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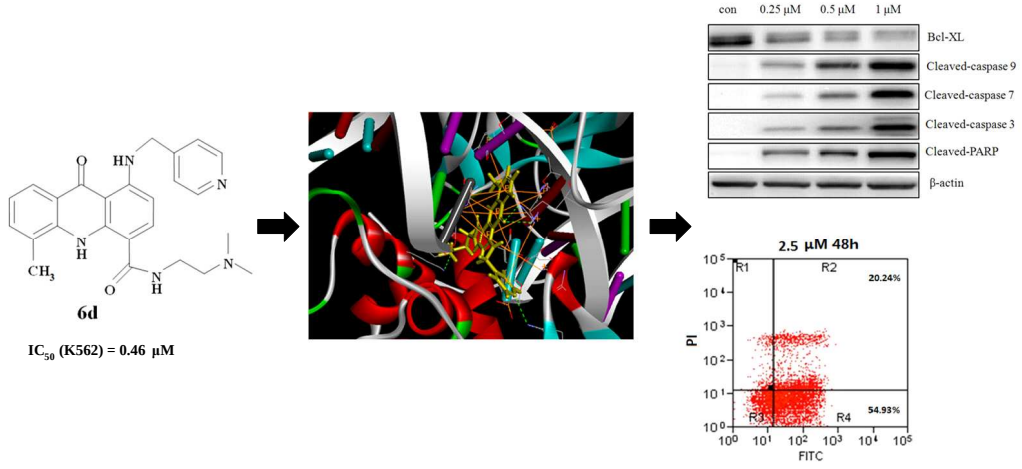

Molecular design, synthesis and biological research of novel pyridyl acridones as potent DNA-binding and apoptosis-inducing agents

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Abstract

A series of novel pyridyl acridone derivatives comprised of a pseudo-five-cyclic system to extend the π-conjugated acridone chromophore, were designed and synthesized as potent DNA binding antitumor compounds. Most synthesized compounds displayed good activity against human leukemia K562 cells in MTT tests, with compound **6d** exhibiting the highest activity with IC_{50} value at 0.46 μM. Moreover, **6d** showed potent activities against solid tumor cell lines (0.16-3.79 μM). Several experimental studies demonstrated that the antitumor mode of action of compound **6d** involves DNA intercalation, topoisomerase I inhibition, and apoptosis induction through the mitochondrial pathway. In summary, compound **6d** represents a novel and promising lead structure for the development of new potent anticancer DNA-binding agents.

Keywords: Acridone; pyridyl; molecular docking; DNA; topoisomerase I; antitumor.

1. Introduction

Cancer is a challenging disease with the second highest mortality rate[1]. While targeted anticancer drugs have been developed and clinically used, chemotherapy remains an important first-line treatment option for various cancers[2-5]. Therefore efforts have been continuously made for the development of more potent and less toxic anticancer chemotherapy drugs[6-11]. As part of the efforts in the discovery of potent antitumor agents, we have developed several series of new compounds such as aminopyrrolidine[12], tricyclic thiophene analogues[13] and acridine/acridone derivatives[14-19] with good antitumor activity. The polycyclic acridine/acridone derivatives have π-conjugated planar structure, which makes them useful in many applications[20-22], such as pigments and dyestuffs, antibacterial agents, antipsoriatic agents[23, 24], anticancer drugs[20, 25, 26]. Acridine/acridones can intercalate into the double stranded DNA base pairs and then inhibit the activity of DNA-related enzymes such as topoisomerases[27, 28] and telomerases[29, 30]. Recently, a number of acridine/acridone drugs have entered clinical trials or preclinical studies[1, 20], such as Imidazoacridone (C-1311), Pyrazoloacridine (PZA), N-[2-(dimethylamino)-ethyl]-acridine-4-carboxamide (DACA), 9-[4-(N,N-dimethylamino)phenylamino]-3,6-bis(3-pyrrolodino-propionamido)acridine (BRACO-19) and Amsacrine (*m*-AMSA) (**Figure 1**), which suggests the usefulness of the relevant molecular scaffolds for developing effective anticancer chemotherapy drugs and indicates the possibility of further exploring these molecular scaffolds for developing agents with more improved properties.

Acridone, which is shown in **Figure 2**, has three planar rings. Earlier studies have suggested that, by adding rings, the resulting acridine/acridone derivatives such as C-1311[31] and PZA[32] may have enhanced DNA-binding capability. Because of the difficulties in adding more rings to acridine/acridone, it is desirable to rationally introduce ring additions that likely increase the activities. By analyzing the structure of acridone, the introduction of amino group at 1 or 8 position and carboxy group at 4 or 6 position may enable the formation of intramolecular hydrogen bonds (IMHBs) with the C=O and N-H groups on the acridone ring respectively. These two IMHBs could form a pseudo-five-cyclic system, which might extend the π-conjugated acridone chromophore and improve the DNA binding ability.

Moreover, the carboxamide group with terminal amino substituents on the acridine ring are beneficial for the DNA binding, as found in molecules such as DACA[33, 34], BRACO-19[35]. We therefore introduced this group to acridones to improve the antitumor activity. We further noted that *m*-AMSA achieves potent anticancer activity by the interaction with DNA and topoisomerase and the substitution pattern of its 9-anilino group participates in the interaction with topoisomerase. Based on the structure of *m*-AMSA, we hypothesized that anilino group at 1 or 8 position may be beneficial for the interaction with topoisomerase. Molecular docking in our study (**Figure 3**) indicated that acridone derivatives with pyridylalkyl amino group have better DNA-topoisomerase I (DNA-topo I) binding affinity than those with anilino groups (Molecular docking studies of the representative acridone compound with anilino group, compound **A** with topo I-DNA

complex model, **Figure 1S**, supporting information).

Therefore, two series of acridone derivatives, **I** and **II** (**Figure 2**) may be introduced. As compound **I** have two big substituents on the two sides of acridone ring (Molecular docking studies of the representative compound **B**, **Figure 2S**, supporting information), which are not good for DNA-topo I interaction, compound **II** was selected to be synthesized and evaluated for their antitumor activity. Denny's group[33] revealed that the substitution pattern of C5 position on acridine ring played an important role in the antitumor activity. Therefore, a series of groups including electron-donating and electron-withdrawing groups were further introduced on C5 position on the acridone scaffold to study the structure-activity relationship (SAR).

Based on the above described rational analysis, we designed and synthesized a series of novel pyridyl acridone derivatives **6a-w** and **7a-c** as our target compounds based on the following attributes: (a) the presence of 4-carboxamide side chain, (b) the presence of 5-substituted group, and (c) introducing pyridinylalkyl group on the C ring on the acridone scaffold. The antiproliferative effects of the pyridyl acridone derivatives on K562 cells were evaluated and the SAR was discussed. The most active compound **6d** was selected to investigate the mode of action. The results showed that the synthesized pyridyl acridones exhibits broad antitumor activity, interacts with DNA and inhibits topo I activity and induces apoptosis through mitochondrial pathway.

2. Results and discussion

2.1. Chemistry

The synthesis of compounds **6a-w** and **7a-c** is shown in **Scheme 1**. First, compounds **3a-e** were obtained from the Ullmann reaction of 2,4-dichlorobenzoic acid **2** with anthranilic acid **1a** or its derivatives **1b-e** in DMF using Cu as the catalyst[16, 17]. Subsequent Friedel-Crafts acylation was carried out in concentrated sulfuric acid at 80 °C for 5 h to give acridone-4-carboxylic acid derivatives **4a-e** in high yields[36], which were then reacted with the corresponding primary aliphatic amines using N,N′-carbonyldiimidazole (CDI) as the condensation agent[37] to afford the intermediates acridone-4-carboxamides **5a-j**. The desired compounds **6a-w** and **7a-c** were produced by the nucleophilic substitution between the corresponding acridone-4-carboxamides and various picolylamines or 4-pyridineethanamine under argon atmosphere. Structures were confirmed by ^{1}H NMR, ^{13}C NMR and high resolution mass spectral data.

2.2. In vitro cytotoxicity

As most acridone and acridine derivatives showed better antitumor activity against leukemia tumor cells than solid tumor cells[20], *the vitro* cytotoxicity of 26 desired pyridyl acridone derivatives against human leukemia K562 cells was first assayed by MTT reduction method. Adriamycin and cisplatin were used as the positive controls. The structures and bioactivity results were shown in **Table 1**. Most of these compounds had better antitumor activity than cisplatin. Compound **6d** showed the highest activity with IC_{50} value at 0.46 μM, which was comparable to adriamycin.

The structure-activity analysis of the data in **Table 1** indicated that the

substitution of the acridone ring played an important role on the antitumor activity. First, the length of alkyl chains between the N,N-dimethylamino group and 4-carboxamide group had great influence on the antitumor activity. Compounds containing two methylene units displayed better antiproliferative activity than those containing three methylene units, suggested by the IC_{50} values of **6b** vs. **6e**, **6c** vs. **6f**, **6d** vs. **6g**, **6k** vs. **6o**. However, when four methylene units existed, the cytotoxicity increased, for example, compound **6q** displayed approximately 2-fold more active than **6u**. In addition, R_2 group on the 4-carboxamide side chain had a great effect on the antitumor activity. Compounds with N,N-dimethylamino group displayed better antitumor activity than those with methoxy group, which can be seen from the IC_{50} values of **6b** and **6h, 6c** and **6i**. Most compounds with methoxy group displayed no cytotoxicity (IC_{50} > 50 μM).

From the biological data of these pyridyl acridone derivatives shown in **Table 1**, most of them had a gratifying antiproliferative activity against K562 cells. These suggested that the position of nitrogen atom on pyridine ring (2-pyridyl, 3-pyridyl, and 4-pyridyl) may have little important influence on the cytotoxicity. In order to evaluate whether the number of pyridyl group has an effect on antitumor activity, we also introduced 2,2′-bispyridyl group to the acridone scaffold (**Table 2**). However, the results led reduction of antitumor activity compared to mono-pyridyl acridone derivatives with N,N-dimethylamino group. For instance, **6k** had significant antitumor activity with IC_{50} of 2.28 μM, while the IC_{50} of **7b** was 13.90 μM. Moreover, the length between the pyridyl group and 1-amino group on the acridone scaffold also had

an important influence on the antiproliferative activity, which can be seen from the the IC_{50} values of **6n** (m = 2) and **6m** (m = 1).

The substitution on C5 position of acridone or acridine ring reportedly plays an important role on the antitumor activity[33]. We therefore synthesized several compounds containing electron-donating or electron-withdrawing groups on C5 position. The testing results suggested that the introduction of methyl group on C5 position of acridone significantly increased the cytotoxicity. Compounds with methyl group (**6d**) showed more than 14-fold better antitumor activity than **6a**. When the more electron-donating group methoxyl was introduced, **6m** resulted in reduced antiproliferative activity compared with **6d**. Introduction of electron-withdrawing group in the case of **6v** (5-trifluoromethyl) and **6w** (5,7-dichloro) also produced less cytotoxicity compared with **6d**. The results indicated that electron-negativity and steric effect on C5 position of acridone may change the cytotoxic profile.

In order to study whether the highly potent compound **6d** have broad antitumor activities against solid tumor cells, we tested it on various cancer cell lines including NCI-H520, U251, A375, A172, Hela, CNE-2, U118-MG, HepG2 and MCF-7 cells with the results shown in **Table 3**. Our testing results showed that **6d** also have potent activities against these solid tumor cells. For example, the IC_{50} values against A375 cells and HepG2 cells were 0.16 μM and 0.32 μM, respectively. Therefore, **6d** was selected to study the antitumor mechanism.

