Studies on Synthesis of Novel N-Heterocycles and Their Antimalarial and Anticancer Evaluations

by

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**Studies on Synthesis of Novel N-Heterocycles and Their Antimalarial and Anticancer Evaluations**

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Chapter 1

Introduction and General Summary
1.1 Introduction

The risk of cancer and malaria infectious diseases is increasing worldwide. Exploring new drugs to meet the needs against drug-resistant diseases is an everlasting demand the therapeutic practice. People health condition is improving globally with the therapy development. In 2009, life expectancy at birth globally was 68 years, ranging from 57 years in low-income countries to 80 years in high-income countries, and it has increased globally by 4 years compared to 1990. But there are still some threatens, like cancer, heart disease, infection, or some other disorders, which are taking peoples lives cruelly every year.\(^1\) Many efforts were devoted to resist such diseases. Meanwhile, the importance of heterocyclic compounds has emerged as a source of anti-cancer and anti-infectious therapeutic agents. Furthermore, new drugs for treatment of diseases are urgently needed to surmount current issues such as low efficacy, resistance and toxicity. Migration, the increase in travel, and global warming a growing risk of spreading disease around the world.

Today, novel development and revolution of technology in health care and environmental conservancy are the first priority issues for the human prosperity in 21st century. For this goal, energy-saving and environment-friendly techniques, and begin methods should be devised. The further-oriented synthetic organic chemistry, namely a sort of green chemistry, would play an important role in performance of these challenges.

Green chemistry, also called sustainable chemistry, is a philosophy of chemical research and engineering that encourage the design of products and processes that minimize the use and generation of hazardous substances.\(^2\) Green chemistry seeks to reduce and prevent pollution from its source. The focus is paid on minimizing the hazard and maximizing the efficiency of any chemical choice.

On the other hand, Microwave heating is attractive technique for achievement of green chemistry.\(^3\) Microwave-assisted organic synthesis is characteristic of spectacular accelerations in many reactions as a consequence of the heating rate, which cannot be reproduced by classical heating.
Higher yields, milder reaction conditions and shorter reaction time can be expected and many processes can be improved. Indeed, even reactions that do not occur by conventional heating can be performed by using microwaves.\textsuperscript{4}

Recently, 1,4-dihydropyridine-based systems have attracted considerable attention due to their wide spectra of biological activities.\textsuperscript{5} For example, cardiovascular agents such as nifedipine (I), used for the treatment of hypertension, contain the dihydropyridyl motif.\textsuperscript{6} NADH coenzymes are comprised of 1,4-dihydropyridine units, which have been explored for their calcium channel activity.\textsuperscript{7}

The author focused on synthesis and biological studies of \(N\)-Amino-substituted 1,2-dihydropyridine by using MW-assisted tandem knoevenagel condensation of enaminone and enals followed by \(6\pi\)-electrocyclization (Figure 1).

![Figure 1. Structures 1,2-dihydropyridines I–II](image)

Research shows also that some products from natural resources have good activities in anticancer or antimalarial treatment. The efforts based on synthetic modification of these naturally products to improve the activities are attracting intensive attention. On the other hand, despite the advances in technology and understanding of biological systems, drug discovery is still a time consuming and money costing process with low rate of new therapeutic discovery. To design and synthesize new compounds for bioactivity screening is still a hard job. Therefore, finding new small molecules with high bioactivities as potential drug candidates is a very important mission of scientists.

Most physiologically active molecules owe their biologically properties to the presence of nitrogen atom in the form of heterocycles. A majority of the known natural products are obtained as heterocycles. Among various nitrogen heterocycles, the quinoline ring system is probably one of
the most common heterocycles motifs in many natural products and biologically active pharmaceuticals. The use of natural products containing quinoline motifs can be traced back to ancient time, that is a well-known story of extracts from cinchona.\(^8\) Some important quinoline-scaffold chemicals are still attracting the scientists' attention today, and the synthesis of this kind of new compound with high bioactivities is still progress.\(^9\)

Quinine which was first extracted from the bark of the cinochina trees native in South America, has been used to treat the pain and deadly fevers associated with malaria since nearly four hundred years ago. Rational attempts to synthesize quinine started early in the first half of the twentieth century, and Woodward and Doering reported a formal synthesis of quinine during World War II, 1944.\(^10\) But, an entirely stereoselective synthesis of quinine was achieved Stork et.al in 2000.\(^11\)

On the other hand, the roots of the West African plant Cryptolepis sanguinolenta have been traditionally used by the Ghanaian healers to treat a variety of health disorders,\(^12\) and since 1974 a decoction of this plant has been used in the clinical therapy of rheumatism, urinary infections, malaria, and other diseases.\(^13\) This plant has proven to be a rich source of indoloquinoline alkaloids as a result of the efforts of several research groups, which are shown in (Figure 2).\(^14\) Two of these teams described independently the isolation of a new alkaloid, 5-methyl-5H-indolo[2,3-b]quinoline (neocryptoline), from the extracts of Cryptolepis sanguinolenta.
Under these circumstances and with these knowledge in mind, the author focused his attention on inventing synthetic methodology for nitrogen-containing molecules such as 1,2-Dihydropyridines, quinoline-indole and N-heterocycles. The products of this thesis should be applicable to urgently required non-toxic and efficient drugs for cancer and malaria infectious diseases.

In this thesis the author has engaged in three research topics directed toward the synthesis of biologically relevant novel nitrogen-containing molecules:

1) Microwave-Irradiated Synthesis and in Vitro Antiproliferative of 1,2-Dihydropyridines

2) Synthetic Access to Poly-Substituted 11H-Pyrido[3,2-a]carbazoles, a DNA-Intercalating Ellipticine Related Structure, and Their Antiproliferative Activity

3) Synthesis and Antimalarial activity of Some Neocryptolepine Analogues Carrying a Multifunctional Linear and Branched Carbon-Side Chains


5) Synthesis, characterization of some new azo-neocryptolpine dyes and Spectroscopic Analysis
1.2 Outline of Chapter 2

Hantzsch reported the first synthesis of dihydropyridine in 1882 in the course of developing his useful synthetic method for pyridine.¹⁵ This reaction produces 1,4-dihydropyridines, as isolable intermediates, that can then be oxidized to pyridines. The outbreak of interest in this class of molecules was stimulated by two major discoveries: 1) the isolation of NADH (1; Figure 3) and its role in biological oxidation–reduction reactions, and 2) the widespread attention gathered by molecules such as nifedipine (2), an antihypertensive drug, with most of the initial studies on this calcium channel blocker being performed in the early 1970s.

From the five theoretically possible isomeric dihydropyridines, the 1,2- and the 1,4-dihydro structures are the most commonly found in known dihydropyridines.¹⁶ The 1,4-dihydropyridines are known to possess a wide range of biological and pharmacological actions and have been used as calcium-channel modulating agents in the treatment of cardiovascular disease, as multidrug-resistance-reversing agents in cancer chemotherapy and as antimycobacterial and anticonvulsant agents.¹⁷

The less studied 1,2-dihydropyridines consist of an important scaffold for the preparation of 2-azabicyclo[2.2.2]octanes (isoquinuclidines).¹⁸ The isoquinuclidine ring system is widely found in natural products such as the alkaloids ibogaine (3) and dioscorine (4; Figure 3), which have a large spectrum of interesting biological properties.¹⁸ It was recently indicated that ibogaine (3) could be used as an anti-addictive and anti-craving agent since it reduces cravings for alcohol and other drugs. Dioscorine (4) has been shown to be a toxic central nervous system depressant and a modulator of the nicotinic acetylcholine receptor.¹⁸
Figure 3. Nature’s reducing agent NADH (1), antihypertensive drug nifedipine (2), and natural product alkaloids ibogaine (3) and dioscorine (4)

The limited availability of synthetic methods for the preparation of 1,2-dihydropyridines and the recent development of resistance against Tamiflu, a potent inhibitor of neuraminidase, led to considerable efforts toward the development of new and efficient methodologies for the synthesis of 1,2-dihydropyridines.

In chapter 2 the author described the convergent access to the poly-substituted 1-(phenylamino)-1,2,7,8-tetrahydroquinolin-5(6H)-ones 3 and N-(5-oxo-5,6,7,8-tetrahydroquinolin-1(2H)-yl)benzamides 4 and 11 by the reaction of the corresponding enaminones 1a and 1b with 2-enals 2. This protocol involves the tandem Knoevenagel condensation, which was readily catalyzed using ethylenediammonium diacetate, and the subsequent 6π-electrocyclization. The reaction feasibility was dependent on the kind of N-substituents and the presence of a C2 substituent on the enals 2. The enaminones 1a from phenylhydrazine showed a slightly higher reactivity than the benzoyl analogues 1b obtained from benzohydrazide. A further study of the biological activities of these products also evaluated.
Scheme 1. Rationale for favorable formation of 3 by the condensation of 1 and 2 catalyzed by EDDA followed by 6π-electrocyclization.
1.3 Outline of chapter 3

In chapter 3, the author developed a synthetic access to the 2-methoxycarbonyl-11H-pyrido[3,2-a]carbazoles 1 by the Fischer indole synthesis of the corresponding 2-methoxycarbonyl-tetrahydroquinolones 2 with phenylhydrazines 3.\textsuperscript{19–21} The method described here would permit formation of 11H-pyrido[3,2-a]carbazole scaffold with an ester group at the C2 and additional option of substituents at the C6 and C8. Availability of poly-substituents in the pyrido[3,2-a]carbazole core could be useful for further SAR study toward prospective anticancer and antimalarial activities (Figure 4). In addition, preliminary biological testing of the some obtained compounds was achieved against MV4-11 cell line (human leukemia).

\textbf{Figure 4.} Synthetic plan to 2-methoxycarbonyl-11H-pyrido[3,2-a]carbazoles by Fischer indole synthesis followed by aromatization.
1.4 Outline of chapter 4

In chapter 4, the author described the synthesis and in vitro antimalarial activity of several neocryptolepine analogues carrying either a linear or branched dibasic side chain at C11.

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 death from malaria. Human suffers malaria when bitten by the female of any one of the 60 species of Anopheles mosquito. 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 5).

![Figure 5. Malaria life cycle](image)

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 is preclinical pipeline. During the structure-activity relationship (SAR) study of CQ (fig 6), 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 resent structure function..
investigations of Krogstad and co-workers\textsuperscript{25-26} which have shown that changes in the length of the aminoalkyl side chain have little influence on activity against chloroquine-sensitive strains of \textit{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.\textsuperscript{22}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{structure.png}
\caption{Proposed structure-function relationships in chloroquine}
\end{figure}

Based on these facts, the author synthesized and evaluated natural product derivatives based on neocryptolpine core, which is minor indolequinone alkaloid from the roots of the West African plants \textit{cryptolepis sanguinolenta}. The author prepared a novel series of neocryptolepine derivatives by systematically varying the structure and length of the linker between the two nitrogen atoms on the neocryptolepine core. All the synthesized compounds showed more potent antiplasmodial activities against CQS parasites (NF54 strain) \textit{in vitro} when compared with neocryptolepaine. A comparative study showed that compounds containing linear-side chains with three carbon atoms spacers between the two distal nitrogen generally present better antiplasmodial activities than those with branched carbon atoms spacers. Further variations in substituents and substitution patterns may be necessary to obtain nontoxic compounds showing better activity and selectivity.
These modified neocryptolpine derivatives were tested for antimalarial activity against CQS parasites (NF54 strain) of plasmodium falciparum in vitro. The evaluation also included cytotoxicity toward mammalian L6 cells.
1.5 Outline of chapter 5

In chapter 5, the author described the synthesis of a series of 11-modified neocryptolepines with branched ω-aminoalkylamino chains of different linker length between the two nitrogen atoms. All the synthesized compounds showed potent in vitro antiproliferative activity against human leukemia MV4-11 cell line over the 11-chloro precursors and also exhibited selective cytotoxicities against A549 (lung cancer), HCT116 (colon cancer) cell lines and normal fibroblast BALB/3T3. As a result of diversification of the ω-aminoalkylamino chains, we found the highest antiproliferative activity with 11-(3-amino-2-hydroxy)propylamino substituted 2h showing a mean IC_{50} value of 0.015 μM. Modification of ω-aminoalkylamino chains to ω-ureido and thioureido derivatives was not the case for the improved activity. Further variations in substituents and their pattern may be necessary to obtain better activity.

Neocryptolepine and cryptolepine are well-known natural indoloquinoline alkaloids as antimalarial drug with cytotoxic properties. They also display potent cytotoxicity against several tumor cell lines, as they are capable of intercalating into DNA at the cytosine-cytosine sites. It was found that 5,11-dimethyl-5H-indolo[2,3-b]quinolin (I, DiMIQ), the analogue of neocryptolepine, is the most promising lead compound for potential anticancer agents among different derivatives of indoloquinolines. The activity of DIMIQ derivatives arises from the ability to intercalate the DNA and create a drug–DNA–topoisomerase II complex.

We have synthesized a series of 2- and 11-position –modified 5-methyl indolo[2,3-b]quinolin as anticancer reagents. The antiproliferative bioactivities against cancer cell lines (human leukemia cell line MV4-11, human lung cancer cell lines A549 and human colon cancer cell line HCT116) and normal mice fibroblast BALB/3T3 cell lines of these compounds were also evaluated. These results indicated that the 11-amino group is important for their activity, especially the (3-amino-2-hydroxy)propylamino group, which could increase the activity against MV4-11 about 67 times more active compared to its precursor (Figure 6). The –NH-group, which might favor the interaction with DNA, was important for activity.
Figure 6. The IC₅₀ of (2h, 1b, 2m, 2n) against MV4-11 cancer cell lines.

The author also observed interesting selectivity of the reagents against normal mice fibroblast BALB/3T3 and against human lung cancer cell line A549 and human colon cancer cell HCT116, as shown in fig. All the tested compounds showed higher antiproliferative activities against the cancer cell than the cisplatin used as the control agent. The compounds 2k and 2h showed higher activity against A549 and HCT116 cell line than other compounds, and they showed selective antiproliferative activity against MV4-11 cell line. The 2-bromo-2n and 2-chloro-2m showed almost the same cytotoxicity against A549 and HCT116 cell lines with normal cell line (BALB/3T3), but 2n showed potent activity against MV4-11 cell line with IC₅₀ 0.078 μM. Compound 2c, and 2f showed low cytotoxicity against normal cell line, and they showed moderate activity against A549 and HCT116 cell lines. It is quite obvious that the introduction of proper
alkylamino substituents into biologically active derivatives can favorably influence their activities and selectivities in DNA binding.

Figure 7. Agents against BALB/3T3, A549 and HCT116 cells line
1.6 Outline of chapter 6

In our previous work, we synthesized a series of neocryptopine and its congers. So we are thinking if the introduction of aryl azo compound linked with neocryptopine structure. The author is focused on the design and synthesis of a series of the neocryptopines bearing azo-dye chromophore and their absorption spectra were discussed for further application as the pigment of textile.
1.5 General Summary

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

(1) In contrast to overwhelming examples of 1,4-dihydropyridine-based systems found widely in biological active compounds such as nifedipine, little attention has been paid to the synthesis and biological evaluation of their double bond regioisomers, i.e., the 1,2-dihydropyridines. The author examined to construct N-amino-substituted 1,2-dihydropyridine motifs using cyclohexane-1,3-diones via the Knoevenagel condensation with enals followed by $6\pi$-electrocyclization using ethylenediammonium diacetate as a catalyst under MW irradiation. A survey of substituents on the N atom indicated that the phenylamino and benzoylamino groups are favorable for formation of 1,2-dihydropyridines, while phenyl, benzyl, and no-substituent are not.

(2) The author described a quick access to poly-substituted 11$H$-pyrido[3,2-$a$]carbazoles 1 by the Fischer indole cyclization on 5-oxo-5,6,7,8-tetrahydroquinoline-3-carboxylate followed by aromatization. Due to high activity of the saturated C5–C6 bond, flanked by two aromatic rings of the resulting 6,11-dihydro-5$H$-pyrido[3,2-$a$]carbazole-2-carboxylate 7, the subsequent DDQ oxidation smoothly proceeded to afford the desired 1 in good yields. When this aromatization was applied to the 6,6-dimethyl derivative 9, the 1,2-rearrangement of a methyl group was induced with SeO$_2$ to produce the corresponding 5,6-dimethylated 11$H$-pyrido[3,2-$a$]carbazole 10. Thus, we developed a new procedure to introduce an alkyl group to the C5 position. Introduction of the phenyl group at C6 is significantly effective to improve the antiproliferative activity.

(3) The author prepared a novel series of neocryptolepine derivatives by systematically varying the nature and length of the linker between the two nitrogen atoms of substituents on the neocryptolepine core. All the synthesized compounds showed potent antiplasmodial activities against CQS (NF54) in vitro over the neocryptolepine. The most potent and selective compound of these derivatives, 14 showed antimalarial activity with an IC$_{50}$ of 2.2 nM and a selectivity index of 1400. The data also demonstrated that the branched structure motif is not superior for the activity over a linear side chain. The compound 15 showed the highest $\beta$-haematin inhibition with IC$_{50}$ value of 10.07 $\mu$M among the tested compounds. While, the compound 15 with branch side-chain has low cytotoxicity with IC$_{50}$ of 7249.3 nM.
(4) The author described the synthesis and antiproliferative evaluation of several neocryptolpine analogues carrying branched dibasic side chain at C11. A set of neocryptolpine analogues having side-chain by varying the nature and length of the linker between the two nitrogen atoms as well as the substitution pattern and basicity of the distal amino group were examined. Many of the prepared neocryptolpine derivatives showed antiproliferative activity of less than μM against the human leukemia MV4-11 cell line. Some 11-(3-amino-2-hydroxy)propylamino derivatives showed the cytotoxicity with a mean IC₅₀ value of 0.042 μM / 0.057μM against MV4-11 cell line, 0.197/0.1988 μM against A549 cell line, and 0.138/0.117 μM against BALB/3T3 cell line.

(5) The author prepared the neocryptolpines bearing azo-dye chromophore and their absorption spectra were discussed for further application as the pigment of textile.
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Chapter 2

Microwave-Irradiated Synthesis and in Vitro Antiproliferative of 1,2- Dihydropyridines
2.1 Abstract

N-Amino-substituted 1,2-dihydropyridine motifs are constructed using cyclohexane-1,3-diones via the Knoevenagel condensation with enals followed by 6π-electrocyclization using ethylenediammonium diacetate as a catalyst under MW irradiation. A survey of substituents on the N atom of the enaminones indicated that the phenylamino and benzoylamino groups are favorable for the reaction, while phenyl, benzyl, and no-substituent are not. The substituent at C2 of enals is crucial for smooth formation of the corresponding adducts and slightly higher yields are obtained with enals bearing an electron-withdrawing aromatic at C3.
2.2 INTRODUCTION

Recently, 1,4-dihydropyridine-based systems have attracted considerable attention due to their wide spectra of biological activities.\(^1\) For example, cardiovascular agents such as nifedipine (I), used for the treatment of hypertension, contain the dihydropyridyl motif (Figure 1).\(^2\) NADH coenzymes are comprised of 1,4-dihydropyridine units, which have been explored for their calcium channel activity.\(^3\) Accordingly, numerous methods have been reported for the synthesis and biological evaluation of 1,4-dihydropyridine derivatives, i.e. IIa, however most of them, have relied on the three-component coupling of 1,3-dicarbonyls (2 equiv.), aldehydes (1 equiv.), and amines (1 equiv.) by the Hantzsch reaction or its modification.\(^4\) Furthermore, the N-aminated derivatives IIb were recently prepared using enamiones derived from arylhydrazines for one component of the Hantzsch reaction,\(^5a,c,f\) and their biological activities were evaluated.\(^5c\)

![Figure 1. Structures of 1,4- and 1,2-dihydropyridines I–III](image)

In contrast to the intensive synthetic and biological studies of 1,4-dihydropyridines,\(^5\) little attention has been paid to the synthesis and biological evaluation of their double bond regioisomer, i.e., the 1,2-dihydropyridines IIIa.\(^6\)
Until now, some synthetic access to the 1,2-dihydropyridines has been explored. The 6π-electron electrocyclic ring closures of the 1-azatriene systems are considered one of the most promising and useful means to form the 1,2-dihydropyridines. Key step to this strategy is the preparation of the functionalized 1-azatriene units. Currently, these units are assembled in situ and directly used to construct the nitrogen heterocycles. For example, the Knoevenagel condensation of iminium ions with enaminones has proven to be a successful strategy for the construction of the 1,2-dihydropyridines. A more direct access to the 1-azatrienes has relied on the reaction of primary amines and 2,4-dienals. Although the use of such cyclic enaminones in a formal [3+3] cycloaddition had already been described by Hsung et al., the reaction conditions were more severe (150 °C in a Sealed tube) and the moisture sensitive α,β-unsaturated imminium salts have to be handled. Besides these procedures, Brønsted acid catalyzed procedures were developed for the formal [3+3] annulation to the cyclohexane-1,3-diones. Therefore, an improved procedure to the 1,2-dihydropyridine structures with the increased choice of Substituents and structural diversity by examination of the kind of amine component is still in needed.

