remarkably insensitivity to wide range of pH (4 to 12) and stable to redox degredation

made the reaction to succeed beyond limit in the presence of all functional groups tested to date. [78]

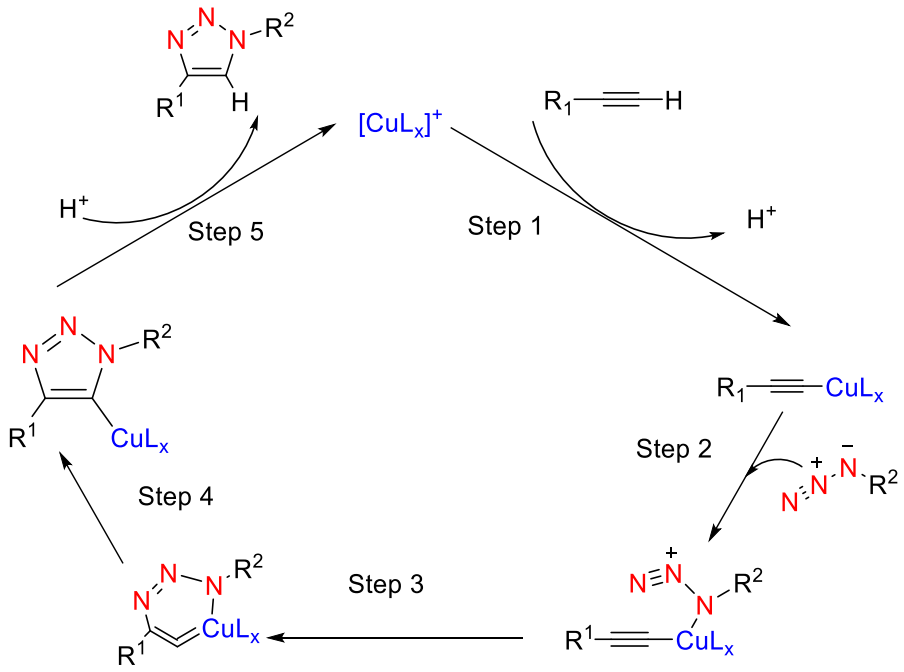


Figure 2.1. Proposed catalytic cycle for the CuAAC reaction

The proposed catalytic mechanism suggests that the 1,3-dipolar cycloaddition of terminal alkynes and azides is catalyzed by copper(I) salts via the formation of copper−acetylide complex, followed by a stepwise or concerted addition to an azide. This mechanism makes the catalysis not to be functional on internal alkynes.[80]

2.5. Applications of Click Chemistry

Click chemistry is originally developed for assisting medicinal chemists to have a triumph over combinational chemistry issues. Based on solution-phase library synthesis, Lexicon Pharmaceuticals synthesised 200,000 compounds of acceptable purity on 25-50

mg scale using click chemistry. The application of click chemistry is wide ranging from drug discovery to material science.[81]

The applicability of click chemistry in bioconjugation involves the attachment of synthetic labels to biomolecular framework covalently. CC enables lengthy bioconjugation processes of 60 steps with more than 99.8% yield. The bioorthagonal properties and solvent tolerance make azides and alkynes very important groups in bioconjugation.[82] The first application of CC in bioconjugation was shown by making 1,4-substituted [1,2,3]-triazoles in peptide backbones.[80] Because the presence of copper in CuAAC may create hinderance to bioorthogonal conjugation in living systems, copper-free click chemistry is applicable using Dibenzcyclooctyl (DBCO) as a source of alkyne.

After introduction of CC, its significance in materials science, specifically in polymer synthesis, was demonstrated by the synthesis of triazole-based dendrimers. The unique properties and application made dendrimers to receive much attention. The main problem in the purification during dendrimer synthesis was solved by the fidelity of CuAAC.[83] The first triazole-based dendrimer was synthesiszed by sequential click reactions on bis-alkynyl scaffold flexible with an azide anion substitution. The ability of polydentate 1,4-disubstituted 1,2,3-triazole ligands to stabilize Cu(I) species under physiologic conditions has proven vital applicaton in biological systems.[84]

Click chemistry has shown to have a cutting-edge application in the demanding world of medicinal chemistry in developing novel approaches to screening compound libraries. One of the main reason which makes CC reliable in drug discovery is that the compounds

can be screened directly from the reaction mixture. This was shown in the discovery of Human r-1,3-fucosyltransferase enzyme when the Cu(I)-catalyzed triazole synthesis was utilized to create library of GDP (guanosine diphosphate)-triazole candidates as the crude aqueous reaction were tested directly.[85] The other applications of click chemistry include glycoscience, peptide chemistry, supramolecular chemistry, oligonucleotide synthesis and others.[82]

2.6. Click chemistry in Drug discovery

It is a challenging task for medicinal chemists to develop new patentable drugs with strong activity and good ADMET properties in short time. The triazole-forming Click chemistry accelerates drug discovery in hit finding and lead optimization due to its reliance on extremely quick reactions in high yield.

Most of triazole containing drugs available so far are enzyme inhibitors, receptor ligands, peptides, and modified natural products. There are few drugs available on the market or in clinical trials including anticancer carboxyamidotriazole (CAI), the nucleoside derivative non-nucloside reverse transcriptase inhibitor Tert-butyldimethylsilylspiroaminooxathioledioxide (TSAO), β-lactum antibiotic Tazobactum and the cephalosporine cefatrizine.[76]

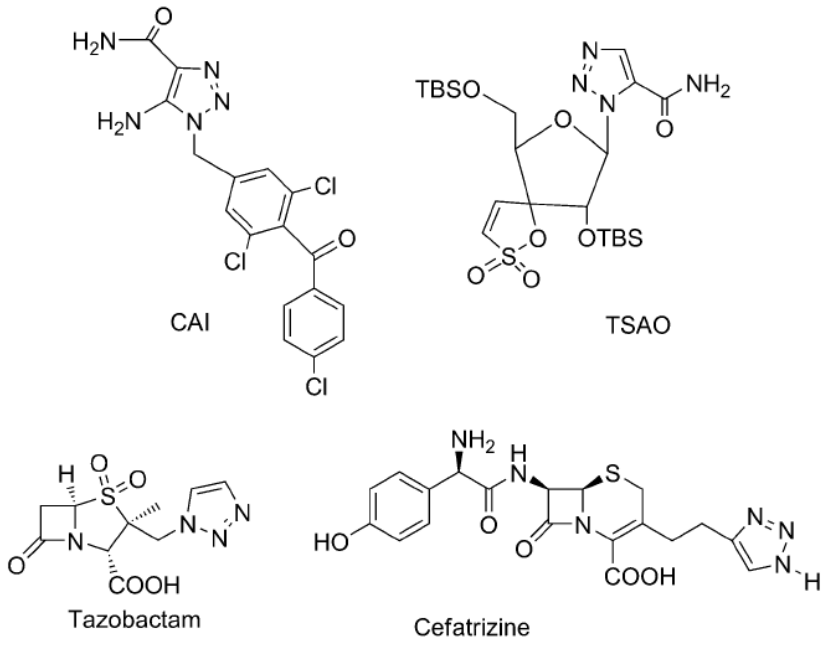
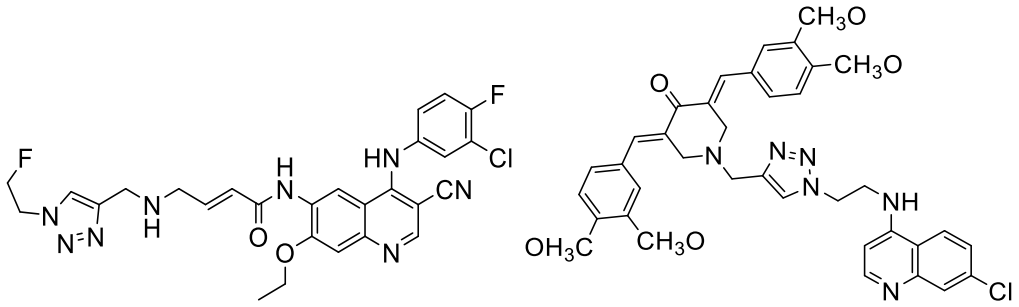


Figure 2.2. Drugs based on 1,2,3-triazoles[76]

Before Click chemistry came into action, several molecules that contain 1,2,3-triazole moiety were synthesized using various methods. These include anticancer, antibacterial, antimalarial, antifungal, antitubercular, antiviral, antihypertensive, and hypocholesterolemic agents.[76]

Pisaneschi et al. synthesized 3-cyanoquinoline-based fluorinated compounds that would make a good imaging agent for epidermal growth factor receptor (EGFR) to facilitate the clinical evaluation of tumors. One of the 20-fluoroethyl-1,2,3-triazoles showed to have good cellular potency, low lipophilicity and good metabolic stability *in vitro* to be a promising radiotracer for imaging of EGFR status.[86] Another new class C5- curcuminoid-4-aminoquinoline molecular hybrids has shown potential anticancer activity when tested on 60 human cancer cell lines at nM concentration.[87]



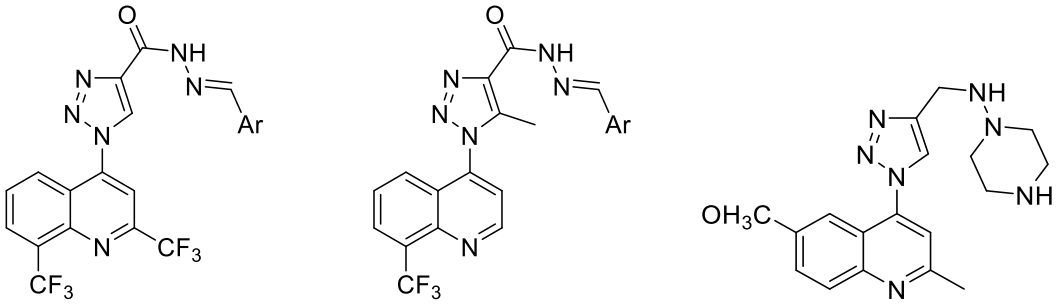
Pisaneschi et al. Rawat et al.

Figure 2.3. Triazole based anticancer molecules

The application of Click chemistry of 1,2,3-triazoles in synthesizing new class of antifungal and antimicrobial agents is becoming prevalent.[76] A series of substituted triazoles were synthesized using 1,2,3-triazole containing quinoline moiety and tested for their antimicrobial and antifungal activity. SAR investigation revealed that the nature of the substituent on the 4-position of the triazole ring influences the antimicrobial activity. When evaluated for *in vitro* activity and compared with standard antifungal Cyclo-piroxolamine, the 3-methylthien-2-yl moiety showed a broad spectrum antimicrobial activity against all tested strains.[88]

Two substituted 1,2,3-triazoles containing quinoline moiety showed good antibacterial and moderate antifungal activity, particularly the compounds with 3-methyl thienyl substituents are found to have increased antimicrobial activity.[89] In other study, Thomas and co-workers reported *in vitro* activity of similar antibacterials and antifungals. The compounds demonstrated moderate to very good activities, when comparable to

Ciprofloxacin and Ciclopiroxolamine respectively. The enhanced activity is due to the presence of active piperazine and its derivatives in their structures.[90]



Sumangala et al Holla et al Thomas et al

Figure 2.4. Triazole based antibacterial and antifungal molecules

Olomola et al synthesized anti-HIV triazole-based hybrids using Cu(I)-catalysed cycloaddition reaction by combining 3-alkynylmethylcoumarins with azidothymidine (AZT). The structurally complex coumarin-AZT conjugates have the potential dual-action as HIV-1 protease and non-nucleoside reverse transcriptase inhibitors.[91]

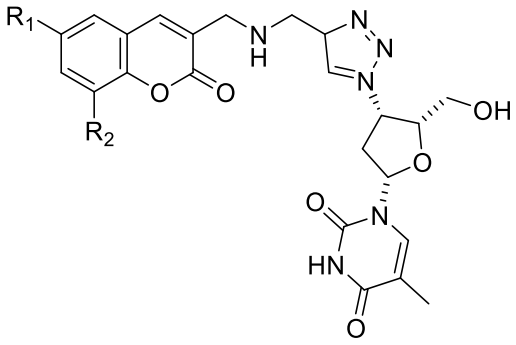


Figure 2.5. Triazole based anti-HIV hybrid molecule

2.7. Triazole based Antimalarials

The modification of well-established antimalarials, particularly chloroquine is very common in making new drugs to counteract drug resistance. Different groups reported the synthesis of 1,2,3-triazole based quinoline moieties using copper(I)-catalysed cycloaddition click chemistry.

A series of 4-aminoquinoline-1,2-3-triazole based hybrids were synthesized and evaluated for their *in vitro* antimalarial acivity against D6 and W2 strains of *P. falciparum*. Some of the compounds have shown promising antimalarial activity without showing any toxicity against Vero cells.[92] On the similar studies done by Mamgain et al., the absence of linear alkyl chain linker have shown to diminish the antimalarial activity.[93]

Pereira et al directly clicked 7-chloroquinoline with different substituents in position C4 of the quinoline at the N1 of the triazole ring. When evaluated for their *in vitro* activity against *P. falciparum* (W2), the compounds showed moderate antimalarial activity and low cytotoxicity to Hep G2A16 cells. The decreased activity was explained by the low resonance contribution of the triazole ring to stabilize the acidic form of the quinoline ring that decreases the pKa of the quinoline nitrogen.[94]

Another set of triazole molecules was made by the Blackie group using the mefloquine pharamacophore by CuAAC chemistry. The *in vitro* biological activity of the compounds on NF54 chloroquine-sensitive strain of *Plasmodium falciparum* was determined to be in the lower l.0 uM range. The authors concluded that the inclusion of the piperazine does

not appear to be advantageous in biological activity because the single basic nitrogen in the side chain seemed to provide sufficient basicity to maintain activity.[95]

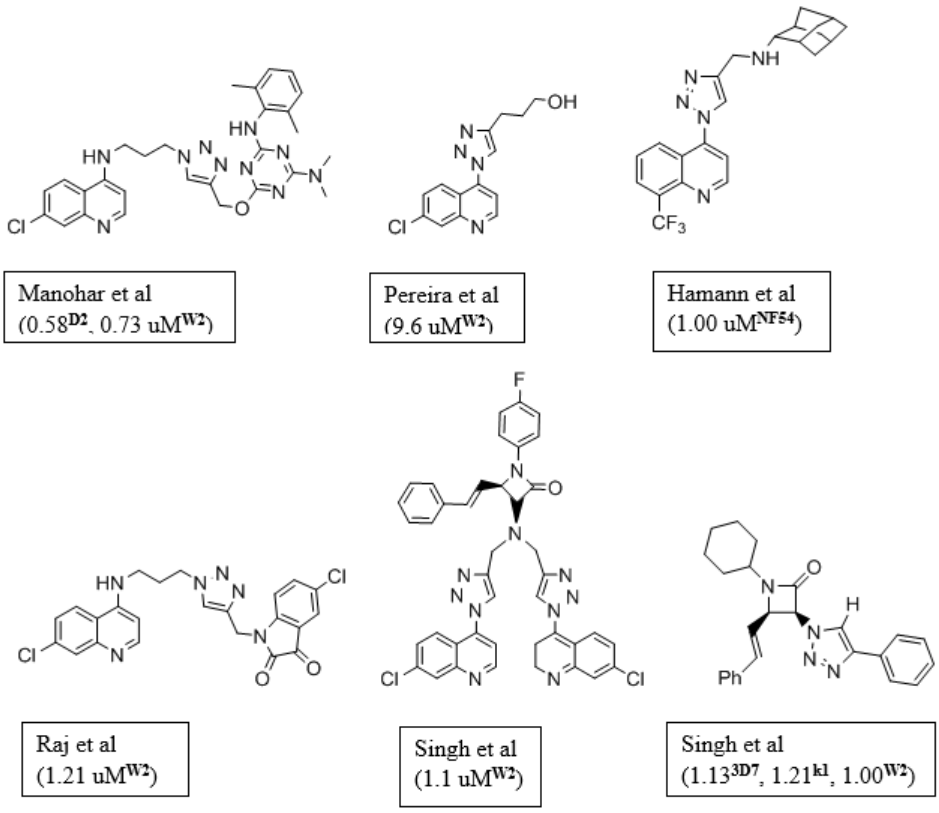


Figure 2.6. Triazole based antimalarials

Many hybrid drugs were synthesized by combining 7-chloroquinoline moiety with other drugs. The synthesis and antimalarial activities of 1H-1,2,3-triazole tethered 7-chloroquinoline-Isatin hybrids was shown by Raj and others. The compound with three carbon alkyl chain and chloro substituent at the C-5 position of the isatin ring displayed the best activity against cultured W2 strain among the test compounds.[96]

1,2,3-Triazole tethered β-lactam and 7-chloroquinoline bifunctional hybrids were synthesized and evaluated against W2 strain of *P. falciparum* for their antimalarial activities. The activity was found to be dependent on the N-substituent of the β-lactam ring and the bis-triazole at the C-3 position, and the p-fluorophenyl substituted analogue showed maximum activity. Docking studies via inhibiting *P. falciparum* dihydofolate reductase (PfDHFR) enzyme was made to explain the activity.[97]

Other non-quinoline based antimalarial compounds having 1,2,3-triazole pharamacophore were made using click chemistry. Triazole-based β-lactams were synthesized and evaluated for their antiplasmodial activity against 3D7, K1, and W2 strains of *Plasmodium falciparum*, and KB cells for their cytotoxicity profiles. The compound with an N-cyclohexyl substituent in the β-lactam ring and a phenyl group on the triazole showed better antiplasmodial activity and cytotoxicity.[98]

2.8. Triazoles as Pharmacophores, Bioisosters and Linkers

The importance of triazolic compounds in medicinal chemistry is undeniable. There are three main groups of 1,2,3- triazoles: monocyclic, benzotriazoles and 1,2,3-triazolium salts. Based on the position of the substituent, the monocyclic 1,2,3- triazoles are subdivided into 1H-, 2H- and 4H-1,2,3-triazoles. The 1H- and 2H- forms are tautomers in equilibrium both in solution and gas phase. The 1H-1,2,3-triazole acts as both weak base (pK_a = 1.17) and weak acid (pK_a = 9.4), and the ring is not protonated at physiological pH.[76, 99]

The acidity and basicity of nitrogen atoms in 1,2,3-triazoles is explained well by their resemblance of having both pyrrole and pyridine-like electronic properties respectively. The pyridine-type nitrogen is weakly σ-donating and strongly π-accepting while the five-membered pyrrole offers an amine-like nitrogen which makes it σ-accepting and π-donating, causing an aromatic system of π-electron excess.[100]

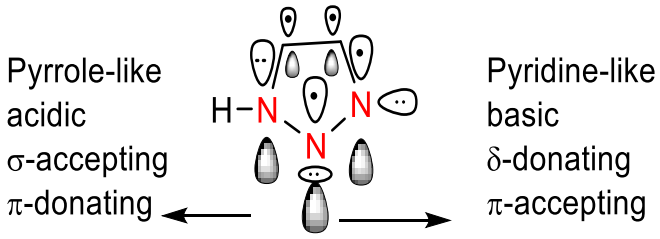


Figure 2.7. Electronic structure of 1H-1,2,3-triazole

The 1,2,3-triazole ring exhibits three resonance forms at which the three N-atoms cause the aromatic π-system to be very polarized on a σ-framework. When both resonance and inductive effect are assessed, the two C-atoms and the N-atom in the 1-position showed positive charges, where as the 2- and 3-Nitrogen atoms indicated partial negative charges. The experimental and computational data showed to be consistent indicating a very large dipole moment of 4.38 and 4.55 D respectively.[100]

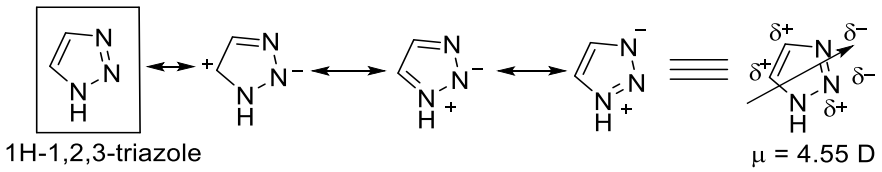


Figure 2.8. Structures, tautomerism, partial charges & dipole moments of the 1H-1,2,3-triazole

Although the incorporation of 1,2,3-triazoles into pharmacologically active molecules had been started decades ago, it became more popular after the introduction of copper-catalyzed click chemistry. The triazole group not only functiones as a passive linker but also as a key part of the pharmacophore. The stabilty to acid-base hydrolysis, oxidative-reductive conditions and resistance to metabolic degradation make triazoles excellent candidates as pharmacophores. Moreover, these heterocycles readily associate with biological targets via H-bonding and dipole–dipole interactions.[74, 76, 101]

According to the arenology principles, triazoles have been employed to substitute labile functional groups such as amides and esters to generate functional, nonclassical bioisosteres.[102] Due to the wide application of peptide chemistry, the bioisosteric replacement of amide bonds with triazole ring is becoming an interest. Depending on the substituent pattern of the triazole, disubstituted 1,2,3-triazoles can replace either trans- or cis-configuration of the amide bond. The presence of both hydrogen bond donor and acceptor at similar positions, and closely related dipole moment make 1,2,3-triazloes ideal bioisosters for amides.[103]

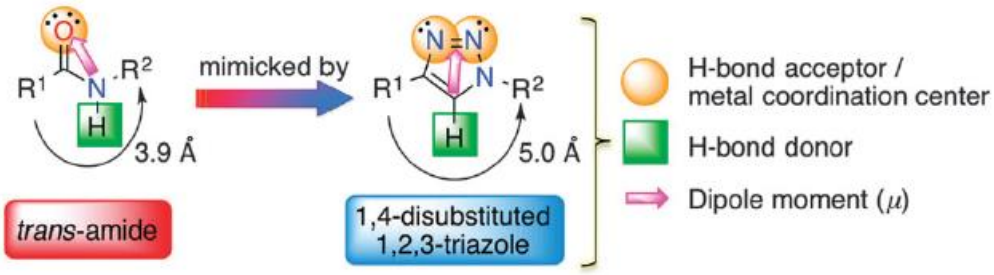


Figure 2.9. Disubstituted trans-amides and 1,2,3-triazoles as bioisosteres[102]

The ability to function as rigid linking units makes 1,2,3-triazoles to be extremely stable to hydrolysis and an excellent replacement for amides. Phillips et al. have replaced the amide group of Linezolid, an oxazolidinone antibiotic, with a 1,2,3-triazole ring and showed the retention of antibacterial activity.[104] In other similar work, 1,2,3-triazole-containing novobiocin analogs were designed and synthesized by replacing the amide moiety. The analogues exhibited comparable antiproliferative activity when evaluated against two breast cancer cell lines.[105]

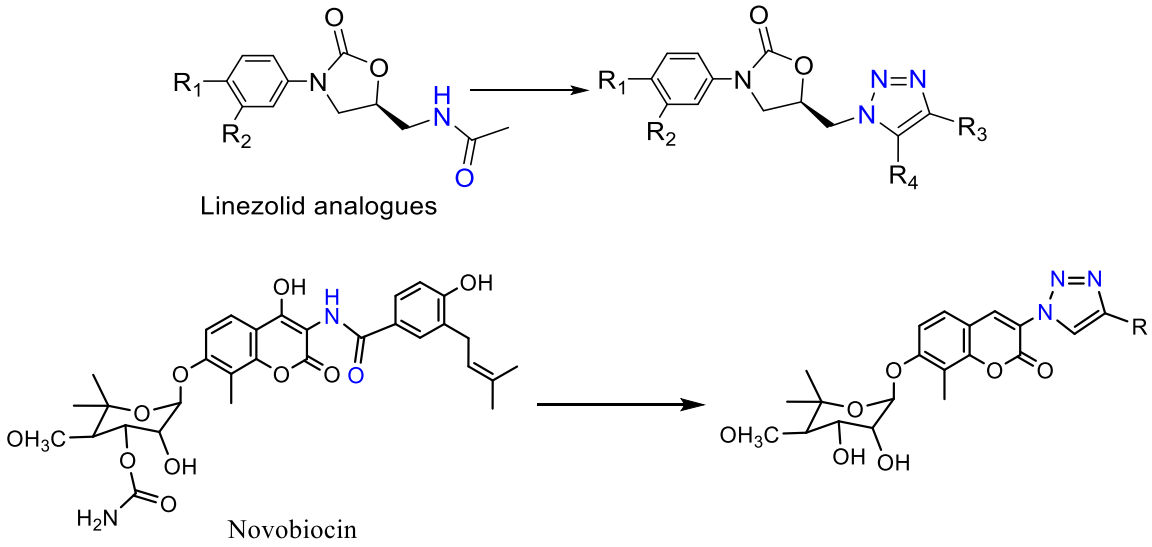


Figure 2.10. 1,2,3-Triazoles used as bioisosters of trans-amides

The triazole moiety has also been shown to act as a non-classical bioisostere of an ester group in arecoline, a muscarinic agonist, for the treatment of Alzheimer's disease.[106] Because of the basic aromacity of 1,2,3-triazoles, they are suitable isosters of heteroaromatic rings. In addition, the triazole moiety is also shown to be the isostere of

double bond. In addition to their pharmacophore role, triazoles have been used as favorable linkers in the synthesis of dimers and bidentate inhibitors.[107]

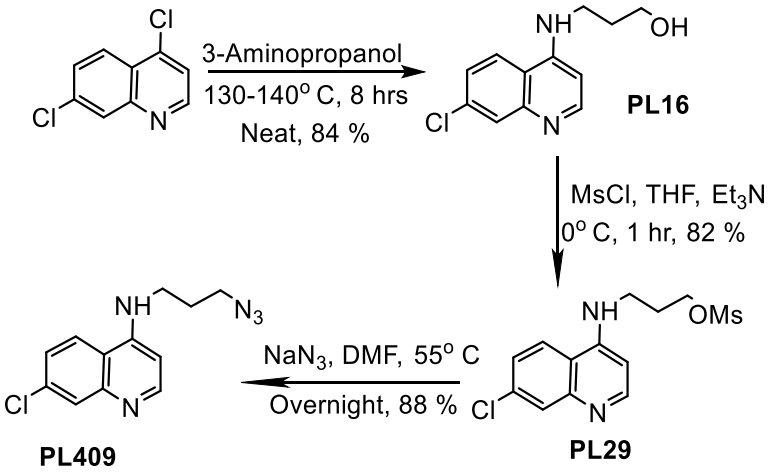
In order to address the challenges of high costs associated with high-throughput screening and chemical synthesis, Durrant and McCammon developed a novel computer algorithm, called AutoClickChem, capable of performing many click chemistry reactions in *silico* that may prove useful in rational drug design and optimization. AutoClickChem, which combines virtual screening and click chemistry, is based on the pymolecule toolbox, a framework that facilitates the development of future python-based programs requiring molecular model manipulation.[108]

CHAPTER 3: Synthesis of 1,2,3-Triazole-based Antimalarials

Series of click compounds were synthesized via copper(I)-catalyzed alkyne-azide cycloaddition reaction using sodium ascorbate as a reducing agent. Based on previous work done in Peyton and other labs, three aliphatic linear chains as linker was considered as optimum length both interms of antimalarial activity and hydrophilicity.[63]

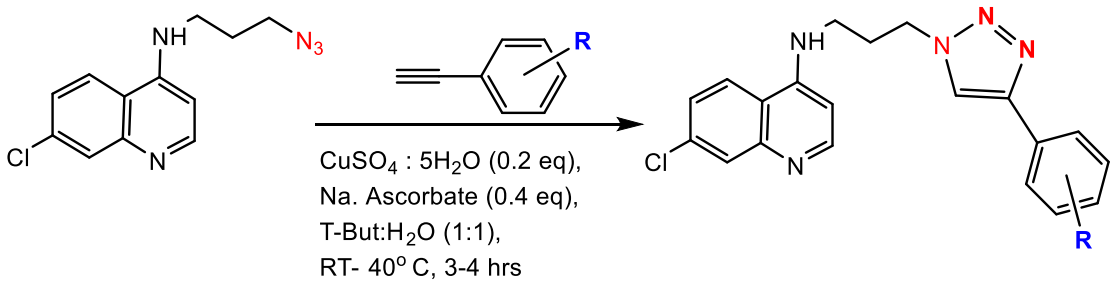
3.1. First-Generation Click Compounds

The azide analogue was prepared in three steps with very good yields as outlined in Scheme 3.1. First, the amino-alcohol quinoline (PL16) was synthesized by nucleophilic aromatic substitution of commercially available starting material 4,7-dichloroquinoline with excess aliphatic chain aminoalcohol. The hydroxyl group was mesylated by methane-sulfonyl chloride and triethylamine base to give mesylated-quinoline (PL29).[62] Finally, the azide analogue (PL409) was prepared by nucleophilic acidification using sodium azide in DMF.[109]



Scheme 3.1. Synthesis of starting material – azide analogue

The first seven click compounds were synthesized by reacting the azide analogue (PL409), which was prepared in large scale, with commercially available terminal alkynes to give 1,4-disubstituted-1,2,3-triazoles. The method used by Mahohar et al. was adapted by using copper sulfate pentahydrate as catalyst and, two-fold excess of sodium ascorbate to reduce the copper (II) to (I).[110] An equimolar ratio of *tert*-butanol and water was used as mixture of solvents. The reactions were run from room temperature with maximum temperature reaching 50^{o}C for 3-4 hours to yield the final target compounds in moderate yield (26 – 76%).



Scheme 3.2. General synthetic pathway of the first generation click compounds

Several substituted terminal alkynes were used as the source of various functional groups. Tri-substituted terminal alkyne with two aromatic groups and a hydroxyl group was of particular interest, due to the similarity with the typical pharmacophore of reversal agents. In addition, both ortho- and meta-methoxy substituted click compounds were synthesized to make a further structure-activity analysis. In order to evaluate the strength of electron-withdrawing groups, monofluoro- and difluoro-substituted click compounds were prepared. Finally, one of the carbons at ortho position of the phenyl group was replaced by nitrogen and the substituted phenyl groups were replaced by a benzyl group.

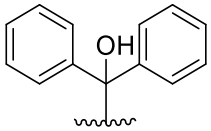
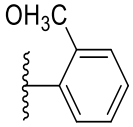
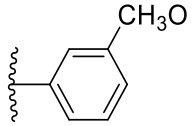
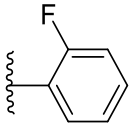
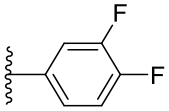
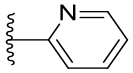
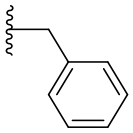
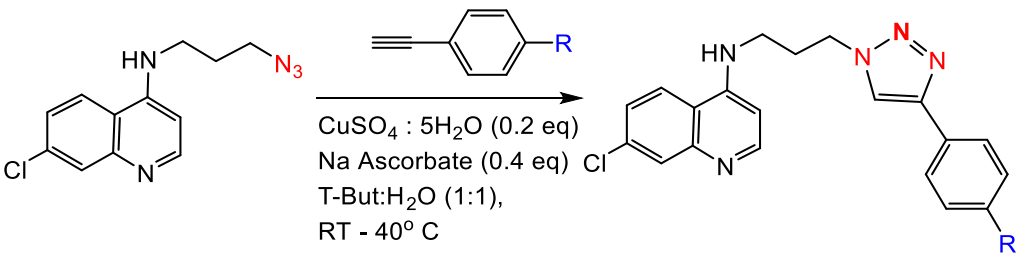
PL No.	Structure of the substituent (R)	% yield
PL404		41
PL405		72
PL406		51
PL407		26
PL408		76
PL412		46
PL414		45

Table 3.1. The first synthesized click compounds

3.2. Para-Phenyl Substituted Triazoles

Based on previous compounds synthesized in the Peyton lab by Bornface Gunsaru et al., the para-substituted quinoline analogues showed better activity than their ortho- and meta-substituted counterparts.[111] Various commercially available p-phenyl substituted terminal alkynes were used to synthesize 10 click compounds using the same synthetic pathway in low to excellent yield (30 – 95%).