2.3. Molecular modeling

Molecular docking studies of the representative compound **6d** with topo I-DNA

complex model (PDB ID: 1K4T) were conducted using the Discovery Studio 3.1.1/Libdock protocol. The LibDockScore is 194.834. As shown in **Figure 3a** and **Figure 3b**, pi-pi interactions were formed between the acridone ring (A-ring, B-ring and C-ring) of **6d** and DNA base pairs (DT10, TGP11, DC112 and DA113). Moreover, specific interactions of **6d** with topo I included: (a) three hydrogen bonds, one formed between the nitrogen atom (N) at the pyridyl ring and Arg488 (N···H-NH), with the distance of 3.0 Å; another formed between C=O at B-ring of acridone and NH_2 of Arg364 (C=O···H-NH), with the distance of 2.9 Å; the other formed between $N(CH_3)_2$ on the 4-carboxamide side chain and NH_2 of Lys751 (N···H-NH), with the distance of 3.1 Å; (b) three pi-pi interactions forming between the three rings at acridone backbone (A-ring, B-ring and C-ring) and Asp533; (c) two cation-pi interaction, one existing between the pyridyl ring and an ammonium cation of Arg364, the other existing between C-ring of acridone and ε-NH_2 of Lys751. **Figure 3c** showed interactions between the receptor and the ligand on 2D diagram. These analyses suggest that **6d** may intercalate into DNA and bind with topo I to contribute or partly contribute to its observed anti-tumor activity.

2.4. UV-visible spectral absorbance

UV-visible spectral absorbance has been universally employed to examine the binding mode of DNA with small molecules [17, 38-40]. In this study, the interactions of **6d** with Calf thymus DNA (ct DNA) were firstly investigated using absorption spectroscopy. The absorption spectra of **6d** in the absence and presence of ct DNA at constant concentration are given in **Figure 4a**. In the absence of ct DNA, **6d** had

strong absorbance peak at about 425 nm. Increasing ct DNA concentration, the absorption bands of **6d** displayed clear hypochromism with a concomitant minor red shift. These phenomena indicated that **6d** probably interact with DNA by intercalation mode, involving a stacking interaction between acridone ring of **6d** and DNA base pairs[38].

In order to further elucidate the binding capability of **6d**, the binding constant K_b was determined by monitoring the changes in absorbance at 425 nm with increasing concentrations of ct DNA. The equation (1) was used to calculate the binding constant K_b[41] (**Figure 4b**). In this equation, [DNA] represents the ct DNA concentrations in base pair, while ε_a, ε_f and ε_b are the extinction coefficient of the compound absorption band at a given ct DNA concentration, the complex free in solution and the complex when fully bound to DNA, respectively. The K_b is calculated by the gradient ratio to the intercept. The value of K_b was derived to be 5.1×10^4 M^{-1}.

$$[DNA] / (\varepsilon_a - \varepsilon_f) = [DNA] / (\varepsilon_b - \varepsilon_f) + 1 / K_b (\varepsilon_b - \varepsilon_f) \quad (1)$$

2.5. Fluorescence emission spectra

Fluorescence spectral technique is an effective method to study the interaction of organic compounds with DNA[17, 38, 42]. The binding mode of DNA with compound **6d** was further investigated using fluorescence emission spectroscopy. Fixed amount of **6d** was titrated with increasing amounts of ct DNA. The results can be seen in **Figure 5a**. Enhanced fluorescence intensity was obtained with increasing

ct DNA concentration indicating a deep intercalation of **6d** with DNA. This result may suggest efficient protection of **6d** from water by the hydrophobic environment inside the DNA helix[41]. In addition, the fluorescence intensity reached saturation gradually with increasing ct DNA concentration, and the terminal concentration of DNA was ten-fold higher than **6d**, the increasing fluorescence trend can be seen in **Figure 5b**.

2.6. DNA-EB displacements

The intercalation mode of **6d** binding to DNA was further investigated using the competitive binding displacement experiment[41]. Ethidium Bromide (EB) is a conjugate planar molecule, and its fluorescence intensity is very weak in the absence of duplex DNA. However, the fluorescence intensity increases greatly when EB intercalates into the base pairs of double-stranded DNA. If there has a compound that can compete with EB for DNA-binding sites, the fluorescence quenching of DNA-EB system is observed. In order to investigate whether **6d** can intercalate into DNA, the competitive binding displacement experiments were carried out, which were shown in **Figure 6a**. The emission intensity of DNA-EB system at about 600 nm decreased distinctly when the concentration of **6d** increased and an isoactinic point appeared at about 500 nm. The phenomenon suggested that **6d** can intercalate into DNA. To confirm the binding affinity of **6d** with DNA quantitatively, the classical Stern-Volmer equation (2) was used to calculate the binding constant[17] (**Figure 6b**).

$$F_0 / F = 1 + K_q [Q] \qquad (2)$$

In the equation above, F_0 is the fluorescence emission intensity in the absence of the quencher, while F is the fluorescence emission intensity in the presence of the quencher and [Q] is the concentration of the quencher. The quenching constant K_q of **6d** was 1.0×10^4 M^{-1}, which is in accordance with the absorption spectra.

2.7. Viscosity experiments

Viscosity experiment is considered as one of the least ambiguous and the most critical tests of the binding modes of compounds to DNA[39, 41]. The viscosity of a DNA solution was sensitive to the addition of compounds bound by intercalation[38]. The relative changes in viscosity were measured using ct DNA with increasing concentrations of **6d**, which were shown in **Figure 7**. In the presence of increased **6d**, DNA viscosity increased, which further confirmed that **6d** could bind to DNA through intercalation binding mode.

2.8. DNA topo I inhibition assay

Most acridine/acridone derivatives can interact with DNA and inhibit the activity of topoisomerases, it is of interest to evaluate whether the synthesized acridone derivatives can also inhibit the activity of topoisomerases. **Figure 8** showed the relative affinity of all synthesized compounds on the relaxtion of plasmid pBR322 DNA mediated by topo I. The results indicated that most of the compounds displayed good topo I inhibitory activity, however, neither pyridyl acridone compounds with methoxy group on the 4-carboxamide side chain (e.g. **6h-j** and **6r-t**) nor bispyridyl acridone derivatives (**7a-c**) showed any topo I inhibitory activities, which were in

accordance with their antitumor activities. All these data suggested that these compounds may have antiproliferative activity by interacting with DNA and then inhibiting topo I.

2.9. Apoptosis induced by compound 6d

The above data indicated that **6d** may bind with DNA and inhibit topo I activity, which was expected to subsequently lead to apoptosis in cancer cells. In order to evaluate this hypothesis, an Annexin-V/PI binding assay was conducted in K562 cells as shown in **Figure 9**. The lower right-hand quadrants (R4) displayed the early stage of apoptotic cells. The upper right-hand quadrants (R2) displayed the late stage of apoptotic or necrotic cells. K562 cells were treated with **6d** at the concentrations of 0, 0.05, 0.25, 0.5, 1 and 2.5 μM for 48 h. As the concentration of **6d** increased, the percentage of early stage apoptotic cells increased from 8.98% to 54.93%. From the results we conclude that **6d** can effectively induce K562 cells apoptosis in a dose-dependent manner.

Apoptosis can be triggered by either intrinsic mitochondrial or death receptor pathways. In order to understand which pathway that **6d** induced apoptosis, the activity of cleaved caspase-9, caspase-3 and caspase-7 was first tested. As shown in **Figure 10**, **6d** at 1 μM for 36 h induced significant activation of cleaved caspase-9, caspase-3 and caspase-7, suggesting that the mitochondrial pathway was involved in the **6d**-induced apoptosis.

PARP (poly ADP-ribose polymerase) is the substrate of caspases. During apoptosis, caspases mediated PARP cleavage, which inactivates the enzyme by

destroying its ability to respond to DNA strand breaks. The results in **Figure 10** revealed the generation of the cleaved PARP. Additionally, Bcl-XL, an important member of Bcl-2 family proteins, plays an important role in extending cellular survival. Compound **6d** at 1 μM effectively decreased the expression of Bcl-XL protein as shown in **Figure 10**, which further confirmed that cell death through apoptosis.

Moreover, the mitochondrial membrane potential (MMP)[43] was tested to confirm compound **6d** induced apoptosis through the mitochondrial pathway. The K562 cells were stained with 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolyl-carbocyanine iodide (JC-1) probe and imaged by fluorescent microscope. JC-1 can aggregate in normal mitochondria and presents red fluorescence, while it produces increased green fluorescence when the potential of mitochondrial membrane is low. Carbonylcyanide-m-chlorophenylhydrazone (CCCP) was used as the positive control. As shown in **Figure 11**, the red fluorescence decreased and the obvious green fluorescence increased with **6d** added. From the results above, **6d** could induce apoptosis through mitochondrial pathway.

3. Conclusion

Based on rational analysis and molecular modeling studies, a series of novel acridone derivatives were synthesized by combination of pyridyl group and the acridone-4-carboxamide scaffold, most of which showed good cytotoxicity against K562 cells. In particular, compound **6d** showed potent activities against K562 cells

(0.46 μM) and nine solid tumor cell lines (0.16-3.79 μM). The DNA-binding properties of **6d** have been investigated by spectrophotometric methods, viscosity measurements and topo I inhibition assay, which demonstrated that **6d** interacts with DNA, inhibits topo I activity and induces apoptosis through the mitochondrial pathway. Our study showed the promising potential of compound **6d** as a potent DNA binding anticancer lead compound and further optimization works were planned for developing it and other analogs into effective anticancer agents.

4. Experimental section

4.1. Synthesis and characterization

See supporting information for synthetic methods and the preparation of compounds **3a-e**, **4a-e** and **5a-j**.

4.1.1. General procedure for compounds 6a-w and 7a-c.

9-oxo-9,10-dihydroacridine-4-carboxamide derivatives (**5a-j**, 0.28mmol) was dissolved in various picolylamines (or 4-Pyridineethanamine, 2.80mmol). The apparatus was flushed with argon and the mixture was stirred at 90 ℃ until the TLC showed the disappearance of the starting material. The mixture was cooled to room temperature and partitioned between CH_2Cl_2 (50 mL) and water (50 mL). The organic layer was worked up to give a residue which was purified by column-chromatography eluted with EtOAC/EtOH (9:1 v/v) to give pure product.

4.1.1.1. N-(2-(dimethylamino)ethyl)-9-oxo-1-((pyridin-4-ylmethyl)amino)-9,10-dihydroacridine-4-carboxamide (6a) yellow solid powder, Yield 10.3%; mp

183-185℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.46 (s, 1H), 11.52 (t, J = 5.7 Hz, 1H), 8.56 (dd, J = 4.6, 1.4 Hz, 2H), 8.37 (d, J = 7.8 Hz, 1H), 7.64 (dd, J = 11.8, 5.0 Hz, 2H), 7.38 (d, J = 8.2 Hz, 1H), 7.33 – 7.28 (m, 2H), 7.25 (s, 1H), 6.98 (s, 1H), 6.03 (d, J = 8.9 Hz, 1H), 4.59 (d, J = 6.0 Hz, 2H), 3.52 (dd, J = 10.9, 5.2 Hz, 2H), 2.59 (t, J = 5.8 Hz, 2H), 2.31 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.10, 169.12, 154.96, 150.19, 147.39, 144.88, 139.41, 133.60, 133.39, 126.06, 122.01, 121.90, 117.37, 107.14, 101.94, 99.74, 57.67, 45.72, 45.05, 36.65; HR-MS(ESI): Calcd for $[M+H]^+$ 416.2087; Found: 416.2097.

4.1.1.2. N-(2-(dimethylamino)ethyl)-5-methyl-9-oxo-1-((pyridine-2-ylmethyl) amino)-9,10-dihydroacridine-4-carboxamide (6b) yellow solid powder, Yield 36.1%; mp 168-170℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.97 (s, 1H), 11.39 (t, *J* = 5.3 Hz, 1H), 8.62 (d, *J* = 4.2 Hz, 1H), 8.41 (t, *J* = 5.3 Hz, 1H), 8.06 (dd, *J* = 17.4, 8.4 Hz, 2H), 7.80 (td, *J* = 7.7, 1.7 Hz, 1H), 7.60 (d, *J* = 7.0 Hz, 1H), 7.44 (d, *J* = 7.8 Hz, 1H), 7.33 (dd, *J* = 6.7, 5.1 Hz, 1H), 7.19 (t, *J* = 7.6 Hz, 1H), 6.35 (d, *J* = 9.0 Hz, 1H), 4.71 (d, *J* = 5.4 Hz, 2H), 3.41 (dd, *J* = 12.5, 6.5 Hz, 2H), 2.53 (s, 3H), 2.49-2.42 (m, 2H), 2.22 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.28, 169.33, 157.84, 154.40, 149.67, 144.58, 138.46, 137.44, 135.09, 134.16, 125.18, 123.78, 122.93, 122.10, 121.77, 121.54, 106.33, 101.52, 100.46, 58.61, 48.19, 45.69, 37.60, 17.02; HR-MS(ESI): Calcd for $[M+H]^+$ 430.2243; Found: 430.2242.