In this study, we report the MW-assisted tandem Knoevenagel condensation of enaminones and enals followed by 6π-azaelectrocyclization, which is affected by kind of substituent on the N atom of the enamines and the substituent on the iminium intermediate from the enals.
2.3 RESULTS AND DISCUSSION

The Knoevenagel condensation of enaminones was performed under the iminium conditions using ethylenediammonium diacetate (EDDA) as a catalyst. Microwave irradiation was used to enhance the sequential condensation and 6π-electron electrocyclization in a short period. The effect of the substituent Y on the enaminones 1 was first examined using the enaminones 1a–1e, prepared by the reaction of cyclohexane-1,3-dione 8a and the respective phenylhydrazine (9a), benzohydrazide (9b), aniline, benzylamine, and ammonia.

Results of the condensation and cyclization of these enaminones 1a–1e with the enal 2g are shown in Table 1. The reaction of the enaminone 1a with 2g smoothly proceeds to produce the corresponding 3g in moderate yield (entry 1). Similarly, the enaminone 1b reacts with 2g under the same conditions in a slightly lesser yield (entry 2). On the other hand, the enaminones 1c and 1d lack the N-N group in the nucleophilic unit result in a decreased reactivity, and produce none of the desired products 5g and 6g (entries 3 and 4). The enamine 1e formed the adduct 7g in a small quantity by the reaction with 2g, which changed to the starting enaminone 1e and the enal 2g during chromatographic purification.

Table 1. MW-Assisted tandem condensation and 6π-azaelectrocyclization by reaction of 1a–1e with 2g using ethylenediammonium diacetate (EDDA). a)

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<td>O</td>
<td>N</td>
<td>Y</td>
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<tr>
<td>1a, Y = NHPh</td>
<td>2g</td>
<td>3g, Y = NHPh</td>
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<td>1b, Y = NHCOPh</td>
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<tr>
<td>5</td>
<td>1e</td>
<td>7g</td>
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a) Carried out using the enaminones 1a–g (1 mmol) and 2g (1 mmol) in DMF (3 mL) with EDDA (5 mol%) at 115 °C under MW irradiation for 5 min. b) Yield based on isolated products.

We then applied this domino condensation-cyclization sequence to the reaction of 1a and 1b with various α-substituted enals 2, and the results are listed in Tables 2 and 3, respectively.

**Table 2.** Synthesis of 1-(phenylamino)-1,2-dihydropyridines 3 from enaminones 1a and enals 2. a)

Reagents: (i) H2O-AcOH (cat), reflux, 2 h. (ii) DMF, ethylenediammonium diacetate (EDDA, 5 mol%), MW irradiation.
<table>
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<tr>
<th>Entry</th>
<th>Enaminone</th>
<th>Product</th>
<th>Yield, %&lt;sup&gt;b)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a</td>
<td>3a</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>1a</td>
<td>3b</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>1a</td>
<td>3c</td>
<td>55</td>
</tr>
<tr>
<td>4</td>
<td>1a</td>
<td>3g</td>
<td>65</td>
</tr>
<tr>
<td>5</td>
<td>1a</td>
<td>3h</td>
<td>22</td>
</tr>
</tbody>
</table>

<sup>a)</sup>Carried out using the enaminones 1a (1 mmol) and 2 (1 mmol) in DMF (3 mL) with EDDA (5 mol%) at 115 °C under MW irradiation for 5 min. <sup>b)</sup>Yield based on isolated products.

**Table 3.** Synthesis of 1-(benzoylamino)-1,2-dihydropyridines 4, 11 from enaminones 1b, 10 and enals 2.<sup>a)</sup>

Reagents: (i) H$_2$O-AcOH (cat), reflux, 2 h. (ii) DMF, EDDA (5 mol%), MW irradiation.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Enaminone</th>
<th>R&lt;sup&gt;1&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Product</th>
<th>Yield, %&lt;sup&gt;b)&lt;/sup&gt;</th>
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</thead>
<tbody>
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<td>1b</td>
<td>a</td>
<td>H</td>
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<td>22</td>
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<tr>
<td>2</td>
<td>1b</td>
<td>b</td>
<td>MeO</td>
<td>4b</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>1b</td>
<td>c</td>
<td>Cl</td>
<td>4c</td>
<td>45</td>
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<tr>
<td>4</td>
<td>1b</td>
<td>d</td>
<td>F</td>
<td>4d</td>
<td>54</td>
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<td>1b</td>
<td>e</td>
<td>MeO&lt;sub&gt;2&lt;/sub&gt;C</td>
<td>4e</td>
<td>45</td>
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<tr>
<td>6</td>
<td>1b</td>
<td>f</td>
<td>CF&lt;sub&gt;3&lt;/sub&gt;</td>
<td>4f</td>
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<td>g</td>
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<td>4g</td>
<td>58</td>
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<tr>
<td>8</td>
<td>10</td>
<td>a</td>
<td>H</td>
<td>Me&lt;sub&gt;1&lt;/sub&gt;a</td>
<td>45</td>
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<td>9</td>
<td>10</td>
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<tr>
<td>11</td>
<td>10</td>
<td>f</td>
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<td>62</td>
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<tr>
<td>13</td>
<td>10</td>
<td>e</td>
<td>MeO&lt;sub&gt;2&lt;/sub&gt;C</td>
<td>Me&lt;sub&gt;1&lt;/sub&gt;f</td>
<td>57</td>
</tr>
</tbody>
</table>

<sup>a</sup>Carried out using the enaminones 1b, 10 (1 mmol) and 2 (1 mmol) in DMF (3 mL) with EDDA (5 mol%) at 115 °C under MW irradiation for 5 min.  
<sup>b</sup>Yield based on isolated products.
The effect of the kinds of aromatic rings at the β-carbon of the α,β-unsaturated aldehydes 2 was observed. Thus, slightly higher yields are obtained using the aromatic R² bearing electron-withdrawing groups (entries 3,4 for 1a in Table 2, and entries 3–7, 9–13 for 1b in Table 3), compared to that of the donating group (entry 2 for 1a in Table 2, and entry 2 for 1b in Table 3). We observed that the yield in Table 2 is better than that in Table 3. Thus, the electron-donating group (NH–NHPPh) in the enaminones is more favorable for the formation of the corresponding 1,2-dihydropyridines than the enaminones with the NH–NHCOPh group bearing an electron-withdrawing group.

In spite of the smooth formation of the 2,3-disubstituted 1,2-dihydropyridine ring onto the cyclic 1,3-diketone monoimines 1a and 1b by the condensation-6π-electrocyclization sequence, the limitation of this method was encountered in the reaction of 1a with cinnamaldehyde (2h) which lacks an α-substituent. The reaction of 1a and cinnamaldehyde (2h) under the iminium conditions described above resulted in a decreased yield of the corresponding annulated adduct 3h (about 22% yield) (Table 2, entry 5).

The reaction mechanism for the formation of 3 can be rationalized as described in Scheme 1 by taking the steric effect of Rⁱ substituent at the C2 of the enals 2 in account. The Knoevenagel condensation through the iminium A and enaminone 1a would lead to the 1-azatrienes B–E, the stereochemical and conformational isomers at equilibrium. The equilibration between B–E would be catalyzed by the employed EDDA. Among them, E (E, s-cis), a sterically favorable configuration for the ensuing cyclization would lead to the desired 3 via spontaneous 6π-electrocyclization.

On the other hand, in all our attempts, the 2H-pyran structure F, which can be available by the 6π-electrocyclization of the favorable configuration B (Z, s-cis), was not detected. This preferable cyclization at the 1-azatriene moiety rather than the 1-oxatriene in the same molecule is good agreement with the results reported by Hsung.⁹
Scheme 1. Rationale for favorable formation of 3 by the condensation of 1 and 2 catalyzed by EDDA followed by 6π-electrocyclization.
2.4 Electro Chemical Oxidation-Reduction measurement of product 3a and 3b

![Diagram of compound II]  

Figure 2. Cyclic voltammetry performed in CH$_2$Cl$_2$ (10 mL) containing nBu$_4$NBF$_4$ (0.3 M) as supporting electrolyte, at 20 °C. Oxidation of product II (5.8 mM) at a gold disk electrode (d = 1 mm) at the scan rate of 0.5 V s$^{-1}$. Epox = + 1.019 V vs SCE
Figure 3. Cyclic voltammetry performed in CH2Cl2 (10 mL) containing nBu4NBF4 (0.3 M) as supporting electrolyte, at 20 °C. Oxidation of product I (5.1 mM) at a gold disk electrode (d = 1 mm) at the scan rate of 0.5 Vs-1. Epox = + 1.022 V vs SCE
2.5 Antiprolifective activity of 1,2- Dihydropyridines against human leukemia MV4-cell line

The result of antiprolifative activity of 3-Methyl-2-phenyl-1-(phenylamino)-1,2,7,8-tetrahydroquinolin-5(6H)-one analogues and N-(3-Methyl-5-oxo-2-phenyl-5, 6, 7, 8-tetrahydroquinolin-1(2H)-yl)benzamide analogues against human leukemia MV4-11 cell line are also summarized in table 4 along with the results of anticancer drugs, cisplatin and doksrubicin HCl. All tested compounds, the compounds 4a, 4b, 4c, 4d, 4e were not cytotoxic against MV4-11 leukemia cells (IC50 higher than 10 μg/ml). We observed also the - (phenylamino)-1,2-dihydropyridines analogues (3a, 3b, 3c, 3g) in their antiproliferative activity is higher than 1-(benzoylamino)-1,2-dihydropyridines derivatives. The highest antiproliferative activity of all test compounds was 3c (IC50 2.20±0.38μM).

Table 4. Yields of 1,2- Dihydropyridines and their Antiproliferative activity against human leukemia MV4-11 Cell line

<table>
<thead>
<tr>
<th>Compound</th>
<th>R¹</th>
<th>R²</th>
<th>Yield</th>
<th>MV4-11 IC50 (μM)</th>
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<tr>
<td>cisplatin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.820±0.450</td>
</tr>
<tr>
<td>Doksrubicin HCl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.006±0.002</td>
</tr>
<tr>
<td>3a</td>
<td>Me</td>
<td>H</td>
<td>50</td>
<td>5.58±1.54</td>
</tr>
<tr>
<td>3b</td>
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<td>3g</td>
<td>Me</td>
<td>NO₂</td>
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<td>3.13±0.93</td>
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<tr>
<td>4a</td>
<td>H</td>
<td>H</td>
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<td>4b</td>
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<td>H</td>
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<td>H</td>
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<td>&gt;10</td>
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<td>F</td>
<td>H</td>
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<td>&gt;10</td>
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<td>3h</td>
<td>H</td>
<td>H</td>
<td>22</td>
<td>3.64±1.48</td>
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</table>
2.6 Experimental Section

2.6.1 General

The $^1$H NMR, $^{13}$C NMR spectra were measured on the Varian INOVA-600 or Varian INOVA-400 spectrometer, using CDCl$_3$ or DMSO-d$_6$ as solvent and tetramethylsilane (TMS) as internal standard. MW reaction was performed with $\mu$Reactor EX, Shikoku Instrumentation Co. Ltd, operated at 2.46 GHz.

**General procedure for the synthesis of 2-(4-Chlorophenyl)-3-methyl-1-(phenylamino)-1,2,7,8-tetrahydroquinolin-5(6H)-one 3c.**

In a side-arm tube flask (10 mm diameter) were introduced N’-(3-oxocyclohex-1-enyl)benzohydrazide (1a, 202 mg, 1 mmol) and 4-chlorophenyl-2-methylacryladehyde (2a, 180 mg, 1 mmol) and EDDA (9 mg, 5% mole) in DMF (2 mL) and the tube was placed into the microwave cavity. The mixture was irradiated under constant microwave for about 5 minutes at controlled temperature of 115 °C. Heating was continued for 3 min under TLC monitoring. The mixture was diluted with cold water and extracted with EtOAc (20 mL, 3 times). Combined organic layer was dried (MgSO$_4$), and concentrated in vacuum. The crude products were purified by flash column chromatography on SiO$_2$ using a mixture of hexane and ethyl acetate with a gradient from 4:1 to 1:4 to obtain the pure product.

![Chemical structure](image-url)

3-Methyl-2-phenyl-1-(phenylamino)-1,2,7,8-tetrahydroquinolin-5(6H)-one (3a) : Yield 165 mg (50%), brown solids; mp 203–205 °C; IR (KBr) $\nu_{\text{max}}$ 3196, 3176, 3020, 2997, 2947, 1593, 1545, 1516, 1496, 1433, 1400, 1377, 1269, 1246, 1188, 1143, 1026, 875, 752, 696 cm$^{-1}$; $^1$H NMR (600MHz, CDCl$_3$) $\delta$ 7.37 (m, 3H), 7.32 (t, $J = 7.2$ Hz, 2H), 7.25 (m, 2H), 6.98 (t, $J = 7.2$ Hz, 1H),
6.74 (d, \( J = 7.80 \text{ Hz}, 2\text{H} \)), 6.59 (s, 1\text{H} ), 5.49 (s, 1\text{H} ), 4.99 (s, 1\text{H} ), 2.63 (t, \( J = 6.6 \text{ Hz}, 2\text{H} \)), 2.34 (t, \( J = 6.6 \text{ Hz}, 2\text{H} \)), 1.92–1.96 (m, 2\text{H} ), 1.47 (s, 3\text{H} ); \(^{13}\text{C} \text{NMR} (151\text{MHz, CDCl}_3) \delta 193.6, 161.4, 145.3, 139.5, 130.8 (2\text{C} ), 130.1 (2\text{C} ), 130.0, 128.9 (2\text{C} ), 125.4, 122.7, 116.3, 114.4 (2\text{C} ), 106.7, 68.2, 36.8, 25.9, 22.2, 21.0. \text{Anal. Calcd for } C_{22}H_{22}N_2O: \text{C, 79.97; H, 6.71; N, 8.48\%}. \text{ Found: C, 79.29; H, 6.51; N, 8.44\%.}

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\includegraphics[width=0.5\textwidth]{image}};
\end{tikzpicture}
\end{center}

\textbf{2-(4-Methoxyphenyl)-3-methyl-1-(phenylamino)-1,2,7,8-tetrahydroquinolin-5(6H)-one (3b):}

\textbf{Yield 125 mg (35\%), brown solids; mp 170–173 °C; IR (KBr) \( \nu_{\text{max}} = 3254, 2933, 1670, 1600, 1546, 1508, 1452, 1400, 1303, 1249, 1184, 1138, 1030, 835, 752, 696 \text{ cm}^{-1} \); \(^{1}H \text{NMR} (600\text{MHz, CDCl}_3) \delta 7.32 (dd, \( J = 8.4, 7.5 \text{ Hz}, 2\text{H} \)), 7.24–7.15 (m, 3\text{H} ), 7.01 (s, 1\text{H} ), 7.01–6.88 (m, 1\text{H} ), 6.76 (d, \( J = 7.6 \text{ Hz}, 2\text{H} \)), 6.60 (s, 1\text{H} ), 5.46 (s, 1\text{H} ), 4.96 (s, 1\text{H} ), 3.81 (s, 3\text{H} ), 2.65 (d, \( J = 4.0 \text{ Hz}, 2\text{H} \)), 2.41 (t, \( J = 6.4 \text{ Hz}, 2\text{H} \)), 1.93 (m, 2\text{H} ), 1.49 (s, 3\text{H} ); \(^{13}\text{C} \text{NMR} (151\text{MHz, CDCl}_3) \delta 192.8, 160.7, 160.4, 144.7, 130.8, 130.1 (2\text{C} ), 129.8, 129.4 (2\text{C} ), 124.9, 121.9, 115.5, 114.7 (2\text{C} ), 113.7 (2\text{C} ), 66.7, 55.6, 36.0, 31.2, 25.2, 21.5. \text{Anal. Calcd for } C_{23}H_{24}N_2O_2: \text{C, 76.64; H, 6.71; N, 7.77\%}. \text{ Found: C, 76.84; H, 6.19; N, 7.79\%.}

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\includegraphics[width=0.5\textwidth]{image}};
\end{tikzpicture}
\end{center}

\textbf{2-(4-Chlorophenyl)-3-methyl-1-(phenylamino)-1,2,7,8-tetrahydroquinolin-5(6H)-one (3c):}

\textbf{Yield 202 mg (55\%), brown solids; mp 200–204 °C; IR (KBr) \( \nu_{\text{max}} = 3257, 2947, 1599, 1548, 1494, 1427, 1398, 1259, 1186, 1138, 1087, 1014, 879, 829, 754 \text{ cm}^{-1} \); \(^{1}H \text{NMR} (600\text{MHz, CDCl}_3) \delta 7.35–7.32 (m, \( J = 8.1, 7.7 \text{ Hz}, 2\text{H} \)), 7.24–7.15 (m, 3\text{H} ), 7.02 (s, 1\text{H} ), 6.78 (d, \( J = 7.6 \text{ Hz}, 2\text{H} \)), 6.60 (s, 1\text{H} ), 5.46 (s, 1\text{H} ), 4.96 (s, 1\text{H} ), 3.80 (s, 3\text{H} ), 2.66 (d, \( J = 4.4 \text{ Hz}, 2\text{H} \)), 2.42 (t, \( J = 7.1 \text{ Hz}, 2\text{H} \)), 1.93 (m, 2\text{H} ), 1.49 (s, 3\text{H} ); \(^{13}\text{C} \text{NMR} (151\text{MHz, CDCl}_3) \delta 192.8, 160.7, 160.4, 144.7, 130.8, 130.1 (2\text{C} ), 129.9, 129.4 (2\text{C} ), 124.9, 121.9, 115.5, 114.7 (2\text{C} ), 113.9 (2\text{C} ), 66.7, 55.6, 36.0, 31.2, 25.2, 21.5. \text{Anal. Calcd for } C_{24}H_{25}N_2ClO: \text{C, 74.30; H, 6.18; N, 7.28\%}. \text{ Found: C, 74.83; H, 6.19; N, 7.36\%.}
\[\delta 7.39-7.27 \text{ (m, 4H)}, 7.25-7.15 \text{ (m, 2H)}, 6.99 \text{ (t, } J = 7.4 \text{ Hz, 1H)}, 6.75 \text{ (d, } J = 7.7 \text{ Hz, 2H)}, 6.60 \text{ (s, 1H)}, 5.49 \text{ (s, 1H)}, 5.00 \text{ (s, 1H)}, 2.63 \text{ (t, } J = 6.2 \text{ Hz, 2H)}, 2.36 \text{ (t, } J = 6.5 \text{ Hz, 2H)}, 2.09-1.89 \text{ (m, 2H)}, 1.49 \text{ (s, 3H)}; ^{13}\text{C NMR (151 MHz, CDCl}_3^3 \delta 192.9, 160.5, 144.4, 137.4, 135.2, 130.1 \text{ (2C), 129.6 \text{ (2C), 129.5 \text{ (2C), 124.3, 122.0, 115.9, 113.5 \text{ (2C), 106.0, 66.8, 36.0, 25.2, 21.4, 20.2. Anal. Calcd for C}_{22}\text{H}_{21}\text{ClN}_2\text{O}: C, 72.42%; H, 5.80%; N, 7.68%. Found: C, 71.82; H, 5.66; N, 7.73%.}

### 3-Methyl-2-(4-nitrophenyl)-1-(phenylamino)-1,2,7,8-tetrahydroquinolin-5(6H)-one (3g):

Yield 245 mg (65%), brown solids; mp 106–108 °C; IR (KBr) \(\nu_{\text{max}}\) = 3240, 2945, 1670, 1599, 1548, 1519, 1398, 1346, 1271, 1247, 1188, 1139 \text{ cm}^{-1}; ^{1}\text{H NMR (600 MHz, CDCl}_3^3 \delta 8.24 \text{ (d, } J = 8.6 \text{ Hz, 2H)}, 7.47 \text{ (d, } J = 8.6 \text{ Hz, 2H)}, 7.33 \text{ (t, } J = 7.9 \text{ Hz, 2H)}, 7.01 \text{ (t, } J = 7.4 \text{ Hz, 1H)}, 6.75 \text{ (d, } J = 7.7 \text{ Hz, 2H)}, 6.66 \text{ (s, 1H)}, 5.46 \text{ (s, 1H)}, 5.17 \text{ (s, 1H)}, 2.66 \text{ (t, } J = 6.2 \text{ Hz, 2H)}, 2.41 \text{ (t, } J = 6.5 \text{ Hz, 2H)}, 1.99-1.92 \text{ (m, 2H)}, 1.51 \text{ (s, 3H)}; ^{13}\text{C NMR (151 MHz CDCl}_3^3 \delta 194.5, 162.0, 150.0, 147.5, 145.6, 131.8 \text{ (2C), 131.7, 130.5 \text{ (2C), 126.1 \text{ (2C), 123.7, 118.1, 114.9 \text{ (2C), 107.9, 68.5, 37.5, 26.7, 22.9, 21.7. Anal. Calcd for C}_{22}\text{H}_{21}\text{N}_3\text{O}_3: C, 70.38%; H, 5.64%; N, 11.19%. Found: C, 69.87; H, 5.19; N, 10.82%.

