Studies on Development of Benign Technologies for Some Organic Transformations with Organic Catalysts and Synthesis of the Substituted Neocryptolepines as Drug Candidates of Antimalarial Agents

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Chapter 1

Introduction and General Summary

1. Introduction

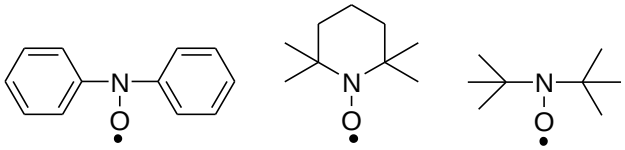
In the last few years there has been placed a strong emphasis on development of so-called "Green Chemistry", which has been employed to protect the environment from pollutants. Many toxic reagents are used in the daily laboratory reactions and their resulting by-product can cause environmental pollution. These chemicals are frequently discharged to the stream of water or air, thus cause either water, air or soil pollutions. As a result, these cause a great threat to our aquatic life as well as different kind of diseases like cancer, chronic lung disease and malaria. So we have two major problems, to minimize this man-made pollution and to explore new drugs to meet the needs of drug-resistant diseases which due to environmental etiologies.

With the rapid industrial development, environmental problems became increasingly serious. Oxidation reactions are among the most numerous and useful of the industrial processes and, at the same time, the most hazardous and polluting ones. They often occur with high E (environment)-factor, defined by Sheldon as the mass of waste, corresponding to all compounds used which are not incorporated in the product, per mass unit of product.[1]

The oxidation of alcohols to their corresponding carbonyl compounds is a pivotal transformation in organic chemistry.[2] Traditionally, oxidation of alcohols is carried out using stoichiometric amounts of metallic oxidants notably chromium(VI) reagents,[3] permanganates,[4] and ruthenium(VIII) oxide,[5] which produce environmentally unacceptable heavy metal wastes.[6] Since the goal of avoiding the use of environmentally unfriendly or toxic metals has always been considered extremely important, the development of efficient metal-free oxidation catalysts has been very actively pursued in the field of organocatalysis. Many of the organic catalysts identified in recent years have also been immobilized on different supports, such as Swern Oxidation, Corey-Kim Oxidation and Dess-Martin oxidation have been developed over the past several decades.[7] Among these, the

2,2,6,6-tetramethyl-1-piperidinyloxy [TEMPO (**2**), a nitroxyl radical] (Figure 1) catalyzed oxidation method has attracted attention in many areas of synthetic organic chemistry because it enables the use of various safe bulk oxidants, thereby enabling a safe and extremely efficient oxidation of alcohols with considerable operational simplicity.[8-9]

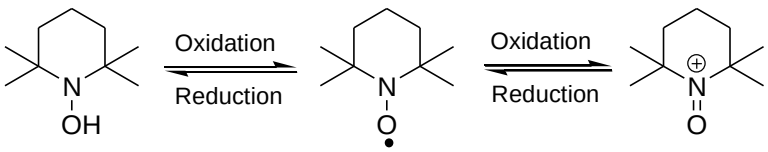
Figure 1. Structures of organic nitroxyl radicals



1 **2** **3**

Organic nitroxyl radicals, such as conjugated nitroxyl radical the diphenylnitroxyl radical (**1**) have been known since the early 20th century.[10] The stable, non-conjugated nitroxyl radicals TEMPO (**2**) and di-*tert*-butylnitroxyl (**3**) were first reported by Lebedev and Kazarnovskii [11] and Hoffmann and Henderson, [12] in 1960 and 1961, respectively. The unpaired electron in these radicals is delocalized over the nitrogen-oxygen bond, as shown in Figure 1, and this accounts for their high stability. Thus, radicals of this type can be stored for long periods of time without decomposition. They dissolve in both polar and non-polar solvents to form brightly colored solutions, e.g., TEMPO is bright orange.

Scheme 1. Oxidation and reduction of TEMPO

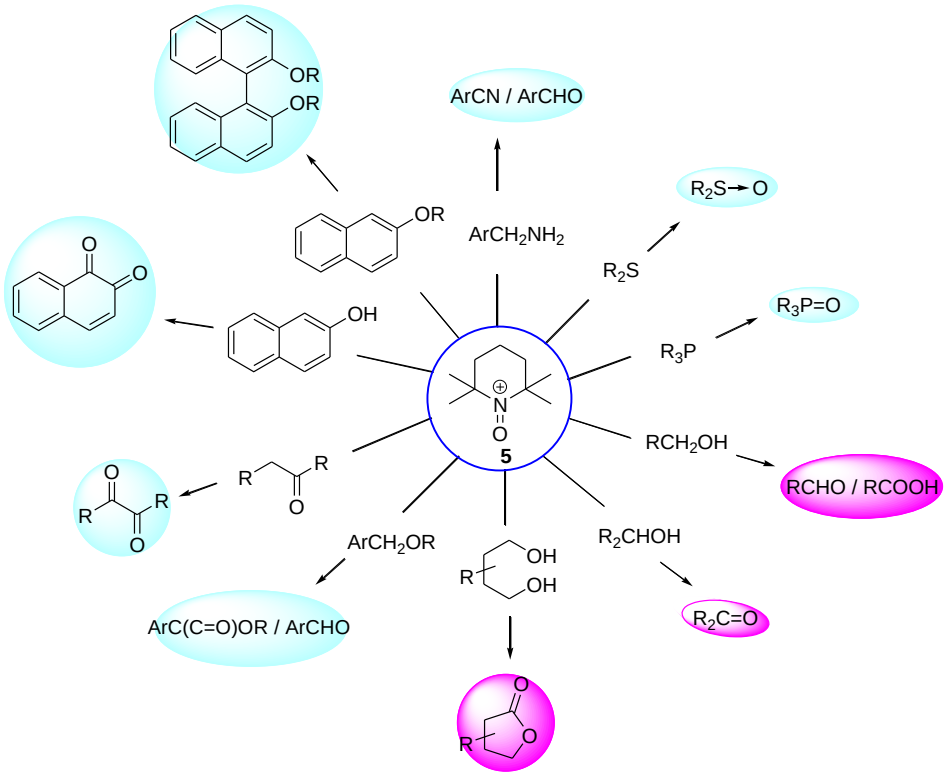


4 **2** **5**

Such nitroxyl radicals (TEMPO) give on oxidation the oxoammonium ion (**5**) and

on reduction hydroxylamines (**4**) (Scheme 1). Nitroxyl radicals are weak oxidants, and by themselves they are limited to the oxidation of ascorbic acid and phenylhydrazine.[13-14] In these redox reactions, TEMPO is converted to the corresponding hydroxylamine. In contrast, when the oxoammonium ion is generated by oxidation of the nitroxyl radical TEMPO, a much stronger oxidant will be resulted, which is capable of oxidizing a large variety of substrates. The important oxidative transformations effected by the oxoammonium ion are shown in the form of a rosette in Scheme 2.[15]

Scheme 2. A rosette of the important oxidative transformations effected by the oxoammonium ion (**5**).



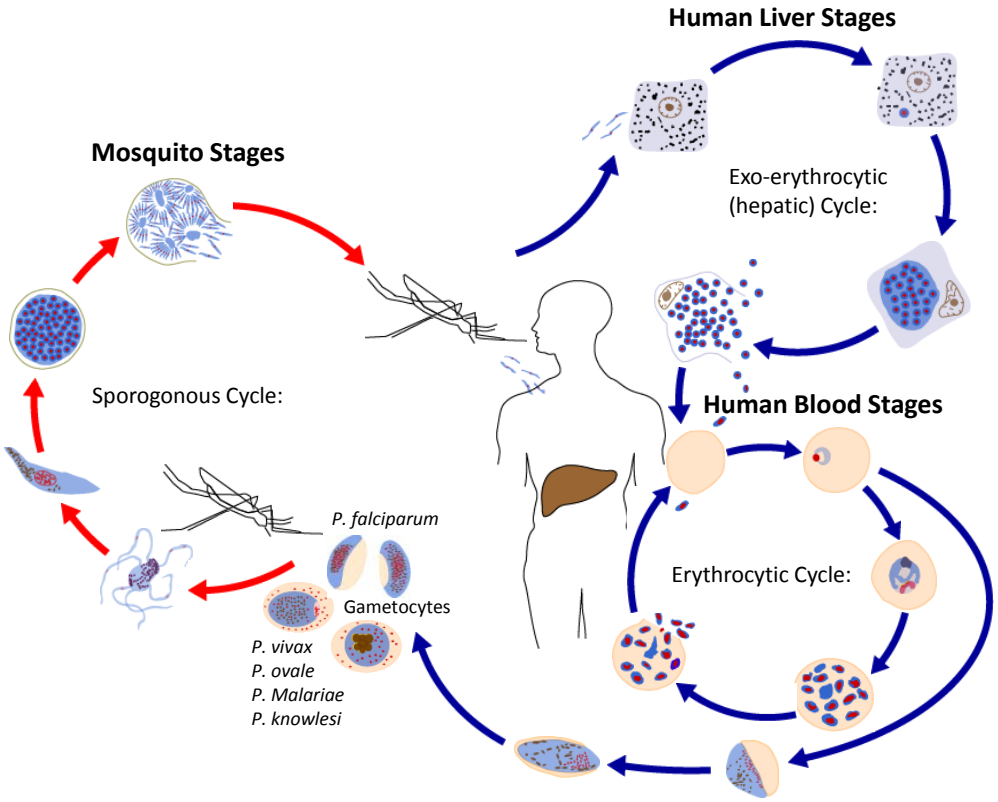
Based on these facts, we can summarize that TEMPO is an environmentally friendly catalyst, because it could be a remarkably stable radical, easily handling, and recyclable and stoichiometric transforming ability for alcohol oxidation.

Meanwhile, exploring new drugs to meet the needs of drug-resistant diseases is still a hot topic in the medicinal chemistry. Especially malaria remains the most

devastating disease in the tropical and subtropical regions where both developing and non-industrialized nations, with staggering infection and mortality statistics. According to the World Health Organization (WHO), this disease led to about 216 million malarial infected cases in 2010, and approximately 0.7 million died due to the non-availability of proper treatment, involving mostly children under 5 years old.[16]

Human malaria is caused by five species of the genus *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P.knowlesi*. The falciparum species is responsible for the majority of human deaths from malaria. Human suffers malaria when bitten by the female of any one of the 60 species of Anopheles mosquito.[17] The life cycle of the parasite from mosquito to human blood, to the human liver, back to the blood, and back to another mosquito is well-known (Figure 2).[17-18]

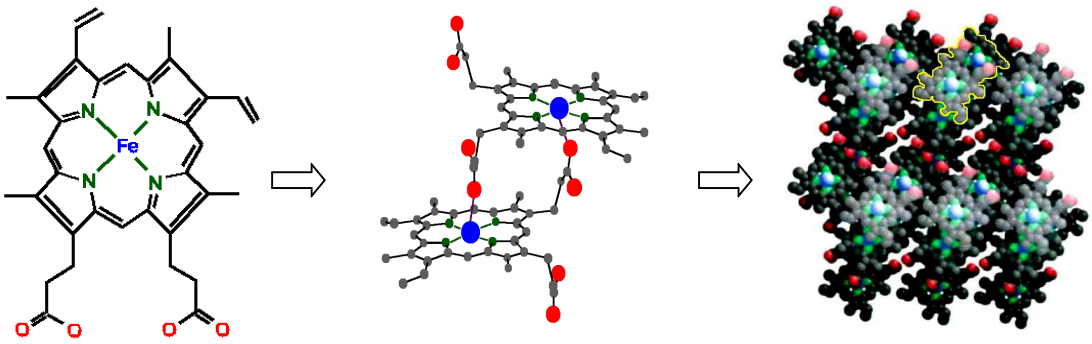
Figure 2. Malaria life cycle



Despite the worldwide public health impact of malaria, neither the mechanism by which the *Plasmodium* parasite detoxifies and sequesters haem, nor the action of

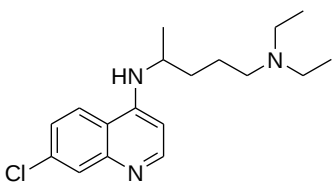
current antimalarial drugs is well understood. A proposal mechanism for *Plasmodium* parasite of detoxification process was reported that parasite converts toxic haem into a non-toxic haemozoin by a mechanism known as haemozoin formation (Figure 3). The toxic haem groups released from the digestion of the haemoglobin of infected red blood cells are oxidized into nontoxic β-haematin dimer by axial ligation of one propionic acid of each Fe(III)-protoporphyrin-IX unit and the β-haematin dimer are aggregated into an insoluble material called haemozoin or malaria pigment.[19]

Figure 3. Proposed mechanism of malarial detoxification process

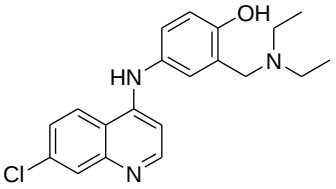


Several types of antimalarial drugs are reported to exhibit antimalarial activity by enhancing free haem toxicity through the inhibition of haemozoin formation. The families belonging to this category are quinoline, zaoles, methylenebule, xanthones, isonitriles and their derivatives. Amongst of them, quinoline and its derivatives represent a very important class of antimalarial drugs that function by targeting the parasite specific haemoglobin breakdown pathway.[20] Important members of this class are chloroquine (CQ), amodiaquine, amopyroquine, mepacrine, quinine, epiquinine, quinidine and bisquinoline, structures and antimalarial activities of which are shown in Figure 4.[21]

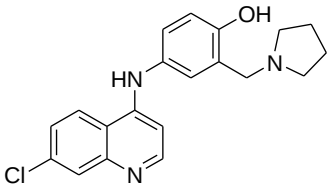
Figure 4. Structures and antimalarial activities[a] of quinoline and its derivatives



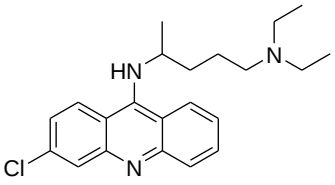
Chloroquine
IC_{50} = 14.0 nM (CQS)
IC_{50} = 192.1 nM (CQR)



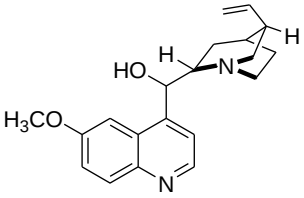
Amodiaquine
IC_{50} = 7.8 nM (CQS)
IC_{50} = 18.5 nM (CQR)



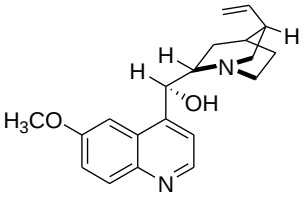
Amopyroquine
IC_{50} = 5.3 nM (CQS)
IC_{50} = 11.5 nM (CQR)



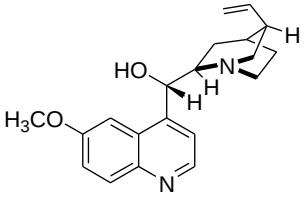
Mepacrine
IC_{50} = 12.9 nM (CQS)
IC_{50} = 43.3 nM (CQR)



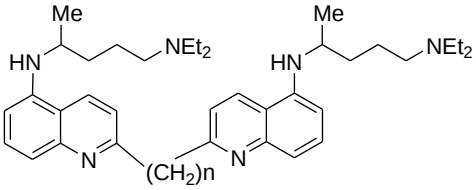
Quinine
IC_{50} = 34.2 nM (CQS)
IC_{50} = 81.2 nM (CQR)



Epiquinine
IC_{50} = 3471 nM (CQS)
IC_{50} = 1179 nM (CQR)



Quinidine
IC_{50} = 21.5 nM (CQS)
IC_{50} = 50.6 nM (CQR)



Bisquinolines (n=8-12)
IC_{50} = 123 nM (CQS)
IC_{50} = 25 nM (CQR)

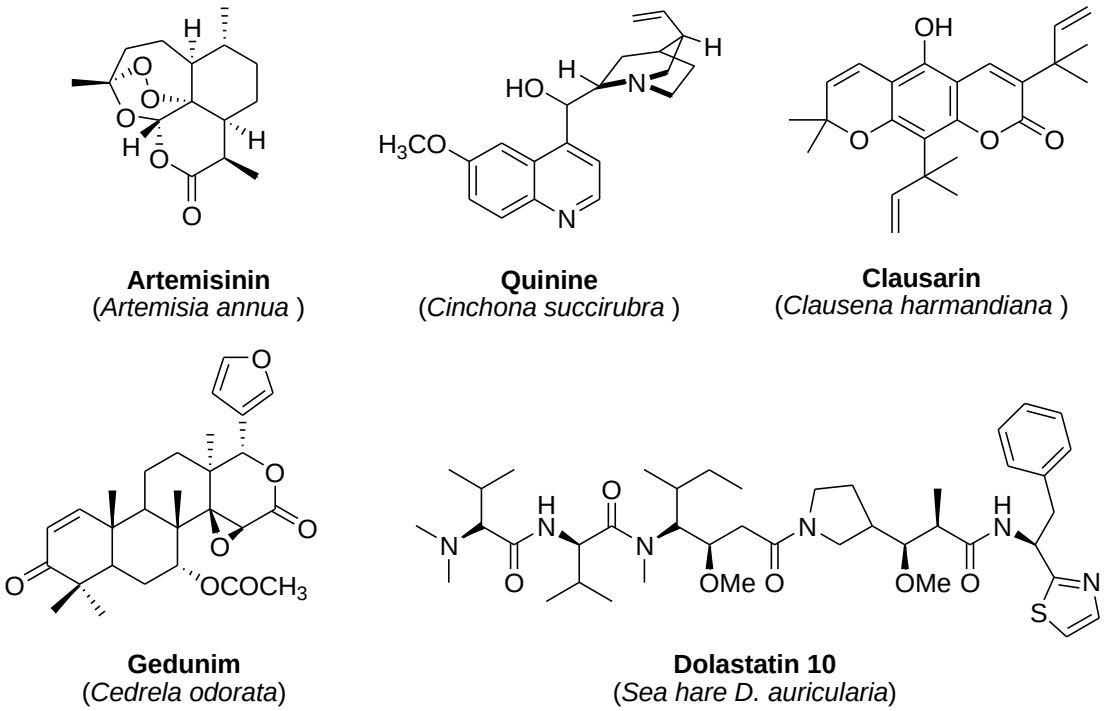
[a] CQS, chloroquine sensitive *P. falciparum*;
CQR, chloroquine resistant *P. falciparum*.

Chloroquine (CQ) is a weakly basic amphipath and accumulated inside the food vacuole. It interacts with the μ-oxo dimer form of oxidized haem and prevents the haemozoin formation. The π–π interaction between chloroquine and the electronic system of haematin governs the formation of adducts. Free haem and haem-chloroquine complexes kill parasites by inducing oxidative stress and this oxidative stress may lead to peroxidation of parasite membrane lipids, damage of DNA, oxidation of protein and finally parasite death.[22]

Malaria is still a major public health problem mainly due to the development of resistance by the most lethal causative parasitic species, *Plasmodium falciparum* to the mainstay drugs like CQ because of its remarkable therapeutic effect and low

cost.[23] Despite the introduction of the artemisinin-based combination therapies (ACTs), new drugs with unique structures and mechanism of action are urgently required to treat sensitive and drug-resistant strains of malaria.

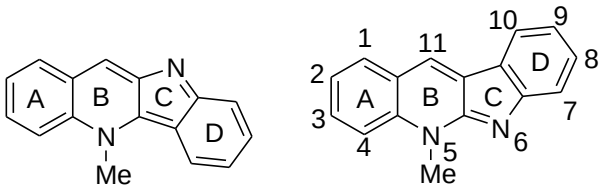
Figure 5. Antimalarial agents from natural products



Meanwhile, natural products are still important resources for discovery of new drugs for antimalarial (Figure 5). Compounds containing novel structure from natural origin also represent a major source for the discovery and development of new drugs for several diseases. Many naturally occurring compounds, including the major alkaloid cryptolepine (**6**) and minor alkaloid neocryptolepine (**7**), were isolated from the roots of the West African plants *Cryptolepis sanguinolenta* (Figure 6). Both of the two tetracyclic heteroaromatic compounds are linearly fused indoloquinolines and exhibit a promising antiplasmodial activity both against chloroquine-sensitive (CQS) and chloroquine-resistant (CQR) *P. falciparum*.[24-25] Further experiments have also indicated that cryptolepine inhibits the β-haematin formation which is responsible for

the treatment of malaria infections, and also has a cytotoxicity due to a DNA intercalation activity.[26-27] Therefore, neocryptolepine could be selected as the lead compound for the development of new antimalarial agents because of its lower affinity for DNA intercalation and topoisomerase II compared to cryptolepine.[28]

Figure 6. Structures of cryptolepine and neocryptolepine.



6, Cryptolepine **7, Neocryptolepine**

Based on these backgrounds, in this thesis, the author has engaged with three research topics toward the environmentally friendly technologies for organic transformations with organic catalysts and design and synthesis of neocryptolepine derivatives for antimalarial agents:

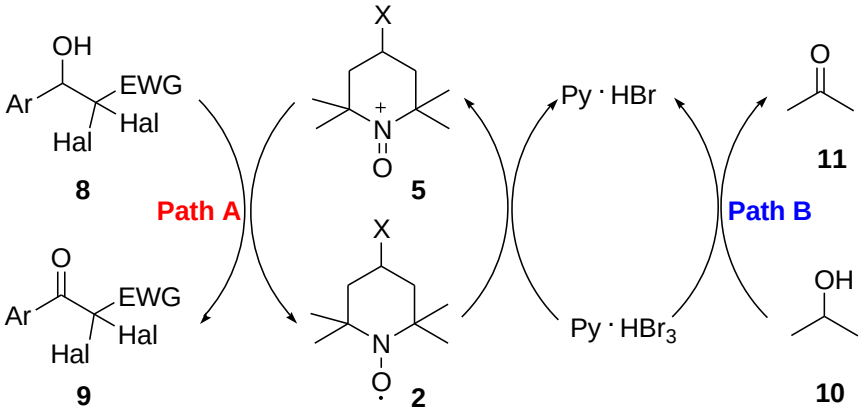
(1) High Performance and Selective Oxidation Method for Secondary Alcohols by Use of Organic Catalyst

(2) Green Procedure for Preparation of Carboxylic Acid by TEMPO Oxidation of Primary Alcohols

(3) Synthesis and Evaluation of Novel Neocryptolepine Derivatives for Developing Antimalarial Agents

In Chapter 2, the author described a combination of Py·HBr_3 as a co-oxidant and the electronically activated TEMPO as a recyclable catalyst is useful for oxidation of not only common alcohols, but also of the electron-deficient secondary alcohols such as $ArCH(OH)CFCl_2$ (Scheme 3). And the enhanced reactivity of the electronically activated TEMPOs was rationalized by the characterization of their redox properties.

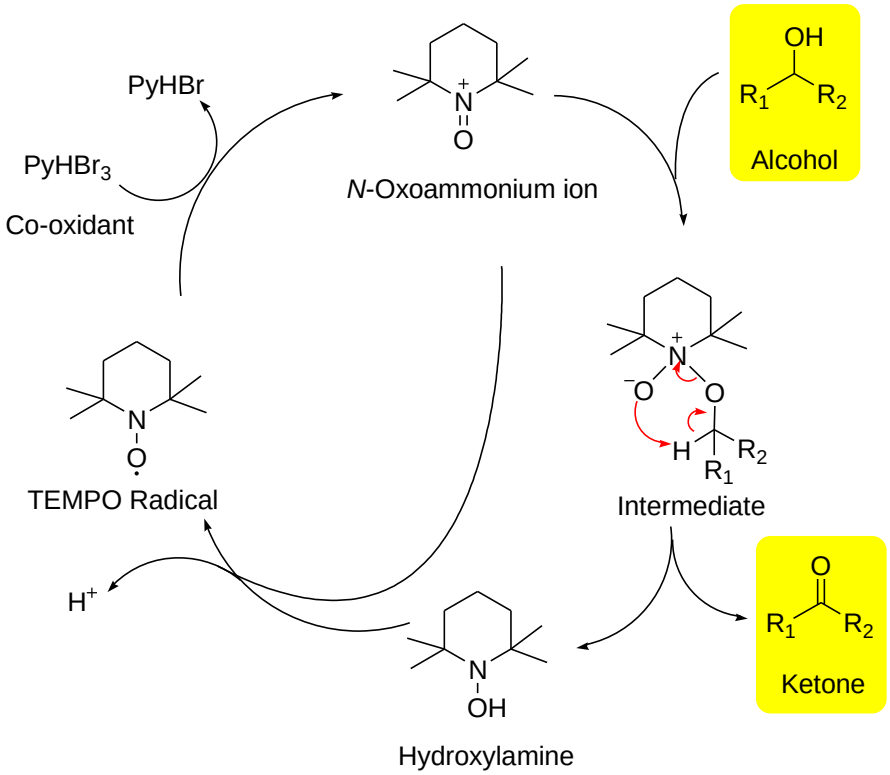
Scheme 3. Reaction paths of alcohol oxidation using EWG-TEMPO and Py·HBr_3



X = Electron□Withdrawing Group
Path A, Mainly about electron-deficient secondary alcohols;
Path B, Possible about electron-rich alcohols.

Oxidation of alcohols to their corresponding carbonyl compounds is a fundamental reaction in synthetic organic chemistry.[29] In this Chapter, the author focused on the TEMPO oxidation of alcohols in combination with a non-toxic co-oxidant since TEMPO is recoverable organic catalyst after the oxidation. Mostly important is that TEMPO oxidation is environmentally friendly procedure in organic chemistry, because TEMPO oxidations are performed in a catalytic manner as a non-metallic reagent. The remarkable acceleration can be explained by the proposed catalytic cycle shown in Scheme 4. TEMPO radical is first oxidized by co-oxidant (Py·HBr_3) to *N*-oxoammonium ion, which rapidly oxidizes the alcohol to the ketone and gives a molecule of the hydroxylamine, finally the hydroxylamine is oxidized to a TEMPO radical and completing the catalytic cycle.

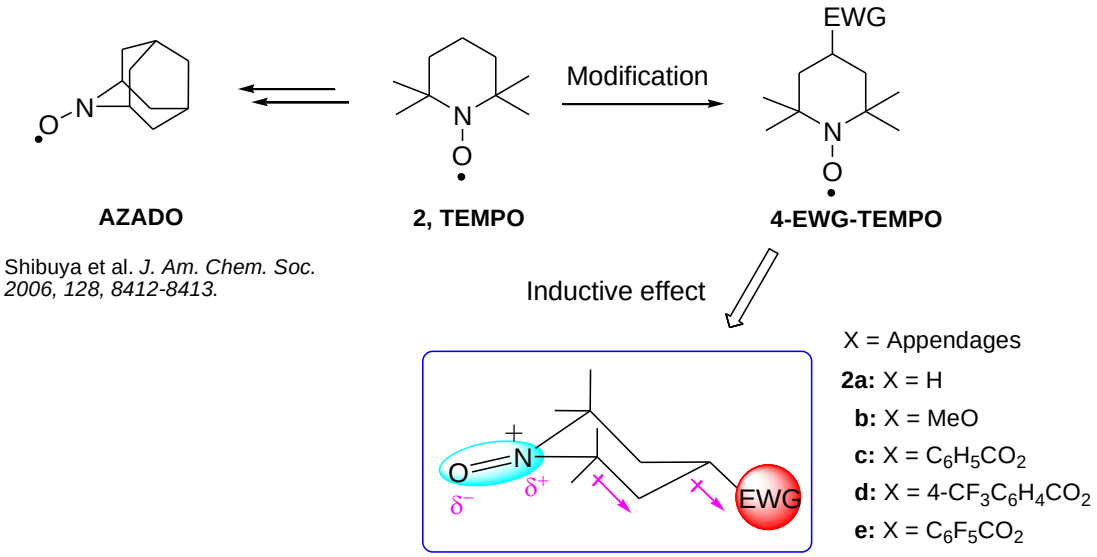
Scheme 4. Proposed mechanism of the TEMPO Oxidation



However, one distinguishing feature of the TEMPO-based method is its capability for the selective oxidation of primary alcohols in the presence of secondary alcohols.[30] The rationale behind such a feature is its reaction mechanism and the catalyst structure of that used, where four methyl groups flanking the nearby catalytic center play key roles in preventing bulky substrates from forming the key intermediate, which collapses to a carbonyl compound and the hydroxylamine (Scheme 4). Paradoxically, TEMPO is inefficient in the oxidation of structurally hindered secondary alcohols, posing a problem in the oxidation of alcohols. Although several modifications by reface of the reaction site of TEMPO have been devised in order to avoid steric repulsion in the nucleophilic addition of alcohol to the *N*-oxoammonium intermediate, as exemplified by Shibuya et al.[31] Examination of effects of the substituent at the C-4 position of TEMPO is still scarce today, so the author examined the effect of the substituents at the C-4 position to increase TEMPO reactivity in oxidation of secondary alcohols by testing four kinds of appendages (X) at the C-4

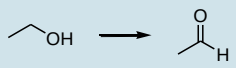
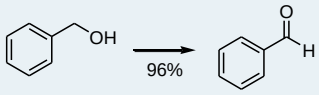
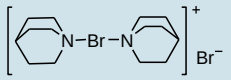
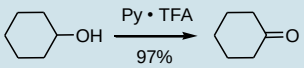
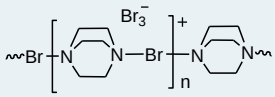
position (Scheme 5). Among these, TEMPOs (**2c**, **2d** and **2e)** are substituted with electron-withdrawing group (EWG) at C-4 position.

Scheme 5. Increasing reactivity of TEMPO



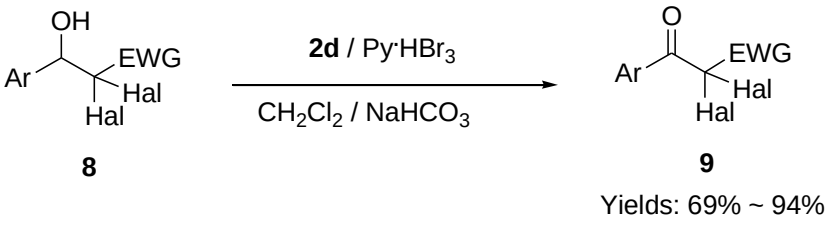
On the other hand, bromine and its amine complexes are capable of oxidizing alcohols, producing the corresponding carbonyls and various methods and bromine-intercalated reagents have been developed (Table 1).[32] In this chapter, the author examined the use of pyridinium hydrobromide perbromide (Py·HBr_3) as a co-oxidant, since this commercially available and stable reagent is less expensive than R_4NBr_3 (tetrabutylammonium tribromide, Bu_4NBr_3), and more advantageously polymer-supported pyridinium hydrobromide perbromide (Poly(N-vinylpyridinium)-hydrotribromide) is now available, though its oxidizing ability is not well characterized until now.[33]

Table 1. Bromine and its derivatives are capable of oxidizing alcohols

Co-oxidants	Reactions	Ref. 32
Br_2		*J. Am. Chem. Soc.* **1949**, *71*, 2829–2833 *J. Am.Chem. Soc.* **1972**, *94*, 6116–6119
$Br_2 + HNO_3 + O_2$		*Synlett* **2004**, 2203–2205
		*J. Org. Chem.* **1992**, *57*, 1600–1603
 Polymeric DABCO-Br_2 complex		*Tetrahedron Lett.* **2006**, *47*, 6635–6636

In this Chapter, the author described that the oxidizing system which carried out by combination of 4-$CF_3C_6H_4CO_2$-TEMPO and Py·HBr_3 in the two phase system of CH_2Cl_2–aqueous $NaHCO_3$ was useful for oxidation of common alcohols and some structurally hindered secondary alcohols, but also of the electron-deficient secondary alcohols (Scheme 6), and attained good yields (69%~94%).