4.1.1.3. N-(2-(dimethylamino)ethyl)-5-methyl-9-oxo-1-((pyridin-3-ylmethyl)

amino)-9,10-dihydroacridine-4-carboxamide (6c) yellow solid powder, Yield 36.1%; mp 149-151℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.93 (s, 1H), 11.22 (t, *J* = 5.5 Hz, 1H), 8.67 (s, 1H), 8.57 – 8.42 (m, 2H), 8.05 (d, *J* = 8.6 Hz, 2H), 7.82 (d, *J* = 7.8 Hz, 1H), 7.60 (d, *J* = 6.9 Hz, 1H), 7.40 (dd, *J* = 7.6, 4.9 Hz, 1H), 7.19 (t, *J* = 7.6 Hz, 1H), 6.37 (d, *J* = 9.0 Hz, 1H), 4.67 (d, *J* = 5.6 Hz, 2H), 3.47-3.43 (m, 2H), 2.70-2.60 (m, 2H), 2.52 (s, 3H), 2.32 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.43, 169.42, 154.51, 149.40, 148.95, 144.53, 138.45, 135.57, 135.21, 134.56, 134.23, 125.18, 124.19, 123.74, 121.83, 121.47, 106.30, 101.76, 100.35, 58.27, 45.22, 43.81, 37.15, 16.95; HR-MS(ESI): Calcd for $[M+H]^+$430.2243; Found: 430.2244.

4.1.1.4. N-(2-(dimethylamino)ethyl)-5-methyl-9-oxo-1-((pyridin-4-ylmethyl) amino)-9,10-dihydroacridine-4-carboxamide (6d) yellow solid powder, Yield 59.4%; mp 220-222℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.62 (s, 1H), 11.53 (t, *J* = 5.7 Hz, 1H), 8.56 (d, *J* = 5.3 Hz, 2H), 8.25 (d, *J* = 8.1 Hz, 1H), 7.64 (d, *J* = 8.9 Hz, 1H), 7.50 (d, *J* = 7.0 Hz, 1H), 7.31 (d, *J* = 5.2 Hz, 2H), 7.19 (t, *J* = 7.6 Hz, 1H), 6.99 (s, 1H), 6.03 (d, *J* = 8.9 Hz, 1H), 4.59 (d, *J* = 6.0 Hz, 2H), 3.52 (dd, *J* = 10.7, 5.2 Hz, 2H), 2.63 (s, 3H), 2.55 (t, *J* = 5.7 Hz, 2H), 2.28 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.38, 169.23, 154.87, 150.13, 147.50, 144.60, 138.54, 133.79, 133.53, 125.02, 123.81, 121.92, 121.75, 121.60, 106.92, 102.08, 99.71, 57.68, 45.70, 45.09, 36.79, 17.14; HR-MS(ESI): Calcd for $[M+H]^+$ 430.2243; Found: 430.2246.

4.1.1.5. N-(3-(dimethylamino)propyl)-5-methyl-9-oxo-1-((pyridin-2-ylmethyl)

amino)-9,10-dihydroacridine-4-carboxamide (6e) yellow solid powder, Yield 56.8%; mp 162-164℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.85 (s, 1H), 11.61 (t, *J* = 5.7 Hz, 1H), 8.78 (s, 1H), 8.64 (d, *J* = 4.2 Hz, 1H), 8.27 (d, *J* = 8.0 Hz, 1H), 7.65 (td, *J* = 7.7, 1.7 Hz, 1H), 7.53 (d, *J* = 8.9 Hz, 1H), 7.49 (d, *J* = 7.0 Hz, 1H), 7.41 (d, *J* = 7.9 Hz, 1H), 7.20 (dd, *J* = 8.0 Hz, 1H), 7.16 (t, *J* = 7.9 Hz, 1H), 6.17 (d, *J* = 8.9 Hz, 1H), 4.72 (d, *J* = 5.9 Hz, 2H), 3.58 (dd, *J* = 10.6, 5.7 Hz, 2H), 2.63 (s, 3H), 2.57-2.45 (m, 2H), 2.33 (s, 6H), 1.79 (dt, *J* = 11.6, 5.9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.35, 169.37, 158.16, 154.86, 149.47, 144.78, 138.64, 136.96, 133.59, 133.26, 125.02, 123.87, 122.24, 121.78, 121.34, 121.09, 106.98, 101.97, 99.94, 59.59, 48.66, 45.41, 40.69, 24.83, 17.23; HR-MS(ESI): Calcd for $[M+H]^+$ 444.2400; Found: 444.2383.

4.1.1.6. N-(3-(dimethylamino)propyl)-5-methyl-9-oxo-1-((pyridin-3-ylmethyl) amino)-9,10-dihydroacridine-4-carboxamide (6f) yellow solid powder, Yield 56.2%; mp 143-145℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.80 (s, 1H), 11.43 (s, 1H), 8.71 (s, 1H), 8.66 (s, 1H), 8.53 (d, J = 3.9 Hz, 1H), 8.20 (d, J = 8.0 Hz, 1H), 7.72 (dd, J = 15.6, 8.4 Hz, 2H), 7.47 (d, J = 6.9 Hz, 1H), 7.15 (t, J = 7.6 Hz, 1H), 6.14 (d, J = 8.9 Hz, 1H), 4.57 (d, J = 5.5 Hz, 2H), 3.59 (d, J = 5.1 Hz, 2H), 2.79 – 2.65 (m, 2H), 2.60 (s, 3H), 2.46 (s, 6H), 1.90 (d, J = 5.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.34, 169.33, 154.71, 149.00, 148.84, 144.74, 138.61, 134.81, 133.71, 133.66, 133.33, 125.05, 123.75, 121.73, 121.43, 106.92, 102.22, 99.58, 59.29, 45.26, 44.31, 40.37, 24.80, 17.18; HR-MS(ESI): Calcd for $[M+H]^+$ 444.2400; Found: 444.2405.

4.1.1.7. N-(3-(dimethylamino)propyl)-5-methyl-9-oxo-1-((pyridin-4-ylmethyl) amino)-9,10-dihydroacridine-4-carboxamide (6g) yellow solid powder, Yield 36.7%; mp 199-201℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.89 (s, 1H), 11.53 (s, 1H), 8.85 (s, 1H), 8.57 (d, *J* = 4.2 Hz, 2H), 8.25 (d, *J* = 7.6 Hz, 1H), 7.52-7.50 (m, 2H), 7.33 (d, *J* = 3.7 Hz, 2H), 7.18 (t, *J* = 7.3 Hz, 1H), 6.03 (d, *J* = 8.8 Hz, 1H), 4.61 (d, *J* = 5.3 Hz, 2H), 3.60-3.50 (m, 2H), 2.64 (s, 3H), 2.55-2.45 (m, 2H), 2.33 (s, 6H), 1.80-1.70 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.44, 169.26, 154.74, 150.21, 147.49, 144.75, 138.66, 133.75, 133.21, 125.13, 123.81, 121.95, 121.74, 121.51, 106.99, 102.47, 99.60, 59.69, 45.70, 45.45, 40.81, 24.75, 17.24; HR-MS(ESI): Calcd for $[M+H]^+$ 444.2400; Found: 444.2397.

4.1.1.8. N-(2-methoxyethyl)-5-methyl-9-oxo-1-((pyridin-2-ylmethyl)amino)-9,10-dihydroacridine-4-carboxamide (6h) yellow solid powder, Yield 57.9%; mp 206-207℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.49 (s, 1H), 11.65 (s, 1H), 8.65 (d, *J* = 4.2 Hz, 1H), 8.27 (d, *J* = 8.1 Hz, 1H), 7.66 (td, *J* = 7.8, 1.5 Hz, 1H), 7.61 (d, *J* = 8.9 Hz, 1H), 7.50 (d, *J* = 6.9 Hz, 1H), 7.40 (d, *J* = 7.8 Hz, 1H), 7.20 (dt, *J* = 15.1, 7.5 Hz, 2H), 6.56 (s, 1H), 6.17 (d, *J* = 8.9 Hz, 1H), 4.73 (d, *J* = 5.7 Hz, 2H), 3.67 (dd, *J* = 9.7, 4.8 Hz, 2H), 3.62-3.53 (m, 2H), 3.41 (s, 3H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.28, 169.29, 157.95, 155.13, 149.46, 144.68, 138.50, 137.01, 133.68, 133.33, 124.92, 123.92, 122.30, 121.85, 121.51, 121.12, 106.92, 101.49, 100.00, 71.26, 58.90, 48.63, 39.41, 17.14; HR-MS(ESI): Calcd for $[M+H]^+$ 417.1927; Found: 417.1927.

4.1.1.9. N-(2-methoxyethyl)-5-methyl-9-oxo-1-((pyridin-3-ylmethyl)amino)-9,10-dihydroacridine-4-carboxamide (6i) yellow solid powder, Yield 57.9%; mp 184-185℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.51 (s, 1H), 11.50 (t, *J* = 5.5 Hz, 1H), 8.67 (d, *J* = 1.7 Hz, 1H), 8.55 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.23 (d, *J* = 8.1 Hz, 1H), 7.74 (d, *J* = 7.8 Hz, 1H), 7.61 (d, *J* = 8.9 Hz, 1H), 7.50 (d, *J* = 7.0 Hz, 1H), 7.31-7.28 (m, 1H), 7.19 (t, *J* = 7.6 Hz, 1H), 6.54 (s, 1H), 6.14 (d, *J* = 8.9 Hz, 1H), 4.60 (d, *J* = 5.7 Hz, 2H), 3.67 (dd, *J* = 9.9, 4.8 Hz, 2H), 3.59 (t, *J* = 4.8 Hz, 2H), 3.41 (s, 3H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.31, 169.21, 154.96, 149.01, 148.90, 144.64, 138.49, 134.80, 133.78, 133.58, 133.33, 124.97, 123.81, 123.76, 121.79, 121.63, 106.88, 101.76, 99.65, 71.22, 58.90, 44.34, 39.41, 17.12; HR-MS(ESI): Calcd for $[M+H]^+$ 417.1927; Found: 417.1920.

4.1.1.10. N-(2-methoxyethyl)-5-methyl-9-oxo-1-((pyridin-4-ylmethyl)amino)-9,10-dihydroacridine-4-carboxamide (6j) yellow solid powder, Yield 82.7%; mp 228-230℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.53 (s, 1H), 11.59 (s, 1H), 8.59 (d, *J* = 4.7 Hz, 2H), 8.27 (d, *J* = 8.1 Hz, 1H), 7.60 (d, *J* = 8.8 Hz, 1H), 7.53 (d, *J* = 7.0 Hz, 1H), 7.33 (d, *J* = 4.5 Hz, 2H), 7.22 (t, *J* = 7.6 Hz, 1H), 6.51 (s, 1H), 6.05 (d, *J* = 8.9 Hz, 1H), 4.62 (d, *J* = 5.7 Hz, 2H), 3.68 (d, *J* = 4.7 Hz, 2H), 3.60 (d, *J* = 4.5 Hz, 2H), 3.41 (s, 3H), 2.64 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.39, 169.17, 155.02, 150.17, 147.42, 144.64, 138.52, 133.86, 133.29, 125.01, 123.85, 121.93, 121.80, 121.69, 106.94, 101.95, 99.72, 71.18, 58.90, 45.72, 39.41, 17.13; HR-MS(ESI): Calcd

for $[M+H]^+$ 417.1927; Found: 417.1932.