Scheme 3.3. General synthetic pathway of para-substituted click compounds

PL No.	**Substituent at para position**	**% yield**
PL403	OCF_3	30
PL411	H	50
PL413	CH_3	56
PL415	NH_2	72
PL416	Br	69
PL417	OCH_3	51
PL418	Cl	76
PL419	CF_3	68
PL420	F	84
PL421	NO_2	95

Table 3.2. Para-substituted click compounds

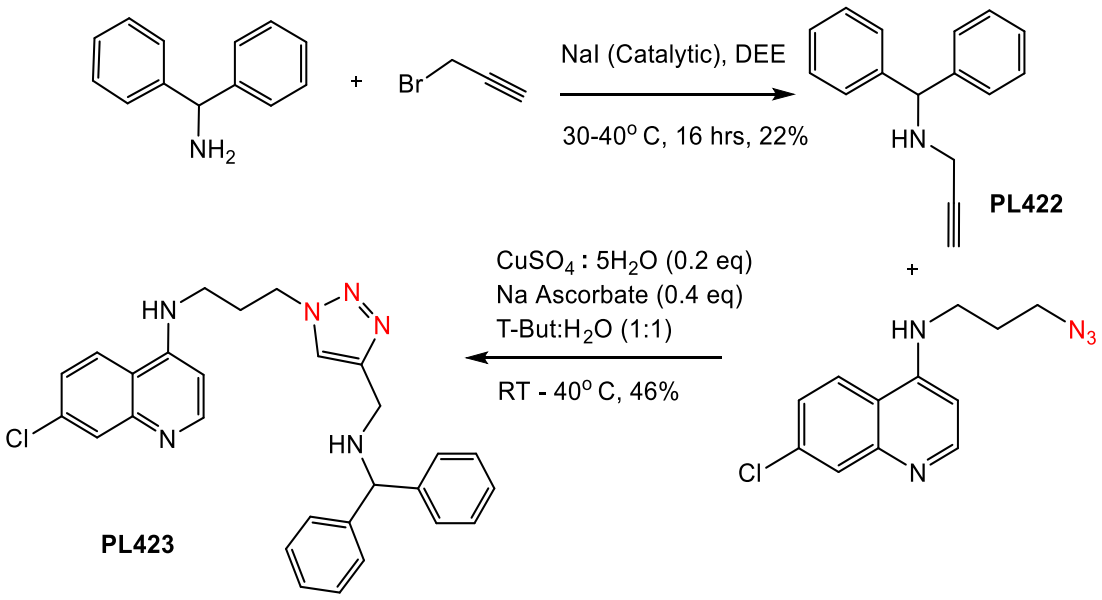
In order to perform structure-activity relationship studies, several click compounds containing electron-donating and electron-withdrawing groups were synthesized. Unsubstituted (H-substituted) analogue was used as a reference. The p-hydroxy phenyl substitution was not synthesized because the starting material was not readily available.

Three electron-donating containing analogues, including amino-, methoxy- ,and methyl were synthesized. In addition, electron-withdrawing groups such as nitro-, trifluoromethyl-, and the halogens were prepared for comparison. The halogens used was fluorine, chlorine, and bromine (but not iodine). This is because the p-iodo substituted terminal alkyne was not commercially available. Finally, a very interesting long-range electron withdrawing substituent, trifluoromethoxy group, was prepared to compare with the methoxy and trifluoromethyl groups.[112]

3.3. Triazole with Reversal Agent Pharmacophore

One additional click compound (PL423) which contains two aromatic rings and nitrogen atom between the triazole and the aromatic rings was made to associate the activity with the reversal agent pharmacophore. First the terminal alkyne (PL422) was synthesized using the *Finkelstein* reaction by replacing bromine with a better leaving group, iodine.[113]

The benzhydrylamine was treated by propargyl bromide using catalytic amount of sodium iodide in diethyl-ether with minimum heating and fair yield. The synthesized terminal alkyne was reacted with the azide analogue (PL409) under the same condition with the primarily synthesized click compounds to give PL423 in a good yield.

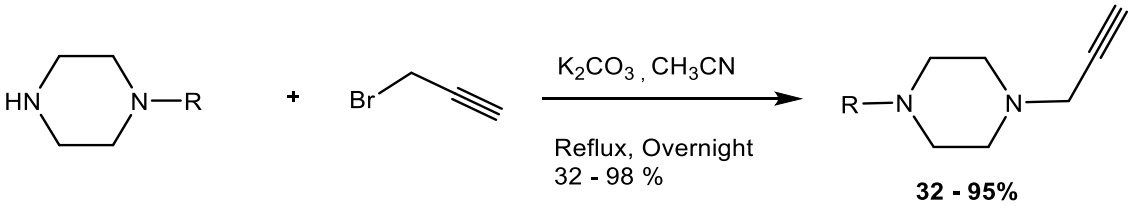


Scheme 3.4. Synthesis of PL423

The first series of 18 click compounds was assessed for their antimalarial activity, and six of them showed better activity than chloroquine against resistant strains of *P. falciparum* (Dd2 and 7G8). Additional compounds containing piperazine ring between the triazole and the aromatic rings were planned to be synthesized in order to incorporate a protonable nitrogen that possesses a weakly basic character.

3.4. Para-Phenyl Substituted Terminal Alkynes

With the intention of making piperazine containing triazoles, 10 para-substituted piperazinyl terminal alkynes were prepared. These intermediate molecules were made by reacting equimolar ratio of readily available p-substituted piperazines with propargyl bromide.[114] The reaction mixture was refluxed overnight using potassium carbonate as a base and acetonitrile as a solvent with moderate to excellent yields.



Scheme 3.5. Synthesis of p-phenyl piperazinyl substituted terminal alkynes

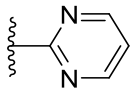
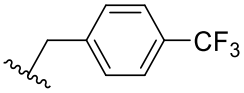
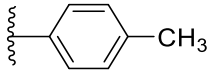
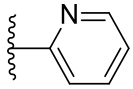
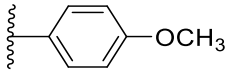
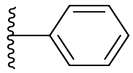
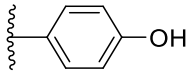
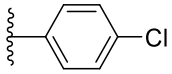
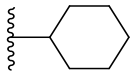
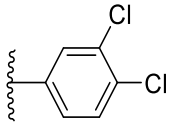
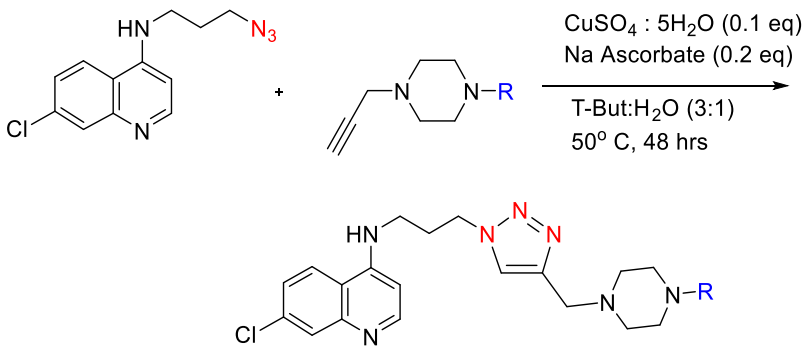
PL No.	Structures (R)	% Yield
PL428		32
PL429		87
PL430		98
PL431		70
PL432		95
PL433		69
PL434		57
PL435		67
PL436		86
PL437		94

Table 3.3. Structures of 10 piperazine-containing terminal alkynes

3.5. **Piperazinyl-linked Triazoles**

The p-substituted piperazines were selected based on different functional groups, heteroatomic replacement, linker length between the piperazine and the aromatic ring, and multiple substitution. The cyclohexyl piperazine was selected for comparison of the aliphatic cyclic compound with the phenyl-substituted piperazine. Consequently, nine very interesting piperazinyl containing triazoles were synthesized using CuAAC.

The concentrations of the copper sulphate catalyst and reducing agent sodium ascorbate were reduced by half from the previous method. This was because of the difficulty in solubility encountered during the work-up (liquid-liquid extraction) and purification (recrystallization) processes of the first series of synthesized molecules.[96, 114] The increased reaction time was the cost of reducing the catalyst and reducing agent concentrations which increased from 3-4 hours to 1-2 days. The amount of *tert*-butanol solvent used was also increased to threefold of the water co-solvent with moderately heating the reaction to 50^{o} C.



Scheme 3.6. Synthesis of piperazine containing triazoles

The triazole-based synthesized compounds contain both electron-donating (methyl, methoxy, and hydroxyl) and electron-withdrawing (trifluoromethyl, chloro, and dichloro). The carbon atom at the ortho position of the phenyl group was mono- and di-substituted by nitrogen to give 2-pyridyl and 2,6-pyrimidyl substituted triazoles.

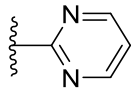
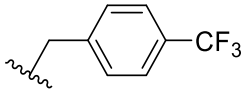
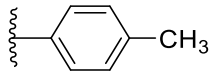
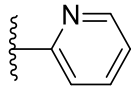
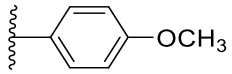
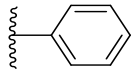
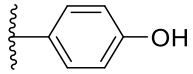
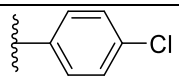
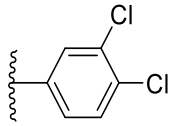
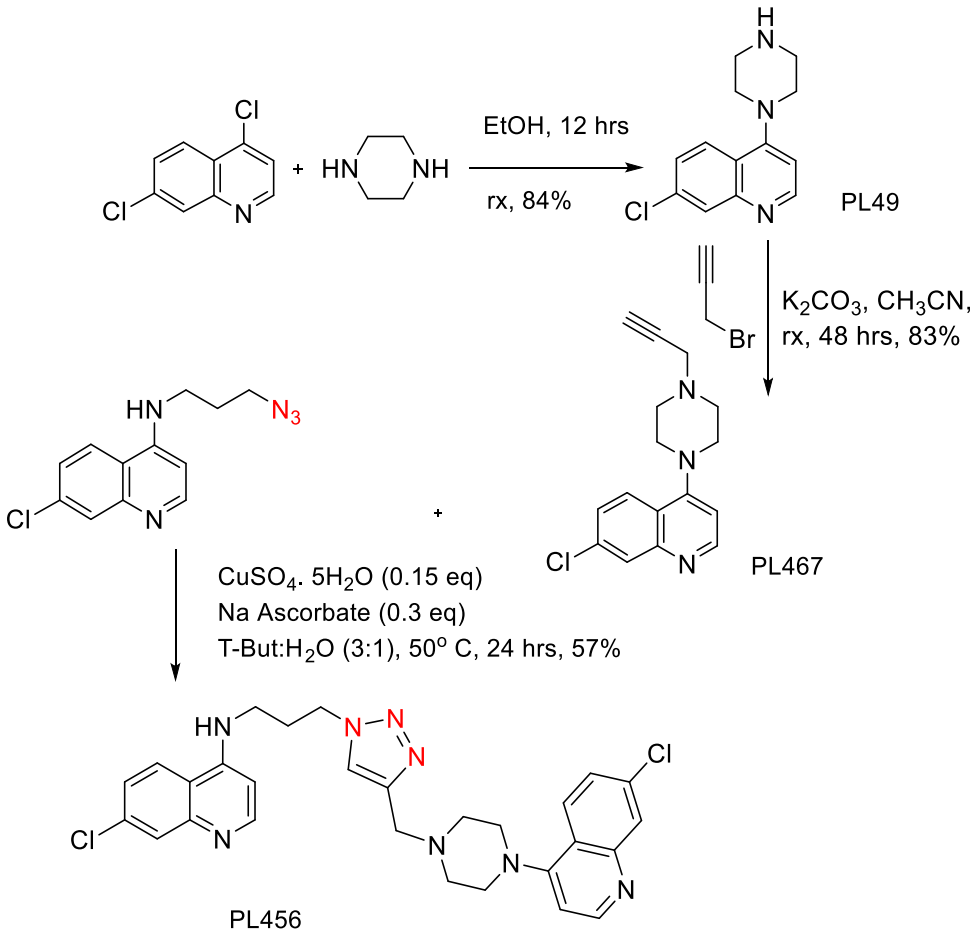
PL No.	Structures (R)	% Yield
PL440		69
PL441		52
PL442		35
PL443		61
PL444		76
PL445		70
PL446		68
PL447		80
PL448		77

Table 3.4. Structures of piperazinyl-triazoles

The p-trifluoromethyl substituent was made with one aliphatic linker between the piperazine and the aromatic ring. The unsubstituted (H-substituted) phenyl analogue was considered as an optimum reference between electron-donating and withdrawing groups. The di-chloro substituted triazole was also synthesized to notice the change it brings in

the strengh of the electron-withdrawing capacity of the ring. The click compound containing cyclohexyl substituent was not successful in synthesizing.

3.6. **Triazole-based Bisquinoline Antimalarial**



Scheme 3.7. Synthesis of bisquinoline containing triazole

As the potent antimalarial activity of bisquinolines was observed in the previously synthesized compounds in Peyton lab, bisquinoline containing 1,2,3-triazole ring was synthesized by copper catalyzed click chemistry (Scheme 3.7).

The target bisquinoline was synthesized in three steps with moderate to very good yields. 4-piperazinyl-quinoline (PL49) was first synthesized by refluxing commercially available starting material 4,7-dichloroquinoline with anhydrous piperazine in ethanol.[115] The terminal alkyne analogue (PL467) was then prepared by reacting PL49 with propargyl bromide using potassium carbonate as a base and acetonitrile as solvent.[114] Finally, the target bisquinoline triazole (PL456) was made by copper catalysed click reaction in optimum catalytic concentration and reaction time than previous click reactions.

CHAPTER 4: Structure-Activity Relationship of Triazoles-Based Antimalarials

The efficiency of synthesizing several triazole-based antimalarials have proven click chemistry to be a fast and efficient route. Antimalarial click compounds were synthesized using copper(I)-catalyzed azide-alkyne cycloaddition reactions and tested for their *in vitro* activity against D6, Dd2, and 7G8 strains of *Plasmodium falciparum*. Several of them have shown better activity against chloroquine-resistant strains (Dd2 and 7G8) than chloroquine.

4.1. **The first generation click compounds**

The first generation click compounds synthesized in Peyton lab contain the triazole ring connected directly to one or two aromatic rings with different substitutions. Six of the triazole-based click compunds showed better activity against chloroquine resistant, Dd2 and 7G8, strains of *P. falciparum*.

Structure-activity relationship studies were made on the synthesized compounds. The phenyl-substituted triazole (PL411) showed an enhanced activity when compared to the 2-pyridyl substituted triazole (PL412) against all the three strains. PL411 gave a better IC_{50} values of 76.8, 87.5 and 151 nM when compared to 420, 960, and 590 nM (d6, Dd2, and 7G8 strains respectively) of PL412.

In another comparison, PL411 showed decreased activity which compared to PL414 which has an additional methyl linker between the phenyl and the triazole rings. Thus addition of a methyl linker was found to be an advantage over direct connection in this case.

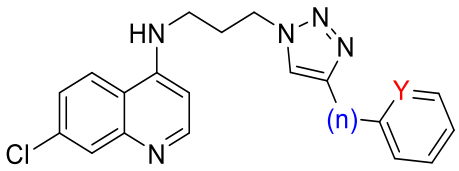


Figure 4.1. The effect of hetroatomic replacement and linker addition

PL No.	Number of linker (n)	Type of atom (Y)	IC_{50} (nM)		
			D6	Dd2	7G8
PL411	0	C	76.8	87.5	151
PL412	0	N	423	696	592
PL414	1	C	9.6	77.7	85.8

Table 4.1. The IC_{50} comparison of phenyl, 2-pyridyl, and benzyl triazoles

The effect of ortho-, meta-, and para-substitutions were also investigated by comparing the electron-donating methoxy group on the phenyl directly connected to the triazole ring. The para-methoxy substituted triazole (PL417) showed an enhanced activity when compared to the meta-substituted (PL406), which inturn also showed a better activity than the ortho-substituted triazole (PL405) against all the three strains.

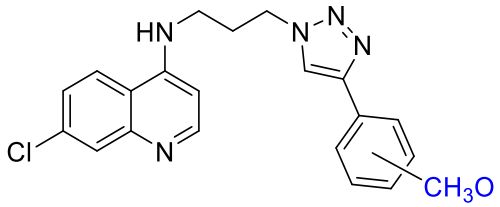
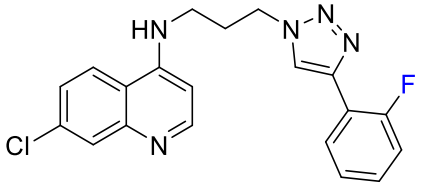


Figure 4.2. The effect of methoxy group substitution

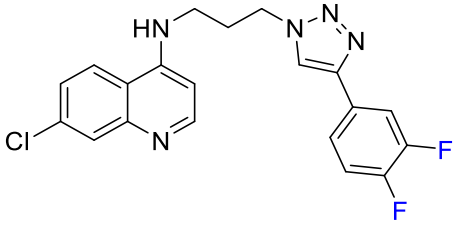
PL No.	Position of methoxy substituent	IC_{50} (nM)		
		D6	Dd2	7G8
PL405	ortho-	98.6	209.8	192.4
PL406	meta-	52.4	120	122.3
PL417	para-	40.5	108.8	140.1

Table 4.2. The IC_{50} comparison of ortho-, meta-, and para-methoxy substitutions

The electron-withdrawing strength effect of halogens were compared by mono- and di-fluorinated substitutions on the phenyl ring directly attached to the triazole. It was found that double halogen (fluorine) substitution (PL408) had improved activity by, almost 2.5 times, for both D6 and Dd2 strains, when compared to mono-fluoro substitution (PL407).



PL407 (81^{D6}, 109^{Dd2}, 129^{7G8})



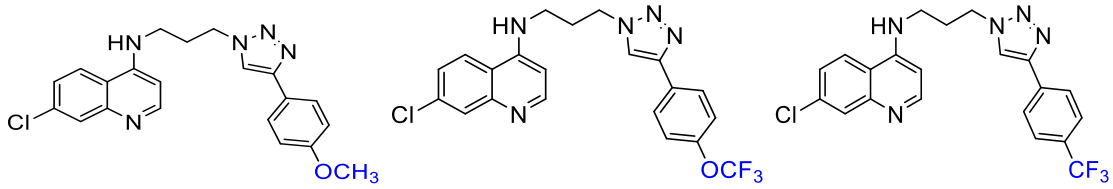
PL408 (23^{D6}, 39^{Dd2}, 96^{7G8})

Figure 4.3. The effect of mono- and di-fluoro substitution

The long-range electro-withdrawing group, the trifluoromethoxy-substituted triazole (PL403) showed the highest activity among all the 18 synthesized first generation triazoles. It showed about 4 times better activity than chloroquine against both CQ-

resistant strains. The IC_{50} values of PL403 are 12.8 and 14.5 nM, while CQ has 54 and 63 for Dd2 and 7G8 strains of *P. falciparum* respectively.

When the para-methoxy substituted triazole (PL417) was compared with PL403, the latter showed a superior activity. The replacement of hydrogen atoms by the most electronegative element fluorine presented an 8 times increase in activity. Moreover, PL403 indicated about 2.5 times increase in activity for Dd2 strain, when compared with a similar electron-withdrawing substituent, trifluoromethyl-substituted triazole (PL419).



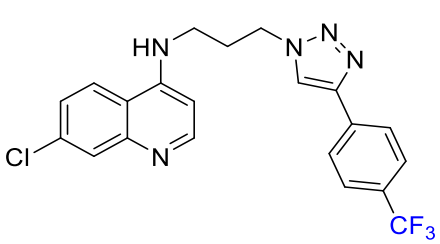
PL417 (41^{D6}, 109^{Dd2}, 140^{7G8}) PL403 (10^{D6}, 13^{Dd2}, 15^{7G8}) PL419 (11^{D6}, 32^{Dd2}, 19^{7G8})

Figure 4.4. Comparison of the IC_{50} values of para-methoxy, trifluoromethoxy and Trifluoromethyl-phenyl substituted click compounds

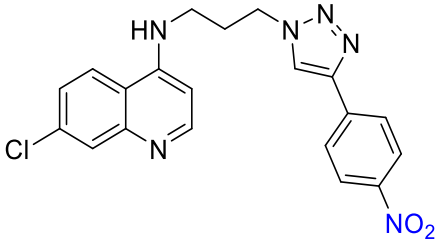
The electron-donating substituents showed inferior activities than chloroquine. These include the para-phenyl substitutions of methyl (PL413), amino (PL415), and methoxy (PL417) functional groups. While the para-methyl substituted triazole presented the best activity from the three electron donating groups, the para-amino showed the least activity against all CQ-sensitive and CQ-resistant strains of *P. falciparum*.

However, the electron-withdrawing groups such as the trifluoromethyl, nitro and the halogens presented better or comparable activity than chloroquine. Most of them

exhibited an improved IC_{50} values than CQ against both resistant strains except the para-fluoro substituted triazole (PL420).



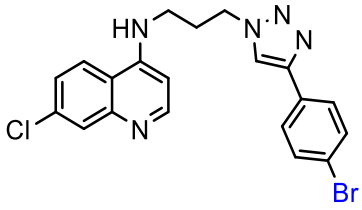
PL419 (11^{D6}, 32^{Dd2}, 19^{7G8})



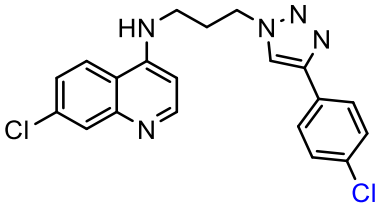
PL421 (19^{D6}, 51^{Dd2}, 45^{7G8})

Figure 4.5. The IC_{50} values of para-phenyl substituted electron-withdrawing groups

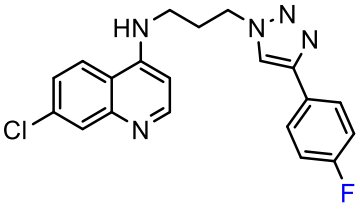
When the halogens are compared, the para-bromophenyl substituted triazole (PL416) showed the best activity against Dd2 strain of *P. falciparum* (IC_{50} of 30.7 nM). The chloro-substituted (PL418) exhibited enhanced activity against both D6 and 7G8 strains (IC_{50} of 18.8 and 36.6 nM respectively) than PL416 and the fluoro-substituted triazole (PL420). The general trend for the halogens indicated that the antimalarial activity increases when electronegativity of the substituent atom decreases.



PL416 (423^{D6}, 31^{Dd2}, 50^{7G8})



PL418 (19^{D6}, 43^{Dd2}, 37^{7G8})



PL420 (40^{D6}, 85^{Dd2}, 80^{7G8})

Figure 4.6. The IC_{50} values comparison of para-halogen substituted triazoles

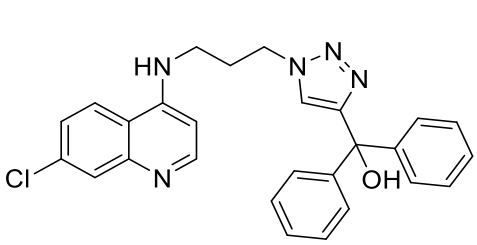
When all the para-phenyl substituted electron-withdrawing groups are compared, the trifluoromethyl and the chloro substitutions showed potent activities, with trifluoromethyl

group possessing the highest. The bromo and nitro groups at para position of the phenyl ring indicated moderate activities, with the bromo group retaining the maximum activity of all against Dd2 strain of *P. falciparum.* The least leaving group, fluorine-substitution, showed the minimum activity of all electron-withdrawing groups.

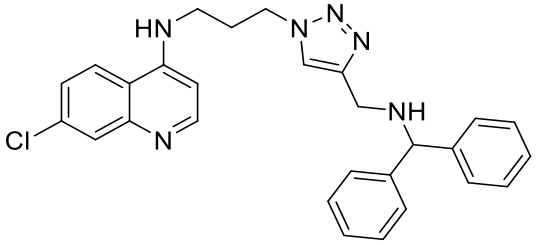
Strains of *Plasmodium falciparum*	**Electron-withdrawing group**
D6	Br < F < NO_2 = Cl < CF_3
Dd2	F < NO_2 < Cl < CF_3 < Br
7G8	F < Br < NO_2 < Cl < CF_3

Table 4.3. Comparison of various electron-withdrawing groups

Surprisingly, the two click compounds that contain two aromatic groups resembling the RA pharmacophore showed poor antimalarial activity. The first compound (PL404) which has a tetra-substituted carbon connecting the triazole ring with two aromatic rings and hydroxyl group, showed better activity only against 7G8 strain of *P. falciparum*. The second click compound (PL423**)** containing a secondary amine between the triazole and two aromatic rings showed poor antimalarial activity against all the strains.



PL404 (25^{D6}, 82^{Dd2}, 48^{7G8})



PL423 (80^{D6}, 160^{Dd2}, 189^{7G8})

Figure 4.7. Click compounds containing pharmacophore similar to the reversal agent

Out of the 18 synthesized first generation click compounds, six were found to be more active than chloroquine against CQ-resistant strains of *P. falciparum*. Although none showed better activity against the CQ-sensitive (D6) strain, the trifluoromethoxy (PL403) and trifluoromethyl (PL419) substituted triazoles showed comparable activity with chloroquine (IC_{50} values of 10.4 and 11 nM respectively).

All six selected compounds have exhibited better activity against Dd2 strain of *P. falciparum* than chloroquine. For the 7G8 strain, five showed an enhanced activity than CQ except the difluoro-phenyl substituted triazole (PL408), which has an IC_{50} value of 95.7 nM when compared to 57 nM of CQ (Refer to Table 4.4).

In conclusion, electron-withdrawing (PL416, PL418, PL419, and PL421) and long-range electron-withdrawing (PL403) substitutions at para positions, and double electron with-drawing (PL408) substitutions were found to be an advantage to have a better antimalarial activity for these group of click compounds.

4.2. Piperazinyl-linked click compounds

"Second generation" of triazole antimalarials were synthesized by incorporating piperazine ring between the triazole and the para-substituted phenyl ring, to yield a protonable nitrogen in the linker which increases the basicity of the molecule. When compared to the first synthesized click compounds, these 9 compounds generally showed enhanced antimalarial activity against all the strains of *P. falciparum*.

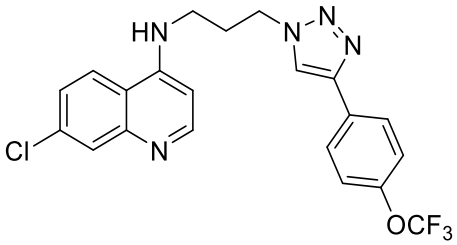
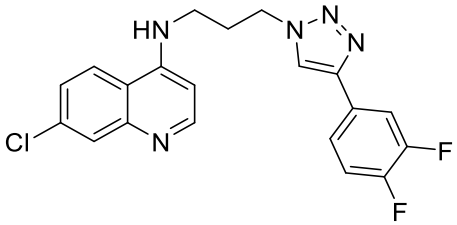
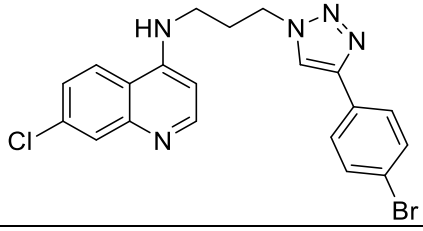
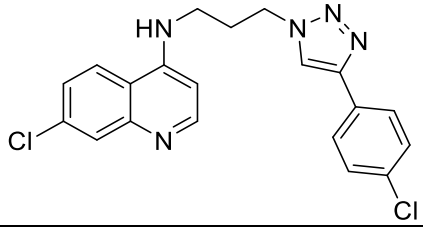
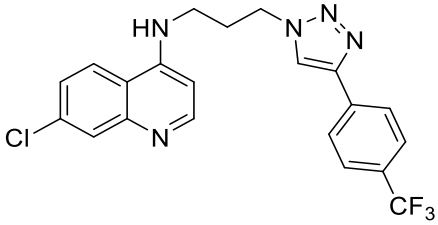
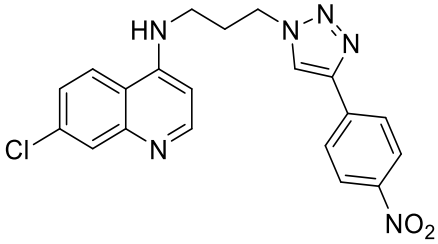
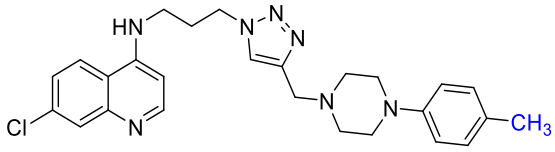
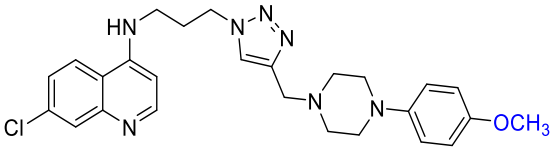
PL No.	Structures	IC_{50} Values		
		D6	Dd2	7G8
CQ	Chloroquine	5.9	54	57
PL403		10.4	12.8	14.5
PL408		22.7	39.1	95.7
PL416		423	30.7	49.6
PL418		18.8	42.7	36.6
PL419		11	31.6	18.7
PL421		18.8	50.5	44.5

Table 4.4. Selected first generation click compounds with better activity than CQ

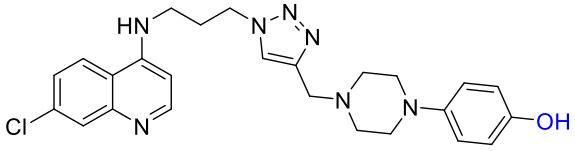
The para-substituted electro-donating groups generally demonstrated better activities than CQ and the first generation triazoles with similar substituents. The para-methyl (PL442) and para-methoxy (PL444) substituted triazoles exhibited an improved activity against Dd2 and 7G8 strains of *P. falciparum* than did CQ. However, the para-hydroxy substituted triazole (PL446) showed poor activity against all the 3 strains of *P. falciparum.*



PL442 (12^{D6}, 37^{Dd2}, 18^{7G8})



PL444 (33^{D6}, 68^{Dd2}, 65^{7G8})



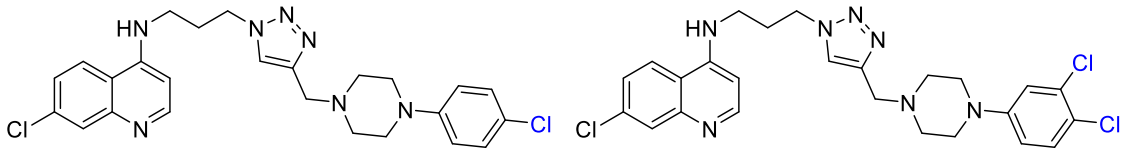
PL446 (155^{D6}, 94^{Dd2}, $>250^{7G8}$)

Figure 4.8. The activity of electron-donating groups substituted at the para position

The 2-pyridyl (PL443) and 2,6-pyrimidyl (PL440) Piperizenyl-substituted triazoles gave poor activity against all the three strains of *P. falciparum* when compared to CQ, with PL440 presenting the lowest activity of all the synthesized triazoles.

As the effect of double substitution of electron-withdrawing groups was shown to improve the antimalarial activity in the first series of compounds using fluorine; the same result was obtained with the piperazinyl-linked triazoles. When the dichloro-substituted

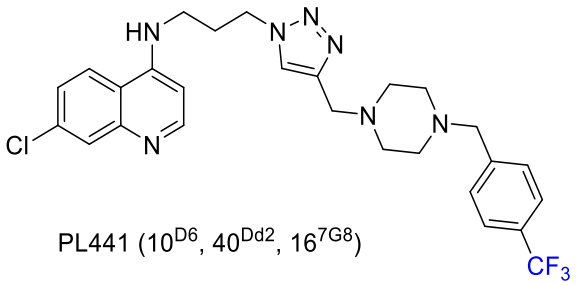
(PL448**)** was compared with monochloro-substituted (PL447) phenyl-piperazinyl triazole, the former showing about 1.6 times increased activity against all the three stains.



PL447 (8.8^{D6}, 26.5^{Dd2}, 19^{7G8}) PL448 (6.8^{D6}, 15.2^{Dd2}, 11^{7G8})

Figure 4.9. The comparison of IC_{50} values of mono and dichloro-substituted triazoles

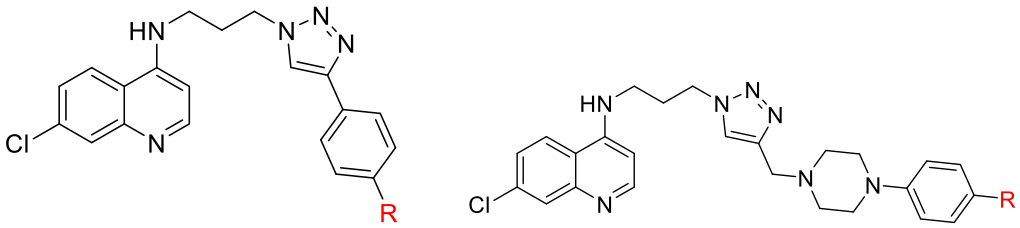
The addition of a single methyl linker between the piperazine and the phenyl ring have shown to retain the antimalarial activity against all the three strains of *P. falciparum* when the phenyl ring was para-substituted by a trifluoromethyl group.