Scheme 6. Oxidation of polyhaloalkylmethanols (**8**) with a combination of **2d** and Py·HBr_3



8 **9**

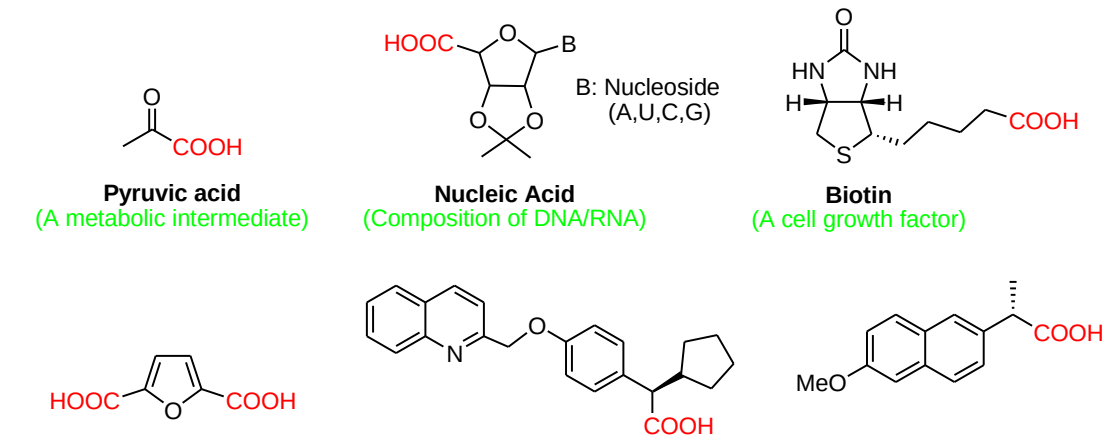
Yields: 69% ~ 94%

In Chapter 3, the author described a benign method for primary alcohol-carboxylic acid conversions with TEMPO-mediated oxidation in a biphasic system composed of aqueous layer and slightly miscible ether solvent, such as tetrahyrdopyran (THP), which is low harmful solvent and shown more efficient compared with cyclopentyl methyl ether (CPME), tetrahydrofuran (THF), diisopropyl ether, methyl tert-butyl ether and CH_2Cl_2. And following easily available co-oxidants such as Py·HBr_3,

Bu_4NBr_3, and electrooxidation were successfully applied to generate *N*-oxoammonium species as a recyclable catalyst, especially Py·HBr_3. The most favorable combination of TEMPO and Py·HBr_3 in THP–aqueous $NaHCO_3$ biphasic system was useful for oxidation of various primary alcohols including aromatic, aliphatic, and carbohydrate derivatives, and yielded the corresponding carboxyl acids.[34]

Synthesis of carboxyl group-containing compounds become an important organic chemistry, as the carboxyl group is widely found not only in natural compounds, such as pyruvic acid and biotin etc., but also common in drugs (Figure 7), [35] which can act as hydrogen bond acceptor in various ways, or as a hydrogen bond donor.[36] On the other hand, oxidation of alcohols to the corresponding carboxylic acids is one of a fundamental operation in organic chemistry.[37]

Figure 7. Structures of carboxyl group containing compounds

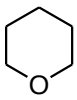
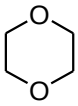


Although many approaches for oxidation of alcohols to the corresponding carboxylic acids have been developed, environmentally friendly procedure of alcohol-carboxylic acid transformations is still a hot topic in chemistry.[38] The author further his endeavor on TEMPO-mediated oxidation of alcohols with combination of electronically activated TEMPO and co-coxiant[39] and replace harmful solvent

(CH_2Cl_2) by ethereal solvent like THP.

THP is an important solvent in organic chemistry, consisting of a saturated six-membered ring containing five carbon atoms and one oxygen atom. THP shows medial physics between 1, 4-dioxane and THF, except for the solubility in water, which has a solubility of only 2.5~8.1 wt% in water (Table 2). Although THP's hydrophobicity is not as good as benzene, it could be considered as an easy to be separated from water. Furthermore, Yasuda, H *et al* have recently demonstrated the excellent stability of THP towards auto-oxidation compared with THF, as a result of their study on tributyltin hydride-mediated radical cyclizaion.[40] The author employed THP as the organic layer of a two-phase system in TEMPO-mediated oxidation of alcohols since THP could be less likely to form peroxide with oxygen during TEMPO oxidation.

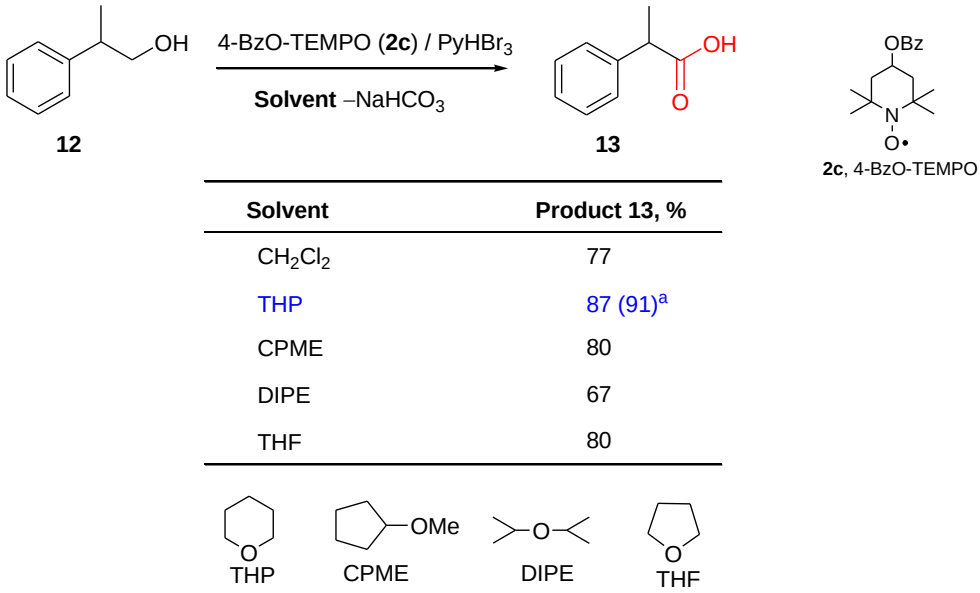
Table 2. Physic properties of THP and other solvents

Solvent	THF	THP	1,4-DIOX	Benzene
Structure				
bp (^oC)	65	88	102	80
mp (^oC)	–109	–45	12	5.5
Azeotropy ($^oC/H_2O$(wt%))	64/6	75/12	88/18	75/15
Solubility (/H_2O(wt%))	∞/∞	2.5/8.1	∞/∞	0.1/0.1
Dielectric Constant (^oC)	7.58	5.61	2.21	2.28

Subsequently, following ethereal solvents were compared with THP (Yield: 91%)

as organic layer by combination of TEMPO and Py·HBr_3 in oxidation of **12** to **13** (Scheme 7): CPME (Yield: 80%), THF (Yield: 80%), diisopropyl ether (Yield: 67%), methyl tert-butylether (Yield: 89%), CH_2Cl_2 (Yield: 77%). Among these solvents, THP should be the best because of its high yield.

Scheme 7. Oxidation of primary alcohol with a combination of 4-BzO-TEMPO and $PyHBr_3$

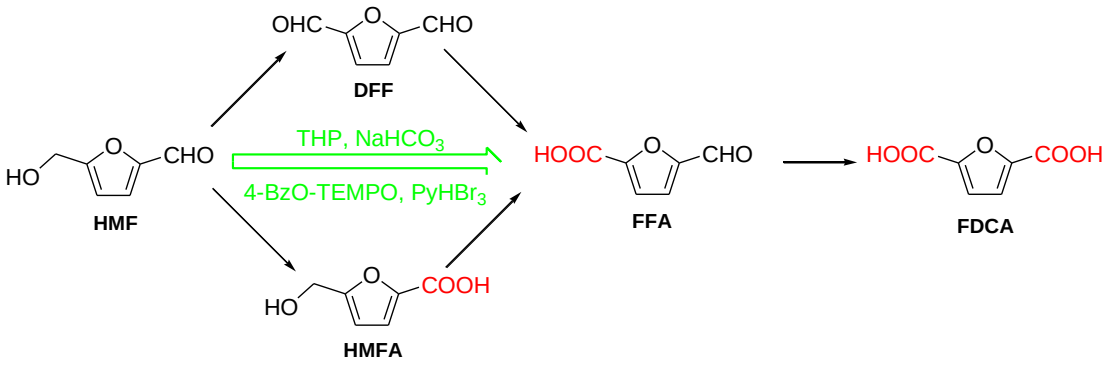


Solvent	Product 13, %
CH_2Cl_2	77
THP	87 (91)[a]
CPME	80
DIPE	67
THF	80

[a] Number in parenthesis is the yield obtained with a large scale operation (**12**, 15 mmol)

Furthermore, the present method by combination of TEMPO and Py·HBr_3 in THP–aqueous $NaHCO_3$ biphasic system was applied not only to aliphatic primary alcohols including acyclic and cyclic structures but also to aromatic and hetero aromatic alcohols, some of which are of significant synthetic value. Such as oxidation of 5-(hydroxymethyl)-2-furaldehyde (HMF), leads selectively to 5-formyl-2-furancarboxylic acid (FFA), useful as a precursor of 2,5-furandicarboxylic acid (FDCA). This method makes it much simple to synthesize FFA compared with other methods reported by Lewkowski J et al,[41] which were carried out by formation 2,5-diformyl furan (DFF) or 5-hydroxymethyl-2-furoic acid (HMFA) as intermediate (Scheme 8).

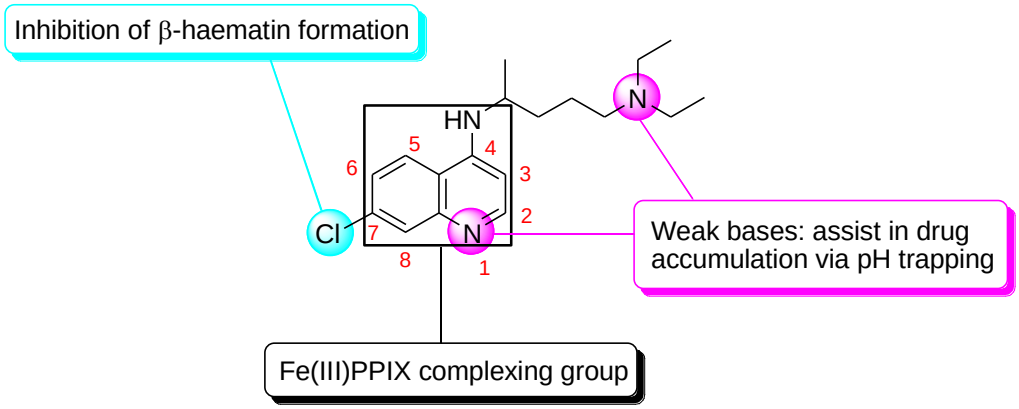
Scheme 8. Oxidation routes of HMF to FDCA



In Chapter 4, the author described synthesis and evaluation of novel neocryptolepine derivatives for developing antimalarial agents.

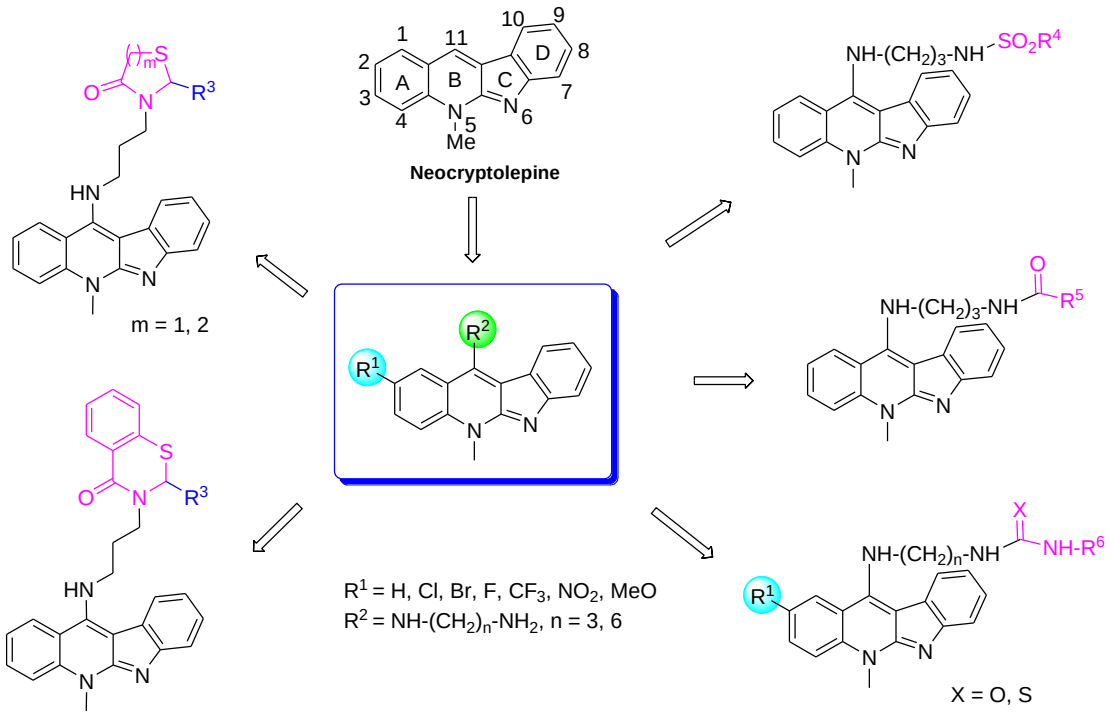
Since the spread of *Plasmodium falciparum* strains resistant to CQ is dramatically increasing over these years, new agents for antimalarial treatment is still urgently need to feed the preclinical pipeline. During the structure-activity relationship (SAR) study of CQ (Figure 8),[42] the author knew that elaborate other molecules which are found to form π–π complexes with Fe (III)PPIX and which inhibit β-haematin formation so as to arrive at novel antimalarials by semirational design. These findings are also of considerable interest when combined with the recent structure function investigations of Krogstad and co-workers[43-44] which have shown that changes in the length of the aminoalkyl side chain have little influence on activity against chloroquine-sensitive strains of *P. falciparum* but a profound influence on activity against chloroquine-resistant strains of the parasite. And further exploration also suggested that sufficiently large changes in the side chain alone could overcome the chloroquine-resistance without having to make changes in the 4-amino-7-haloquinoline template responsible for the Fe (III) PPIX complexation and inhibition of β-haematin formation.[42]

Figure 8. Proposed structure-function relationships in chloroquine



Based on these facts, the author synthesized and evaluated natural product derivatives based on neocryptolepine core, which is minor indolequinone alkaloid from the roots of the West African plants *cryptolepis sanguinolenta*. The author designed various novel neocryptolepine derivatives for improving antimalarial activity by modifications of the side chain at the C-11 position of neocryptolepine core under varying the substituents at the C-2 position with electron-withdrawing or electron-donating groups, and for further variation, the aminoalkylamino substituents were transformed into the corresponding acyclic or cyclic carbamates or thiocarbamates (Scheme 9).

Scheme 9. Modification of neocryptolepine analogues



These modified neocryptolepine derivatives were tested for antimalarial activity against CQR (K1) and CQS (NF54) of *Plasmodium falciparum in vitro*. The evaluation also included cytotoxicity toward mammalian L6 cells.

All the synthesized neocryptolepine derivatives showed potent antiplasmodial activities against CQR (K1) and CQS (NF54) *in vitro*. Some tested compounds showed more potent activity than CQ (Figure 9 and Table 3).

Figure 9. Structures of some modified neocryptolepine derivatives

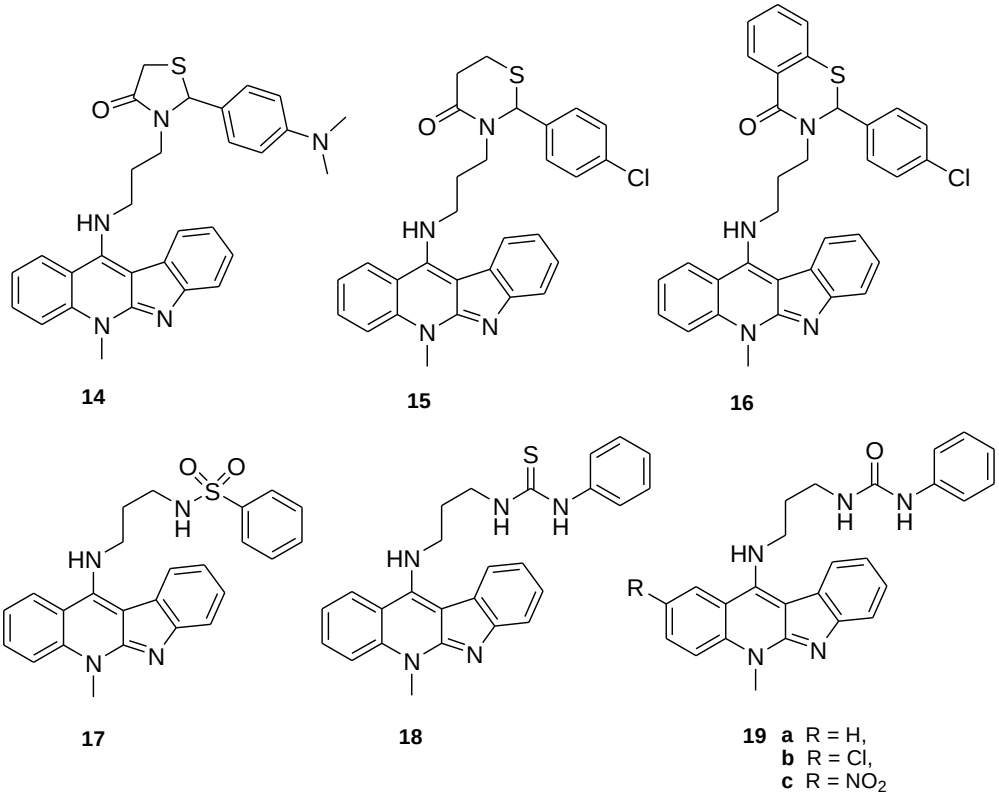


Table 3. Antimalarial activities of the modified neocryptolepine derivatives

	CQS (NF54) IC $_{50}$ (nM)	CQR (K1) IC $_{50}$ (nM)	Cytotoxicity (L6) IC $_{50}$ (nM)	SI[a] (L6/NF54)	SI[a] (L6/K1)	RI[b] (K1/NF54)
14	54.9	143.2	3551	64.7	24.8	2.6
15	52.4	38.8	3359	64.1	86.6	0.7
16	26.6	21.3	1099	41.3	51.6	0.8
17	63	123.7	65.2	1.0	0.5	2.0
18	9.1	111.5	1228	134.9	11.0	12.3
19a	21.3	9.4	1244	58.4	132.3	0.4
19b	2.2	-	3079	1400	-	-
19c	2.1	-	2732	1301	-	-
CQ	9.4	209.5	-	-	-	22.3

[a], Selectivity Index is the ratio of IC_{50} for cytotoxicity versus antiplasmodial activity (L6/P.f.)

[b], Resistance Index is the ratio of IC_{50} for the resistant versus the sensitive strain (K1/NF54)

As shown in Table 3, among these tested compounds, **19b** showed a 4 times more potent activity than CQ for CQS (NF54) with an IC_{50} of 2.2 nM and a selectivity index of 1400, similarly high antimalarial activity was shown by **19c**. Furthermore, **19a** showed a 22 times more potent activity than CQ for CQR (K1) with an IC_{50} of 9.4 nM, an electivity index of 132.3 and a resistance index of 0.4 with K1/NF54. These present findings are sufficient to establish that the methodical variation of the side chain of the neocryptolepine core provides a promising entry point toward affordable haem-targeted antimalarials that overcome the ever-increasing problem of worldwide drug resistance.

2. General Summary

In conclusion, the author summarized the present contributions as follows:

(1) A high performance oxidation method of alcohols with TEMPO substituted with an EWG at the C-4 position, which is useful for the electron-deficient secondary alcohols such as $ArCH(OH)CFCl_2$, has been developed by using $Py{\cdot}HBr_3$ as a co-oxidant. Reactivity of $Py{\cdot}HBr_3$ was discussed in terms of efficiency and selectivity in comparison with similar bromine compounds such as Bu_4NBr_3, and the method was easily extended to the polymer supported bromine reagents as a co-oxidant. Inductive activation of TEMPO by the appendage of electron-withdrawing group at the C-4 position was shown to facilitate the reaction rate, which was rationalized by measuring the cyclic voltammetry of the 4-substituted TEMPOs.

(2) The author developed an efficient primary alcohol-carboxylic acid conversion by employing TEMPO oxidation in ethereal solvent such as THP–aqueous layer instead of often used harmful solvents like CH_2Cl_2–aqueous layer. During the TEMPO mediated oxidation of primary alcohols, many easily available oxidants, such as $Py{\cdot}HBr_3$ and electrooxidation with bromide ion were successfully applied as co-oxidants. The method could easily be applied to various primary alcohols including aromatic, aliphatic, and carbohydrate derivatives, some of which are of significant synthetic value.

(3) A novel series of new neocryptolepine derivatives have been developed by systematically varying the 2-substituents of the neocryptolepine core and simply modifying the terminal amino group of the C-11 aminoalkylamino side chain. All the synthesized neocryptolepine derivatives were tested for antimalarial activity against CQR (K1) and CQS (NF54) of *Plasmodium falciparum in vitro* and cytotoxicity toward mammalian L6 cells. All tested compounds showed potent antiplasmodial activities against CQR (K1) and CQS (NF54) *in vitro* and some compounds, such as **19a-c**, showed much greater potency than CQ. These present findings are sufficient to

establish that the methodical variation of the side chain of the neocryptolepine core provides a promising entry point toward affordable haem-targeted antimalarials that overcome the ever-increasing problem of worldwide drug resistance.

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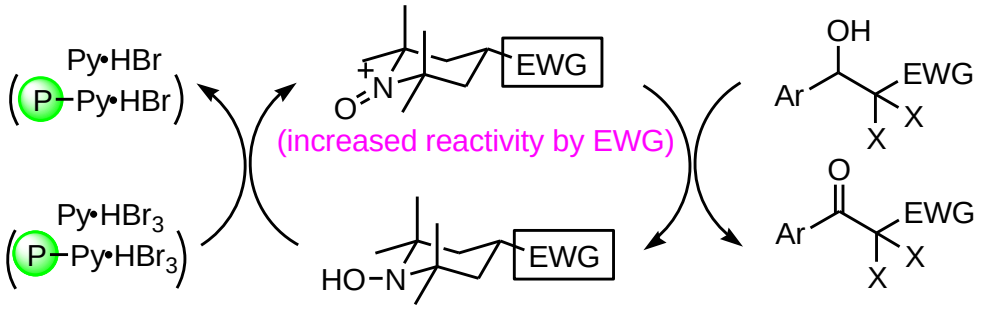
Chapter 2

High Performance and Selective Oxidation Method for Secondary Alcohols by Using of Organic Catalyst

2.1 Abstract

A new TEMPO-mediated catalytic oxidation method in combination with Py•HBr_3 (stoichiometric) is developed for oxidation of secondary alcohols to the corresponding ketones. The performance of this oxidizing system is better compared with that of TEMPO method combined with R_4NBr_3. Poly (4-vinylpyridine) •HBr_3 can be used in place of Py•HBr_3. The electron-withdrawing substituent at the C-4 position of TEMPO increases the reactivity of TEMPO significantly in the oxidation of electron-deficient alcohols such as polyhaloalkylmethanols. Inductive effect of the substituent of TEMPO is discussed through the characterization of the redox potential of N–O radical by cyclic voltammetry.



Keywords: Oxidation, TEMPO, Co-oxidant, Redox, Cyclic voltammetry

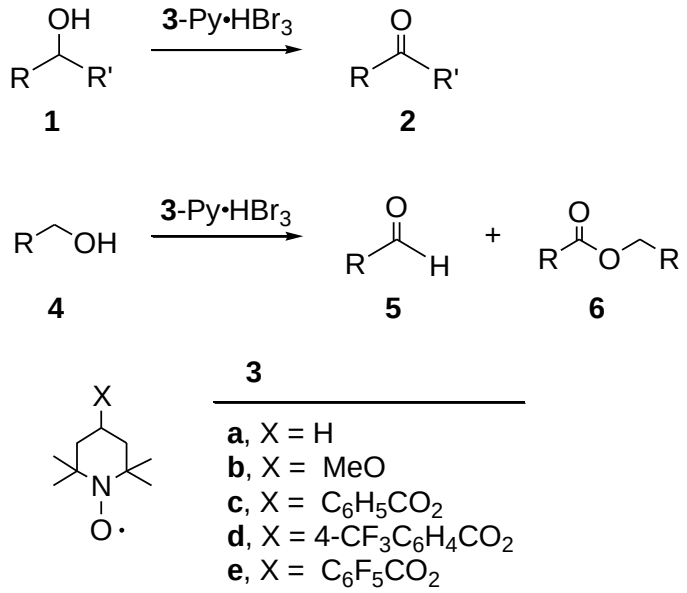
2.2 Introduction

Various oxidation methods of alcohols with metallic and non-metallic reagents,[1] performed in a stoichiometric or catalytic manner,[2,3] meeting with the demand in synthetic transformations have been developed. However, the examples of catalytic oxidation methods relied on non-metallic reagent are scarce in spite of increasing importance in view of green chemistry. In this context, the use of TEMPO in combination with a non-toxic co-oxidant[4] is practically meaningful especially for process chemistry producing pharmaceutical substances,[5] since the TEMPO is recoverable organic catalyst after the oxidation. In addition, the TEMPO and its *N*-oxoammonium intermediate, an active form for alcohol oxidation, can be featured by fair durability and safety in conducting the operation at ambient temperature.[6]

Although TEMPO is selective in the oxidation of primary hydroxy group in the presence of secondary ones,[6c] which is cumbersome to the oxidation of sterically hindered secondary alcohols. Accordingly, several modifications by reface of the reaction site of TEMPO have been devised in order to avoid steric repulsion in the nucleophilic addition of alcohol to the *N*-oxoammonium intermediate, as exemplified by using 2,2,6-trimethylpiperidine assembled in the adamantane framework[7] or 2,6-dialkylpiperidine on the 9-azabicylo[3.3.1]nonane structure as well as its homologues,[8] and acyclic derivatives.[9] However, the activation of 2,2,6,6-tetramethylpiperidine-*N*-oxoammonium intermediate through an inductive effect of electron-withdrawing appendage at the C-4 position would be viable approach for a high performance oxidation of alcohols because of commercial availability of 4-hydroxyTEMPO. Although the effects of the substituent such CN and amides at the C-4 position of TEMPO was examined on reduction rate of ascorbate,[10] the activation of the *N*-oxoammonium intermediate for oxidations based on the same protocol has not been attempted so far. Thus, we examined the effect of the substituent at the C4 position to attain smooth oxidations of secondary alcohols.

Previously, we developed convenient catalytic oxidation methods of alcohols with TEMPO in combination with following activation procedures and co-oxidants: (a) electrooxidation in the presence of bromide ion,[11a] (b) aerobic oxidation in the presence of ruthenium catalyst,[11b] and cheap co-oxidants such as (c) $NaBrO_2$,[11c] (d) R_4NBr_3,[11d] and (e) $Ca(OCl)_2$.[11c] Now, we examined the use of pyridinium hydrobromide perbromide (Py•HBr_3) as a co-oxidant,[12a] since this commercially available and stable reagent is less expensive than R_4NBr_3,[12b] and more advantageously polymer supported pyridinium hydrobromide perbromide is now available, though its oxidizing ability is not well characterized until now.[13]

Bromine and its amine complexes are capable of oxidation of alcohols, producing the corresponding carbonyls and thus far various methods and bromine-intercalated reagents have been developed.[14,15] In general, the bromine-oxidation of alcohol proceeds slowly even with respect to electron rich secondary alcohols. Accordingly, in executing the catalytic oxidation of alcohols with TEMPO by the aid of Py•HBr_3 (stoichiometric) as a co-oxidant, concurrent oxidation of the substrate with Py•HBr_3 is issue to be discussed (Scheme 1).



Scheme 1. TEMPO-mediated oxidation of alcohols with 3-Py•HBr_3.

2.3 Results and Discussion

2.3.1 TEMPO-mediated oxidation of aliphatic secondary alcohols

We firstly examined the reactivity of Py•HBr_3 as a co-oxidant in the TEMPO-mediated catalytic oxidation of secondary alcohols. As shown in Figure 1, the reaction of 2-undecanol (**1a**, R = C_9H_{19}, R' = CH_3) with a mixture of 4-BzOTEMPO (**3c**,[16] X = $C_6H_5CO_2$, 10 mol%) and Py•HBr_3 (1.5 equivalent) in a CH_2Cl_2-aqueous $NaHCO_3$ system completes within 15 min to form the corresponding 2-undecanone (**2a**, R = C_9H_{19}, R' = CH_3) in 99% (curve a). The amount of **3c** can be reduced to 1 mol% for this conversion (curve b), though the reaction becomes somewhat sluggish. The oxidation of **1a** to **2a**, which is presumably due to oxidizing ability of Py•HBr_3, was observed in the reaction system that lacks the presence of **3c** (curve c), though being of no synthetic potential. Noteworthy is that the oxidation of **1a** to **2a** by the combination of **3c** and R_4NBr_3 as co-oxidant proceeds much slower compared with Py•HBr_3 (curve d).[17]

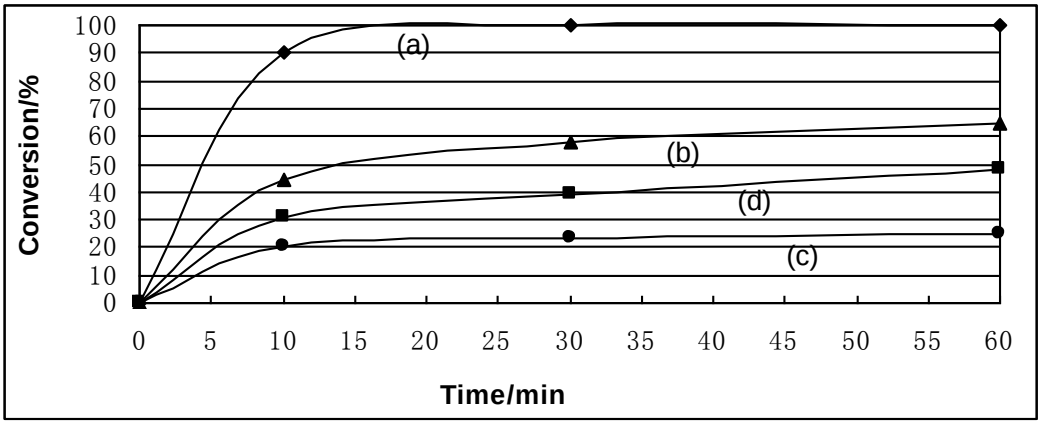
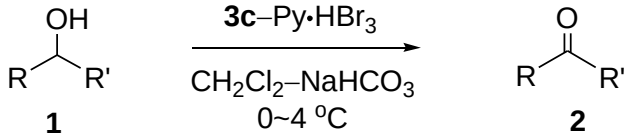
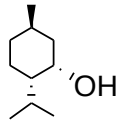
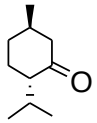
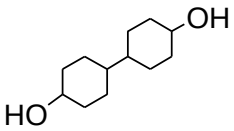
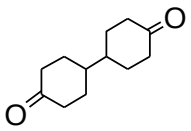
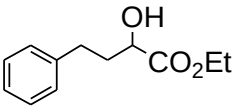
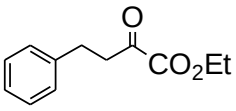
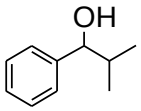
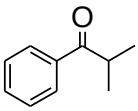
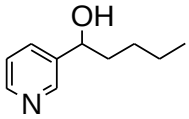
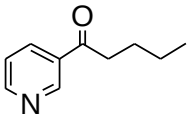
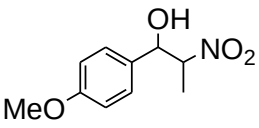
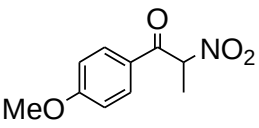


Figure 1.
Time-conversion curves for oxidation of **1a** to **2a** under varying the amount of **3c** with Py•HBr_3 (1.5 equiv.) at 0-4 ^{o}C. Symbols are as follows: **3c**, (a) = 10 mol%; **3c**, (b) = 1.0 mol%; **3c**, (c) = no addition; **3c**, (d) = (10 mol%)-Bu_4NBr_3. Data points were obtained by GC analyses.

The present oxidizing system comprised of **3c** (3~10 mol %) and Py•HBr_3 (1.5 equivalent) was applied to the oxidation of various secondary alcohols **1**. As shown in Table 1, most of secondary alcohols **1** can be oxidized at 0–4 °C, giving the corresponding ketones **2** in good yields. The reaction of sterically hindered alcohol such as menthol (**1b**) is best achieved at room temperature (entry 2). In place of chromate[18] or Swern oxidation methods, 2-nitroalcohol **1g**, accessible by Henry reaction, was smoothly oxidized to synthetically useful 2-nitroketone **2g** by the present method, though a small of amount of bromination at the C-2 position was accompanied (ca. 5%, entry 7).

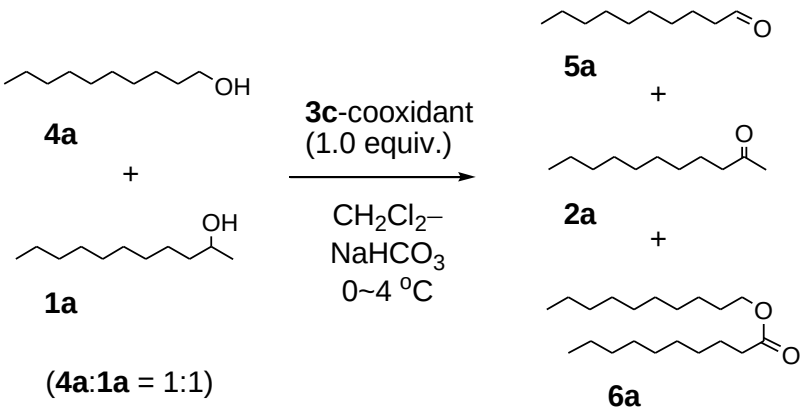
Table 1.
Oxidation of secondary alcohols with a combination of 4-BzOTEMPO (**3c**) and Py•HBr_3[a]



Entry	Alcohol **1**		Product **2**	Yield (%)[b,d]
1		**a**, R = C_9H_{19}		79 (78)
2[c]		**b**		76
3		**c**		93
4		**d**		81 (69)
5		**e**		80 (64)
6		**f**		84 (74)
7		**g**		87[e]

[a] Carried out by the reaction of **1** (1 mmol) with **3c** (5 mol%) and Py•HBr_3 (1.5~2 equiv.) at 0~4 °C.
[b] Based on isolated products after column chromatography.
[c] Carried out at room temperature.
[d] Numbers in parenthesis are the data obtained with a **3c**–poly(4-vinylPy)•HBr_3 system.
[e] Bromination at the C-2 was accompanied (ca. 5%).