### 2-Phenyl-1-(phenylamino)-1,2,7,8-tetrahydroquinolin-5(6H)-one (3h):

Yield 70 mg (22%), brown solids; mp 180–183 °C; IR (KBr) \(\nu_{\text{max}}\) = 3211, 3176, 3024, 2945, 1735, 1643, 1593, 1531, 1494, 1433, 1348, 1278, 1190, 1028 \text{ cm}^{-1}; ^{1}\text{H NMR (600 MHz, CDCl}_3^3 \delta 7.38-7.30 \text{ (m, 2H), }
7.29–7.25 (m, 5H), 6.99 (t, $J = 12.1$, Hz, 1H), 6.97 (m, 3H), 5.58 (s, 1H), 5.33 (m, 1H), 5.28 (d, $J = 4.8$ Hz, 1H), 2.86–2.62 (m, 2H), 2.36 (t, $J = 6.6$ Hz, 2H), 1.96–1.88 (m, 2H); $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 192.5, 162.7, 144.2, 139.9, 129.8 (2C), 129.2 (2C), 129.0, 127.7 (2C), 121.7, 119.0, 116.6, 113.4 (2C), 105.4, 63.4, 35.7, 25.1, 21.1. Anal. Calcd for C$_{21}$H$_{20}$N$_2$O: C, 79.72; H, 6.37; N, 8.85%. Found: C, 78.87; H, 6.09; N, 9.01%.

\[
\begin{align*}
&N-(3\text{-Methyl-5-oxo-2-phenyl-5, 6, 7, 8-tetrahydroquinolin-1(2H)-yl)benzamide (4a)}:
&\text{Yield 72.5 mg (22%), yellow solids; mp 130–133 °C; IR (KBr) } v_{\text{max}} = 3176, 2947, 2360, 1683, 1591, 1543, 1523, 1491, 1433, 1396, 1375, 1352, 1265, 1193, 1166, 1139, 1089, 1028, 896, 806, 738, 698 \text{ cm}^{-1};
&\text{H NMR (600 MHz, CDCl}_3\text{) } \delta 7.62 \text{ (d, } J = 7.4 \text{ Hz, 2H), 7.54 (dd, } J = 13.0, 4.3 \text{ Hz, 1H), 7.42 (t, } J = 7.6 \text{ Hz, 3H), 7.34–7.28 \text{ (m, 5H), 6.51 (s, 1H), 5.26 (s, 1H), 2.49 (m, 2H), 2.28 (m, 2H), 1.88–1.79 (m, 2H), 1.45 (s, 3H). Anal. Calcd for C}_{23}\text{H}_{22}\text{N}_2\text{O}_2\text{: C, 77.07; H, 6.19; N, 7.82%. Found: C, 76.89; H, 5.76; N, 7.86%.}
\end{align*}
\]

\[
\begin{align*}
&N-(2-(4\text{-Methoxyphenyl)-3-methyl-5-oxo-5,6,7,8-tetrahydroquinolin-1(2H)-yl)benzamide (4b)}:
&\text{Yield 114 mg (33%), yellow solids; mp 116–118 °C; IR (KBr) } v_{\text{max}} = 3198, 2951, 1681, 1668, 1608,
\end{align*}
\]
1510, 1404, 1354, 1251, 1174, 1139, 1028, 895, 835 cm\(^{-1}\); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 7.65 (m, 2H), 7.53 (m, 1H), 7.37 (t, \(J = 7.6\) Hz, 2H), 7.24 (m, 3H), 6.82 (d, \(J = 3.9\) Hz, 2H), 6.43 (s, 1H), 5.18 (s, 1H), 3.75 (s, 3H), 2.47 (m, 2H), 2.17 (t, \(J = 6.5\) Hz, 2H), 1.87–1.80 (m, 2H), 1.44 (s, 3H). HRMS (ESI) calcd for C\(_{24}\)H\(_{24}\)N\(_2\)O\(_3\) [M+H]\(^+\) Exact Mass: 388.18, found 388.18

![Molecule](image1)

\(N\)-(2-(4-chlorophenyl)-3-methyl-5-oxo-5,6,7,8-tetrahydroquinolin-1(2H)-yl) benzamide (4c):

Yield 158.5 mg (45 %), yellow solids; mp 143–146 °C; IR (KBr) \(\nu_{\text{max}}\) = 3196, 2945, 2362, 1686, 1599, 1556, 1489, 1435, 1402, 1354, 1288, 1263, 1193, 1089, 1014, 895, 831 cm\(^{-1}\); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 7.58 (d, \(J = 7.8\) Hz, 2H), 7.50 (t, \(J = 7.2\) Hz, 1H), 7.36 (t, \(J = 7.2\)Hz, 2H), 7.28 (m, 3H), 7.28 (d, \(J = 8.4\) Hz, 2H), 6.33 (s, 1H), 5.25 (s, 1H), 2.47 (t, \(J = 6.5\) Hz, 2H), 2.03 (t, \(J = 10.6\) Hz, 2H), 1.81 (m, 2H), 1.42 (s, 3H). HRMS (ESI) calcd for C\(_{23}\)H\(_{21}\)ClN\(_2\)O\(_2\) [M+H]\(^+\) Exact Mass: 392.13, found 392.13

![Molecule](image2)

\(N\)-(2-(4-Fluorophenyl)-3-methyl-5-oxo-5,6,7,8-tetrahydroquinolin-1(2H)-yl) benzamide (4d):

Yield 206 mg (54%), yellow solids; mp 140–142 °C; IR (KBr) \(\nu_{\text{max}}\) = 3192, 2947, 1681, 1602, 1552, 1506, 1402, 1266, 1222, 1193, 1155, 1139, 1087, 1028, 896, 839, 694 cm\(^{-1}\); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 7.67 (d, \(J = 7.3\) Hz, 2H), 7.54 (m, 1H), 7.42 (t, \(J = 7.4\) Hz, 2H), 7.33–7.26 (m, 3H), 7.01
(s, 2H), 6.43 (s, 1H), 5.25 (s, 1H), 2.50 (m, 2H), 2.26 (t, $J = 4.8$ Hz, 2H), 2.00–1.83 (m, 2H), 1.46 (s, 3H). Anal. Calcd for $\text{C}_{23}\text{H}_{21}\text{FN}_2\text{O}_2$: C, 73.39; H, 5.62; N, 7.44 %. Found: C, 73.53; H, 5.43; N, 7.24 %.

**Methyl 4-(1-benzamido-3-methyl-5-oxo-1,2,5,6,7,8-hexahydroquinolin-2-yl)benzoate (4e):**

Yield 186 mg (45%), yellow solid; mp 131–133 °C (decomp.); IR (KBr) $\nu_{\text{max}} = 3192, 2947, 1681, 1670, 1602, 1552, 1506, 1437, 1402, 1354, 1265, 1222, 1139, 1087, 1028, 896, 839, 694 \text{ cm}^{-1}$; $^1$H NMR (400 MHz CDCl$_3$) $\delta$ 7.94 (d, $J = 8.1$ Hz, 2H), 7.68 (d, $J = 7.3$ Hz, 2H), 7.53 (t, $J = 7.5$ Hz, 2H), 7.44–7.35 (m, 4H), 6.49 (s, 1H), 5.35 (s, 1H), 3.86 (s, 3H), 2.52 (dd, $J = 9.6$, 5.8 Hz, 2H), 2.25 (s, 2H), 1.90 (d, $J = 5.3$ Hz, 2H), 1.46 (s, 3H). HRMS (ESI) calcd for $\text{C}_{25}\text{H}_{24}\text{N}_2\text{O}_4$ [M+H]$^+$ Exact Mass: 416.17, found 416.17

**N-(3-Methyl-5-oxo-2-(4-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinolin-1(2H)-yl)benzamide (4f):**

Yield 241 mg (56%), yellow solids; mp 125–127 °C; IR (KBr) $\nu_{\text{max}} = 3176, 2947, 1681, 1591, 1543, 1433, 1396, 1265, 1253, 1193, 1139, 1091, 1028, 896, 698, 698. \text{ cm}^{-1}$; $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.69–7.60 (d, $J = 6$ Hz, 2H), 7.54 (t, $J = 8.0$ Hz, 3H), 7.47 (d, $J = 7.8$ Hz, 2H),
7.40 (t, $J = 6$ Hz, 2H), 6.45 (s, 1H), 5.36 (s, 1H), 2.51 (dt, $J = 15.3$, 6.2 Hz, 2H), 2.16 (t, $J = 6.5$ Hz, 2H), 2.02–1.71 (m, 2H), 1.46 (s, 3H). Anal. Calcd for C$_{24}$H$_{21}$F$_3$N$_2$O$_2$: C, 67.60; H, 4.96; N, 6.57%. Found: C, 66.98; H, 4.82; N, 6.49%.

**N-(3-Methyl-2-(4-nitrophenyl)-5-oxo-5,6,7,8-tetrahydroquinolin-1(2H)-yl)benzamide (4g):**
Yield 241 mg (58%), yellow solids; mp 125–127 °C; IR (KBr) $\nu_{\text{max}}$ = 3182, 3026, 2945, 1645, 1595, 1552, 1516, 1491, 1437, 1400, 1344, 1290, 1193, 1149, 1107, 1084, 956, 920, 856 cm$^{-1}$; $^1$H NMR (600 MHz, DMSO-d$_6$) $\delta$ 8.23 (d, $J = 6.0$ Hz, 2H), 7.88 (d, $J = 6.0$ Hz, 1H), 7.72 (d, $J = 6.2$ Hz, 2H), 7.60 (d, $J = 6.2$ Hz, 2H), 7.55–7.43 (m, 3H), 6.94 (s, 1H), 6.38 (s, 1H), 5.39 (s, 1H), 2.47 (m, 2H), 2.16 (s, 2H), 1.81 (m, 2H), 1.44 (m, 3H). HRMS (ESI) calcd for C$_{23}$H$_{21}$N$_3$O$_4$ [M+H]$^+$ Exact Mass: 403.15, found 403.17

**N-(3,7,7-Trimethyl-5-oxo-2-phenyl-5,6,7,8-tetrahydroquinolin-1(2H)-yl)benzamide (11a):**
Yield 172 mg (45%), yellow solids; mp 198–201 °C; IR(KBr) $\nu_{\text{max}}$ = 3157, 2956, 2362, 1680, 1597, 1585, 1552, 1519, 1489,1437, 1404, 1301, 1292, 1251, 1186, 1151, 1089, 1070, 1028, 1001, 966, 927, 910,881, 806, 763, 694 cm$^{-1}$; $^1$H NMR (600 MHz, CDCl3) $\delta$ 7.70 (d, $J = 6.0$ Hz, 2H),
7.51 (d, J = 6 Hz, 1H), 7.39 (d, J = 6.1 Hz, 2H), 7.32 (s, 4H), 6.37 (s, 1H), 5.25 (s, 1H), 2.45 (dd, J = 18.0, 18.0 Hz, 2H), 1.94 (s, 2H), 1.45 (s, 3H), 0.97 (d, J = 6.0 Hz, 6H). Anal. Calcd for C_{25}H_{26}N_{2}O_{2}: C, 77.69; H, 6.78; N, 7.25%. Found: C, 77.55; H, 6.05; N, 6.82%.

\[ \text{N-(2-(4-Chlorophenyl)-3,7,7-trimethyl-5-oxo-5,6,7,8-tetrahydroquinolin-1(2H)-yl)benzamide (11b):} \]

Yield 255 mg (60%), yellow solid; mp 125–127 °C; IR (KBr) \( \nu_{\text{max}} = 3188, 2958, 2360, 1681, 1600, 1552, 1487, 1435, 1402, 1288, 1257, 1149, 1089, 1014, 883, 833, 694 \text{ cm}^{-1} \); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \( \delta \) 7.71 (d, \( J = 6.0 \) Hz, 2H), 7.51 (d, \( J = 6.0 \) Hz, 1H), 7.38 (t, \( J = 12 \) Hz, 2H), 7.28–7.24 (m, 4H), 6.35 (s, 1H), 5.24 (s, 1H), 7 (dd, \( J = 18.0, 18.0 \) Hz, 2H), 1.93 (s, 2H), 1.43 (s, 3H), 0.95 (d, \( J = 4.2 \) Hz, 6H). Anal. Calcd for C\(_{25}\)H\(_{25}\)ClN\(_2\)O\(_2\): C, 71.33; H, 5.99; N, 6.66%. Found: C, 70.93; H, 5.75; N, 6.67%.

\[ \text{N-(3,7,7-Trimethyl-2-(4-nitrophenyl)-5-oxo-5,6,7,8-tetrahydroquinolin-1(2H)-yl)benzamide (11c):} \]

Yield 305 mg (70%), yellow solids; mp 158–160 °C; IR (KBr) \( \nu_{\text{max}} = 3192, 2958, 1685, 1600, 1554, 1519, 1437, 1346, 1247, 1182, 1149, 1070, 1028, 1014, 968, 922, 889, 812, 754, 694 \text{ cm}^{-1} \); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \( \delta \) 8.12 (d, \( J = 6.0 \) Hz, 2H), 7.77 (d, \( J = 6.0 \) Hz, 2H), 7.52 (m, 3H), 7.42 (t, \( J = 12.0 \) Hz, 2H), 6.38 (s, 1H), 5.37 (s, 1H), 27 (dd, \( J = 18.0, 18.0 \) Hz, 2H), 2.1 (s, 2H), 1.46
(s, 3H), 1.01 (d, J = 12.0 Hz, 6H). Anal. Calcd for C_{25}H_{25}N_{3}O_{4}: C, 69.59; H, 5.84; N, 9.74%. Found: C, 68.86; H, 5.64; N, 9.54%.

N-(3,7,7-Trimethyl-5-oxo-2-(4-(trifluoromethyl)phenyl)-5,6,7,tetrahydroquinolin-1(2H)-yl)benzamide (11d): Yield 297 mg (65%), yellow solids; mp 128–130 °C; IR (KBr) \( \nu_{\text{max}} = 3201, 2960, 1681, 1600, 1556, 1435, 1325, 1249, 1165, 1126, 1066, 1018, 883, 844, 798, 761, 694 \text{ cm}^{-1} \); \(^1\text{H} \text{NMR} \) (600 MHz, CDCl\(_3\)) \( \delta \) 7.69 (d, \( J = 7.6 \text{ Hz}, 2\text{H} \)), 7.64–7.51 (m, 2H), 7.47–7.41 (m, 5H), 6.44 (s, 1H), 5.34 (s, 1H), 2.37 (dd, \( J = 18.0, 18.0 \text{ Hz}, 2\text{H} \)), 2.05 (s, 2H), 1.46 (s, 3H), 0.99 (m, 6H). Anal. Calcd for C\(_{26}\)H\(_{25}\)F\(_3\)N\(_2\)O\(_2\): C, 68.71; H, 5.54; N, 6.16%. Found: C, 68.76; H, 5.29; N, 5.86%.

N-(2-(4-fluorophenyl)-3,7,7-trimethyl-5-oxo-5,6,7,8-tetrahydroquinolin-1(2H)-yl)benzamide (11e): Yield 252 mg (62 %), yellow solids; mp 204–206 °C; IR (KBr) \( \nu_{\text{max}} = 3201, 2960, 1681, 1600, 1556, 1435, 1325, 1249, 1165, 1126, 1066, 1018, 883, 844, 798, 761, 694 \text{ cm}^{-1} \); \(^1\text{H} \text{NMR} \) (600 MHz, CDCl\(_3\)) \( \delta \) 7.71 (d, \( J = 7.4 \text{ Hz}, 2\text{H} \)), 7.53 (d, \( J = 7.4 \text{ Hz}, 1\text{H} \)), 7.41 (dd, \( J = 12.0, 6.0 \text{ Hz}, 2\text{H} \)), 7.31–7.25 (m, 2H), 7.01 (s, 2H), 6.40 (s, 1H), 5.25 (s, 1H), 2.46–2.23 (dd, \( J = 18.0, 18.0 \text{ Hz}, 2\text{H} \)), 2.01 (d, \( J = 3.6 \text{ Hz}, 2\text{H} \)), 1.45 (s, 3H), 0.97 (d, \( J = 30.0 \text{ Hz}, 6\text{H} \)). \text{HRMS} \text{ (ESI)} \text{ calcd for } \text{C}_{25}\text{H}_{25}\text{F}_{2}\text{N}_{2}\text{O}_{2} \text{ [M+H]}^+ \text{ Exact Mass: } 404.19, \text{ found } 404.19.
Methyl 4-(1-benzamido-3,7,7-trimethyl-5-oxo-1,2,5,6,7,8-hexahydroquinolin-2-yl) benzoate (11f): Yield 250 mg (57%), Yellow solids; mp 130–133 °C; IR (KBr) $\nu_{\text{max}} = 3209, 2955, 1724, 1681, 1600, 1554, 1435, 1413, 1402, 1280, 1261, 1149, 1105, 966$ cm$^{-1}$; $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.91 (d, $J = 8.1$ Hz, 2H), 7.73 (d, $J = 7.3$ Hz, 2H), 7.42 (dt, $J = 15.8, 8.2$ Hz, 1H), 7.33–7.20 (m, 4H), 6.38 (s, 1H), 5.34 (s, 1H), 3.81 (d, $J = 6.3$ Hz, 3H), 2.33 (dd, $J = 18.0, 18.0$ Hz, 2H), 1.97 (t, $J = 6.0$ Hz, 2H), 1.42 (s, 3H), 0.96 (d, $J = 5.8$ Hz, 6H). Anal. Calcd for C$_{27}$H$_{28}$N$_2$O$_4$: C, 72.95; H, 6.35; N, 6.30%. Found: C, 72.93; H, 6.30; N, 6.06%.