PL441 (10^{D6}, 40^{Dd2}, 16^{7G8})

Figure 4.10. Triazole having methyl linker between piperazine and phenyl rings

Improved antimalarial activity was observed when the first series of triazoles were compared with piperazine linked for the same substituents. This increase in activity is attributed to the integration of the piperazine linker, which provides additional proton for this second generation click triazoles.



R = CH_3,	PL413 (41^{D6}, 61^{Dd2}, 60^{7G8})	PL442 (12^{D6}, 37^{Dd2}, 18^{7G8})
R = CH_3O,	PL417 (41^{D6}, 109^{Dd2}, 140^{7G8})	PL444 (33^{D6}, 68^{Dd2}, 65^{7G8})
R = H,	PL411 (77^{D6}, 88^{Dd2}, 151^{7G8})	PL445 (17^{D6}, 67^{Dd2}, 74^{7G8})
R = Cl,	PL418 (19^{D6}, 43^{Dd2}, 37^{7G8})	PL447 (8.8^{D6}, 26.5^{Dd2}, 19^{7G8})

Figure 4.11. The comparison of the selected first and second generation triazoles for similar substituents

When the electron-donating substituents, methyl and methoxy, were compared, PL442 and PL444 indicated more potency than their respective piperazine omitted counterparts PL413 and PL417 against D6, Dd2 and 7G8 strains. Moreover, PL447, which has an electron-withdrawing chloro-substituent, indicated two-fold activity when compared to PL418. The same trend was observed for the unsubstituted (H-substituted) triazoles against all the three strains (PL411 vs PL445**).**

In summary, six of the nine synthesized piperazinyl-linked triazoles were found to have more potent activity than chloroquine against CQ-resistant strains of *P. falciparum.* Although none of them showed better activity against CQ-sensitive strain, the para-chloro (PL447) and dichloro (PL448) substituted click compounds exhibited comparable activity with chloroquine with an IC_{50} values of 8.8 and 6.8 nM respectively (Refer to table 4.5).

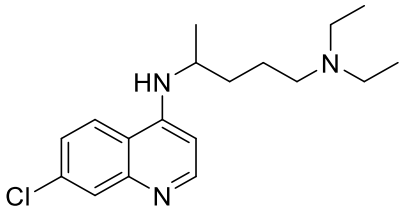
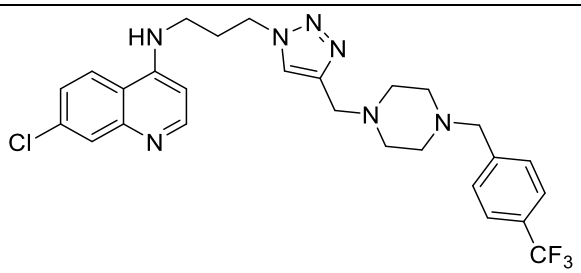
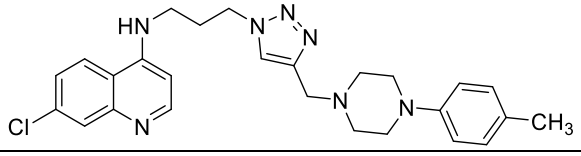
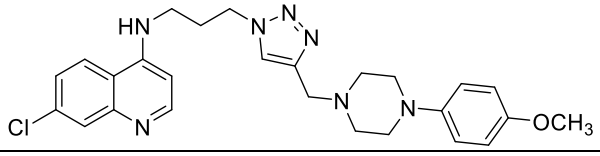
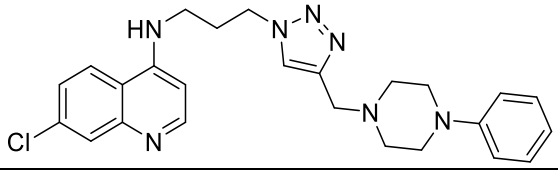
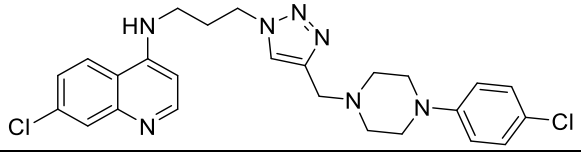
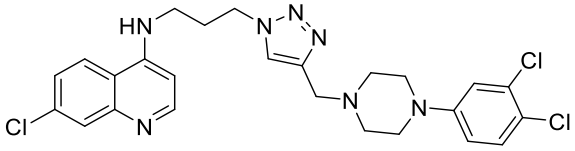
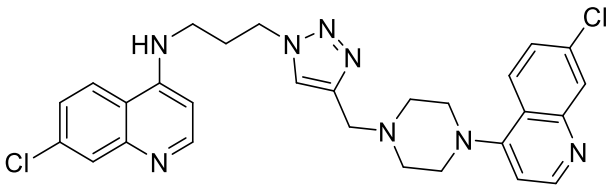
PL No.	Structures	IC_{50} Values		
		D6	Dd2	7G8
CQ		5	69	47
PL441		10	40	16
PL442		12	37	18
PL444		33	68	65
PL445		17	67	74
PL447		8.8	26.5	19
PL448		6.8	15.2	11

Table 4.5. Selected piperazine containing click compounds having better activity than Chloroquine against CQ-resistant strains

All the six selected compounds possessed better potency against Dd2 strain of *P. falciparum* than chloroquine. When tested against 7G8 strain, PL441, PL442, PL447 and PL448 showed enhanced activity but not the para-methoxy (PL444) and the unsubstituted (PL445) triazoles. Generally, the para and di-substitutions with electron-withdrawing groups were found to be beneficial for having better antimalarial activity for these types of piperazine-linked click compounds. PL448 has proved to possess the best activity from all the second generation triazoles.

4.3. The Activity of Triazole-based Bisquinoline

The triazole-based bisquinoline, PL456, synthesized by linking two chloroquinoline moieties by triazole ring, was tested for *in vitro* antimalarial activity against all the three strains of *P. falciparum.* Unlike the other click compounds, it showed excellent activity against CQ-sensitive strain (D6). In addition, PL456 exhibited a potent activity against both CQ-resistant strains (Dd2 and 7G8) when compared to chloroquine.



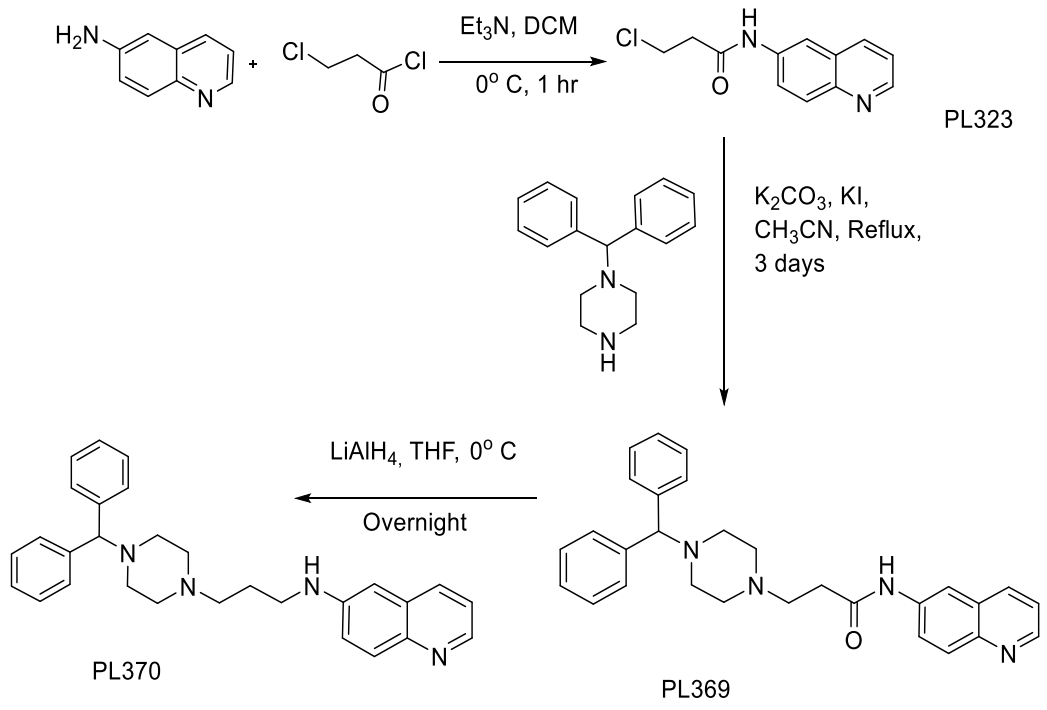
Compounds	**IC_{50} (nM)**		
	D6	**Dd2**	**7G8**
Chloroquine	9	143	127
PL456	0.5	10	11

Table 4.6. The structure and activity of triazole-based bisquinoline

CHAPTER 5: Reversed Chloroquines

5.1. The 6-Aminoquinoline antimalarials

Although the 4- and 8-quinoline substitutions are very common on the quinoline ring, there are few antimalarials containing functional groups on the 6-position. An electron-donating, methoxy group, is a very common substituent on the 6-position of the quinoline ring. Common antimalarial drugs such as quinine and primaquine all have a methoxy functional group substitution on the 6-position but the main linkers are on the 4- and 8-quinoline substitutions respectively.



Scheme 5.1. Synthesis of 6-aminoquinoline antimalarial, PL370

A reversed quinoline hybrid molecule was synthesized in Peyton lab by linking 6-quinoline moiety with benzhydryl-piperazine which has the reversal agent pharmacophore. First, PL323 was prepared by treating 6-aminoquinoline with 3-chloropropionyl-chloride in a cold reaction using triethylamine as a base and dichloromethane as a solvent.[116] The amide was reacted with benzhydryl-piperazine to form PL369**,** which is further reduced using $LiAlH_4$ in cold reaction to get the target molecule, PL370 in a good yield.

When tested for antimalarial activity against D6, Dd2 and 7G8 strains of *P. falciparum*, PL370 showed poor antimalarial activty against both CQ-sensitive and CQ-resistant strains with an IC_{50} value of >250 nm when compared to chloroquine.

5.2. Fluoxetine as Reversal Agent

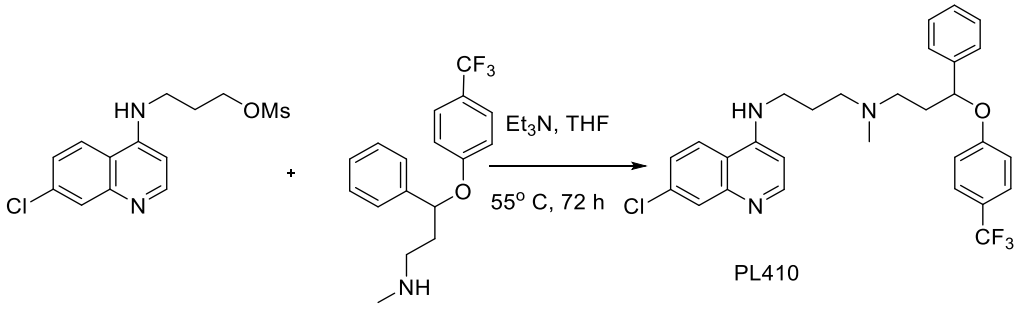
Among the common reversal agents, the SSRI antidepresant, fluoxetine which doesn't possess any intrinsic antimalarial activity when used alone, showed to potentiate the effect of chloroquine. Fluoxetine showed to modulate chloroquine resistance of CQ-resistant W2 clone by 66% at a concentrations of 500 nM with verapamil showing only 61%.[54] In other recent studies, fluoxetine showed to reverse both chloroquine and mefloquine resistance against Dd2 strain of *P. falciparum* although it indicated to be more synergistic with chloroquine than mefloquine.[117]

In addition, fluoxetine has proven to be a highly effective chemosensitizer in multidrug resistance (MDR) for cancer chemotherapy. *In vitro*, fluoxetine enhanced 10-100 fold cytotoxicity of many anticancer drugs in drug-resistant cells by increasing drug

accumulation within MDR-cells and inhibiting drug efflux from those cells. *In vivo*, fluoxetine enhanced 12-fold accumulation of doxorubicin within tumors with unaltered pharmacokinetics.[118]

In other *in vitro* and *in vivo* findings, fluoxetine was found to be a chemosensitizer of the multipump type and considered as a fourth-generation chemosensitizer.[119] Considering the effect of fluoxetine as a better chemosentisizer, reversed chloroquine molecule using fluoxetine as a reversal agent was synthesized in Peyton lab.

The free-base was reacted with PL29 using triethylamine as a base in tetrahydrofuran to give PL410 in moderate yield. The molecule was potent antimalarial and showed about 20-fold activity when compared to chloroquine. It has an IC_{50} values of < 2.5 nm for Dd2 and 7G8 strains of *P. falciparum* while CQ has 54 and 57 nM respectively.

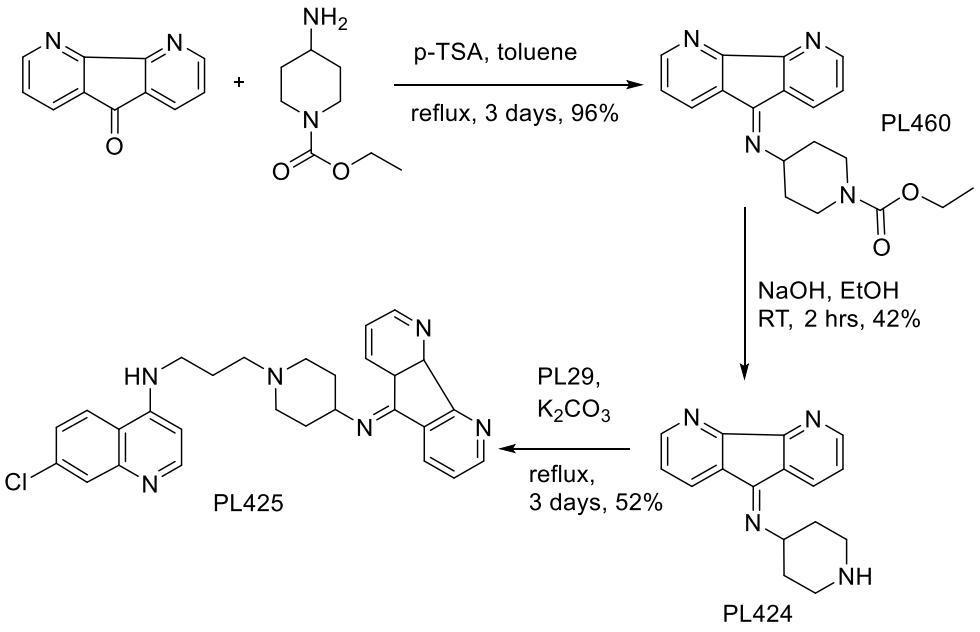


Scheme 5.2. Synthesis of reversed chloroquine using fluoxetine as reversal agent

5.3. Meta-substituted Analogue of PL69

The first hit compound, PL01 which was synthesized as a hybrid drug by using haloquinoline moiety and imipramine as a reversal agent was optimized to increase its hydrophilicity. PL69 was the result of lead optimization that is both potent antimalarial and has better hydrophilicity than the first analogue. In order to see the effect of a substituent on the activity, PL425, which has the nitrogens on the meta positions of the aromatic rings of the reversal agent instead of on the ortho positions was synthesized.

First, 1,8-Diazafluoren-9-one (DFO) was reacted with protected piperidine (Ethyl-4-amino-1-piperidine carboxylate) by using catalytic amount of p-toluenesulfonic acid to



Scheme 5.3. Synthesis of m-substituted analogue of PL69

form PL460 in an excellent yield. The piperidine was deprotected in basic condition by treating with NaOH in ethanol to prepare the intermediate product PL424**.** Finally, the

reversal agent was reacted with PL29 using potassium carbonate base to give the target compound, PL425 in a good yield.

The meta-substituted compound showed better antimalarial activity than CQ, only against Dd2 strain of *P. falciparum* with an IC_{50} value 32.5 nM when compared to 54 nM of chloroquine. However, for the D6 and 7G8 strains, PL425 was found to be less active than CQ.

CHAPTER 6: Summary and Conclusion

Malaria is considered as one of the most prevalent and debilitating diseases affecting humans. There are 3.4 billion people at risk of malaria transmission with more than half a million deaths, many of them are children. *Plasmodium falciparum* is the most virulent form and responsible for about 80% of human malaria worldwide. The *plasmodium* parasite developed resistance to several antimalarial drugs.

Chloroquine is one of the most successful antimalarials developed that is effective, cheap, and safe to children and pregnant women. However, due to the emergence of drug resistance by *P. falciparum*, its use has been limited. In order to circumvent chloroquine resistance, reversed chloroquines that reverse the resistance by inhibiting the efflux of chloroquine were designed in Peyton lab. These hydrid drugs has been found to be potent against both chloroquine-sensitive and resistant strains of *P. falciparum* both *in vitro* and *in vivo*.

It's a challenging task for medicinal chemists to develop new patentable drugs with strong activity and good pharmacokinetic properties in short time. Click chemistry has shown to have a cutting-edge advantage in demanding world of medicinal chemistry in hit finding and lead optimization due to its reliance on extremely quick reactions. Particularly, the copper(I)-catalyzed cycloaddition reaction between azides and terminal alkynes to give 1,4-disubstituted-1,2,3-triazoles has become the benchmark of 'click chemistry' by its reliability, selectivity and biocompatibility.

The incorporation of 1,2,3-Triazoles into pharmacologically active molecules became more popular after the introduction of copper-catalyzed click chemistry. The stabilty to acid-base hydrolysis, oxidative-reductive conditions and metabolic degradation make triazoles not only good passive linkers but also excellent candidates as pharmacophores and bioisosters. Moreover, these heterocycles readily associate with biological targets via H-bonding and dipole–dipole interactions.

Series of click compounds were synthesized via copper(I)-catalyzed alkyne-azide cycloaddition reaction by modifying the aliphatic chains terminal of chloroquine. Three aliphatic linear chains as linker between the chloroquinoline and the triazole rings were chosen as optimum length both interms of antimalarial activity and hydrophilicity. The 28 antimalarial click compounds were tested for their *in vitro* activity against D6, Dd2, and 7G8 strains of *P. falciparum*.

The first 18 click compounds was synthesized by reacting the azide analogue with commercially available terminal alkynes. The first generation click compounds synthesized contain the triazole ring connected directly to one or two aromatic rings with different substitutions. Six of the triazole-based click compunds showed better activity against chloroquine resistant, Dd2 and 7G8, strains of *P. falciparum*. Particularly, PL403 showed about 4 times better activity than chloroquine with an IC_{50} values of 12.8 and 14.5 nM against CQ-resistant Dd2 and 7G8 strains respectively.

The second generation triazole antimalarials were synthesized by incorporating piperazine ring between the triazole and the p-substituted phenyl ring to have a

protonable nitrogen which increases the basicity of the molecule. The modification has brought an enhanced antimalarial activity when compared to the first series of compounds. Six of the nine synthesized piperazinyl-linked triazoles were found to have potent activity than chloroquine against CQ-resistant strains. The dichloro substituted triazole, PL448**,** has proved to possess an excellent activity with IC_{50} values of 15.2 (Dd2) and 11 (7G8) nM.

In addition to click compounds, other reversed chloroquines were synthesized. A reversed quinoline hybrid molecule was synthesized by linking 6-quinoline moiety with benzhydryl-piperazine as a reversal agent. When tested for antimalarial activity, PL370 showed poor antimalarial activity against all strains of *P. falciparum.* By considering the effect of an SSRI antidepressant, fluoxetine (Prozac), as a very good chemosentisizer in reversing both chloroquine and mefloquine resistance, PL410 was synthesized. This hit was found to be about 20-fold potent than chloroquine with IC_{50} values of < 2.5 nM for Dd2 and 7G8 strains of *P. falciparum*.

In conclusion, the efficiency of synthesizing several triazole-based antimalarials have proven click chemistry to be fast and efficient reaction. Several of the synthesized triazole-based antimalarials possessed better activity against chloroquine resistant strains than chloroquine. Generally, para-substitutions and di-substitutions with short-range and long-range electron-withdrawing groups were found to be beneficial for having better antimalarial activity for these group of click compounds.

CHAPTER 7: Experimental Materials and Methods

7.1. Instrumentation

All starting chemicals were purchased from Sigma-Aldrich Chemical Co. and TCI America. Nomenclature was done using ChemBioDraw Ultra. Thin layer chromatography was mostly done on Alumina basic column using ethyl-acetate:methanol (90:10) as mobile phase and visualized by UV lamp (254 nm). The purification was commonly done by CombiFlash® Rf-200 chromatography using silica column at UV absorbance of 254 and 280 nM with solid loading, and collecting only the peaks. The eluents used are chloroform:methanol (90:10) for target compunds and hexane:ethyl-acetate for intermediate compounds.

The compounds were characterized by NMR and Mass spectroscopy. ^{1}H-, ^{13}C-, COSY, HSQC, HMBC and NOESY experiments were run on Bruker 400 MHz instrument, using the standard pulse sequences provided at 25^{0}C. High resolution mass spectrometry was performed on Bruker micrOTOF-Q instrument. Results were obtained using electospray ionization (ESI) in the positive mode, at a flow rate of 0.4 mL/min with methanol or acetonitrile.

7.2. General Synthetic Procedures

PL16 (3-((7-chloroquinolin-4-yl)amino)propan-1-ol)

Purified 4,7-dichloroquinoline (1eq, 5 g, 25.2 mmol) and 3-amino-1-propanol (12.3 eq, 23.7 ml, 309.9 mmol) was healted slowly for 1 hour at 70^{o} C and the temperature was

increased to 135^{o} C and stirred for 7 additional hours. After the completion was confirmed by TLC, the reaction mixture was poured to ice-cold H_2O (100ml). The white precipitate formed was filtered by vaccum filtration, washed with water and dried. After drying, the solid was recrystallized using EtOAC (50ml). The solution was filtered, and after cooling down, vaccum-filtered and washed with water to give pure, white-yellowish solid (5.02 g, 84%). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.39 (d, J = 5.5 Hz, 1H), 8.25 (d, J = 9.0 Hz, 1H), 7.78 (d, J = 2.2 Hz, 1H), 7.44 (dd, J = 9.0, 2.3 Hz, 1H), 7.30 (t, J = 5.4 Hz, 1H), 6.48 (d, J = 5.4 Hz, 1H), 3.55 (t, J = 6.2 Hz, 2H), 3.39 – 3.28 (m, 7H), 1.88 – 1.76 (m, 2H).

PL28 (2-((7-chloroquinolin-4-yl)amino)ethan-1-ol)

A mixture of purified 4,7-dichloroquinoline (1 eq, 5 g, 25.25 mmol) and ethanolamine (10 eq, 15.24 ml, 252.5 mmol) was heated with stirring overnight at 130 ^{o}C. After the reaction mixture was cooled to RT, it was poured to pre-cooled water (150 ml). The white precipitate formed was filtered and dried. After drying, the solid was recrystallized by methanol (100 ml), cooled in ice and filtered by vaccum-filtration to give a pure white solid (4.48 g, 80 %). ^{1}H NMR (600 MHz, DMSO-d6) δ 8.39 (d, J = 5.4 Hz, 1H), 8.27 (d, J = 9.0 Hz, 1H), 7.79 (d, J = 2.3 Hz, 1H), 7.45 (dd, J = 9.0, 2.4 Hz, 1H), 7.27 (t, J = 5.6 Hz, 1H), 6.50 (d, J = 5.5 Hz, 1H), 3.67 (t, J = 6.1 Hz, 2H), 3.36 (q, J = 5.8 Hz, 2H).

PL29 (3-((7-chloroquinolin-4-yl)amino)propyl methanesulfonate)

To a suspension of PL16 (1 eq, 5 g, 21.2 mmol) in anhydrous THF (100ml) stirring under N_2 and cooled in an ice-bath, triethylamine (2 eq, 5.9 ml, 42.3 mmol) was added. After

the mixture was cooled to 0 ^{o}C, methane-sulfonyl chloride (1.3 eq, 2.13 ml, 27.6 mmol) was added very slowly keeping the temperature below 10 ^{o}C and the reaction was stirred for 1 hour. After the completion was confirmed by TLC, work-up was done by liquid-liquid extraction by adding saturated $NaHCO_3$ (100ml). After separating the organic layer, the aqueous layer was extracted with diethyl-ether (200ml and then 2*100ml). The orgainic layers were mixed and dried by anhydrous $MgSO_4$, concentrated by rotovap and dried to give white-yellowish solid (5.4 g, 82%). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.43 (d, J = 5.3 Hz, 1H), 8.28 (d, J = 9.0 Hz, 1H), 7.82 (d, J = 2.2 Hz, 1H), 7.48 (dd, J = 9.0, 2.3 Hz, 1H), 7.36 (t, J = 5.5 Hz, 1H), 6.52 (d, J = 5.4 Hz, 1H), 4.37 (t, J = 6.2 Hz, 2H), 3.51 – 3.33 (m, 2H), 3.21 (s, 3H), 2.10 (p, J = 6.5 Hz, 2H).

PL30 (2-((7-chloroquinolin-4-yl)amino)ethyl methanesulfonate)

PL28 (1 eq, 5 g, 22.5 mmol) was dissolved in dry acetone (100 ml) and triethylamine (1.5 eq, 4.7 ml, 33.7 mmol) was added. The mixture was chilled to 0 ^{o}C in an ice-bath and the reaction was carried under N_2. Methane-sulfonylchloride (1.2 eq, 2.1 ml, 27 mmol) was added drop-wise keeping the temperature below 10 ^{o}C. The reaction was continued to run with stirring for 5 hours. After the completion, the solid was filtered and the solvent was evaporated. The two solids were combined and washed by saturated $NaHCO_3$ (100ml). The orgainic layer was dried by anhydrous $MgSO_4$, filtered and concentrated to give white solid (5.8 g, 85%). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.44 (d, J = 5.4 Hz, 1H), 8.26 (d, J = 9.0 Hz, 1H), 7.85 – 7.76 (m, 1H), 7.55 – 7.43 (m, 2H), 6.60 (d, J = 5.5 Hz, 1H), 4.44 (t, J = 5.4 Hz, 2H), 3.68 (q, J = 5.4 Hz, 2H), 3.17 (s, 3H).

PL49 (7-chloro-4-(piperazin-1-yl)quinoline)

4,7-dichloroquinline (1 eq, 2.5 g, 12.62 mmol) and anhydrous piperazine (7 eq, 7.6 g, 88.36 mmol) were refluxed in ethanol (37.5 ml) for 12 hours. After completion, the solvent was evaporated and the solid was dissolved in dichloromethane (50 ml). The solution was washed with saturated $NaHCO_3$ (4 * 25 ml). Finally the organic layer was separated, dried by anhydrous $MgSO_4$, filtered and concentrated. The compound was pure enough to be used in the next step without purification (2.62 g, 84 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (d, J = 5.0 Hz, 1H), 8.03 (d, J = 2.1 Hz, 1H), 7.95 (d, J = 8.9 Hz, 1H), 7.42 (dd, J = 9.0, 2.1 Hz, 1H), 6.83 (d, J = 5.0 Hz, 1H), 3.23 – 3.12 (m, 9H), 1.79 (s, 1H, NH).

PL107 (N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine)

4,7-dichloroquinoline (1 eq, 2.3 g, 11.61 mmols) and 1,3-diaminopropane (4.4 eq, 4.27 ml, 51.1 mmols) were stirred neatly for 1.5 hours at 80 ^{o}C and 6 hours at 145 ^{o}C. After the completion of the reaction was confirmed by TLC, it was cooled to room temperature. NaOH (10%, 7.2 ml) was added and the mixture was stirred on a sonicator. The precipitate was filtered by vaccum-filtration and washed with excess water and dried to give white solid (2.5 g, 91 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.38 (t, J = 5.3 Hz, 1H), 8.27 (dd, J = 21.6, 9.1 Hz, 1H), 7.78 (d, J = 2.3 Hz, 1H), 7.44 (ddd, J = 8.8, 6.1, 2.3 Hz, 1H), 6.49 (dd, J = 16.2, 5.4 Hz, 1H), 3.43 (q, J = 6.4 Hz, 1H), 3.33 (t, J = 6.9 Hz, 2H), 3.07 (s, 2H), 2.70 (t, J = 6.5 Hz, 2H), 1.75 (p, J = 6.7 Hz, 2H).

PL190 (N-(piperidin-4-yl)-1,1-di(pyridin-2-yl)methanimine)

2, 2'-dipyridyl-ketone (1 eq, 3.0 g, 16.3 mmol) was dissolved in toluene (70 ml). 4-aminopiperidine (1.15 eq, 1.98 ml, 18.7 mmol) was added, followed by p-toluenesulfonic-acid (0.1 g). The reaction was refluxed while stirring and using dean-stark apparatus to continuously remove the water by-product. After the completion of the reaction, the solvent was evaporated and purified by combi-flash chromatography on silica column (1.27 g, 29 %). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.72 (dt, J = 5.0, 1.3 Hz, 1H), 8.55 – 8.46 (m, 1H), 8.15 (dt, J = 8.0, 1.2 Hz, 1H), 7.77 (dtd, J = 26.2, 7.8, 1.8 Hz, 2H), 7.38 – 7.09 (m, 3H), 3.39 (tt, J = 9.4, 4.5 Hz, 1H), 3.18 – 3.02 (m, 2H), 2.79 – 2.48 (m, 2H), 1.87 – 1.65 (m, 4H).

PL294 (7-chloro-N-(piperidin-4-yl)quinolin-4-amine)

4,7-dichloroquinoline (1 eq, 5 g, 25 mmol) and 4-aminopiperidine (5 eq, 13.4 ml, 126 mmol) in N,N-diisopropylethylamine (100 ml) was heated at 100 ^{o}C for 20 hours. The DIPEA was evaporated and the crude was purified by combi-flash chromatography in silica column and using DCM:MeOH:Et_3N (8.9:1:0.1) as eluents to give a pure solid (3.10 g, 47 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.62 (d, J = 5.2, 1H), 7.96 (d, J = 2.0, 1H), 7.85 (d, J = 8.9, 1H), 7.35 (dd, J = 8.9, 2.0, 1H), 6.75 (d, J = 5.2, 1H), 5.24 (s, 1H), 3.48 (d, J = 12.0, 2H), 2.89 (m, 1H), 2.82 (t, J = 11.6, 2H), 1.97 (d, J = 12.0, 2H), 1.61 (m, 4H).

PL323 (3-chloro-N-(quinolin-6-yl)propanamide)

6-aminoquinoline (1 eq, 5.0 g, 34.68 mmol) and triethylamine (1.2 eq, 5.8 ml, 41.62 mmols) were mixed in anhydrous dichloromethane (40 ml) on ice-bath. 3-Chloropropionyl-chloride (1.05 eq, 3.47 ml, 36.41 mmols) was added dropwise keeping the temperature below 10 ^{o}C. The reaction was stirred for 1 hour. Saturated $NaHCO_3$ (25 ml) was added and the aqueous layer was extracted by dichloromethane (3 * 10ml). The organic layer was dried by anhydrous $MgSO_4$, filtered and dried. The crude was purified by combi-flash chromatography using silica column and EtOAC:MeOH as eluents to give a yellow solid (2.49 g, 50 %). ^{1}H NMR (400 MHz, Chloroform-d) δ ^{1}H NMR (400 MHz, DMSO-d6) δ 10.66 (s, 1H), 8.81 (dd, J = 4.2, 1.7 Hz, 1H), 8.41 – 8.29 (m, 2H), 8.00 (d, J = 9.0 Hz, 1H), 7.81 (dd, J = 9.1, 2.4 Hz, 1H), 7.50 (dd, J = 8.3, 4.2 Hz, 1H), 3.93 (t, J = 6.3 Hz, 2H), 2.90 (t, J = 6.3 Hz, 2H).