In contrast to high performance in secondary alcohols, the oxidation of primary alcohol **4a** (R = $C_{10}H_{21}$) with a **3c** (catalytic)-Py•HBr_3 (stoichiometric) system led to a mixture of the desired undecanal (R = $C_{10}H_{21}$, **5a**) and the corresponding dimeric ester **6a** in a ratio of 4:1 in 90% yield. For further insight into the effect of co-oxidant, two bromine compounds, i.e., Py•HBr_3 and Bu_4NBr_3,[11d] were compared in the competitive oxidation of primary and secondary alcohols. As shown in Scheme 2, the oxidation of a mixture of **4a** and **1a** with a **3c**-Py•HBr_3 (1.0 equiv.) system produces a mixture of the corresponding aldehyde **5a**, ketone **2a**, and dimeric ester **6a** in a ratio of 94:2:4, while the same run with a **3c**-Bu_4NBr_3 (1.0 equiv.) system afforded **5a**, selectively (**5a**/**6a** = 99:1). Thus, the problem forming dimeric ester **6a** from primary alcohol with a **3c**-Py•HBr_3 can be avoided by employing the Bu_4NBr_3 as a co-oxidant.



Cooxidant	Product / ratio **5a** : **2a** : **6a**
Py•HBr_3	94 : 2 : 4
Bu_4NBr_3	99 : 1 : 0

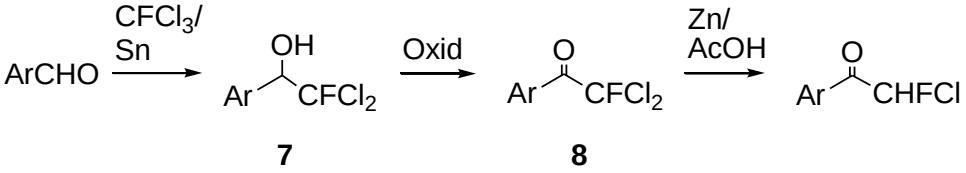
Scheme 2. Competitive oxidation of primary and secondary alcohols.

Merit of Py•HBr_3 as a co-oxidant lies in its easy extension to the polymer supported derivative,[19] poly (4-vinylPy)•HBr_3, which is commercially available. Thus, we examined the use of this polymer-supported reagent in place of Py•HBr_3 and the results for oxidation of secondary alcohols are shown in the parenthesis of Table 1.

Although slightly inferior results than that with Py•HBr_3 are obtained with this supported reagent, the present TEMPO (**3c**)-mediated oxidation were smoothly performed and the solid pyridine support was recovered quantitatively only by filtration.

2.3.2 TEMPO-mediated oxidation of aryl polyhaloalkyl alcohols

In the course of our study on synthesis of fluorine-containing building blocks (Scheme 3), we met with somewhat low yields in the oxidation of 1-aryl-2,2-dichloro-2-fluoroethyl alcohols **7** to the corresponding ketones **8** with conventional methods such as Swern and chromium(VI) oxidation.[20] Since these unfavorable results seemed to be due to strong electron–withdrawing nature of dichlorofluromethyl group, we attempted to employ TEMPOs bearing an EWG group at the C4 position as an appendage which would result in enhancement of electronic polarity of reaction site of *N*-oxoammonium intermediate.



Scheme 3. Synthesis of α-fluoroketones from fluorohaloalkanes.

Thus, effect of the appendage on TEMPOs is examined by dictating the time-course of the conversion of **7a** (Ar = C_6H_5) to **8a** (Ar = C_6H_5) by changing kind of substituent at the C-4 position. As shown in Figure 2, the oxidation of **7a** is fairly facilitated by appendage of an arenecarboxy group on TEMPO, curves (c), (d), and (e), compared with the TEMPO bearing no appendage, curve (a). Among them, the most favorable conversion was attained with the 4-(4-trifluoromethylbenzoyl)-substituted **3d** (curve (d)), prepared by 4-trifluoromethylbenzoylation of 4-hydroxyTEMPO. Similar

enhancement in the conversion was also observed in the oxidation of 2-octanol, being classified as electron-rich alcohol compared with 2,2-dichloro-2-fluroethyl derivatives, in which the best conversion was also attained with **3d**.

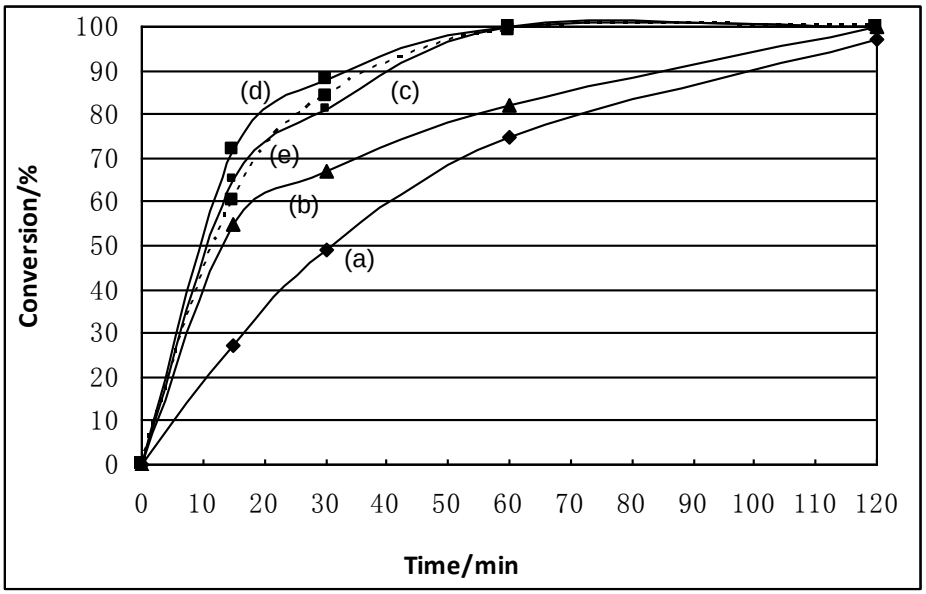
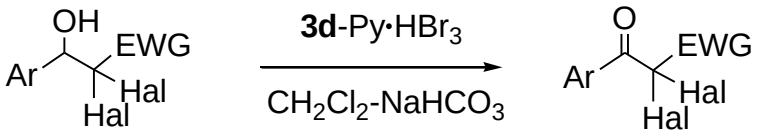
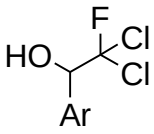
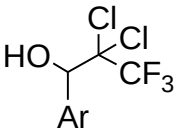
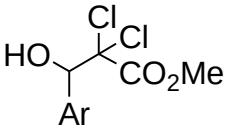
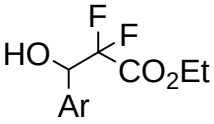
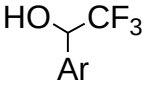


Figure 2.
Time-conversion curves for oxidation of **7a** to **8a** under various TEMPO catalysts **3a**-**d**. Carried out by reaction of **7a** (1 mmol) with **3** (5 mol%) and Py HBr_3 (1.5~2 equiv.) at room temperature. Symbols are as follows: (a) = **3a**, (b) = **3b**, (c) = **3c**, (d) = **3d**, and (e, dotted line) = **3e**. Data points were obtained by GC analyses.

Based on these results, we next attempted the oxidation of carbinol with an electron-withdrawing group. As shown in Table 2, the dichlorofluoro and dichlorotrifluoromethyl, dichlorocarboxy alcohols[21] are cleanly oxidized under the conditions developed above.

Table 2.
Oxidation of (aryl)polyhaloalkylmethanols with a combination of 4-(4-CF_3BzO)TEMPO (**3d**) and Py•HBr_3[a]



Entry	Alcohol		Ar =	Product	Yield (%)[b]
1		**7a**	C_6H_5	**8a**	91
2		**8b**	4-$MeOC_6H_5$	**8b**	94
3		**8c**	4-ClC_6H_5	**8c**	92
4		**8d**	4-MeC_6H_5	**8d**	93
5		**9a**	C_6H_5	**10a**	69
6		**9b**	4-$MeOC_6H_5$	**10b**	86
7		**11a**	C_6H_5	**12a**	79
8		**11b**	4-$MeOC_6H_5$	**12b**	78
9		**13a**	C_6H_5	**14a**	80
10		**13b**	4-$MeOC_6H_5$	**14b**	72
11		**15**	4-$MeOC_6H_5$	**16**	70

[a] Carried out by reaction of polyhalocarbinol (1 mmol) with **3d** (5 mol%) and Py•HBr_3 (1.5~2 equiv.) at room temperature.
[b] Isolated yield based on separated products.

2.3.3 Characterization of redox properties of C4-substituted TEMPOs

The enhanced reactivity of the electronically activated TEMPOs was rationalized by the characterization of their redox properties. The cyclic voltammetry of C4-substituted TEMPOs (**3a-e**) was performed in dichloromethane, the same solvent as in the catalytic reactions. All exhibited an oxidation peak and the reduction peak of C4-substituted $TEMPO^+$ on the reverse scan, in a reversible system at the scan rate of 0.5 Vs^{-1} (Table 3). From the potentials values, it emerges that the redox properties of C4-substituted TEMPOs are affected by the electronic properties of the substituents. The oxidation peak potentials of TEMPOs substituted by ester groups (**3c-e**) are very similar and are more positive than for H and OMe substituents (**3a, b**). The reduction peak potentials of their oxidized forms, $N\text{-}O^+$ are also more positive for esters substituents. Consequently, the $TEMPO^+$ substituted by the esters groups at the C4 position generated by the oxidation of the C4-substituted TEMPOs (**3c-e**) are more powerful oxidants for the oxidation of alcohols than those substituted by H or MeO. This is in agreement with the results of the catalytic reactions in which Py•HBr_3 acts as an oxidant for the TEMPOs.

Table 3.
Oxidation peak potentials of C4-Z-substituted TEMPO (2 mM) in CH_2Cl_2 (containing nBu$_4$NBF$_4$, 0.3 M) and reduction peak potentials of C4-Z-substituted TEMPO+.

3	Z	Z-TEMPO	Z-TEMPO$^+$	
		E^p_{ox}	E^p_{red}	E^0
		(V vs SCE)[a]	(V vs SCE)[a]	(V vs SCE)[a]
a	H	+0.852	+0.766	+0.809
b	MeO	+0.885	+0.803	+0.844
c	$C_6H_5CO_2$	+0.970	+0.887	+0.928
d	4-CF_3-$C_6H_4CO_2$	+0.966	+0.885	+0.925
e	$C_6F_5CO_2$	+0.974	+0.886	+0.930

a) Potentials were determined at a gold disk electrode (d = 0.5 mm), at the scan rate of 0.5 V $^{-1}$ at 22 ^{o}C.

2.4 Conclusion

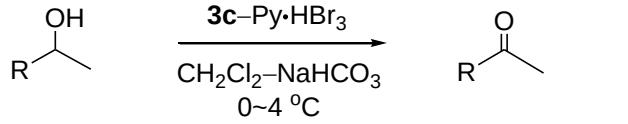
In summary, a high performance oxidation method of alcohols with TEMPO substituted with an EWG at the C-4, which is useful for the electron-deficient secondary alcohols such as $ArCH(OH)CFCl_2$, has been developed by using $Py{\bullet}HBr_3$ as a co-oxidant. Reactivity of $Py{\bullet}HBr_3$ was discussed in terms of efficiency and selectivity in comparison with similar bromine compounds such as Bu_3NBr_3, and the method was easily extended to the polymer supported bromine reagents as a co-oxidant. Inductive activation of TEMPO by the appendage of electron-withdrawing group at the C4 position was shown to facilitate the reaction rate, which was rationalized by measuring the cyclic voltammetry of the 4-substituted TEMPOs.

2.5 Experimental Section

2.5.1 General

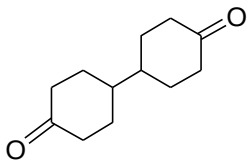
IR spectra were obtained with a Shimazu, Model FT-IR 8400, and only major absorptions are cited. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on Varian instruments with $CDCl_3$ as a solvent unless otherwise indicated.

2.5.2 General procedure for oxidation of secondary alcohols to the ketones



1a, R = C_9H_{19} **2a**, R = C_9H_{19}

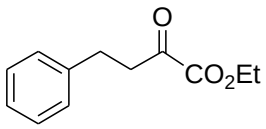
A solution of 2-undecanol (**1a**, 172 mg, 1 mmol) and **3c** (28 mg, 0. 1 mmol) in CH_2Cl_2 (6 mL) was covered with aqueous saturated $NaHCO_3$ (12 mL). To this biphase mixture was added portionwise Py•HBr_3 (480 mg, 1.5 mmol) under a vigorous stirring at 0–4 °C. The mixture was stirred for an additional 30 min. The reaction was quenched with aqueous 5% $Na_2S_2O_3$. The products were extracted with CH_2Cl_2 and the aqueous layer was again extracted with AcOEt. Extracts were washed separately with aqueous NH_4Cl, dried ($MgSO_4$), and concentrated. The combined crude product was purified by column chromatography (SiO_2, hexane-AcOEt 10:1 to 5:1) to give 135 mg (79% yield) of **2a** (R_f = 0.79, hexane-AcOEt 3:1); IR (neat): 1719, 1466, 1410, 1358, 1228, 1163, 758, 719 cm^{-1}; ^{1}H NMR (300 MHz): δ 0.87 (t, *J* = 7.4 Hz, 3H), 1.26 (brs, 12H), 1.56 (m, 2H), 2.13 (s, 3H), 2.41 (t, *J* = 7.4 Hz, 2H); ^{13}C NMR (75.5 MHz): δ□13□9□□22□5□ 23.9, 29.1, 29.2, 29.31, 29.33, 29.6, 31.8, 43.7, 208.9.



2c

4,4'-Bicyclohexanone (2c):

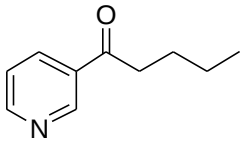
Yield 93% (R_f = 0.47, hexane-AcOEt 1:2); mp 113-115 °C (from hexane) (lit.[22] 112-116 °C); IR (KBr): 1705, 1464, 1439, 1418, 1354, 1333, 1321, 1302, 1281, 1267, 1244, 1215, 1167, 1155, 1115, 1069, 1011, 980, 932, 858, 816, 762, 704 cm^{-1}; ^1H NMR (300 MHz): δ 1.45–1.60 (m, 4H), 1.63–1.79 (m, 2H), 2.02–2.12 (m, 4H), 2.27–2.46 (m, 8H); ^{13}C NMR (75.5 MHz): δ□29□8 (4C)□ □40.3 (2C), 40.7 (4C), 211.0 (2C).



2d

Ethyl 2-Oxo-4-phenylbutanoate (2d):

Yield 81% (R_f = 0.5, hexane-AcOEt 5:1); IR (neat): 1728, 1605, 1497, 1454, 1400, 1370, 1304, 1271, 1250, 1190, 1067, 1030, 856, 750, 700 cm^{-1}; ^1H NMR (300 MHz): δ 1.35 (t, *J* = 7.1 Hz, 3H), 2.96 (t, *J* = 7.7 Hz, 2H), 3.18 (t, *J* = 7.7 Hz, 2H), 4.31 (q, *J* = 7.1 Hz, 2H), 7.18–7.32 (m 5H); ^{13}C NMR (75.5 MHz): δ□13.9, 28.9, 40.7, 62.1, 125.9, 127.9 (2C), 128.1 (2C), 139.6, 160.4, 192.8.



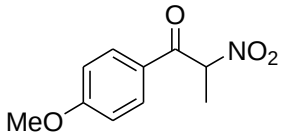
2f

3-Pentanoylpyridine (2f):

Yield 84% (R_f = 0.41, hexane-AcOEt 1:1); IR (neat): 1690, 1586, 1466, 1458, 1420, 1374, 1350, 1269, 1223, 1117, 1011, 970, 797, 704 cm^{-1}; ^1H NMR (300 MHz): δ 0.94

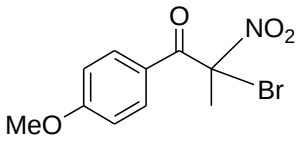
(t, *J* = 7.9 Hz, 3H), 1.41 (m, 2H), 1.72 (m, 2H), 2.97 (t, *J* =7.2 H, 2H), 7.41 (d,d,d, *J* = 7.9, 4.7, 1.1 Hz, 1H), 8.23 (d,d,d, *J* = 7.9, 2.2, 2.2 Hz, 1H), 8.76 (d,d, *J* = 4.9, 1.6 Hz, 1H), 9.15 (d, *J* = 2.2 Hz, 1H); ^{13}C NMR (75.5 MHz): δ□13.6, 22.2, 26.0, 38.4, 123.4, 132.2, 135.1, 149,4, 153.1, 198.9.

2-Nitro-1-(4-methoxyphenyl)propanone (2g) and 2-bromo-2-nitro-1-(4-methoxy phenyl)propanone (byproduct):



2g

2g: yield 87% (R_f = 0.31, hexane-AcOEt 3:1); IR (neat): 3534, 2843, 1686, 1601, 1560, 1512, 1452, 1389, 1364, 1325, 1269, 1229, 1175, 1123, 1026, 966, 845, 752, 683 cm^{-1}; ^{1}H NMR (300 MHz) (absorptions based on major isomer) [23]: δ 1.82 (d, *J* = 7.1 Hz, 3H), 3.90 (s, 3H), 6.13 (q, *J* =7.1 H, 2H), 6.99 (d, *J* = 8.8 H, 2H), 7.94 (d, *J* = 8.8 H, 2H); ^{13}C NMR (75.5 MHz) (absorptions based on major isomer): δ□16.0, 55.6, 84.5, 114.4 (2C), 126.4, 131.2 (2C), 164.8, 188.1.



2-Bromo-2-nitro-1-phenylpropanone: yield 5% (R_f = 0.65, hexane-AcOEt 3:1); IR (neat): 2843, 1686, 1601, 1560, 1512, 1458, 1441, 1424, 1381, 1333, 1317, 1258, 1180, 1140, 1121, 1080, 1028, 957, 845 cm^{-1}; ^{1}H NMR (300 MHz): δ 2.49 (s, 3H), 3.88 (s, 3H), 6.92 (d, *J* = 9.1 H, 2H), 7.94 (d, *J* = 9.1 H, 2H); ^{13}C NMR (75.5 MHz): δ□30.2, 55.6, 92.2, 114.2 (2C), 123.8, 132.1 (2C), 164.4, 183.1.

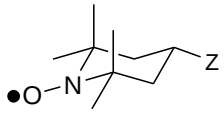
2.5.3 Time-course for the oxidation of 2a with a 3c-cooxidants system

A mixture of **1a** (172 mg, 1 mmol), **3c** (28 mg, 0. 1 mmol), and Py•HBr_3 (480 mg, 1.5 mmol) in CH_2Cl_2 (6 mL)-aqueous saturated $NaHCO_3$ (12 mL) was allowed to react and the aliquots at the prescribed time were analyzed by GC and the selectivity was calculated based on the peak areas **(**Figure 1**)**. Similarly, the time-course of the oxidation of **7a** was achieved by using Py•HBr_3 in combination with various **3a-d (**Figure 2**)**.

2.5.4 A typical procedure for oxidation of secondary alcohols to the ketones with poly(4-vinylPy)•HBr_3

A solution of **1a** (86 mg, 0.5 mmol) and **3c** (28 mg, 0. 1 mmol) in CH_2Cl_2 (3 mL) was covered with aqueous saturated $NaHCO_3$ (6 mL). To this biphase mixture was added portionwise poly(4-vinylpyridinium tribromide) (300 mg) under a vigorous stirring at room temperature. The stirring was continued at room temperature until **1a** was consumed, for about 2 h as monitored with TLC. The mixture was filtered off to leave poly(4-vinylpyridine) (107 mg) and the filtrate was worked up in the usual manner to give 67 mg (78% yield) of **2a** after purification by column chromatography.

2.5.5 Preparation of 4-(4-trifluoromethylbenzoyloxy)-2,2,6,6-tetramethyl piperidine-1-oxyl (**3d**)

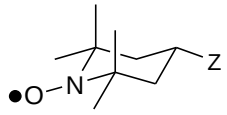


3d, Z=4-CF_3-$C_6H_4CO_2$

To a solution of 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (1.72 g, 10 mmol) and pyridine (1.62 mL, 20 mmol) in THF (10 mL) was added dropwise a solution of 4-trifluoromethylbenzoyl chloride (1.63 mL, 11 mmol) in THF (3 mL) at 0–4 °C. The mixture was stirred at room temperature overnight and worked up in the usual manner. The crude product was purified by column chromatography (SiO_2, hexane-AcOEt

10:1 to 5:1) to give 3.2 g (93% yield) of **3d** as solids: mp 74–75°C (from hexane) (R_f = 0.55 hexane-AcOEt 3:1); IR (KBr): 1721, 1585, 1512, 1466, 1412, 1331, 1283, 1242, 1167, 1138, 1128, 1101, 1067, 1017, 963, 862, 775, 705 cm^{-1}; ^1H NMR, treated with $PhNHNH_2$ (300 MHz): δ 1.176 and 1.181 (s, 12H), 1.68 (m 2H), 1.94-2.00 (m, 2H), 5.23 (m, 1H), 7.60 (d, *J* = 8.2 Hz, 2H), 8.03 (d, *J* = 8.24 Hz, 2H); ^{19}F NMR, treated with $PhNHNH_2$ (282.3 MHz): δ –63.3 (s). HRMS (ESI) calcd for $C_{11}H_{14}NO_4$ (MH^+) 224.0923, found 224.0928 (MH^+). HRMS (ESI) calcd for $C_{17}H_{21}F_3NO_3$ (M^+) 344.1474, found 344.1499 (M^+).

2.5.6 Preparation of 4-(2,3,4,5,6-pentafluorobenzoyloxy)-2,2,6,6-tetramethyl piperidine-1-oxyl (3e)



3e, $Z=C_6F_5CO_2$

Compound **3e** was prepared by the reaction of 2,3,4,5,6-pentafluorobenzoic acid and 4-hydroxy-2,2,6,6-tetramethylpiperinine-1-oxyl in the presence of carbon tetrabromide, PPh_3, pyridine in CH_2Cl_2: mp 102–104 °C (from hexane-AcOEt 10:1) (R_f = 0.64 hexane-AcOEt 3:1); IR (KBr): 1728, 1651, 1526. 1495, 1416, 1368, 1337, 1232, 1177, 1107, 1092, 1069, 1007, 957, 770 cm^{-1}; ^1H NMR, treated with $PhNHNH_2$ (300 MHz): δ 1.152 and 1.158 (s, 12H), 1.64 (m 2H), 1.93-1.99 (m, 2H), 5.24 (m, 1H); ^{19}F NMR, treated with $PhNHNH_2$ (282.3 MHz): δ –160.6 (m), –149.0 (m), –138.9 (m). HRMS (ESI) calcd for $C_{11}H_{14}NO_4$ (MH^+) 224.0923, found 224.0928 (MH^+). HRMS (ESI) calcd for $C_{16}H_{17}F_5NO_3$ (M^+) 366.1129, found 366.1121 (M^+).

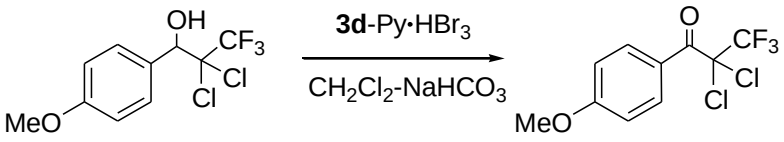
2.5.7 Electrochemical set-up and electrochemical procedure for cyclic voltammetry

Cyclic voltammetry was performed with a home made potentiostat and a

wave-form generator, PAR Model 175. The cyclic voltammograms were recorded on a Nicolet 3091 digital oscilloscope. Experiments were carried out in a three-electrode cell. The working electrode was a steady gold disk electrode (d = 0.5 mm). The counter electrode was a platinum wire of ca 1 cm^2 apparent surface area. The reference was a saturated calomel electrode separated from the solution by a bridge filled by 2 mL of dichloromethane containing nBu$_4$BF$_4$ (0.3 M). 15 mL of distilled and degassed dichloromethane containing nBu$_4$BF$_4$ (0.3 M) was poured into the cell, followed by 4.68 mg (0.03 mmol, 2 mM) of TEMPO (**3a**). The cyclic voltammetry was performed at the scan rate of 0.5 Vs^{-1} in the potential range between 0 and +1.2 V.

Similar experiments were performed from **3b** (5.6 mg), **3c** (8 mg), **3d** (10 mg), **3e** (11 mg).

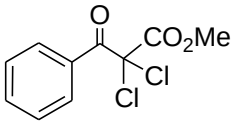
2.5.8 General procedure for oxidation of polyhaloalkyl alcohols to the ketones with 3d-Py•HBr$_3$



9b **10b**

A solution of 2,2-dichloro-3,3,3-trifluoro-1-(4-methoxyphenyl)propanol[18] (**9b**, 288 mg, 1.0 mmol) and 4-(4-$CF_3C_6H_4CO_2$)TEMPO (**3d**, 35 mg, 0.1 mmol) in CH_2Cl_2 (6 mL) was covered with aqueous 5% $NaHCO_3$ (12 mL). To this biphase mixture was added portionwise Py•HBr_3 (480 mg, 1.5 mmol) under a vigorous stirring at room temperature. The mixture was stirred for an additional 1.5 h and the reaction was quenched with aqueous 5% $Na_2S_2O_3$ (5 mL). The products were extracted with CH_2Cl_2 and the aqueous layer was again extracted with AcOEt. Extracts were separately washed with brine, dried ($MgSO_4$), and concentrated. The combined crude product was purified by column chromatography (SiO_2, hexane-AcOEt 10:1 to 3:1) to

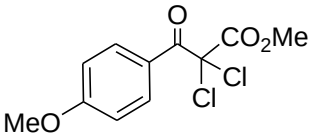
give 245 mg (86% yield) of **10b** (R_f = 0.65, hexane-AcOEt 5:1): IR (neat): 2845, 1697, 1601, 1574, 1512, 1460, 1425, 1316, 1261, 1207, 1180, 1124, 1045, 1026, 930, 870, 847, 829, 737, 702, 673 cm^{-1}; ^1H NMR (300 MHz): δ 3.91 (s, 3H), 6.97 (d, *J* = 9.2 Hz, 2H), 8.27 (d, *J* = 9.2 Hz, 2H); ^{13}C NMR (75.5 MHz): δ 55.5, 78.7 (q, $^2J_{CF}$ = 31.1 Hz), 113.8 (2C), 121.3 (q, $^1J_{CF}$ = 283.3 Hz), 122.7, 133.4 (2C), 164.7, 181.3; ^{19}F NMR (282.3 MHz): δ –75.2 (s).



12a

Methyl 2,2-Dichloro-3-oxo-3-phenylpropanoate (12a):

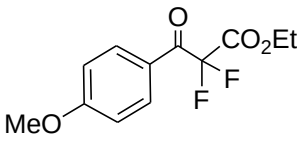
Yield 79% (R_f = 0.53, hexane-AcOEt 5:1); IR (neat): 2957, 1769, 1746, 1713, 1690, 1597, 1449, 1437, 1252, 1217, 1186, 1015, 864, 824, 795, 689 cm^{-1}; ^1H NMR (300 MHz): δ 3.87 (s, 3H), 7.45–7.52 (m, 2H), 7.60–7.65 (m, 1H), 8.02–8.07 (m, 2H); ^{13}C NMR (75.5 MHz): δ 54.8, 81.6, 128.6 (2C), 130.0 (2C), 130.8, 134.1, 164.5, 183.3. HRMS (ESI) calcd for $C_{10}H_9Cl_2O_3$ (MH^+) 246.9929, found 246.9887 (MH^+).



12b

Methyl 2,2-Dichloro-3-oxo-3-(4-bromophenyl)propanoate (12b):

Yield 78% (R_f = 0.62, hexane-AcOEt 5:1); IR (neat): 1769, 1746, 1713, 1690, 1584, 1485, 1437, 1398, 1250, 1217, 1184, 1074, 1007, 928, 868, 824, 760, 725 cm^{-1}; ^1H NMR (300 MHz): δ 3.89 (s, 3H), 7.63 (d, *J* = 8.8 Hz, 2H), 7.92 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (75.5 MHz): δ□55.0. 81.4, 129.6, 129.7, 131.5 (2C), 132.1 (2C), 164.3, 182.7. HRMS (ESI) calcd for $C_{10}H_7BrCl_2O_3$ (MH^+) 324.9034, found 324.8992 (MH^+).



14b

Ethyl 2,2-Difluoro-3-(4-methoxyphenyl)-3-oxopropanoate (14b):

Yield 72% (R_f = 0.59, hexane-AcOEt 5:1); IR (neat): 2845, 1771, 1694, 1690, 1600, 1573, 1514, 1464, 1447, 1427, 1395, 1373, 1316, 1269, 1182, 1159, 1122, 1099, 1076, 1026, 924, 910, 847, 791, 712, 698 cm-1; ^{1}H NMR (300 MHz): δ 1.32 (t, *J* = 7.1 Hz, 3H), 3.90 (s, 3H), 4.38 (q, *J* = 7.1 Hz, 2H), 6.98 (d, *J* = 9.1 Hz, 2H), 8.07 (d, *J* = 9.1 Hz, 2H); ^{13}C NMR (75.5 MHz): δ□13.7, 55.6, 63.5, 110.1 (t, $^{1}J_{CF}$ = 264.3 Hz), 114.3 (2C), 124.0, 132.5 (t, $^{4}J_{CF}$ = 2.9 Hz) (2C), 162.0 (t, $^{2}J_{CF}$ = 30.5 Hz), 165.1, 183.8 (t, $^{2}J_{CF}$ = 27.1 Hz); ^{19}F NMR (282.3 MHz): δ –107.6 (s). HRMS (ESI) calcd for $C_{12}H_{13}F_2O_4$ (MH^+) 259.0782, found 259.0749 (MH^+).

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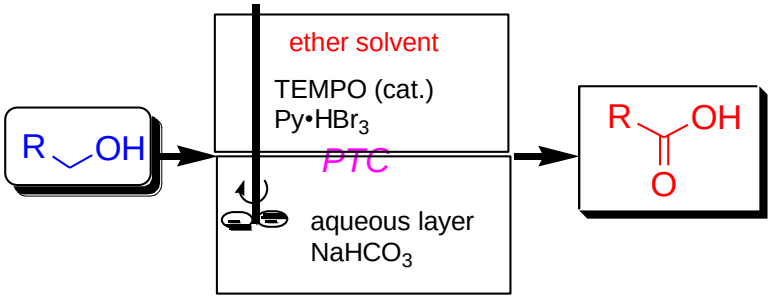
Chapter 3

Green Procedure for Preparation of Carboxylic Acids by TEMPO Oxidation of Primary Alcohols

3.1 Abstract

Expeditious and benign methods for primary alcohol-carboxylic acid conversions with TEMPO were developed in a biphasic system composed of a slightly miscible ether (THP) and aqueous layer. Easily available co-oxidants such as Py•HBr_3, Bu_4NBr_3, and electro-oxidation were successfully applied to generate *N*-oxoammonium species as a recyclable catalyst.