2.6.2 Cell line

Established in vitro, human cell line: MV4-11 (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 (IET, Poland) supplemented with 2 mM L-glutamine, 1.0 mM sodium pyruvate and 10% fetal bovine serum (all from Sigma-Aldrich, Germany). The culture medium was supplemented with 100 units/ml penicillin (Sigma-Aldrich, Germany), and 100 µg/ml streptomycin (Polfa Tarchomin S.A., Poland). MV4-11 cells were grown at 37°C with 5% CO2 humidified atmosphere.

2.6.3 Anti-proliferative assay in vitro

Test solutions of the 10 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 $1 \times 10^4$ 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$_{50}$ values were calculated for each experiment separately and mean values ± SD are presented in the Table 4. Each compound in each concentration was tested in triplicate in a single experiment, which was repeated 3-5 times.

### 2.6.4 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: 5 mg/ml, Sigma-Aldrich, 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-Aldrich, 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 Synergy H4 photometer (BioTek Instruments, USA) at 570 nm wavelength. Each compound in given concentration was tested in triplicates in each experiment, which was repeated 3-5 times.
2.7 References and Notes


Chapter 3

Synthetic Access to Poly-Substituted 11H-Pyrido[3,2-a]carbazoles, a DNA-Intercalating Ellipticine Related Structure, and Their Antiproliferative Activity
3.1 Abstract

The facile procedure for the synthesis of the 11H-pyrido[3,2-a]carbazole structure involving the Fischer indole cyclization on tetrahydroquinolinones, available from enaminones and methyl 2-formyl-3-oxopropanoate, followed by the aromatization of the resulting 5,6-dihydro derivatives is described. This method allows for the introduction of substituents at C2, C6, and C8 to the scaffold by choice of the starting materials. In the biological testing, introduction of the phenyl group at C6 is significantly effective to improve the antiproliferative activity.
3.2 INTRODUCTION

The natural plant alkaloids with a linearly condensed aromatic ring system show interesting antitumor activities due to their DNA intercalating ability. For example, ellipticine (5,11-dimethyl-6H-pyrido[4,3-b]carbazole, I, Figure 1), an alkaloid isolated from Apocyanaceae plants, exhibits significant antitumor, anti-HIV, and anti-malarial activities. Accordingly, the overlapping interaction of the 6H-pyridocarbazole aromatic rings with that of a DNA base pair was precisely demonstrated. Nevertheless, ellipticine I and its derivatives are mutagenic to the salmonella typhimurium Ames tester strains, neurospora crassa, and mammalian cells.

![Figure 1. Structures of biologically relevant natural and synthetic pyridocarbazoles.](image)

The DNA intercalating and antitumor ability of the related structures, such as 11H-pyrido[3,2-a]-, [4,3-a]-, [3,4-a]-, and [2,3-a]-carbazoles III~VI were subsequently tested, indicating that the cytotoxicity of the 11H-pyridocarbazoles, measured on L1210 cells in vitro, is much lower than those of the 6H- and 7H-pyridocarbazole analogues I, II. However, a comparative study of the interaction of the pyridocarbazole analogues with DNA topoisomerase II has not been well examined. Furthermore, the 3-substituted 11H-pyrido[3,2-a]carbazole derivatives were shown to possess slight antidepressant and L-dopa-potentiating effects.

Syntheses of ellipticines and their related pyridocarbazole analogues were mainly relied on cyclisation of hydrazinoquinoline with cyclohexanone via the Fischer-Borsche indole synthesis.
followed by aromatization or the Skraup reaction of aminocarbazoles with acrolein.\textsuperscript{9,10} A Doebner reaction was applied to form 1-carboxy-3,5-dimethyl-7H-pyrido[2,3-c]carbazole from 3-amino-2-methylcarbazole, acetaldehyde, and pyruvic acid.\textsuperscript{11} The C ring of ellipticine core are constructd by the cyclization of o-quinodimethane intermediate, generated by thermolysis of the 2-alkyl-3-[\(\alpha\)-(3-pyridyl)vinyl]indole.\textsuperscript{12,13} Regioselective acylation of a 3-lithio-l-(phenylsulfonyl)indole with 3,4-pyridinedicarboxylic anhydride to the corresponding quinone was demonstrated.\textsuperscript{14} A one-step procedure was developed by the Diels-Alder reaction between 1,4-dimethylpyrano[3,4-b]indol-3-one and 3,4-didehydropyridine to construct ellipticine core.\textsuperscript{15}

For the synthesis of the 11\(H\)-pyridocarbazoles core, the Fischer indole cyclization of the 7-(azonaphatalenyl)hydrazines with 4-methoxycyclohexanone followed by aromatization,\textsuperscript{16–19} or the reaction of 7,8-dihydroquinoline-2,5(1\(H\),6\(H\))-dione with phenylhydrazine followed by aromatization,\textsuperscript{8} or the electrocyclization of the 1-(3-indolyl-2-(pyridyl)propenes under UV irradiation have been reported.\textsuperscript{20,21} However, these procedures suffer from low yields during the aromatization steps and are not feasible to supply the corresponding pyridocarbazoles with various substituents in the further structure–activity relationship (SAR) studies.

![Figure 2](image)

**Figure 2.** Synthetic plan to 2-methoxycarbonyl-11\(H\)-pyrido[3,2-a]carbazoles by Fischer indole synthesis followed by aromatization.

In this study, we developed a synthetic access to the 2-methoxycarbonyl-11\(H\)-pyrido[3,2-a]carbazoles 1 by the Fischer indole synthesis of the corresponding 2-methoxycarbonyl-tetrahydroquinolones 2 with phenylhydrazines 3.\textsuperscript{22–24} The method described here would permit formation of 11\(H\)-pyrido[3,2-a]carbazole scaffold with an ester group at the C2 and additional
option of substituents at the C6 and C8. Availability of poly-substituents in the pyrido[3,2-a]carbazole core could be useful for further SAR study toward prospective anticancer and antimalarial activities (Figure 2). In addition, preliminary biological testing of the some obtained compounds was achieved against MV4-11 cell line (human leukemia).
3.3 Results and Discussion

Synthesis of tetrahydroquinolinone derivatives and their biological property as group I mGluR antagonists are claimed in the patent by Jirgensons et al.\textsuperscript{25} However, we prepared the starting 2-methoxycarbonyl-tetrahydroquinolinones 2 by condensation of the enaminones 5 with methyl 2-formyl-3-oxopropanoate (7), according to the method reported by us.\textsuperscript{26}

Thus, 5 were formed by the reaction of the 5-substituted cyclohexane-1,3-diones 4 with ammonium acetate while heating in toluene.\textsuperscript{27,28} Their counterpart 7 was prepared by the Claisen condensation of methyl 3,3-dimethoxypropionate 6 with methyl formate using NaH in ether followed by hydrolysis with 10% HCl. The two-component [3+3] cycloannulation process for the rapid formation of the desired tetrahydroquinolinones 2 was achieved in an one-pot operation by the tosylation of 7 with TsCl-Et\textsubscript{3}N forming the corresponding 2-formyl-3-(tosyloxy)acrylate, followed by the addition of 5 in the presence of pyridine, affording 2-methoxycarbonyl-tetrahydroquinolones 2 in moderate yields (Scheme 1).

Scheme 1. Synthesis of tetrahydroquinolinones 2. Reagent and conditions: (i) AcONH\textsubscript{4}, toluene; (ii) (a) HCO\textsubscript{2}Me, NaH, ether, (b) HCl; (iii) (a) 7, Et\textsubscript{3}N, then pTsCl in DMF, (b) 5, pyridine, DMF.
The Fischer indole cyclization of 2a ($R^2 = H$) using phenylhydrazine 3 ($R^3 = H$) was performed by heating in acetic acid with 36% HCl, affording the desired tetracyclic 6,11-dihydro-5H-pyrido[3,2-a]carbazoles 8a in 72% yield. A similar indole synthesis on 2a ($R^2 = H$) was successfully achieved with 4-BrC$_6$H$_4$NHNH$_2$ 3b ($R^3 = Br$), while the use of 4-NO$_2$C$_6$H$_4$NHNH$_2$ 3d ($R^3 = NO_2$) resulted in no desired indole structure. The reaction of 4-MeOC$_6$H$_4$NHNH$_2$ 3c ($R^3 = OMe$) with 2a ($R^2 = H$) was carried out in a mixed solution of AcOH-TMSCl (4:1 v/v) with heating for 10 h, affording the desired 8e in 48% yield. The oxidative dehydrogenation of 8a to the desired 11H-pyrido[3,2-a]carbazole 1a was achieved by the treatment with DDQ in benzene in 74% yield. The substituent $R^2$ affected the conversion of the DDQ oxidation; a high yield was attained with Ph group (8d, 99% yield), while a slightly lesser yield with Me group (8c, 85%). Oxidation of 8e with DDQ resulted in formation of an unidentified mixture (Scheme 2).

Scheme 2. Synthesis of substituted 11H-pyrido[3,2-a]carbazoles 1. Reagent and conditions: (i) AcOH-36% HCl, heated (for 8a and 8b); (ii) (a) AcOH-36%HCl, heated, (b) MeOH-SOCl$_2$, reflux (for 8c and 8d); (iii) AcOH-TMSCl, heated (for 8e); (iv) DDQ, benzene, heated.

We subsequently examined the preparation of 5,6-dimethyl-11H-pyrido[3,2-a]carbazole 11 through the 1,2-rearrangement of the gem-dimethyl group of the precursor 10 during the
aromatization step.\textsuperscript{29-31} Thus, the tetrahydroquinolinone 9, derived from the dimedone in a manner described above, was converted to the corresponding 6,6-dimethyl-6,11-dihydro-5\textit{H}-pyrido[3,2-\textit{a}]carbazole 10 by the Fischer indole cyclization with hydrazine 3\textit{a}. The oxidation of 10 with SeO\textsubscript{2} (2 equiv.) in MeCN-H\textsubscript{2}O afforded the desired 11 (36\%) and the ketone 12 (54\%) as a by-product. In all our attempts, the oxidation of 10 with DDQ, the reaction was sluggish and a hardly separable mixture of the starting 10 and the aromatized 11 was produced. The formation of 11 can be explained by the Wagner-Meerwein type rearrangement of the methyl group followed by aromatization during the oxidation process with SeO\textsubscript{2} (Scheme 3).

\begin{equation}
\text{Scheme 3. Synthesis of 5,6-dimethyl-11\textit{H}-pyrido[3,2-\textit{a}]carbazole 11. Reagent and conditions: (i) a) 3\textit{a}, AcOH-36\%HCl, heated, b) MeOH-SOCl\textsubscript{2}, reflux; (ii) SeO\textsubscript{2}, MeCN, heated.}
\end{equation}

In connection of search for finding biologically significant derivatives, the 11\textit{H}-pyrido[3,2-\textit{a}]carbazoles 1\textit{a} was converted its pyridinium salt 13 by treatment with MeI in DMF on heating, quantitatively (Scheme 4).

\begin{equation}
\text{Scheme 4. Formation of pyridinium salt 13.}
\end{equation}
3.4 Antiproliferative activity in vitro against MV4-11 cell line

In order to study the biological activity of $11H$-pyrido[3,2-a]carbazoles, we submitted the compounds prepared here to the testing of antiproliferative activity against human leukemia MV4-11 cells and the results are summarized in Table 1. The results of the cytotoxic activity in vitro were expressed as $IC_{50}$ (in μM), the concentration of the compound that inhibits proliferation of the cells by 50% as compared to the untreated control cells. Except for 1d and 12, other compounds show no activity against MV4-11 cells. We modified structure of 1a by introducing Br group at C8 and Me group at C6, but those groups are not effective. Introduction of the Ph group at C6 is significantly effective to improve the antiproliferative activity. The presence of two methyl groups at C5 and C6 is also not effective. Some literatures reported that introduction Me group at indolo-N or quinoline-N can improve the biological activity of the compound, but the compound 13, pyridinium bearing the methyl group at the N4 of the quinoline ring, still showed no activity. Change the ester to carboxy group at C2 also is not useful to improve the activity. However, 5-ketone compound 12 showed antiproliferative activity. According to SAR study, introduction amino group is promising method to improve the biological activity, so in the future, we will modify the $11H$-pyrido[3,2-a]carbazole core with the amino group and varying other substituent at C8 or C6.

Table 1. Antiproliferative activity of neocryptolepine analogues against MV4-11 human leukemia cell line. IC 50 value, μM.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC$_{50}$, μM (ug/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>NA$^a$</td>
</tr>
<tr>
<td>1b</td>
<td>NA$^a$</td>
</tr>
<tr>
<td>1c</td>
<td>NA$^a$</td>
</tr>
<tr>
<td>1d</td>
<td>6.75±0.60 μM (2.38±0.21)</td>
</tr>
<tr>
<td>11</td>
<td>NA$^a$</td>
</tr>
<tr>
<td>12</td>
<td>15.17±3.28 μM (4.86±1.05)</td>
</tr>
<tr>
<td>13</td>
<td>NA$^a$</td>
</tr>
<tr>
<td>Carboxylic acid of 1a$^b$</td>
<td>NA$^a$</td>
</tr>
</tbody>
</table>

$^a$NA- not active, IC$_{50}$ above 10 μg/ml

$^b$Carboxylic acid of 1a: $11H$-pyrido[3,2-a]carbazole-2-carboxylic acid
3.5 Experimental Section

The $^1$H NMR, $^{13}$C NMR spectra were measured on the Varian INOVA-600 or Varian INOVA-400 spectrometer, using CDCl$_3$ or DMSO-d$_6$ as solvent and tetramethylsilane (TMS) as internal standard. MW reaction was performed with µReactor EX, Shikoku Instrumentation Co. Ltd, operated at 2.46 GHz.

Preparation of methyl 6,11-dihydro-5H-pyrido[3,2-a]carbazole-2-carboxylate (8a), a general procedure (Method A for 8a and 8b):

A mixture of 2a (205 mg, 1.0 mmol) and phenylhydrazine (3a, 130 mg, 1.2 mmol) in AcOH (4 mL)-36% HCl (1 mL) was heated under reflux for 5 h. After the reaction completed, a small amount of ice water was added, then the mixture was neutralized with aqueous saturated NaHCO$_3$. Products were extracted with ethyl acetate (30 mL x 3), and the extracts were washed with brine, dried over Na$_2$SO$_4$, and concentrated on an evaporator. The crude product was used for the next step without further purification.

Fischer indole cyclization and the subsequent esterification, giving 8c, 8d, and 10 (Method B):

A mixture of 2b (1.0 mmol) and 3a (1.2 mmol) were dissolved in AcOH-HCl (4:1, 5 mL) and heated for 5 h under reflux. After the reaction completed, the yellow solids were collected by filtration. The solids thus obtained were dissolved in MeOH (10 mL) and SOCl$_2$ (238 mg, 2 mmol) was added. The mixture was stirred under reflux for 20 h. The solvents were evaporated under reduced pressure, a small amount of water was added, and neutralize to pH 8 with 1N NaOH. The solids 7c obtained by filtration was dried in vacuum overnight.
Methyl 6,11-dihydro-5H-pyrido[3,2-a]carbazole-2-carboxylate (8a): Pale yellowish solids in 72% yield, mp: 239–241 °C (after LC); IR (KBr) $\nu_{\text{max}}$ = 3225, 2947, 2889, 2839, 1937, 1898, 1821, 1728, 1601, 1551, 1481, 1441, 1440, 1362, 1317, 1294, 1244, 1119, 1051, 1009, 993, 978, 922, 910, 870, 810, 760, 748 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.93 (s, 1H), 8.39 (s, 1H), 8.16 (s, 1H), 7.58 (d, $J$ = 8.0 Hz, 1H), 7.41 (d, $J$ = 8.4 Hz, 1H), 7.25 (t, $J$ = 7.2 Hz, 1H), 7.16 (t, $J$ = 7.2 Hz, 1H), 3.98 (s, 3H), 3.34 (t, $J$ = 8.0 Hz, 2H), 3.15 (t, $J$ = 8.0 Hz, 2H); $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 165.9, 161.9, 147.1, 138.3, 130.9, 127.6, 126.6, 125.3, 124.5, 123.0, 119.8, 119.4, 112.5, 112.1, 52.8, 32.4, 18.9. HRMS (ESI) calcd for $\text{C}_{17}\text{H}_{15}\text{N}_{2}\text{O}_2$ (MH$^+$): 279.1134, found: 279.1128 (MH$^+$).

Methyl 8-bromo-6,11-dihydro-5H-pyrido[3,2-a]carbazole-2-carboxylate (8b): Pale yellowish solids in 74% yield, mp: 293–295 °C (after LC); IR (KBr) $\nu_{\text{max}}$ = 3229, 2953, 2841, 1886, 1822, 1726, 1616, 1601, 1550, 1487, 1443, 1397, 1360, 1312, 1296, 1248, 1220, 1128, 1041, 1013, 990, 972, 934, 968, 812, 764 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 12.00 (br s, 1H), 8.79 (s, 1H), 8.48 (s, 1H), 7.72 (s, 1H), 7.35 (d, $J$ = 8.4 Hz, 1H), 7.23 (d, $J$ = 8.4 Hz, 1H), 3.91 (s, 3H), 3.21 (t, $J$ = 8.0 Hz, 2H), 3.03 (t, $J$ = 8.0 Hz, 2H); $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 165.8, 162.1, 147.6, 136.9, 132.4, 128.4, 128.1, 125.3, 124.8, 124.5, 121.7, 114.0, 112.3, 112.0, 51.8, 28.4, 27.8. HRMS calcd for $\text{C}_{17}\text{H}_{13}\text{BrN}_{2}\text{O}_2$: 356.0160, found: 356.0201.
Methyl 6-methyl-6,11-dihydro-5H-pyrido[3,2-a]carbazole-2-carboxylate (8c): yellow solids in 91% yield of the two steps, mp: 163–165 °C (after LC); IR (KBr) νmax = 3555, 3142, 2953, 2482, 2012, 1892, 1730, 1713, 1605, 1553, 1479, 1441, 1412, 1364, 1323, 1308, 1290, 1277, 1233, 1136, 1076, 1049, 117, 980, 922, 882, 812, 764, 743 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 11.80 (s, 1H), 8.81 (d, J = 1.6 Hz, 1H), 8.56 (d, J = 1.6 Hz, 1H), 7.61 (d, J = 7.6 Hz, 1H), 7.40 (d, J = 8.0 Hz, 1H), 7.15 (d, J = 7.6 Hz, 1H), 7.03 (d, J = 7.6 Hz, 1H), 3.92 (s, 3H), 3.46–3.35 (m, 2H), 3.03 (dd, J = 16.4, 7.2 Hz, 1H), 1.24 (d, J = 6.0 Hz, 3H); ¹³C NMR (100 MHz, DMSO-d₆) δ 165.7, 160.7, 146.4, 141.5, 138.5, 129.6, 128.1, 126.0, 125.1, 124.9, 123.1, 119.9, 119.7, 118.0, 112.2, 52.9, 26.2, 21.4. HRMS calcd for C₁₈H₁₆N₂O₂: 292.1212, found: 292.1182.