PL326 (N-(3-chloropropyl)quinolin-6-amine)

PL323 (1 eq, 1.87 g, 7.97 mmol) was dissolved in tetrahydrofuran (20ml). $LiAlH_4$ in 1M THF (3eq, 23.9ml, 23.9 mmol) was slowly added under N_2 in an ice-bath. An additional 30ml of THF was added and the reaction was allowed to stir overnight . After completion was confirmed by TLC, the reaction mixture was quenched with EtOAc (20ml) and MeOH (20ml), and then by citric acid solution. After filtering the yellow precipitate, the solution was extracted using diethyl-ether (2 * 20ml). The organic layer was separated, dried by anhydrous $MgSO_4$, filtered and concentrated to get a brownish-oily compound (1.01g, 57.4%). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.61 – 8.53 (m, 1H), 7.94 – 7.87

(m, 1H), 7.84 (d, J = 9.1 Hz, 1H), 7.23 (dt, J = 8.3, 4.2 Hz, 1H), 7.06 (dd, J = 9.1, 2.6 Hz, 1H), 6.66 (dd, J = 8.0, 2.6 Hz, 1H), 3.17 (t, J = 7.2 Hz, 2H), 1.72 – 1.66 (m, 2H), 1.04 (t, J = 7.4 Hz, 2H).

PL334 (2-chloro-N-(quinolin-6-yl)acetamide)

6-aminoquinoline (1 eq, 1.0 g, 6.94 mmol) and triethylamine (1.2 eq, 1.16 ml, 14.15 mmols) were mixed in anhydrous dichloromethane (40 ml) on ice-bath. Chloro-acetylchloride (1.05 eq, 0.58 ml, 7.29 mmols) was added dropwise keeping the temperature below 10^{o}C. The reaction was stirred for 1 hour. Saturated $NaHCO_3$ (25 ml) was added and the aqueous layer was extracted by DCM (3 * 10ml). The organic layer was dried by anhydrous $MgSO_4$, filtered and dried. The crude was purified by combi-flash chromatography using silica column and EtOAC:MeOH as eluents to give a pure solid (1.10 g, 66 %). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.87 (dd, J = 4.2, 1.7 Hz, 1H), 8.53 (s, 1H), 8.35 (d, J = 2.4 Hz, 1H), 8.18 – 8.13 (m, 1H), 8.10 (d, J = 9.0 Hz, 1H), 7.65 (dd, J = 9.0, 2.5 Hz, 1H), 7.42 (dd, J = 8.3, 4.2 Hz, 1H), 4.26 (s, 2H).

PL369 (3-(4-benzhydrylpiperazin-1-yl)-N-(quinolin-6-yl)propanamide)

PL323 (1eq, 2.0g, 8.53 mmol) was dissolved in acetonitrile (40 ml). 1-benzhydrylpiperazine (1.05 eq, 2.26 g, 8.96 mmol), anhydrous K_2CO_3 (2.4 eq, 2.97 g, 21.50 mmol) and KI (1 eq, 1.42 g, 8.53 mmol) were all added to the solution. The reaction was refluxed with stirring for 3 days. After completion was confirmed, the acetonitrile solvent was evaporated and the slurry was taken up by EtOAC (20 ml) and water (40 ml). The aqueous phase was extracted by EtOAC (2 * 10 ml) and the organic

layer was washed with saturated $NaHCO_3$ (15 ml), and then washed with brine (20 ml). The organic layer was dried by anhydrous $MgSO_4$, filtered and concentrated. The crude was purified by combi-flash chromatography using silica column and and EtOAC:MeOH (85:15) as eluents to give a yellowish solid (1.6 g, 80 %). ^{1}H NMR (400 MHz, Chloroform-d) δ 11.41 (s, 1H), 8.81 (dd, J = 4.3, 1.7 Hz, 1H), 8.44 (d, J = 2.3 Hz, 1H), 8.14 – 8.09 (m, 1H), 8.02 (d, J = 9.0 Hz, 1H), 7.48 – 7.43 (m, 4H), 7.41 – 7.34 (m, 2H), 7.31 (dd, J = 8.3, 6.8 Hz, 4H), 7.25 – 7.19 (m, 2H), 4.38 (s, 1H), 2.50–2.81 (ddd, J = 80.7, 7.0, 5.2 Hz, 8H). ^{13}C NMR (101 MHz, Chloroform-d) δ 171.0, 149.1, 145.3, 142.1, 136.6, 135.9, 130.1, 128.6, 128.0, 127.2, 123.1, 121.5, 115.6, 75.9, 53.6, 52.6, 52.0, 32.5. m/z 450.25 M + H (calculated 450.59).

PL370 (N-(3-(4-benzhydrylpiperazin-1-yl)propyl)quinolin-6-amine)

PL369 (1 eq, 1.6 g, 3.66 mmol) was dissolved in tetrahydrofuran (20 ml). Under N_2, $LiAlH_4$ in 2.5M THF (5 eq, 7.33 ml, 18.31 mmol) was added into the reaction mixture. The reaction was allowed to run at 0 ^{o}C overnight. After completion, the solution was quenched by citric acid solution. To the yellow-greenish suspension formed, NaOH (2.75 M, 1 ml) and saturated $NaHCO_3$ (20 ml) was added, and the aqueous phase was extracted with diethylether (2 * 15 ml). The organic phase was washed with water (10 ml) and salted out with brine (10 ml). Finally, the organic layer was dried by anhydrous $MgSO_4$, filtered and evaporated to give a brownish, oily solid. The crude (0.76 g) was purified by combi-flash chromatography by dissolving in acetone and small amount of methanol. Silica gel sorbent (7.6 g) was added and the suspension was stirred on sonicator before evaporating the solvent. The solid was purified in silica column (40 g) using

EtOAC:MeOH (85:15) as eluents by collecting only the peaks for 20 minutes twice (0.42 g, 56 %). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.58 (dd, J = 4.3, 1.7 Hz, 1H), 7.91 – 7.81 (m, 2H), 7.47 – 7.39 (m, 4H), 7.32 – 7.15 (m, 8H), 7.02 (dd, J = 9.1, 2.6 Hz, 1H), 6.62 (d, J = 2.6 Hz, 1H), 4.28 (s, 1H), 3.26 (t, J = 6.2 Hz, 2H), 2.61 – 2.34 (m, 10H), 1.84 (p, J = 6.3 Hz, 2H). ^{13}C NMR (101 MHz, Chloroform-d) δ 149.1, 145.3, 142.1, 136.6, 135.9, 130.1, 128.6, 128.0, 127.2, 123.1, 121.5, 115.6, 75.9, 53.6, 52.6, 52.0, 41.1, 32.5. m/z 436.27 M + H (calculated 436.60).

PL403 (7-chloro-N-(3-(4-(4-(trifluoromethoxy)phenyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

To a stirred solution of PL409 (1 eq, 0.16 g, 0.61 mmol), 4-(trifluoromethoxy)phenylacetylene (1.07 eq, 0.1 ml, 0.65 mmol) in *tert*-butanol (7 ml), was added a solution of $CuSO_4$. $5H_2O$ (0.2 eq, 0.030 g, 0.12 mmol) and sodium ascorbate (0.4 eq, 0.048 g, 0.24 mmol) in water (7 ml), and the mixture was stirred for 4 hours at 40 ^{o}C. After completion, $CHCl_3$ (20 ml) was added and the mixture was washed with water (2 * 100 ml). Finally the organic layer was dried by anhydrous $MgSO_4$, filtered and concentrated to give a white solid (0.08 g, 30 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.68 (s, 1H), 8.41 (s, 1H), 8.26 (d, J = 9.0 Hz, 1H), 7.99 – 7.90 (m, 2H), 7.79 (s, 1H), 7.50 – 7.41 (m, 3H), 7.36 (t, J = 5.4 Hz, 1H), 6.49 (d, J = 4.9 Hz, 1H), 4.58 (t, J = 6.9 Hz, 2H), 3.37 (d, J = 6.5 Hz, 2H), 2.30 (p, J = 6.9 Hz, 2H), 13C NMR (101 MHz, DMSO-d6) δ 145.0, 133.4, 130.2 ,126.9 , 124.1, 122.0, 121.6, 47.7, 40.1, 28.3. m/z 447.11 M + H (calculated 447.85).

PL404 ((1-(3-((7-chloroquinolin-4-yl)amino)propyl)-1H-1,2,3-triazol-4-yl)diphenylmethanol)

To a stirred solution of PL409 (1 eq, 0.50 g, 1.9 mmol), 1,1-diphenyl-2-propyn-1-ol (1.07 eq, 0.42 g, 2.84 mmol) in *tert*-butanol (23 ml), a solution of $CuSO_4$. $5H_2O$ (0.2 eq, 0.095 g, 0.38 mmol) and sodium ascorbate (0.4 eq, 0.15 g, 0.76 mmol) in water (23 ml) was added, and the mixture was stirred for 3 hours at 40 ^oC. After completion, the reaction mixture was cooled to RT and taken by $CHCl_3$ (60 ml) and washed with water (3 * 300 ml). Finally the organic layer was dried by anhydrous $MgSO_4$, filtered and concentrated The crude was purified using combi-flash chromatography in silica column by eluting using $CHCl_3$:MeOH (90:10) to give a pure white-yellowish solid (0.35 g, 41 %). ^1H NMR (400 MHz, DMSO-d6) δ 8.37 (d, J = 5.4 Hz, 1H), 8.26 (d, J = 9.0 Hz, 1H), 7.88 (s, 1H), 7.79 (d, J = 2.3 Hz, 1H), 7.46 (dd, J = 9.0, 2.3 Hz, 1H), 7.40 – 7.17 (m, 10H), 6.54 (s, 1H), 6.40 (d, J = 5.4 Hz, 1H), 4.51 (t, J = 6.9 Hz, 2H), 3.33 – 3.24 (m, 2H), 2.22 (p, J = 7.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 153.9 , 151.9 , 149.9 , 149.0 , 147.1 , 133.4 , 127.5 (d, J = 3.6 Hz), 127.0 , 126.6 , 124.1 (d, J = 3.8 Hz), 123.4 , 117.5 , 98.6 , 75.7 , 59.7 , 47.2 , 38.9 , 28.5 , 14.1. m/z 469.17 M + H (calculated 469.97).

PL405 (7-chloro-N-(3-(4-(2-methoxyphenyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

To a stirred solution of PL409 (1 eq, 0.50 g, 1.9 mmol), 2-ethynylanisole (1.07 eq, 0.26 ml, 2.04 mmol) in *tert*-butanol (19 ml), was added $CuSO_4$. $5H_2O$ (0.2 eq, 0.095 g, 0.38 mmol) and sodium ascorbate (0.4 eq, 0.15 g, 0.76 mmol) in water (19 ml), and the mixture was stirred for 3 hours at 40 ^oC. After completion, the crude mixture was taken

by $CHCl_3$ (25 ml) and washed with water (3 * 120 ml). Finally the organic layer was dried by anhydrous $MgSO_4$, filtered and concentrated. The crude was purified using combi-flash chromatography in silica column by eluting using $CHCl_3$:MeOH (90:10) to give a pure solid (0.54 g, 72 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.42 (s, 1H), 8.34 – 8.26 (m, 1H), 8.13 (dd, J = 7.7, 1.8 Hz, 1H), 7.81 (s, 1H), 7.45 (dd, J = 13.8, 7.3 Hz, 2H), 7.33 (td, J = 8.0, 1.8 Hz, 1H), 7.16 – 6.99 (m, 3H), 6.51 (s, 1H), 4.58 (t, J = 6.9 Hz, 2H), 3.89 (s, 3H), 3.34 (q, J = 6.4 Hz, 2H), 2.29 (p, J = 6.7 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 155.3 , 141.7 , 133.5 , 128.8 , 126.5 , 124.2 , 124.0 , 120.6 , 119.1 , 111.5 , 55.4 , 47.4 , 40.1 , 28.5. m/z 393.14 M + H (calculated 393.88).

PL406 (7-chloro-N-(3-(4-(3-methoxyphenyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

To a stirred solution of PL409 (1 eq, 0.50 g, 1.9 mmol), 3-ethynylanisole (1.07 eq, 0.26 ml, 2.04 mmol) in *tert*-butanol (19 ml), was added $CuSO_4$. $5H_2O$ (0.2 eq, 0.095 g, 0.38 mmol) and sodium ascorbate (0.4 eq, 0.15 g, 0.76 mmol) in water (19 ml) was added and the mixture was stirred for 3 hours at 40 ^{o}C. After completion, the crude mixture was taken by $CHCl_3$ (25 ml) and washed with water (3 * 120 ml). Finally the organic layer was dried by anhydrous $MgSO_4$, filtered and concentrated. The crude was purified using combi-flash chromatography in silica column by eluting using $CHCl_3$:MeOH (90:10) to give a pure white-yellowish solid (0.38 g, 51 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.64 (s, 1H), 8.29 (d, J = 8.9 Hz, 1H), 7.81 (s, 1H), 7.54 – 7.31 (m, 5H), 6.90 (ddd, J = 8.1, 2.6, 1.2 Hz, 1H), 6.53 (s, 1H), 4.56 (t, J = 6.9 Hz, 2H), 3.81 (s, 3H), 3.36 (q, J = 6.5 Hz, 2H), 2.30 (p, J = 7.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 159.6 , 150.2 , 146.2 ,

133.7 , 132.1 , 130.0 , 124.2, 121.7 , 117.4 , 113.5 , 110.3 , 55.1 , 47.6 , 38.9 , 28.3. m/z 393.14 M + H (calculated 393.88).

PL407 (7-chloro-N-(3-(4-(2-fluorophenyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

To a stirred solution of PL409 (1 eq, 0.50 g, 1.9 mmol), 2-fluorophenylacetlene (1.07 eq, 0.24 ml, 2.04 mmol) in tert-butanol (15 ml), was added $CuSO_4$. $5H_2O$ (0.2 eq, 0.095 g, 0.38 mmol) and sodium ascorbate (0.4 eq, 0.15 g, 0.76 mmol) in water (15 ml) was added and the mixture was stirred for 3 hours at 40 ^oC. After completion, the mixture of solvents was evaporated and the solid was purified using combi-flash chromatography in silica column by eluting using $CHCl_3$:MeOH (90:10) to give a pure white-yellowish solid (0.10 g, 26 %). ^1H NMR (400 MHz, DMSO-d6) δ 8.50 (d, J = 3.7 Hz, 1H), 8.41 (d, J = 5.3 Hz, 1H), 8.26 (dd, J = 9.0, 5.6 Hz, 1H), 8.13 (td, J = 7.6, 1.7 Hz, 1H), 7.79 (d, J = 2.4 Hz, 1H), 7.45 (dd, 1H), 7.40 (dt, 1H), 7.34(t, 3H), 6.48 (dd, J = 11.0, 5.4 Hz, 1H), 4.62 (t, J = 6.7 Hz, 2H), 3.32 (s, 2H), 2.30 (p, J = 6.8 Hz, 2H).^{13}C NMR (101 MHz, DMSO-d6) δ 151.9, 149.9, 149.0 , 133.4 , 127.4, 124.9 , 124.1 , 117.5 , 98.7 , 48.5 , 47.6 , 40.1 , 28.4 , 27.1. m/z 381.12 M + H (calculated 381.84).

PL408 (7-chloro-N-(3-(4-(3,4-difluorophenyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

To a stirred solution of PL409 (1 eq, 0.50 g, 1.9 mmol), 3,4-difluorophenylacetlene (1.07 eq, 0.25 ml, 2.04 mmol) in *tert*-butanol (15 ml), $CuSO_4$. $5H_2O$ (0.2 eq, 0.095 g, 0.38 mmol) and sodium ascorbate (0.4 eq, 0.15 g, 0.76 mmol) in water (15 ml) was added and the mixture was stirred for 3 hours at 40 ^oC. After completion, the mixture of solvents

was evaporated and the solid was purified using combi-flash chromatography in silica column by eluting using $CHCl_3$:MeOH (90:10) to give a pure white-yellowish solid (0.58 g, 76 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.66 (s, 1H), 8.40 (d, 1H), 8.24 (d, 1H), 7.84 (dt, 1H), 7,78 (d, 1H), 7.67 (dtd, J = 10.6, 4.6, 3.6, 1.9 Hz, 1H), 7.60 – 7.29 (m, 3H), 6.49 (d, 1H), 4.56 (t, 2H), 3.36 (dd, J = 14.3, 7.7 Hz, 2H), 2.29 (p, J = 7.2 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 151.9 , 149.9, 148.9, 133.4, 127.5 , 124.1, 122.1 , 118.2 , 98.8 , 47.7 , 40.1 , 28.2. m/z 399.11 M + H (calculated 399.83).

PL409 (N-(3-azidopropyl)-7-chloroquinolin-4-amine)

PL29 (1 eq, 7.0 g, 22.2 mmol) was dissolved in anhydrous dimethylformamide (100ml). Sodium azide (2 eq, 2.9 g, 44.5 mmol) was added and the mixture was stirred at 55 ^{o}C for 12 hours. After completion, De-ionized water (135 ml) was added and the aqueous layer was extracted with toluene (3*330ml). The organic layer was dried with brine (130ml), dried by anhydrous $MgSO_4$, filtered and evaporated to give yellow solid (5.1 g, 88 %). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.55 (d, J = 5.3 Hz, 1H), 7.96 (d, J = 2.1 Hz, 1H), 7.67 (d, J = 8.9 Hz, 1H), 7.36 (dd, J = 9.0, 2.2 Hz, 1H), 6.42 (d, J = 5.3 Hz, 1H), 5.35 (t, J = 5.4 Hz, 1H), 3.57 – 3.40 (m, 4H), 2.02 (p, J = 6.6 Hz, 2H).

PL410 (N^1-(7-chloroquinolin-4-yl)-N3-methyl-N3-(3-phenyl-3-(4-(trifluoromethyl)phenoxy)propyl)propane-1,3-diamine)

First, the free base fluoxetine was prepared by dissolving fluoxetine HCl (1 eq, 0.55 g, 1.59 mmol) in water (7ml). Solid $NaHCO_3$ (2.28 eq, 0.30 g, 3.62 mmol) was added and the mixture was extracted with dichloromethane (9ml * 3). The organic phase was dried with anhydrous $MgSO_4$, filtered and evaporated to give yellow oliy liquid (0.51g, 93%).

The free-base fluoxetine (1 eq, 0.51 g, 1.65 mmol) was dissolved in tetrahydrofuran (18ml), followed by addition of PL29 (1.33 eq, 0.69 g, 2.19 mmol) and triethylamine (2.9 eq, 0.67 ml, 4.79 mmol) and the reaction mixture was stirred for 72 hours at 55 ^{o}C. After the reaction completion was confirmed by TLC on alumina plate, the reaction was cooled to RT and THF was evaporated by rotovap. The crude was washed with EtOAC (25ml) and saturated $NaHCO_3$ (25ml). The organic layer was separated and the aqueous layer was washed with EtOAC (2*15ml). The organic layer was finally dried by anhydrous $MgSO_4$, filtered and evaporated. The crude was purified by combi-flash chromatography on alumina column using EtOAC:Hexane as eluents to get a yellow solid (0.6g, 69%). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.37 (d, J = 5.4 Hz, 1H), 8.21 (d, J = 9.0 Hz, 1H), 7.78 (d, J = 2.2 Hz, 1H), 7.56 – 7.38 (m, 3H), 7.37 – 7.19 (m, 6H), 6.98 (d, J = 8.6 Hz, 2H), 6.40 (d, J = 5.5 Hz, 1H), 5.44 (dd, J = 8.2, 4.8 Hz, 1H), 3.49 – 3.37 (m, 2H), 3.23 (dtd, J = 19.6, 12.8, 6.4 Hz, 2H), 2.57 – 2.26 (m, 2H), 2.19 (s, 3H), 2.16 – 1.86 (m, 2H), 1.86 – 1.70 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 160.5, 151.9, 150.1, 149.0, 141.0, 133.3, 128.5, 127.6, 126.9 – 126.6 (m), 125.9 , 124.0 (d, J = 4.8 Hz), 120.8 , 117.4 , 116.0 , 98.6 , 77.4 , 54.8 , 53.2 , 41.9 , 40.7 , 35.7 , 25.3 , 22.3. m/z 527.20 M + H (calculated 528.02).

PL411 (7-chloro-N-(3-(4-phenyl-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

To a stirred solution of PL409 (1 eq, 0.50 g, 1.9 mmol), Ethynyl-benzene (1eq, 0.21ml, 1.9mmol) in *tert*-butanol (5ml), $CuSO_4$. $5H_2O$ (0.2eq, 0.095g, 0.38mmol) and sodium ascorbate (0.4 eq, 0.15 g, 0.76 mmol) in water (5ml) was added and the mixture was stirred for 4 hours at 40 ^{o}C. After completion, the mixture of solvents was evaporated and

the solid was purified using combi-flash chromatography in silica column by eluting using $CHCl_3$:MeOH (90:10) to give a pure white-yellowish solid (0.34g, 50%). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.62 (s, 1H), 8.40 (d, J = 5.4 Hz, 1H), 8.27 (d, 1H), 7.94 – 7.74 (m, 3H), 7.50 – 7.28 (m, 5H), 6.48 (dd, J = 5.5, 3.7 Hz, 1H), 4.59 (dt, J = 11.1, 6.9 Hz, 2H), 3.34 (dt, J = 13.4, 6.9 Hz, 2H), 2.30 (q, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 151.9, 149.9 (d, J = 5.9 Hz), 149.5, 148.9, 146.3, 137.1, 133.4, 130.8, 128.9, 127.8, 127.5, 125.1, 124.1 (d, J = 3.6 Hz), 122.9, 121.5, 119.3, 117.5, 98.7, 47.6, 40.1, 28.34. m/z 363.13 M + H (calculated 363.85).

PL412 (7-chloro-N-(3-(4-(pyridin-2-yl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

To a stirred solution of PL409 (1 eq, 0.25 g, 1.9 mmol), 2-Ethynylpyridine (1eq, 0.1ml, 1.9 mmol) in *tert*-butanol (5ml), $CuSO_4$. $5H_2O$ (0.2eq, 0.095g, 0.38mmol) and sodium ascorbate (0.4 eq, 0.15 g, 0.76 mmol) in water (5ml) was added and the mixture was stirred for 4 hours at 40 ^{o}C. After completion, the mixture of solvents was evaporated and the solid was purified using combi-flash chromatography in silica column by eluting using $CHCl_3$:MeOH (90:10) to give a yellow solid (0.16g, 46%). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.48 (s, 2H), 7.45 (s, 1H), 7.37 (s, 1H), 6.76 (s, 1H), 4.70 (s, 2H), 3.17 (d, J = 5.0 Hz, 2H), 2.09 (s, 2H).

PL413 (7-chloro-N-(3-(4-(p-tolyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

To a stirred solution of PL409 (1 eq, 0.50 g, 1.9 mmol), 4-Ethynyltoluene (1 eq, 0.24 ml, 1.9 mmol) in *tert*-butanol (5ml), $CuSO_4$. $5H_2O$ (0.2eq, 0.095g, 0.38mmol) and sodium ascorbate (0.4 eq, 0.15 g, 0.76 mmol) in water (5ml) was added and the mixture was

stirred for 4 hours at 40 ^{o}C. After completion, the mixture of solvents was evaporated and the solid was recrystallized by $CHCl_3$:MeOH (90:10) to give a pure solid (0.4g, 56%). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.55 (s, 1H), 8.41 (s, 1H), 8.29 (d, 1H), 7.81 (d, 1H), 7.71 (d, 2H), 7.46 (d, 1H), 7.43 (t, 1H), 7.25 (d, 2H), 6.52 (d, 1H), 4.55 (t, 2H), 3.43 (d, 2H), 2.32 (s, 3H), 2.27 (q, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 137.1, 129.4, 128.0, 125.0, 121.1, 47.5, 28.1, 20.8. m/z 377.14 M + H (calculated 377.88).

PL414 (N-(3-(4-benzyl-1H-1,2,3-triazol-1-yl)propyl)-7-chloroquinolin-4-amine)

To a stirred solution of PL409 (1 eq, 0.50 g, 1.9 mmol), 3-phenyl-1-propyne (1 eq, 0.24 ml, 1.9 mmol) in *tert*-butanol (5ml), $CuSO_4$. $5H_2O$ (0.2eq, 0.095g, 0.38mmol) and sodium ascorbate (0.4 eq, 0.15 g, 0.76 mmol) in water (5ml) was added and the mixture was stirred for 4 hours at 40 ^{o}C. After completion, the mixture of solvents was evaporated and the solid was recrystallized by $CHCl_3$:MeOH (90:10) to give a pure solid (0.33 g, 45%). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.31 (d, J = 6.1 Hz, 1H), 7.88 (s, 1H), 7.82 (d, 1H), 7.46 (dd, J = 10.5, 7.1 Hz, 2H), 7.35 – 7.15 (m, 6H), 6.48 (s, 1H), 4.46 (t, J = 6.9 Hz, 2H), 3.98 (s, 2H), 3.28 (q, J = 6.5 Hz, 2H), 2.20 (p, J = 6.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 150.1, 146.1, 139.6, 133.6, 128.4(d, J = 8.9 Hz), 126.1, 124.3, 122.6, 47.2, 38.9, 31.3, 28.4. m/z 377.14 M + H (calculated 377.88).

PL415 (N-(3-(4-(4-aminophenyl)-1H-1,2,3-triazol-1-yl)propyl)-7-chloroquinolin-4-amine)

To a stirred solution of PL409 (1 eq, 0.50 g, 1.9 mmol), 4-ethynylaniline (1 eq, 0.22 g, 1.9 mmol) in *tert*-butanol (5 ml), $CuSO_4$. $5H_2O$ (0.2 eq, 0.095 g, 0.38 mmol) and sodium ascorbate (0.4 eq, 0.15 g, 0.76 mmol) in water (5ml) was added and the mixture was

stirred for 4 hours at 40 ^{o}C. After completion, the mixture of solvents was evaporated and the solid was recrystallized by $CHCl_3$: MeOH (90:10) to give a pure solid (0.52 g, 72 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.40 (s, 1H), 8.28 (d, J = 20.1 Hz, 2H), 7.80 (d, J = 2.4 Hz, 1H), 7.53 – 7.35 (m, 4H), 6.62 (m, 2H), 6.48 (d, J = 5.4 Hz, 1H), 5.22 (s, 2H), 4.51 (t, J = 6.9 Hz, 2H), 3.35 (m, 2H), 2.25 (dq, J = 11.5, 5.8, 4.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 151.8, 150.0, 148.5, 147.3, 133.5, 127.3, 126.1, 124.1 (d, J = 8.6 Hz), 119.3, 118.4, 113.9, 98.8, 47.3, 38.8, 28.4. m/z 378.14 M + H (calculated 378.86).

PL416 (N-(3-(4-(4-bromophenyl)-1H-1,2,3-triazol-1-yl)propyl)-7-chloroquinolin-4-amine)

To a stirred solution of PL409 (1 eq, 0.25 g, 0.95 mmol), 1-Bromo-4-Ethynylbenzene (1 eq, 0.17 g, 0.95 mmol) in *tert*-butanol (4 ml), $CuSO_4$. $5H_2O$ (0.2 eq, 0.047 g, 0.19 mmol) and sodium ascorbate (0.4eq, 0.075 g, 0.38 mmol) in water (4 ml) was added and the mixture was stirred for 4 hours at 40 ^{o}C. After completion, the mixture of solvents was evaporated and the solid was recrystallized by $CHCl_3$: MeOH (90:10) to give a pure solid (0.29 g, 69 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.65 (s, 1H), 8.58 (s, 1H), 7.78 (d, J = 8.1 Hz, 2H), 7.68 – 7.55 (m, 3H), 7.45 (d, J = 8.6 Hz, 1H), 4.56 (t, J = 6.8 Hz, 2H), 3.34 (d, J = 5.9 Hz, 2H), 2.35 – 2.19 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 145.3, 131.8, 129.9, 127.1, 124.6, 121.9, 120.8, 47.6, 40.1, 38.8, 28.1. m/z 443.04 M + H (calculated 442.75).

PL417 (7-chloro-N-(3-(4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

To a stirred solution of PL409 (1 eq, 0.25 g, 0.95 mmol), 4-Ethynylanisole (1 eq, 0.13 ml, 0.95 mmol) in *tert*-butanol (4 ml), $CuSO_4$. $5H_2O$ (0.2 eq, 0.047 g, 0.19 mmol) and sodium ascorbate (0.4eq, 0.075 g, 0.38 mmol) in water (4 ml) was added and the mixture was stirred for 4 hours at 40 ^oC. After completion, the mixture of solvents was evaporated and the solid was recrystallized by $CHCl_3$: MeOH (90:10) to give a pure solid (0.19 g, 51 %). ^1H NMR (400 MHz, DMSO-d6) δ 8.49 (s, 1H), 8.33 (s, 1H), 7.84 (s, 1H), 7.79 – 7.70 (m, 2H), 7.54 – 7.43 (m, 2H), 7.05 – 6.97 (m, 2H), 6.57 (s, 1H), 4.54 (t, J = 6.9 Hz, 2H), 3.79 (d, J = 1.2 Hz, 3H), 3.34 (d, J = 6.4 Hz, 2H), 2.27 (tt, J = 12.9, 5.7 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 158.9, 146.2, 126.4, 124.3, 120.5, 114.3, 55.1, 47.5, 38.8, 28.3. m/z 393.14 M + H (calculated 393.88).

PL418 (7-chloro-N-(3-(4-(4-chlorophenyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

To a stirred solution of PL409 (1 eq, 0.25 g, 0.95 mmol), 1-Chloro-4-Ethynylbenzene (1 eq, 0.13 g, 0.95 mmol) in *tert*-butanol (4 ml), $CuSO_4$. $5H_2O$ (0.2 eq, 0.047 g, 0.19 mmol) and sodium ascorbate (0.4eq, 0.075 g, 0.38 mmol) in water (4 ml) was added and the mixture was stirred for 4 hours at 40 ^oC. After completion, the mixture of solvents was evaporated and the solid was recrystallized by $CHCl_3$: MeOH (90:10) to give a pure solid (0.28 g, 76 %). ^1H NMR (400 MHz, DMSO-d6) δ 8.64 (s, 1H), 7.84 (d, J = 8.3 Hz, 2H), 7.48 (dd, J = 26.9, 8.3 Hz, 4H), 6.91 (s, 1H), 4.57 (t, J = 6.7 Hz, 2H), 3.34 (p, J = 5.4, 4.4

Hz, 2H), 2.36 – 2.19 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 145.2, 132.2, 129.6, 128.9, 126.8, 124.5, 121.8, 47.6, 38.8, 28.9, 28.1. m/z 397.09 M + H (calculated 398.29).

PL419 (7-chloro-N-(3-(4-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

To a stirred solution of PL409 (1 eq, 0.25 g, 0.95 mmol), 1-Ethynyl-4-(trifluoromethyl) benzene) (1 eq, 0.16 ml, 0.95 mmol) in *tert*-butanol (4 ml), $CuSO_4$. $5H_2O$ (0.2 eq, 0.047 g, 0.19 mmol) and sodium ascorbate (0.4eq, 0.075 g, 0.38 mmol) in water (4 ml) was added and the mixture was stirred for 4 hours at 40 ^{o}C. After completion, the mixture of solvents was evaporated and the solid was recrystallized by $CHCl_3$: MeOH (90:10) to give a pure solid (0.28 g, 68 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.77 (s, 1H), 8.34 (s, 1H), 8.04 (d, J = 8.0 Hz, 2H), 7.81 (d, J = 8.0 Hz, 2H), 7.58 (t, J = 5.3 Hz, 1H), 7.46 (d, J = 8.8 Hz, 1H), 6.63 (s, 1H), 4.60 (t, J = 6.7 Hz, 2H), 3.39 (q, J = 6.0 Hz, 2H), 2.32 (p, J = 6.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 144.9, 134.7, 133.8, 127.8, 125.9 – 125.7 (m), 125.6, 124.4, 122.8 (d, J = 9.9 Hz), 47.7, 38.8, 28.1. m/z 431.12 M + H (calculated 431.85).

PL420 (7-chloro-N-(3-(4-(4-fluorophenyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

To a stirred solution of PL409 (1 eq, 0.50 g, 1.9 mmol), 1-Ethynyl-4-Fluorobenzene (1 eq, 0.23 g, 1.9 mmol) in *tert*-butanol (5 ml), $CuSO_4$. $5H_2O$ (0.2 eq, 0.095 g, 0.38 mmol) and sodium ascorbate (0.4eq, 0.15 g, 0.76 mmol) in water (5 ml) was added and the mixture was stirred for 4 hours at 50 ^{o}C. After completion, the mixture of solvents was evaporated and the solid was recrystallized by $CHCl_3$: MeOH (90:10) to give a pure solid

(0.61 g, 84 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.60 (s, 1H), 8.45 (s, 1H), 7.92 – 7.81 (m, 2H), 7.45 (d, J = 8.4 Hz, 2H), 7.29 (t, J = 8.8 Hz, 2H), 6.72 (s, 1H), 4.57 (t, J = 6.8 Hz, 2H), 3.44 – 3.29 (m, 2H), 2.36 – 2.19 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 145.5, 133.4, 127.4 – 126.9 (m), 124.3, 121.4, 115.9, 115.7, 47.6, 38.8, 28.2. m/z 381.12 M + H (calculated 381.84).