Keywords: Oxidation, TEMPO, Co-oxidant, Ether

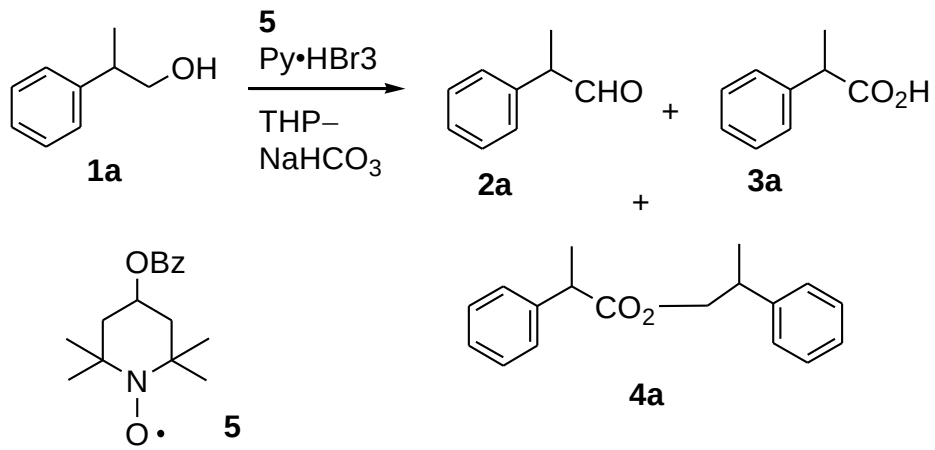
3.2 Introduction

Oxidation of alcohols to the corresponding carboxylic acids is a fundamental operation in organic chemistry.[1,2] TEMPO oxidations, achieved in a catalytic manner in coordination with stoichiometric amount of co-oxidants, have been recognized to be inherently benign and useful for selective transformations of alcohols.[3] Conventionally, TEMPO oxidations are executed in an aqueous-organic two-phase system and moderately or highly water-miscible solvents such as CH_2Cl_2 and acetonitrile are often employed.[4] Inspired by recent development in devising environmentally friendly procedures,[5] we further examined the improvement of TEMPO oxidation by tuning the reaction media to replace harmful solvents and by changing the co-oxidant. Thus, ethereal solvents like tetrahydropyran (THP)[6] were employed as the organic layer of a two-phase system for the exhaustive conversion of primary alcohols to carboxylic acids. In this respect, easy recoverability after reaction and stability toward air-oxidation of the ethereal solvent should be a criteria for practical application.

3.3 Results and Discussion

3.3.1 Catalytic oxidation of primary alcohols with a combination of 4-BzOTEMPO (5) and Py•HBr_3

We employed 4-benzoyloxy-2,2,6,6-tetramethyl-piperidine-1-oxyl (4-BzOTEMPO, **5**) for the recyclable catalyst from various TEMPO derivatives because **5** is slightly activated by the electron-withdrawing benzoyloxy group at the C4 position.[7] As the first step, we examined the oxidation of **1a** with a combination of **5** (5 mol%) and a co-oxidant in THP-aqueous saturated $NaHCO_3$ (1:1 v/v) containing a quaternary ammonium salt such as BenzylEt_3NCl and acetylcholine chloride as a phase-transfer catalyst (PTC). Thus, the addition of Py•HBr_3 (3 equiv.) as a co-oxidant to the reaction media afforded the corresponding carboxylic acid **3a** in 91% yield and formation of dimeric ester **4a** was negligible (~1%). The presence of quaternary ammonium salts in the two-phase system was not crucial in comparison with a run without these reagents, since pyridinium salts in this reaction media could participate as a PTC.[4d]



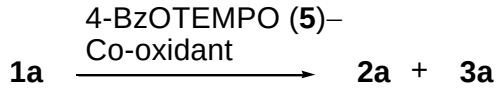
Scheme 1. Catalytic oxidation of primary alcohols with a combination of 4-BzOTEMPO (**5**) and Py•HBr_3

3.3.2 Optimized reaction conditions

Subsequently, we surveyed suitable ethereal solvents in addition to THP for the two-phase system, and the following results were obtained in the oxidation of **1a** to **3a**: cyclopentyl methyl ether (CPME) (80%), tetrahydrofuran (THF) (80%), diisopropyl ether (67%), methyl *tert*-butyl ether (89%), and CH_2Cl_2 (77%). It appears that solvents slightly miscible in water are useful as an organic layer.[8] However, among these solvents, THP should be the best in terms of high yield, easy handling, and recoverability. Furthermore, Yasuda, Ryu, et al. have recently demonstrated the excellent stability of THP towards hydrogen abstraction from the oxygen-substituted carbon, as compared with THF, as a result of their study on tributyltin hydride-mediated radical cyclization.[9] This finding favors the use of THP since it is less likely to form peroxide with oxygen during TEMPO oxidation.

Subsequently, we searched for a favorable co-oxidant for the conversion of **1a** to **3a** in a two-phase system comprised of THP-aqueous $NaHCO_3$. As shown in Table 1, entries 3~5, it turns out that bromine salts such as Py•HBr_3,[7] Bu_4NBr_3,[10] and electrolysis with bromide ion[11] are the appropriate choice of co-oxidizing reagent because of high yield and high catalytic performance. The runs employing NaOCl[4e] and $Ca(OCl)_2$[10] in combination with KBr were less effective than those with the above bromine compounds (entries 1 and 2).

Table 1.
Search for Co-oxidant in TEMPO-mediated oxidation of **1a** to **3a**.[a]



entry	Co-oxidant	Product, %[b]	
		3a	**2a**
1	NaOCl (6)	33	48
2	$Ca(OCl)_2$ (4.5)	59	23
3	$Py{\bullet}HBr_3$ (3)	87	--
4	Bu_4NBr_3 (3)	72	trace
5	Electrolysis (10 F)[c]	84	trace

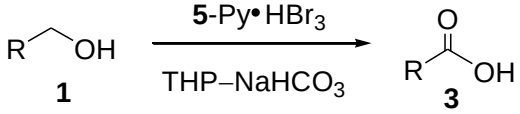
[a]Carried out with **5** and co-oxidant in THP (8 mL)–sat. $NaHCO_3$ (8 mL) in the presence of PTC.
[b]Based on the isolated products.
[c]Electricity passed.

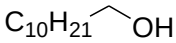
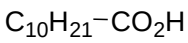
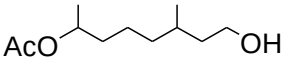
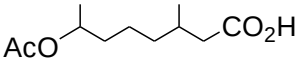
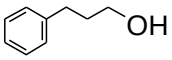
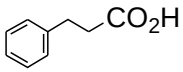
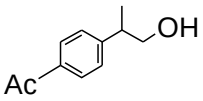
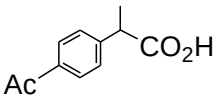
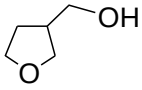
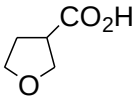
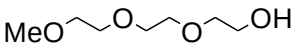
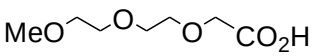
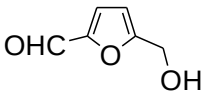
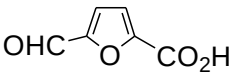
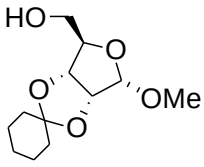
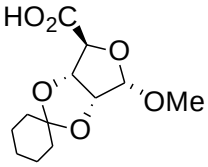
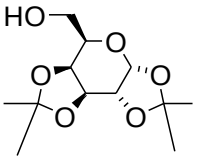
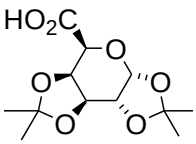
This reaction can be performed on a scale of 15 mmol of **1a** in THP (120 mL)-aqueous $NaHCO_3$ (120 mL) in the presence of PTC at room temperature for 5 h, giving **3a** in 91% yield, in which 83% of THP used for the reaction media and workup solvent was recovered on a rotary evaporator after separation of the aqueous layer.

As shown in Table 2, the present method can readily be applied not only to aliphatic primary alcohols **1** including acyclic (entries 1, 2, and 6) and cyclic structures (entry 5), but also to aromatic (entries 3 and 4) and hetero aromatic alcohols (entry 7), producing the corresponding carboxylic acids **3** respectively. The oxidation of 5-(hydroxymethyl)-2-furaldehyde (HMF, **1h**), a value-added chemical available from biomass,[12] leads selectively to 5-formyl-2-furancarboxylic acid (**3h**),[13,14] useful as a precursor of 2,5-furandicarboxylic acids. Carbohydrate derivatives **1i**, **j** with primary hydroxy groups are easily oxidized to the corresponding uronic acids **3i**, **j** in good yields (entries 8 and 9).

Table 2.

Oxidation of primary alcohols with a combination of 4-BzOTEMPO (**5**) and Py•HBr_3[a]



Entry	Alcohol **1**		Product **3**	Yield (%)[b]
1		**b**		70
2		**c**		74
3		**d**		92
4[c]		**e**		92
5		**f**		82
6		**g**		87[c]
7		**h**		85
8		**i**		97
9		**j**		97

[a] Carried out by the reaction of **1** (1 mmol) with **5** (5~10 mol%) and Py•HBr_3 (2.5~3.5 equiv.) at room temperature.

[b] Based on isolated products after column chromatography.

[c] Yield based on the crude product.

3.4 Conclusions

In summary, we developed an efficient primary alcohol-carboxylic acid conversion by employing TEMPO oxidation in ethereal solvent such as THP-aqueous layer, in which bromine complexes such as Py•HBr_3 and electrooxidation with bromide ion were useful as co-oxidants. The method could easily be applied to various primary alcohols including aromatic, aliphatic, and carbohydrate derivatives, some of which are of significant synthetic value.

3.5 Experimental Section

3.5.1 Materials and methods

Reagents were obtained commercially and used without further purification unless stated otherwise. Solvent was removed under reduced pressure and the residue obtained was chromatographed on a silica gel column (230-400 mesh) using a gradient solvent system (*n*-hexane / ethyl acetate as the eluant unless specified otherwise). ^{1}H and ^{13}C spectra were measured on a Mercury-300 spectrometer. Chemical shifts (δ ppm) were determined with tetramethylsilane (TMS) as internal reference. High resolution mass spectra were obtained on a JEOL JMS-700 spectrometer. IR spectra were recorded on a Shimadzu FTIR-8400S spectrometer. Optical rotations were measured on a Horiba SEPA-300 Polarimeter.

3.5.2 A typical procedure for oxidation of primary alcohols to the carboxylic acid

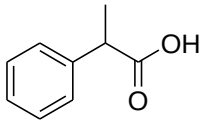
To a two phase mixture of THP (8 mL) and aqueous saturated $NaHCO_3$ (8 mL) containing $PhCH(Me)CH_2OH$ (**1a**, 137 mg, 1 mmol), 4-BzOTEMPO (**5**, 28 mg, 0. 1 mmol), and PTC (acetylcholine chloride, 0.1 mmol) was added Py•HBr_3 (3.0 mmol) in portions under vigorous stirring at room temperature. The mixture was stirred for an additional 120 min with TLC monitoring, and quenched with aqueous 5% $Na_2S_2O_3$. The mixture was acidified with aqueous tartaric acid and extracted with EtOAc. The usual workup followed by purification of the residue by column chromatography (SiO_2, hexane-EtOAc) gave 131mg (87% yield) of the carboxylic acid **3a** and dimeric ester **4a** (trace amount).

3.5.3 Large scale operation and the recoverability of THP

To a two phase mixture of THP (120 mL) and aqueous saturated $NaHCO_3$ (120 mL) containing **1a** (2.04 g, 15 mmol), **5** (415 mg, 1.5 mmol), and PTC (acetylcholine chloride, 1.5 mmol) was added Py•HBr_3 (45 mmol) in portions under vigorous stirring at room temperature. The mixture was stirred for an additional 5 h with TLC monitoring. The mixture was worked up in the manner described above by using THP (170 mL) as an extractive solvent. The extract was separated on a rotary evaporator for the recovery of THP (240 mL) and the crude material was purified by column chromatography (SiO_2, hexane-EtOAc) to give 2.07g (91% yield) of the carboxylic acid **3a**.

3.5.4 A typical procedure for electrochemical oxidation of primary alcohols to the carboxylic acid

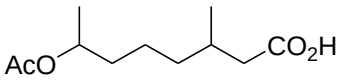
Two platinum foils were immersed to the lower layer of a two phase mixture comprised of THP (8 mL) and aqueous saturated $NaHCO_3$ (8 mL)-25% NaBr which contained **1a** (128 mg, 1 mmol), **5** (28 mg, 0. 1 mmol), and PTC (acetylcholine chloride, 0.1 mmol). The mixture was electrolyzed under a constant current of 20 mA/cm^2 (applied voltage: 1.5~2.5 V) with a moderate stirring. The electrolysis was continued until the starting material was consumed. It required about 10 F/mol of electricity. The mixture was worked up in the manner described to give 84% of **3a** after column chromatography. Spectral data and physical properties of selected products in Table 2 are included here.



3a

2-Phenylpropanoic Acid (3a):

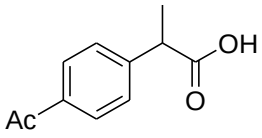
Yield 92%; R_f = 0.59 (hexane-EtOAc 1:1); IR (neat) 3088, 2982, 1705, 1601, 1497, 1454, 1414, 1263, 1233,1064, 939, 860, 760, 727, 698 cm^{-1}; ^1H NMR (300 MHz) δ 1.52 (d, *J* = 7.1 Hz, 3H), 3.74 (q, *J* = 7.1 Hz, 1H), 7.25–7.34 (m, 5H); ^{13}C NMR (75.5 MHz) δ 18.0, 45.4, 127.3, 127.6 (2C), 128.6 (2C), 139.8, 180.8.



3c

7-Acetoxy-3-methyloctanoic Acid (3c):

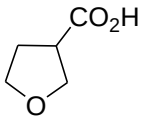
Yield 74%; R_f = 0.35 (hexane-EtOAc 1:1); IR (neat) 2938, 2874, 1736, 1707, 1458, 1375, 1246, 1130, 1032, 949 cm^{-1}; ^1H NMR (600 MHz) δ 0.96 (d, *J* = 6.6 Hz, 3H), 1.20 (d, *J* = 6.6 Hz, 3H), 1.20-1.40 (m, 4H), 1.46 (m, 1H), 1.56 (m, 1H), 1.95 (m, 1H), 2.03 (s, 3H), 2.16 (d,d,d, *J* = 15.1, 8.1, 2.4 Hz, 1H), 2.33 (d,d,d *J* = 15.1, 6.1, 3.2 Hz, 1H), 4.88 (m, 1H); ^{13}C NMR (75.5 MHz) δ 19.48 + 19.51, 19.8, 19.9, 21.3, 22.6, 35.8, 36.20 + 36.22, 41.39 + 41.45, 70.84 + 70.87, 170.9, 179.2. HRMS (ESI) calcd for $C_{11}H_{21}O_4$ (MH^+) 217.1440, found 217.1436 (MH^+).



3e

2-(4-Acetylphenyl)propanoic Acid (3e):

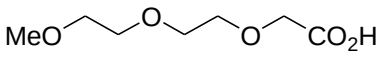
Yield 92%; R_f = 0.37 (hexane-EtOAc 1:1); IR (neat) 2970, 1709, 1680, 1605, 1456, 1420, 1358, 1329, 1271, 1246, 1188, 1119, 1082, 997, 961, 932, 866, 851, 833, 764, 692 cm^{-1}; ^1H NMR (300 MHz) δ 1.53 (d, *J* = 7.1 Hz, 3H), 2.58 (s, 3H), 3.80 (q, *J* = 7.1 Hz, 1H), 7.41 (d, *J* = 8.2 Hz, 2H), 7.91 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR (75.5 MHz) δ 17.8, 26.4, 45.2, 127.8 (2C), 128.6 (2C), 135.9, 145.2, 179.0, 198.1.



3f

3-Oxolanecarboxylic acid (3f):

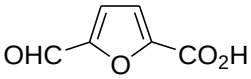
Yield 82% (R_f = 0.32, hexane-EtOAc 1:1); IR (neat) 2982, 2884, 1736, 1710, 1417, 1211, 1188, 1065, 905 cm^{-1}; ^1H NMR (300 MHz) δ 2.10–2.29 (m, 2H), 3.09–3.18 (m, 1H), 3.79–3.95 (m, 2H), 3.99 (d, *J* = 6.6 Hz, 2H); ^{13}C NMR (75.5 MHz) δ 29.3, 43.4, 68.1, 69.4, 179.0.



3g

3,6,9-Trioxadecanoic Acid (3g):

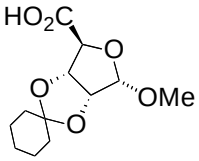
Yield 87%; not R_f = 0.59, hexane-EtOAc 5:1); IR (neat) 2928, 2891, 1717, 1452, 1356, 1316, 1277, 1248, 1200, 1177, 1113, 1072, 1026, 852, 718, 684 cm^{-1}; ^1H NMR (300 MHz) δ 3.39 (s, 3H), 3.56–3.59 (m, 2H), 3.68–3.72 (m, 4H), 3.76–3.79 (m, 2H), 4.12 (s, 2H).



3h

5-Formyl-2-furancarboxylic acid (3h):

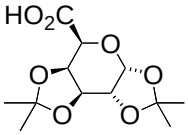
Yield 85%; IR (KBr) 3144, 3107, 2930, 1674, 1568, 1518, 1435, 1397, 1346, 1294, 1260, 1221, 1165, 1043, 963, 849, 783 cm^{-1}; ^1H NMR (300 MHz in methanol-D_6) δ 7.38 (d, *J* = 3.8 Hz, 1H), 7.50 (d, *J* = 3.8 Hz, 1H), 9.79 (s, 1H); ^{13}C NMR (75.5 MHz in methanol-D_6) δ 119.0, 121.1, 148.4, 154.4, 158.6, 179.3, 179.3.



3i

1-O-Methyl-3,4-O-cyclohexylidene-α-D-ribouronic Acid (3i):

Yield 97%; R_f = 0.4 (hexane-EtOAc 1:2); $[\alpha]_D^{31}$ –50.2° (*c* 0.9, $CHCl_3$); IR (neat) 2937, 2862, 2673, 1715, 1450, 1417, 1368, 1273, 1223, 1165, 1115, 1047, 949, 924, 853, 833, 800, 752, 673, 1211, 1188, 1065, 905 cm^{-1}; ^1H NMR (300 MHz) δ 1.39 (m, 2H), 1.55 (m, 4H), 1.61 (m, 2H), 1.70 (m, 2H), 3.43 (s, 3H), 4.57 (d, *J* = 5.2 Hz, 1H), 4.67 (brs, 1H), 5.08 (s, 1H), 5.17 (d, *J* = 5.7 Hz, 1H); ^{13}C NMR (75.5 MHz) δ 23.7, 23.9, 24.9, 34.4, 36.0, 55.6, 81.7, 83.62, 83.70, 109.6, 113.7, 175.1. HRMS (ESI) calcd for $C_{12}H_{19}O_6$ (MH^+) 259.1182, found 259.1151 (MH^+).



3j

1,2:3,4-Di-O-isopropylidene-α-D-galactopyranuronic Acid (3j):

Yield 97%; R_f = 0.2 (hexane-EtOAc 1:5); mp 149–50 °C (from hexane-EtOAc) (lit.[4d] 152°C); $[\alpha]_D^{22}$ –91.2° (*c* 1.4, $CHCl_3$) (ref.[4d] –102.9°); ^1H NMR (300 MHz) δ 1.35 (s, 6H), 1.45 (s, 3H), 1.53 (s, 3H), 4.40 (d,d, *J* = 4.95, 2.5 Hz, 1H), 4.46 (d, *J* = 2.2 Hz, 1H), 4.63 (d,d, *J* = 7.7, 2.2 Hz, 1H), 4.69 (d,d, *J* = 7.7, 2.5 Hz, 1H), 5.65 (d, *J* = 4.95 Hz, 1H).

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17. Higher reactivity of Py•HBr_3 than that of Bu_4NBr_3 may be ascribable to lower basicity of pyridine compared with Bu_3N.

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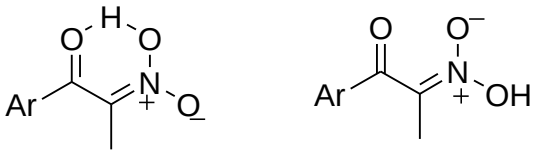
23. Two isomers **i** and **ii** are conceived for **2g**.



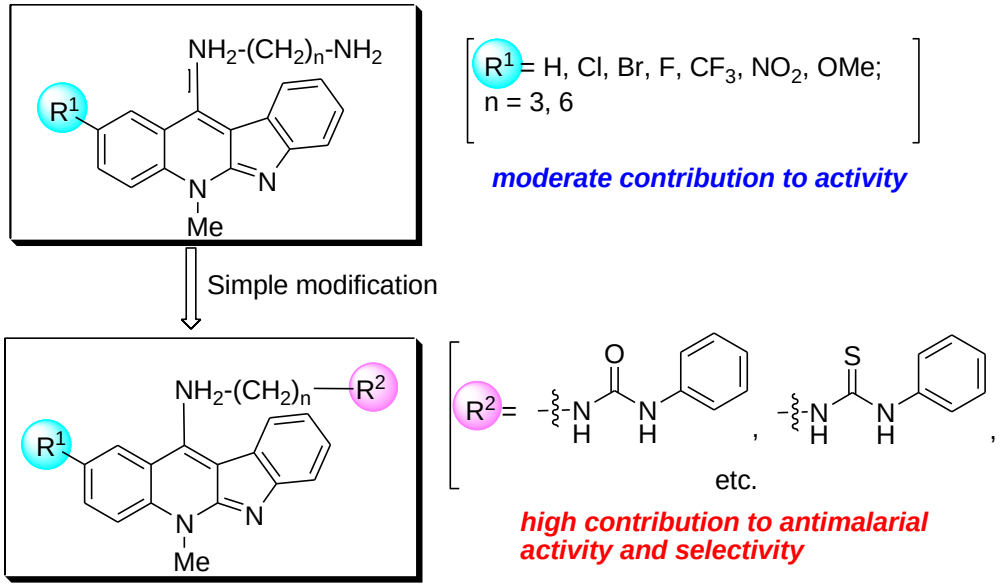
i **ii**

Chapter 4

Synthesis and Evaluation of Novel Neocryptolepine Derivatives for Developing Antimalarial Agents

4.1 Abstract

In order to obtain a high antimalarial activity with neocrytolepine derivatives, modifying and changing the side chains at the C-11 position with varying the substituents of an electron-withdrawing or electron-donating nature at the C-2 position for a SAR study were executed. As the strategic scaffold of this study, improved synthesis of the 11-chloroneocrytolepines was developed in three steps using *N*-methylanilines and indole-3-carboxylate as the counterpart. Installation of alkylamino and ω-aminoalkylamino groups at the C-11 position of the neocrytolepine core was achieved by the reaction of the 11-chloroneocryptolepines and the appropriate amines. For further variation, the aminoalkylamino subsituents were transformed into the corresponding acyclic or cyclic carbamides or thiocarbamides. These side-chain modified neocryptolepine derivatives were tested for antimalarial activity against CQS (NF54) and CQR (K1) of *Plasmodium falciparum* in vitro. The evaluation also included cytotoxicity toward mammalian *L6* cells. In particular, among the tested compounds, the 11-((3-(3-phenylureido)propyl)amino)-substituted **17f**, bearing an additional Cl at the C-2 position, showed antimalarial activity 4 times more potent than chloroquine (CQ) for CQS (NF54) with an IC_{50} of 2.2 nM and a selectivity index of 1400, and **17c** without substituents at the C-2 position, showed a 22 times greater potency than CQ for CQR (K1) with an IC_{50} of 9.4 nM, a selectivity index of 131.8 and a resistance index of 0.4 by K1/NF54.



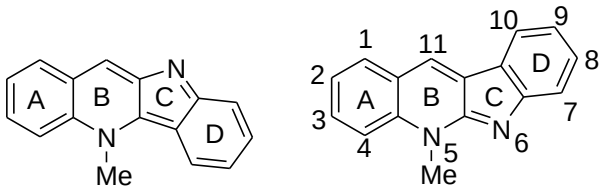
Keywords: Indolo[2,3-*b*]quinolines, side modification, antimalarial activity

4.2 Introduction

Malaria is still one of the most frightening parasitic diseases in the tropical and subtropical regions where both developing and non-industrialized nations are confronted with them. According to the World Health Organization (WHO), this disease led to about 216 million malarial infected cases in 2010, and approximately 0.7 million died due to the non-availability of proper treatment, involving mostly children under 5 years old.[1] These facts justify its classification as a dreaded infectious disease along with tuberculosis and AIDS.[2-3] Malaria is caused by five main species of malaria parasites, which are *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P.knowlesi*, and the most serious form is the *Plasmodium falciparum*. Fortunately, chloroquine (CQ), which was discovered in the 1940s, is used as the main antimalarial drug until today because of its remarkable therapeutic effect as an antimalarial drug and its low cost.[4] However, the spread of *Plasmodium falciparum* (Pf) strains resistant to CQ is dramatically increasing over these years in the endemic areas. Despite the introduction of the artemisinin-based combination therapies (ACTs), the development of new chemotherapeutic agents for the treatment of malaria is still urgently needed to help to ensure the availability of new compounds to feed the preclinical pipeline.[5]

Plants are still an important resource for the discovery of new drugs. The potential of natural compounds as a new candidate of drugs is demonstrated by the introduction of artemisinin and its derivatives as antimalarial agents.[6] These important roles of natural products isolated from plants have stimulated us to evaluate natural resources in endemic areas. Many naturally occurring compounds, including the major alkaloid cryptolepine and minor alkaloid neocryptolepine, were isolated from the roots of the West African plants *Cryptolepis sanguinolenta* (Figure 1).[7-9] Both of the two tetracyclic heteroaromatic compounds are linearly fused indoloquinolines and exhibit a promising antiplasmodial activity both against chloroquine-sensitive (CQS) and

chloroquine-resistant (CQR) *P. falciparum*.[10-11] Further experiments have also indicated that cryptolepine inhibits the β-haematin formation which is responsible for the treatment of malaria infections, and also has a cytotoxicity due to a DNA intercalation activity.[12-13] Therefore, we selected neocryptolepine as the lead compound for the development of new antimalarial agents because of its lower affinity for DNA intercalation and topoisomerase II compared to cryptolepine.[14]



Cryptolepine **Neocryptolepine**

Figure 1. Structures of cryptolepine and neocryptolepine.

On the other hand, during the study of the structure-activity relationship (SAR) of CQ, the importance of the 4-aminopyridine substructure for haematin binding and antimalarial activity was illustrated by both experimental and molecular modeling studies by Cheruku et al.[15] They also suggested that sufficiently large changes in the side chain alone could overcome the chloroquine-resistance without having to make changes in the 4-amino-7-haloquinoline template responsible for the Fe(III)PPIX complexation and inhibition of β-haematin formation.[16] Since various methodical modifications of the CQ side chain have been reported to date (Figure 2),[17-19] a systematic variation in the side chain structure and basicity seems to be more promising for the development of new antimalarial agents based on the indolequinoline derivatives.

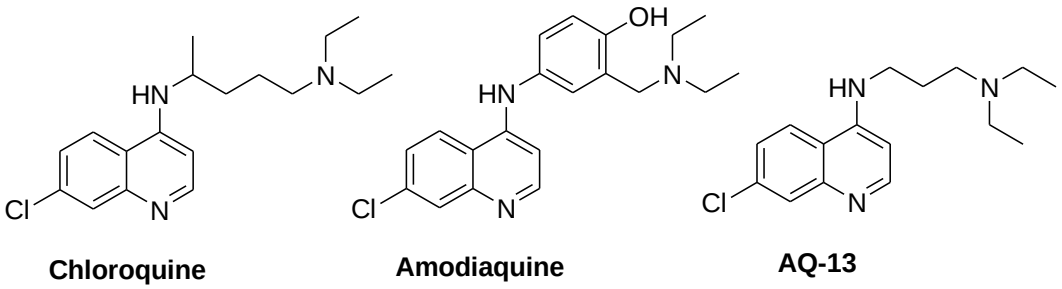


Figure 2. Structures of antimalarial 4-aminoquinolines.

In this study, we focused our attention on the side chain modification of the neocryptolepine core for development of antimalarial agents. For this, introduction of various aminoalkylamino groups into the C-11 position and further selective modifications of the pendent amino group with different functional groups were examined (Figure 3). Our methods are based on the fact that the thiazolidin-4-one （Figure 3, compounds **9** and **11**）is a biologically privileged scaffold and well tolerated in human subjects.[20] The sulfonamide (Figure 3, compound **13**)， thiophene-2-carboxamide (Figure 3, compound **15**) and urea/thiourea (Figure 3, compound **17**) were incorporated with the idea of improving the solubility properties and *in vitro* antiparasitic activities according to literature data of related compounds with them.[21-22] We also envisioned that the introduction of electron-withdrawing substituents, such as halogen and nitro groups, and electron-donating substituents, such as the methoxy group, at the C-2 position of the neocryptolepine core would reduce the cytotoxicity of the parent compounds. We now report the synthesis and evaluation of the antimalarial activity of novel 11-amino substituted neocryptolepine derivatives with further modifications of the amino group at the terminal side chain.

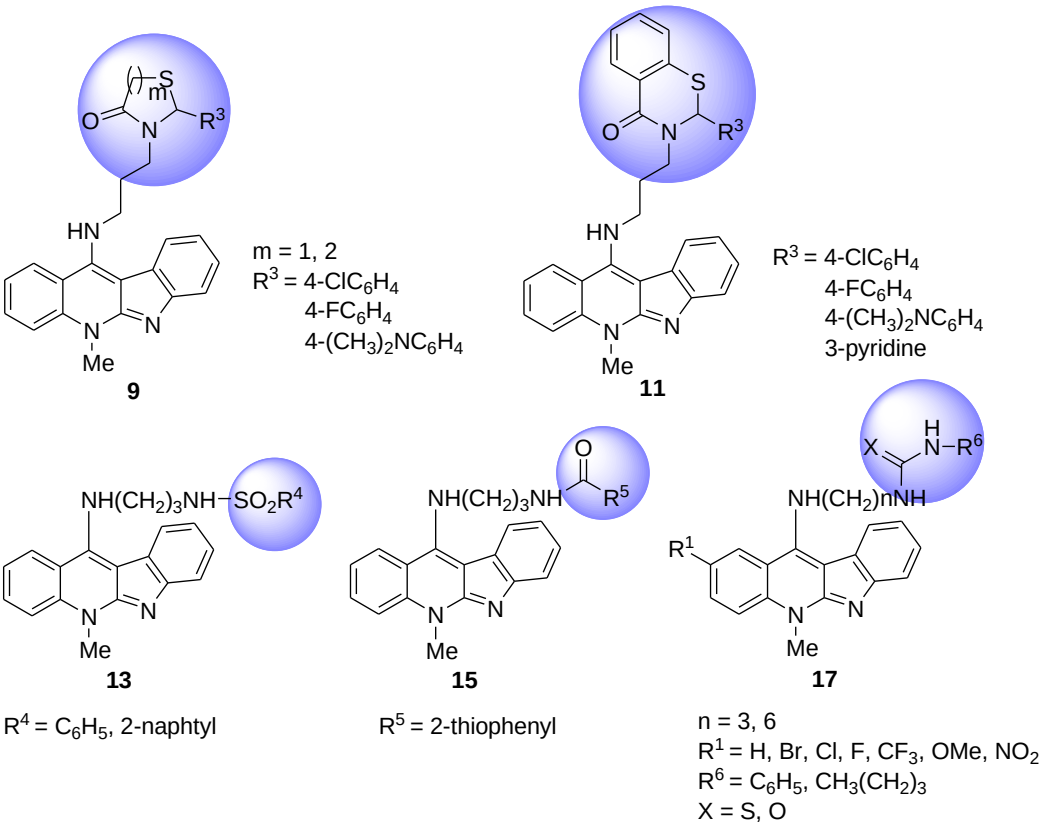


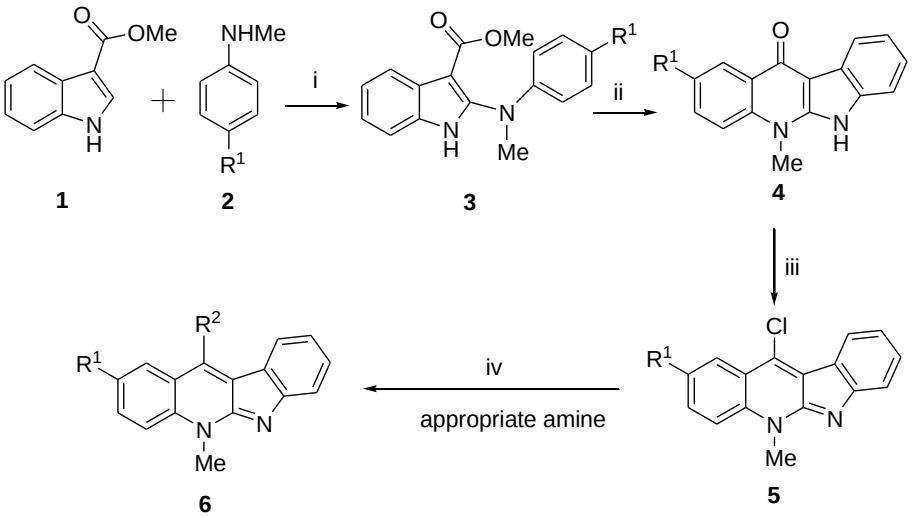
Figure 3.
Structures of terminal amino group of the side chain modified neocrytolepine derivatives.