Methyl 6-phenyl-6,11-dihydro-5H-pyrido[3,2-a]carbazole-2-carboxylate (8d): yellow solids in 95% yield of the two steps, mp: 233–235 °C (after LC); IR (KBr) νmax = 3096, 2499, 2002, 1890, 174, 1630, 1564, 1497, 1454, 1439, 1417, 1352, 1325, 1292, 1215, 1126, 109, 985920, 895, 814, 743 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 12.05 (s, 1H), 8.77 (d, J = 13.6 Hz, 2H), 7.42 (d, J = 8.4 Hz, 1H), 7.24–7.18 (m, 6H), 6.99 (d, J = 8.0 Hz, 1H), 6.88 (t, J = 7.6 Hz, 1H), 4.70 (t, J = 7.2 Hz, 1H), 3.95 (s, 3H), 3.71 (dd, J = 16.8, 7.6 Hz, 1H), 3.45 (dd, J = 16.8, 6.4 Hz, 1H); ¹³C NMR (100 MHz, DMSO-d₆) δ 165.1, 144.4, 138.7, 130.4, 129.6 (2C), 129. (2C), 127.8 (2C), 127.1, 126.1 (2C), 125.7, 123.5, 120.0, 119.9, 115.8, 112.3, 53.1 (2C), 38.4, 37.2. HRMS calcd for C₂₃H₁₈N₂O₂: 354.1368, found: 354.1369.
**Methyl 8-methoxy-6,11-dihydro-5H-pyrido[3,2-a]carbazole-2-carboxylate (8e):** Pale yellowish solids in 48% yield, mp: 263–265 °C (after LC); IR (KBr) *v*<sub>max</sub> = 3240, 3194, 2991, 2935, 2825, 2359, 1728, 1629, 1558, 1512, 1491, 1435, 1369, 1323, 1294, 1263, 1247, 1217, 1109, 1055, 1033, 999, 813, 790, 765, 810, 760 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 11.56 (s, 1H), 8.75 (s, 1H), 8.43 (s, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 6.84 (d, *J* = 8 Hz, 1H), 6.78 (t, *J* = 4 Hz, 1H), 3.89 (d, *J* = 8 Hz, 3H), 3.75 (s, 3H), 3.18 (t, *J* = 4 Hz, 2H), 3.01 (t, *J* = 8.0 Hz, 2H); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 165.9, 161.8, 154.9, 146.9, 133.4, 131.4, 127.4, 126.9, 124.4, 120.9, 114.2, 112.8, 112.3, 100.7, 55.7, 52.7, 32.4, 24.2.

**Methyl 6,6-dimethyl-6,11-dihydro-5H-pyrido[3,2-a]carbazole-2-carboxylate (10):** Pale yellowish solids in 74% yield, mp: 217–220 °C (after LC); IR (KBr) *v*<sub>max</sub> = 3433, 3096, 2949, 2498, 2021, 1904, 1744, 1630, 1560, 1499, 1456, 1416, 1325, 1304, 1267, 1223, 1194, 1136, 1119, 1096, 1034, 1013, 980, 924, 882, 814, 760 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 12.03 (s, 1H), 8.80 (s, 1H), 8.77 (s, 1H), 7.75 (s, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.16 (t, *J* = 6.6 Hz, 1H), 7.02 (t, *J* = 8.6 Hz, 1H), 3.93 (s, 3H), 3.21 (s, 2H), 1.41 (s, 6H); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 164.8, 158.8, 143.4, 138.8, 130.1, 128.3, 126.2, 126.0, 125.2, 123.4, 121.9, 121.0, 120.1, 112.5, 53.2, 46.3, 33.4, 28.9 (2C). HRMS calcd for C<sub>19</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub>: 306.1368, found: 306.1319.
Preparation of methyl 11H-pyrido[3,2-a]carbazole-2-carboxylate (1a), a general procedure: A mixture of 8a (140 mg, 0.5 mmol) and DDQ (227 mg, 1.0 mmol) was dissolved in benzene (10 mL) and then heated at reflux for 4 h under the monitoring of TLC (hexane:THF = 2:1). After the reactions completed, the reaction was quenched with aqueous saturated NaHCO$_3$ (30 mL) and extracted with ethyl acetate (30 mL x 3). The organic phase was collected and washed with saturated NaHCO$_3$ and brine to give 1a.

Methyl 11H-pyrido[3,2-a]carbazole-2-carboxylate (1a): Pale yellowish solids in 74% yield, mp: 272–275 °C (after LC) ; IR (KBr) $\nu_{\text{max}}$ = 3185, 2949, 1865, 1788, 1732, 1614, 1597, 1564, 1524, 1497, 1456, 1439, 1412, 1366, 1329, 1290, 1254, 1233, 1206, 1125, 1053, 1011, 991, 926, 854, 831, 810, 748 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.47 (s, 1H), 9.25 (s, 1H), 9.20 (br s, 1H), 8.49 (d, $J$ = 8.8 Hz, 1H), 8.17 (d, $J$ = 8.8 Hz, 1H), 7.99 (d, $J$ = 8.8 Hz, 1H), 7.65 (d, $J$ = 8.4 Hz, 1H), 7.52 (t, $J$ = 7.6 Hz, 1H), 7.38 (t, $J$ = 7.6 Hz, 1H), 4.06 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 165.9, 149.1, 148.3, 139.7, 135.6, 133.2, 126.3, 125.8, 122.9, 121.8, 120.6, 120.3, 120.1, 118.7, 115.4, 112.1, 52.9. HRMS calcd for C$_{17}$H$_{12}$N$_2$O$_2$: 276.0899, found: 276.0853.

Methyl 8-bromo-11H-pyrido[3,2-a]carbazole-2-carboxylate (1b): Pale yellowish solids in 81% yield, mp: 307–310 °C (after LC) ; IR (KBr) $\nu_{\text{max}}$ = 3163, 2953, 2214, 1881, 1794, 1724, 1614, 1593, 1564, 1522, 1479, 1445, 1408, 1366, 1333, 1292, 1273, 1258, 1229, 1206, 1115, 1044, 984,
926, 856, 831, 814, 764, 737 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 12.86 (s, 1H), 9.53 (d, \(J = 2.0\) Hz, 1H), 9.29 (d, \(J = 2.0\) Hz, 1H), 8.60 (d, \(J = 8.8\) Hz, 1H), 8.47 (s, 1H), 7.78 (d, \(J = 8.8\) Hz, 1H), 7.62–7.56 (m, 1H), 7.56–7.54 (m, 1H), 3.98 (s, 3H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 165.8, 149.3, 148.6, 138.4, 136.2, 133.2, 128.2, 126.5, 124.8, 123.2, 121.9, 120.5, 117.8, 115.4, 114.1, 112.5, 52.9. HRMS calcd for C\(_{17}\)H\(_{11}\)BrN\(_2\)O\(_2\): 354.0004, found: 353.9977.

**Methyl 6-methyl-11H-pyrido[3,2-\(a\)]carbazole-2-carboxylate (1c):** Pale yellowish solids in 85% yield, mp: 245–248 °C (after LC); IR (KBr) \(\nu_{\text{max}}\) = 3381, 3061, 2955, 1879, 1690, 1620, 1564, 1524, 1460, 1439, 1402, 1366, 1336, 1310, 1269, 1240, 1209, 1159, 1134, 1090, 1036, 988, 928, 856, 812, 772 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 12.71 (s, 1H), 9.49 (d, \(J = 1.2\) Hz, 1H), 9.23 (d, \(J = 1.2\) Hz, 1H), 8.20 (d, \(J = 8.0\) Hz, 1H), 7.68 (d, \(J = 8.0\) Hz, 1H), 7.55 (s, 1H), 7.47 (t, \(J = 7.6\) Hz, 1H), 7.29 (t, \(J = 7.6\) Hz, 1H), 3.97 (s, 3H), 2.94 (s, 3H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 165.9, 148.9, 148.4, 139.7, 139.5, 135.4, 132.7, 125.3, 123.2, 122.3, 120.9, 120.3, 120.1, 118.0, 113.7, 112.1, 52.7, 21.7. HRMS calcd for C\(_{18}\)H\(_{14}\)N\(_2\)O\(_2\): 290.1055, found: 290.1040.

**Methyl 6-phenyl-11H-pyrido[3,2-\(a\)]carbazole-2-carboxylate (1d):** Pale yellowish solids in 98% yield, mp: 263–266 °C (after LC); IR (KBr) \(\nu_{\text{max}}\) = 3377, 3057, 2953, 2361, 1723, 1694, 1620, 1559, 1528, 1495, 1454, 1362, 1323, 1310, 1275, 1238, 1198, 1123, 1103, 995, 934, 866, 812, 773, 745 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 12.98 (s, 1H), 9.66 (d, \(J = 2.0\) Hz, 1H), 9.32 (d, \(J = 2.0\) Hz, 1H), 7.70–7.59 (m, 7H), 7.41 (t, \(J = 7.6\) Hz, 1H), 7.31 (d, \(J = 8.0\) Hz, 1H), 7.04 (t, \(J = 8.0\) Hz, 1H), 4.00 (s, 1H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 165.9, 148.9, 148.6, 142.6, 140.1, 140.0, 136.1,
133.1, 129.2, 129.1, 128.8, 125.6, 122.4, 121.7, 121.6, 120.4, 120.0, 116.6, 114.5, 112.3, 52.9. 
HRMS calcd for C$_{23}$H$_{16}$N$_2$O$_2$ (MH$^+$): 352.1212, found: 352.1248.

**Oxidation of 10 with SeO$_2$ and rearrangement to form 11.**

To a solution of 10 (153 mg, 0.5 mmol) dissolved in an MeCN:H$_2$O (3:1, 40 mL), heated to reflux, was added SeO$_2$ (277 mg, 2.5 mmol) was added portion by portion (five times every one hour) and the reaction was continued for 12 h, as monitored by TLC (hexane:THF = 2:1). After the reaction finished, MeCN was removed under vacuum. The residue was extracted with ethyl acetate (50 mL x 3), and the organic layer was collected and washed with saturated NaHCO$_3$ and brine to give 11 and 12.

**Methyl 5,6-dimethyl-11H-pyrido[3,2-a]carbazole-2-carboxylate (11):** Pale yellowish solids in 36% yield, mp: 294–296 °C (after LC); IR (KBr) $\nu$$_{max}$ = 3362, 3051, 2922, 2854, 1871, 1703, 1609, 1566, 1508, 1449, 1418, 1373, 1350, 1327, 1302, 1265, 1229, 1134, 1109, 1086, 1049, 1028, 941, 873, 810 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 12.63 (s, 1H), 9.55 (d, $J$ = 1.6 Hz, 1H), 8.33 (d, $J$ = 2.4 Hz, 1H), 8.31 (d, $J$ = 8.0 Hz, 1H), 7.68 (d, $J$ = 8.0 Hz, 1H), 7.45 (d, $J$ = 8.0 Hz, 1H), 7.29 (t, $J$ = 7.6 Hz, 1H), 4.00 (s, 3H), 3.00 (s, 3H), 2.81 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 166.1, 147.5, 147.4, 139.8, 137.0, 133.7, 132.9, 125.1, 124.3, 123.3, 122.6, 120.4, 120.0, 118.1, 113.8, 112.0, 52.7, 18.2, 13.4. HRMS calcd for C$_{19}$H$_{16}$N$_2$O$_2$: 304.1212, found: 304.1263.
Methyl 6,6-dimethyl-5-oxo-6,11-dihydro-5H-pyrido[3,2-a]carbazole-2-carboxylate (12): yellow solid in 54% yield, mp: 265–268 °C (after LC); IR (KBr) $\nu_{\text{max}} = 3364, 3281, 1732, 1711, 1688, 1603, 1551, 1439, 1408, 1366, 1325, 1302, 1261, 1248, 1134, 1092, 1005, 924, 878, 810, 750 $ cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 12.06 (s, 1H), 9.04 (d, $J = 2.0$ Hz, 1H), 8.93 (d, $J = 2.0$ Hz, 1H), 7.79 (d, $J = 8.0$ Hz, 1H), 7.47 (d, $J = 8.0$ Hz, 1H), 7.21 (d, $J = 7.6$ Hz, 1H), 7.06 (t, $J = 7.6$ Hz, 1H), 3.98 (s, 3H), 1.59 (s, 6H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 201.3, 165.1, 148.4, 146.1, 139.0, 131.2, 130.3, 129.2, 125.4, 123.8, 120.7, 120.3, 112.8, 53.3, 46.3, 26.9. HRMS calcd for $\text{C}_{19}\text{H}_{16}\text{N}_{2}\text{O}_{3}$: 320.1161, found: 320.1141.

![Chemical structure of Methyl 6,6-dimethyl-5-oxo-6,11-dihydro-5H-pyrido[3,2-a]carbazole-2-carboxylate (12)](image)

Synthesis of 2- (methoxycarbonyl)-4-methyl-11H-pyrido[3,2-a]carbazol-4-ium iodide (13). A solution of 1a (40 mg, 0.145 mmol) and MeI (82.2 mg, 0.58 mmol) in DMF (1 mL) was stirred at 60 °C for 9 h. Removal of the volatile under reduced pressure, the solid were washed with ethyl acetate and hexane several times to obtain the gray solids, 51 mg, 84% yield; mp: >350 °C; IR (KBr) $\nu_{\text{max}} = 3451, 3078, 1721, 1632, 1601, 1547, 1503, 1458, 1435, 1393, 1368, 1333, 1294, 1273, 1252, 1221, 1165, 1126, 1105, 991, 937, 922, 797, 754 $ cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 10.32 (s, 1H), 9.95 (s, 1H), 9.19 (d, $J = 8.8$ Hz, 1H), 8.45 (d, $J = 6.8$ Hz, 1H), 8.20 (d, $J = 7.2$ Hz, 1H), 7.94 (s, 1H), 7.83 (d, $J = 8.0$ Hz, 1H), 7.66–7.64 (m, 1H), 7.46–7.44 (m, 1H), 4.77 (s, 3H), 4.09 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 163.1, 147.9, 140.9, 140.0, 138.1, 135.5, 132.1, 127.7, 121.8, 121.6, 121.5, 121.4, 120.8, 116.7, 112.6, 108.8, 53.8, 46.8. HRMS calcd for $\text{C}_{18}\text{H}_{15}\text{IN}_{2}\text{O}_{2}$: 418.0184, found: 418.0217.

3.6 Antitumor screening test. Anti-proliferative assays in vitro were performed in the same manner as described in previous Chapter 2.
References


Chapter 4

Synthesis and Antimalarial activity of Some Neocryptolepine Analogues Carrying a Multifunctional Linear and Branched Carbon-Side Chains
4.1 Abstract

The synthesis and *in vitro* antimalarial activity of several neocryptolepine analogues carrying either a linear or branched dibasic side chain at C11 were described. Many of these neocryptolepine analogues have subnanomolar antimalarial activity against the chloroquine-sensitive *P. falciparum* (NF54). The data also demonstrated that the branched structure motif is not superior for the activity over a linear side chain, but their thioureido derivatives showed lower cytotoxicity than the linear one. Ureido and thioureido derivatives showed also higher β-haematin inhibition than the corresponding free amines.
4.2 INTRODUCTION

The emergence and spread of chloroquine-resistant *Plasmodium falciparum* parasites has been a major global health problem and contributes significantly to the continued high prevalence of malaria.\(^1\)\(^2\) New, safe, and effective drugs active against multidrug resistant *P. falciparum* strains are thus urgently needed.\(^1\)\(^3\) Medicinal plants have long been used for treating parasitic diseases, including malaria, and constitute an important source of new molecules for lead optimization programs, as exemplified by the success of artemisinin and its derivatives.\(^4\)\(^-\)\(^9\) Neocryptolepine, \(\text{1}\) (Figure 1), is an indolo[2,3-\(b\)]quinoline alkaloid isolated as a minor alkaloid alongside with its major regio-isomer cryptolepine \(\text{2}\) from the roots of *Cryptolepis sanguinolenta*, a shrub used in traditional medicine for the treatment of malaria in Central and West Africa.\(^10\)\(^11\)

![Figure 1. Indoloquinolines from *Cryptolepis sanguinolenta*](image)

In addition, neocryptolepine \(\text{1}\) also exerts a broad range of potential biological applications\(^12\)\(^-\)\(^22\) and appeared to have lower cytotoxicity compared to cryptolepine.\(^23\)\(^-\)\(^24\) However, several neocryptolepine analogues have been described for DNA interfering activity and are reported as anticancer drugs.\(^21\)\(^22\) Based on our recent findings, it was speculated that substitution of neocryptolepine could be favorable for more potent and selective antimalarial activities and several series of substituted neocryptolepines have been synthesized.\(^12\)\(^-\)\(^20\) These promising results prompted us to investigate structure-activity relationships (SAR) to the structural requirements of side-chains at C-11 of the neocryptolepine scaffold for improved antiplasmodial activity and selectivity relative to the lead compound, neocryptolepine \(\text{1}\). In this study, we tried to explore a set of neocryptolepine analogues having diversified side-chain by varying the structure and length of the linker between the two nitrogen atoms as well as the substitution pattern and basicity of the distal amino group.
4.3 Results and Discussion

4.3.1 Chemistry

The synthetic strategy of neocryptolepine analogues 8a–g was based on the nucleophilic aromatic substitution ($S_{N}$Ar) reaction of the key intermediate 11-chloro-substituted neocryptolepines 7 obtained via Scheme 1. This method was used for synthesis of neocryptolepines with substitutions on B ring (C-11 position). Thus, a series of neocryptolepines with different side chains at C-11 were prepared starting from methyl 1H-indole-3-carboxylate 3 and N-methylaniline derivatives 4. The intermediate methyl 2-(phenylamino)-1H-indole-3-carboxylates 5 were obtained via chlorination with N-chlorosuccinimide in the presence of 1,4-dimethylpiperazine followed by addition of the aniline derivative as trichloroacetate salt. Cyclization of 5 was achieved by heating in boiling diphenyl ether to afford 5,6-dihydro-11H-indolo[2,3-b]quinolin-11-ones 6, which was dehydrochlorinated with POCl$_3$ to give 11-chloroneocryptolepines 7, the key intermediate for the diversification. Subsequent amination of 7 with various 1,2-diaminoethanes and 1,3-diaminopropanes in DMF by heating yielded the target compounds 8a–g as depicted in Scheme 1.

Scheme 1: Synthesis of indolo[2,3-b]quinolones with substituents at C2 and C11. Reagents and conditions: (i) (a) N-chlorosuccinimide, 1,4-dimethylpiperazine, CH$_2$Cl$_2$, 0°C, 2h; (b) trichloroacetic acid, RT, 2 h; (ii) diphenyl ether, reflux, 1–3 h; (iii) POCl$_3$, toluene, reflux, 6–12 h; (iv) appropriate amines, 120 °C. 4h.
4.3.2 Antiplasmodial Activity and Cytotoxicity

The synthesized compounds 8a–g were evaluated for their in vitro antimalarial activity against the chloroquine-sensitive *P. falciparum* (NF54). The corresponding IC$_{50}$ values together with their cytotoxicity determined using mammalian L6 cells are presented in Table 1. In this study, we have chosen the side chain of branched structure for the aminoalkylamino substituent at the C11, because incorporation of such shorter side chain variant proved to be of important element for the antimalarial activity.$^{25,26}$ The side chains at C11 of 8 were introduced by the reaction of 7 with branched 1,2-aminoethane or 1,3-aminopropanes, and their in vitro antimalarial activity was compared with that of 7 with no branched aminoalkylamino substituent at C-11. As it appears from the data, IC$_{50}$ values for compounds 8a–g range from 11.8 to 232.5 nM, which represent a significant improvement in antiplasmodial activity over the neocryptolepine 1 (1580 nM), but not as good as the well-known antimalarial drugs, artemisinin (4.3 nM) and chloroquine (9.4 nM).