PL421 (7-chloro-N-(3-(4-(4-nitrophenyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

To a stirred solution of PL409 (1 eq, 0.50 g, 1.9 mmol), 1-Ethymyl-4-Nitrobenzene (1 eq, 0.28 g, 1.9 mmol) in *tert*-butanol (5 ml), $CuSO_4$. $5H_2O$ (0.2 eq, 0.095 g, 0.38 mmol) and sodium ascorbate (0.4 eq, 0.15 g, 0.76 mmol) in water (5 ml) was added and the mixture was stirred for 4 hours at 40 ^{o}C. After completion, the mixture of solvents was evaporated and the solid was recrystallized by $CHCl_3$: MeOH (90:10) to give a pure solid (0.74 g, 95 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.85 (s, 1H), 8.36 – 8.25 (m, 3H), 8.12 – 8.04 (m, 2H), 7.82 (s, 1H), 7.43 (dd, J = 9.9, 5.6 Hz, 2H), 6.56 (s, 1H), 4.61 (t, J = 6.9 Hz, 2H), 3.37 (q, J = 6.1 Hz, 2H), 2.37 – 2.12 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 144.4, 125.8, 124.3 (d, J = 11.4 Hz), 123.6, 47.9, 38.8, 28.9, 28.1. m/z 408.11 M + H (calculated 408.85).

PL422 (N-benzhydrylprop-2-yn-1-amine)

A solution of benzhydramine (1.4 eq, 3 ml, 17 mmol) in anhydrous ether (40ml) was treated with propargyl bromide in 80% toluene (1 eq, 1.35 ml, 12.2 eq), and catalytic amount of NaI. The reaction was heated with stirring at 35 ^{o}C for 16 hours. After completion, the reaction mixture was washed with water (2*20ml) and the organic layer

was dried by anhydrous $MgSO_4$, filtered and concentrated. The crude was purified using combi-flash chromatography in silica column by eluting in Hexanes:EtOAC (95:5) mixture to giving a pure yellow solid (1.1 g, 42 %). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.45 – 7.40 (m, 4H), 7.32 – 7.27 (m, 4H), 7.25 – 7.18 (m, 2H), 5.10 (s, 1H), 3.36 (d, J = 2.5 Hz, 2H), 2.25 (t, J = 2.4 Hz, 1H), 1.82 – 1.67 (m, 1H).

PL423 (N-(3-(4-((benzhydrylamino)methyl)-1H-1,2,3-triazol-1-yl)propyl)-7-chloroquinolin-4-amine)

To a stirred solution of PL409 (1 eq, 0.55 g, 2.11 mmol), PL422 (1.07 eq, 0.50 g, 2.26 mmol) in *tert*-butanol (10 ml), a solution of $CuSO_4$. $5.H_2O$ (0.2 eq, 0.10 g, 0.42 mmol) and sodium ascorbate (0.4 eq, 0.17 g, 0.84 mmol) in water (10ml) was added. The mixture was stirred for 4 hours at 40 ^oC. After completion was confirmed by TLC, chloroform (25 ml) was added and the organic layer was washed by DI-water (3*125 ml), and then with brine (50 ml). Finally, the organic layer was dried by anhydrous $MgSO_4$, filtered and concentrated. The crude was purified in combi-flash using silica column and $CHCl_3$: MeOH (95:5) as eluents obtaining pure solid (0.47 g, 46 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.40 (s, 1H), 8.28 (d, J = 9.1 Hz, 1H), 8.02 (s, 1H), 7.80 (d, 1H), 7.46 (dd, J = 9.1 Hz, 1H), 7.40 (t, 4H), 7.35 (t, 1H), 7.28 (dt, 4H), 7.18 (t, 2H), 6.45 (d, J = 5.3 Hz, 1H), 5.10 (s, 1H), 4.83 (s, 1H), 4.50 (t, J = 6.9 Hz, 2H), 3.66 (s, 2H), 3.29 (q, J = 6.5 Hz, 2H), 2.23 (p, J = 6.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 152.3, 124.6, 124.1, 123.3, 127.9, 128.5, 124.6, 127.4, 128.7, 127.5, 129.4, 128.6, 127.1, 127.6, 99.2, 65.9, 59.7, 47.7, 42.9, 40.0, 28.9. m/z 482.20 M + H (calculated 483.02).

PL424 (N-(piperidin-4-yl)-5H-cyclopenta[2,1-b:3,4-b']dipyridin-5-imine)

To the protected amine (1 eq, 0.88 g, 2.62 mmol), solid NaOH (10 eq, 1.05 g, 26.2 mmol) in ethanol (20 eq, 3.1 ml, 52.3 mmol) was added and stirred at room temperature for 2 hours. The solvent was evaporated, and the crude was taken by EtOAC (40 ml) and water (20 ml). The aqueous layer was extracted with $CHCl_3$ (3 * 20 ml). The two organic layers were mixed and dried with anhydrous $MgSO_4$, filtered and concentrated to give a brownish solid (0.37 g, 42 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.67 (ddd, J = 8.6, 4.9, 1.5 Hz, 2H), 8.11 (dd, J = 8.0, 1.5 Hz, 1H), 7.95 (dd, J = 7.7, 1.6 Hz, 1H), 7.42 (ddd, J = 20.5, 7.7, 4.9 Hz, 2H), 4.70 (tt, J = 9.1, 4.1 Hz, 1H), 3.97 (ddd, J = 16.4, 7.8, 4.1 Hz, 2H), 3.86 (s, 2H), 2.85 (dq, J = 12.3, 3.7 Hz, 2H), 1.67 (m, 2H).

PL425 (N-(3-(4-((5H-cyclopenta[2,1-b:3,4-b']dipyridin-5-ylidene)amino)piperidin-1-yl)propyl)-7-chloroquinolin-4-amine)

PL424 (1.5 eq, 1.1 g, 4.16 mmol) was dissolved in acetonitrile (15 ml), and PL29 (1 eq, 0.87 g, 2.77 mmol) and K_2CO_3 (2 eq, 0.77 g, 5.44 mmol) were all added. The reaction mixture was refluxed at 70 ^{o}C while stirring for 3 days. After completion, the solvent was evaporated and the residue was slurred in water (30ml) and vaccum-filtered. The crude was recrystallized from Toluene:Hexane (50:50) mixture to recover a brownish solid (0.7 g, 52 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.75 (t, J = 5.7 Hz, 4H), 8.38 (dd, J = 27.1, 6.6 Hz, 4H), 8.27 (d, J = 9.0 Hz, 2H), 8.14 (d, J = 7.5 Hz, 2H), 7.79 (d, J = 2.4 Hz, 2H), 7.56 – 7.40 (m, 10H), 7.30 – 7.08 (m, 8H), 6.52 (d, J = 5.4 Hz, 2H), 4.48 (tt, J = 9.4, 4.7 Hz, 2H), 3.30 (s, 6H), 2.96 (dd, J = 9.7, 5.3 Hz, 3H), 2.49 (s, 1H), 2.29 (d, J = 6.1

Hz, 9H), 1.97 – 1.78 (m, 11H), 1.30 – 1.21 (m, 1H), 0.84 (dt, J = 10.8, 6.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d6) δ 160.33 , 158.29 , 155.65 , 152.25 , 151.9, 151.7, 150.1, 149.1, 134.9, 133.3, 132.9, 129.7, 128.9, 128.2, 127.5, 125.3, 124.6, 124.4, 123.9, 117.4, 98.6, 55.9, 51.3, 40.9, 32.9, 25.2, 21.0. m/z 482.20 M + H (calculated 483.02).

PL426 (7-chloro-N-(pyrrolidin-3-yl)quinolin-4-amine)

4,7-dichloroquinoline (1 eq, 2.5 g, 12.5 mmol) was dissolved in N,N-Diisopropylethylamine (50ml). 3-Aminopyrrolidine dihydrochloride (2 eq, 3.98 g, 25 mmol) and $CsCO_3$ (4 eq, 16.45 g, 50 mmol) was added slowly with stirring and the mixture was refluxed for 4 hours. The solvent was evaporated and the crude was purified in combi-flash by DCM:MeOH:Et_3N (8.9:1:0.1) as mixture of solvents to give orange-colored solid (1.35 g, 44 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.69 (d, J = 4.8, 1H), 8.02 (d, J = 2.0, 1H), 7.90 (d, J = 8.8, 1H), 7.42 (dd, J = 8.8, 2.0, 1H), 6.82 (d, J = 4.8, 1H), 3.83 (m, 1H), 3.68 (m, 1H), 3.42 (m, 1H), 3.14 s, 1H), 2.14 (m, 1H), 1.85 (m, 1H).

PL427 (N-(2-azidoethyl)-7-chloroquinolin-4-amine)

PL30 (1 eq, 6.25 g, 20.8 mmol) was dissolved in dimethylformamide (65ml). Sodium azide (2 eq, 2.70 g, 41.6 mmol) was added, and the reaction mixture was stirred overnight at 55 ^{o}C. After completion, DI-water (85 ml) was added and the aqueous layer was extracted with toluene (3 * 250 ml). The organic layer was dried by anhydrous $MgSO_4$, filtered and concentrated giving pure solid (5.02 g, 98 %). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.54 (d, J = 5.4 Hz, 4H), 8.01 (s, 5H), 7.96 (d, J = 2.2 Hz, 4H), 7.83 (d, J = 9.0 Hz, 4H), 7.34 (dd, J = 9.0, 2.2 Hz, 4H), 7.30 – 7.18 (m, 1H), 7.17 (dt, J = 7.9, 2.2

Hz, 1H), 6.42 (d, J = 5.3 Hz, 4H), 5.94 (t, J = 5.7 Hz, 4H), 3.66 (dd, J = 6.4, 5.0 Hz, 8H), 3.53 (q, J = 5.6 Hz, 9H), 2.95 (s, 14H), 2.88 (s, 14H), 2.74 (s, 3H), 2.35 (s, 1H).

General procedure for the synthesis of terminal alkynes:

The 10 terminal alkynes PL428, PL429, PL430, PL431, PL432, PL433, PL434, PL435, PL436 and PL437 were prepared according to the following procedure:

To a mixture of N-substituted piperazine (1 eq, 3.2 mmol) and anhydrous K_2CO_3 (1 eq, 0.44 g, 3.2 mmol) in anhydrous acetonitrile (5 mL), propargyl bromide (80% W/V solution in toluene, 1 eq , 0.36 ml, 3.2 mmol) was added. The mixture was stirred overnight under reflux, and then filtered and evaporated. The residue was purified by combiflash chromatography using silica column eluting by Hexanes:EtOAc mixture.

PL428 (2-(4-(prop-2-yn-1-yl)piperazin-1-yl)pyrimidine)

0.21 g, 32 %. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.47 (dd, J = 11.1, 2 Hz, 11H), 6.79 (dt, J = 14.5, 4.9 Hz, 1H), 4.68 (s, 2H), 4.17 (s, 1H), 4.14 (dd, J = 13.0, 8.0 Hz, 4H), 3.67 (t, J = 5.1 Hz, 4H).

PL429 (1-(prop-2-yn-1-yl)-4-(4-(trifluoromethyl)benzyl)piperazine)

0.78 g, 87 %. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.56 (dd, 2H), 7.47 (dd, J = 11.2, 8.0 Hz, 2H), 3.96 (t, J = 5.1 Hz, 2H), 3.56 (d, J = 18.7 Hz, 2H), 3.30 (d, J = 2.5 Hz, 2H), 3.09 – 2.93 (m, 2H), 2.68 – 2.58 (m, 4H), 2.26 (t, J = 2.4 Hz, 1H).

PL430 (1-(prop-2-yn-1-yl)-4-(p-tolyl)piperazine)

0.68 g, 99 %. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.14 – 7.02 (m, 2H), 6.93 – 6.74 (m, 2H), 3.45 – 3.23 (m, 2H), 3.22 – 3.10 (m, 4H), 2.78 – 2.70 (m, 4H), 2.27 (d, J = 6.0 Hz, 3H).

PL431 (1-(prop-2-yn-1-yl)-4-(pyridin-2-yl)piperazine)

0.45 g, 70 %. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.19 (tt, J = 4.9, 2.1 Hz, 1H), 7.63 – 7.43 (m, 1H), 6.86 – 6.59 (m, 2H), 4.12 – 3.95 (m, 2H), 3.90 (t, J = 5.2 Hz, 2H), 3.71 – 3.44 (m, 2H), 3.36 (d, J = 2.5 Hz, 2H), 2.73 – 2.65 (m, 2H), 2.28 (t, J = 2.5 Hz, 1H).

PL432 (1-(4-(oxo-λ^{6}-methyl)phenyl)-4-(prop-2-yn-1-yl)piperazine)

1-(4-methoxyphenyl)-piperazine dihydrochloride (1 eq, 2 g, 7.5 mmol) was dissolved in water (20ml), and solid $NaHCO_3$ (2.3 eq, 1.4 g, 17.1 mmol) was added with stirring. After addition of dichloromethane (25 ml), the aqueous phase was extracted with DCM (3 * 25ml) and the organic phase was dried by anhydrous $MgSO_4$, filtered and concentrated by rotovap to give the free base starting material.

0.7 g, 95 %. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.02 – 6.78 (m, 4H), 3.76 (d, J = 2.5 Hz, 3H), 3.36 (d, J = 2.5 Hz, 2H), 3.17 – 2.98 (m, 4H), 2.78 – 2.55 (m, 4H), 2.28 (t, J = 2.4 Hz, 1H).

PL433 (1-phenyl-4-(prop-2-yn-1-yl)piperazine)

0.56 g, 88 %. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.33 – 7.20 (m, 2H), 6.98 – 6.80 (m, 3H), 3.36 (d, J = 2.5 Hz, 2H), 3.27 – 3.20 (m, 4H), 2.77 – 2.70 (m, 4H), 2.28 (t, J = 2.4 Hz, 1H).

PL434 (4-(4-(prop-2-yn-1-yl)piperazin-1-yl)phenol)

0.39 g, 57 %. ^{1}H NMR (400 MHz, DMSO-d6) δ 6.91 – 6.59 (m, 4H), 4.62 (d, J = 2.6 Hz, 2H), 4.15 (t, 1H), 3.68 (t, J = 5.0 Hz, 2H), 3.39 (t, J = 4.8 Hz, 2H), 3.17 (s, 1H), 3.13 – 3.01 (m, 4H).

PL435 (1-(4-chlorophenyl)-4-(prop-2-yn-1-yl)piperazine)

0.5 g, 67 %. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.27 – 7.15 (m, 2H), 6.91 – 6.78 (m, 2H), 3.37 (d, J = 2.4 Hz, 2H), 3.26 – 3.16 (m, 4H), 2.78 – 2.69 (m, 4H), 2.28 (t, J = 2.5 Hz, 1H).

PL436 (1-cyclohexyl-4-(prop-2-yn-1-yl)piperazine)

0.57 g, 86 %. ^{1}H NMR (400 MHz, Chloroform-d) δ 3.34 (d, J = 2.5 Hz, 2H), 3.10 (dtd, 4H), 2.42 – 2.26 (m, 3H), 1.95 (m, 2H), 1.53 (qd, J = 12.1, 3.4 Hz, 4H), 1.27 (m, 6H).

PL437 (1-(3,4-dichlorophenyl)-4-(prop-2-yn-1-yl)piperazine)

0.81 g, 94 %. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.39 – 7.22 (m, 1H), 7.01 – 6.84 (m, 1H), 6.74 (ddd, J = 8.9, 4.9, 2.9 Hz, 1H), 3.41 – 3.29 (m, 2H), 3.25 – 3.10 (m, 4H), 2.80 – 2.68 (m, 4H), 2.29 (t, J = 2.4 Hz, 1H).

PL439 (N^1,N^3-bis(7-chloroquinolin-4-yl)propane-1,3-diamine)

To PL426 (1.5 eq, 0.88 g, 3.57 mmol) dissolved in acetonitrile (30ml), PL29 (1 eq, 0.75 g, 2.38 mmol) and cesium carbonate (2 eq, 1.55 g, 4.76 mmol) was added. The reaction mixture was refluxed for 3 days. The solvent was evaporated and the crude was slurred in water (80 ml) in a sonicator for 30 minutes. The solid was filtered by vaccum-filtration and purified on silica column using combi-flash chromatography eluting by EtOAC:MeOH. One of the peaks (collection 3) was collected and concentrated to give a yellow solid (0.1 g, 67 %). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.74 (d, J = 5.2 Hz, 1H), 8.58 – 8.43 (m, 1H), 8.12 (d, J = 8.9 Hz, 1H), 8.05 (d, J = 2.0 Hz, 1H), 8.01 – 7.91 (m, 1H), 7.66 (d, J = 9.0 Hz, 1H), 7.45 (dd, J = 8.9, 2.1 Hz, 1H), 7.40 – 7.26 (m, 1H), 6.73 (d, J = 5.3 Hz, 1H), 6.49 (d, J = 5.4 Hz, 1H), 5.43 (s, 1H), 4.38 (t, J = 5.6 Hz, 2H), 3.69 (q, J = 6.3 Hz, 2H), 2.43 (p, J = 6.3 Hz, 2H). m/z 397.08 M + H (calculated 397.30).

General procedure for the synthesis of piperazine-linked triazoles:

The 9 compounds PL440, PL441, PL442, PL443, PL444, PL445, PL446, PL447 and PL448 were prepared according to the following procedure:

To a stirred solution of PL409 (1 eq, 0.25 g, 0.95 mmol) and synthesized N-piperazinyl-substituted terminal alkynes (1.05 eq, 1.0 mmol) in *tert*-butanol (6.8 ml), $CuSO_4$. $5H_2O$ (0.1 eq, 0.024 g, 0.095 mmol) and sodium ascorbate (0.2 eq, 0.038 g, 0.19 mmol) in water (2.3 ml) was added. The mixture was stirred for 48 hours at 50 ^oC. After completion, the mixture of solvents was evaporated and the solid was purified using combi-flash chromatography in silica column by eluting using $CHCl_3$:MeOH (90:10).

PL440 (7-chloro-N-(3-(4-((4-(pyrimidin-2-yl)piperazin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

0.32 g, 70 %. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.39 (d, J = 5.4 Hz, 1H), 8.34 (d, J = 4.8 Hz, 2H), 8.26 (d, J = 9.0 Hz, 1H), 8.07 (s, 1H), 7.79 (d, J = 2.3 Hz, 1H), 7.47 (dd, J = 9.0, 2.0 Hz, 1H), 7.35 (t, J = 5.4 Hz, 1H), 6.61 (t, J = 4.8 Hz, 1H), 6.42 (d, J = 5.4 Hz, 1H), 4.49 (t, J = 6.9 Hz, 2H), 3.69 (t, J = 5.0 Hz, 4H), 3.60 (s, 2H), 3.28 (q, J = 6.5 Hz, 2H), 2.44 (t, J = 5.0 Hz, 4H), 2.24 (h, J = 7.4, 7.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 157.1, 141.3, 136.2, 129.7, 123.9, 110.1, 52.0, 47.2, 43.4, 38.9, 28.2 (d, J = 20.9 Hz). m/z 463.20 M + H (calculated 463.97).

PL441 (7-chloro-N-(3-(4-((4-(4-(trifluoromethyl)benzyl)piperazin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

0.16 g, 52 %. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.39 (d, J = 5.4 Hz, 1H), 8.26 (d, J = 9.0 Hz, 1H), 8.01 (s, 1H), 7.79 (d, J = 2.3 Hz, 1H), 7.66 (d, J = 8.0 Hz, 2H), 7.50 (d, J = 8.0 Hz, 2H), 7.47 (dd, J = 9.0, 2.0 Hz, 1H), 7.36 (t, J = 5.3 Hz, 1H), 6.41 (d, J = 5.5 Hz, 1H), 4.49 (t, J = 6.9 Hz, 2H), 3.54 (d, J = 5.2 Hz, 4H), 3.27 (q, J = 6.5 Hz, 2H), 2.36 (s, 8H), 2.22 (p, J = 6.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 151.7, 150.0, 148.8, 143.2, 133.5, 129.3, 127.3, 125.0, 124.2, 124.0, 123.9, 120.9, 117.4, 98.7, 61.2, 52.4, 52.1, 47.2, 28.4. m/z 543.22 M + H (calculated 544.02).

PL442 (7-chloro-N-(3-(4-((4-(p-tolyl)piperazin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

0.17 g, 35 %. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.40 (d, J = 5.4 Hz, 1H), 8.26 (d, J = 9.0 Hz, 1H), 8.06 (s, 1H), 7.80 (s, J = 2.3 Hz, 1H), 7.47 (dd, J = 9.0, 2.0 Hz, 1H), 7.37 (t, J =

5.2 Hz, 1H), 7.00 (d, J = 8.3 Hz, 2H), 6.84 – 6.76 (m, 2H), 6.42 (t, J = 6.4 Hz, 1H), 4.49 (q, J = 6.8 Hz, 2H), 3.60 (s, 2H), 3.27 (s, 2H), 3.06 – 2.99 (m, 4H), 2.50 (d, J = 1.8 Hz, 4H), 2.38 (d, J = 13.8 Hz, 2H), 2.32 – 2.16 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d6) δ 151.0, 150.0, 133.5, 128.9, 124.2, 118.8, 115.3, 52.2, 48.1, 47.3, 28.4. m/z 475.23 M + H (calculated 476.03).

PL443 (7-chloro-N-(3-(4-((4-(pyridin-2-yl)piperazin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

0.28 g, 61 %. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.43 – 8.37 (m, 1H), 8.27 (d, J = 9.0 Hz, 1H), 8.17 – 8.05 (m, 2H), 7.80 (d, J = 2.3 Hz, 1H), 7.56 – 7.39 (m, 3H), 6.79 (d, J = 8.6 Hz, 1H), 6.62 (dd, J = 7.0, 4.9 Hz, 1H), 6.44 (d, J = 5.5 Hz, 1H), 4.50 (t, J = 6.9 Hz, 2H), 3.60 (s, 2H), 3.44 (t, J = 4.9 Hz, 4H), 3.29 (s, 2H), 2.47 (d, J = 5.0 Hz, 4H), 2.23 (p, J = 6.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 147.5, 137.5, 124.3, 113.0, 107.1, 47.3, 40.1, 38.8, 28.3. m/z 462.21 M + H (calculated 462.99).

PL444 (7-chloro-N-(3-(4-((4-(4-methoxyphenyl)piperazin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

0.37 g, 76 %. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.41 (s, 1H), 8.29 (d, J = 9.1 Hz, 1H), 8.06 (s, 1H), 7.80 (d, J = 2.3 Hz, 1H), 7.55 – 7.46 (m, 2H), 6.90 – 6.75 (m, 4H), 6.46 (d, J = 5.4 Hz, 1H), 4.50 (t, J = 6.8 Hz, 2H), 3.68 (d, J = 9.3 Hz, 3H), 3.60 (s, 2H), 3.24 – 3.15 (m, 2H), 2.97 (t, J = 4.9 Hz, 4H), 2.53 (d, J = 4.9 Hz, 4H), 2.24 (p, J = 6.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 124.3, 117.3, 114.2, 55.1, 38.8. m/z 491.22 M + H (calculated 492.02).

PL445 (7-chloro-N-(3-(4-((4-phenylpiperazin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

0.32 g, 70 %. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.40 (d, J = 5.4 Hz, 1H), 8.34 – 8.23 (m, 1H), 8.07 (s, 1H), 7.80 (d, J = 2.3 Hz, 1H), 7.47 (dd, J = 9.0, 2.2 Hz, 1H), 7.37 (t, J = 5.3 Hz, 1H), 7.27 – 7.14 (m, 2H), 6.90 (d, J = 8.2 Hz, 2H), 6.76 (t, J = 7.2 Hz, 1H), 6.43 (d, J = 5.4 Hz, 1H), 4.51 (t, J = 6.9 Hz, 2H), 3.60 (s, 2H), 3.27 (d, J = 6.5 Hz, 2H), 3.08 (t, J = 4.9 Hz, 4H), 2.52 (t, J = 4.9 Hz, 4H), 2.24 (p, J = 6.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 151.0, 150.0, 133.5, 128.9, 124.2, 118.8, 115.3, 52.2, 48.1, 47.3, 28.4. m/z 461.21 M + H (calculated 462.00).

PL446 (4-(4-((1-(3-((7-chloroquinolin-4-yl)amino)propyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenol)

0.33 g, 68 %. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.79 (s, 1H), 8.42 – 8.23 (m, 3H), 8.06 (d, J = 5.2 Hz, 1H), 7.79 (d, J = 2.3 Hz, 1H), 7.47 (dd, J = 9.0, 2.2 Hz, 1H), 7.36 (t, J = 5.5 Hz, 1H), 6.84 – 6.70 (m, 1H), 6.67 – 6.57 (m, 1H), 6.42 (d, J = 5.4 Hz, 1H), 4.50 (td, J = 6.9, 2.1 Hz, 2H), 3.69 (t, J = 5.0 Hz, 2H), 3.59 (d, J = 3.8 Hz, 2H), 2.91 (t, J = 4.9 Hz, 4H), 2.44 (t, J = 5.1 Hz, 2H), 2.23 (p, J = 6.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 157.8, 149.8, 133.5, 124.12 (d, J = 11.1 Hz), 117.7, 115.4, 56.6, 52.4, 49.8, 47.3, 38.7, 28.4. m/z 477.21 M + H (calculated 478.00).

PL447 (7-chloro-N-(3-(4-((4-(4-chlorophenyl)piperazin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

0.40 g, 80 %. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.80 (s, 1H), 8.42 – 8.36 (m, 3H), 8.27 (d, J = 9.1 Hz, 3H), 8.06 (d, J = 2.2 Hz, 3H), 7.79 (d, J = 2.3 Hz, 3H), 7.47 (dd, J = 9.0,

2.2 Hz, 4H), 7.36 (t, J = 5.1 Hz, 4H), 7.28 – 7.17 (m, 4H), 7.00 – 6.87 (m, 4H), 6.79 – 6.70 (m, 2H), 6.67 – 6.58 (m, 2H), 6.42 (d, J = 5.4 Hz, 3H), 5.78 – 5.73 (m, 1H), 4.50 (t, J = 6.9 Hz, 2H), 3.59 (d, J = 3.7 Hz, 2H), 3.26 (s, 2H), 3.08 (t, J = 5.0 Hz, 4H), 2.91 (dd, J = 9.5, 4.5 Hz, 2H), 2.52 (s, 2H), 2.24 (h, J = 6.7 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 150.2, 149.7, 133.7, 128.5, 127.5, 127.1, 124.2 (d, J = 8.4 Hz), 123.9, 122.3, 116.8, 52.4, 52.0, 47.9, 47.3, 38.8, 28.4. m/z 495.17 M + H (calculated 496.44).

PL448 (7-chloro-N-(3-(4-((4-(3,4-dichlorophenyl)piperazin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

0.41 g, 77 %. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.40 (s, 1H), 8.27 (d, J = 9.0 Hz, 1H), 8.06 (s, 1H), 7.79 (d, J = 2.3 Hz, 1H), 7.51 – 7.34 (m, 3H), 7.10 (d, J = 2.9 Hz, 1H), 7.00 – 6.87 (m, 1H), 6.43 (d, J = 5.4 Hz, 1H), 4.50 (t, J = 6.9 Hz, 2H), 3.56 (d, J = 26.0 Hz, 2H), 3.32 – 3.24 (m, 2H), 3.13 (t, J = 5.0 Hz, 4H), 2.56 (s, 4H), 2.24 (h, J = 6.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 156.2, 152.2, 150.3, 133.5, 128.0, 127.2, 126.1, 125.7, 124.1, 123.9, 109.4, 52.0, 51.7, 47.3, 38.9, 28.3. m/z 530.14 M + H (calculated 530.88).

PL456 (7-chloro-N-(3-(4-((4-(7-chloroquinolin-4-yl)piperazin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine)

The purified PL467 (1.07 eq, 1.15 g, 4.02 mmol) and PL409 (1 eq, 0.99 g, 3.76 mmol) dissolved in *tert*-butanol (15 ml) were mixed with $CuSO_4$. $5H_2O$ (0.15 eq, 0.14 g, 0.56 mmol) and sodium ascorbate (0.3 eq, 0.22 g, 1.13 mmol) in water (5 ml) for 24 hours at 50 ^{o}C. After evaporating the solvent, the crude was purified using combi-flash chromatography on silica by eluting in $CHCl_3$:MeOH (90:10) to give the target

compound (1.2 g, 57 %). ^{1}H NMR (400 MHz, CDCl3) δ 8.70 (d, J = 5.0 Hz, 1H), 8.47 (d, J = 5.3 Hz, 1H), 8.03 (d, J = 2.1 Hz, 1H), 7.94 (d, J = 2.2 Hz, 1H), 7.91 (d, J = 8.9 Hz 1H), 7.75 (d, J = 9.0 Hz, 1H), 7.57 (s, 1H), 7.41 (dd, J = 9.0, 2.1 Hz, 1H), 7.40 (dd, J = 9.0, 2.3 Hz, 1H), 6.80 (d, J = 5.0 Hz, 1H), 6.36 (d, J = 5.4 Hz, 1H), 5.87 (t, J = 5.5 Hz, 1H), 4.57 (t, J = 6.2 Hz, 2H), 3.78 (s, 2H), 3.47 (d, 2H), 3.20 (t, 4H), 2.77 (t, 4H), 2.39 (p, J = 6.5 Hz, 2H). 13C NMR (101 MHz, DMSO-d6) δ 156.8, 151.8, 150.9, 150.1, 149.8, 144.8, 135.5, 134.9, 128.8, 126.2, 125.8, 125.1, 123.6, 123.2, 121.9, 121.5, 108.9, 98.6, 53.2, 52.8, 51.9, 48.1, 40.3, 28.3. m/z 547.19 M + H (calculated 547.49).

PL457 (7-chloro-4-isothiocyanatoquinoline)

A mixture of 4,7-dichloroquinoline (1 eq, 1.8 g, 9.09 mmol) and silverthiocyanate (2 eq, 3.02 g, 18.18 mmol) was stirred in anhydrous toluene (15 ml) for 12 hours. The reaction was carried in disposable screw cap culture tube (100 ml) using fishr PTFE disposable bar stirrer. The hot mixture was filtered and the filtrate was washed with chloroform (3 * 37.5 ml) and concentrated by rotovap to give a yellow solid (1.88 g , 94 %). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.86 (d, J = 4.7 Hz, 1H), 8.12 (d, J = 2.0 Hz, 1H), 8.03 (d, J = 8.9 Hz, 1H), 7.58 (dd, J = 8.9, 2.1 Hz, 1H), 7.26 (d, J = 4.7 Hz, 1H).

PL459 (Methyl (3-((7-chloroquinolin-4-yl)amino)propyl)carbamodithioate)

The intermediate product was synthesized in one-pot reaction by dissolving PL107 (1 eq, 2.0 , 8.49 mmol) in dimethylsulfoxide (4 ml) and stirring at room temperature. Carbon disulfide (1.25 eq, 0.64 ml, 10.61 mmol) and 10 % NaOH (1.25 eq, 3.78 ml, 10.61 mmol) were added dropwise over 30 minutes. Then the mixture was stirred for another 30

minutes. After 1 hour, dimethylsulfate (1 eq, 0.81 ml, 8.49 mmol) was added at 5-10 ^{o}C, and the stirring continued for 3 hours. The sticky-brownish suspension formed was added to ice-water (50 ml) and transferred to sep-funnel. The aqueous layer was extracted by chloroform (3 * 20 ml). The organic layer was washed by saturated $NaHCO_3$ (25 ml) and then salted out by brine (20 ml). Finally the organic layer was dried by anhydrous $MgSO_4$, filtered and concentrated to give brownish solid (0.8 g, 30 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 9.99 (t, J = 5.2 Hz, 1H), 8.40 (d, J = 5.4 Hz, 1H), 8.25 (d, J = 9.0 Hz, 1H), 7.80 (d, J = 2.3 Hz, 1H), 7.52 – 7.27 (m, 2H), 6.49 (t, J = 6.0 Hz, 1H), 3.66 (dtd, J = 43.9, 6.9, 4.3 Hz, 2H), 3.32 (q, J = 6.6 Hz, 2H), 2.52 (d, J = 2.4 Hz, 3H), 1.99 (m, 2H).