4.3 Chemistry

Several synthetic strategies of the neocryptolepine core have been developed in recent years. Timaril *et al*. reported the synthesis of neocryptolepine from 3-bromoquinoline using the Suzuki procedure,[23] Molina *et al*. reported the synthesis of neocryptolepine via the Staudinger, aza-Wittig and electrocyclization reactions,[24] Ho *et al*. synthesized neocryptolepine from the common intermetidates 1,3-bis-(2-nitrophenyl)propan-2-one, by transition-metal mediated reductive cyclization,[25] and the Perkin reaction and double reduction-double cyclization as the main steps were used for the synthesis of neocryptolepine by Parvatkar *et al.*,[26] the heteroatom directed photoannulation technique reported by Dhanabal *et al.*,[27] the Graebe-Ullmann reaction by Peczynska-Czoch *et al.*,[28] and other miscellaneous methods.[29-32]

In a previous study, we developed an improved procedure for the syntheses of the 11-chloroneocryptolepine core and its 6-methyl congener with substitutes at the C-2~4 positions, of which started from methyl 1*H*-indole-3-carboxylate (**1**) and *N*-methylanilines **2** or anilines (Scheme 1).[33-34] Thus, the intermediate, 2-*N*-(*N*-methylanilino)indole-3-carboxylate (**3**), was obtained from **1** by chlorination with *N*-chlorosuccinimide in the presence of 1,4-dimethylpiperzine followed by the addition of a mixture of **2** and trichloroacetic acid. The Cyclization of **3** was carried out at 250 ^{o}C in diphenyl ether to form the tetracyclic ketone **4**, which was converted to 11-chloroneocrypotolepine **5** by dehydrative chlorination with $POCl_3$. Subsequently, the amination of **5** with appropriate amines by heating in DMF yielded the aminoalkylamino-substituted neocryptolepine derivatives **6**.

Scheme 1. Synthesis of neocryptolepines with substituents at the C-2 and C-11 positions

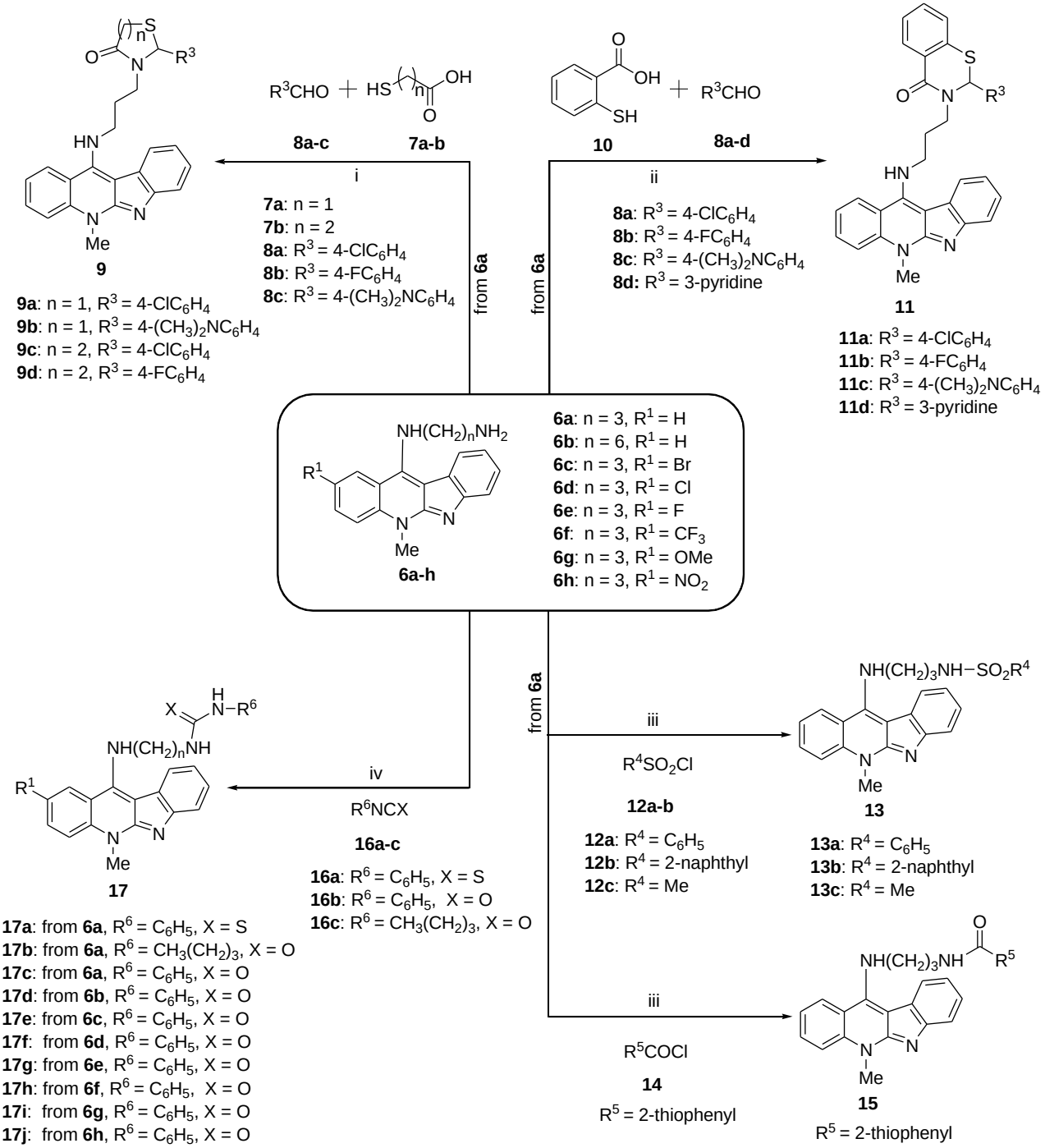


R^1 = H, Br, Cl, F, CF_3, OMe, NO_2
R^2 = Table1 and Table 4

Reagents and conditions:
(i) a, *N*-Chlorosucccinimide, 1,4-dimethylpiperazine, CH_2Cl_2, 0 ^oC, 2 h; b,trichloroacetic acid, RT, 2 h;
(ii) diphenyl ether, reflux, 1-3 h; (iii) $POCl_3$, toluene, reflux. 6-12 h; (iv) appropriate amine, DMF, 135 ^oC.

Further modifications of the 11-amino neocryptolepine derivatives **6** at the terminal amino group of the lateral attachment were carried out as outlined in Scheme 2. The amino group was transformed into the thiazolidin-4-one skeleton by the one-pot three-component condensation with an aldehyde and mercapto acid.[35] Thus, the neocryptolepine derivatives bearing 2-substituted thiazolidin-4-ones **9a-d** were obtained from the *N*-(3-aminopropyl)-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine **6a** by heatment with the substituted aldehydes **8a-c** and mercapto acids **7a-b** in the presence of DCC in dry THF at room temperature in good yields. On the other hand, due to the insolubility of the 2-mercaptobenzoic acid **10** in THF, the synthesis of the neocryptolepine derivatives of the 2-substituted 2,3-dihydrobenzo[e][1,3]thiazin-4-ones **11a-d** were carried out by heating a mixture of the appropriate amine **6a**, the substituted aldehydes **8a-d**, and **10** in the presence of DCC in dry toluene.

Scheme 2. Synthesis of neocryptolepine derivatives by further modifications of the lateral amino group



Reagents and conditions:
(i) DCC, THF, RT; (ii) DCC, toluene, reflux; (iii) DMF, Et_3N, RT; (iv) CH_2Cl_2, RT.

The neocryptolepine derivatives of the 2-substituted sulfonamides **13a-c** and 2-substituted thiophene-2-carboxamide **15** were easily synthesized by sulfonylation of the appropriate amine **6a** with sulfonyl chloride **12a-b** and with thiophene-2-carbonyl chloride **14** in DMF in the presence of Et_3N at room temperature, respectively. Furthermore, the neocryptolepine derivatives of the 2-substituted thiourea **17a** and 2-substituted urea **17b-j** could also be easily obtained by mixing of the appropriate amine **6a-h** with isothiocyanate **16a** and isocyanate **16b-c** in dry CH_2Cl_2 at room temperature, respectively.

All the synthesized neocryptolepine derivatives for biological screening tests are listed in Tables 1, 2, 3, 4 and 5

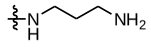
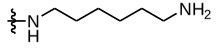
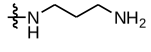
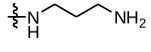
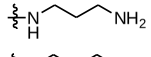
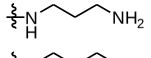
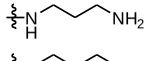
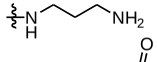
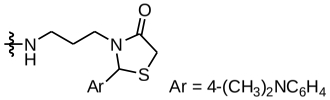
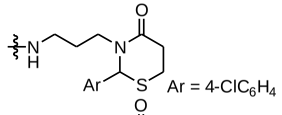
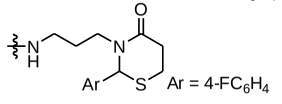
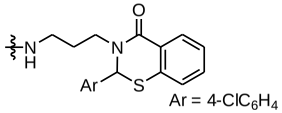
4.4 Results and Discussion

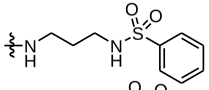
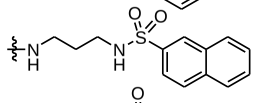
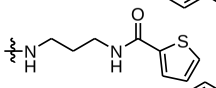
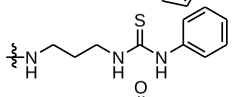
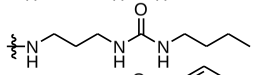
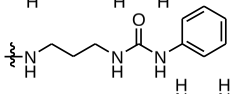
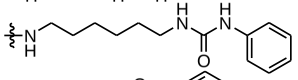
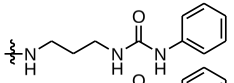
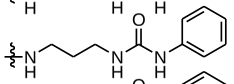
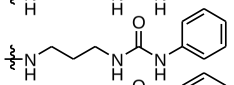
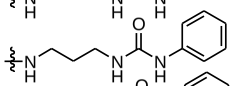
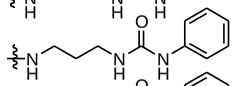
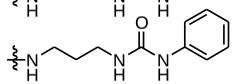
4.4.1 Antimalarial Activity against *P.falciparum* (CQS: NF54 and CQR: K1) and cytotoxicity toward *L6* cells

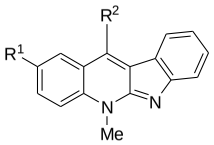
The introduction of 3-amioalkylamino group, such as 3-aminoalkylamino and *N*,*N*-diethyl-5-amino-pentyl-2-amio groups at the C-11 on the 5-methylindole[2,3-*b*]quinoline (neocryptolepine) core, significantly increased the antiproliferatice activities.[36] Recently, the aminoalkylamino-substituted neocryptolepine was shown to be 1500-fold more efficacious when compared to the natural product itself against the chloroquine-sensitive *P. falciparum* Ghana strain.[34] Based on these facts, we introduced the 3-aminopropylamino groups at the C-11 position by the varying kinds of substituents at the C-2 positions for the SAR study of the neocryptolepine core.

We first examined the antimalarial activity of the 2-substituted 11-(3-aminopropylamino) neocryptolepines against the CQS (NF54). As shown in Table 1, the compounds **6c**, **6d**, **6f** substituted with halogens, such Br, Cl, and electron-withdrawing CF_3, increased the activity against the NF54, compared to the non-substituted **6a**. No remarkable increase in the activity was found using the F-substituted **6e**. Noteworthy is the remarkably increased activity of **6h** substituted with NO_2 at the C-2 position. On the other hand, the longer chain-substituted **6b** with the 6-aminohexylamino group showed a slightly decreased activity compared to the shorter one, such as 1,3-diamino-tri(methylene) **6a**. Accordingly, the 3-aminopropylamino group was then further modified to produce a higher activity.

Table 1. Antiplasmodial activity against *P.falciparum*(NF54) and cytotoxicity toward L6 cells of neocryptolepine derivatives

NO	R^1	R^2	Cytotoxicity (*L6* cells) IC_{50} nM[b]	*P.falciparum* (NF54) IC_{50} nM[b]	SI[a] (*L6*/NF54)
6a	2-H		279.2	78.8	3.5
6b	2-H		692.7	98.1	7.1
6c	2-Br		258.3	10.4	24.8
6d	2-Cl		268.6	11.8	22.8
6e	2-F		338.1	49.6	6.8
6f	2-CF_3		923.8	10.7	86.0
6g	2-OMe		382.8	74.8	5.1
6h	2-NO_2		208.9	11.4	18.3
9a	2-H	Ar = 4-ClC_6H_4	3473	64	54.4
9b	2-H	 Ar = 4-$(CH_3)_2NC_6H_4$	3551	54.9	64.7
9c	2-H	 Ar = 4-ClC_6H_4	3359	52.4	64.1
9d	2-H	 Ar = 4-FC_6H_4	2728	38.1	71.6
11a	2-H	 Ar = 4-ClC_6H_4	1099	26.6	41.3
Podophylotoxin			14.5		
Chloroquine				9.4	

NO	R^1	R^2	Cytotoxicity (*L6* cells) IC_{50} nM[b]	*P.falciparum* (NF54) IC_{50} nM[b]	SI[a] (*L6*/NF54)
13a	2-H		65.2	63.0	1.0
13b	2-H		18.2	14.2	1.3
15	2-H		4463	258.1	17.3
17a	2-H		1228	9.1	134.9
17b	2-H		1740	104.1	16.7
17c	2-H		1244	21.3	58.4
17d	2-H		397	25.8	15.4
17e	2-Br		2826	4.0	706.5
17f	2-Cl		3079	2.2	1400
17g	2-F		3556	24.9	142.8
17h	2-CF_3		1282	4.1	312.7
17i	2-OMe		2734	4.4	621.4
17j	2-NO_2		2732	2.1	1301
Podophylotoxin			14.5		
Chloroquine				9.4	



[a] Selectivity Index is the ratio of IC_{50} for cytotoxicity versus antiplasmodial activity (*L6*/P.f.)

[b] The IC_{50} values are the means of two independent assays, the individual values vary less than a factor 2.

In the 7-chloroquinoline core of CQ, the 4-(*n*-aminoalkylamino) side chains were modified in order to explore the antiplasmodial activity of the derivatives having a thiazolidin-4-one nucleus at the terminal side chain amino group. Furthermore, this type of modification would prevent dealkylation without adversely affecting the lipophilicity and antimalarial activity of the molecule as described in the literature.[37] In our study, we have designed compounds wherein the 3-aminoalkylamino side chain of **6a** was further transformed into the thiazolidine-4-one, i.e., the [1,3]thiazinan-4-one groups, without varying the substituent at the C-2 position. The thiazolidine-4-one substituted **9a-d** and **11** showed slightly increased activities compared to **6a**. Thus, it turned out that a protected non-basic nitrogen at the terminal of the 3-aminoalkylamino side chain significantly affected the antimalarial activity. These derivatives with non-basic substituents showed higher SI data compared to the free terminal amine derivative **6a**.

Similarly, the terminal nitrogen of the 3-aminoalkylamino side chain of **6a** was simply and straightforwardly transferred into the corresponding sulfonamides **13a-b**, amide **15** and urea derivatives **17a-c**. The naphthalenesulfonyl derivative **13b** showed a higher activity than its benzenesulfonyl derivative **13a** with a similar SI value. A decreasing antimalarial activity was obtained by the thiophenecarbonyl derivative **15**, giving the higher IC_{50} value of 258.1 nM for CQS (NF54) than **6a** (IC_{50} = 78.8 nM), but an improved SI value from 3.5 to 17.3. An impressive antimalarial activity was also observed with the urea derivatives **17a-c**, and amongst them, **17a** showed the highest antimalarial activity (IC_{50} = 9.1 nM) for CQS (NF54) and higher than CQ (IC_{50} = 9.4 nM) with the high SI value of 134.9. Similar results were also reported during the study of the 4-aminoquinolines by Dominguez *et al.*,[38] in which urea derivatives have been identified as inhibitors of β-haematin formation which are digested by malaria parasites as they grow within their host red blood cells. The urea derivatives **17d** with a long chain (6-aminohexylamino group) showed a slightly

decreased activity compared to the shorter one, such as 1,3-diamino-tri(methylene) **17c**. This result also agreed with the comparison of **6a** and **6b**.

In this study, we also investigated the effect of substituents at the C-2 position of the urea derivatives **17c** by varying the substituents at the C-2 position. Among these compounds **17e-j**, several compounds with 2-substituent showed much higher antimalarial activities than **17c** without a substituent at the C-2 position. Interestingly, both the electron-withdrawing group, such as CF_3 and NO_2, and electron-donating group, such as OMe, at the C-2 position of the urea derivatives **17c** obviously showed an increased antimalarial activity against CQS (NF54) compared to **17c**. A similarly increased antimalarial activity was also obtained with **17e** and **17f**, when substituted with halogens (Br, Cl). Especially, the 2-Cl substituted **17f** showed the highest SI value of 1400 and IC_{50} value of 2.2 nM for CQS (NF54) amongst all the synthesized compounds.

Table 2. Antiplasmodial activities and cytotoxicity of the tested compounds

NO	Cytotoxicity (L6 cells)	NF54	SI[a]	K1	SI[a]	RI[b]
	IC_{50} nM[c]	IC_{50} nM[c]	(L6/NF54)	IC_{50} nM[c]	(L6/K1)	(K1/NF54)
9b	3551	54.9	64.7	143.2	24.8	2.6
9c	3359	52.4	64.1	38.8	86.6	0.7
11a	1099	26.6	41.3	21.3	51.6	0.8
13a	65.2	63.0	1.0	123.7	0.5	2.0
17a	1228	9.1	134.9	111.5	11.0	12.3
17c	1244	21.3	58.4	9.4	131.8	0.4
Podophylotoxin	14.5					
Chloroquine		9.4		209.5		22.3
Artemisinin		4.3		2.8		0.7

[a], Selectivity Index is the ratio of IC_{50} for cytotoxicity versus antiplasmodial activity (L6/P.f.).

[b], Resistance Index is the ratio of IC_{50} for the resistant versus the sensitive strain (K1/NF54).

[c],The IC_{50} values are the means of two independent assays; the individual values vary less than a factor 2.

As shown in Table 2, six compounds with different substituents at the terminal amino group were submitted for measurement involving the CQS (NF54) and CQR (K1) strains. Several tested compounds showed promising antimalarial activities against both strains and a low resistance index (RI). The RI provides a quantitative measurement of the antiplasmodial acitivity against the CQR strains relative to that against CQS strains and reveals promising drug discovery leads.[39] Gratifyingly, we found that all six compounds were significantly more active against the CQR (K1) than CQ, giving IC_{50} values between 9.4 nM and 143.2 nM for K1 (CQ: IC_{50} = 209.5 nM) and RI values ranging from 0.4 to 12.3 (CQ: RI = 22.3), especially, **17c** had an IC_{50} value of 9.4 nM for K1 and RI value of 0.4.

Table 3. Antiprotozoal activity and cytotoxicity of tested compounds

NO	Cytotoxicity (L6 cells)	T.b.rhodesiense	T.cruzi.	L.donovani (axenic amastigotes)
	IC_{50} nM[a]	IC_{50} nM[a]	IC_{50} nM[a]	IC_{50} nM[a]
9b	3551	1373	2727	26095
9c	3359	547.5	3184	12950
11a	1099	868.4	2362	9749
13a	65.2	164.2	1597	58036
17a	1228	589.2	6120	79167
17c	1244	606.8	10602	97518
Podophylotoxin	14.5			
Melarsoprol		5.0		
Benznidazole			1606	
Miltefosine				360.7

[a] The IC_{50} values are the means of two independent assays; the individual values vary less than a factor 2.

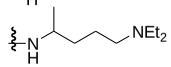
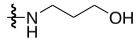
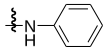
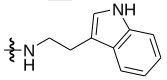
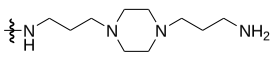
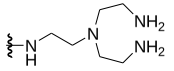
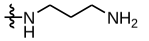
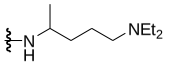
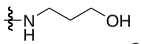
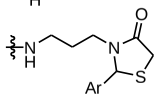
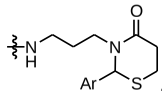
These six compounds were also selected for testing against three parasitic protozoa, *Trypanosoma brucei rhodesiense* (*T.b.rhodesiense*), *Trypanosoma cruzi* (*T.cruzi*) and *Leishmania donovani* (*L.donovani*) (Table 3). Several of these compounds showed antiprotozoal activities against *T.b.rhodesiense* and *T.cruzi.*, but all these neocryptolepine derivative also showed cytotoxicity and thus no selectivity.

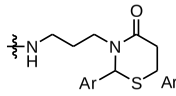
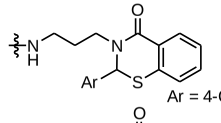
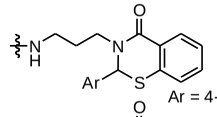
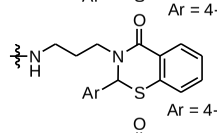
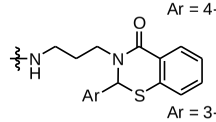
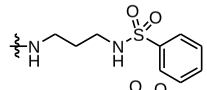
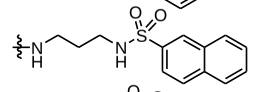
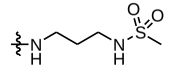
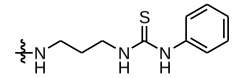
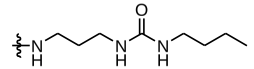
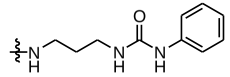
4.4.2 Antiproliferative activity of neocrytpolepine derivatives against human leukemia MV4-11 cells

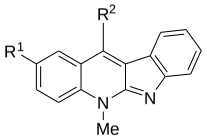
Some synthesized neocryptolepine derivatives were also tested by antiproliferative activity of against human leukemia MV4-11 cells, and all results are summrized in Tabel 4. Among these, some compounds showed high cytotoxic against the MV4-11 cells, such as **6a**, **6c**, **6j**, **6l**, **6n** and **13b**.

IC_{50}-concentration of tested compound leading to 50% inhibition of cell proliferation was obtained in a standard 72h MTT assay. The IC_{50} values were calculated for each experiment and the mean values ±SD were calculated from at least 3-5 independent experiments.

Table 4. Antiproliferative activity of neocryptolepine derivatives against human leukemia MV4-11 cells

NO	R^1	R^2	MV4-11 IC_{50} μg/ml	MV4-11 SD
Cisplatin			0.846	0.135
Neocryptolepine			0.509	0.119
5a	2-H	Cl	0.350	0.070
5b	2-Br	Cl	0.280	0.050
6a	2-H		0.020	0.007
6i	2-H		0.280	0.060
6j	2-H		0.052	0.003
6k	2-H		0.218	0.043
6l	2-H		0.052	0.015
6m	2-H		0.340	0.100
6n	2-H		0.045	0.002
6o	2-H		1.828	0.501
6c	2-Br		0.0046	0.0008
6p	2-Br		0.270	0.020
6q	2-Br		0.270	0.034
9b	2-H	 Ar = 4-$(CH_3)_2NC_6H_4$	2.749	0.484
9c	2-H	 Ar = 4-ClC_6H_4	2.140	0.230

NO	R^1	R^2	MV4-11 IC_{50} μg/ml	MV4-11 SD
Cisplatin			0.846	0.135
Neocryptolepine			0.509	0.119
9d	2-H	 Ar = 4-FC_6H_4	2.990	0.290
11a	2-H	 Ar = 4-ClC_6H_4	1.830	0.220
11b	2-H	 Ar = 4-FC_6H_4	2.110	0.290
11c	2-H	 Ar = 4-$(CH_3)_2NC_6H_4$	1.150	0.280
11d	2-H	 Ar = 3-pyridine	2.890	0.330
13a	2-H		0.338	0.058
13b	2-H		0.050	0.041
13c	2-H		0.317	0.032
17a	2-H		1.050	0.200
17b	2-H		0.414	0.078
17c	2-H		0.483	0.105



4.4.3 Antiproliferative activity of neocrytpolepine derivatives against human lung cancer cell line A549, colon cancer cell line HCT116 and normal mice fibroblast Balb/3T3 cell

Some neocryptolepine derivatives shown high antiproliferative activity against MV4-11 cells were chosen for antiproliferative activity against human lung cancer cell line (A549), colon cancer cell line (HCT 116) and normal mice fibroblast (Balb/3T3). As shown in Table 5, compound **13b** showed the most active against A549 and the activity against HCT116 cancer line and normal fibroblast Balb/3T3 was 7-9 time lower. Compound **6a** showed high antiproliferative activity against A549 and HCT116, but also showed lower cytotoxicity. Antiproliferative activity against normal fibroblast Balb/3T3 was 3-4 time lower.

Table 5. Antiproliferative activity of neocrytpolepine derivatives against Balb/3T3, A549 and HCT116

NO	Balb/3T3		A549		HCT116	
	IC_{50} μg/ml	SD	IC_{50} μg/ml	SD	IC_{50} μg/ml	SD
Cisplatin	2.610	0.291	2.961	0.720	2.550	0.162
Neocryptolepine	3.090	0.230	3.410	1.900	0.610	0.190
6a	0.269	0.035	0.0623	0.024	0.092	0.017
6c	0.333	0.007	0.208	0.098	0.105	0.019
6j	0.194	0.058	0.297	0.073	0.218	0.026
6l	0.510	0.198	0.479	0.130	0.570	0.088
6n	0.316	0.019	0.591	0.167	0.325	0.052
6q	0.460	0.310	0.610	0.280	0.380	0.090
13a	3.050	0.390	2.030	0.960	1.460	0.490
13b	2.008	0.593	0.350	0.088	2.865	0.222
13c	2.610	0.200	1.980	0.790	0.630	0.190
17b	3.170	0.210	2.440	3.500	0.640	0.140
17c	3.410	0.210	2.610	0.580	1.380	0.550

4.5 Conclusion

The author prepared a novel series of new neocryptolepine derivatives by systematically varying the 2-substituents of the neocryptolepine core and modifying the terminal amino group of the C-11 aminoalkylamino side chain. All the synthesized neocryptolepine derivatives showed potent antiplasmodial activities against CQS (NF54) and CQS (K1) in vitro. A comparison with CQ clearly revealed that **17a**, **17e-f** and **17h-j** had increased activities against CQS (NF54), **9b-c**, **11**, **13a**, **17a** and **17c** were also clearly observed to afford superior activities against CQR (K1). In particular，among the tested compounds, **17f** showed a 4 times more potent activity than CQ for CQS (NF54) with an IC_{50} of 2.2 nM and a selectivity index of 1400, similarly high antimalarial activity was showed by **17j**. On the other hand, **17c** showed a 22 times more potent activity than CQ for CQR (K1) with an IC_{50} of 9.4 nM, a selectivity index of 131.8 and a resistance index of 0.4 by K1/NF54. These present findings are sufficient to establish that the methodical variation of the side chain of the neocryptolepine core provides a promising entry point toward affordable heme-targeted antimalarials that overcome the ever increasing problem of worldwide drug resistance.

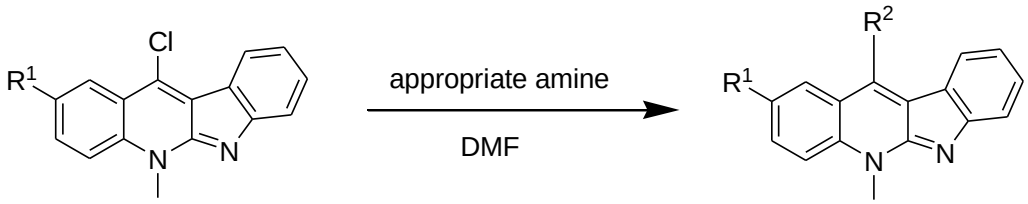
4.6 Experimental Section

4.6.1 Chemistry

Materials and methods

Column chromatographies were achieved on a silica gel column (230-400 mesh) using a gradient solvent system (*n*-hexane / ethyl acetate as the eluant unless otherwise specified). The ^{1}H, ^{13}C and ^{19}F NMR spectra were taken on a Varian INOVA-600 spectrometer with $CDCl_3$ or DMSO-d_6 as the solvent unless otherwise indicated. Chemical shifts (δ ppm) were determined using tetramethylsilane (TMS) as the internal reference. Melting points were determined on a J-Science RFS-10 hot stage microscope. IR spectra were recorded on a Shimadzu FTIR-8400S spectrometer. High resolution mass spectra were obtained on a Bruker micrOTOF II-SKA spectrometer.

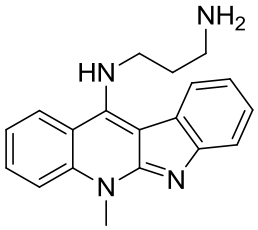
General procedure for the synthesis of 11-aminoneocryptolepines 6a-q



5 **6**

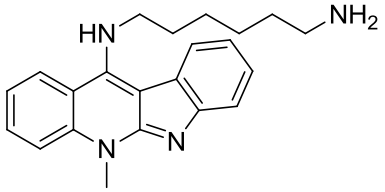
R^1 = H,Br, Cl, F, CF_3, OMe, NO_2

11-Chloroindoloquinolines **5** (0.3 mmol) and an excess of the appropriate aminoalkylamine (3.0 mmol) were heated together at 135–155 °C for 1–4 h. TLC monitoring was used to ensure the completion of reaction. The resulting brown crude oil was purified by flash chromatography using AcOEt-2N ammonia in MeOH (9:1) as an eluent to yield pure **6** as yellowish-orange solids.



N-(3-Aminopropyl)-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (6a):

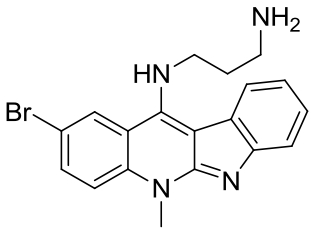
Yield: 96%, yellow solids. Mp: 69–71 ^{o}C; IR (KBr) 3435, 2928, 2868, 2359, 2342, 1622, 1559, 1489, 1441, 1418, 1287, 1248, 1057, 750, 669 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.80 (quint, *J* = 6.0 Hz, 2H), 3.01 (t, *J* = 6.0 Hz, 2H), 4.01 (t, *J* = 6.0 Hz, 2H), 4.22 (s, 3H), 7.17 (t, *J* = 7.2 Hz, 1H), 7.18 (br. s., 1H), 7.31 (t, *J* = 7.2 Hz, 1H), 7.41 (t, *J* = 7.2 Hz, 1H), 7.61 (d, *J* = 9.0 Hz, 1H), 7.67 (td, *J* = 7.2, 1.2 Hz, 1H), 7.76 (d, *J* = 7.8 Hz, 1H), 7.95 (d, *J* = 7.8 Hz, 1H), 8.13 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 32.64, 32.66, 41.26, 49.25, 105.61, 114.40, 115.86, 116.94, 118.49, 120.35, 121.37, 124.06, 124.12, 125.16, 130.08, 137.79, 148.52, 151.94, 156.55. HRMS (ESI) calcd for $C_{19}H_{21}N_4$ $[M+H]^+$ Exact Mass: 305.1761, found 305.1765.



N-(6-Aminohexyl)-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (6b):

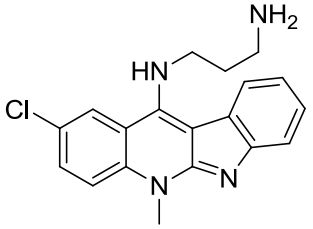
Yield: 96%, yellow solids. Mp: 54–56 ^{o}C; IR (KBr) 3350, 2928, 2855, 1622, 1593, 1568, 1489, 1441, 1422, 1281, 1246, 1200, 1142, 882, 752 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.19 (m, 5H), 1.36 (m, 1H), 1.68 (m, 2H), 2.38 (t, *J* = 6.6 Hz, 1H), 2.95 (t, *J* = 6.6 Hz, 1H), 3.83 (m, 2H), 4.16 (s, 3H), 6.99 (br. s., 1H), 7.07 (t, *J* = 7.2 Hz, 1H), 7.28 (t, *J* = 7.2 Hz, 1H), 7.41 (t, *J* = 7.8 Hz, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.79 (t, *J* = 7.8 Hz, 1H), 7.84 (d, *J* = 7.8 Hz, 1H), 7.90 (d, *J* = 7.8 Hz, 1H), 8.53 (d, *J* = 7.2 Hz, 1H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 22.58, 26.24, 26.27, 30.98, 32.66, 41.20, 48.13, 104.68, 115.41, 115.82, 116.79, 118.47, 121.12, 122.22, 124.20

(2C), 124.98, 131.13, 137.61, 148.78, 152.10, 156.59. HRMS (ESI) calcd for $C_{22}H_{27}N_4$ $[M+H]^+$ Exact Mass: 347.2230, found 347.2235.