Inspection of the data in Table 1 allows the following conclusions to be drawn. First, compounds containing linear 3-aminopropylamino group with a three carbon spacer, e.g., 8a, 8c, generally present better antiplasmodial activity than those with the corresponding branched carbon atoms, e.g., 8b, 8d. The type of pendant group residing on the spacer also has influence on antiplasmodial activity, for example, replacing the dimethyl group in 8d with hydroxy group as in 8e substantially improved the antiplasmodial activity. Also the number of the pendant methyl groups residing on the spacer has an influence as it appears when the results of side-chain with two carbon atom spacer and branched with one methyl group, e.g., 8e, and two methyl groups, e.g., 8g, are compared. Thus, the results showed that such variation has a slight effect on activity but induce a remarkable effect on cytotoxicity and subsequently improved selectivity index (SI) against the parasite, namely, SI of 8g is over 100. It should also be noted that the antiplasmodial activity as well as the selectivity indices increased by adding a chlorine substituent at C-2 on the A-ring in combination with 11-aminoalkylamino side-chain on neocryptolepine core, as in compounds 8c and 8d, when compared with the corresponding no chlorinated analogues 8a and 8b.
Table 1. The antiplasmodial activity against *P. falciparum* (CQS: NF54) and cytotoxicity towards mammalian L6 cells of the neocryptolepine derivatives 8a–g

<table>
<thead>
<tr>
<th>Compound</th>
<th>R&lt;sup&gt;1&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Yield %</th>
<th>L6 cells IC&lt;sub&gt;50&lt;/sub&gt; nM&lt;sup&gt;b&lt;/sup&gt;</th>
<th>NF 54 IC&lt;sub&gt;50&lt;/sub&gt; nM&lt;sup&gt;b&lt;/sup&gt;</th>
<th>SI&lt;sup&gt;a&lt;/sup&gt;</th>
<th>β-Heamatin inhibition IC&lt;sub&gt;50 &lt;/sub&gt;μM&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neocryptolepine</td>
<td>H</td>
<td>H</td>
<td>-</td>
<td>3194</td>
<td>1580</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>H</td>
<td>Cl</td>
<td>-</td>
<td>1459</td>
<td>2055</td>
<td>0.7</td>
<td>-</td>
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<tr>
<td>8a</td>
<td>H</td>
<td>NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>96</td>
<td>279.2</td>
<td>78.8</td>
<td>3.5</td>
<td>-</td>
</tr>
<tr>
<td>8b</td>
<td>H</td>
<td>NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>84</td>
<td>1475.0</td>
<td>232.5</td>
<td>6.3</td>
<td>293.90</td>
</tr>
<tr>
<td>8c</td>
<td>Cl</td>
<td>NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>92</td>
<td>268.6</td>
<td>11.8</td>
<td>22.8</td>
<td>-</td>
</tr>
<tr>
<td>8d</td>
<td>Cl</td>
<td>NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>95</td>
<td>2175.5</td>
<td>108.2</td>
<td>20.1</td>
<td>748.50</td>
</tr>
<tr>
<td>8e</td>
<td>Cl</td>
<td>NH&lt;sub&gt;2&lt;/sub&gt;OH</td>
<td>82</td>
<td>1352.8</td>
<td>69.9</td>
<td>19.4</td>
<td>73.02</td>
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<tr>
<td>8f</td>
<td>Cl</td>
<td>NH&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;</td>
<td>87</td>
<td>675.9</td>
<td>27.4</td>
<td>24.7</td>
<td>156.90</td>
</tr>
<tr>
<td>8g</td>
<td>Cl</td>
<td>NH&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;</td>
<td>90</td>
<td>2644.1</td>
<td>26.1</td>
<td>101.3</td>
<td>138.10</td>
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<tr>
<td>Podphylo toxin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>14.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Artemisinin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.3</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup>Selectivity Index is the ratio of IC<sub>50</sub> for cytotoxicity versus antiplasmodial activity (L6/P.f.).<sup>b</sup>The IC<sub>50</sub> values are the means of two independent assays; the individual values vary less than a factor 2.

Based on the above results, we have further studied the influence of substituent modification around the terminal nitrogen atom in compounds 8 on antimalarial activity. In the previous paper, we demonstrated that the installation of rigid ureido functionality in the terminal of 3-aminopropylamino-substituent at C11 of neocryptolepine core improved both the antimalarial activity and selectivity index. Thus, the ureido derivative from 8c showed IC<sub>50</sub> 2.2 nM and SI 1400, respectively, which are 5.3 and 61 times higher than those of 8c, respectively. Accordingly, a series of ureido and thioureido derivatives 9, 10 were prepared in high yields by modification of...
free terminal amines of 8 with phenylisocyanate and isothiocyanate in dry CH$_2$Cl$_2$ at room temperature, respectively, as shown below.

**Table 2.** The antiplasmodial activity against *P. falciparum* (CQS: NF54) and cytotoxicity towards mammalian L6 cells of the neocryptolepine derivatives 9a–g

<table>
<thead>
<tr>
<th>Compound</th>
<th>R$^1$</th>
<th>R$^3$</th>
<th>Yield, %</th>
<th>L6 cells IC$_{50}$ nM$^b$</th>
<th>NF 54 IC$_{50}$ nM$^b$</th>
<th>SI$^a$</th>
<th>L6/NF54 IC$_{50}$ μM$^b$</th>
<th>β-Heamatin inhibition IC$_{50}$ μM$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>9a</td>
<td>H</td>
<td>Me</td>
<td>91</td>
<td>5647.1</td>
<td>68.7</td>
<td>82.2</td>
<td>26.34</td>
<td>10.07</td>
</tr>
<tr>
<td>9b</td>
<td>Cl</td>
<td>Me</td>
<td>84</td>
<td>1179.0</td>
<td>19.1</td>
<td>61.7</td>
<td>10.07</td>
<td></td>
</tr>
<tr>
<td>10a</td>
<td>H</td>
<td>Me</td>
<td>90</td>
<td>7249.3</td>
<td>57.3</td>
<td>126.5</td>
<td>19.97</td>
<td></td>
</tr>
<tr>
<td>10b</td>
<td>Cl</td>
<td>Me</td>
<td>79</td>
<td>3266.5</td>
<td>19.9</td>
<td>164.1</td>
<td>34.11</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Selectivity Index is the ratio of IC$_{50}$ for cytotoxicity versus antiplasmodial activity (L6/P.f.).

The antiplasmodial activity of the compounds 9 and 10 was measured against *P. falciparum* (CQS: NF54) and the results are listed in Table 2. All compounds within this series showed antimalarial activity against CQS strains (NF54) with high selectivity indices and reveals promising drug discovery leads. More importantly, ureido and thioureido derivatives 9b, and 10b with branched 3-carbon spacer in combination with chlorine atom at C2 on neocryptolepine core showed the best
activity in this series. It should be noted that compounds with a thioureido functionality are more potent than the corresponding ureido analogues. Comparing 9b with 10b, the antiplasmodial activities of them were almost same, but 10b bearing thioureido group shows the 3 times higher SI.

4.3.3 β-Haematin Inhibition Testing

Some studies revealed that the CQ effective as antimalarial drug might be ascribed to its capability of docking with the fastest growing face of the haemozoin crystal. The quinoline ring of CQ interacts with ferriprotoporphyrin IX (Fe(III)PPIX) by π-π stacking, as well as the 4-amino group of CQ interacts with heamtin by electrostatic interaction. This heamatin-CQ complex directly exerts a toxic effect on the parasite. With these backgrounds, we tested the β-haematin inhibition of the 11-(ω-aminoalkylamino)indolo[2,3-b]quinolones and their ureido and thioureido derivatives. The compounds 8b, d, f, g with a branched ω-aminoalkylamino substituent showed weak β-haematin inhibition. The compound 8e with a hydroxylated pendant showed slightly increased β-haematin inhibition, which may be attributed to the improved hydrophilicity of the polar hydroxy group. The compound 9 and 10 with ureido and thioureido functionality showed higher β-haematin inhibition, especially 9b showed the highest β-haematin inhibition with IC₅₀ value of 10.07 μM.

Figure 2. Antiplamodial activity and β-haematin inhibition
4.4 EXPERIMENTAL

The commercially obtained reagents were directly used without further purification. The $^1$H NMR, $^{13}$C NMR spectra were measured on the Varian INOVA-600 or Varian INOVA-400 Varian or INOVA-300 spectrometer, using CDCl$_3$ or DMSO-d$_6$ as solvent and tetramethylsilane (TMS) as the internal standard. HRMS were obtained on a Bruker microTOF II-SKA spectrometer. Melting points were determined on a J-Science RFS-10 hot stage microscope. Compound 8a and 8b was described in details in our previous work.

General procedure for the synthesis of 11-aminoneocryptolpines 8a-8g

11-chloroindoloquinolines 7 (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 yellow crude product was purified by flash chromatography using AcOEt-2N ammonia in MeOH (9:1v/v) as an eluent to yield pure yellowish solids product.

\[
\text{N-(3-Amino-2,2-dimethylpropyl)-5-methyl-5\text{H}-indolo[2,3-b]quinolin-11-amine (8b): Yield 280 mg (84%), yellow solids; mp 112–114 °C; }^1\text{H NMR (300MHz, CDCl}_3) \delta 8.39 (\text{br s, 1H}), 8.11 (d, J = 8.3 Hz, 1H), 7.76 (d, J = 8.0 Hz, 1H), 7.61 (dt, J = 17.0, 8.5 Hz, 2H), 7.39–7.33 (m, 1H), 7.29–7.24 (m, 1H), 7.19–7.16 (m, 1H) 4.20 (s, 3H), 3.83 (s, 2H), 2.83 (s, 2H), 0.86 (s, 6H). \]
\[
^1\text{C NMR (151MHz, CDCl}_3) \delta 157.56, 152.53, 149.42, 138.20, 130.25 (d), 125.02, 124.20, 123.58, 122.22, 120.55, 118.59, 117.32, 116.20, 114.73, 104.53, 61.79, 53.35, 36.03, 32.95 (d), 23.92 (d) (2C). HRMS (ESI) calcd for C$_{21}$H$_{24}$N$_4$ [M-H]. Exact mass: 332.4421, found 331.4462.
\]
**N-(3-Amino-2,2-dimethylpropyl)-2-chloro-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (8d):**

Yield 248 mg (95%), yellow solids; mp 177–179 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.07 (d, $J$ = 1.9 Hz, 1H), 7.97(d, $J$ = 8 Hz, 1H), 7.76 (d, $J$ = 7.9 Hz, 1H), 7.54 (dd, $J$ = 5.0, 4.1 Hz, 1H), 7.49 (t, $J$ = 7.4 Hz, 1H), 7.41 (t, $J$ = 7.6 Hz, 1H), 7.18 (t, $J$ = 7.5 Hz, 1H), 4.18 (s, 3H), 3.78 (s, 2H), 2.84 (s, 2H), 0.86 (s, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 148.44, 136.82, 130.40 (2C), 126.31, 125.70 (2C), 124.69, 124.02 (2C), 122.47 (2C), 119.21, 117.54, 116.36, 62.08, 53.49, 36.01, 33.41, 24.09 (2C). HRMS (ESI) calcd for C$_{21}$H$_{23}$ClN$_4$ [M-H]$^-$. Exact mass: 366.1611, found 365.1624.

**1-Amino-3-(2-chloro-5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propan-2-ol (8e):** Yield 290 mg (82%), yellow solids; mp 150–152 °C; $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.56 (d, $J$ = 2.1 Hz, 1H), 7.95 (d, $J$ = 7.8 Hz, 1H), 7.85 (d, $J$ = 9.2 Hz, 1H), 7.78 (dd, $J$ = 9.1, 2.1 Hz, 1H), 7.50 (d, $J$ = 7.8 Hz, 1H), 7.28 (t, $J$ = 7.5 Hz, 1H), 7.07 (d, $J$ = 14.2 Hz, 1H), 4.13 (s, 3H), 3.91–3.87 (m, 1H), 3.79 (dd, $J$ = 12.8, 6.5 Hz, 2H), 3.69–3.66 (m, 2H). $^{13}$C NMR (101MHz, DMSO-d$_6$) $\delta$ 156.17, 152.45, 147.55, 136.10, 130.17, 124.92 (2C), 123.91, 123.40, 122.21, 118.19, 117.01, 116.77, 116.67, 105.46, 70.97, 52.32, 45.41, 32.34. HRMS (ESI) calcd for C$_{19}$H$_{19}$ClN$_4$O [M-H]$^-$. Exact mass: 354.1247, found 353.1275.
N-(2-chloro-5-methyl-5H-indolo[2,3-b]quinolin-11-yl)propane-1,2-diamine (8f): Yield 297 mg (87%), yellow solids; mp 95–79 °C; $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.10 (d, $J = 5.7$ Hz, 2H), 7.76 (d, $J = 7.9$ Hz, 1H), 7.61–7.59 (m, 1H), 7.54 (d, $J = 9.1$ Hz, 1H), 7.44 (t, $J = 7.6$ Hz, 1H), 7.22 (t, $J = 7.5$ Hz, 1H), 6.48 (br, 1H), 4.24–4.18 (m, 3H), 3.79 (d, $J = 11.8$ Hz, 1H), 3.40 (t, $J = 6.0$ Hz, 1H), 3.12 (dd, $J = 4.4$, 1.7 Hz, 1H), 1.10 (d, $J = 6.3$ Hz, 6H). $^{13}$C NMR (151MHz, CDCl$_3$) $\delta$ 156.16, 152.66, 147.82(d), 136.95(d), 130.52, 130.41 (d), 127.01, 126.22 (d), 124.45, 121.75(d), 119.41(d), 117.57, 116.40 (d), 107.63, 55.20 (d), 47.90 (d), 33.17(d), 22.95(d). HRMS (ESI) calcd for C$_{19}$H$_{19}$ClN$_4$ [M-H]$^-$. Exact mass: 338.1298, found 337.1232.

N-(2-Amino-2-methylpropyl)-2-chloro-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (8g): Yield 320 mg (90%), yellow solids; mp 108–110 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.15 (dd, $J = 12.5$, 4.9 Hz, 2H), 7.76 (d, $J = 8.0$ Hz, 1H), 7.62 (dt, $J = 19.8$, 5.3 Hz, 2H), 7.44 (t, $J = 7.6$ Hz, 1H), 7.21 (t, $J = 7.5$ Hz, 1H), 6.73 (br, 1H), 4.22 (d, $J = 0.4$ Hz, 3H), 3.58 (d, $J = 4.5$ Hz, 2H), 1.11 (s, 6H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 156.47, 152.91, 148.22, 137.17, 130.43 (d), 126.12 (2C), 124.58(2C), 121.64 (2C) 119.40(d), 117.67, 116.83(d), 107.63, 59.14, 51.50, 33.16, 29.50(2C). HRMS (ESI) calcd for C$_{20}$H$_{21}$ClN$_4$ [M-H]$^-$. Exact mass: 352.1455, found 351.1422.

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General procedure for the synthesis of Compounds 9a-9f.

2-Substituted 5-methyl-5H-indolo[2,3-b]quinolone-11-amine (8a-g, 50 mg) was completely dissolved in dry CH₂Cl₂ (1 mL), and then a solution of isocyanate or isothiocyanate (1.1equiv) and dry CH₂Cl₂ (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, and the reaction mixture was evaporated to dryness under reduced pressure. The crude product was purified by flash chromatography using AcOEt-2N ammonia in MeOH (9:1v/v) as an eluent to yield pure products as yellowish solids.

1-(2,2-dimethyl-3-(5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-3-phenylurea (9a):
Yield 412 mg (91%), yellow solids; mp 154–156 °C; ¹H NMR (600 MHz, CDCl₃)  δ 9.25 (s, 1H), 8.42 (d,  J = 5.7 Hz, 1H), 7.74 – 7.70 (m, 2H), 7.64 (d,  J = 5.6 Hz, 1H), 7.47 (s, 2H), 7.40 – 7.35 (m, 2H), 7.24 – 7.22 (m, 2H), 7.14 (d,  J = 2.8 Hz, 1H), 6.97 (d,  J = 2.9 Hz, 2H), 6.77 (s, 1H), 6.64 (s, 1H), 4.03 (d,  J = 2.8 Hz, 3H), 3.60 (s, 2H), 3.25 (s, 2H), 2.29 (br, 1H), 0.63 (d,  J = 2.2 Hz, 6H). ¹³C NMR (151MHz, CDCl₃)  δ 158.01, 157.18, 151.56, 149.62, 140.05, 137.95, 130.96, 129.53(2C), 126.04, 124.40, 124.25, 123.19, 122.67, 121.99, 120.00 (2C), 119.73, 116.93, 116.59, 115.04, 106.85, 55.71, 48.02, 38.61, 33.30, 24.07(2C). HRMS (ESI) calcd for C₂₈H₂₉N₆O [M-H]⁻. Exact mass: 451.2372, found 450.2364.
1-(3-(2-chloro-5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)-2,2-dimethylpropyl)-3-phenylurea (9b): Yield 410 mg (84%), yellow solids; mp 232–234 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.37 (s, 1H), 8.13 (br, 1H), 7.73 – 7.70 (m, 2H), 7.60 – 7.57 (m, 1H), 7.45 (dd, $J = 8.0$, 4.7 Hz, 3H), 7.39 (t, $J = 7.4$ Hz, 1H), 7.14 – 7.06 (m, 2H), 7.31 (t, $J = 7.2$ Hz, 2H), 6.53 (br, 1H), 5.91 (br, 1H), 4.12 (d, $J = 5.5$ Hz, 3H), 3.57 (d, $J = 6.2$ Hz, 2H), 3.31 (d, $J = 6.0$ Hz, 2H), 0.73 (s, 2H), 0.73 (s, 6H).$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 157.75, 156.80, 148.24, 139.72, 136.45, 130.92, 129.70(2C), 127.36, 126.62, 124.04(2C), 123.94, 123.75, 122.68, 120.69 (2C), 120.01, 117.92, 116.98, 116.46, 106.85, 55.97, 48.31, 38.44, 33.49, 24.18(2C). HRMS (ESI) calcd for C$_{28}$H$_{28}$ClN$_5$O $[M-H]^-$. Exact mass: 485.1982, found 482.1918.