PL460 (Ethyl 4-((5H-cyclopenta[2,1-b:3,4-b']dipyridin-5-ylidene)amino)piperidine-1-carboxylate)

1,8-Diazafluoren-9-one (1 eq, 0.5 g, 2.74 mmol) and Ethyl-4-amino-1-piperidine carboxylate (1.15 eq, 0.54 ml, 3.15 mmol) was dissolved in anhydrous toluene (15 ml). Catalytic amount of p-toluene-sulfonic acid was added and the reaction was performed using dean-stark apparatus to remove the water continuously forming as a by-product. The reaction was refluxed stirring for 3 days. After the mixture was cooled to RT, the solvent was evaporated, and the crude was used in the next reaction prior to purification (0.88 g, 96 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.75 (ddd, J = 8.6, 4.9, 1.5 Hz, 2H), 8.42 (dd, J = 8.0, 1.5 Hz, 1H), 8.12 (dd, J = 7.7, 1.6 Hz, 1H), 7.49 (ddd, J = 20.5, 7.7, 4.9 Hz, 2H), 4.70 (tt, J = 9.1, 4.1 Hz, 1H), 4.09 (q, J = 7.1 Hz, 2H), 3.99 (ddd, J = 16.4, 7.8, 4.1 Hz, 2H), 3.27 (s, 2H), 1.91 (dq, J = 12.3, 3.7 Hz, 2H), 1.80 – 1.62 (m, 2H), 1.20 (dt, J = 16.9, 7.1 Hz, 3H).

PL467 (7-chloro-4-(4-(prop-2-yn-1-yl)piperazin-1-yl)quinoline)

PL49 (1 eq, 2.5 g, 10.1 mmol), propargyl bromide in 80 % W/V toluene (1 eq, 0.96 ml, 10.1 mmol) and K_2CO_3 (1 eq, 1.4 g, 10.1 mmol) in acetonitrile (15 ml) were refluxed for 48 hours. After the completion of the reaction was confirmed by TCL on alumina plate using EtOAC:MeOH (90:10) as eluents, the solvent was evaporated by rotovap. The crude was taken up by dichloromethane (40 ml) and washed by saturated $NaHCO_3$ (3 * 20 ml). All the aqueous phase were mixed and and extracted by DCM (30 ml). The two organic phases were mixed and dried by anhydrous $MgSO_4$, filtered and concentrated. The crude was purified using combi-flash chromatography on silica using Hexanes:EtOAC as eluents give brownish solid (2.41 g, 83 %). ^{1}H NMR (400 MHz, CDCl3) δ 8.71 (d, J = 5.0 Hz, 1H), 8.03 (d, J = 2.1 Hz, 1H), 7.94 (d, J = 8.9 Hz, 1H), 7.41 (dd, J = 9.0, 2.1 Hz, 1H), 6.83 (d, J = 5.0 Hz, 1H), 3.44 (d, 2H), 3.27 (t, 4H), 2.86 (t, 4H), 2.35 (t, 1H).

7.3. *In Vitro* Drug Susceptibility Assays

Both CQS (D6) and CQR (Dd2 and 7G8) *P. falciparum* maintained continuously in culture were used. Asynchronous cultures were diluted with uninfected RBCs and complete medium (RPMI-1640 with 0.5% Albumax II) to achieve 0.2% parasitemia and 2% hematocrit. In 96-well microplates, CQ (positive control) or click compounds diluted in complete medium from 10 mM stock in DMSO were added to the cell mixture to yield triplicate wells with drug concentrations ranging from 0 to 10^{-4} M in a final well volume of 100 µL. After 72 h of incubation under standard culture conditions, plates were

harvested and read by the SYBR Green I fluorescence-based method using a 96-well fluorescence plate reader (Gemini-EM, Molecular Devices), with excitation and emission wavelengths at 497 and 520 nm, respectively. The fluorescence readings were plotted against log[drug], and the IC_{50} values were obtained from curve fitting performed by nonlinear regression using either Prism (GraphPad) or Excel (Microsoft) software. The values obtained for each cell line are normalized to CQ values of 5.9 nM for D6, 54 nM for Dd2, and 57 nM for 7G8. Errors were estimated to be ± 30%. This was determined by looking at the variability in IC_{50} values of 4 compounds, each with 3 or 4 sets of results per IC_{50} determination, and by taking into account the estimated uncertainties resulting from the weighing of the compounds.

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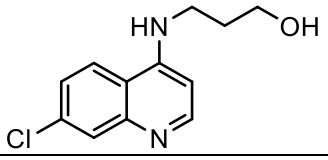
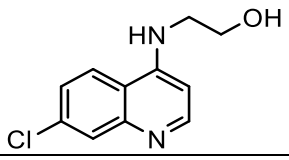
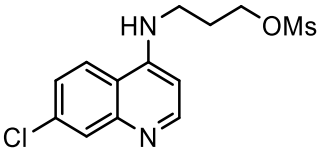
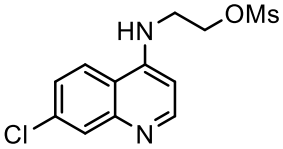
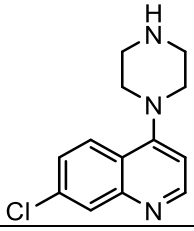
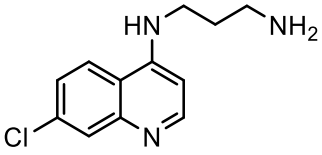
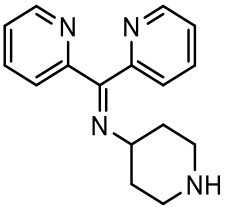
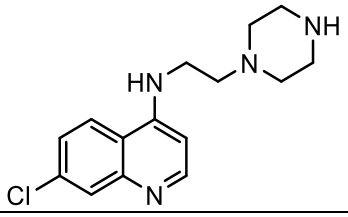
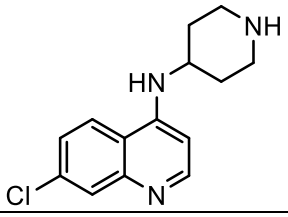
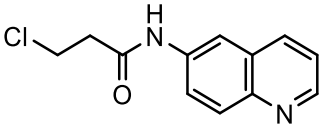
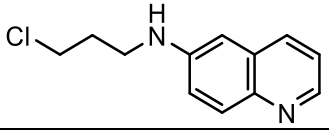
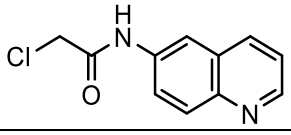
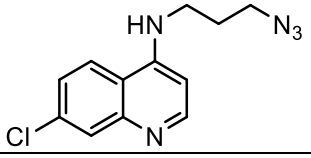
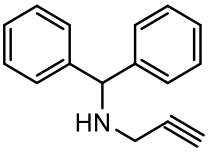
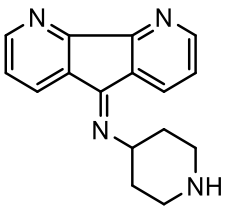
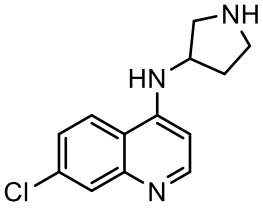
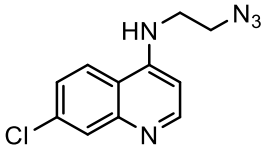
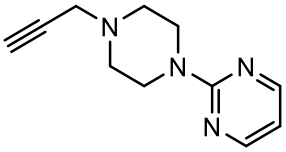
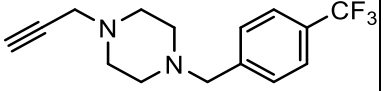
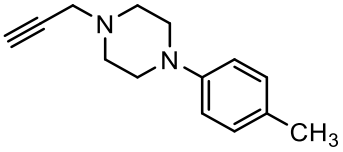
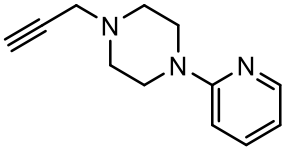
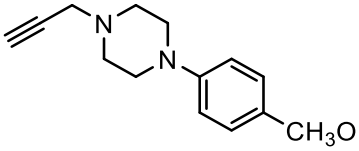
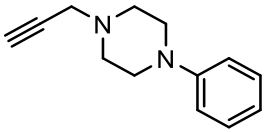
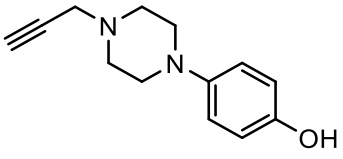
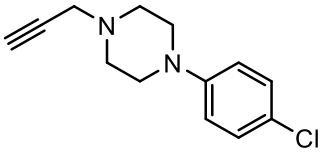
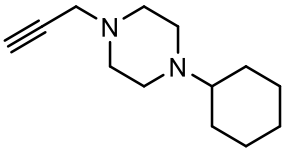
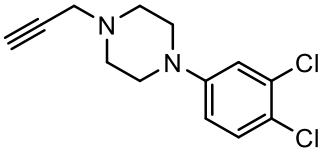
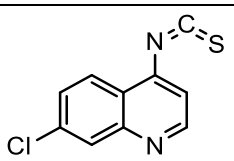
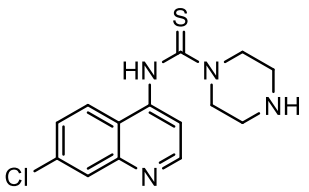
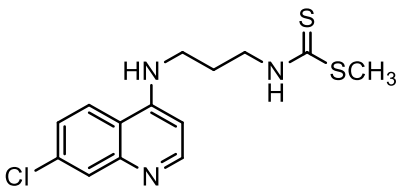
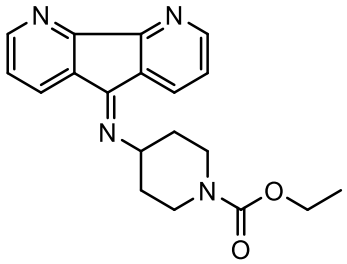
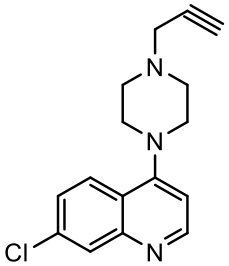
APPENDICES

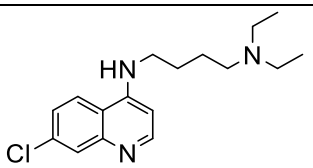
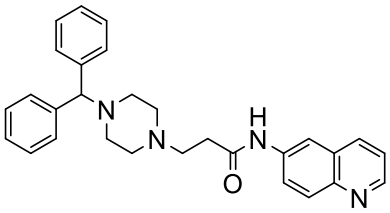
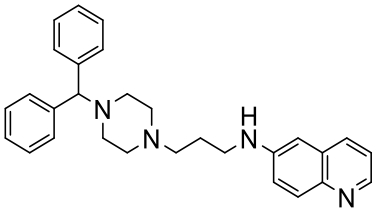
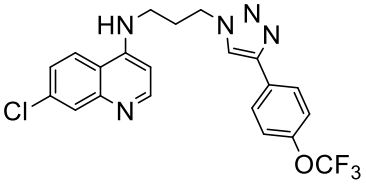
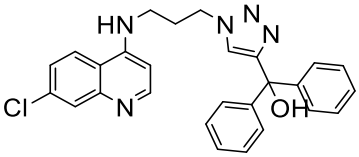
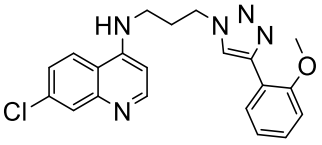
Appendix A: List of Intermediate Compounds Synthesized

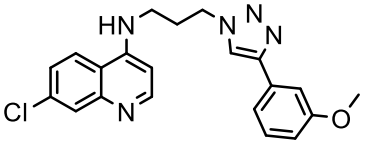
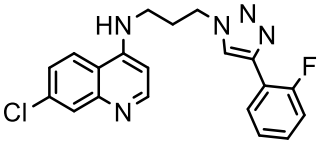
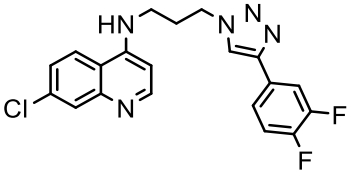
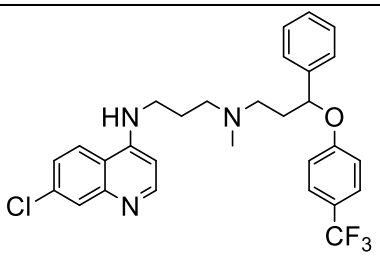
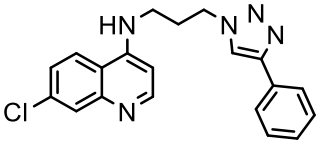
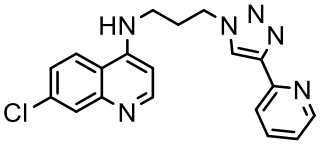
PL No.	Structure	PL No.	Structure
PL16		PL28	
PL29		PL30	
PL49		PL107	
PL190		PL46	
PL294		PL323	
PL326		PL334	
PL409		PL422	

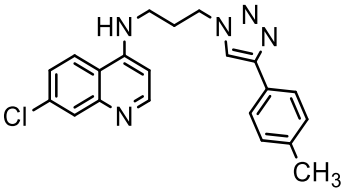
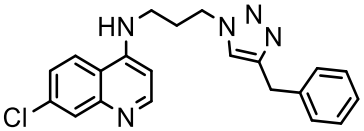
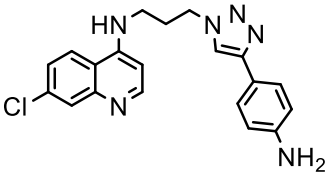
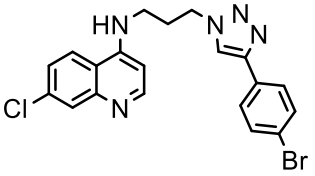
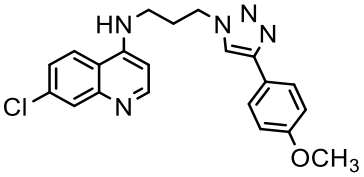
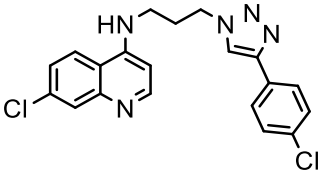
PL424		PL426	
PL427		PL428	
PL429		PL430	
PL431		PL432	
PL433		PL434	
PL435		PL436	
PL437		PL457	

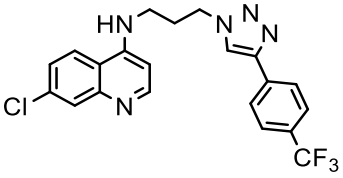
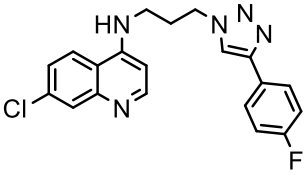
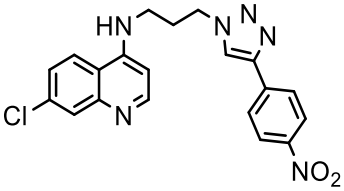
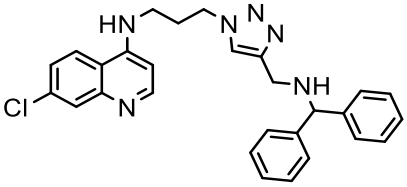
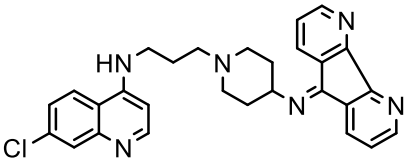
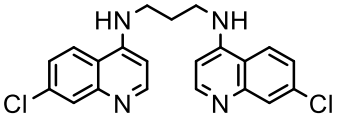
PL458		PL459	
PL460		PL467	

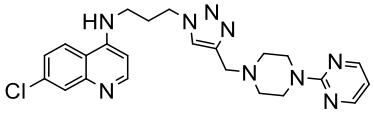
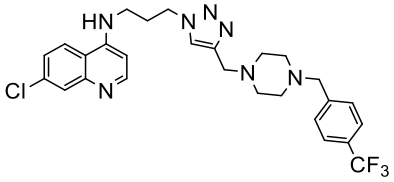
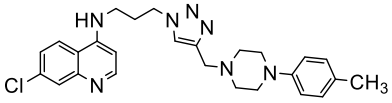
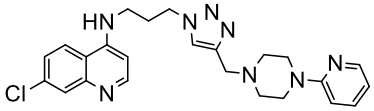
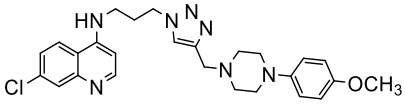
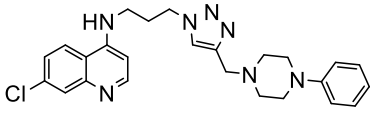
Appendix B: List of Target Compounds Synthesized

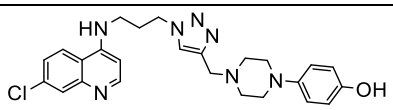
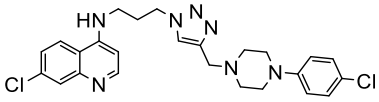
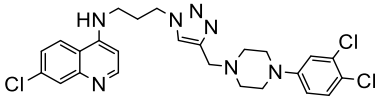
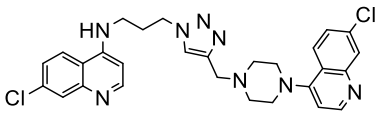
PL No.	Click Compound	IC_{50} (D6, Dd2, 7G8)	HBD/ HBA	NRot	CLogP	Mol.Wt
CQ		5.9 54 57	1/3	8	4.75	305.85
369		>250 >250 >250	1/5	8	5.63	450.59
370		>250 >250 >250	1/4	8	6.37	436.60
403		10.4 12.8 14.5	1/6	8	6.28	447.85
404		25 81.6 48	2/6	8	4.84	469.97
405		98.6 210 192	1/6	7	4.61	393.88

406		52.4 120 122	1/6	7	5.17	393.88
407		81.0 109 129	1/5	6	5.37	381.84
408		22.7 39.1 95.7	1/5	6	5.44	399.83
410		9.6 <2.5 <2.5	1/4	12	8.97	528.02
411		76.8 87.5 151	1/5	6	5.19	363.85
412		423 696 592	1/6	6	4.07	364.84

413		40.5 61.4 60	1/5	6	5.69	377.88
414		9.6 77.7 85.8	1/5	7	4.93	377.88
415		76.8 345 238	2/6	6	4.05	378.86
416		423 30.7 49.6	1/5	6	6.09	442.75
417		40.5 109 140	1/6	8	5.17	393.88
418		18.8 42.7 36.6	1/5	6	5.94	398.29

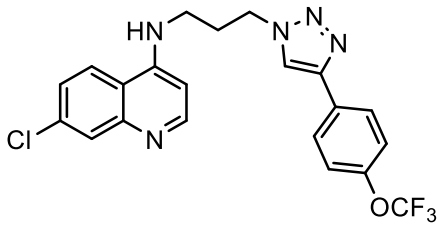
419		11 31.6 18.7	1/5	7	6.13	431.85
420		39.6 85.2 80.3	1/5	6	5.37	381.84
421		18.8 50.5 44.5	1/8	7	5.01	408.85
423		80.3 160 189	2/6	10	4.96	483.02
425		20.5 32.5 86	1/6	6	4.08	483.02
439		2.5 4 2.5	2/4	6	6.87	397.30

440		67 >250 >250	1/9	8	3.11	463.97
441		10 40 16	1/7	10	6.00	544.02
442		12 37 18	1/7	8	5.33	476.03
443		41 >250 >250	1/8	8	3.88	462.99
444		33 68 65	1/8	10	4.85	492.02
445		17 67 74	1/7	8	4.83	462.00

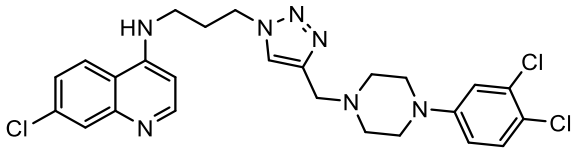
446		155 94.4 >250	2/8	8	4.16	478.00
447		8.8 26.5 19	1/7	8	5.71	496.44
448		6.8 15.2 11	1/7	8	6.36	530.88
456		0.5 10 11	1/8	8	6.04	547.49

Appendix C: Selected Spectral and Chromatography Data

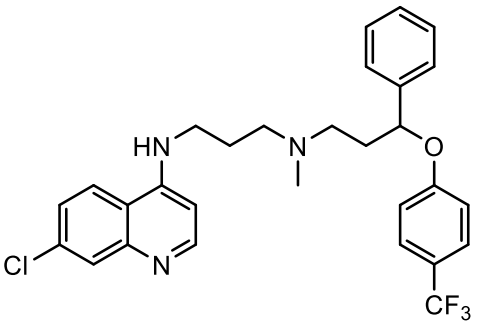
^{1}H-Proton spectroscopy, COSY, HSQC, HMBC, NOESY, ^{13}C-Spectroscopy, mass spectroscopy and combiflash chromatography data of selected compounds:



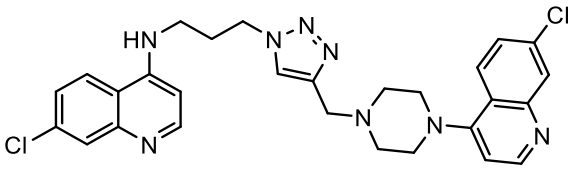
PL403



PL448



PL410



PL456

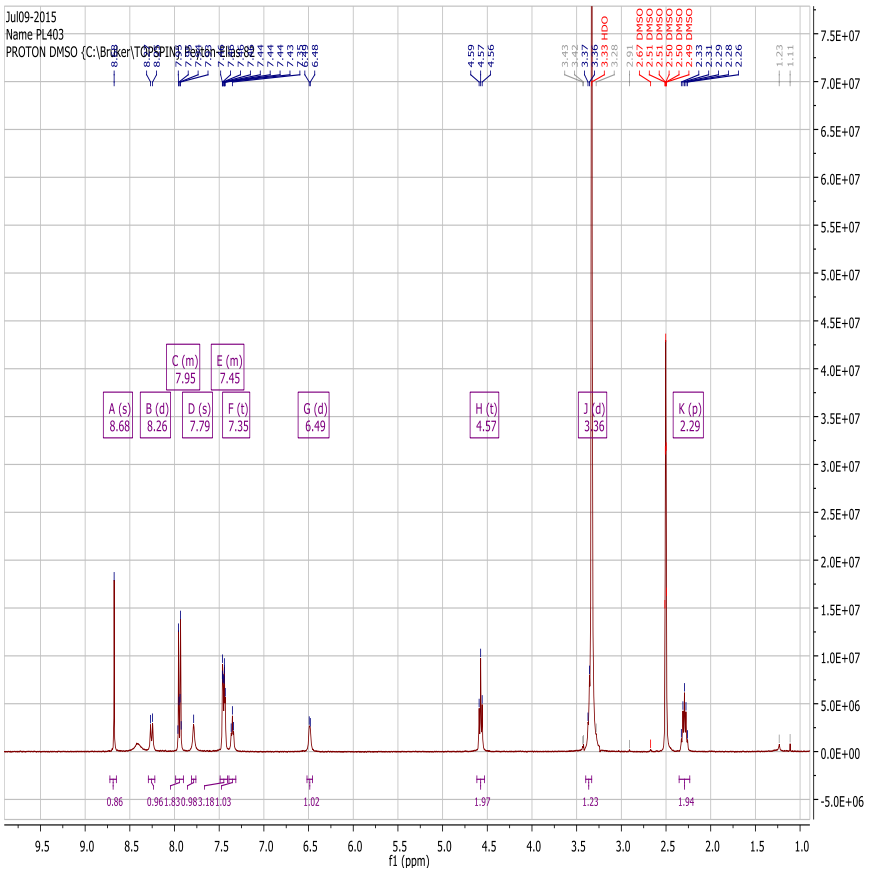


Figure 1.1. ^{1}H-proton spectrum of PL403

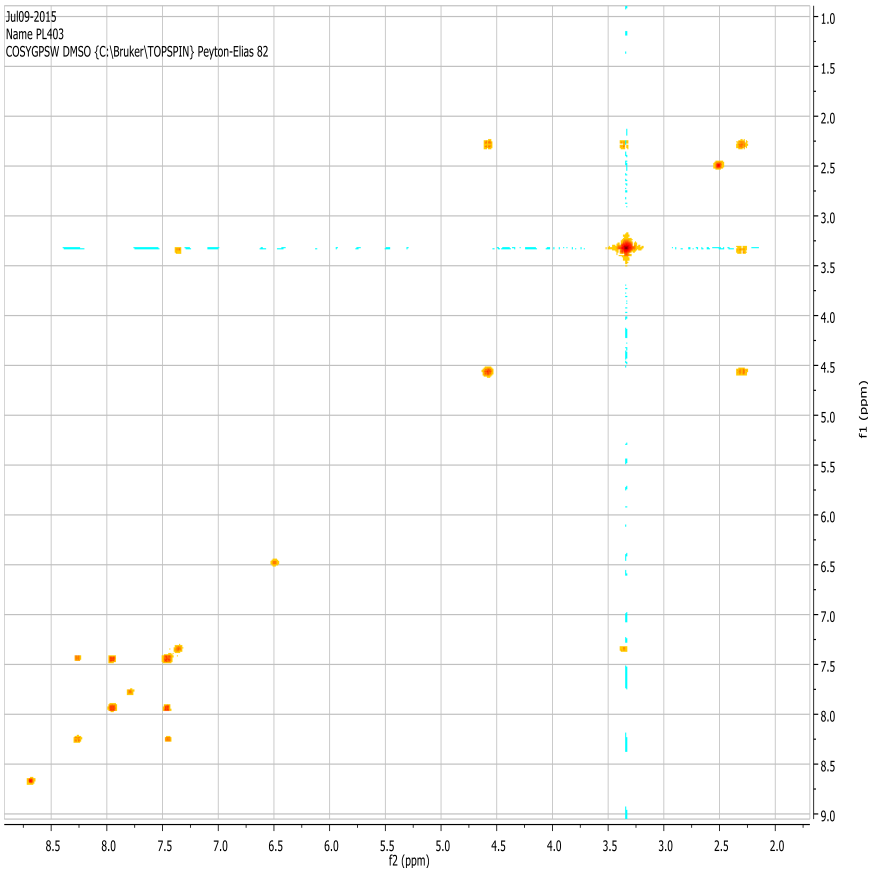


Figure 1.2. COSY spectrum of PL403

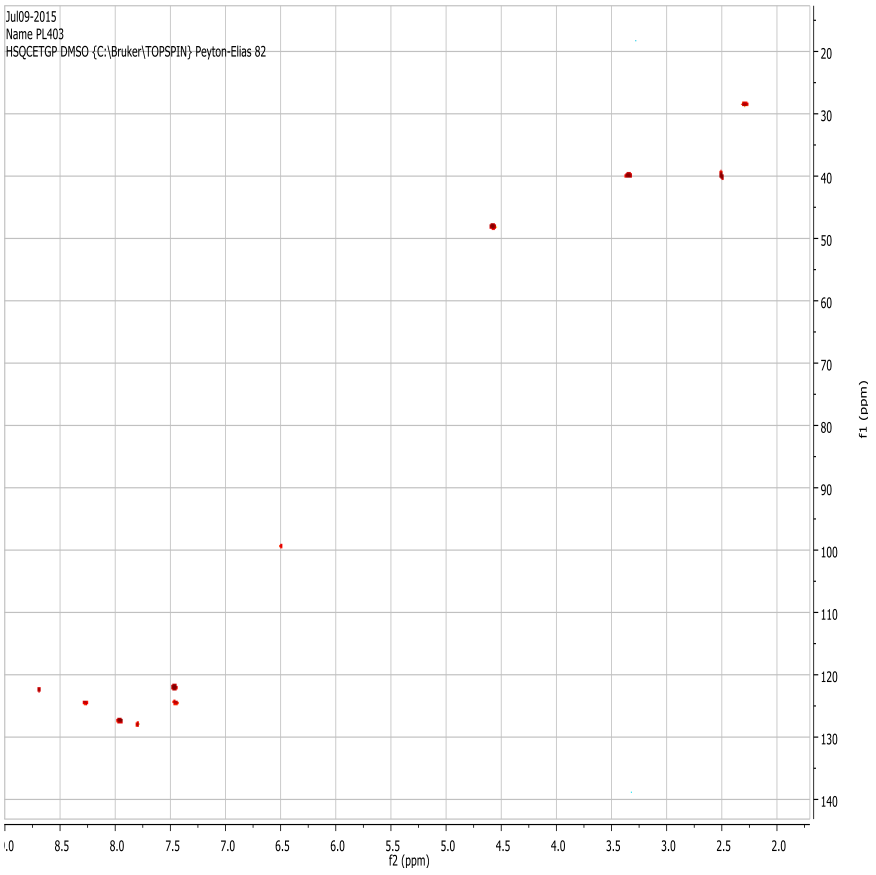


Figure 1.3. HSQC spectrum of PL403

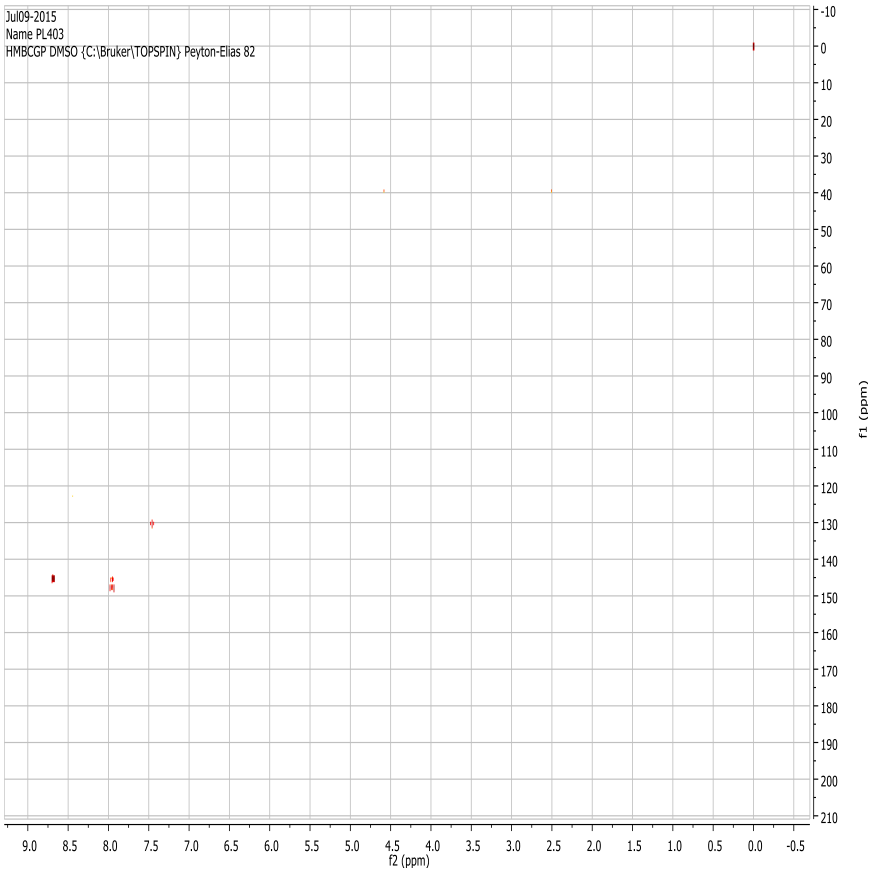


Figure 1.4. HMBC spectrum of PL403

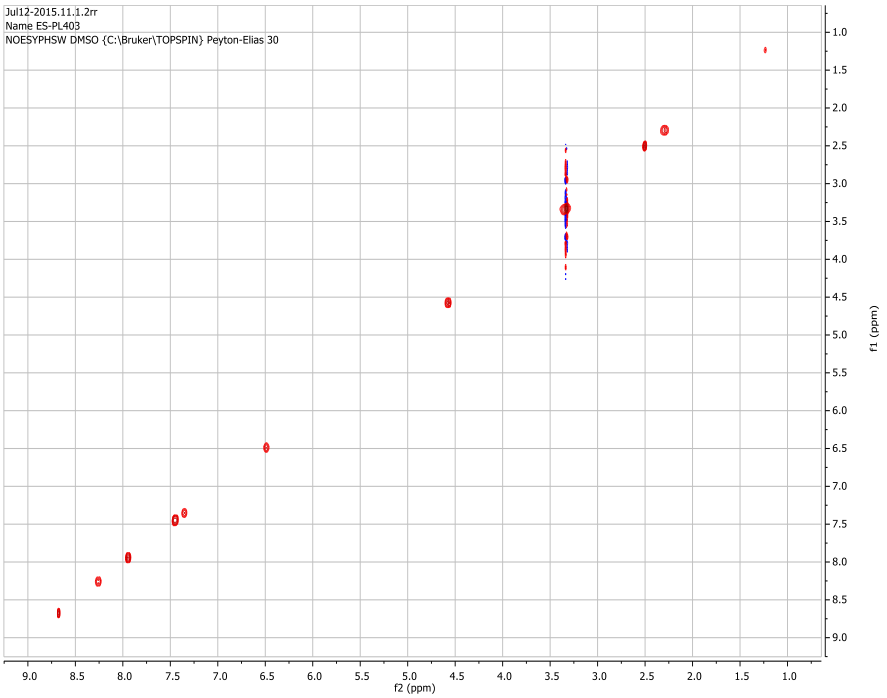


Figure 1.5. NOESY spectrum of PL403

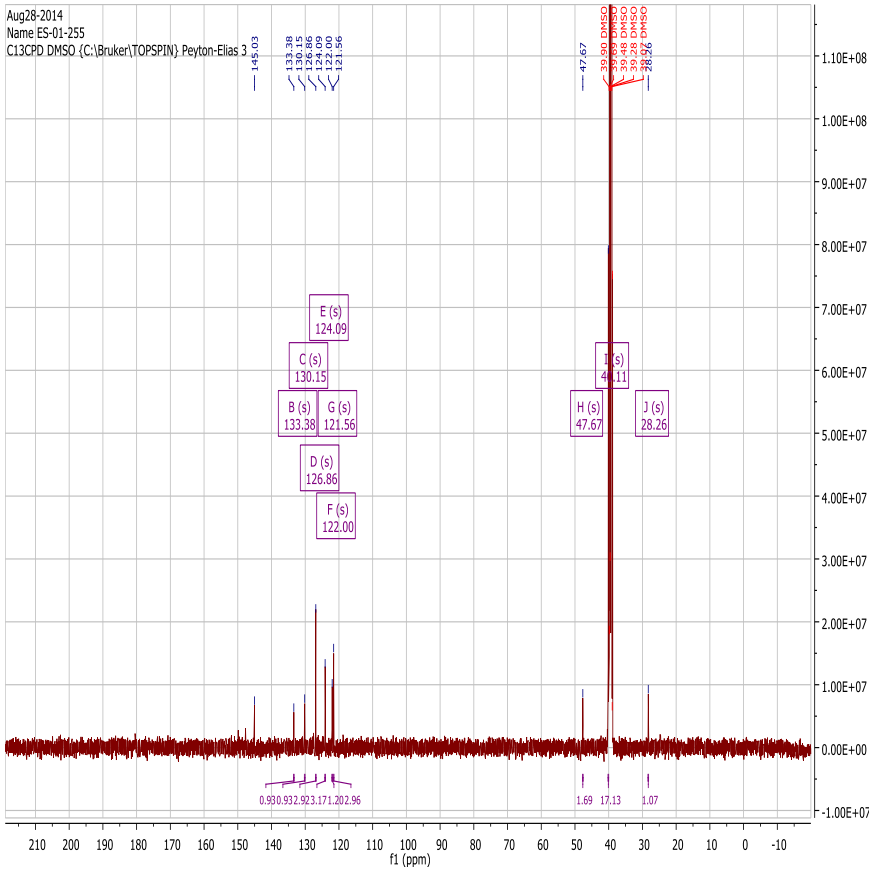


Figure 1.6. ^{13}C-spectrum of PL403

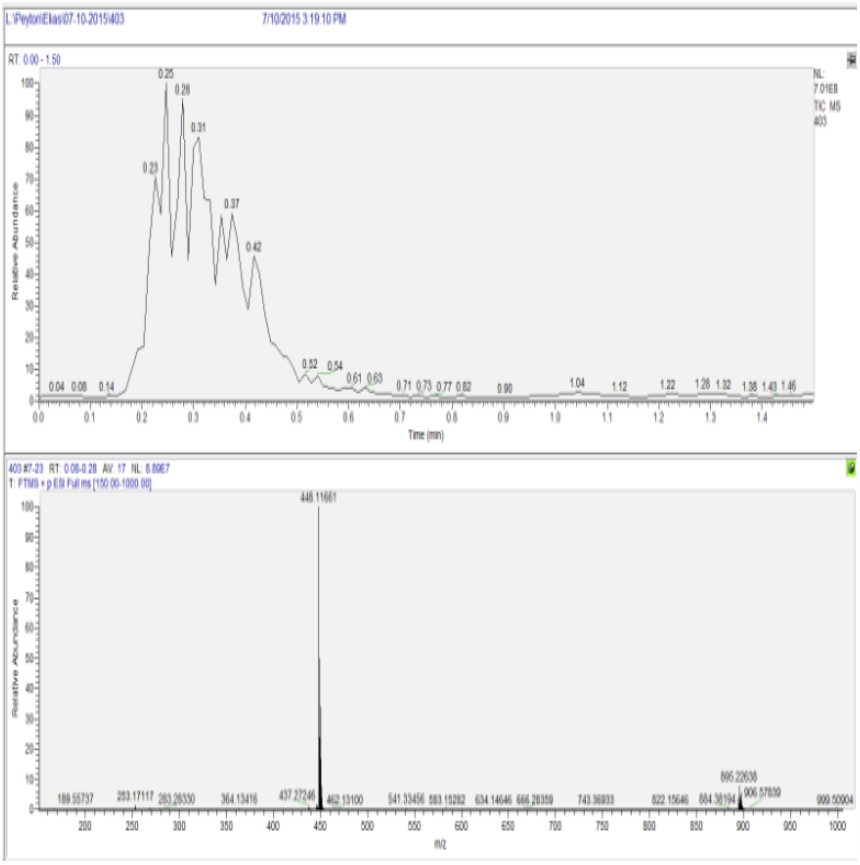


Figure 1.7. Mass spectrum of PL403

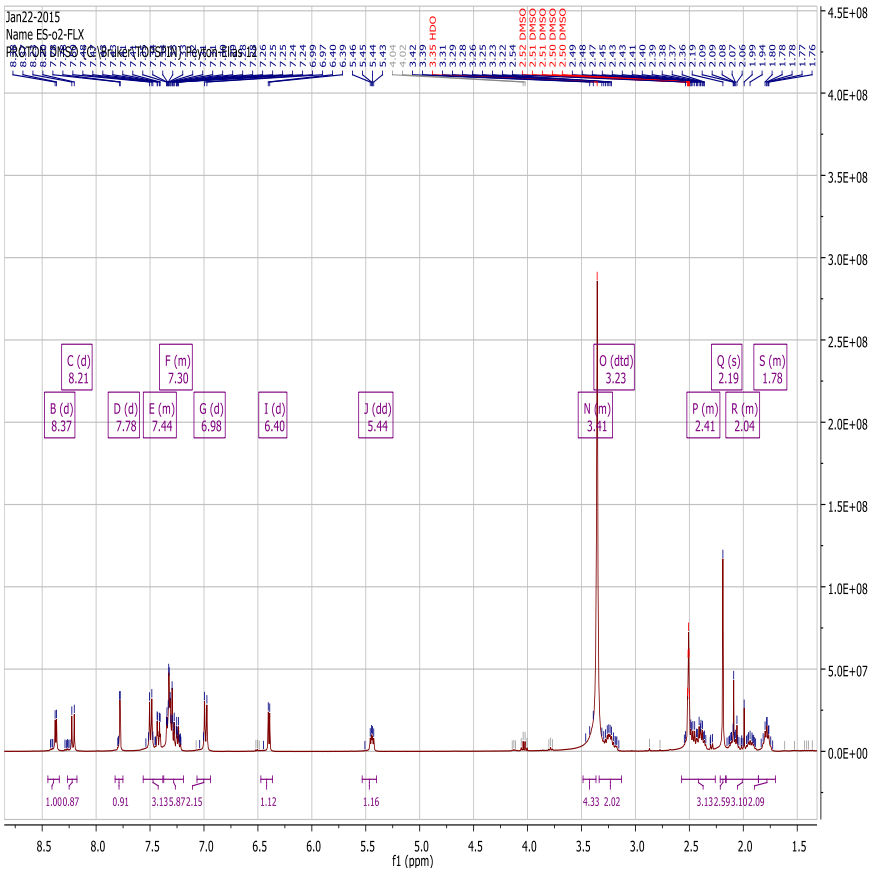


Figure 2.1. ^{1}H-proton spectrum of PL410

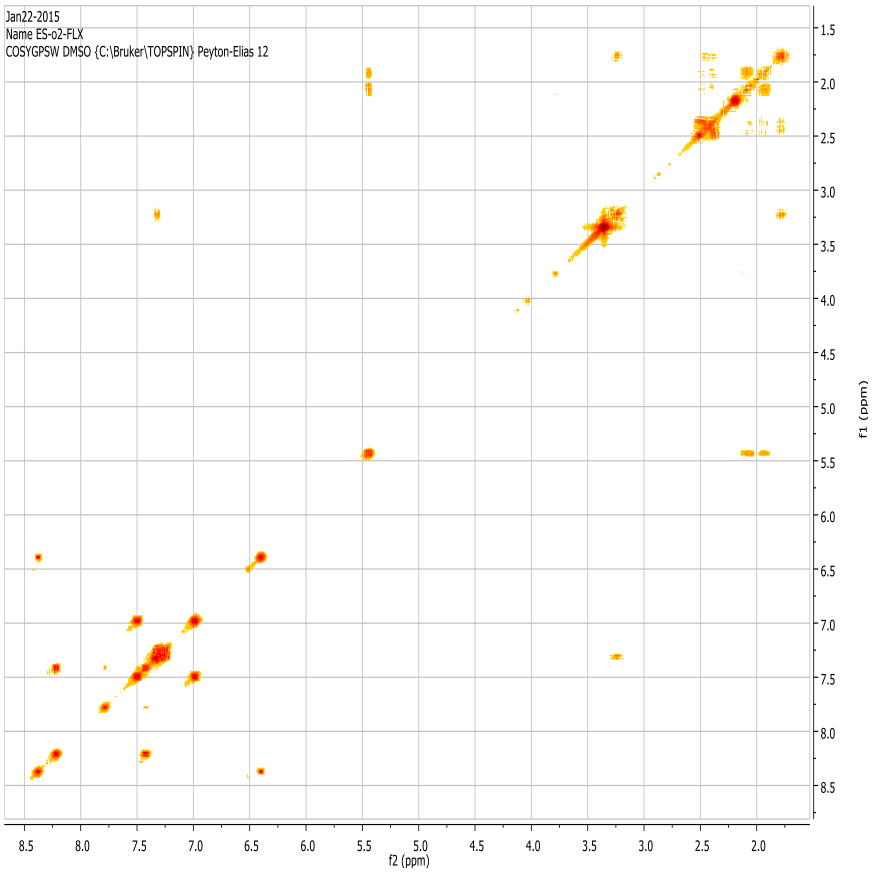


Figure 2.2. COSY spectrum of PL410

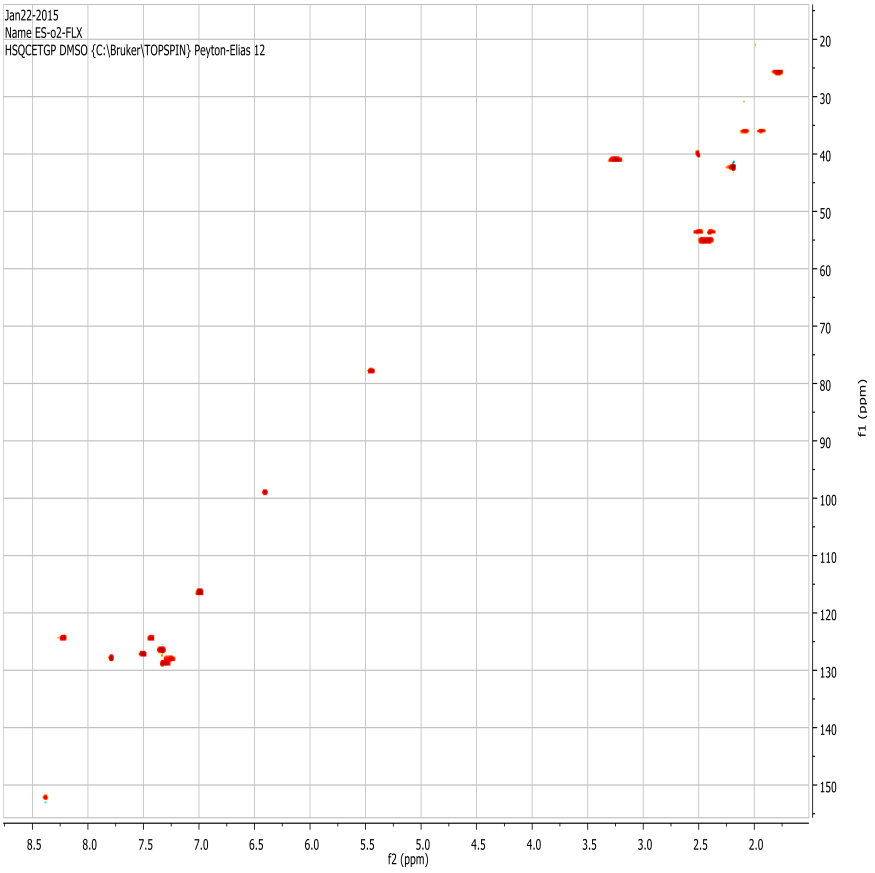


Figure 2.3. HSQC spectrum of PL410

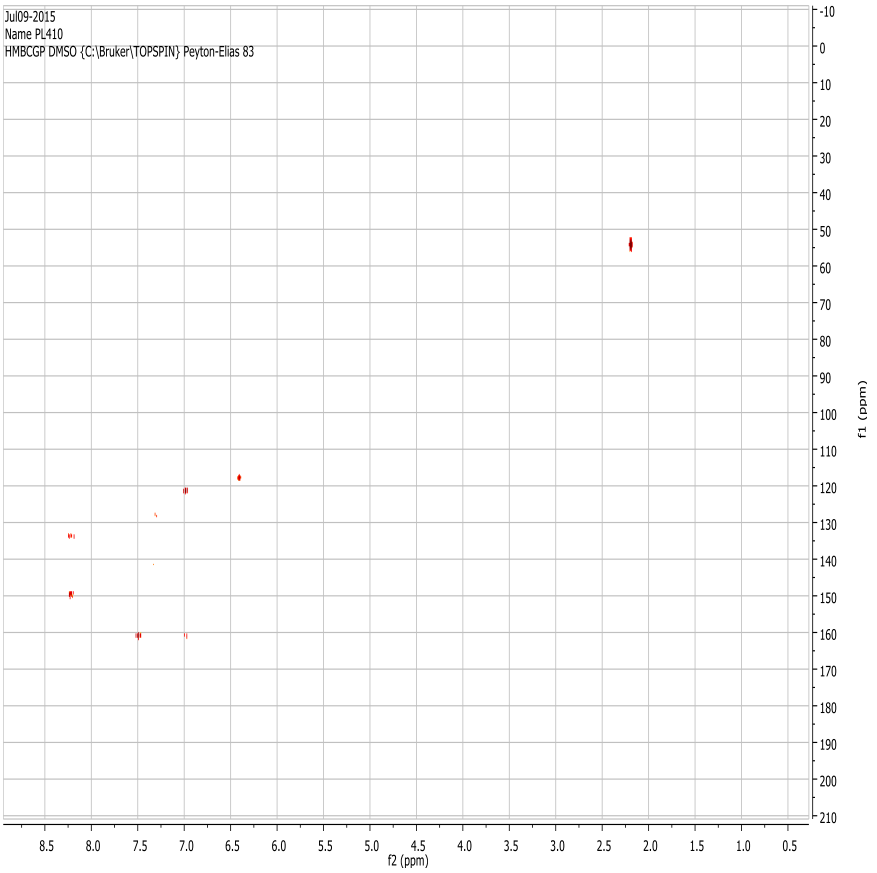


Figure 2.4. HMBC spectrum of PL410

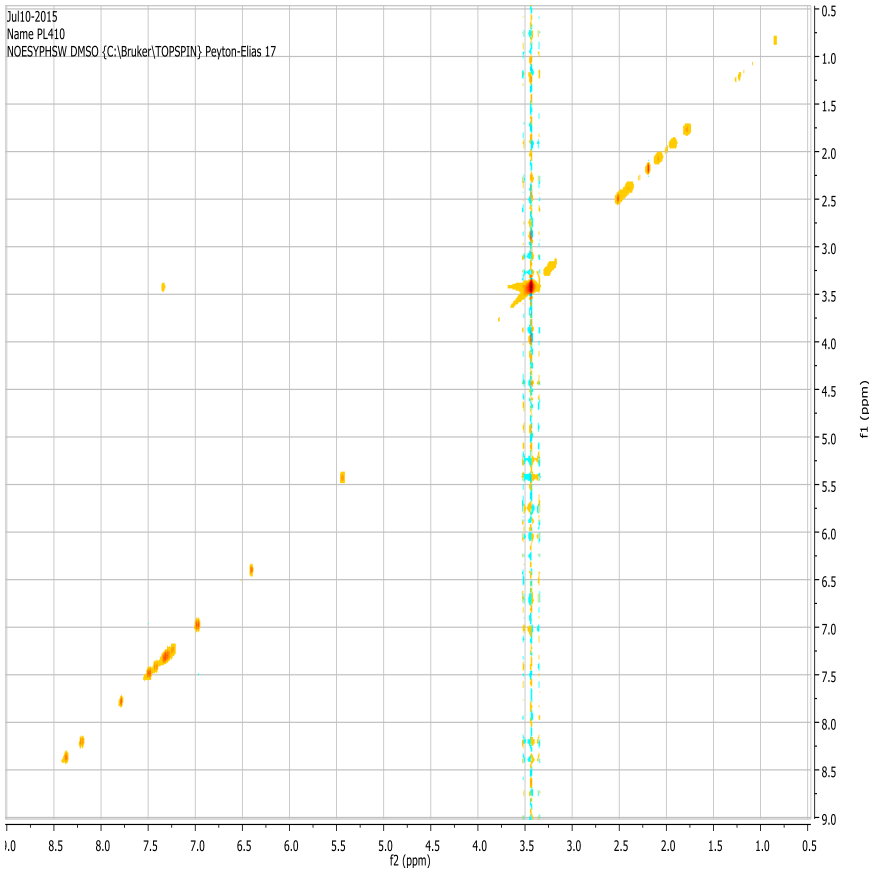


Figure 2.5**.** NOESY spectrum of PL410

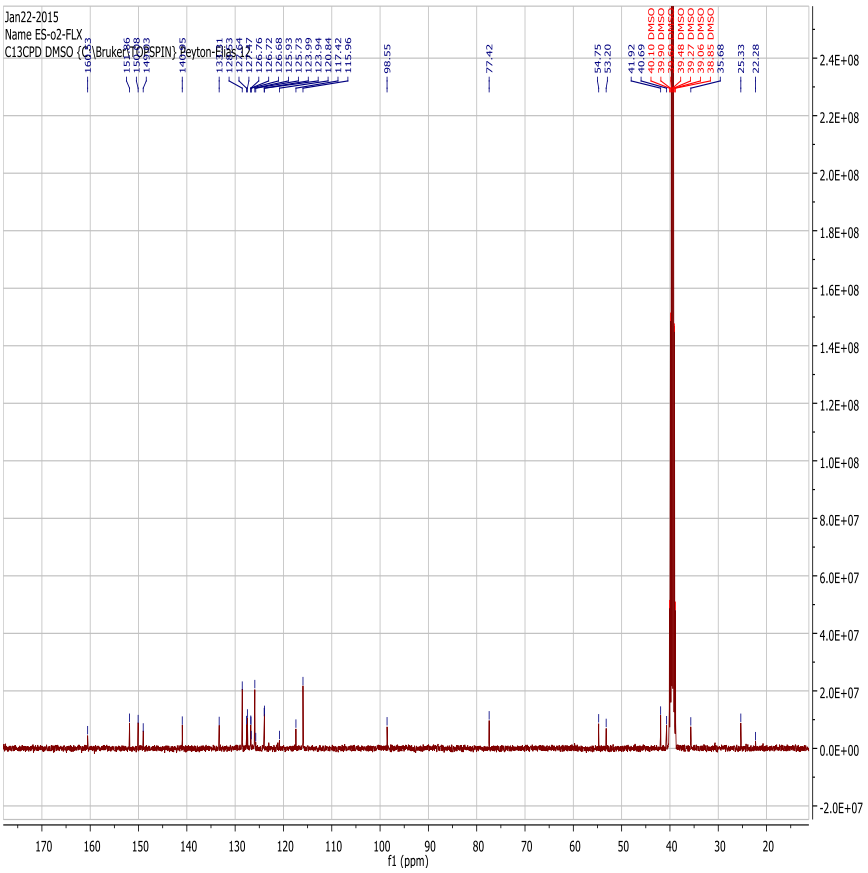


Figure 2.6. ^{13}C-spectrum of PL410

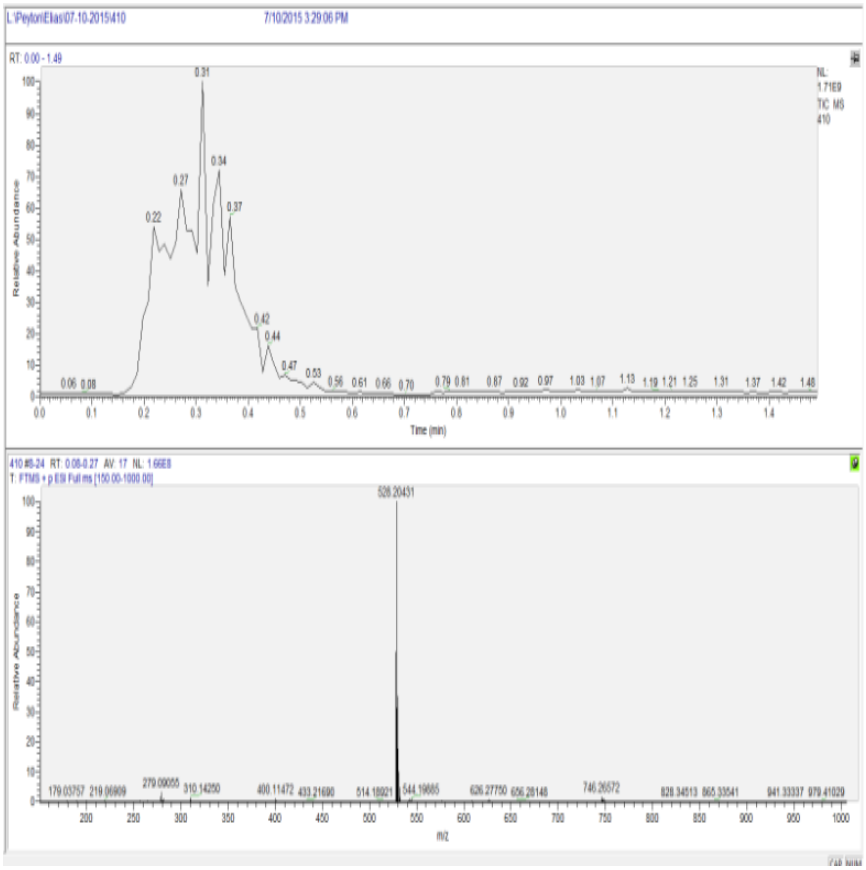


Figure 2.7. Mass spectrum of PL410

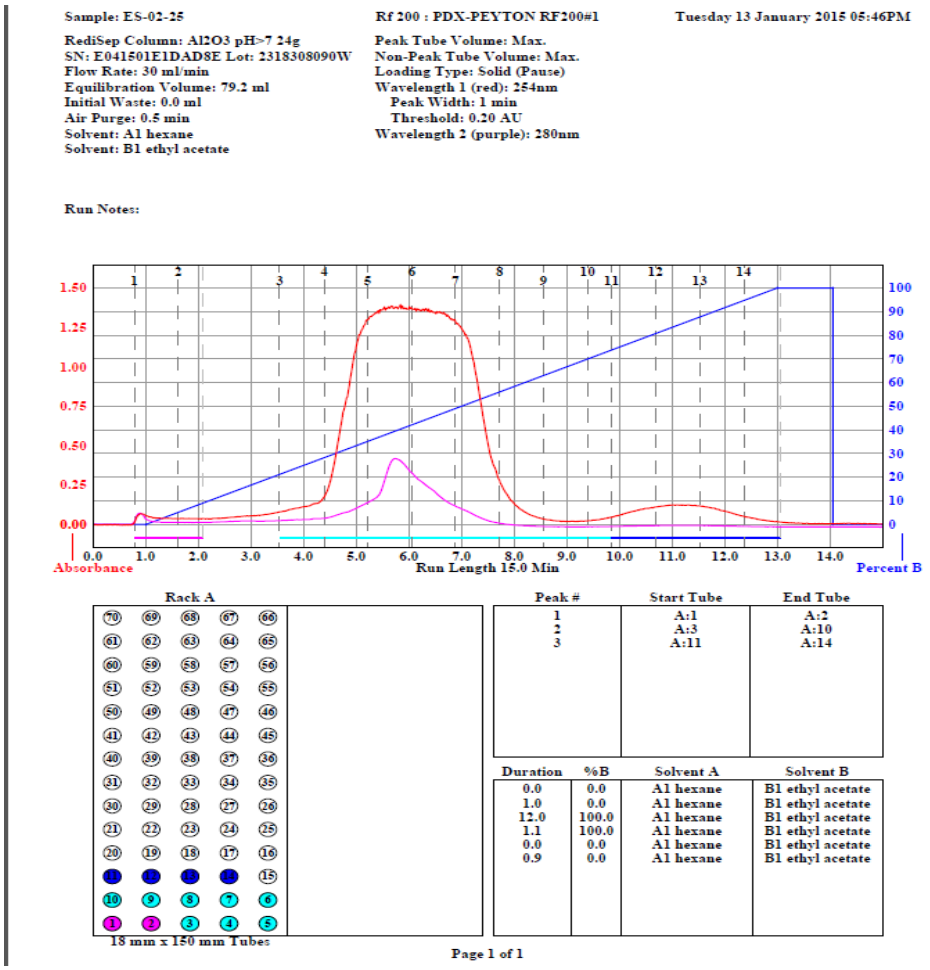


Figure 2.8. Combiflash chomatography of PL410