***N-(3-Aminopropyl)-2-bromo-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (6c)*:**

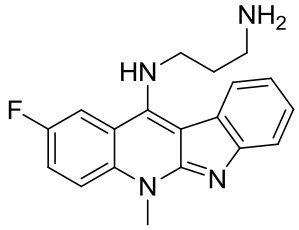
Yield: 94%, yellow solids. Mp: 137–139 ^oC; IR (KBr) 3229, 3152, 3067, 2940, 2909, 2857, 1622, 1589, 1557, 1505, 1487, 1441, 1418, 1389, 1281, 1238, 1209, 1109, 1057, 876, 800, 762, 743 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ ppm 1.82 (quint, *J* = 6.0 Hz, 2H), 3.08 (t, *J* = 6.0 Hz, 2H), 4.03 (m, 2H), 4.21 (s, 3H), 7.17 (t, *J* = 7.2 Hz, 1H), 7.42 (t, *J* = 7.2 Hz, 1H), 7.46 (br. s., 1H), 7.50 (d, *J* = 9.0 Hz, 1H), 7.72–7.75 (m, 2H), 7.97 (d, *J* = 7.8 Hz, 1H), 8.30 (d, *J* = 2.4 Hz, 1H); ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 32.23, 32.68, 41.51, 49.68, 106.28, 113.02, 116.07, 117.29, 117.52, 118.70, 121.67, 124.17, 125.56, 126.72, 132.57, 136.66, 147.17, 152.59, 156.65. HRMS (ESI) calcd for $C_{19}H_{18}BrN_4$ $[M–H]^–$ Exact Mass: 381.0720, found 381.0710.



N-(3-Aminopropyl)-2-chloro-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (6d):

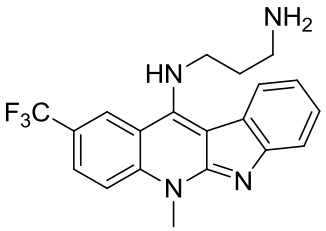
Yield: 92%, yellow solids. Mp: 132–134 ^oC; IR (KBr) 3420, 3264, 3050, 2934, 2870, 1618, 1587, 1559, 1491, 1443, 1418, 1341, 1290, 1279, 1248, 1217, 1115, 1065, 876, 797, 756, 731 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.74 (quint, *J* = 6.6 Hz, 2H), 2.59 (t, *J* = 6.6 Hz, 2H), 3.91 (t, *J* = 6.6 Hz, 2H), 4.14 (s, 3H), 7.08 (t, *J* = 7.2 Hz, 1H), 7.29 (t, *J* = 7.2 Hz, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.77–7.81 (m, 1H), 7.85 (m, 1H), 7.92 (d, *J* = 7.8 Hz, 1H), 8.59 (d, *J* = 2.4 Hz, 1H); ^{13}C NMR (150.8 MHz,

DMSO-d_6) δ ppm 32.40, 33.53, 39.46, 46.75, 104.57, 116.70, 116.80, 117.11, 118.18, 122.38, 123.09, 124.11, 124.79, 125.04, 130.24, 136.06, 147.10, 152.38, 156.34. HRMS (ESI) calcd for $C_{19}H_{20}ClN_4$ $[M+H]^+$ Exact Mass: 339.1371, found 339.1382.



N-(3-Aminopropyl)-2-fluoro-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (6e):

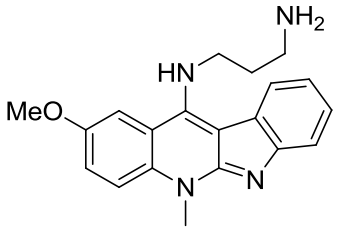
Yield: 89%, yellow solids. Mp: 87–88 ^oC; IR (KBr) 3283, 3055, 2932, 2359, 1614, 1601, 1568, 1489, 1445, 1424, 1344, 1281, 1242, 1136, 856, 795, 760, 739 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.73 (quint, J = 6.6 Hz, 2H), 2.60 (t, J = 6.6 Hz, 2H), 3.92 (t, J = 6.6 Hz, 2H), 4.16 (s, 3H), 7.07 (t, J = 7.2 Hz, 1H), 7.29 (t, J = 7.2 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.68–7.71 (m, 1H), 7.88 (m, 1H), 7.93 (d, J = 7.8 Hz, 1H), 8.36 (dd, J = 11.4, 2.4 Hz, 1H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 32.48, 33.53, 39.46, 46.79, 104.78, 109.10 (d, J = 24.1 Hz), 116.31 (d, J = 7.8 Hz), 116.53, 117.16 (d, J = 8.4 Hz), 117.97, 118.49 (d, J = 24.1 Hz), 122.35, 123.86, 124.79, 134.20, 147.32 (d, J = 3.3 Hz), 152.52, 156.46, 156.58 (d, J = 236.8 Hz); ^{19}F NMR (564 MHz, DMSO-d_6) δ ppm –121.26. HRMS (ESI) calcd for $C_{19}H_{20}FN_4$ $[M+H]^+$ Exact Mass: 323.1667, found 323.1670.



N-(3-Aminopropyl)-5-methyl-2-(trifluoromethyl)-5H-indolo[2,3-b]quinolin-11-amine (6f):

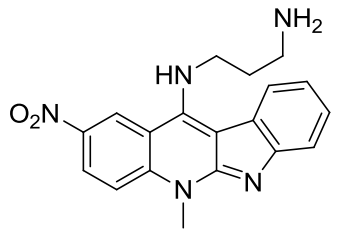
Yield: 87%, yellow solids. Mp: 64–65 ^oC; IR (KBr) 3430, 2930, 2351, 1634, 1595, 1568, 1505, 1443, 1429, 1402, 1333, 1279, 1246, 1146, 1117, 1088, 814, 762, 746

cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.76 (quint, J = 6.0 Hz, 2H), 2.63 (t, J = 6.0 Hz, 2H), 3.97 (t, J = 6.6 Hz, 2H), 4.18 (s, 3H), 7.11 (t, J = 7.2 Hz, 1H), 7.30 (m, 1H), 7.53 (d, J = 7.2 Hz, 1H), 7.96 (d, J = 7.8 Hz, 1H), 8.00 (t, J = 6.6 Hz, 1H), 8.04 (m, 1H), 8.86 (s, 1H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 32.51, 33.24, 39.56, 46.98, 104.22, 115.25, 116.17, 116.93, 118.53, 123.21 (q, J = 268.1 Hz), 121.79, 124.12, 124.78, 124.89, 126.37, 139.37, 147.60, 152.10, 156.41; ^{19}F NMR (564 MHz, DMSO-d_6) δ ppm –58.64. HRMS (ESI) calcd for $C_{20}H_{20}F_3N_4$ $[M+H]^+$ Exact Mass: 373.1635, found 373.1646.



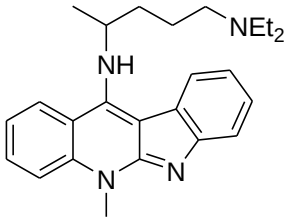
N-(3-Aminopropyl)-2-methoxy-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (6g):

Yield: 99%, yellow solids. Mp: 105–106 ^{o}C; IR (KBr) 3381, 3268, 2934, 1614, 1593, 1568, 1489, 1445, 1424, 1348, 1288, 1246, 1184, 1140, 1038, 937, 810, 760 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.72 (quint, J = 6.6 Hz, 2H), 2.64 (t, J = 6.6 Hz, 2H), 3.91 (s, 3H), 3.94 (t, J = 6.6 Hz, 2H), 4.14 (s, 3H), 7.04 (t, J = 7.2 Hz, 1H), 7.26 (t, J = 7.2 Hz, 1H), 7.43–7.47 (m, 2H), 7.79 (d, J = 9.6 Hz, 1H), 7.92 (m, 2H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 32.23, 33.44, 39.82, 47.18, 55.80, 104.47, 105.88, 116.18, 116.28, 116.43, 117.47, 119.52, 122.15, 123.98, 124.43, 132.18, 147.73, 152.50, 153.55, 156.26. HRMS (ESI) calcd for $C_{20}H_{23}N_4O$ $[M+H]^+$ Exact Mass: 335.1866, found 335.1864.

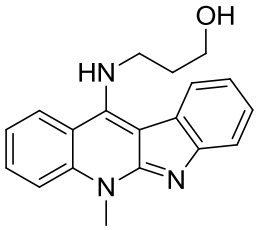


N-(3-Aminopropyl)-5-methyl-2-nitro-5H-indolo[2,3-b]quinolin-11-amine (6h):

Yield: 83%, red solids. Mp: 159–162 ^{o}C; IR (KBr) 3430, 3069, 2940, 2868, 1616, 1570, 1505, 1441, 1424, 1329, 1294, 1246, 1121, 941, 826, 760, 739 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.78 (quint, J = 6.6 Hz, 2H), 2.67 (t, J = 6.6 Hz, 2H), 4.00 (t, J = 6.6 Hz, 2H), 4.20 (s, 3H), 7.13 (t, J = 7.2 Hz, 1H), 7.31 (t, J = 7.2 Hz, 1H), 7.55 (d, J = 7.8 Hz, 1H), 7.98 (t, J = 7.8 Hz, 2H), 8.52 (m, 1H), 9.45 (d, J = 2.4 Hz, 1H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 32.85, 32.96, 39.90, 47.21, 103.96, 115.06, 116.11, 117.26, 119.04, 121.08, 122.41, 124.21, 124.56, 124.88, 140.18, 141.06, 147.60, 151.90, 156.17. HRMS (ESI) calcd for $C_{19}H_{18}N_5O_2$ [M–H]$^-$ Exact Mass: 348.1466, found 348.1485.

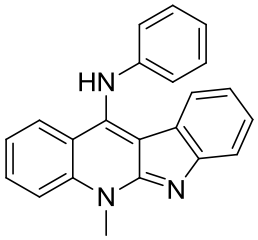


N-(5-(Diethylamino)pentan-2-yl)-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (6i): Yield: 99%, yellow solids. Mp: 70–73 ^{o}C; IR (KBr) 3242, 3048, 2965, 2801, 2620, 1591, 1566, 1549, 1487, 1441, 1422, 1404, 1373, 1277, 1244, 1192, 1142, 1061, 748 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 0.92 (t, J=7.2 Hz, 6 H), 1.36 (d, J=6.0 Hz, 3 H), 1.50–1.60 (m, 2 H), 1.64–1.78 (m, 2 H), 2.34 (m, 2 H), 2.41 (q, J=7.2 Hz, 4 H), 4.29 (s, 3 H), 4.30 (m, 1 H), 4.91 (d, J=10.8 Hz, 1 H), 7.21 (td, J=7.8, 0.6 Hz, 1 H), 7.38 (t, J=7.2 Hz, 1 H), 7.46 (t, J=7.8 Hz, 1 H), 7.68–7.73 (m, 2 H), 7.77 (d, J=7.8 Hz, 1 H), 7.91 (d, J=7.8 Hz, 1 H), 8.16 (d, J=8.4 Hz, 1 H); ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 11.42 (2C), 22.35, 23.71, 32.74, 37.20, 46.73 (2C), 52.66, 54.34, 110.75, 114.77, 116.55, 117.50, 118.96, 120.60, 120.67, 124.09, 124.67, 126.32, 130.38, 138.15, 148.49, 153.15, 156.58. HRMS (ESI) calcd for $C_{25}H_{31}N_4$ [M–H]$^-$ Exact Mass: 387.2554, found 387.2583.



3-(5-Methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propan-1-ol (6j):

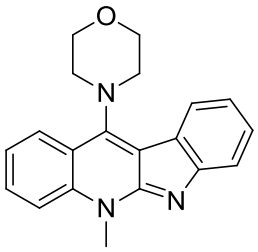
Yield: 94%, yellow solids. Mp: 187–188 ^{o}C; IR (KBr) 3391, 3080, 3042, 2922, 2859, 2359, 1912, 1618, 1591, 1570, 1514, 1443, 1416, 1341, 1292, 1248, 1225, 1074, 1063, 864, 748, 714 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.95 (quint, *J*=6.0 Hz, 2 H), 3.96–4.02 (m, 4 H), 4.20 (s, 3 H), 6.20 (br. s., 1 H), 7.14 (t, *J*=7.2 Hz, 1 H), 7.31 (td, *J*=7.8, 1.2 Hz, 1 H), 7.37 (t, *J*=7.8 Hz, 1 H), 7.60 (d, *J*=8.4 Hz, 1 H), 7.67 (t, *J*=7.2 Hz, 1 H), 7.73 (d, *J*=8.4 Hz, 1 H), 7.91 (d, *J*=7.8 Hz, 1 H), 8.11 (d, *J*=7.8 Hz, 1 H); ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 32.74, 32.87, 48.39, 62.20, 107.05, 114.63, 115.87, 117.08, 118.73, 120.50, 121.10, 124.00, 124.29, 125.58, 130.27, 138.06, 148.51, 152.30, 156.56. HRMS (ESI) calcd for $C_{19}H_{18}N_3O$ $[M–H]^-$ Exact Mass: 304.1455, found 304.1485.



5-Methyl-N-phenyl-5H-indolo[2,3-b]quinolin-11-amine (6k):

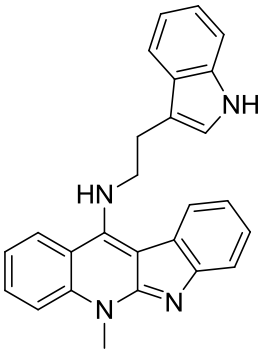
Yield: 95%, yellow solids. Mp: 187–188 ^{o}C; IR (KBr) 3296, 3053, 2943, 1620, 1589, 1568, 1524, 1497, 1485, 1439, 1416, 1402, 1317, 1261, 1242, 1171, 1142, 1099, 897, 858, 746, 721, 692 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 4.27 (s, 3 H), 6.89 (m, 3 H), 7.00 (t, *J*=7.20 Hz, 2 H), 7.19–7.25 (m, 3 H), 7.33 (t, *J*=7.20 Hz, 1 H), 7.43 (t, *J*=7.8 Hz, 1 H), 7.68 (m, 1 H), 7.73 (m, 2 H), 8.05 (d, *J*=7.80 Hz, 1 H); ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 32.99, 114.60, 114.88, 117.07, 117.16, 118.21 (2C), 119.32, 121.14, 122.25, 123.21, 123.45, 124.72, 127.44, 129.42 (2C), 130.54, 137.75, 140.05, 142.50, 153.30, 156.75. HRMS (ESI) calcd for $C_{22}H_{18}N_3$ $[M+H]^+$ Exact Mass:

324.1495, found 324.1509.



5-Methyl-11-morpholino-5H-indolo[2,3-b]quinoline (6l):

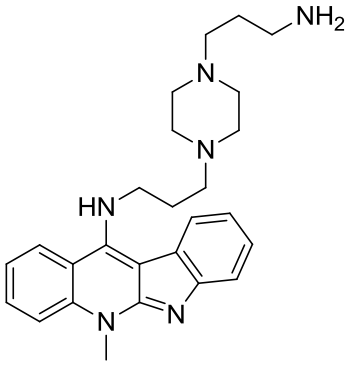
Yield: 77%, orange solids. Mp: 205–207 ^{o}C; IR (KBr) 3046, 2951, 2849, 1734, 1614, 1599, 1564, 1520, 1489, 1437, 1416, 1387, 1366, 1292, 1269, 1242, 1194, 1171, 1105, 1063, 1009, 856, 746, 721 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 3.58 (t, *J*=4.80 Hz, 4 H), 4.09 (t, *J*=4.80 Hz, 4 H), 4.36 (s, 3 H), 7.27 (m, 1 H), 7.46 (m, 1 H), 7.55 (t, *J*=7.20 Hz, 1 H), 7.74–7.78 (m, 3 H), 8.36 (d, *J*=7.80 Hz, 1 H), 8.57 (d, *J*=8.40 Hz, 1 H); ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 33.15, 49.34 (2C), 67.68 (2C), 114.41, 117.60, 119.49, 120.82, 121.38, 122.78, 123.44, 124.91, 126.20, 128.50, 130.46, 138.03, 149.93, 154.55, 157.81. HRMS (ESI) calcd for $C_{20}H_{20}N_3O$ $[M+H]^+$ Exact Mass: 318.1601, found 318.1621.



N-(2-(1H-Indol-3-yl)ethyl)-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (6m):

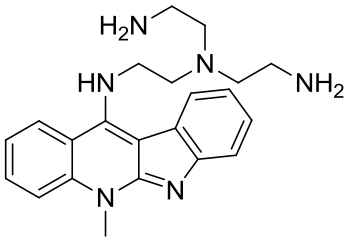
Yield: 92%, yellow solids. Mp: 198–200 ^{o}C; IR (KBr) 3410, 3053, 2918, 2862, 1717, 1622, 1593, 1568, 1499, 1441, 1418, 1343, 1290, 1248, 1109, 1071, 887, 745 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 3.18 (t, *J*=6.6 Hz, 2 H), 4.20–4.23 (m, 2 H), 4.22 (s, 3 H), 5.44 (br. s., 1 H), 7.04 (m, 2 H), 7.11 (t, *J*=7.8 Hz, 1 H), 7.23 (t, *J*=8.4 Hz, 1 H),

7.29 (t, *J*=7.8 Hz, 1 H), 7.36 (t, *J*=7.8 Hz, 1 H), 7.42 (dd, *J*=8.4, 0.6 Hz, 1 H), 7.48 (d, *J*=8.4 Hz, 1 H), 7.58–7.62 (m, 2 H), 7.68 (t, *J*=7.8 Hz, 1 H), 7.75 (d, *J*=8.4 Hz, 1 H), 8.02 (d, *J*=8.4 Hz, 1 H), 8.54 (br. s., 1 H); ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 26.62, 32.52, 48.61, 103.99, 110.81, 111.35, 115.18, 115.70, 115.96, 118.14, 118.25, 118.38. 120.87, 120.94, 122.02, 122.97 (2C), 123.62, 124.14, 124.73, 126.99, 130.84, 136.12, 137.26, 148.60, 155.31. HRMS (ESI) calcd for $C_{26}H_{23}N_4$ $[M+H]^+$ Exact Mass: 391.1917, found 391.1943.



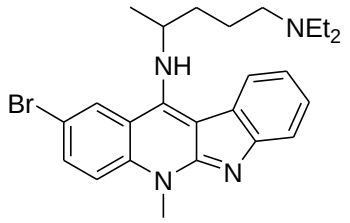
N-(3-(4-(3-Aminopropyl)piperazin-1-yl)propyl)-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (6n):

Yield: 95%, yellow solids. Mp: 90–94 ^{o}C; IR (KBr) 3368, 3242, 3051, 2936, 2816, 1616, 1589, 1562, 1493, 1443, 1422, 1406, 1288, 1248, 1142, 876, 754 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.68 (quint, *J*=6.6 Hz, 2 H), 1.90 (m, 2 H), 2.14 (br. s., 2 H), 2.40–2.83 (m, 14 H), 3.97 (m, 2 H), 4.24 (s, 3 H), 7.04 (br. s., 1 H), 7.16 (t, *J*=7.2 Hz, 1 H), 7.34 (t, *J*=7.2 Hz, 1 H), 7.41 (t, *J*=7.2 Hz, 1 H), 7.64–7.75 (m, 3 H), 7.93 (d, *J*=7.8 Hz, 1 H), 8.20 (d, *J*=7.8 Hz, 1 H); ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 26.29, 30.10, 32.73, 40.82, 49.40, 53.10 (2C), 53.75 (2C), 56.71, 58.31, 106.20, 114.59, 116.12, 117.19, 118.62, 120.48, 121.69, 124.14, 124.21, 125.41, 130.24, 137.82, 148.56, 152.33, 156.98. HRMS (ESI) calcd for $C_{26}H_{33}N_6$ $[M–H]^–$ Exact Mass: 429.2772, found 429.2756.



N-(2-(bis(2-Aminoethyl)amino)ethyl)-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (6o):

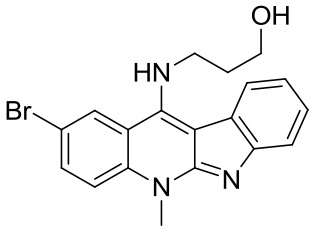
Yield: 78%, yellowish-orange solids. Mp: 69 ^{o}C; IR (KBr) 3354, 3048, 2934, 2855, 2357, 2189, 1616, 1591, 1564, 1495, 1489, 1418, 1279, 1246, 1142, 1103, 1024, 882, 853, 752 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 2.41 (br. s., 4 H), 2.61 (t, *J*=6.6 Hz, 4 H), 2.72 (t, *J*=6.0 Hz, 2 H), 2.80 (t, *J*=6.0 Hz, 4 H), 3.94 (t, *J*=5.4 Hz, 2 H), 4.23 (s, 3 H), 7.16 (t, *J*=7.2 Hz, 1 H), 7.33 (t, *J*=7.2 Hz, 1 H), 7.41 (t, *J*=7.2 Hz, 1 H), 7.63–7.69 (m, 2 H), 7.74 (d, *J*=7.8 Hz, 1 H), 7.95 (d, *J*=7.2 Hz, 1 H), 8.31 (d, *J*=8.4 Hz, 1 H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ ppm 32.73, 39.71 (2C), 45.66, 55.29, 56.56 (2C), 106.73, 114.68, 115.94, 117.18, 118.60, 120.45, 121.31, 123.99, 124.15, 125.57, 130.35, 137.97, 148.38, 152.37, 156.67. HRMS (ESI) calcd for $C_{22}H_{27}N_6$ $[M–H]^-$ Exact Mass: 375.2303, found 375.2324.



2-Bromo-N-(5-(diethylamino)pentan-2-yl)-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (6p):

Yield: 82%, red solids. Mp: 39 ^{o}C; IR (KBr) 3296, 3050, 2967, 2934, 2799, 1622, 1559, 1489, 1443, 1418, 1379, 1273, 1242, 1188, 1107, 1059, 909, 802, 760, 741 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 0.93 (t, *J*=7.2 Hz, 6 H), 1.35 (d, *J*=6.6 Hz, 3 H), 1.58 (m, 2 H), 1.65–1.78 (m, 2 H), 2.37 (m, 2 H), 2.44 (q, *J*=7.2 Hz, 4 H), 4.21 (m, 1 H), 4.25 (s, 3 H), 4.87 (d, *J*=10.8 Hz, 1 H), 7.22 (td, *J*=7.8, 1.2 Hz, 1 H), 7.47 (t, *J*=7.8 Hz, 1 H), 7.56 (d, *J*=9.0 Hz, 1 H), 7.73–7.80 (m, 2 H), 7.90 (d, *J*=7.2 Hz, 1 H), 8.25 (d,

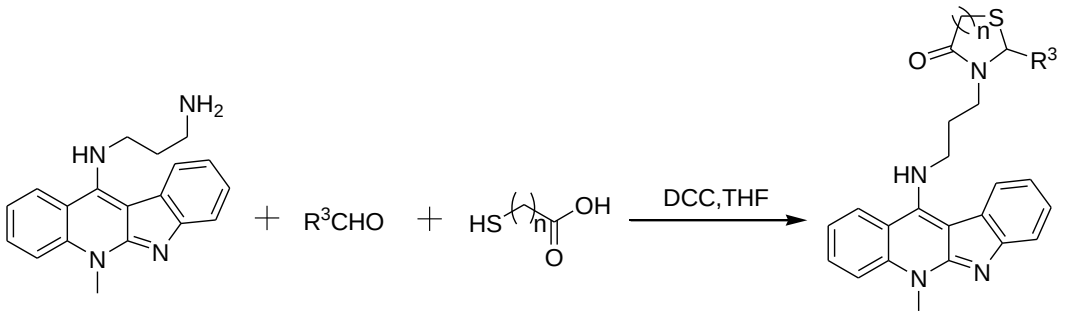
J=2.4 Hz, 1 H); ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 11.38 (2C), 22.37, 23.65, 32.82, 37.14, 46.74 (2C), 52.57, 54.47, 111.65, 113.45, 116.40, 117.71, 118.13, 119.29, 120.83, 123.95, 126.79, 127.17, 132.98, 136.90, 147.18, 153.35, 156.30. HRMS (ESI) calcd for $C_{25}H_{32}BrN_4$ $[M+H]^+$ Exact Mass: 467.1805, found 467.1807.



3-(2-Bromo-5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propan-1-ol (6q):

Yield: 90%, yellow solids. Mp: 238–240 ^{o}C; IR (KBr) 3312, 3048, 2955, 2918, 2754, 2681, 1618, 1589, 1568, 1557, 1485, 1443, 1422, 1343, 1281, 1238, 1219, 1190, 1072, 1045, 924, 799, 760, 737 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.84 (quint, *J*=6.0 Hz, 2 H), 3.43 (t, *J*=6.0 Hz, 2 H), 3.90 (m, 2 H), 4.14 (s, 3 H), 4.58 (br. s., 1 H), 7.08 (t, *J*=4.8 Hz, 1 H), 7.15 (br. s., 1 H), 7.29 (t, *J*=7.2 Hz, 1 H), 7.50 (d, *J*=7.8 Hz, 1 H), 7.79 (d, *J*=9.0 Hz, 1 H), 7.88–7.93 (m, 2 H), 8.74 (d, *J*=1.8 Hz, 1 H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 32.32, 33.51, 45.66, 58.30, 104.90, 112.81, 116.68, 117.31, 117.32, 118.16, 122.36, 124.11, 124.81, 125.93, 132.88, 136.34, 147.02, 152.42, 156.28. HRMS (ESI) calcd for $C_{19}H_{19}BrN_3O$ $[M+H]^+$ Exact Mass: 384.0706, found 384.0718.

General procedure for the synthesis of compounds 9a-d



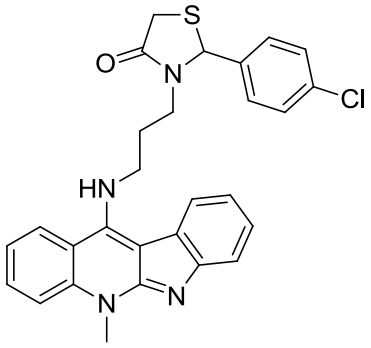
6a **8** **7** **9**

8a: R^3 = 4-ClC_6H_4
b: R^3 = 4-FC_6H_4
c: R^3 = 4-$(CH_3)_2NC_6H_4$

7a: n=1
b: n=2

9a: n=1, R^3 = 4-ClC_6H_4
b: n=1, R^3 = 4-$(CH_3)_2NC_6H_4$
c: n=2, R^3 = 4-ClC_6H_4
d: n=2, R^3 = 4-FC_6H_4

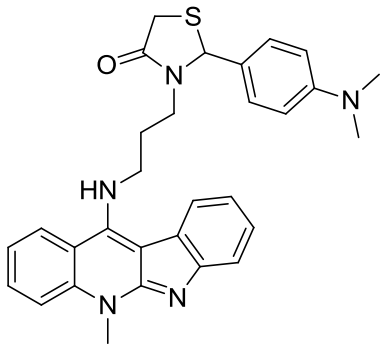
N-(3-Aminopropyl)-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (**6a**, 100 mg) and 2.0 equiv. of benzaldehyde (**8a**-**c**) was stirred in dry THF under ice-cold conditions for 5 min, and then mercaptopropionic acid (**7a**-**b**) (3.0 equiv) was added. After 5 min, DCC (1.2 equiv.) was added to the reaction mixture at 0 ℃ and the reaction mixture was stirred for 1–3 h at room temperature. DCU was removed by filtration and the filtrate was concentrated to dryness under reduced pressure and the residue was taken up in chloroform. The organic layer was successively washed with aq. 5% $NaHCO_3$ and then finally with brine. The organic layer was dried over $MgSO_4$ and evaporated to give a crude product which was purified by flash chromatography using ethyl acetate AcOEt-2N ammonia in MeOH (9:1 v/v) as an eluent to yield pure products as yellowish-orange solids (**9a**-**d**).



2-(4-Chlorophenyl)-3-(3-(5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)thiazolidin-4-one (9a):

Yield: 30.4%, yellowish-orange solids. Mp: 151–154 ^oC; IR (KBr) 3368, 3057, 2930,

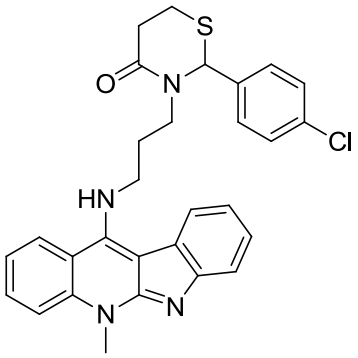
2864, 1668, 1616, 1593, 1489, 1462, 1408, 1316, 1261, 1217, 1088, 1013, 856, 802, 756 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.86 (quint, *J* = 7.2 Hz, 2H), 2.60 (m, 1H), 3.37–3.46 (m, 2H), 3.77 (dd, *J* = 15.6, 1.8 Hz, 1H), 3.82–3.92 (m, 2H), 4.20 (s, 3H), 5.75 (d, *J* = 1.2 Hz, 1H), 7.27 (m, 2H), 7.34 (m, 3H), 7.49 (t, *J* = 7.2 Hz, 1H), 7.64 (m, 2H), 7.98 (t, *J* = 8.4 Hz, 2H), 8.08 (d, *J* = 9.0 Hz, 1H), 8.15 (br. s., 1H), 8.61 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 28.26, 32.97, 36.41, 40.03, 44.55, 63.37, 100.58, 113.49, 115.67, 116.45, 119.81, 121.40, 122.16, 124.17, 125.16, 126.21, 128.82 (2C), 129.45 (2C), 132.56, 135.44, 136.70, 137.22, 137.80, 147.42, 151.77, 172.75. HRMS (ESI) calcd for $C_{28}H_{24}ClN_4OS$ $[M–H]^-$ Exact Mass: 499.1365, found 499.1374.



2-(4-(Dimethylamino)phenyl)-3-(3-(5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino) propyl)thiazolidin-4-one (9b):

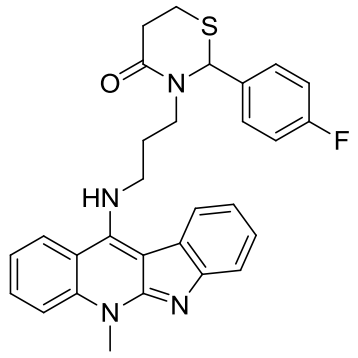
Yield: 65%, yellow solids. Mp: 102–104 ^{o}C; IR (KBr) 3356, 2928, 1661, 1614, 1593, 1564, 1524, 1493, 1441, 1418, 1352, 1281, 1246, 1184, 1167, 1063, 945, 799, 752 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.49 (m, 1H), 1.69 (m, 1H), 2.94 (s, 6H), 3.15 (m, 1H), 3.58 (m, 1H), 3.69 (m, 1H), 3.77–3.94 (m, 3H), 4.28 (s, 3H), 5.56 (s, 1H), 6.63 (d, *J* = 9.0 Hz, 2H), 6.91 (br. s., 1H), 7.19 (m, 3H), 7.38 (t, *J* = 7.2 Hz, 1H), 7.46 (t, *J* = 7.2 Hz, 1H), 7.66 (d, *J* = 9.0 Hz, 1H), 7.74 (t, *J* = 7.8 Hz, 1H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.84 (d, *J* = 7.8 Hz, 1H), 8.41 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 28.36, 33.19, 33.96, 39.94, 40.23 (2C), 43.71, 64.58, 105.16, 112.13 (2C), 114.90, 116.10, 116.43, 119.80, 121.76, 121.96, 122.63, 124.01, 124.48, 125.87, 128.57 (2C), 131.01, 137.38, 147.94, 149.30, 151.15, 154.07, 172.54. HRMS (ESI)

calcd for $C_{30}H_{30}N_5OS$ $[M–H]^-$ Exact Mass: 508.2171, found 508.2197.



2-(4-Chlorophenyl)-3-(3-(5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-1, 3-thiazinan-4-one (9c):

Yield: 42%, yellow solids. Mp: 87–89 ^oC; IR (KBr) 3420, 2934, 1622, 1593, 1568, 1489, 1441, 1420, 1314, 1285, 1246, 1202, 1144, 1092, 1013, 829, 754 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ ppm 1.95 (m, 2H), 2.53–2.65 (m, 5H), 3.81 (m, 1H), 3.85–3.95 (m, 2H), 4.18 (s, 3H), 5.76 (s, 1H), 7.12 (d, *J* = 8.4 Hz, 2H), 7.21 (t, *J* = 7.2 Hz, 1H), 7.33–7.42 (m, 3H), 7.51 (t, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.62 (br. s., 1H), 7.88 (t, *J* = 7.8 Hz, 1H), 7.96 (d, *J* = 7.8 Hz, 2H), 8.56 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 20.83, 28.50, 33.48, 33.82, 44.37, 45.45, 59.97, 114.63, 115.73, 115.93, 119.81, 122.06, 122.38, 122.55, 124.12, 125.22, 128.12 (2C), 128.33 (2C), 129.39, 131.20, 131.53, 132.21, 137.01, 138.88, 149.75, 168.60, 171.47. HRMS (ESI) calcd for $C_{29}H_{26}ClN_4OS$ $[M–H]^-$ Exact Mass: 513.1521, found 513.1535.