1-(2,2-dimethyl-3-(5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propyl)-3phenylthiourea (10a): Yield 420 mg (90%), yellow solids; mp 172–174 °C; $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.54 (d, $J = 7.7$ Hz, 1H), 7.86 (d, $J = 7.0$ Hz, 1H), 7.76 (dd, $J = 15.1$, 7.2 Hz, 2H), 7.66 (d, $J = 8.1$ Hz, 1H), 7.46 (t, $J = 6.7$ Hz, 1H), 7.39 (dd, $J = 17.1$, 8.2 Hz, 3H), 7.33 (d, $J = 6.4$ Hz, 2H), 7.24 (dd, $J = 18.7$, 8.2 Hz, 3H).
11.6 Hz, 1H), 6.89 (s, 1H), 4.24 (s, 3H), 3.79 (d, \( J = 4.2 \) Hz, 2H), 3.73 (d, \( J = 4.2 \) Hz, 2H), 1.96 (br, 1H), 0.72 (s, 6H). \(^{13}\)C NMR (151MHz, CDCl\(_3\)) \( \delta \) 182.53, 153.42, 151.07, 145.53, 138.59, 137.49, 132.00, 129.50(2C), 126.44, 126.24, 125.25 (2C), 125.01, 123.39, 123.16, 122.73, 121.14, 117.18, 115.42, 115.19, 103.55, 54.75, 51.84, 39.27 (d), 34.68, 24.30(d) (2C).

\[ C_{28}H_{29}N_5S [M-H]^- \]

Exact mass: 467.2144, found 466.2170

\[ 1-(3-(2-chloro-5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)-2,2-dimethylpropyl)-3-phenylthiourea (10b): \]

Yield 401 mg (79%), yellow solids; mp 155–157 °C; \(^1\)H NMR (600 MHz, CDCl\(_3\)) \( \delta \) 8.56 (br, 1H), 8.50 (d, \( J = 3.8 \) Hz, 1H), 7.87 – 7.85 (m, 1H), 7.73 – 7.71 (m, 1H), 7.60 (dd, \( J = 8.7, 6.3 \) Hz, 1H), 7.51 (dd, \( J = 9.0, 6.2 \) Hz, 1H), 7.42 – 7.37 (m, 3H), 7.31 (ddd, \( J = 10.8, 9.8, 4.6 \) Hz, 3H), 7.19 (dd, \( J = 13.6, 6.8 \) Hz, 1H), 6.61 (br, 1H), 4.15 (d, \( J = 6.2 \) Hz, 3H), 3.76 (d, \( J = 5.9 \) Hz, 2H), 3.65 (t, \( J = 5.8 \) Hz, 2H), 1.99 (br, 1H), 0.66 (d, \( J = 6.0 \) Hz, 6H). \(^{13}\)C NMR (151MHz, CDCl\(_3\)) \( \delta \) 182.15, 157.42, 152.91, 147.77, 136.64, 136.55, 130.86, 130.67 (2C), 128.15, 127.22, 126.51, 126.00 (2C), 124.11(2C), 122.96, 119.60, 117.89, 117.53, 116.46, 108.04, 54.79, 52.92, 39.13, 33.42, 24.15 (2C). HRMS (ESI) calcd for C\(_{28}\)H\(_{28}\)ClN\(_5\)S [M-H]^- . Exact mass: 501.1754, found 500.1777
4.5 REFERENCES (AND NOTES)


Chapter 5

Synthesis and in Vitro Antiproliferative Activity of 11-Modified Neocryptolepine with Branched $\omega$-Aminoalkylamino Chains
5.1 Abstract

The present report describes the synthesis and antiproliferative evaluation of several neocryptolepine analogues carrying branched, functionalized dibasic side chain at C11. These 2-substituted 5-methyl indolo[2,3-b]quinoline derivatives were prepared by nucleophilic aromatic substitution ($S_N$Ar) reaction of 11-chloroneocryptolepines with appropriate 1,2- and 1,3-diamines. Some of the 11-(ω-aminoalkylamino) derivatives were further transformed into 11-ureido and thioureido analogues. Many of the prepared neocryptolepine derivatives showed antiproliferative activity of less than μM against human leukemia MV4-11 cell line. Among them, 11-(3-amino-2-hydroxy)propylamino derivative 2h and 2k were the most cytotoxic with a mean IC$_{50}$ value of 0.042 μM / 0.057μM against MV4-11 cell line, 0.197/0.1988 μM against A549 cell line, and 0.138/0.117 μM against BALB/3T3 cell line.

\[
\begin{align*}
R^1 &= \text{Cl} \\
R^2 &= \text{OH}, R^3 = \text{H} \\
\text{high anticancer activity} \\
n &= 0 \text{ or } 1
\end{align*}
\]
5.2 Introduction

The tetracyclic indoloquinoline ring systems constitute an important structural motif in natural products exhibiting numerous biological activities.\textsuperscript{1-4} For example, cryptolepine (I, indolo[3,2-b]quinolone) and neocryptolepine (II, indolo[2,3-b]quinolone) are representative alkaloids isolated from the roots of the African plant \textit{Cryptolepis sanguinolenta}.\textsuperscript{5-7} Notably, an aqueous macerate or decoction of this plant is used in traditional medicine against malaria.\textsuperscript{8} These two alkaloids, which only differ by the respective orientation of their indole and quinoline component part, display potent antiparasitic properties.\textsuperscript{9-12} Due to the linearly arranged tetracyclic plane structure, cryptolepine I and neocryptolepine II are DNA intercalating agents and inhibit topoisomerase II, showing a high level of cytotoxicity.\textsuperscript{13-16}

\begin{figure}[h]
\centering
\includegraphics[width=0.7\textwidth]{indoloquinolines.png}
\caption{Indoloquinolines from \textit{Cryptolepis sanguinolenta}}
\end{figure}

Our previous SAR study about the antiproliferative activity of 5-Me-indolo[2,3-b]quinoline series revealed that the \(\omega\)-aminoalkylamino substituent at C11 is important element for their bioactivity. For example, the 3-aminopropylamino group on 2 (\(R^1 = H\)) could increase the antiproliferative activity against human leukemia MV4-11 cell line about 20 times compared with that of 11-chloro precursor 1 (\(R^1 = H\)).\textsuperscript{17,18} Based on these findings, we further discussed in this work the effect of the \(\omega\)-aminoalkylamino substituent at C11 in the 5-Me-indolo[2,3-b]quinoline core by diversifying the side-chain structure, i.e., changing of the length, branching of the linker between the two nitrogen atoms, etc. We also examined whether a hydroxy group residing on the spacer exert influence on antiproliferative activity.
5.3 Chemistry

Synthesis

The synthetic strategy for the 5-Me-indolo[2,3-b]quinoline derivatives 2 was based on the nucleophilic aromatic substitution (S_NAr) reaction of 11-chloroneocryptolepines 1 with appropriate amine as shown in Scheme 1. The 11-chloroneocryptolepines 1, the key intermediate for the diversification, was derived starting from various substituted N-methylanilines and methyl 1H-indole-3-carboxylate in three steps in good yields. The amination of 1 with various 1,2-diaminoethanes and 1,3-diaminopropanes using excess amount in DMF under heating yielded the corresponding 11-aminated 2, smoothly. Subsequently, a series of ureido 3 and thioureido derivatives 4 were prepared in high yields by modification of free terminal amines of 2 with phenylisocyanate and thioisocyanate in dry CH_2Cl_2 at room temperature, respectively.


5.4 Biological evaluation

Antiproliferative activity in vitro against MV4-11 cell line

The synthesized compounds 2 bearing various ω-aminoalkylamino groups at C11 were tested against the human leukemia MV4-11 cell line under varying the substituents R^1 at C2. As shown in Table 1, all the assayed compounds were cytotoxic against the MV4-11 leukemia cells (IC_{50} below 0.9 μM), and exhibited higher antiproliferative activities than the reference anticancer drug cisplatin.
(IC$_{50}$ 2.820 µM), but lesser than that of doksorubicinHCl (IC$_{50}$ 0.006 µM). Amongst the compounds 2a–d, bearing the two methyl groups pendant on the amino side chain of three carbon atom spacer, Cl atom at the C2 favored over Me group (i.e., 2c vs. 2b), and Br atom (i.e., 2c vs. 2d). But, amongst the compounds 2e–g with two carbon atom spacer, the compound 2f bearing Me group at C2 was more effective than 2g with Cl atom at C2. Compound 2m bearing the only one methyl group pendant on two carbon atom spacer was more active than 2g with two methyl group pendant. Compound 2m and 2n bearing a Cl and Br atom at C2 respectively show the almost same activity. Amongst the tested compounds, 2h, modified with a 3-amino-2-hydroxypropylamino group at C11 and a chlorine atom at C2, show the highest antiproliferative activity (IC$_{50}$ 0.042 µM).

Subsequently, the ureido and thioureido derivatives 3 and 4 were tested for the antiproliferative activity against the human leukemia MV4-11 cell line. However, unfortunately, the compounds 3 and 4 showed slightly lesser antiproliferative activities, Compared with the compounds 2 bearing terminal free amino group.

**Table 1.** Antiproliferative activity of 11-aminoalkylamino-5-methyl-indolo[2,3-b]quinolines against human leukemia MV4-11 cell line.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R$^1$</th>
<th>11-Substituent$^a$</th>
<th>Yield of amination</th>
<th>MV4-11 IC$_{50}$ µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>cisplatin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.820±0.450</td>
</tr>
<tr>
<td>Doksrubicin HCl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.006±0.002</td>
</tr>
<tr>
<td>1a</td>
<td>H</td>
<td>Cl</td>
<td>-</td>
<td>1.312±0.262</td>
</tr>
<tr>
<td>1b</td>
<td>Br</td>
<td>Cl</td>
<td>-</td>
<td>0.810±0.145</td>
</tr>
<tr>
<td>2a</td>
<td>H</td>
<td>2_\text{HN} _\text{NH}$_\text{2}$</td>
<td>84</td>
<td>0.150±0.060</td>
</tr>
<tr>
<td>2b</td>
<td>Me</td>
<td>2_\text{HN} _\text{NH}_2</td>
<td>86</td>
<td>0.288±0.075</td>
</tr>
<tr>
<td>2c</td>
<td>Cl</td>
<td>2_\text{HN} _\text{NH}_2</td>
<td>95</td>
<td>0.119±0.043</td>
</tr>
<tr>
<td>2d</td>
<td>Br</td>
<td>2_\text{HN} _\text{NH}_2</td>
<td>86</td>
<td>0.308±0.102</td>
</tr>
<tr>
<td>Compound</td>
<td>Substituent</td>
<td>Yield</td>
<td>Yield Value</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-------------</td>
<td>-------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>2e</td>
<td>H</td>
<td>92</td>
<td>0.392±0.188</td>
<td></td>
</tr>
<tr>
<td>2f</td>
<td>Me</td>
<td>88</td>
<td>0.105±0.027</td>
<td></td>
</tr>
<tr>
<td>2g</td>
<td>Cl</td>
<td>90</td>
<td>0.453±0.209</td>
<td></td>
</tr>
<tr>
<td>2h</td>
<td>Cl</td>
<td>82</td>
<td>0.042±0.014</td>
<td></td>
</tr>
<tr>
<td>2k</td>
<td>Br</td>
<td>76</td>
<td>0.057±0.015</td>
<td></td>
</tr>
<tr>
<td>2m</td>
<td>Cl</td>
<td>87</td>
<td>0.103±0.014</td>
<td></td>
</tr>
<tr>
<td>2n</td>
<td>Br</td>
<td>90</td>
<td>0.078±0.020</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>H</td>
<td>91</td>
<td>0.549±0.108</td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td>Me</td>
<td>83</td>
<td>0.427±0.092</td>
<td></td>
</tr>
<tr>
<td>3c</td>
<td>Cl</td>
<td>84</td>
<td>0.790±0.302</td>
<td></td>
</tr>
<tr>
<td>3d</td>
<td>Cl</td>
<td>89</td>
<td>0.464±0.141</td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>H</td>
<td>90</td>
<td>0.680±0.215</td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>Cl</td>
<td>84</td>
<td>2.330±1.015</td>
<td></td>
</tr>
</tbody>
</table>

*The substituents at C11 of compounds 2–4. Yield of ureidation of the precursor amine. Yield of thioureidation of the precursor amine.*
The compounds 2a, 2c, 2f, 2h, 2k, 2m, and 2n of high antiproliferative activity against MV4-11 were chosen as candidates for further studies on their antiproliferative activity against non-small cell lung cancer (A549) and colon cancer (HCT116) cell lines, along with normal murine fibroblasts (BALB/3T3), and the testing results were listed in the Table 2. All the tested compounds showed higher antiproliferative activities against the cancer cell than the cisplatin used as the control agent. The compounds 2k and 2h showed higher activity against A549 and HCT116 cell line than other compounds, and they showed selective antiproliferative activity against MV4-11 cell line. The 2-bromo-2n and 2-chloro-2m showed almost the same cytotoxicity against A549 and HCT116 cell lines with normal cell line (BALB/3T3), but 2n showed potent activity against MV4-11 cell line with IC$_{50}$ 0.078 μM. Compound 2c, and 2f showed low cytotoxicity against normal cell line, and they showed moderate activity against A549 and HCT116 cell lines. It is quite obvious that the introduction of proper alkylamino substituents into biologically active derivatives can favorably influence their activities and selectivities in DNA binding.

Table 2. Antiproliferative activity of 11-alkylaminated 5-methyl indolo[2,3-b]quinolines against normal mice fibroblast BALB/3T3 and against cancer cell lines of A549 and HCT 116.

<table>
<thead>
<tr>
<th>Compound</th>
<th>BALB/3T3 IC$_{50}$ μM</th>
<th>A549 IC$_{50}$ μM</th>
<th>HCT 116 IC$_{50}$ μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>cisplatin</td>
<td>8.700±0.097</td>
<td>9.870±2.400</td>
<td>8.500±0.540</td>
</tr>
<tr>
<td>Dokso rubicin HCl</td>
<td>1.078±0.033</td>
<td>0.329±0.097</td>
<td>0.390±0.098</td>
</tr>
<tr>
<td>2a</td>
<td>4.789±2.018</td>
<td>1.512±0.198</td>
<td>1.262±0.361</td>
</tr>
<tr>
<td>2c</td>
<td>9.131±0.844</td>
<td>1.455±0.168</td>
<td>1.373±0.351</td>
</tr>
<tr>
<td>2f</td>
<td>10.558±0.330</td>
<td>1.795±0.270</td>
<td>2.370±0.481</td>
</tr>
<tr>
<td>2h</td>
<td>0.896±0.042</td>
<td>0.197±0.028</td>
<td>0.138±0.050</td>
</tr>
<tr>
<td>2k</td>
<td>0.864±0.015</td>
<td>0.190±0.027</td>
<td>0.117±0.055</td>
</tr>
<tr>
<td>2m</td>
<td>1.018±0.017</td>
<td>1.269±0.118</td>
<td>1.204±0.283</td>
</tr>
<tr>
<td>2n</td>
<td>0.939±0.018</td>
<td>0.988±0.164</td>
<td>0.842±0.367</td>
</tr>
</tbody>
</table>
5.5 Spectroscopic characterization of neocryptolepine derivative 2d interacting with salmon fish sperm DNA

The DNA binding studies of compound 2d was performed using UV-Vis absorption spectroscopy with salmon fish sperm DNA in phosphate buffer of pH 7.0 at 20 °C. The red shift and hypochromic effect were observed in the absorption spectra while the DNA solution was gradually added to the solution of the compound 2d. The results in Figure 1 showed that the absorption band at 256 nm for the 2d (a) decreased while increasing the DNA concentration. The maximum absorption shifted from 256 nm to a longer wavelength at 289 nm. It illustrated that the mode of 2d binding to DNA was intercalation. Then the binding constant of 2d-DNA was calculated as $4.12 \times 10^5$ L·mol$^{-1}$, according to double-reciprocal equation.

![Figure 2](image)

Figure 2 (a) UV-Vis absorption spectra of compound 2d at 20 °C. $C_{2d}=50\mu$mol/L, CDNA= 0.0, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.2, 0.225, 0.25 μmol/L for curve 1-11 in pH 7.0 phosphate buffer solution; (b) The plot of $1/\Delta A$ vis $1/[DNA]$ for 2d-DNA
5.6 EXPERIMENTAL

5.6.1 GENERAL

The commercially obtained reagents were directly used without further purification. The $^1$H NMR, $^{13}$C NMR spectra were measured on the Varian INOVA-600 or Varian INOVA-400 Varian or INOVA-300 spectrometer, using CDCl$_3$ or DMSO-d$_6$ as solvent and tetramethylsilane (TMS) as the internal standard. HRMS were obtained on a Bruker microTOF II-SKA spectrometer. Melting points were determined on a J-Science RFS-10 hot stage microscope. Some compounds were described in details in last chapter.

**General procedure for the synthesis of 11-aminoneocryptolpines (2a–2m):**

11-Chloroindoloquinolines 1 (0.3 mmol) and an excess of the appropriate aminoalkylamine (3.0 mmol) were heated together at 120 °C for 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 v/v) as an eluent to yield pure yellowish solids product.

![Chemical structure](image)

$N$-(3-amino-2,2-dimethylpropyl)-2,5-dimethyl-5H-indolo[2,3-b]quinolin-11-amine (2b) : Yield 300 mg (86 %), yellow solids; mp 173–175 °C; $^1$H NMR (300MHz, CDCl$_3$) $\delta$ 8.19 (br s, 1H), 7.98 (d, $J$ = 8.0 Hz, 1H), 7.91 (s, 1H), 7.74 (d, $J$ = 8.0 Hz, 1H), 7.47 (q, $J$ = 8.0 Hz, 2H), 7.38 (t, $J$ = 8.0 Hz, 1H), 7.16 (t, $J$ = 4.0 Hz, 1H), 4.19 (s, 3H), 3.82 (d, $J$ = 2.8 Hz, 2H), 2.84 (s, 2H), 2.49 (s, 3H) 0.87 (s, 6H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 157.28, 152.25, 149.58, 136.52, 131.90, 130.22, 125.25, 124.82, 124.16, 122.20, 118.72, 117.22, 116.29, 114.90, 104.99, 100.36, , 61.66, 53.40, 36.29, 33.17, 24.06 (2C), 21.74.
N-(3-Amino-2,2-dimethylpropyl)-2-bromo-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (2d): Yield 355 mg (86 %), yellow solids; mp 184–186 °C; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.99 (t, \(J = 6.0\) Hz, 2H), 7.76 (d, \(J = 6.0\) Hz, 1H), 7.56 (dd, \(J = 3.0, 3.0\) Hz, 1H), 7.40 (t, \(J = 9.0\) Hz, 1H), 7.24 (d, \(J = 9.0\) Hz, 1H), 7.19 (t, \(J = 9.0\) Hz, 1H), 4.06 (s, 3H), 3.66 (s, 2H), 2.68 (s, 2H), 0.76 (s, 6H). \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 157.12, 152.48, 148.07, 136.89, 132.71, 126.78, 125.42, 124.84, 122.57, 118.95, 117.71, 117.49, 116.38, 113.31, 104.67, 62.02, 53.24, 35.72, 33.14, 23.95 (2C).

N-(2-Amino-2-methylpropyl)-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (2e): Yield 290 mg (92%), yellow solids; mp 130–132°C; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 8.21–8.12 (m, 2H), 7.78–7.68 (m, 3H), 7.46–7.38 (m, 1H), 7.36–7.30 (m, 1H), 7.23 (t, \(J = 6.9\)Hz, 1H) 6.79 (s, 1H), 4.26 (s, 3H), 3.64 (d, \(J = 4.7\) Hz, 2H), 1.11 (s, 6H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 156.48, 152.69, 149.55, 138.74, 130.60 (d), 125.70, 125.18, 124.66, 121.51, 120.62, 119.28 (d), 117.51, 115.88, 115.03, 106.90, 59.41, 51.62, 33.07, 29.48 (2C).
**N-(2-Amino-2-methylpropyl)-2,5-dimethyl-5H-indolo[2,3-b]quinolin-11-amine (2f):** Yield 295 mg (88 %), yellow solids; mp 132–134°C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.13 (d, $J = 7.8$ Hz, 1H), 7.95 (s, 1H), 7.76 (d, $J = 8.0$ Hz, 1H), 7.56 (q, $J = 10.0$ Hz, 2H), 7.42 (q, $J = 7.9$ Hz, 1H), 7.19 (t, $J = 7.5$ Hz, 1H), 6.71 (s, 1H), 4.23 (s, 3H), 3.64 (d, $J = 4.5$ Hz, 2H), 2.55 (s, 3H), 1.10 (s, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 157.05, 153.01, 149.67, 137.08, 132.18, 130.29, 125.86, 124.75, 124.68, 121.64, 119.02, 117.52, 116.13, 115.15, 107.48, 59.76, 51.90, 33.18, 29.61 (2C), 21.74.

**1-Amino-3-(2-bromo-5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propan-2-ol (2k):** Yield 302 mg (76%), yellow solids; mp 162–164 °C; $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.76 (s, 1H), 7.94 (t, $J = 8.0$ Hz, 2H), 7.81 (d, $J = 8.0$ Hz, 1H), 7.52 (d, $J = 8.0$, 1H), 7.30 (t, $J = 7.8$ Hz, 1H), 7.11 (t, $J = 8.0$ Hz, 1H), 4.13 (s, 3H), 3.87–3.82 (m, 1H), 3.81 (dd, $J = 12.0$, 6.5 Hz, 2H), 3.68–3.65 (m, 2H). $^{13}$C NMR (101MHz, DMSO-d$_6$) $\delta$ 156.18, 152.42, 147.56, 136.40, 132.89, 126.33 (2C), 124.95, 123.99, 122.29, 118.26, 117.30, 116.72, 112.78, 105.29, 70.93, 52.36, 45.41, 32.35.
N-(2-amino-2-methylpropyl)-2-bromo-5-methyl-5H-indolo[2,3-b]quinolin-11-amine (2n): Yield 345 mg (90%), yellow solids; mp 101–103°C; 1H NMR (600 MHz, CDCl₃) δ 8.21 (d, J = 2.1 Hz, 1H), 8.08 (d, J = 7.6 Hz, 1H), 7.75 (d, J = 7.9 Hz, 1H), 7.71 (dd, J = 9.1, 2.0 Hz, 1H), 7.48–7.43 (m, 2H), 7.20 (t, J = 7.5 Hz, 1H), 6.46 (s, 1H), 4.17 (d, J = 14.0 Hz, 3H), 3.74 (d, J = 11.9 Hz, 1H), 3.37 (dd, J = 11.8, 7.8 Hz, 1H), 3.11–3.08 (m, 1H), 1.09 (d, J = 6.3 Hz, 3H). 13C NMR (75 MHz, cdcl₃) δ 156.13, 152.67, 147.85, 137.33, 133.13, 127.56, 127.21, 126.29, 124.29, 121.54 (d), 119.48, 117.62 (d), 116.65(d), 113.36, 108.06, 55.18, 47.96 (d), 33.20, 22.96.