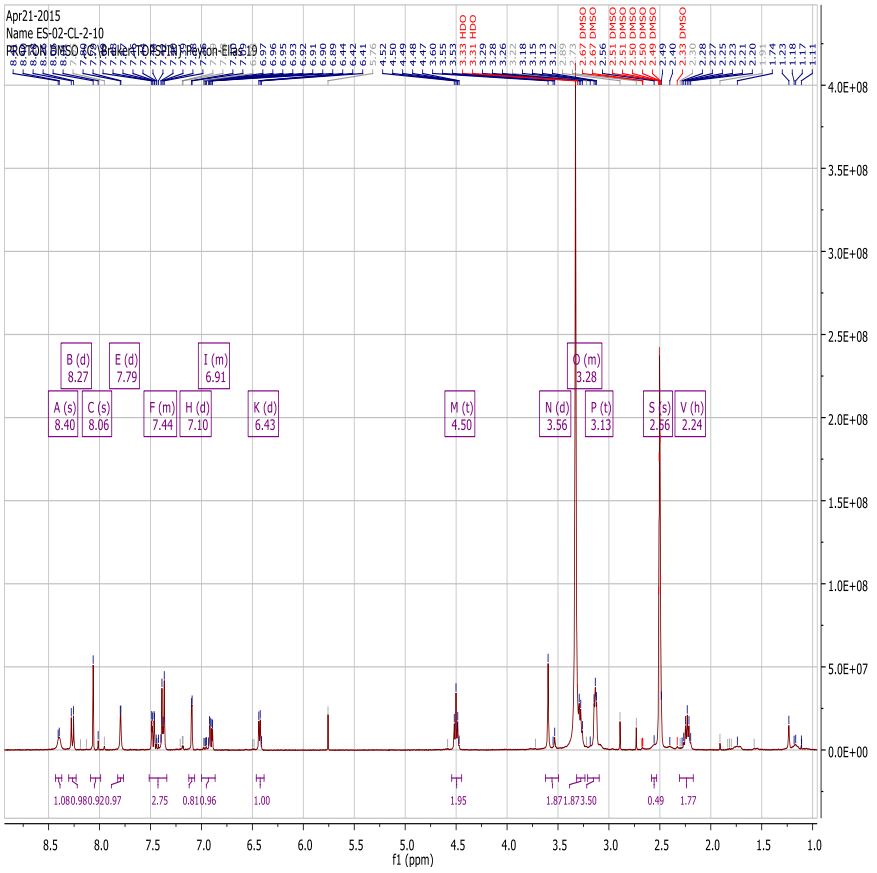


Figure 3.1. ^{1}H-proton spectrum of PL448

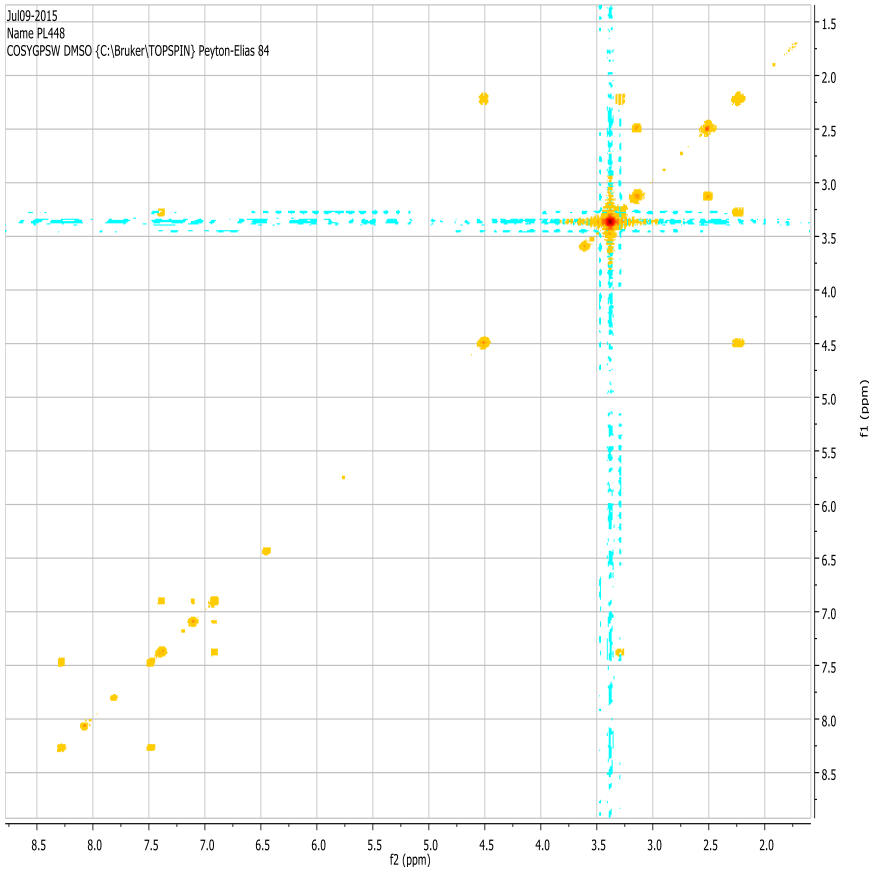


Figure 3.2. COSY spectrum of PL448

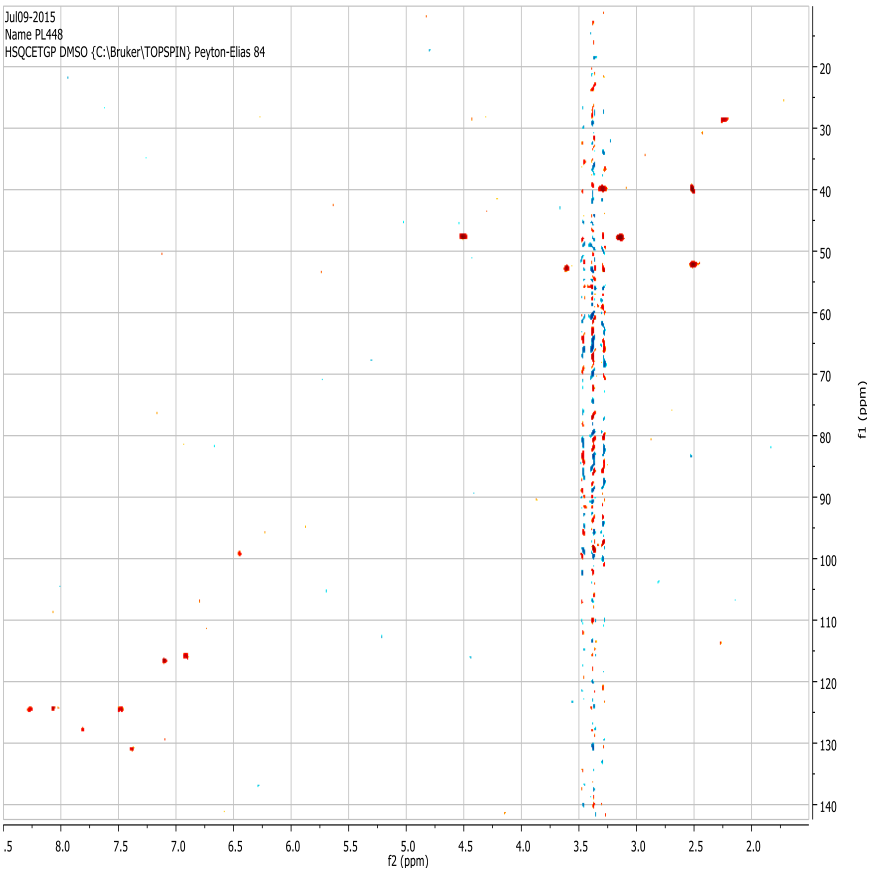


Figure 3.3. HSQC spectrum of PL448

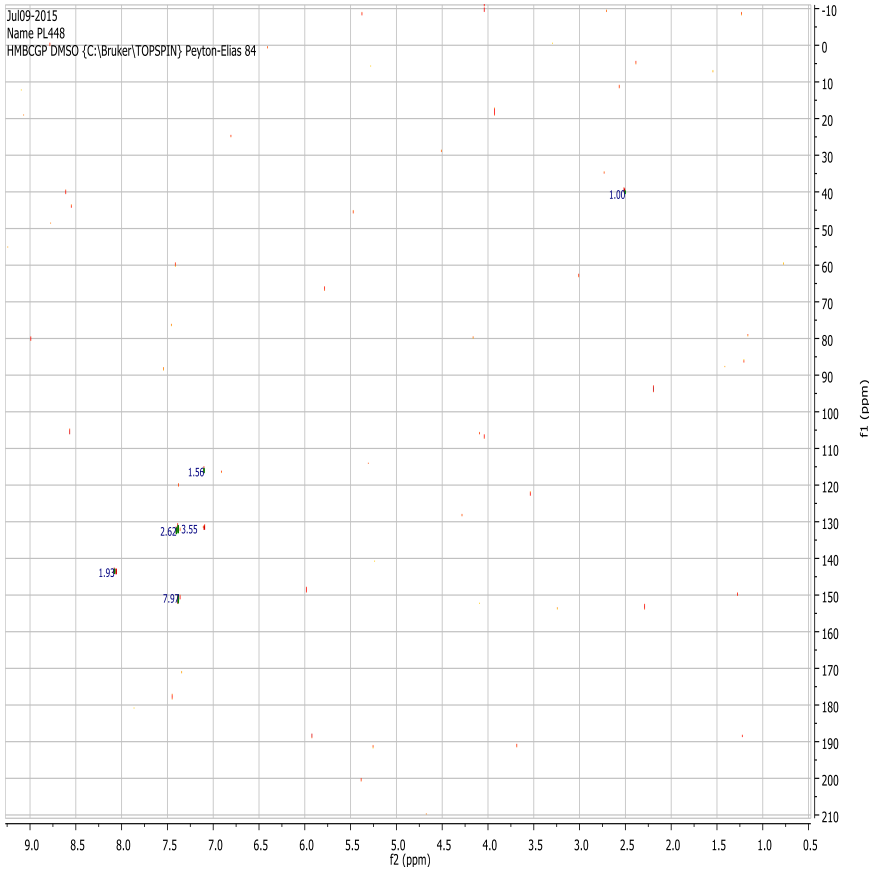


Figure 3.4. HMBC spectrum of PL448

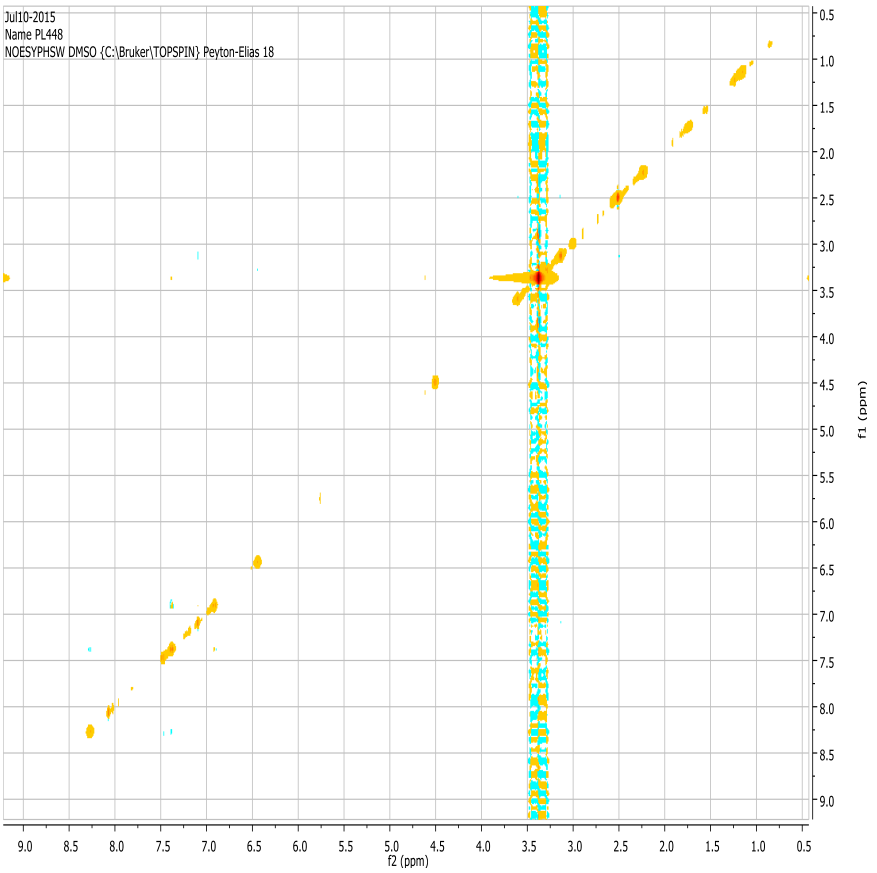


Figure 3.5. NOESY spectrum of PL448

Figure 3.6. ^{13}C-Specrum of PL448

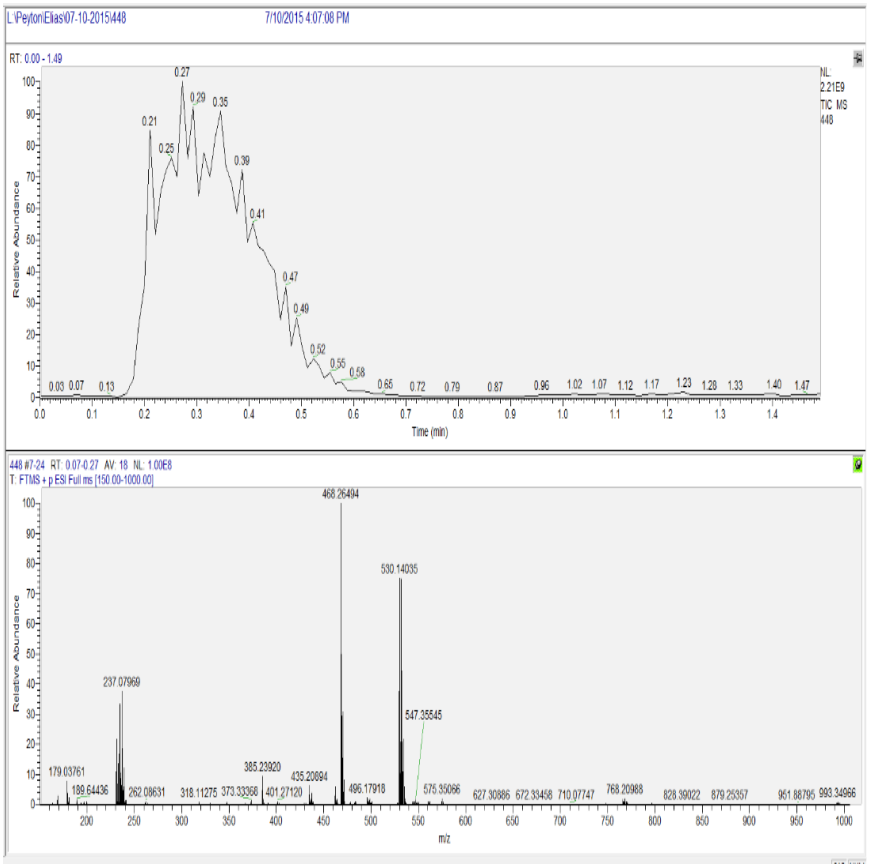


Figure 3.7. Mass spectrum of PL448

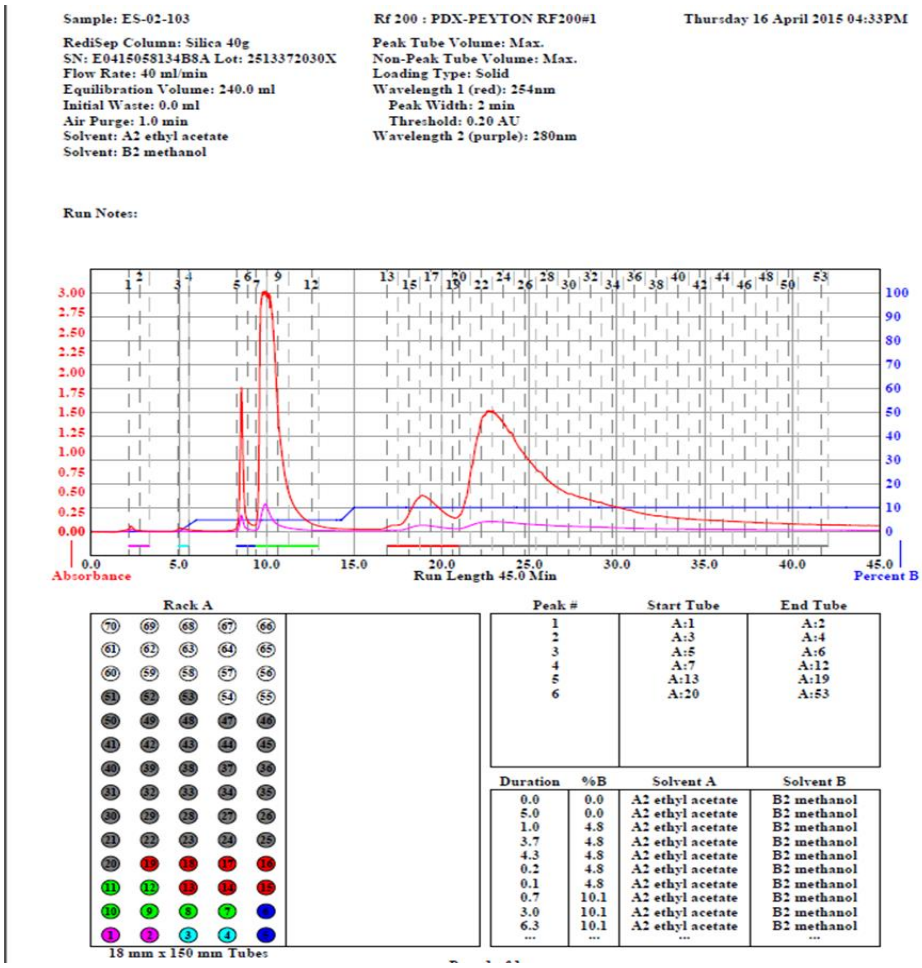


Figure 3.8. Combiflash chromatography of PL448

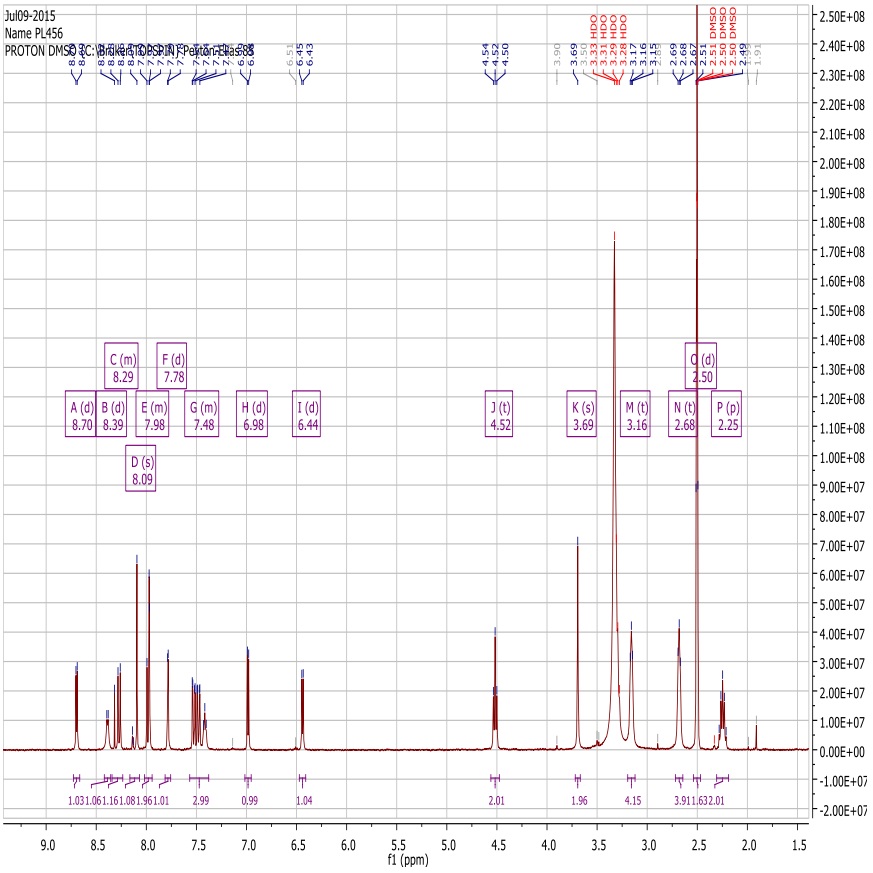


Figure 4.1. ^{1}H-proton spectrum of PL456

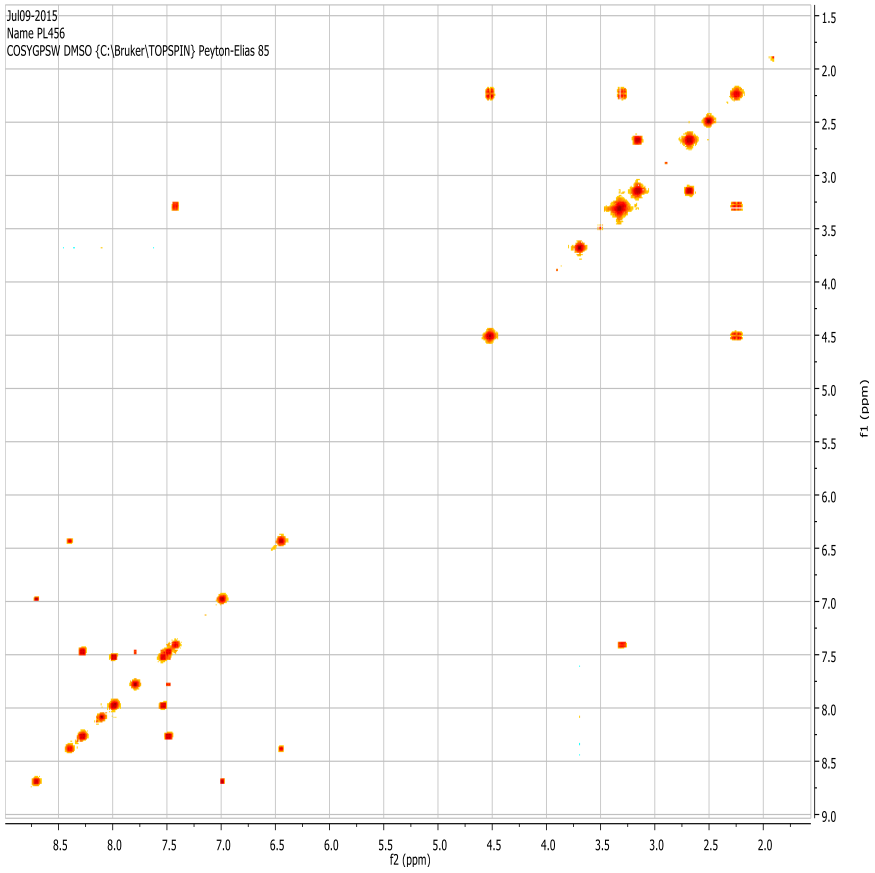


Figure 4.2. COSY spectrum of PL456

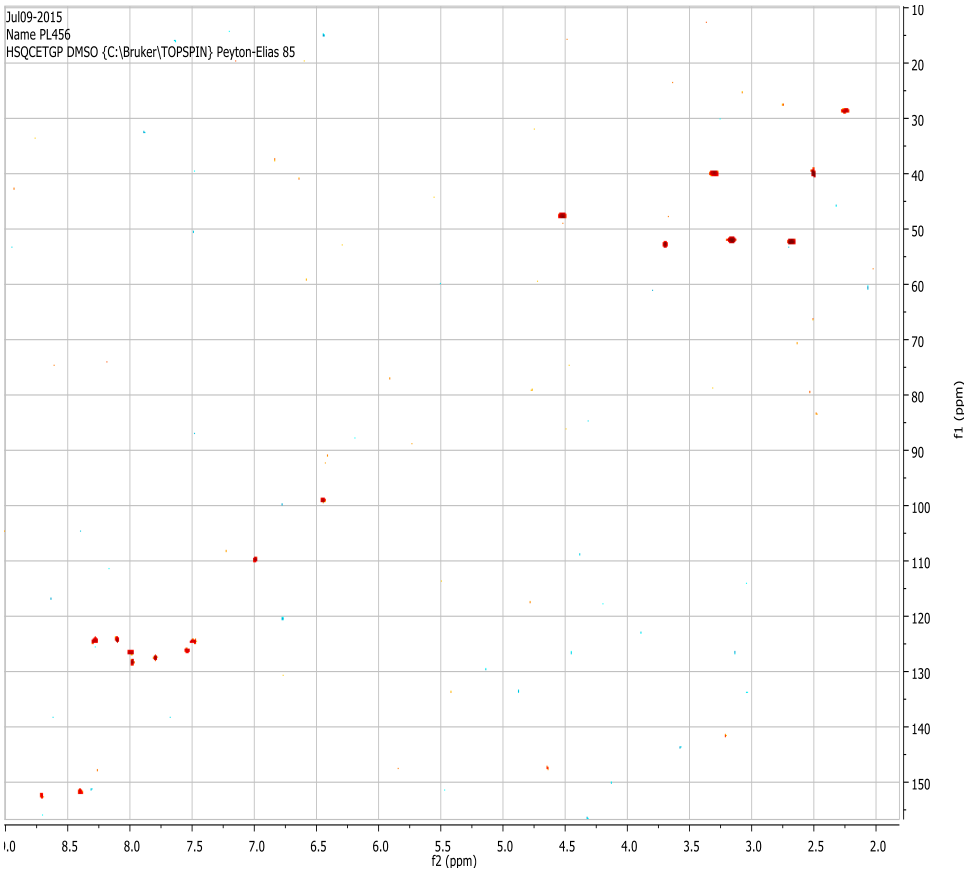


Figure 4.3. HSQC spectrum of PL456

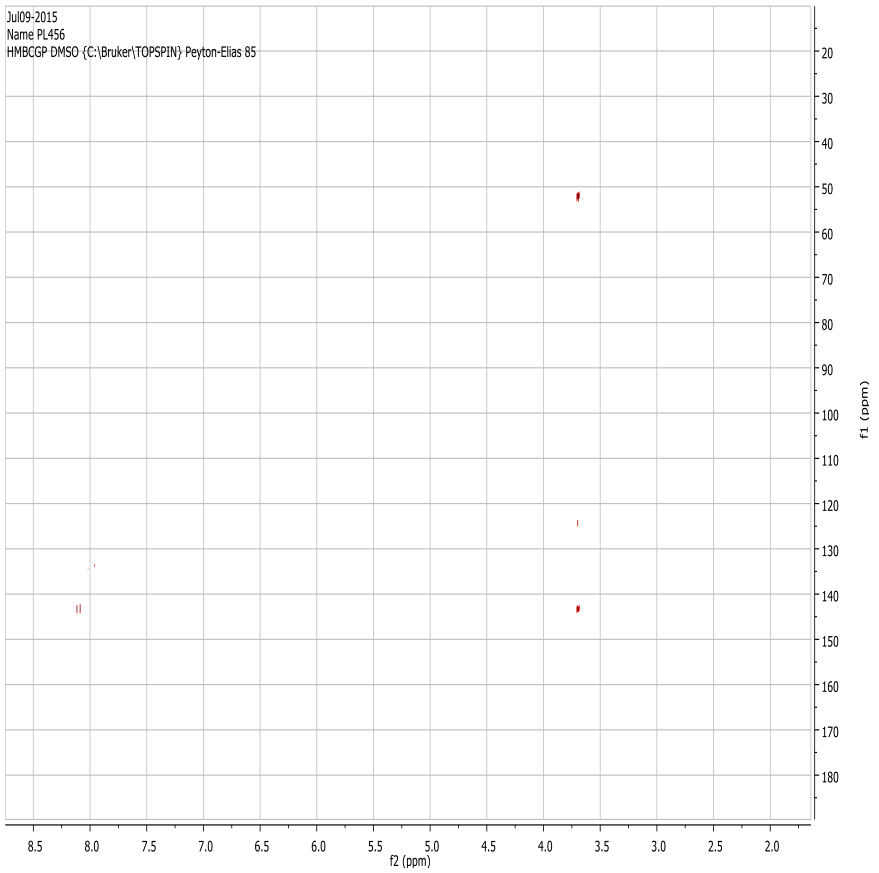


Figure 4.4. HMBC spectrum of PL456

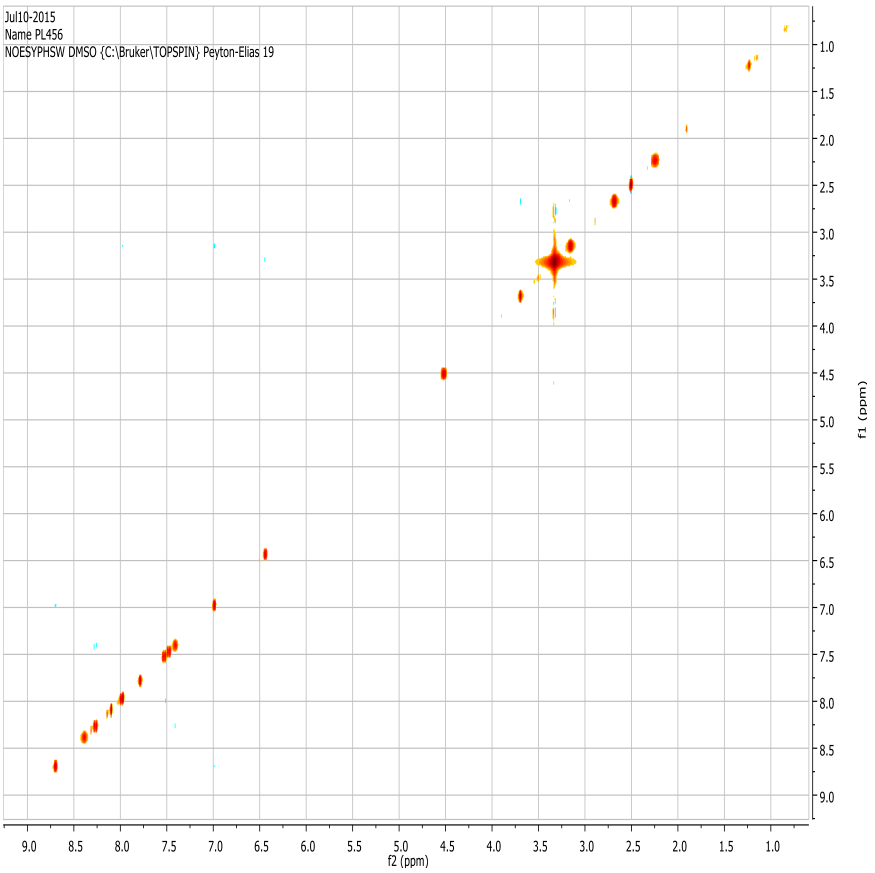


Figure 4.5. NOESY spectrum of PL456

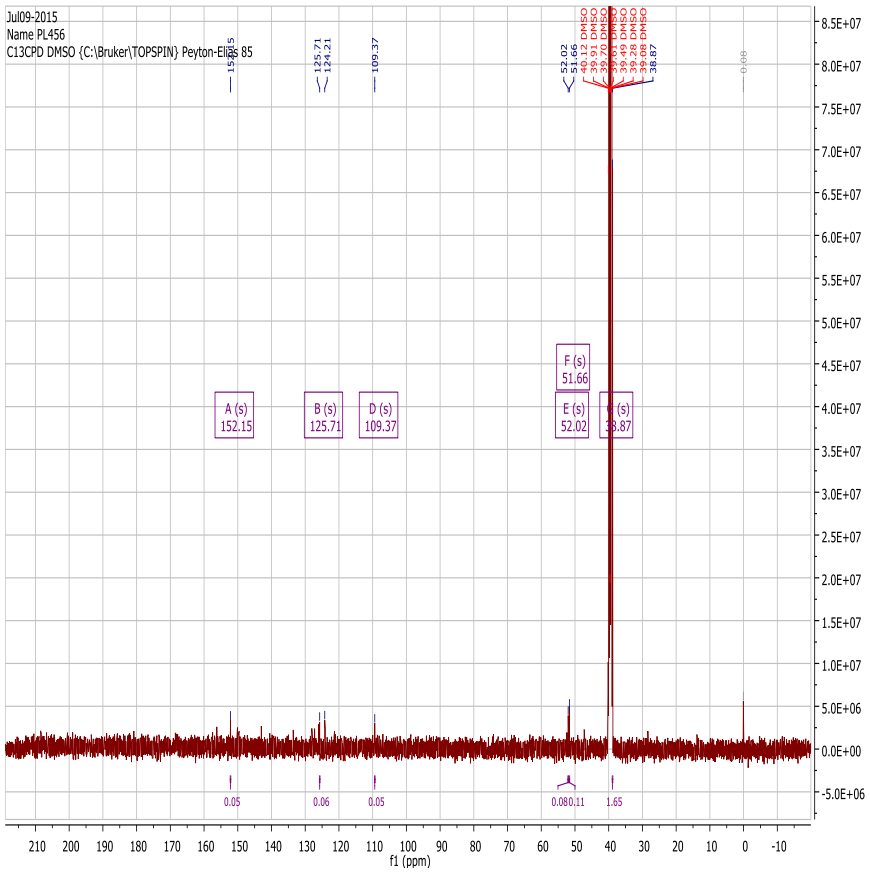


Figure 4.6. ^{13}C-Spectrum of PL456

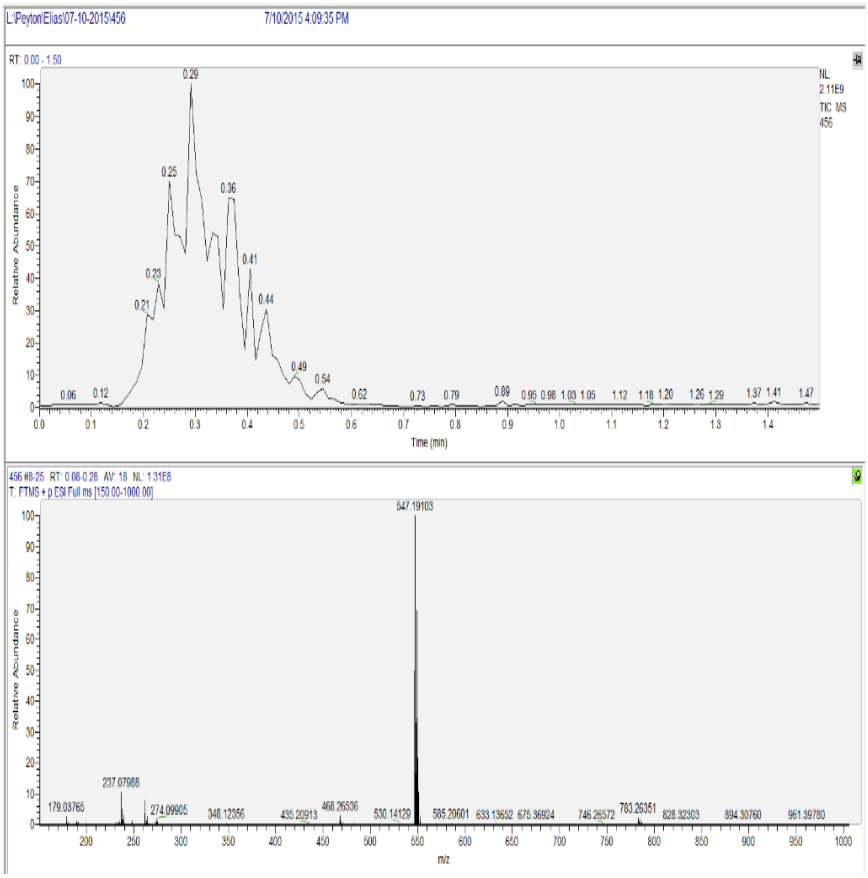


Figure 4.7. Mass spectrum of PL456

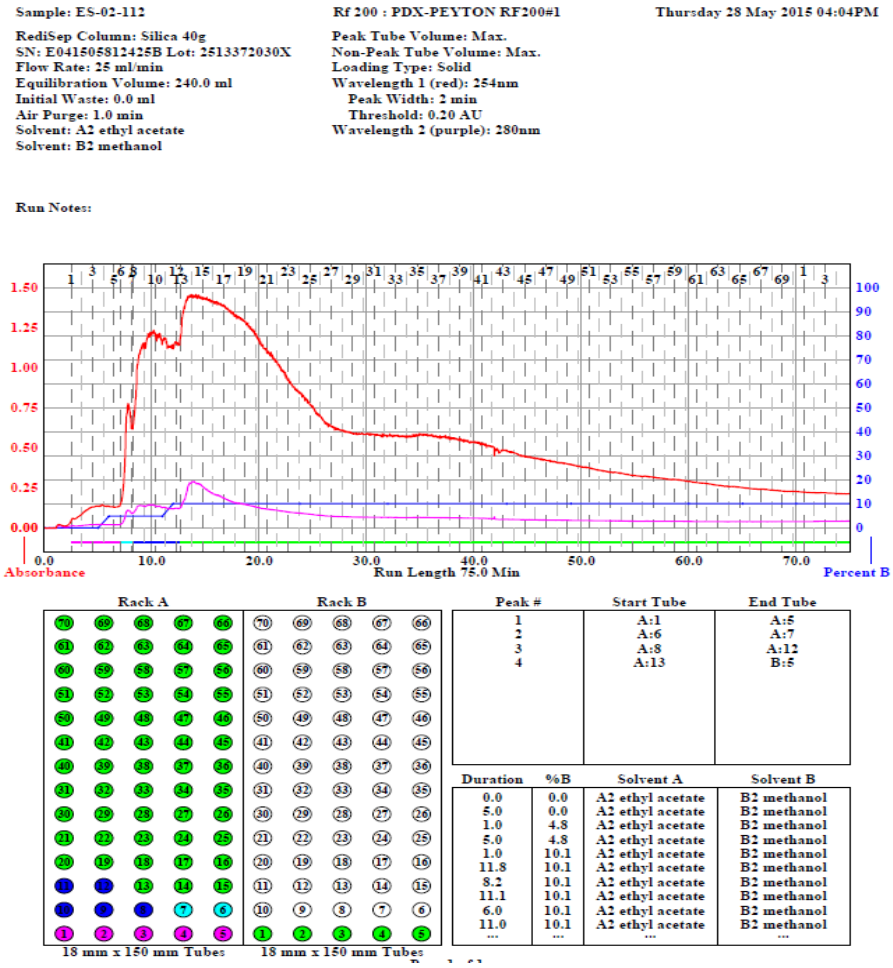


Figure 4.8. Combiflash Chromatography of PL456