4.1.1.11. 1-(bis(pyridin-2-ylmethyl)amino)-N-(2-methoxyethyl)-5-methyl-9-oxo-9,10-dihydroacridine-4-carboxamide (7a) yellow solid powder, Yield 33.9%; mp 180-182℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.19 (s, 1H), 8.47 (d, J = 4.1 Hz, 2H), 8.25 (d, J = 8.0 Hz, 1H), 7.65 – 7.55 (m, 3H), 7.52 (d, J = 7.6 Hz, 2H), 7.47 (d, J = 6.9 Hz, 1H), 7.19 – 7.13 (m, 1H), 7.13 – 7.06 (m, 2H), 6.78 (s, 1H), 6.60 (d, J = 8.7 Hz, 1H), 4.72 (s, 4H), 3.72 – 3.60 (m, 2H), 3.60 – 3.47 (m, 2H), 3.37 (s, 3H), 2.59 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.11, 169.01, 158.25, 154.91, 148.91, 145.30, 138.14, 136.89, 133.46, 131.57, 124.60, 124.57, 122.89, 122.81, 122.10, 121.36, 112.39, 109.21, 107.03, 71.08, 59.71, 58.84, 45.86, 39.47; HR-MS(ESI): Calcd for $[M+H]^+$ 508.2349; Found: 508.2363.

4.1.1.12. N-(2-(dimethylamino)ethyl)-5-methoxy-9-oxo-1-((pyridin-2-ylmethyl) amino)-9,10-dihydroacridine-4-carboxamide (6k) yellow solid powder, Yield 41.9%; mp 201-203℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.60 (s, 1H), 11.64 (s, 1H), 8.65-8.64 (m, 1H), 7.97 (d, *J* = 8.0 Hz, 1H), 7.68-7.64 (m, 2H), 7.41 (d, *J* = 7.6 Hz, 1H), 7.19 (d, *J* = 7.8 Hz, 2H), 7.10 (d, *J* = 7.4 Hz, 1H), 6.97 (s, 1H), 6.18 (d, *J* = 8.8 Hz, 1H), 4.73 (d, *J* = 5.7 Hz, 2H), 4.10 (s, 3H), 3.56 (d, *J* = 4.9 Hz, 2H), 2.74-2.46 (m, 2H), 2.31 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.91, 169.09, 158.11, 155.09, 149.51, 147.94, 144.15, 136.93, 133.65, 131.02, 122.46, 122.23, 121.15, 121.05, 117.17, 111.31, 107.37, 102.06, 99.94, 57.76, 56.28, 48.70, 45.08, 36.73;

HR-MS(ESI): Calcd for $[M+H]^+$ 446.2192; Found: 446.2190.

4.1.1.13. N-(2-(dimethylamino)ethyl)-5-methoxy-9-oxo-1-((pyridin-3-ylmethyl) amino)-9,10-dihydroacridine-4-carboxamide (6l) yellow solid powder, Yield 53.9%; mp 222-224℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.83 (s, 1H), 11.21 (t, *J* = 5.7 Hz, 1H), 8.66 (s, 1H), 8.50 (d, *J* = 4.6 Hz, 1H), 8.31 (s, 1H), 8.00 (d, *J* = 8.9 Hz, 1H), 7.82 (d, *J* = 7.6 Hz, 1H), 7.75 (d, *J* = 8.1 Hz, 1H), 7.40 (dd, *J* = 7.6, 4.9 Hz, 1H), 7.31 (d, *J* = 7.8 Hz, 1H), 7.20 (t, *J* = 8.0 Hz, 1H), 6.36 (d, *J* = 8.9 Hz, 1H), 4.66 (d, *J* = 5.7 Hz, 2H), 4.04 (s, 3H), 3.41-3.38 (m, 2H), 2.43 (t, *J* = 6.8 Hz, 2H), 2.19 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.05, 168.87, 154.45, 149.40, 148.93, 147.94, 143.94, 135.57, 135.12, 134.59, 130.71, 124.18, 122.05, 121.80, 116.95, 112.84, 106.73, 102.30, 100.25, 58.69, 56.88, 45.77, 43.83, 37.72; HR-MS(ESI): Calcd for $[M+H]^+$ 446.2192; Found: 446.2207.

4.1.1.14. N-(2-(dimethylamino)ethyl)-5-methoxy-9-oxo-1-((pyridin-4-ylmethyl) amino)-9,10-dihydroacridine-4-carboxamide (6m) yellow solid powder, Yield 83.8%; mp 190-192℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.61 (s, 1H), 11.54 (s, 1H), 8.57 (d, *J* = 4.8 Hz, 2H), 7.95 (d, *J* = 8.2 Hz, 1H), 7.69 (d, *J* = 8.9 Hz, 1H), 7.32 (d, *J* = 5.2 Hz, 2H), 7.21 (t, *J* = 8.0 Hz, 1H), 7.12 (t, *J* = 7.0 Hz, 1H), 7.08 (s, 1H), 6.03 (d, *J* = 8.8 Hz, 1H), 4.59 (d, *J* = 5.9 Hz, 2H), 4.10 (s, 3H), 3.58 (dd, *J* = 10.5, 5.1 Hz, 2H), 2.62 (t, *J* = 5.6 Hz, 2H), 2.34 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.98, 169.02, 154.92, 150.15, 147.96, 147.50, 144.07, 133.70, 131.02, 122.38, 121.94, 121.33,

117.07, 111.44, 102.50, 100.00, 99.69, 57.82, 56.30, 45.73, 45.00, 36.63; HR-MS(ESI): Calcd for $[M+H]^+$ 446.2192; Found: 446.2209.

4.1.1.15. 1-(bis(pyridin-2-ylmethyl)amino)-N-(2-(dimethylamino)ethyl)-5-methoxy-9-oxo-9,10-dihydroacridine-4-carboxamide (7b) yellow solid powder, Yield 38.7%; mp 97-98℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.25 (s, 1H), 8.46 (s, 2H), 7.93 (d, J = 7.8 Hz, 1H), 7.84 (d, J = 8.7 Hz, 1H), 7.65 (s, 1H), 7.61 – 7.43 (m, 4H), 7.14 (t, J = 7.8 Hz, 1H), 7.07 (dd, J = 12.4, 6.7 Hz, 3H), 6.61 (d, J = 8.6 Hz, 1H), 4.70 (s, 4H), 4.04 (s, 3H), 3.66 (br, 2H), 2.81 (br, 2H), 2.48 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.61, 168.97, 158.35, 155.02, 149.02, 147.76, 144.88, 136.75, 132.29, 130.55, 123.47, 122.73, 122.02, 120.95, 117.86, 112.62, 111.27, 109.22, 107.13, 59.73, 58.08, 56.27, 44.63, 36.11; HR-MS(ESI): Calcd for $[M+H]^+$ 537.2614; Found: 537.2595.

4.1.1.16. N-(2-(dimethylamino)ethyl)-5-methoxy-9-oxo-1-((2-(pyridin-4-yl)ethyl) amino)-9,10-dihydroacridine-4-carboxamide (6n) yellow solid powder, Yield 25.4%; mp 208-210℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.57 (s, 1H), 11.17 (s, 1H), 8.54 (d, *J* = 5.7 Hz, 2H), 7.90 (d, *J* = 8.1 Hz, 1H), 7.78 (d, *J* = 8.9 Hz, 1H), 7.23 (d, *J* = 5.6 Hz, 2H), 7.17 (t, *J* = 8.0 Hz, 1H), 7.13 (s, 1H), 7.07 (d, *J* = 7.7 Hz, 1H), 6.23 (d, *J* = 8.9 Hz, 1H), 4.07 (s, 3H), 3.65-3.55 (m, 4H), 3.06 (t, *J* = 7.3 Hz, 2H), 2.72-2.61 (m, 2H), 2.39 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.74, 169.16, 155.02, 149.98, 147.99, 147.91, 144.26, 133.83, 130.97, 124.10, 122.44, 121.14, 117.12, 111.34,

106.99, 101.62, 99.03, 58.11, 56.26, 44.96, 43.23, 36.51, 34.73; HR-MS(ESI): Calcd for $[M+H]^+$ 460.2349; Found: 460.2362.

4.1.1.17. N-(3-(dimethylamino)propyl)-5-methoxy-9-oxo-1-((pyridin-2-ylmethyl) amino)-9,10-dihydroacridine-4-carboxamide (6o) yellow solid powder, Yield 67.4%; mp 204-206℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.87 (s, 1H), 11.53 (t, *J* = 5.8 Hz, 1H), 8.76 (s, 1H), 8.57 (dd, *J* = 4.5, 1.5 Hz, 2H), 7.94 (d, *J* = 7.9 Hz, 1H), 7.52 (d, *J* = 8.9 Hz, 1H), 7.33 (d, *J* = 5.9 Hz, 2H), 7.19 (t, *J* = 8.0 Hz, 1H), 7.09 (dd, *J* = 7.8, 0.9 Hz, 1H), 6.02 (d, *J* = 8.9 Hz, 1H), 4.60 (d, *J* = 6.0 Hz, 2H), 4.09 (s, 3H), 3.60 (dd, *J* = 10.5, 5.7 Hz, 2H), 2.58-2.51 (m, 2H), 2.32 (s, 6H), 1.79 (dt, *J* = 11.5, 5.9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.92, 169.02, 158.25, 154.86, 149.45, 148.01, 144.23, 136.90, 133.19, 131.13, 122.41, 122.18, 121.06, 120.99, 117.14, 111.24, 107.43, 102.49, 99.82, 59.79, 56.24, 48.71, 45.50, 40.81, 24.99; HR-MS(ESI): Calcd for $[M+H]^+$ 460.2349; Found: 460.2343.

4.1.1.18. N-(3-(dimethylamino)propyl)-5-methoxy-9-oxo-1-((pyridin-3-ylmethyl) amino)-9,10-dihydroacridine-4-carboxamide (6p) yellow solid powder, Yield 56.2%; mp 220-222℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.83 (s, 1H), 11.46 (s, 1H), 8.70 (s, 1H), 8.67 (s, 1H), 8.54 (d, *J* = 3.8 Hz, 1H), 7.91 (d, *J* = 8.1 Hz, 1H), 7.75 (d, *J* = 7.6 Hz, 1H), 7.60 (d, *J* = 8.8 Hz, 1H), 7.30 (s, 1H), 7.17 (t, *J* = 7.9 Hz, 1H), 7.08 (d, *J* = 7.5 Hz, 1H), 6.14 (d, *J* = 8.9 Hz, 1H), 4.59 (d, *J* = 5.3 Hz, 2H), 4.08 (s, 3H), 3.60 (d, *J* = 4.5 Hz, 2H), 2.65-2.55 (m, 2H), 2.38 (s, 6H), 1.86-1.76 (m, 2H); ^{13}C NMR

(100 MHz, $CDCl_3$) δ 180.93, 169.06, 154.70, 149.01, 148.84, 147.99, 144.17, 134.84, 133.72, 133.45, 131.05, 123.78, 122.32, 121.16, 116.98, 111.30, 107.33, 102.59, 99.56, 59.17, 56.26, 45.19, 44.32, 40.17, 24.80; HR-MS(ESI): Calcd for $[M+H]^+$ 460.2349; Found: 460.2343.

4.1.1.19. N-(3-(dimethylamino)propyl)-5-methoxy-9-oxo-1-((pyridin-4-ylmethyl) amino)-9,10-dihydroacridine-4-carboxamide (6q) yellow solid powder, Yield 65.8%; mp 212-214℃; ^1H NMR (400 MHz, $CDCl_3$) δ 13.84 (s, 1H), 11.62 (t, *J* = 5.8 Hz, 1H), 8.73 (s, 1H), 8.66-8.59 (m, 1H), 7.95 (d, *J* = 8.1 Hz, 1H), 7.64 (td, *J* = 7.7, 1.8 Hz, 1H), 7.52 (d, *J* = 8.9 Hz, 1H), 7.41 (d, *J* = 7.9 Hz, 1H), 7.18 (dd, *J* = 15.3, 7.5 Hz, 2H), 7.08 (dd, *J* = 7.8, 1.0 Hz, 1H), 6.16 (d, *J* = 8.9 Hz, 1H), 4.72 (d, *J* = 5.9 Hz, 2H), 4.08 (s, 3H), 3.59 (dd, *J* = 10.6, 5.7 Hz, 2H), 2.58-2.48 (m, 2H), 2.34 (s, 6H), 1.83-1.72 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.93, 169.05, 158.22, 154.85, 149.46, 147.99, 144.23, 136.94, 133.28, 131.09, 122.38, 122.21, 121.06, 121.02, 117.10, 111.21, 107.40, 102.41, 99.87, 59.67, 56.25, 48.69, 45.44, 40.69, 24.88; HR-MS(ESI): Calcd for $[M+H]^+$ 460.2349; Found: 460.2336.