General procedure for the synthesis of Compounds (3a-4b):
2-Substituted 5-methyl-5H-indolo[2,3-b]quinolone-11-amine 2 (50 mg) was completely dissolved in dry CH₂Cl₂ (1 mL), and then a solution of phenylisocyanate or phenylisothiocyanate (1.1 equiv) and dry CH₂Cl₂ (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. 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 solids.
1-(3-(2,5-Dimethyl-5H-indolo[2,3-b]quinolin-11-ylamino)-2,2-dimethylpropyl)-3-phenylurea (3b) : Yield 390 mg (83%), yellow solids; mp 228–230°C; $^1$H NMR (300 MHz, CDCl$_3$) δ 9.18 (s, 1H), 8.13 (s, 1H), 7.69 (d, $J$ = 7.9 Hz, 1H), 7.59 (d, $J$ = 7.8 Hz, 1H), 7.47 (d, $J$ = 7.8 Hz, 3H), 7.34 (dd, $J$ = 13.8, 8.1 Hz, 2H), 7.22 (d, $J$ = 8.2 Hz, 2H), 7.08 (t, $J$ = 7.5 Hz, 1H), 6.99 (t, $J$ = 7.4 Hz, 1H), 6.49 (s, 1H), 6.10 (s, 1H), 3.97 (s, 3H), 3.54 (d, $J$ = 6.5 Hz, 2H), 3.35 (d, $J$ = 6.2 Hz, 2H), 2.58 (s, 3H), 0.77 (s, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 157.99, 156.47, 150.75, 149.74, 140.36, 136.05, 132.50, 131.67, 129.42 (2C), 125.96, 124.22 (2C), 124.04, 122.82, 122.68, 119.67 (2C), 116.86, 116.24, 114.96, 106.52, 55.72, 48.02, 38.60, 33.38, 24.11(2C), 21.78.

\[ \text{Structure Image} \]

1-(2-Methyl-1-(5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)propan-2-yl)-3-phenylurea (3d): Yield 420 mg (89%), yellow solids; mp 175–177°C; $^1$H NMR (600 MHz, CDCl$_3$) δ 8.89 (s, 1H), 8.32 (s, 1H), 7.73 (dd, $J$ = 18.9, 7.7 Hz, 2H), 7.51 (t, $J$ = 10.8 Hz, 4H), 7.37 (d, $J$ = 8.3 Hz, 2H), 7.30 (t, $J$ = 7.4 Hz, 2H), 7.10 (t, $J$ = 7.2 Hz, 1H), 7.04 (t, $J$ = 7.0 Hz, 1H), 6.05 (s, 1H), 4.07 (s, 3H), 3.77 (s, 2H), 1.18 (s, 6H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 157.26, 156.96, 151.92, 148.72, 139.88, 136.53, 130.93, 129.60 (2C), 129.55, 127.16, 126.26, 124.39, 123.39, 122.32, 119.95 (2C), 119.78, 117.40, 117.06, 116.32, 106.43, 60.18, 54.62 (d), 33.45, 26.36 (2C).
1-(1-(2-Chloro-5-methyl-5H-indolo[2,3-b]quinolin-11-ylamino)-2-methylpropan-2-yl)-3-phenylthiourea (4b): Yield 410 mg (84%), yellow solids; mp 147–149 °C; $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.43 (s, 1H), 8.09 (d, $J = 2.1$ Hz, 1H), 7.82 (d, $J = 7.7$ Hz, 1H), 7.68 (d, $J = 7.9$ Hz, 1H), 7.58 (dt, $J = 10.7$, 5.3 Hz, 1H), 7.48 (d, $J = 9.1$ Hz, 1H), 7.38 (t, $J = 7.6$ Hz, 1H), 7.33–7.29 (m, 2H), 7.22 (t, $J = 7.5$ Hz, 1H), 7.11 (dd, $J = 12.9$, 7.5 Hz, 3H), 6.13 (s, 1H), 5.88 (s, 1H), 4.41 (d, $J = 5.5$ Hz, 2H), 4.06 (s, 3H), 1.22 (s, 6H). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 180.66, 156.54, 152.85, 147.96, 136.89(d), 130.89, 130.44, 127.67, 126.80, 126.69, 125.53 (2C), 124.54, 124.45, 123.94, 121.72, 118.43, 117.63, 117.17, 116.63, 109.12, 60.86, 58.74, 55.50, 33.31, 26.77 (2C).

5.6.2 Cell line

Established in vitro, human cell line: MV4-11 (leukemia), A549 (lung cancer), HCT116 (colon cancer) and normal mice fibroblast (Balb/3T3) was used. This 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.

MV4-11 cells were cultured in the RPMI 1640 (IET, Poland) supplemented with 2 mM L-glutamine, 1.0 mM sodium pyruvate and 10% fetal bovine serum (all from Sigma-Aldrich, Germany), HCT 116 and A549 cells were cultured in the RPMI 1640+OptiMEM (50:50) medium (IET, Poland) supplemented with 2 mM L-glutamine and 5% fetal bovine serum (all from Sigma-Aldrich Germany), BALB/3T3 cells were cultured in Dulbecco medium (IET, Poland) supplemented with 2 mM L-glutamine and 1.0 mM sodium pyruvate, 10% fetal bovine serum (all from Sigma-Aldrich, Germany). All culture medium was supplemented with 100 units/ml penicillin (Sigma-Aldrich, Germany), and 100 µg/ml streptomycin (Polfa Tarchomin S.A., Poland). All cell lines were grown at 37°C with 5% CO$_2$ humidified atmosphere.
5.6.3 Anti-proliferative assay in vitro

Test solutions of the 27 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 $1 \times 10^4$ 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 (MV4-11) or SRB (A549, HCT116 and Balb/3T3) 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 1 and 2. Each compound in each concentration was tested in triplicate in a single experiment, which was repeated 3--5 times.

5.6.4 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: 5 mg/ml, Sigma-Aldrich, 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-Aldrich, 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 Synergy H4 photometer (BioTek Instruments, USA) at 570 nm wavelength. Each compound in given concentration was tested in triplicates in each experiment, which was repeated 3-5 times.
5.6.5 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 [1]. 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 0.1% sulforhodamine B (SRB, Sigma, Germany) dissolved in 1% acetic acid (POCh, Gliwice, Poland) for 30 minutes. Unbound dye was removed by rinsing (4x) 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) on Synergy H4 photometer (BioTek Instruments, USA).
References

Chapter 6

Synthesis, characterization of some new azo-neocryptolpine dyes and spectroscopic studies
6.1 Abstract

Azo chromophores have versatile applicability ranging from textile dyeing, leather dyeing, coloring of plastics and polymer to advanced applications such as liquid crystal displays, biological and medical studies and advanced application in organic synthesis. Also, they contribute greatest production volume of the dyestuff industry due to simplistic mode of their synthesis with high yield. A new simple and fast method was presented as a new novel azo-neocryptolpine derivatives with spectroscopic absorption by reaction 5-methyl-5H-indolo[2,3-b] quinoline-1,3-diamine with Azo benzene sulfoniy chloride to obtain of their corresponding azo-neocryptolpine dyes, A further study for application of this derivatives as pigment of textile is currently underway.

\[
\begin{align*}
R^2, R^3 &= \text{Me}; H \\
R^1 &= \text{H, F} \\
N &= 0 \text{ or } 1, 2
\end{align*}
\]
6.2 INTRODUCTION

The chemical properties of quinoline and its derivatives have been widely discussed because of their biological relevance, coordination capacity and their use as metal extracting agent\textsuperscript{1}. They have attracted special interest due to their therapeutic properties. On the other hand, indoloquinoline have been used in treatment of cancer, tuberculosis and malaria\textsuperscript{2}. Several quinoline derivatives possess chemotherapeutic activity and act as antimalaria and antiallergic agents\textsuperscript{3}. They show broad-spectrum efficiency against multiple herpes viruses and they have a potential role for the treatment of a variety of infections\textsuperscript{4}. On the other hand, azo dyes are a class of compounds containing a N=N double bond and, due to their ability to absorb visible light and ease of synthesis, have been extensively used in the textile, fiber, leather, paint and printing industries for more than a century\textsuperscript{5}. In addition, azo compounds based on indoloquinoline derivatives will play a central role in textile industry in the future. Although many papers described the synthesis and some properties of carbocyclic azo hydroxquinoline dyes\textsuperscript{6-8}, only few hetarylazo indoloquinoline or hydroxquinoline compounds were synthesized\textsuperscript{9-12}.

We therefore interested in the synthesis of a series of a monoazo dyes base on neocryptolpine analogues which we have achieved in our previous work and evaluation of their spectrophotometric and pharmacological activities. Hence, the author has focused on the synthesis of novel arylazoindoloquinolene dyes and the examination of these derivatives as pigment of textile is currently underway.
6.3 RESULTS AND DISCUSSION

Azo chromophore–linked neocryptopline analogues were synthesized according to the protocol described in Scheme 1. As described in Scheme 1, the 2-Substituted 5-methyl-5H-indolo[2,3-b]quinolone-11-amine (2) was completely dissolved in dry DMF, and then a mixture Azo benzene Sulfonyl chloride and dry DMF were added drop by drop under stirring and finally triethylamine was added. Compound 5a–5f was already successfully synthesized and summarized in table 1.
<table>
<thead>
<tr>
<th>Compound</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>n</th>
<th>Yield %</th>
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<tbody>
<tr>
<td>5a</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>1</td>
<td>75</td>
</tr>
<tr>
<td>5b</td>
<td>H</td>
<td>CH₃</td>
<td>CH₃</td>
<td>1</td>
<td>72</td>
</tr>
<tr>
<td>5c</td>
<td>Br</td>
<td>CH₃</td>
<td>CH₃</td>
<td>1</td>
<td>61</td>
</tr>
<tr>
<td>5d</td>
<td>F</td>
<td>H</td>
<td>H</td>
<td>1</td>
<td>56</td>
</tr>
<tr>
<td>5e</td>
<td>H</td>
<td>H</td>
<td>CH₃</td>
<td>0</td>
<td>54</td>
</tr>
<tr>
<td>5f</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>2</td>
<td>71</td>
</tr>
</tbody>
</table>

$n = 0 \text{ or } 1, 2$

R₁ = H, F, Br

R₂, R₃ = Me, H

Azo group
6.4 Spectroscopic characterization of Azo neocryptolepine derivative 5b in Ethanol at different concentration.


6.5 EXPERIMENTAL

The commercially obtained reagents were directly used without further purification. The $^1$H NMR, $^{13}$C NMR spectra were measured on the Varian INOVA-600 or Varian INOVA-400 Varian or INOVA-300 spectrometer, using CDCl$_3$ or DMSO-d$_6$ as solvent and tetramethylsilane (TMS) as the internal standard. Melting points were determined on a J-Science RFS-10 hot stage microscope.

General procedure for the synthesis of Compounds 5a-5f.

2-Substituted 5-methyl-5H-indolo[2,3-b]quinolone-11-amine (5a, 50 mg) was completely dissolved in dry DMF (2 mL), and then a mixture Azo benzene Sulfonyl chloride (1.2equiv) 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. After reaction was finished. The crude product was purified by flash chromatography using AcOEt-2N ammonia in MeOH (9:1v/v) as an eluent to yield pure products as orange solids.

(E)-N-(3-((5-methyl-5H-indolo[2,3-b]quinolin-11-yl)amino)propyl)-4-(phenyldiazenyl)benzenesulfonamide (5a): Yield 415 mg (75%), orange solids; mp 180–182 °C; $^1$H NMR (600MHz, DMSO-d$_6$) $\delta$ 8.48 (d, $J$ = 8 Hz, 2H), 7.94 –7.90 (m, 2H), 7.89 (m, 2H), 7.79 (d, $J$ = 8.0 Hz, 2H), 7.64 (dt, $J$ = 17.0, 8.5 Hz, 3H), 7.53–7.48 (m, 1H), 7.33 –7.29 (m, 1H), 7.20 (t, $J$ = 4 Hz, 1H), 7.11 (m 1H) 6.89 (d, $J$ = 4 Hz, 1H), 6.79 (d, $J$ = 4 Hz, 1H) 4.14 (s, 3H), 3.84 (m, 2H), 2.76
m, 2H), 1.84–1.80 (m, 2H). $^{13}$C NMR (101MHz, CDCl$_3$) $\delta$ 153.54, 151.84, 142.05, 137.14, 132.43, 131.17, 129.66, (2C) 129.50, 127.79 (2C) 127.28, 126.77, 125.07, 124.13, 123.12, 123.05 (2C) 122.92, 122.63, 122.29, 122.16, 121.49, 120.15, 118.88, 115.86, 115.44, 45.50, 36.03, 32.98, 30.57.

(E)-N-(2,2-dimethyl-3-((5-methyl-5H-indolo[2,3-b]quinolin-11-yl)amino)propyl)-4-(phenyldiazenyl)benzenesulfonamide (5b): Yield 420 mg (72%), orange solids; mp 162–164 °C; $^1$H NMR (600MHz, DMSO-d$_6$) $\delta$ 8.49 (d, $J = 4$ Hz, 2H), 7.98–7.94 (m, 2H), 7.89 (d, $J = 4.0$ Hz, 2H), 7.83 (dt, $J = 8$, 8.5 Hz, 3H), 7.66–7.64 (m, 2H), 7.52 (d, $J = 4$ Hz, 2H), 7.49 (d, $J = 4$ Hz, 1H), 7.33 (t, $J = 4$ Hz, 1H), 7.14 (t, $J = 4$ Hz, 1H), 6.81 (d, $J = 4$ Hz, 1H) 4.16 (s, 3H), 3.74 (d, $J = 4$ Hz, 2H), 2.60 (d, $J = 4$ Hz, 2H), 0.73 (2, 6H). $^{13}$C NMR (101MHz, CDCl$_3$) $\delta$ 152.03, 151.12, 141.05, 136.14, 131.14, 130.17, 129.12, (2C) 128.50, 126.50 (2C) 125.24, 125.02, 124.90, 124.12, 123.50, 123.03 (2C), 122.80, 122.61, 122.27, 122.04, 121.45, 120.12, 118.41, 115.12, 115.02, 50.12, 47.03, 32.06, 22.12 (2C)
(E)-N-(3-(2-bromo-5-methyl-5H-indolo[2,3-b]quinolin-11-yl)amino)-2,2-dimethylpropyl)-4-(phenyldiazenyl)benzenesulfonamide (5c): Yield 401 mg (61%), orange solids; mp 184–186 °C; $^1$H NMR (400MHz, DMSO-$d_6$) δ 8.78 (d, $J = 4$ Hz, 2H), 7.90–7.86 (m, 2H), 7.85 (d, $J = 4.0$ Hz, 2H), 7.58 (dt, $J = 4$, 8.5 Hz, 4H), 7.33 (d, $J = 4$ Hz, 2H), 7.16–7.10 (m, 2H), 6.91 (d, $J = 4$ Hz, 1H), 6.80 (d, $J = 4$ Hz, 1H), 4.12 (s, 3H), 3.57 (s, 2H), 2.78 (d, $J = 4$ Hz, 2H), 1.86 (s, 2H). $^{13}$C NMR (101MHz, CDCl$_3$) δ 153.99, 152.27, 148.82, 142.78, 137.57, 132.81, 131.16 (2C), 130.05, 129.22 (2C) 128.27, 127.75 (2C), 125.08, 124.41, 124.30, 123.52 (2C), 123.33, 122.48, 121.19, 120.56, 118.62, 116.70, 116.03, 115.53, 42.70, 32.79, 28.31, 26.84

(E)-N-(3-(2-fluoro-5-methyl-5H-indolo[2,3-b]quinolin-11-yl)amino)propyl)-4(phenyldiazenyl)benzenesulfonamide (5d): Yield 401 mg (56 %), orange solids; mp 201–203 °C; $^1$H NMR (400MHz, DMSO-$d_6$) δ 8.50 (d, $J = 4$ Hz, 2H), 7.80 (m, 2H), 7.23 (d, $J = 4.0$ Hz, 2H), 7.02 (dt, $J = 4$, 8.5 Hz, 4H), 6.98 (d, $J = 4$ Hz, 2H), 6.90 (m, 2H), 6.78 (d, $J = 4$ Hz, 1H), 6.70 (d, $J = 4$ Hz, 1H),
4.02 (s, 3H), 3.13 (s, 2H), 2.51 (d, J = 4 Hz, 2H), 1.78 (s, 2H). $^{13}$C NMR (101MHz, CDCl$_3$) $\delta$ 152.80, 151.27, 148.79, 141.18, 136.52, 132.77, 131.14 (2C), 130.02, 129.56 (2C) 128.88, 127.80 (2C), 125.89, 124.69, 124.44, 123.45 (2C), 123.31, 122.42, 121.02, 120.54, 118.90, 116.80, 116.01, 115.44, 41.77, 33.74, 27.33, 25.88

(E)-N-(1-(5-methyl-5H-indolo[2,3-b]quinolin-11-yl)amino)propan-2-yl)-4-(phenyldiazenyl)benzenesulfonamide (5e): Yield 348 mg (54 %), orange solids; mp 190–193 °C; $^1$H NMR (400MHz, DMSO-d$_6$) $\delta$ 8.46 (d, J = 8 Hz, 2H), 7.94 –7.90 (m, 4H), 7.80 –7.76 (m, 3H), 7.61 (d, J = 4 Hz, 2H), 7.51 (t, J = 8 Hz, 2H), 7.39 (t, J = 8 Hz, 1H), 7.10 –7.06 (m, 2H), 6.77 (d, J = 4 Hz, 1H), 4.12 (s, 3H), 3.85 –3.78 (m, 2H), 0.81 (s, 3H). $^{13}$C NMR (101MHz, CDCl$_3$) $\delta$ 162.72, 156.47, 153.94, 152.29, 148.38, 144.0, 137.68, 132.78, 131.12, 130.04 (2C), 128.06 (2C), 125.26, 124.34, 124.11, 123.52 (2C), 123.32 (2C), 122.53, 121.20, 118.64, 116.83, 115.76, 115.46, 104.95, 54.0, 50.59, 32.77, 18.77
(E)-N-(4-((5-methyl-5H-indolo[2,3-b]quinolin-11-yl)amino)butyl)-4-(phenyldiazenyl) benzenesulfonamide (5f): Yield 402 mg (71 %), orange solids; mp 214–216 °C; $^1$H NMR (400MHz, DMSO-d$_6$) δ 8.51 (d, $J$ = 8 Hz, 2H), 7.97 –7.92 (m, 4H), 7.88 –7.75 (m, 3H), 7.62 (t, $J$ = 8.0 Hz, 1H), 7.56 (dd, $J$ = 8, 8 Hz, 2H), 7.41 (t, $J$ = 8 Hz, 1H), 7.28 –7.21 (m, 1H), 7.07 (t, $J$ = 4 Hz, 1H), 6.92 (d, $J$ = 4 Hz, 1H), 6.80 (d, $J$ = 4 Hz, 1H) 4.14 (s, 3H), 3.78 (t, $J$ = 8 Hz, 2H), 2.71 (t, $J$ = 4 Hz, 2H), 1.68 (t, $J$ = 8 Hz, 2H), 1.34 (t, $J$ = 8 Hz, 2H). $^{13}$C NMR (101MHz, CDCl$_3$) δ 153.99, 152.27, 148.82, 142.78, 137.75, 132.81, 131.16, 130.05 (2C), 129.22 (2C) 128.27, 127.75, 125.08, 124.41, 124.30, 123.52 (2C), 123.33 (2C), 122.48, 121.19, 120.56, 118.62, 116.70, 116.03, 115.53, 47.44, 42.70, 32.79, 28.31, 26.84
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