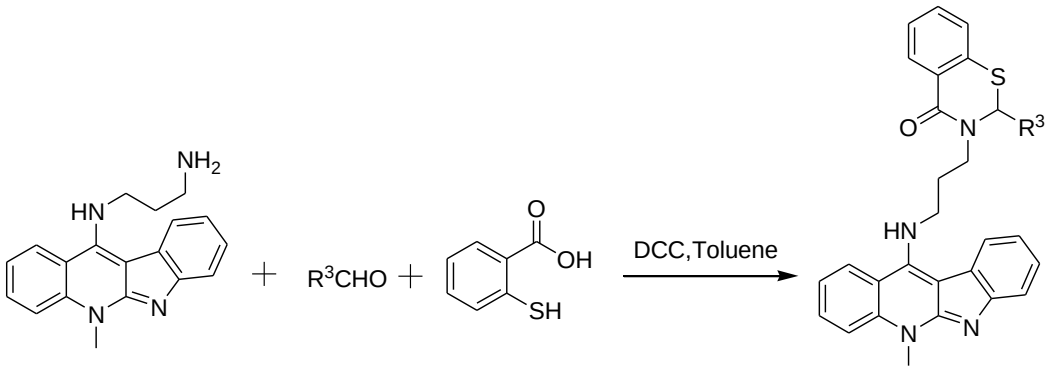


2-(4-Fluorophenyl)-3-(3-(5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-1,

3-thiazinan-4-one (9d):

Yield: 29.3%, yellowish-orange solids. Mp: 92–94 ^{o}C; IR (KBr) 3420, 3063, 2932, 1616, 1593, 1564, 1506, 1464, 1435, 1402, 1323, 1225, 1157, 1103, 1065, 835, 754 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.85 (m, 1H), 1.96 (m, 1H), 2.70 (m, 1H), 2.77–2.84 (m, 2H), 2.91 (m, 2H), 3.62–3.67 (m, 1H), 4.13–4.18 (m, 1H), 4.27 (s, 3H), 4.29–4.35 (m, 1H), 5.54 (s, 1H), 7.05 (t, *J* = 8.4 Hz, 2H), 7.16–7.23 (m, 3H), 7.33 (t, *J* = 7.2 Hz, 1H), 7.42 (br. s., 1H), 7.51 (t, *J* = 7.2 Hz, 1H), 7.64 (d, *J* = 8.4 Hz, 1H), 7.77 (t, *J* = 7.8 Hz, 1H), 7.83 (t, *J* = 9.0 Hz, 2H), 8.48 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 21.62, 28.67, 29.66, 34.22, 34.76, 44.00, 61.11, 103.37, 115.17, 115.75 (d, *J* = 21.9 Hz, 2C), 116.56, 120.68, 121.74, 122.79, 124.31, 125.99, 128.16 (2C), 128.21 (2C), 131.53, 134.25, 134.27, 137.11, 150.23, 161.61, 163.26, 170.82; ^{19}F NMR (564 MHz, $CDCl_3$) δ ppm –113.09. HRMS (ESI) calcd for $C_{29}H_{26}FN_4OS$ $[M–H]^-$ Exact Mass: 497.1817, found 497.1820.

General procedure for the synthesis of compound 11a-d



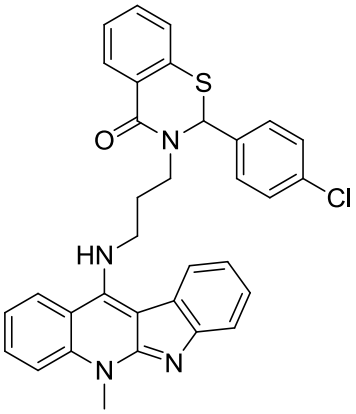
6a **8** **10** **11**

8a: R^3 = 4-ClC_6H_4
8b: R^3 = 4-FC_6H_4
8c: R^3 = 4-$(CH_3)_2NC_6H_4$
8d: R^3 = 3-pyridine

11a: R^3 = 4-ClC_6H_4
11b: R^3 = 4-FC_6H_4
11c: R^3 = 4-$(CH_3)_2NC_6H_4$
11d: R^3 = 3-pyridine

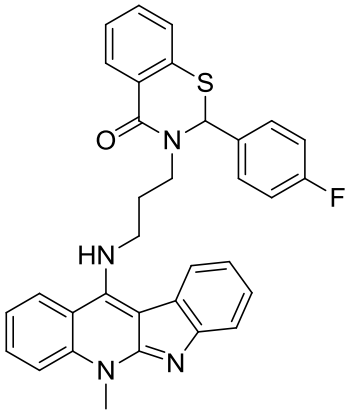
N-(3-Aminopropyl)-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (**6a**, 100 mg) and 2.0 equiv. of benzaldehyde (**8a**) was stirred in dry toluene at 70–80 ℃ for 5min, followed by addition of thiosalicylic acid (**10**) (3.0 equiv) and DCC (1.2 equiv). Then

the reaction mixture was heated to reflux for 10–15 h. The reaction mixture was cooled to room temperature and concentrated to dryness under reduced pressure and the residue was taken up in chloroform and washed with aq. 5% $NaHCO_3$ and then finally with brine. The organic layer was dried over and evaporated to get a crude product that was purified by flash chromatography using ethyl acetate (AcOEt)–2N ammonia in MeOH (9:1 v/v) as the eluent to yield pure products as yellowish-orange solids (**11**).



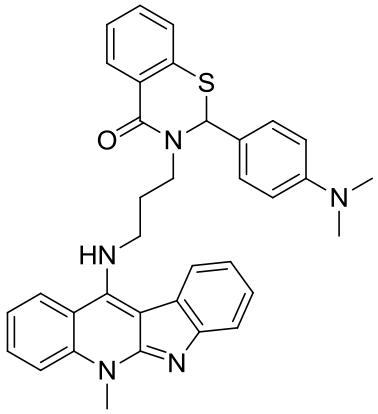
2-(4-Chlorophenyl)-3-(3-(5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-2, 3-dihydrobenzo[e][1,3]thiazin-4-one (11a):

Yield: 49.7%, yellow solids. Mp: 124–126 ^{o}C; IR (KBr) 3349, 3055, 2932, 1622, 1591, 1564, 1489, 1456, 1441, 1422, 1310, 1277, 1246, 1204, 1146, 1092, 1013, 841, 748 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 2.06 (m, 1H), 2.13 (m, 1H), 3.23 (dt, *J* = 15.0, 5.4 Hz, 1H), 3.82 (m, 1H), 4.22 (m, 1H), 4.34 (s, 3H), 4.51 (m, 1H), 5.73 (s, 1H), 7.11 (d, *J* = 7.8 Hz, 1H), 7.15–7.22 (m, 5H), 7.29 (t, *J* = 7.8 Hz, 1H), 7.32–7.36 (m, 2H), 7.58 (t, *J* = 7.2 Hz, 1H), 7.68 (d, *J* = 9.0 Hz, 1H), 7.70 (br. s., 1H), 7.81 (t, *J* = 7.8 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.91 (d, *J* = 7.8 Hz, 1H), 8.16 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.63 (d, *J* = 8.4 Hz, 1 H); ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 29.56, 35.64, 44.08, 45.23, 61.03, 102.48, 114.67, 115.41, 116.73, 120.94, 121.41, 121.77, 123.46, 124.67, 126.25, 126.71, 127.45 (2C), 127.79, 128.49, 128.82 (2C), 129.01, 129.95, 131.99, 132.53, 132.74, 134.51, 136.77, 136.97, 150.09, 151.09, 165.22. HRMS (ESI) calcd for $C_{33}H_{26}ClN_4OS$ $[M–H]^-$ Exact Mass: 561.1516, found 561.1517.



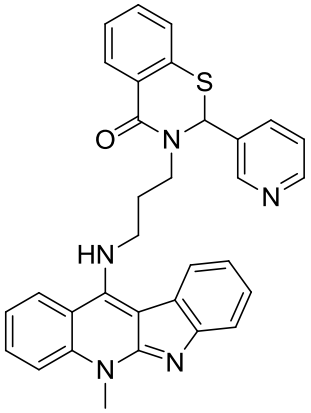
2-(4-Fluorophenyl)-3-(3-(5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-2, 3-dihydrobenzo[e][1,3]thiazin-4-one (11b):

Yield: 69%, yellow solids. Mp: 104–108 ^{o}C; IR (KBr) 3349, 3055, 2934, 1622, 1593, 1564, 1506, 1456, 1441, 1422, 1279, 1244, 1229, 1159, 1098, 845, 746 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 2.13 (m, 1 H), 2.19 (m, 1 H), 3.25 (dt, *J*=14.4, 4.8 Hz, 1 H), 3.85 (m, 1 H), 4.22 (m, 1 H), 4.35 (s, 3 H), 4.50 (m, 1 H), 5.82 (s, 1 H), 6.91 (t, *J*=8.4 Hz, 2 H), 7.10 (d, *J*=7.8 Hz, 1 H), 7.16–7.33 (m, 5 H), 7.35 (t, *J*=7.2 Hz, 1 H), 7.61 (t, *J*=7.8 Hz, 1 H), 7.67 (d, *J*=8.4 Hz, 1 H), 7.82 (t, *J*=7.8 Hz, 2 H), 7.93 (d, *J*=7.8 Hz, 1 H), 8.10 (m, 1 H), 8.14 (d, *J*=8.4 Hz, 1 H), 8.68 (d, *J*=8.4 Hz, 1 H); ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 29.52, 36.51, 44.33, 45.07, 60.98, 100.67, 113.75, 115.63, 115.64 (d, *J*=21.9 Hz, 2C), 116.77, 120.01, 121.72, 122.21, 124.23, 125.02, 126.31, 126.61, 127.78, 127.92 (d, *J*=8.3 Hz, 2C), 128.43, 129.88, 132.50, 132.72, 132.74, 133.92, 136.68, 138.05, 147.76, 151.99, 161.75, 165.25; ^{19}F NMR (564 MHz, $CDCl_3$) δ ppm –112.96. HRMS (ESI) calcd for $C_{33}H_{26}FN_4OS$ $[M–H]^-$ Exact Mass: 545.1817, found 545.1842.



2-(4-(Dimethylamino)phenyl)-3-(3-(5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino) propyl)-2,3-dihydrobenzo[e][1,3]thiazin-4-one (11c):

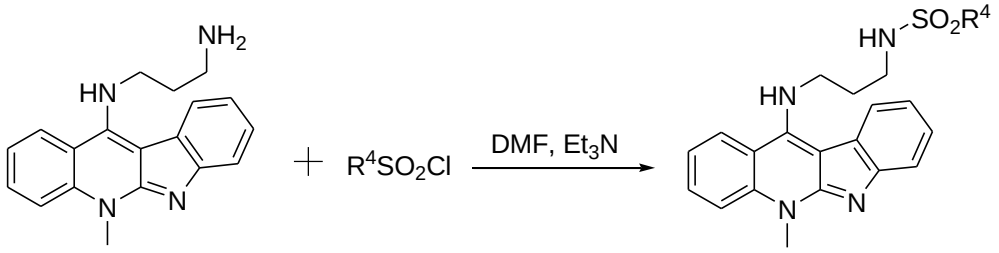
Yield: 81%, yellow solids. Mp: 119–121 ^{o}C; IR (KBr) 3364, 3053, 2934, 1614, 1593, 1564, 1520, 1495, 1456, 1441, 1420, 1360, 1281, 1246, 1188, 1165, 1065, 947, 748 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.82–1.94 (m, 2 H), 2.88 (s, 6 H), 3.32 (m, 1 H), 3.74 (m, 1 H), 4.11 (m, 1 H), 4.28 (s, 3 H), 4.36 (m, 1 H), 5.61 (s, 1 H), 6.54 (d, *J*=8.4 Hz, 2 H), 7.01 (br. s., 1 H), 7.09 (d, *J*=9.0 Hz, 2 H), 7.14 (d, *J*=7.8 Hz, 1 H), 7.20 (t, *J*=7.2 Hz, 1 H), 7.28 (t, *J*=7.2 Hz, 1 H), 7.34 (t, *J*=7.2 Hz, 1 H), 7.41 (t, *J*=7.2 Hz, 1 H), 7.46 (t, *J*=7.2 Hz, 1 H), 7.65 (m, 1 H), 7.73 (m, 1 H), 7.81 (d, *J*=7.8 Hz, 1 H), 7.95 (d, *J*=7.8 Hz, 1 H), 8.25 (dd, *J*=7.8, 1.2 Hz, 1 H), 8.53 (d, *J*=8.4 Hz, 1 H); ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 29.70, 33.42, 40.14 (2C), 43.71, 44.59, 62.10, 106.04, 111.85 (2C), 114.66, 116.48, 116.58, 119.29, 121.49, 121.95, 123.27, 124.04, 124.55, 125.68, 126.14, 127.25 (2C), 127.59, 128.69, 130.00, 130.64, 132.24, 133.82, 137.52, 149.05, 149.89, 150.40, 155.38, 165.34. HRMS (ESI) calcd for $C_{35}H_{32}N_5OS$ $[M–H]^-$ Exact Mass: 570.2333, found 570.2346.



3-(3-(5-Methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-2-(pyridin-3-yl)-2,3-dihydrobenzo[e][1,3]thiazin-4-one (11d):

Yield: 90%, yellow solids. Mp: 96–97 ^{o}C; IR (KBr) 3418, 3053, 2934, 1622, 1591, 1564, 1495, 1456, 1441, 1418, 1283, 1246, 1202, 1153, 1024, 795, 748, 710 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.92–2.02 (m, 2 H), 3.16 (dt, *J*=14.4, 5.4 Hz, 1 H), 3.75 (m, 1 H), 4.17 (m, 1 H), 4.25 (s, 3 H), 4.52 (m, 1 H), 5.63 (s, 1 H), 6.80 (br. s., 1 H), 7.09–7.19 (m, 3 H), 7.29 (t, *J*=7.8 Hz, 1 H), 7.33 (t, *J*=7.8 Hz, 1 H), 7.38 (t, *J*=7.8 Hz, 1 H), 7.46 (t, *J*=7.2 Hz, 1 H), 7.51 (d, *J*=7.8 Hz, 1 H), 7.65 (d, *J*=8.4 Hz, 1 H), 7.73 (t, *J*=7.2 Hz, 1 H), 7.77 (d, *J*=7.8 Hz, 1 H), 7.92 (d, *J*=7.8 Hz, 1 H), 8.20 (d, *J*=8.4 Hz, 1 H), 8.44 (d, *J*=4.2 Hz, 1 H), 8.48 (d, *J*=7.8 Hz, 1 H), 8.51 (d, *J*=2.4 Hz, 1 H); ^{13}C NMR (150.8 MHz, $CDCl_3$) δ ppm 29.66, 33.49, 43.81, 45.51, 59.65, 106.35, 114.78, 116.43, 116.49, 119.49, 121.65, 121.86, 123.07, 123.22, 124.06, 125.91, 126.87, 127.83, 128.66, 130.13, 130.80, 132.06, 132.69, 133.47, 134.25, 137.52, 147.49 (2C), 148.96, 149.67, 155.09, 164.85. HRMS (ESI) calcd for $C_{32}H_{26}N_5OS$ $[M–H]^-$ Exact Mass: 528.1864, found 528.1870.

General procedure for the synthesis of compounds 13a-c

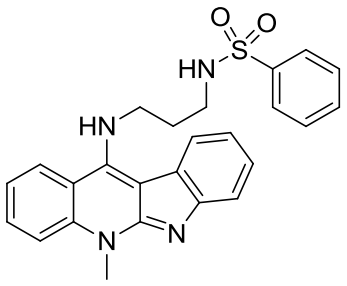


6a **12** **13**

12a: $R^4 = C_6H_5$
12b: R^4 = 2-naphthyl
12c: R^4 = Me

13a: $R^4 = C_6H_5$
13b: R^4 = 2-naphthyl
13c: R^4 = Me

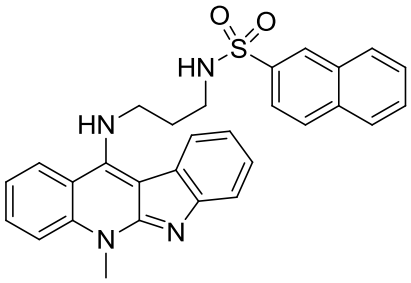
N-(3-Aminopropyl)-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (**6a**, 50mg) was completely dissolved in dry DMF (2 mL), and then a mixture of arenesulfonyl chloride (**12a**-**c**) (1.2 equiv.) and dry DMF (1 mL) were added drop by drop with stirring, and finally 2.0 equiv of triethylamine was added, the reaction was carried out at room temperature for 2–4 h. TLC monitoring was used to ensure the completion of reaction. The crude product was purified by flash chromatography using AcOEt-2N ammonia in MeOH (9:1 v/v) as the eluent to yield pure products as yellowish-orange solids.



N-(3-(5-Methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)benzenesulfonamide (13a):

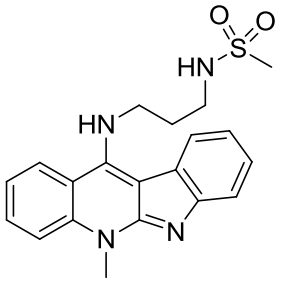
Yield: 68%, yellow solids. Mp: 189–192 ^{o}C; IR (KBr) 3389, 3055, 2932, 1626, 1568, 1489, 1445, 1420, 1308, 1281, 1246, 1153, 1092, 878, 860, 743, 691 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.79 (quin, *J* = 7.2 Hz, 2H), 2.70 (q, *J* = 6.6 Hz, 2H), 3.80 (q, *J* = 6.6 Hz, 2H), 4.16 (s, 3H), 6.88 (t, *J* = 5.4 Hz, 1H), 7.06 (t, *J* = 6.6 Hz, 1H), 7.29 (t, *J* = 7.8 Hz, 1H), 7.40 (t, *J* = 6.6 Hz, 1H), 7.45 (t, *J* = 7.8 Hz, 2H), 7.49 (d, *J* = 7.2 Hz, 1H), 7.55 (t, *J* = 7.8 Hz, 1H), 7.60 (m, 3H), 7.80 (t, *J* = 7.2 Hz, 1H), 7.85 (d, *J*

= 9.0 Hz, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 8.45 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 30.80, 32.24, 40.10, 45.42, 105.16, 115.04, 115.07, 115.62, 116.48, 118.04, 120.59, 122.07, 123.97, 124.73, 126.28 (2C), 129.06 (2C), 130.64, 132.25, 137.34, 140.12, 148.08, 152.20, 156.28. HRMS (ESI) calcd for $C_{25}H_{25}N_4O_2S$ $[M+H]^+$ Exact Mass: 445.1693, found 445.1718.



N-(3-(5-Methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)naphthalene-2-sulfon amide (13b):

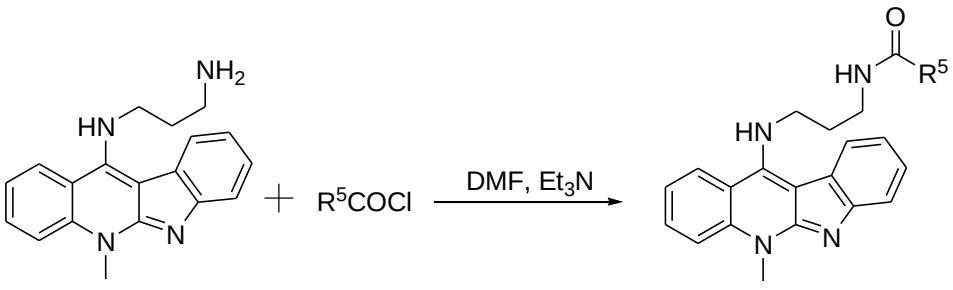
Yield: 76%, yellow solids. Mp: 198 ^{o}C; IR (KBr) 3393, 3055, 2924, 2874, 2359, 1738, 1626, 1574, 1489, 1443, 1424, 1314, 1283, 1242, 1144, 1103, 1080, 878, 862, 814, 745 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.81 (quin, *J* = 7.2 Hz, 2H), 2.75 (q, *J* = 6.0 Hz, 2H), 3.80 (q, *J* = 7.2 Hz, 2H), 4.13 (s, 3H), 6.86 (t, *J* = 6.0 Hz, 1H), 7.03 (t, *J* = 7.8 Hz, 1H), 7.27 (t, *J* = 7.8 Hz, 1H), 7.33 (t, *J* = 7.8 Hz, 1H), 7.48 (d, *J* = 7.8 Hz, 1H), 7.62–7.71 (m, 4H), 7.75 (t, *J* = 7.2 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 7.86 (d, *J* = 7.2 Hz, 1H), 7.99 (d, *J* = 8.4 Hz, 2H), 8.05 (d, *J* = 8.4 Hz, 1H), 8.29 (s, 1H), 8.41 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 30.84, 32.19, 40.16, 45.44, 105.21, 114.98, 115.57, 116.52, 117.98, 120.48, 122.04, 122.06, 123.92, 123.98, 124.70, 127.25, 127.50, 127.79, 128.62, 129.10, 129.28, 130.58, 131.62, 134.04, 137.15, 137.31, 148.02, 152.35, 156.34. HRMS (ESI) calcd for $C_{29}H_{27}N_4O_2S$ $[M+H]^+$ Exact Mass: 495.1849, found 495.1878.



N-(3-(5-Methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)methanesulfonamide (13c):

Yield: 76%, yellow solids. Mp: 191–195 ^{o}C; IR (KBr) 3439, 3393, 2926, 2361, 2342, 1624, 1570, 1489, 1420, 1325, 1310, 1148, 781, 745 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.88 (quint, *J*=7.2 Hz, 2 H), 2.74 (s, 3 H), 2.92 (q, *J*=6.0 Hz, 2 H), 3.87 (q, *J*=6.6 Hz, 2 H), 4.16 (s, 3 H), 6.94 (t, *J*=6.0 Hz, 1 H), 6.98 (t, *J*=6.0 Hz, 1 H), 7.07 (t, *J*=7.2 Hz, 1 H), 7.29 (t, *J*=7.2 Hz,1 H), 7.41 (t, *J*=7.2 Hz, 1 H), 7.49 (d, *J*=7.8 Hz, 1 H), 7.79 (t, *J*=6.6 Hz, 1 H), 7.84 (d, *J*=7.8 Hz,1 H), 7.93 (d, *J*=7.8 Hz, 1 H), 8.51 (d, *J*=8.4 Hz, 1 H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 31.06, 32.21, 38.96, 39.99, 45.55, 105.06, 115.05, 115.62, 116.54, 118.00, 120.57, 122.11, 123.99, 124.06, 124.70, 130.65, 137.37, 148.12, 152.35, 156.40. HRMS (ESI) calcd for $C_{20}H_{23}N_4O_2S$ $[M+H]^+$ Exact Mass: 383.1536, found 383.1555.

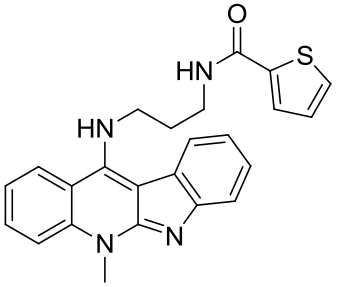
General procedure for the synthesis of compound 15



6a **14,** R^5 = 2-thiophenyl **15,** R^5 = 2-thiophenyl

N-(3-aminopropyl)-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (**6a,** 50mg) was completely dissolved in dry DMF (2 mL), and then a mixture solution of acyl chloride (**14**) (1.2 equiv.) and dry DMF (1 mL) were added drop by drop under stirring, and

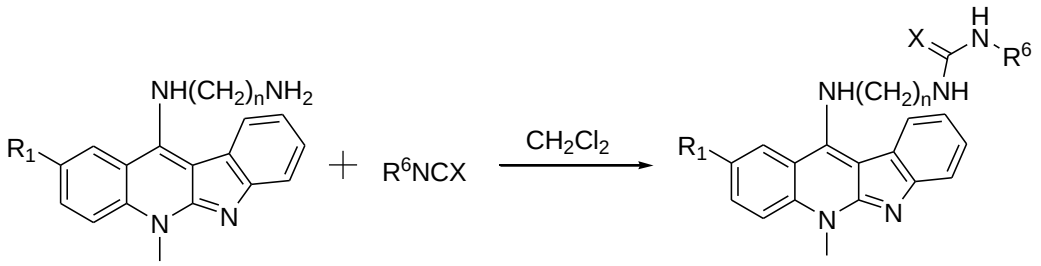
finally 2.0 equiv of triethylamine was added, the reaction was carried out at room temperature for 2–4 h. TLC monitoring was used to ensure the completion of reaction. The crude product was purified by flash chromatography using AcOEt-2N ammonia in MeOH (9:1 v/v) as an eluent to yield pure products as yellowish-orange solids.



N-(3-(5-Methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)thiophene-2-carboxamide (15):

Yield: 95%, yellow solids. Mp: 204–205 ^{o}C; IR (KBr) 3424, 3333, 3059, 2930, 1732, 1622, 1593, 1566, 1545, 1499, 1439, 1418, 1358, 1294, 1248, 1204, 1144, 1071, 862, 745, 719 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.92 (quint, J = 7.2 Hz, 2H), 3.26 (q, J = 6.6 Hz, 2H), 3.88 (q, J = 7.2 Hz, 2H), 4.16 (s, 3H), 7.02–7.07 (m, 2H), 7.10 (dd, J = 4.8, 2.4 Hz, 1H), 7.27 (t, J = 7.2 Hz, 1H), 7.41 (t, J = 7.2 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.64 (dd, J = 3.6, 1.2 Hz, 1H), 7.72 (dd, J = 5.4, 1.2 Hz, 1H), 7.79 (td, J = 7.2 1.2 Hz, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 7.2 Hz, 1H), 8.52 (d, J = 6.6 Hz, 2H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 30.92, 32.22, 36.69, 45.39, 105.08, 115.06, 115.65, 116.55, 118.00, 120.58, 122.08, 123.97, 124.02, 124.68, 127.81, 127.93, 130.64 (2C), 137.39, 139.84, 148.14, 152.38, 156.40, 161.33. HRMS (ESI) calcd for $C_{24}H_{23}N_4OS$ $[M+H]^+$ Exact Mass: 415.1587, found 415.1597.

General procedure for the synthesis of compounds 17a-j

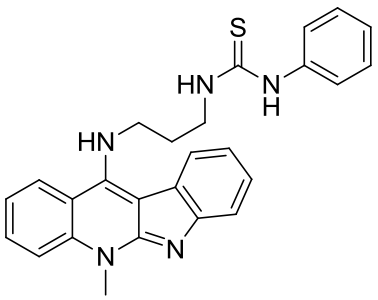


6a-h **16** **17**

16a: $R^6 = C_6H_5$, X = S
b: $R^6 = C_6H_5$, X = O
c: $R^6 = CH_3(CH_2)_3$, X = O

17a: from **6a**, $R^6 = C_6H_5$, X = S
17b: from **6a**, $R^6 = CH_3(CH_2)_3$, X = O
17c: from **6a**, $R^6 = C_6H_5$, X = O
17d: from **6b**, $R^6 = C_6H_5$, X = O
17e: from **6c**, $R^6 = C_6H_5$, X = O
17f: from **6d**, $R^6 = C_6H_5$, X = O
17g: from **6e**, $R^6 = C_6H_5$, X = O
17h: from **6f**, $R^6 = C_6H_5$, X = O
17i: from **6g**, $R^6 = C_6H_5$, X = O
17j: from **6h**, $R^6 = C_6H_5$, X = O

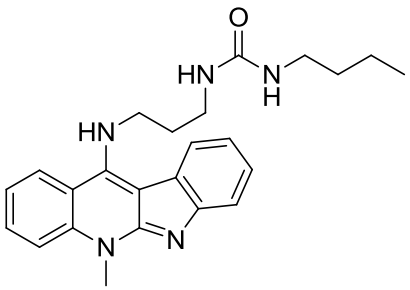
2-Substituted 5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine (**6a-h,** 50 mg) was completely dissolved in dry CH_2Cl_2 (1 mL), and then a solution of isocyanate (**16a-c**) (1.1 equiv.) and dry CH_2Cl_2 (1 mL) were added drop by drop under stirring at room temperature for 2–4 h. TLC monitoring was used to ensure the completion of reaction. After reaction was finished, the reaction mixture was evaporated to dryness under reduced pressure. The crude product was purified by flash chromatography using AcOEt-2N ammonia in MeOH (9:1 v/v) as an eluent to yield pure products as yellowish-orange solids.



1-(3-(5-Methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-3-phenylthiourea (17a):

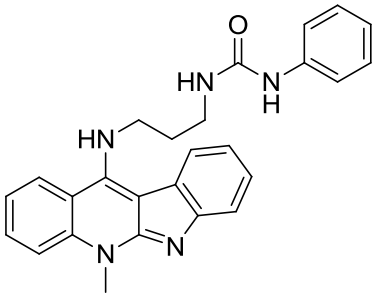
Yield: 99%, yellow solids. Mp: 201–203 ^{o}C; IR (KBr) 3335, 3208, 3055, 2938, 1622,

1595, 1568, 1539, 1512, 1485, 1441, 1422, 1406, 1310, 1277, 1242, 1190, 1142, 1103, 1072, 955, 858, 748, 733 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.98 (quint, *J* = 6.6 Hz, 2H), 3.51 (m, 2H), 3.89 (q, *J* = 6.6 Hz, 2H), 4.16 (s, 3H), 7.06–7.11 (m, 3H), 7.24 (d, *J* = 4.2 Hz, 4H), 7.30 (t, *J* = 7.2 Hz, 1H), 7.43 (t, *J* = 7.8 Hz, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.73 (br. s., 1H), 7.80 (t, *J* = 7.2 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.96 (d, *J* = 7.8 Hz, 1H), 8.55 (d, *J* = 8.4 Hz, 1H), 9.46 (br. s., 1H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 30.36, 32.33, 41.39, 45.60, 104.58, 115.09, 115.68, 116.35, 118.22, 120.69, 122.17 (2C), 123.16, 123.93, 124.04 (2C), 124.21, 124.69, 128.66, 130.73, 137.33, 138.87, 148.28, 151.74, 156.11, 180.24. HRMS (ESI) calcd for $C_{26}H_{24}N_5S$ $[M–H]^-$ Exact Mass: 438.1758, found 438.1751.



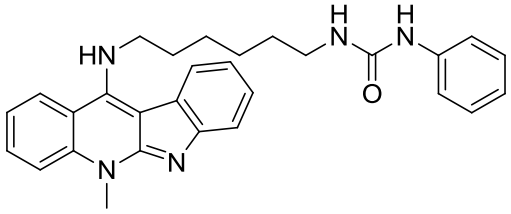
1-Butyl-3-(3-(5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)urea (17b):

Yield: 86%, yellow solids. Mp: 80–81 ^{o}C; IR (KBr) 3360, 3312, 2953, 2920, 2870, 1622, 1593, 1568, 1520, 1489, 1443, 1416, 1400, 1288, 1250, 1196, 1065, 1022, 841, 750 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 0.86 (t, *J* = 7.8 Hz, 3H), 1.31 (sext, *J* = 7.8 Hz, 2H), 1.45 (quint, *J* = 7.2 Hz, 2H), 1.68 (quint, *J* = 6.0 Hz, 2H), 3.19 (q, *J* = 6.6 Hz, 2H), 3.36 (q, *J* = 6.6 Hz, 2H), 3.85 (q, *J* = 6.0 Hz, 2H), 4.10 (s, 3H), 5.49 (br. s., 1H), 5.79 (br. s., 1H), 6.92 (m, 1H), 7.13 (t, *J*=7.2 Hz, 1H), 7.31–7.38 (m, 2H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.63–7.67 (m, 2H), 7.82 (d, *J* = 7.8 Hz, 1H), 8.39 (dd, *J* = 9.0, 1.2 Hz, 1H); ^{13}C NMR (150.8MHz, $CDCl_3$) δ ppm 13.77, 20.04, 32.33, 32.38, 33.07, 36.60, 40.19, 44.25, 105.39, 114.51, 116.07, 116.41, 119.25, 121.44, 121.83, 123.48, 124.28, 125.44, 130.64, 137.45, 149.30, 152.45, 155.40, 159.77. HRMS (ESI) calcd for $C_{24}H_{28}N_5O$ $[M–H]^-$ Exact Mass: 402.2299, found 402.2319.



1-(3-(5-Methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-3-phenylurea (17c):

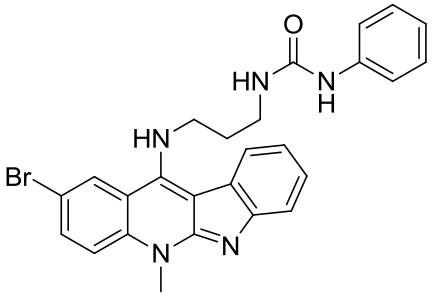
Yield: 99%, yellow solids. Mp: 215 ^{o}C; IR (KBr) 3341, 3048, 3024, 2969, 2930, 1694, 1620, 1591, 1557, 1489, 1443, 1406, 1314, 1275, 1227, 1177, 1144, 891, 758, 718, 692 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.86 (quint, *J* = 6.6 Hz, 2H), 3.14 (q, *J* = 6.6 Hz, 2H), 3.86 (q, *J* = 6.6 Hz, 2H), 4.16 (s, 3H), 6.18 (t, *J* = 6.0 Hz, 1H), 6.87 (t, *J* = 7.2 Hz, 1H), 7.06 (m, 2H), 7.20 (t, *J* = 7.8 Hz, 2H), 7.27 (t, *J* = 7.2 Hz, 1H), 7.35 (d, *J* = 7.8 Hz, 2H), 7.42 (t, *J* = 7.2 Hz, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.79 (t, *J* = 7.2 Hz, 1H), 7.85 (d, *J* = 8.4 Hz, 1H), 7.96 (d, *J* = 7.8 Hz, 1H), 8.42 (s, 1H), 8.54 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 31.62, 32.20, 36.48, 45.43, 105.02, 115.06, 115.62, 116.55, 117.72 (2C), 117.98, 120.55, 121.03, 122.05, 124.00, 124.07, 124.65, 128.59 (2C), 130.63, 137.40, 140.40, 148.23, 152.38, 155.52, 156.40. HRMS (ESI) calcd for $C_{26}H_{24}N_5O$ $[M–H]^-$ Exact Mass: 422.1986, found 422.2003.