4.1.1.20. 1-(bis(pyridin-2-ylmethyl)amino)-N-(3-(dimethylamino)propyl)-5-methoxy-9-oxo-9,10-dihydroacridine-4-carboxamide (7c) yellow solid powder, Yield 39.9%; mp 90-92℃; ^1H NMR (400 MHz, $CDCl_3$) δ 13.55 (s, 1H), 8.85 (s, 1H), 8.50 (d, *J* = 4.4 Hz, 2H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.63-7.54 (m, 4H), 7.52 (d, *J* = 8.8 Hz, 1H), 7.16 (t, *J* = 7.9 Hz, 1H), 7.13-6.99 (m, 3H), 6.60 (d, *J* = 8.7 Hz, 1H), 4.73 (s,

4H), 4.07 (s, 3H), 3.58 (d, *J* = 4.8 Hz, 2H), 2.58-2.46 (m, 2H), 2.29 (s, 6H), 1.82-1.65 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.70, 168.66, 158.47, 154.59, 148.94, 147.87, 144.89, 136.80, 131.35, 130.66, 123.38, 122.81, 122.03, 120.88, 117.76, 112.91, 111.17, 109.20, 108.00, 59.75, 59.47, 56.24, 45.41, 40.68, 24.86; HR-MS(ESI): Calcd for $[M+H]^+$ 551.2771; Found: 551.2793.

4.1.1.21. 5-methoxy-N-(2-methoxyethyl)-9-oxo-1-((pyridin-2-ylmethyl)amino)-9,10-dihydroacridine-4-carboxamide (6r) yellow solid powder, Yield 83.5%; mp 218-220℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.48 (s, 1H), 11.65 (s, 1H), 8.64 (d, *J* = 4.6 Hz, 1H), 7.95 (d, *J* = 7.9 Hz, 1H), 7.66 (t, *J* = 7.1 Hz, 1H), 7.60 (d, *J* = 8.9 Hz, 1H), 7.40 (d, *J* = 7.7 Hz, 1H), 7.20 (dd, *J* = 16.1, 7.9 Hz, 2H), 7.09 (d, *J* = 7.7 Hz, 1H), 6.47 (s, 1H), 6.17 (d, *J* = 8.9 Hz, 1H), 4.73 (d, *J* = 5.5 Hz, 2H), 4.08 (s, 3H), 3.67 (d, *J* = 4.9 Hz, 2H), 3.58 (d, *J* = 4.8 Hz, 2H), 3.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.90, 168.95, 157.96, 155.13, 149.28, 147.90, 144.11, 137.15, 133.37, 130.97, 122.45, 122.32, 121.22, 121.16, 117.15, 111.35, 107.36, 101.97, 99.93, 71.29, 58.88, 56.29, 48.56, 39.35; HR-MS(ESI): Calcd for $[M+H]^+$ 433.1876; Found: 433.1862.

4.1.1.22. 5-methoxy-N-(2-methoxyethyl)-9-oxo-1-((pyridin-3-ylmethyl)amino)-9,10-dihydroacridine-4-carboxamide (6s) yellow solid powder, Yield 75.2%; mp 252-253℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.48 (s, 1H), 11.49 (s, 1H), 8.66 (s, 1H), 8.54 (d, J = 3.9 Hz, 1H), 7.91 (d, J = 8.1 Hz, 1H), 7.73 (d, J = 7.6 Hz, 1H), 7.60 (d, J = 8.9 Hz, 1H), 7.29 – 7.27 (m, 1H), 7.18 (t, J = 8.0 Hz, 1H), 7.08 (d, J = 7.7 Hz, 1H),

6.50 (s, 1H), 6.11 (d, J = 8.9 Hz, 1H), 4.58 (d, J = 5.5 Hz, 2H), 4.08 (s, 3H), 3.75 – 3.62 (m, 2H), 3.62 – 3.50 (m, 2H), 3.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.88, 168.90, 154.95, 149.03, 148.89, 147.92, 144.06, 134.78, 133.59, 133.34, 130.97, 123.72, 122.41, 121.30, 117.06, 111.43, 107.31, 102.29, 99.54, 71.27, 58.85, 56.28, 44.35, 39.37; HR-MS(ESI): Calcd for $[M+H]^+$ 433.1876; Found: 433.1881.

4.1.1.23. 5-methoxy-N-(2-methoxyethyl)-9-oxo-1-((pyridin-4-ylmethyl)amino)-9,10-dihydroacridine-4-carboxamide (6t) yellow solid powder, Yield 79.3%; mp 235-236℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.48 (s, 1H), 11.56 (t, *J* = 5.7 Hz, 1H), 8.57 (d, *J* = 5.1 Hz, 2H), 7.94 (d, *J* = 8.2 Hz, 1H), 7.58 (d, *J* = 8.9 Hz, 1H), 7.34 (d, *J* = 5.2 Hz, 2H), 7.19 (d, *J* = 8.0 Hz, 1H), 7.10 (d, *J* = 7.8 Hz, 1H), 6.48 (s, 1H), 6.01 (d, *J* = 8.9 Hz, 1H), 4.60 (d, *J* = 5.9 Hz, 2H), 4.09 (s, 3H), 3.67 (dd, *J* = 10.0, 5.1 Hz, 2H), 3.57 (t, *J* = 4.9 Hz, 2H), 3.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.95, 168.85, 154.99, 150.15, 147.94, 147.42, 144.04, 133.30, 130.99, 122.41, 121.92, 121.36, 117.09, 111.48, 107.36, 102.48, 99.59, 71.24, 58.85, 56.30, 45.74, 39.37; HR-MS(ESI): Calcd for $[M+H]^+$ 433.1876; Found: 433.1881.

4.1.1.24. N-(4-(dimethylamino)butyl)-5-methoxy-9-oxo-1-((pyridin-4-ylmethyl) amino)-9,10-dihydroacridine-4-carboxamide (6u) yellow solid powder, Yield 74.2%; mp 213-215℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.86 (s, 1H), 11.26 (t, *J* = 5.8 Hz, 1H), 8.54 (d, *J* = 4.0 Hz, 2H), 8.43 (s, 1H), 7.98 (d, *J* = 9.0 Hz, 1H), 7.77 (d, *J* = 7.9 Hz, 1H), 7.39 (d, *J* = 5.2 Hz, 2H), 7.32 (d, *J* = 7.5 Hz, 1H), 7.21 (t, *J* = 8.0 Hz,

1H), 6.22 (d, *J* = 9.0 Hz, 1H), 4.69 (d, *J* = 5.8 Hz, 2H), 4.04 (s, 3H), 3.30-3.20 (m, 2H), 2.21 (t, *J* = 7.0 Hz, 2H), 2.11 (s, 6H), 1.54 (dd, *J* = 14.2, 7.1 Hz, 2H), 1.46 (dd, *J* = 14.2, 7.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.98, 169.16, 154.67, 150.13, 147.99, 147.45, 143.94, 133.51, 131.06, 122.35, 121.96, 121.23, 117.04, 111.38, 107.40, 103.20, 99.25, 59.34, 56.26, 45.72, 45.32, 39.90, 27.40, 25.69; HR-MS(ESI): Calcd for $[M+H]^+$ 474.2505; Found: 474.2495.

4.1.1.25. N-(2-(dimethylamino)ethyl)-9-oxo-1-((pyridin-4-ylmethyl)amino)-5-(trifluoromethyl)-9,10-dihydroacridine-4-carboxamide (6v) yellow solid powder, Yield 18.2%; mp 178-180℃; ^{1}H NMR (400 MHz, MeOD) δ 8.58 (d, *J* = 8.2 Hz, 1H), 8.50 (d, *J* = 5.9 Hz, 2H), 8.05 (d, *J* = 7.5 Hz, 1H), 7.93 (d, *J* = 9.0 Hz, 1H), 7.49 (d, *J* = 5.4 Hz, 2H), 7.38 (t, *J* = 7.9 Hz, 1H), 6.28 (d, *J* = 9.1 Hz, 1H), 4.73-4.76 (m, 2H), 3.56 (t, *J* = 6.7 Hz, 2H), 2.64 (t, *J* = 6.6 Hz, 2H), 2.37 (s, 6H). ^{19}F NMR (376 MHz, DMSO-d_6) δ -61.63; HR-MS(ESI): Calcd for $[M+H]^+$484.1960; Found: 484.1940.

4.1.1.26. 5,7-dichloro-N-(2-(dimethylamino)ethyl)-9-oxo-1-((pyridin-4-ylmethyl) amino)-9,10-dihydroacridine-4-carboxamide (6w) yellow solid powder, Yield 28.1%; mp 114-116℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.01 (s, 1H), 11.23 (s, 1H), 8.57 (d, *J* = 5.7 Hz, 2H), 8.23 (d, *J* = 2.2 Hz, 1H), 7.74 (d, *J* = 9.0 Hz, 1H), 7.69 (d, *J* = 2.2 Hz, 1H), 7.46 (s, 1H), 7.29 (d, *J* = 5.5 Hz, 2H), 6.04 (d, *J* = 9.0 Hz, 1H), 4.56 (d, *J* = 5.6 Hz, 2H), 3.64 (d, *J* = 5.2 Hz, 2H), 2.80-2.70 (m, 2H), 2.44 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.24, 169.03, 154.69, 150.20, 146.97, 144.41, 135.25, 134.56,

132.65, 126.87, 124.39, 123.28, 122.48, 121.87, 106.77, 102.09, 100.76, 57.97, 45.71, 44.45, 35.96; HR-MS(ESI): Calcd for $[M+H]^+$ 484.1307; Found: 484.1317.

4.2. Molecular docking

The molecular modeling of organic compounds were performed with Discovery Studio 3.1.1/Libdock protocol (Accelrys Software Inc.) according to the reported process[16, 44]. Three dimensional structures of human topo I-DNA complex (PDB ID: 1K4T) was downloaded from Protein Data Bank (PDB). The general procedure is as followed: (a) removing the water molecules which co-crystallized with the original protein structure; (b) preparing ligand and receptor and then finding the candidate binding site; (c) deleting small molecular docking in candidate binding site on the target protein and then docking the designed compound into the candidate binding site; (d) molecular modeling based on the above docking data.

4.3. Bioassay

4.3.1. Cell culture

K562 (suspension cells line), NCI-H520 (adherent cell line), Hela (adherent cell line), were cultured followed by the instruction in reference RPMI-1640, HepG-2,MCF-7, CNE-2, A375, A172 and U118-MG (adherent cell lines) were cultured in DMEM, U251 (adherent cell line) was cultured in MEM-EBSS, with 10% fetal bovine serum (FBS) in humidified air at 37 ºC with 5% CO_2.

4.3.2. Cell growth inhibition assay

The K562 cells were seeded into 96-well plates at 1.5×10^4 cells/well, treated with the synthesized compounds with the final concentrations at 50, 25, 10, 1 and 0.1

μM. After 48 h treatment, the cells were incubated with 15 μL MTT (3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide from Sigma) solution (5 mg/mL) for 4 h at 37 ºC, 5% CO_2. The adherent cells (NCI-H520, U251, A375, A172, Hela, CNE-2, U118-MG, HepG2 and MCF-7 cells) were seeded into 96-well plates at 6×10^3 cells/well, treated with the synthesized compounds at different concentrations after these adherent cells hatched for 12 hours at 37 ºC, 5% CO_2. (The final concentrations of these compounds were 50, 25, 10, 1 and 0.1 μM). After 48 h treatment, the cells were incubated with 10 μL MTT solution (5 mg/mL) for 4 h at 37 ºC, 5% CO_2.The formazan precipitates were dissolved in 100 μL DMSO. At 490 nm, the absorbance was measured by Infinite M1000 PRO (TECAN).

4.4. Biophysical evaluation

4.4.1. Materials

Concentrated stock solutions of compounds were prepared by dissolving them in DMSO. Calf thymus DNA (ct DNA) and EB were obtained from Sigma Chemical Co. All the measurements involving the interactions of tested compound **6d** with ct DNA were carried out in doubly distilled water buffer containing 5 mM Tris and 50 mM NaCl, and adjusted to pH 7.2 with hydrochloric acid. Stock solutions of ct DNA were prepared in buffer and concentration was determined by UV absorbance by employing an extinction coefficient of 6600 $M^{-1}cm^{-1}$ at 260 nm.

4.4.2. UV-visible Absorption spectra

UV-visible absorption spectra were all recorded on a computer controlled Beckman Coulter DU 800 spectrophotometer by using a quartz cell having 1.0 cm

pathway. The solution of tested compound **6d** (1 mM in DMSO, 20 μL) and the buffer above were transferred to the quartz, and then a known volume (0-30) μL of ct DNA solution (2 mM in Tris-HCl buffer) was also added. The solution was incubated for 5 min and then tested.

4.4.3. Fluorescence emission spectra

The emission spectra were carried out on Fluorolog spectrometer. 20 μL of tested compound **6d** (1 mM in DMSO) was incubated in Tris-HCl buffer solution (the final concentration of the tested compound was 10 μM), and then ct DNA solution (2 mM in Tris-HCl buffer) was also added with the final concentration from 0 to 100 μM. The excitation wavelength was set at 425 nm. The incubating time before testing was 5 min.

4.4.4. Fluorescence spectra of DNA-EB in the presence of 6d

The emission spectra were carried out on Fluorolog spectrometer. 20 μL of EB (1 mM in Tris-HCl buffer) and 40 μL ct DNA solution (2 mM in Tris-HCl buffer) were incubated inTris-HCl buffer solution (the final concentration of EB was 10 μM), and then a known volume (0-60) μL of tested compound **6d** (2.5 mM in DMSO) was also added with the final concentration from 0 to 30 μM. The excitation wavelength was set at 366 nm. The incubating time before testing was 5 min.

4.4.5. Viscosity experiments

Viscosity experiments were carried out on a Pinkevitch viscometer maintained at a constant temperature at 25.0 ºC. A known volume (12-72) μL of tested compound **6d** (2.5 mM in DMSO) was introduced into DNA solution (200 μM in Tris-HCl buffer)

present in the viscometer. Flow time was measured with a digital stopwatch, and each sample was measured three times, and an average flow time was calculated. Relative viscosities for DNA in the presence and absence of compounds were calculated from the relation $\eta = (t - t_0)/t_0$, where t is the observed flow time of the DNA-containing solution and t_0 is the flow time of buffer alone. Data were presented as $(\eta/\eta_0)^{1/3}$ versus binding ratio of the concentration of the tested compound **6d** to DNA, where η is the viscosity of DNA in the presence of compound, and η_0 is the viscosity of DNA alone.

4.5. DNA topo I inhibition assay

The solutions of a mixture of 100 ng of plasmid DNA pBR322 (commercial available from Takara), 1.0 units of recombinant human DNA topo I (from Takara) and with compounds of different amount were incubated at 37 ºC for 30 min in the relaxation buffer (35 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 72 mM KCl, 0.01% bovine serum albumin, 2 mM spermidine, 5 mM dithiothreitol). DNA samples were then electrophoresed on a 1% agarose gel at 100 V for 25 min with a running Buffer (Tris-Acetic acid-EDTA). Gels were visualized by EB staining under ultraviolet light.

4.6. Western blot analysis

K562 cells were cultured in 6 cm dishes, followed by treatment with **6d** for different concentration-periods for 36 h. Then the cells were centrifuged and treated with lysis buffer at 0 ºC for 0.5 h, followed by centrifugation at 20,000 g for 10 min. Protein concentrations in the supernatant were determined using bicinchoninic acid (BCA). Lysate proteins were subjected to 12% sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), and electrophoretically transferred

to PVDF membrane (amcBiobind NT-200). After blotting, the membrane was blocked in 5% milk for 1 h, and incubated with the specific primary antibody for overnight at 4 ºC. Protein bands were detected using the BIO-RAD GelDoc XR after hybridization with the antibody.

4.7. Mitochondrial membrane potential detection

The mitochondrial membrane potential detection was tested using the Mitochondrial membrane potential detection kit (Beyotime Company), 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolyl-carbocyanine iodide (JC-1) as fluorescent cationic dye was used to measure mitochondrial membrane potential, while CCCP was used as the positive control.

4.8. Flow cytometry assay

Phosphatidylserine externalization was determined with the scheme which is designed as the instructions of the manufacturer by AnnexinV-FITC/PI apoptosis detection kit (Beyotime Company).

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Supplementary data

Supplementary data (molecular docking analysis of compound **A** and **B** with topo I-DNA complex model, general methods and the preparation of compounds **3a-e**, **4a-e** and **5a-j**, ^{1}H NMR, ^{13}C NMR and High resolution mass spectrometry of synthetic compounds) associated with this article can be found, in the online version.

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Captions

Figure 1. The structures of C-1311, DACA, PZA, BRACO-19 and *m*-AMSA as typical acridine/acridone derivatives

Figure 2. The structure of representative compound **6d** with two intramolecular hydrogen bonds which could form a pseudo-five-cyclic system

Figure 3. (a) Molecular modeling of compound **6d** binding to topo I-DNA complex. (b) Hydrogen bonds and cation-pi interactions existed between **6d** and amino acid residues of topo I (ARG364, ARG488, LYS751, LYS532 and ASP533), pi-pi interactions existed between the acridone ring of **6d** and DNA base pairs (DT10, TGP11, DC112 and DA113). (c) Interactions between the receptor and the ligand on 2D diagram. Compound **6d** is represented by the yellow color and hydrogen bonds are represented by green dotted lines, while pi-pi interactions and cation-pi interactions are represented by orange lines.

Figure 4. (a) UV-visible absorption spectra of tested compound **6d** (10 μM) in the presence of increasing amounts of ct DNA; [DNA] = 0, 2.5, 5, 7.5, 10, 15, 20, 30 μM. DNA titration of the compound was performed in 5 mM Tris-HCl buffer containing 50 mM NaCl at pH 7.2. The arrow indicates the absorbance changes upon increasing DNA concentrations. (b) The plot of [DNA]/($\square_a$ - $\square_f$) versus [DNA] for the titration

of DNA to **6d**. The binding constant $K_b = 5.1 \times 10^4\ M^{-1}$.

Figure 5. (a) Spectrofluorimetric titration of compound **6d** (10 μM) in 5 mM Tris-HCl buffer containing 50 mM NaCl (pH 7.2) by increasing the concentrations of ct DNA; [DNA] = 0, 10, 20, 30, 40 ,50, 60, 70, 80, 90, 100 μM; λ_{ex} = 425 nm. The arrow indicates the fluorescence emission changes upon increasing DNA concentrations. (b) The plot of F/F_0 versus [DNA]/[compound] for the titration of DNA to **6d**. F_0 was the fluorescence intensity of tested compound **6d** in Tris-HCl buffer without adding ct DNA.

Figure 6.(a) Fluorescence emission spectra of DNA-EB in the presence of 0, 5, 10, 20, 30 μM of **6d**; [EB] = 10 μM; [DNA] = 40 μM; λ_{ex} = 366 nm. (b) The Stern-Volmer quenching plots of the fluorescence titration. The quenching constant $K_q = 1.0 \times 10^4\ M^{-1}$.

Figure 7. Effect of increasing amounts of **6d** on the relative viscosity of ct DNA at 25.0 ºC, [DNA] = 200 μM, [**6d**] = 6, 12, 18, 24, 30, 36 μM.

Figure 8. Effect of the compounds on the relaxation of plasmid DNA by human topo I. (a) Lane 1, DNA pBR322; Lane 2, topo I + DNA pBR322 + DMSO; Lanes 3-17, DNA pBR322 relaxation by topo I and **6b-j**, **7a**, **6k-m**, **7b**, **6o** at concentrations of 50 μM, respectively. (b) Lane 1, DNA pBR322; Lane 2, topo I + DNA pBR322 +DMSO;

Lanes 3-17, DNA pBR322 relaxation by topo I and **6p-q**, **7c**, **6r-u**, **6n**, **6a**, **6v-w** at concentrations of 50 μM, respectively.

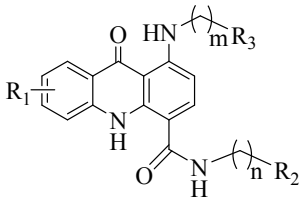
Figure 9. Flow cytometric analysis of phosphoatidylserine externalization (Annexin-V binding) and cell membrane integrity (PI staining). K562 cells were treated with **6d** at 0, 0.05, 0.25, 0.5, 1, 2.5 μM, respectively.

Figure 10. K562 cells were treated with different concentrations of compound **6d**, and Western blot analysis was used to evaluate the levels of C-caspase-9, C-caspase-7, C-caspase-3, C-PARP and Bcl-XL.

Figure 11. Detection of the mitochondrial membrane potential using JC-1 (a) K562 cells with no compound added as blank group; (b) K562 cells were treated with CCCP (10 μM) as the positive control group; (c) K562 cells were treated with **6d** at 1 μM for 24 h.

Scheme 1. Reagents and conditions: (i) K_2CO_3, Cu, DMF, 130 ºC, overnight; (ii) H_2SO_4, 80 ºC, 5 h; (iii) CDI, various N,N-dimethyldiamines or 2-methoxyethanamine, DMF, r.t.; (iv) various picolylamine or 4-Pyridineethanamine, under the protection of Ar, 90 ºC.

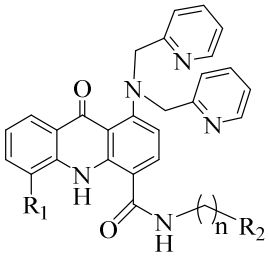
Table 1. Antiproliferative activity of pyridyl acridone derivatives aganist K562 cells



6a-w

Compd	R_1	R_2	R_3	m	n	K562 IC_{50}(μM)
Adriamycin						0.55 ± 0.019
Cisplatin						20.92 ± 2.828
6a	H	$N(CH_3)_2$	4-pyridyl	1	2	6.33 ± 3.579
6b	5-CH_3	$N(CH_3)_2$	2-pyridyl	1	2	1.71 ± 0.145
6c	5-CH_3	$N(CH_3)_2$	3-pyridyl	1	2	1.09 ± 0.277
6d	5-CH_3	$N(CH_3)_2$	4-pyridyl	1	2	0.46 ± 0.206
6e	5-CH_3	$N(CH_3)_2$	2-pyridyl	1	3	6.45 ± 1.079
6f	5-CH_3	$N(CH_3)_2$	3-pyridyl	1	3	11.77 ± 1.844
6g	5-CH_3	$N(CH_3)_2$	4-pyridyl	1	3	8.25 ± 2.143
6h	5-CH_3	OCH_3	2-pyridyl	1	2	> 50
6i	5-CH_3	OCH_3	3-pyridyl	1	2	> 50
6j	5-CH_3	OCH_3	4-pyridyl	1	2	> 50
6k	5-OCH_3	$N(CH_3)_2$	2-pyridyl	1	2	2.28 ± 0.538
6l	5-OCH_3	$N(CH_3)_2$	3-pyridyl	1	2	0.93 ± 0.038
6m	5-OCH_3	$N(CH_3)_2$	4-pyridyl	1	2	1.36 ± 0.363
6n	5-OCH_3	$N(CH_3)_2$	4-pyridyl	2	2	3.08 ± 0.181
6o	5-OCH_3	$N(CH_3)_2$	2-pyridyl	1	3	6.12 ± 0.857
6p	5-OCH_3	$N(CH_3)_2$	3-pyridyl	1	3	11.27 ± 0.632
6q	5-OCH_3	$N(CH_3)_2$	4-pyridyl	1	3	11.94 ± 2.932
6r	5-OCH_3	OCH_3	2-pyridyl	1	2	> 50
6s	5-OCH_3	OCH_3	3-pyridyl	1	2	> 50
6t	5-OCH_3	OCH_3	4-pyridyl	1	2	> 50
6u	5-OCH_3	$N(CH_3)_2$	4-pyridyl	1	4	6.07 ± 0.666
6v	5-CF_3	$N(CH_3)_2$	4-pyridyl	1	2	0.89 ± 0.055
6w	5,7-dichloro	$N(CH_3)_2$	4-pyridyl	1	2	0.97 ± 0.224