1-(6-(5-Methyl-5H-indolo[2,3-b]quinolin-11-ylamino)hexyl)-3-phenylurea (17d):

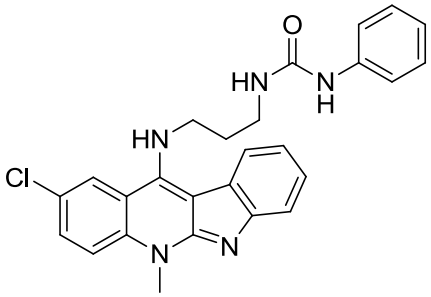
Yield: 89%, yellow solids. Mp: 101–104 ^{o}C; IR (KBr) 3356, 3053, 2930, 2855, 1668, 1622, 1595, 1559, 1499, 1441, 1420, 1312, 1279, 1244, 1071, 750, 694 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.19–1.25 (m, 4H), 1.31 (quint, *J* = 6.6 Hz, 2H), 1.68 (quint, *J* = 6.6 Hz, 2H), 2.95 (q, *J* = 6.0 Hz, 2H), 3.83 (q, *J* = 6.6 Hz, 2H), 4.16 (s, 3H), 6.03 (t, *J* = 6.0 Hz, 1H), 6.86 (t, *J* = 7.2 Hz, 1H), 7.01 (t, *J* = 5.4 Hz, 1H), 7.08 (t,

J = 7.2 Hz, 1H), 7.19 (t, *J* = 7.2 Hz, 2H), 7.28 (t, *J* = 7.2 Hz, 1H), 7.34 (d, *J* = 7.2 Hz, 2H), 7.42 (t, *J* = 7.8 Hz, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.78 (t, *J* = 7.8 Hz, 1H), 7.84 (d, *J* = 7.8 Hz, 1H), 7.91 (d, *J* = 7.8 Hz, 1H), 8.34 (s, 1H), 8.53 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 25.90, 26.03, 29.60, 30.75, 32.22, 38.82, 48.00, 104.48, 115.06, 115.61, 116.50, 117.52 (2C), 117.96, 120.59, 120.86, 121.98, 123.98, 124.09, 124.59, 128.61 (2C), 130.66, 137.37, 140.58, 148.35, 152.10, 155.15, 156.34. HRMS (ESI) calcd for $C_{29}H_{30}N_5O$ $[M–H]^-$ Exact Mass: 464.2456, found 464.2480.



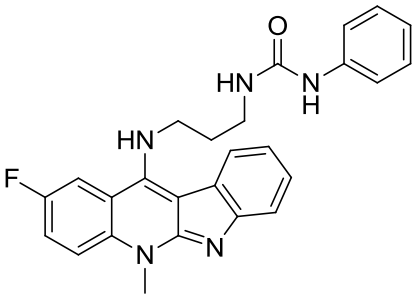
1-(3-(2-Bromo-5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-3-phenylurea (17e):

Yield: 80%, yellow solids. Mp: 132–135 ^{o}C; IR (KBr) 3347, 3050, 2938, 1680, 1616, 1591, 1557, 1499, 1487, 1443, 1424, 1314, 1281, 1246, 1200, 1111, 1086, 885, 799, 760, 741, 694 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.88 (quint, *J* = 6.6 Hz, 2H), 3.14 (q, *J* = 6.0 Hz, 2H), 3.84 (q, *J* = 6.6 Hz, 2H), 4.13 (s, 3H), 6.18 (t, *J* = 6.0 Hz, 1H), 6.87 (t, *J* = 7.2 Hz, 1H), 7.07 (t, *J* = 7.2 Hz, 1H), 7.19 (t, *J* = 7.2 Hz, 3H), 7.28 (t, *J* = 7.2 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.80 (m, 1H), 7.90 (d, *J* = 7.2 Hz, 2H), 8.41 (s, 1H), 8.78 (d, *J* = 1.8 Hz, 1H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 31.46, 32.37, 36.50, 45.38, 105.23, 112.92, 116.74, 117.35, 117.38, 117.70 (2C), 118.28, 121.01, 122.37, 124.02, 124.94, 125.92, 128.59 (2C), 132.94, 136.32, 140.40, 146.99, 152.49, 155.48, 156.32. HRMS (ESI) calcd for $C_{26}H_{23}BrN_5O$ $[M–H]^-$ Exact Mass: 500.1091, found 500.1087.



1-(3-(2-Chloro-5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-3-phenylurea (17f):

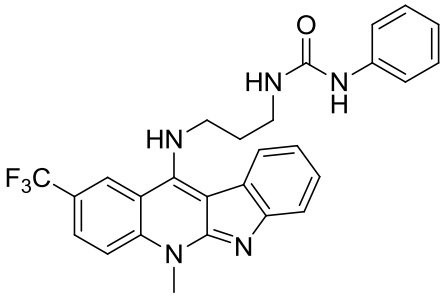
Yield: 83%, yellow solids. Mp: 130–133 ^{o}C; IR (KBr) 3347, 3051, 2938, 1680, 1622, 1595, 1557, 1499, 1443, 1422, 1314, 1281, 1246, 1200, 1119, 991, 800, 760, 743, 694 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.89 (quint, J = 6.6 Hz, 2H), 3.14 (q, J = 6.0 Hz, 2H), 3.84 (q, J = 6.6 Hz, 2H), 4.14 (s, 3H), 6.17 (t, J = 6.0 Hz, 1H), 6.87 (t, J = 7.2 Hz, 1H), 7.07 (t, J = 7.2 Hz, 1H), 7.17 (m, 3H), 7.29 (t, J = 7.2 Hz, 1H), 7.34 (d, J = 7.8 Hz, 2H), 7.50 (d, J = 7.8 Hz, 1H), 7.80 (dd, J = 9.0, 2.4 Hz, 1H), 7.86 (d, J = 9.6 Hz, 1H), 7.91 (d, J = 7.2 Hz, 1H), 8.40 (s, 1H), 8.67 (d, J = 2.4 Hz, 1H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 31.45, 32.41, 36.49, 45.41, 105.32, 116.70, 116.89, 117.10, 117.69 (2C), 118.27, 121.00, 122.37, 123.02, 123.96, 124.95, 125.14, 128.57 (2C), 130.25, 136.01, 140.39, 147.06, 152.45, 155.46, 156.32. HRMS (ESI) calcd for $C_{26}H_{25}ClN_5O$ $[M+H]^+$ Exact Mass: 458.1742, found 458.1747.



1-(3-(2-Fluoro-5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-3-phenylurea (17g):

Yield: 87%, yellow solids. Mp: 218–220 ^{o}C; IR (KBr) 3339, 3053, 2926, 1690, 1613, 1599, 1566, 1499, 1487, 1445, 1400, 1317, 1281, 1234, 1144, 878, 795, 760, 692 cm^{-1};

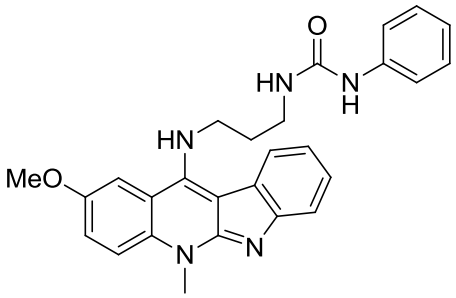
^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.84 (quint, J = 6.6 Hz, 2H), 3.11 (q, J = 6.0 Hz, 2H), 3.82 (q, J = 6.6 Hz, 2H), 4.14 (s, 3H), 6.13 (t, J = 6.0 Hz, 1H), 6.84 (t, J = 7.2 Hz, 1H), 6.99 (t, J = 6.0 Hz, 1H), 7.04 (t, J = 7.2 Hz, 1H), 7.16 (t, J = 7.8 Hz, 2H), 7.26 (t, J = 7.2 Hz, 1H), 7.31 (dd, J = 8.4, 1.2 Hz, 2H), 7.47 (d, J = 7.8 Hz, 1H), 7.67 (m, 1H), 7.86 (dd, J = 9.6, 4.8 Hz, 1H), 7.89 (d, J = 7.8 Hz, 1H), 8.37 (s, 1H), 8.42 (dd, J = 10.8, 2.4 Hz, 1H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 31.49, 32.50, 36.49, 45.42, 105.66, 109.04 (d, J = 24.1 Hz), 116.44 (d, J = 7.8 Hz), 116.55, 117.16 (d, J = 8.4 Hz), 117.69 (2C), 118.08, 118.53 (d, J = 23.5 Hz), 121.00, 122.36, 123.73, 124.99, 128.57 (2C), 134.15, 140.39, 147.27 (d, J = 2.9 Hz), 152.65, 155.44, 156.45, 156.65 (d, J = 237.2 Hz); ^{19}F NMR (564 MHz, DMSO-d_6) δ ppm –121.25. HRMS (ESI) calcd for $C_{26}H_{23}FN_5O$ $[M–H]^-$ Exact Mass: 440.1892, found 440.1890.



1-(3-(5-Methyl-2-(trifluoromethyl)-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-3-phenylurea (17h):

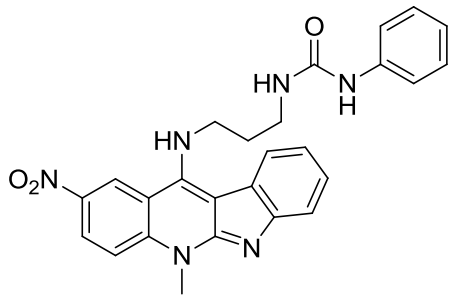
Yield: 77%, yellow solids. Mp: 204–205 ^{o}C; IR (KBr) 3335, 3055, 2938, 1688, 1614, 1597, 1553, 1499, 1443, 1435, 1333, 1317, 1277, 1242, 1196, 1148, 1119, 1090, 912, 816, 766, 752, 725 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.90 (quint, J = 6.6 Hz, 2H), 3.15 (q, J = 6.0 Hz, 2H), 3.88 (q, J = 6.6 Hz, 2H), 4.19 (s, 3 H), 6.17 (t, J = 6.0 Hz, 1H), 6.87 (t, J = 7.2 Hz, 1H), 7.10 (t, J = 7.2 Hz, 1H), 7.19 (t, J = 7.8 Hz, 2H), 7.29–7.34 (m, 3H), 7.46 (t, J = 6.0 Hz, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 7.8 Hz, 1H), 8.00 (d, J = 9.0 Hz, 1H), 8.05 (dd, J = 9.0, 1.2 Hz, 1H), 8.39 (s, 1H), 8.94 (s, 1H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 31.47, 32.55, 36.45, 45.34, 105.04, 115.37, 116.16, 116.97, 117.70 (2C), 118.66, 120.95, 121.03, 121.73, 121.75, 123.22

(q, *J* = 244.6 Hz), 123.80, 124.98, 126.38, 128.57 (2C), 139.33, 140.39, 147.61, 152.25, 155.52, 156.46; ^{19}F NMR (564 MHz, DMSO-d_6) δ ppm –58.57. HRMS (ESI) calcd for $C_{27}H_{23}F_3N_5O$ $[M–H]^-$ Exact Mass: 490.1860, found 490.1889.



1-(3-(2-Methoxy-5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-3-phenylurea (17i):

Yield: 98%, yellow solids. Mp: 122–124 ^{o}C; IR (KBr) 3383, 3050, 2936, 1682, 1614, 1597, 1568, 1532, 1499, 1445, 1314, 1288, 1246, 1142, 1036, 804, 758, 694 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.84 (quint, *J* = 6.6 Hz, 2H), 3.15 (q, *J* = 6.6 Hz, 2H), 3.85 (q, *J* = 6.6 Hz, 2H), 3.94 (s, 3H), 4.15 (s, 3H), 6.19 (t, *J* = 6.0 Hz, 1H), 6.88 (t, *J* = 7.2 Hz, 1H), 7.02–7.06 (m, 2H), 7.20 (t, *J* = 7.8 Hz, 2H), 7.27 (t, *J* = 7.2 Hz, 1H), 7.35 (d, *J* = 7.2 Hz, 2H), 7.45–7.49 (m, 2H), 7.81 (d, *J* = 9.6 Hz, 1H), 7.93 (d, *J* = 7.8 Hz, 1H), 8.00 (d, *J* = 2.4 Hz, 1H), 8.43 (s, 1H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 31.74, 32.31, 36.45, 45.19, 55.87, 105.69, 105.86, 116.32, 116.35, 116.50, 117.65, 117.76 (2C), 119.65, 121.06, 122.19, 123.81, 124.75, 128.59 (2C), 132.20, 140.38, 147.72, 152.65, 153.66, 155.60, 156.21. HRMS (ESI) calcd for $C_{27}H_{26}N_5O_2$ $[M–H]^-$ Exact Mass: 452.2092, found 452.2102.



1-(3-(5-Methyl-2-nitro-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-3-phenylurea (17j):

Yield: 92%, orange solids. Mp: 222–225 ^{o}C; IR (KBr) 3360, 2930, 1649, 1616, 1568, 1501, 1439, 1424, 1323, 1290, 1240, 1121, 1071, 814, 754, 739, 694 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.92 (quint, *J* = 6.6 Hz, 2H), 3.15 (q, *J* = 6.0 Hz, 2H), 3.90 (q, *J* = 6.6 Hz, 2H), 4.17 (s, 3H), 6.15 (t, *J* = 6.0 Hz, 1H), 6.87 (t, *J* = 7.2 Hz, 1H), 7.12 (t, *J* = 7.2 Hz, 1H), 7.18 (t, *J* = 7.8 Hz, 2H), 7.30 (m, 3H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.65 (t, *J* = 6.0 Hz, 1H), 7.94–7.97 (m, 2H), 8.36 (s, 1H), 8.50 (dd, *J* = 9.6, 2.4 Hz, 1H), 9.50 (d, *J*=2.4 Hz, 1H); ^{13}C NMR (150.8 MHz, DMSO-d_6) δ ppm 31.31, 32.87, 36.48, 45.54, 104.83, 115.20, 116.10, 117.28, 117.66 (2C), 119.15, 121.00, 121.02, 122.41, 124.12, 124.55, 125.07, 128.56 (2C), 140.28, 140.37, 141.00, 147.68, 152.05, 155.42, 156.24. HRMS (ESI) calcd for $C_{26}H_{23}N_6O_3$ $[M–H]^-$ Exact Mass: 467.1837, found 467.1835.

4.6.2 Biological testing assay

Activity against *Plasmodium falciparum*

In vitro activity against erythrocytic stages of *P. falciparum* was determined using a ^{3}H-hypoxanthine incorporation assay[40-41], using the chloroquine and pyrimethamine resistant *P. falciparum* K1 strain that originate from Thailand (Thaitong et al. 1983)[42] and strain susceptible to known antimalarial drugs (*P. falciparum* NF54) (Ponnudurai et al. 1981),[43] and all the test compounds were compared for activity with the standard drug chloroquine (Sigma C6628). Compounds were dissolved in DMSO at 10 mg/mL and added to parasite cultures incubated in RPMI 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g/l), $NaHCO_3$ (2.1 g/l), neomycin (100 U/mL), Albumax^{R} (5 g/l) and washed human red cells A^+ at 2.5% haematocrit (0.3% parasitaemia). Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared. The 96-well plates were incubated in a

humidified atmosphere at 37 °C; 4% CO_2, 3% O_2, 93% N_2. After 48 h 50 µl of ^{3}H-hypoxanthine (=0.5 µCi) was added to each well of the plate. The plates were incubated for a further 24 h under the same conditions. The plates were then harvested with a Betaplate™ cell harvester (Wallac, Zurich, Switzerland), and the red blood cells transferred onto a glass fibre filter then washed with distilled water. The dried filters were inserted into a plastic foil with 10 mL of scintillation fluid, and counted in a Betaplate™ liquid scintillation counter (Wallac, Zurich, Switzerland). IC_{50} values were calculated from sigmoidal inhibition curves by linear regression (Huber 1993)[44] using Microsoft Excel.

Activity against *Trypanosoma brucei rhodesiense*

Trypanosoma brucei rhodesiense, STIB900 strain. And the standard drugs, melarsoprol, were used for the assay. This stock was isolated in 1982 from a human patient in Tanzania and after several mouse passages cloned and adapted to axenic culture conditions (Baltz et al 1985)[45] Minimum Essential Medium (50 µl) supplemented with 25 mM HEPES, 1g/l additional glucose, 1% MEM non-essential amino acids (100x), 0.2 mM 2-mercaptoethanol, 1mM Na-pyruvate and 15% heat inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 µg/mL were prepared. Then 4×103 bloodstream forms of T. b. rhodesiense STIB 900 in 50 µl was added to each well and the plate incubated at 37 °C under a 5% CO2 atmosphere for 70 h. 10 µl Alamar Blue (resazurin, 12.5 mg in 100 mL double-distilled water) was then added to each well and incubation continued for a further 2–4 h (Raz et al 1997).[46] Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave length of 588 nm. The IC_{50} values were calculated by linear regression (Huber 1993)[44] from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices

Cooperation, Sunnyvale, CA, USA).

Activity against *Trypanosoma cruzi*

Rat skeletal myoblasts (L6 cells) were seeded in 96-well microtitre plates at 2000 cells/well in 100 µL RPMI 1640 medium with 10% FBS and 2 mM l-glutamine. After 24 h the medium was removed and replaced by 100 µL per well containing 5000 trypomastigote forms of *T. cruzi* Tulahuen strain C2C4 containing the β-galactosidase (Lac Z) gene (Buckner et al. 1996).[47] After 48 h the medium was removed from the wells and replaced by 100 µl fresh medium with or without a serial drug dilution of eleven 3-fold dilution steps covering a range from 100 to 0.002 µg/mL. After 96 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterility. Then the substrate CPRG/Nonidet (50 µl) was added to all wells. A color reaction developed within 2–6 h and could be read photometrically at 540 nm. Data were analyzed with the graphic programme Softmax Pro (Molecular Devices), which calculated IC_{50} values by linear regression (Huber 1993)[44] from the sigmoidal dose inhibition curves.

Activity against *Leishmania donovani (axenic amastigotes)*

Amastigotes of *L. donovani* strain MHOM/ET/67/L82 were grown in axenic culture at 37 °C in SM medium (Cunnigham et al. 1977)[48] at pH 5.4 supplemented with 10% heat-inactivated fetal bovine serum under an atmosphere of 5% CO_2 in air. One hundred microlitres of culture medium with 10^5 amastigotes from axenic culture with or without a serial drug dilution were seeded in 96-well microtitre plates. Serial drug dilutions of eleven 3-fold dilution steps covering a range from 90 to 0.002 µg/mL were prepared. After 70 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. 10 µl of Alamar Blue (12.5 mg resazurin dissolved in 100 mL distilled water) (Mikus and Steverdig.

2000)[49] were then added to each well and the plates incubated for another 2 h. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave length of 588 nm. Data were analyzed using the software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA). Decrease of fluorescence (= inhibition) was expressed as percentage of the fluorescence of control cultures and plotted against the drug concentrations. From the sigmoidal inhibition curves the IC_{50} values were calculated by linear regression (Huber 1993).[44]

Cytotoxicity against *L6* cells

In vitro cytotoxicity against *L6* cells. Assays were performed in 96-well microtiter plates, each well containing 100 µl of RPMI 1640 medium supplemented with 1% L-glutamine (200 mM) and 10% fetal bovine serum, and 4000 L6 cells (a primary cell line derived from rat skeletal myoblasts) (Page et al., 1993 and Ahmed et al., 1994).[50-51] Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 µg/mL were prepared. After 70hours of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. 10 µl of Alamar Blue was then added to each well and the plates incubated for another 2 hours. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave length of 588 nm. The IC_{50} values were calculated by linear regression (Huber 1993)[44] from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Cooperation, Sunnyvale, CA, USA).

Antiproliferative activity against human leukemia MV4-11 cells: Materials and methods

Cell line

Established *in vitro,* human leukemia cell line: MV4-11 (biphenotypic B myelomonocytic leukemia) was used. This line was obtained from American Type Culture Collection (Rockville, Maryland, USA) and is being maintained at the Institute of Immunology and Experimental Therapy, Wroclaw, Poland.

MV4-11 cells were cultured in the RPMI 1640 medium (Gibco, Scotland, UK) supplemented with 2 mM L-glutamine and 1.0 mM sodium pyruvate, 10% fetal bovine serum (all from Sigma-Aldrich Chemie GmbH, Steinheim, Germany), 100 units/ml penicillin, and 100 μg/ml streptomycin (both from Polfa, Tarchomin S.A., Poland). The cell line was grown at 37 °C with 5% CO_2 humidified atmosphere.

Antiproliferative assay in vitro

Test solutions of the compounds tested (1 mg/ml) were prepared by dissolving the substances in 100 μl of DMSO completed with 900 μl of tissue culture medium. Afterwards, the tested compounds were diluted in culture medium to reach the final concentrations of 10, 1, 0.1, 0.01 and 0.001 μg/ml.

Twenty four hours prior to the addition of the tested compounds, the cells were plated in 96-well plates (Sarstedt, Germany) at a density of 1×10^4 cells per well. The assay was performed after 72 h of exposure to varying concentrations of the tested agents. The *in vitro* cytotoxic effect of all agents was examined using the MTT assay.

The results were calculated as an IC_{50} (inhibitory concentration 50) – the dose of tested agent which inhibits proliferation of 50% of the cancer cell population. IC values were calculated for each experiment separately and mean values ± SD are presented in the tables 1. Each compound in each concentration was tested in triplicate in a single experiment, which was repeated 3~5 times.

MTT assay

This technique was applied for the cytotoxicity screening against leukemia cells growing in suspension culture. An assay was performed after 72 hours exposure to

varying concentrations (from 0.001 to 10 μg/ml) of the tested agents. For the last 3-4 hours of incubation 20 μl of MTT solution were added to each well (MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; stock solution: 5mg/ml, Sigma, Germany). The mitochondria of viable cells reduce the pale yellow MTT to a navy blue formazan: the more viable cells are present in well, the more MTT will be reduced to formazan. When incubation time was completed, 80 μl of the lysing mixture were added to each well (lysing mixture: 225 ml dimethylformamide, POCh, Gliwice, Poland, 67.5 g sodium dodecyl sulphate, Sigma, Germany, and 275 ml of distilled water). After 24 h, when formazan crystals had been dissolved, the optical densities of the samples were read on a Multiskan RC photometer at 570 nm wavelength.

Antiproliferative activity of neocryptolepine derivatives against A549, HCT116 and Balb/c 3T3 cell lines: Materials and methods

Cell line

Established *in vitro*, human cell line: A549 (lung cancer), HCT116 (colon cancer) and normal mice fibroblast (Balb/c 3T3) was used. These lines were obtained from American Type Culture Collection (Rockville, Maryland, USA) and are being maintained at the Institute of Immunology and Experimental Therapy, Wroclaw, Poland.

HCT116 and A549 cells were cultured in the RPMI 1640+OptiMEM (50:50) medium (Gibco, Scotland, UK) supplemented with 2 mM L-glutamine and 5% fetal bovine serum (all from Sigma-Aldrich Chemie GmbH, Steinheim, Germany), Balb/c 3T3 cells were cultured in Dulbecco medium (IIET) supplemented with 2 mM L-glutamine and 1.0 mM sodium pyruvate, 10% fetal bovine serum (all from Sigma-Aldrich Chemie GmbH, Steinheim, Germany). All culture medium was supplemented with 100 units/ml penicillin and 100 μg/ml streptomycin (both from

Polfa, Tarchomin S.A., Poland). All cell lines were grown at 37 °C with 5% CO_2 humidified atmosphere.

Antiproliferative assay in vitro

Test solutions of the compounds tested (1 mg/ml) were prepared by dissolving the substances in 100 μl of DMSO completed with 900 μl of tissue culture medium. Afterwards, the tested compounds were diluted in culture medium to reach the final concentrations of 10, 1, 0.1, 0.01 and 0.001 μg/ml.

Twenty four hours prior to the addition of the tested compounds, the cells were plated in 96-well plates (Sarstedt, Germany) at a density of 1×10^4 cells per well. The assay was performed after 72 h of exposure to varying concentrations of the tested agents. The *in vitro* cytotoxic effect of all agents was examined using the SRB assay. The results were calculated as an IC_{50} (inhibitory concentration 50) – the dose of tested agent which inhibits proliferation of 50% of the cancer cell population. IC values were calculated for each experiment separately and mean values ± SD are presented in the Table 2. Each compound in each concentration was tested in triplicate in a single experiment, which was repeated 3~5 times.

SRB assay

This technique was applied for the cytotoxicity screening against cells growing in adherent culture. The details of this technique were described by Skehan.[8] The cytotoxicity assay was performed after 72 hour exposure of the cultured cells to varying concentrations (from 0.01 to 10 μg/ml) of the tested agents. The cells attached to the plastic were fixed by gently layering cold 50% TCA (trichloroacetic acid, Aldrich-Chemie, Germany) on the top of the culture medium in each well. The plates were incubated at 4 °C for 1 h and then washed five times with tap water. The background optical density was measured in the wells filled with culture medium, without the cells. The cellular material fixed with TCA was stained with 0.4% sulforhodamine B (SRB, Sigma, Germany) dissolved in 1% acetic acid (POCh,

Gliwice, Poland) for 30 minutes. Unbound dye was removed by rinsing (4×) with 1% acetic acid. The protein-bound dye was extracted with 10 mM unbuffered Tris base (Sigma, Germany) for determination of optical density (at 540 nm) in a computer-interfaced, 96-well microtiter plate reader Multiskan RC photometer (Labsystems, Helsinki, Finland).

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List of Publications

Refereed Papers

1. Zhen-Wu Mei, Wei Peng, Wen-Jie Lu, Cui-Qing Pang, Tsukasa Maeda, Li Wang, Marcel Kaiser, Ibrahim El-Tantawy El-Sayed, Tsutomu Inokuchi. Synthesis and in vitro antimalarial testing of neocryptolepines: SAR study for improved activity by introduction and modifications of side chains at C2 and C11 on indolo[2,3-*b*]quinolines. Submitted for publication.
2. Zhen-Wu Mei, Li-Jian Ma, Hiroyuki Kawafuchi, Takumi Okihara, Tsutomu Inokuchi. TEMPO-mediated oxidation of primary alcohols to carboxylic acids by exploitation of ethers in an aqueous-organic biphase system. *Bulletin of the Chemical Society of Japan*, 2009, 82(8), 1000–1002.
3. Zhen-Wu Mei, Takumi Omote, Mounir Mansour, Hiroyuki Kawafuchi, Yutaka Takaguchi, Anny Jutand , Sadao Tsuboi, Tsutomu Inokuchi. A high performance oxidation method for secondary alcohols by inductive activation of TEMPO in combination with pyridine-bromine complexes. *Tetrahedron*, 2008, 64(47), 10761–10766.
4. Wei Peng, Marta Świtalska, Zhen-Wu Mei, Li Wang, Yoshiki Edazawa, Cui-Qing Pang, Ibrahim El-Tantaway El-Sayed, Joanna Wietrzyk, Tsutomu Inokuchi. Synthesis and in vitro antiproliferative activity of new 11-aminoalkylamino-substituted chromeno[2,3-*b*]indoles. *European Journal of Medicinal Chemistry*, 2012, accepted.
5. Li Wang, Marta Świtalskab, Zhen-Wu Mei, Wen-Jie Lu, Yoshito Takahara, Xing-Wen Feng, Ibrahim El-Tantawy El-Sayed, Joanna Wietrzyk, Tsutomu Inokuchi. Synthesis and in vitro antiproliferative activity of new 11-aminoalkylamino-substituted 5*H*- and 6*H*-indolo[2,3-*b*]quinolines; structure-activity relationships of neocryptolepines and 6-methyl congeners.

Bioorganic & Medicinal Chemistry, 2012, 20(15), 4820–4829.

6. Li Wang, Wen-Jie Lu, Tomohito Odawara, Zhen-Wu Mei, Wei Peng, Ibrahim El-Tantaway El-Sayed, Tsutomu Inokuchi. Improved synthesis and reaction of 11-chloroneocryptolepines, strategic scaffold for antimalaria agent, and their 6-methyl congener from indole-3-carboxylate. *Journal of Heterocyclic Chemistry*, 2012, in press.
7. Li-Jian Ma, Zhen-Wu Mei, Keisuke Toyohara, Hiroyuki Kawafuchi, Junzo Nokami, Tsutomu Inokuchi. Diastereoselective Michael addition of magnesium amide to *O*-(2-alkenoyl)TEMPOs and comparison of reactivity with acyl substituent-modified carboxylic analogues. *Bulletin of the Chemical Society of Japan*, 2010, 83(12), 1545–1547.

Patents

1. JP Patent 「インドールキノリン誘導体、該誘導体の製造方法、該誘導体を含む抗マラリア剤および抗がん剤」井口 勉、Ibrahim EI-Sayed、佐々木 健二、梅 振武、王 力、陸 文傑. 特願：2012-136013.（出願日：平成24年6月15日）
2. JP Patent 「新規な抗マラリア活性剤インドールキノリン誘導体」井口 勉、Ibrahim EI-Sayed、佐々木 健二、梅 振武. 特願：2011-237348.（出願日：平成23年10月28日）
3. JP Patent 「ベンゾピランインドール誘導体、該誘導体の製造方法および該誘導体を含む抗ガン剤」井口 勉、彭 維、梅 振武. 特願：2012-37710.（出願日：平成24年2月23日）

Oral and Poster Presentation

1. Zhen-Wu Mei, Li Wang, Wen-Jie Lu, Ming-Qi Wang, Ibrahim El-Sayed, Tsutomu Inokuchi. Benign synthesis of poly-substituted indolequinolines and application

to antimalarial agents. *The Japanese Society for Process Chemistry, 2012 Summer Symposium*, 2P-49, July 19-20, 2012, Kyoto, Japan.

2. Li Wang, Wen-Jie Lu, Yoshito Takahara, Cui-Qing Pang, Zhen-Wu Mei, Hiroyuki Kawafuchi, Tsutomu Inokuchi. High performance synthesis of indolequinoline for antimalaria agents. *The 92nd Annual Meeting of the Chemical Society of Japan*, oral presentation, 4K5-06, March 25-28, 2012, Yokohama, Japan.
3. Li Wang, Wen-Jie Lu, Yoshito Takahara, Zhen-Wu Mei, Tomohito Odawara, Ibrahim El-Sayed, Tsutomu Inokuchi. Synthesis and reaction of 11-chloroneocryptolepines, strategic scaffold for antimalarial agent, and their 6-methyl congener from indole-3-carboxylate. *The 5th International Symposium for Future Technology Creating Better Human Health and Society Development*, P-12, March 15-16, 2012, Okayama, Japan.
4. Masashi Okada, Zhen-Wu Mei, Tomonari Kasai, Akifumi Mizutani, Takayuki Kudoh, Ibrahim El-Sayed, Masaharu Seno. New alkaloid derivatives inhibiting caspase-like activity of 20S proteasome. *The 34th Annual Meeting of the Molecular Biology Society of Japan*, December 13-16, 2011, Yokohama, Japan.
5. Li Wang, Wen-Jie Lu, Ryuhei Misumi, Zhen-Wu Mei, Ibrahim El-Sayed, Tsutomu Inokuchi. Benign access to indolequinolines as a strategic scaffold for antiinfectious agent. *The 10th International Symposium on Organic Reactions (ISOR10)*, PP-46, November 21-24, 2011, Yokohama, Japan.
6. Li Wang, Wen-Jie Lu, Ryuhei Misumi, Zhen-Wu Mei, Yoshiki Edazawa, Ibrahim El-Sayed, Tsutomu Inokuchi. Synthesis of F-Substituted 11-Chloroneocypotolepines from Indole-3-carboxylate and N-Methylanilines. *The 35th Fluorine Conference of Japan*, P-8, September 26-27, 2011, Okayama, Japan.
7. Tsutomu Inokuchi, Ibrahim El-Sayed, Zhen-Wu Mei, Li Wang, Wei Peng, Wen-Jie Lu. Phosphorylation of dienone pharmacophore for targeted design of highly effective and fluorescent cytostatics. *International Congress on Organic Chemistry (the Butlerov's Congress)*, oral presentation, O-53, September 18-23,

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