Table 2. Antiproliferative activity of 2,2′-bispyridyl acridone derivatives aganist K562 cells

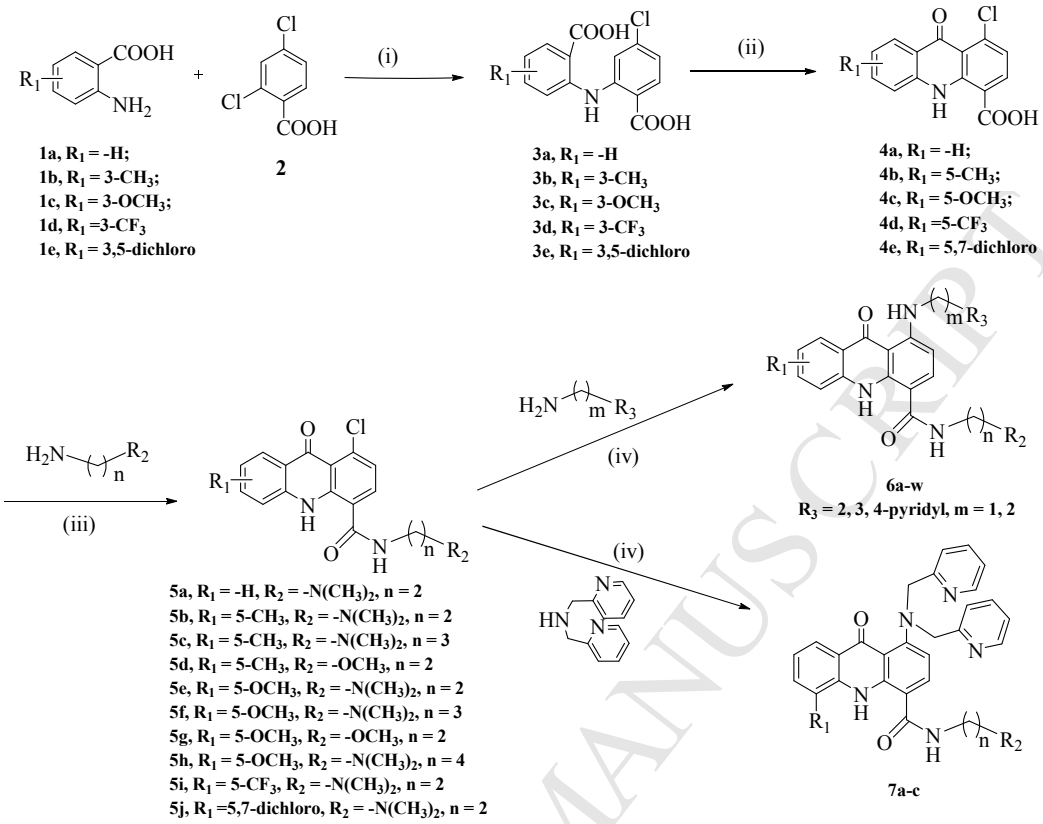


7a-c

Compd	R_1	R_2	n	K562 IC_{50}(μM)
Adriamycin				0.55 ± 0.019
Cisplatin				20.92 ± 2.828
7a	CH_3	OCH_3	2	11.20 ± 2.198
7b	OCH_3	$N(CH_3)_2$	2	13.90 ± 3.509
7c	OCH_3	$N(CH_3)_2$	3	13.53 ± 1.456

Table 3. Antiproliferative activities of compound **6d** aganist various cancer cell lines

Cell Lines	**NCI-H520**	**U251**	**A375**	**A172**	**Hela**	**CNE-2**	**U118-MG**	**HepG2**	**MCF-7**
IC_{50} (μM)	1.03 ± 0.078	0.79 ± 0.492	0.16 ± 0.012	2.20 ± 0.583	3.79 ± 0.347	0.98 ± 0.202	1.08 ± 0.036	0.32 ± 0.036	2.55 ± 0.049



Scheme 1

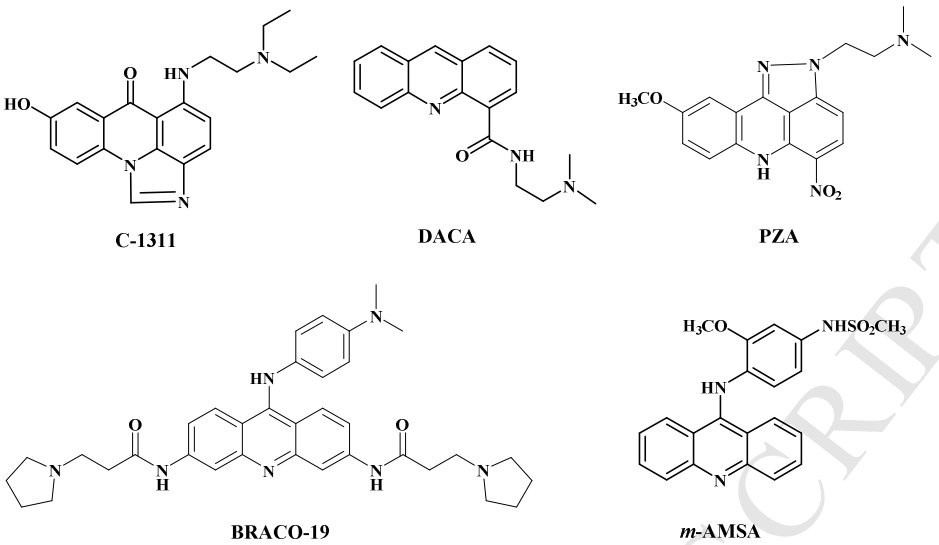


Figure 1

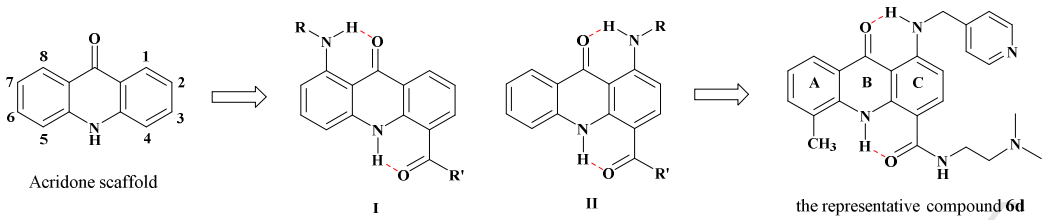


Figure 2

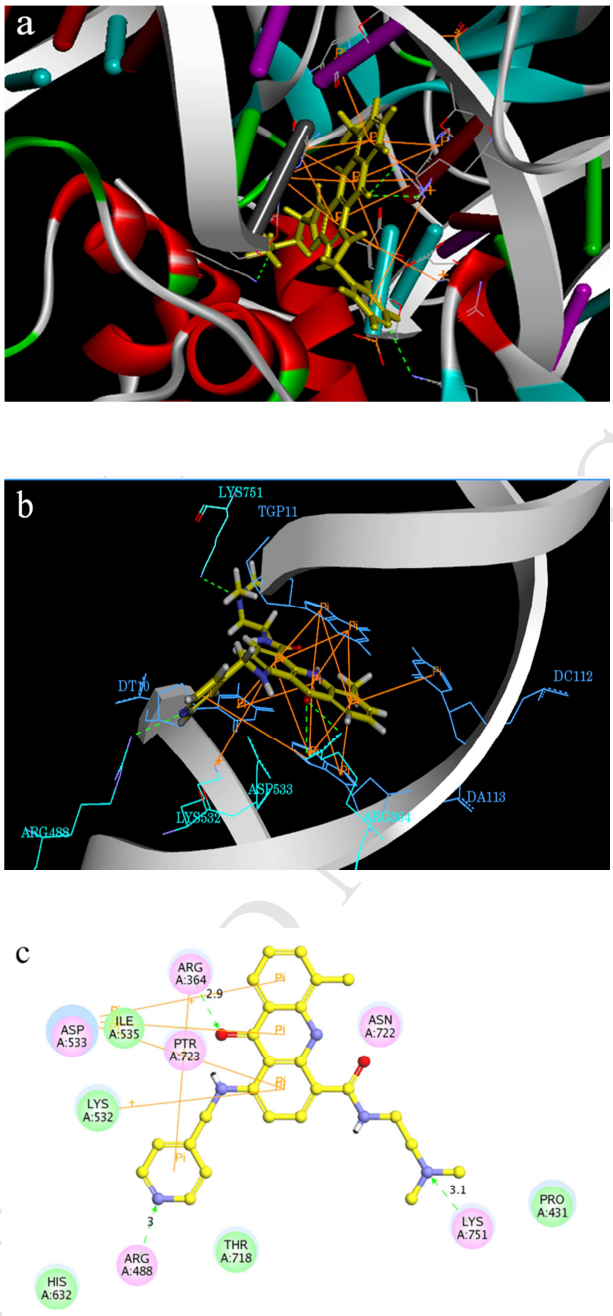

a
b
LYS751
TGP11
DT10
DC112
ASP533
LYS532
ARG488
DA113
c
ARG A:364
2.9
ASP A:533
ILE A:535
PTR A:723
ASN A:722
LYS A:532
3.1
PRO A:431
LYS A:751
3
ARG A:488
THR A:718
HIS A:632

Figure 3

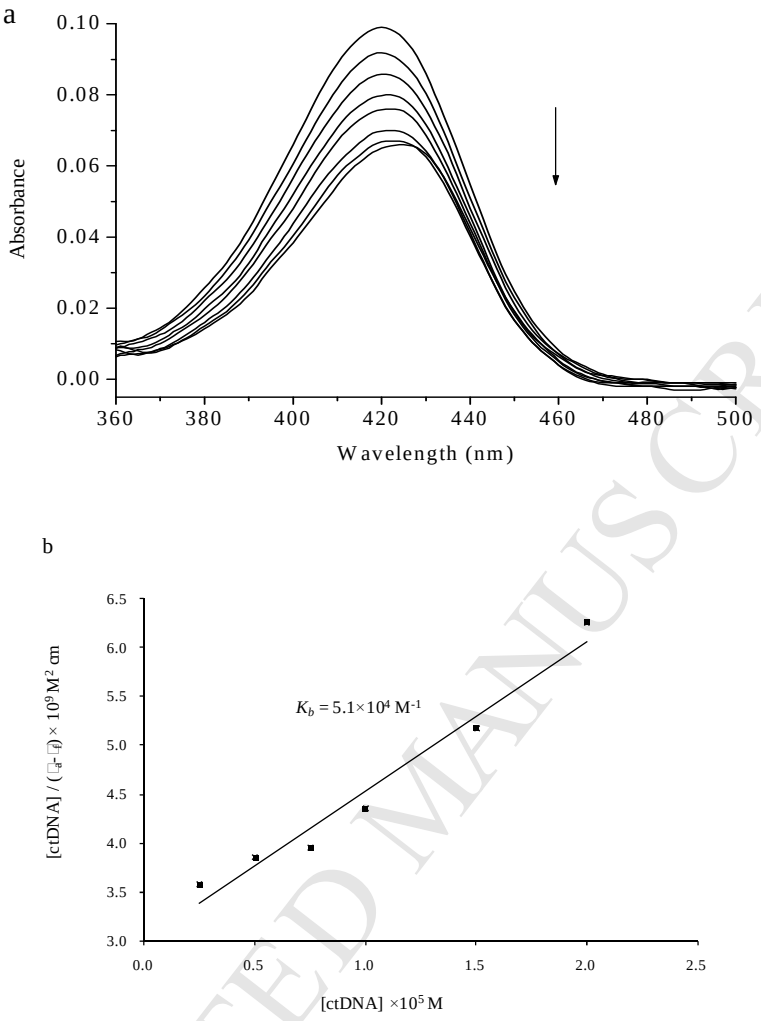


Figure 4

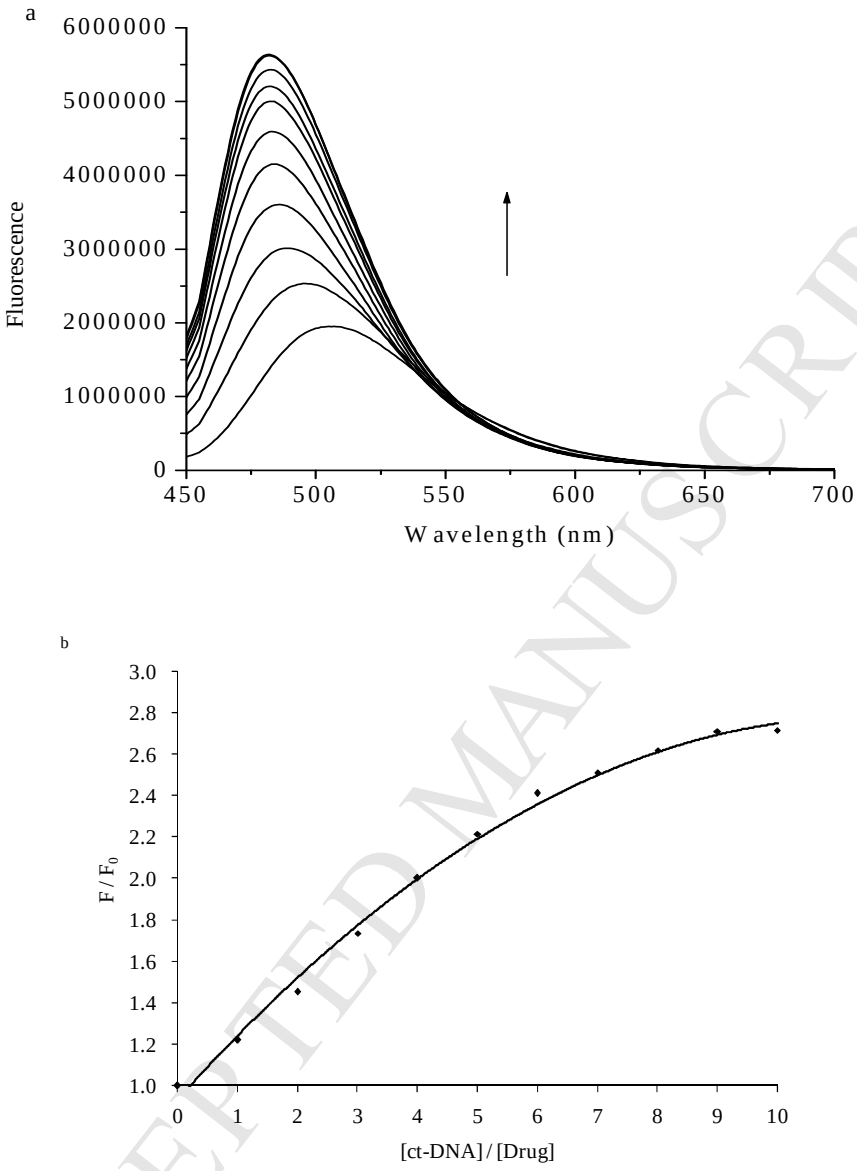


Figure 5

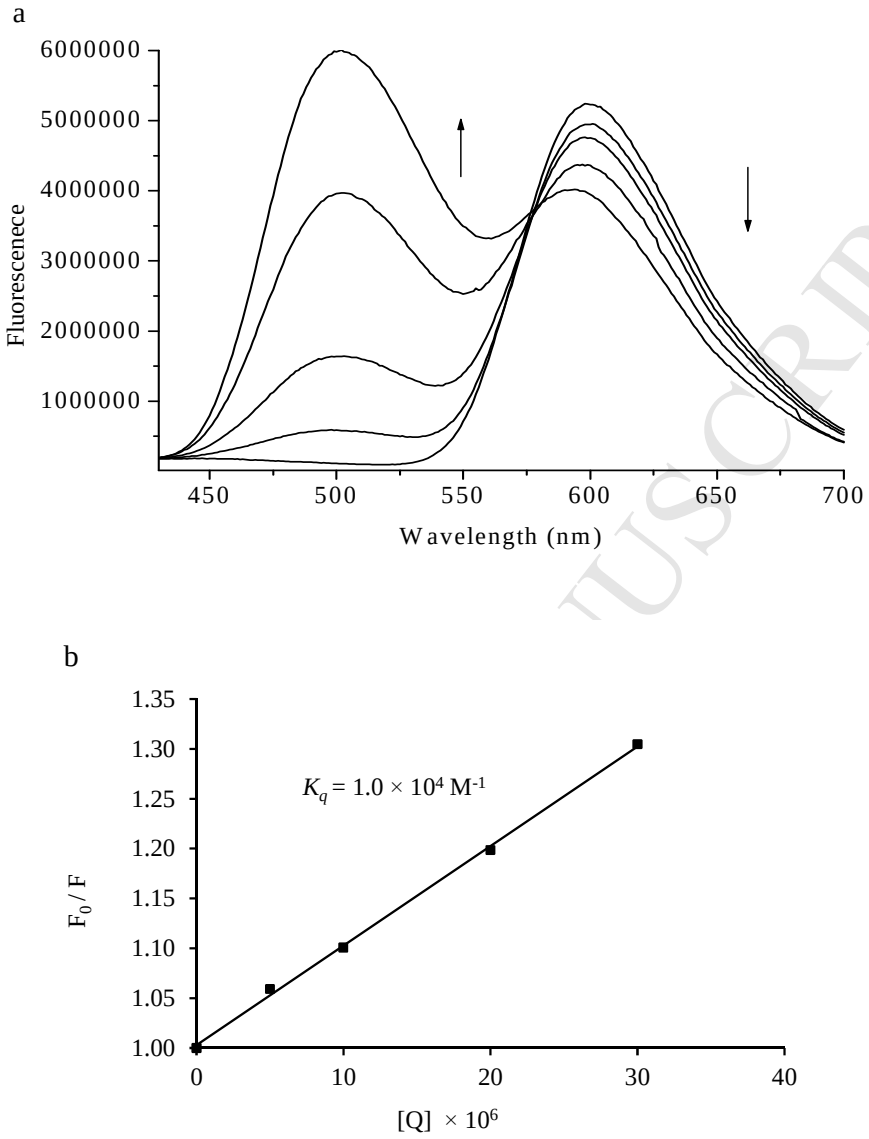


Figure 6

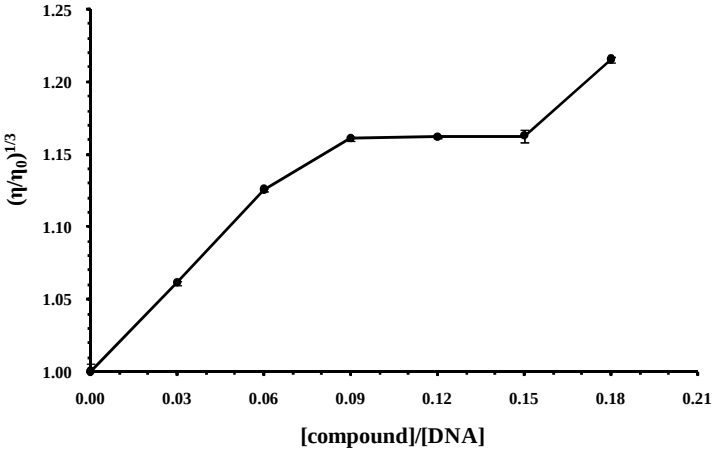


Figure 7

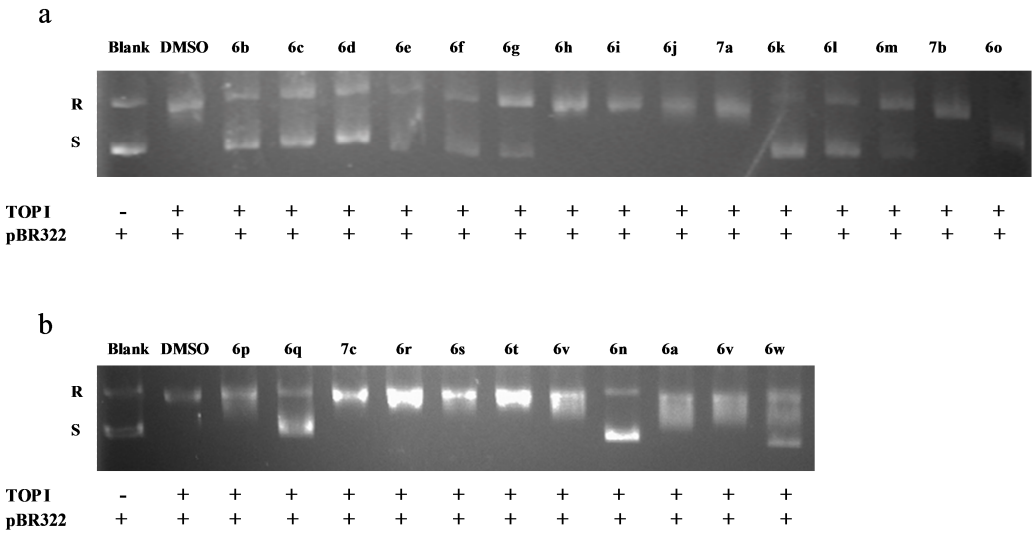


Figure 8

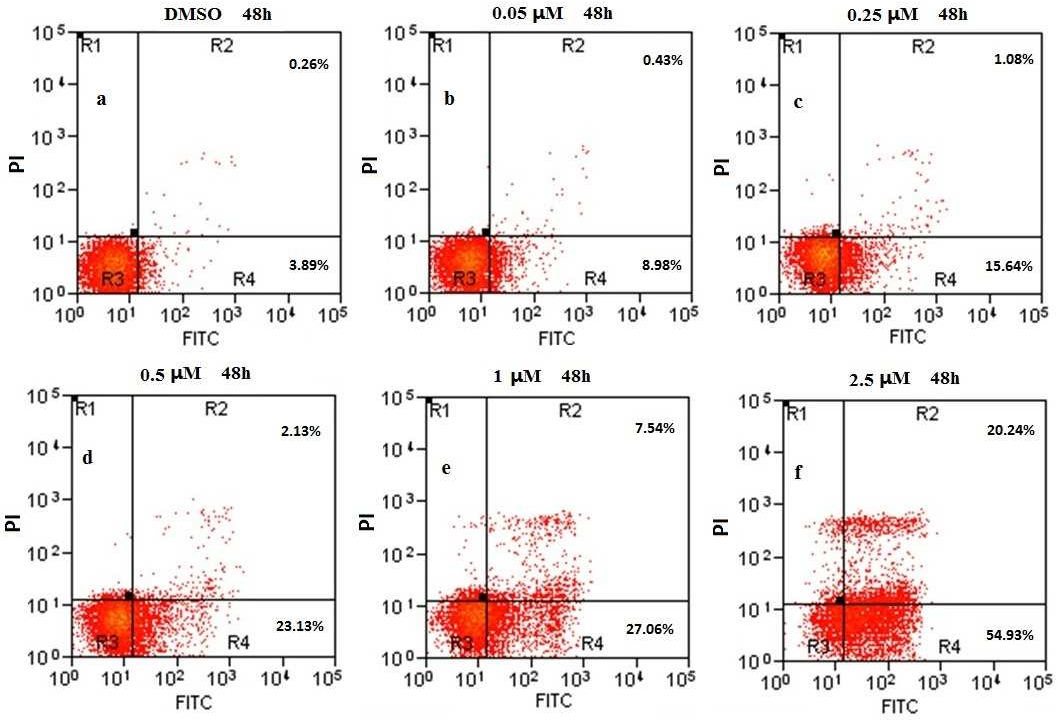


Figure 9

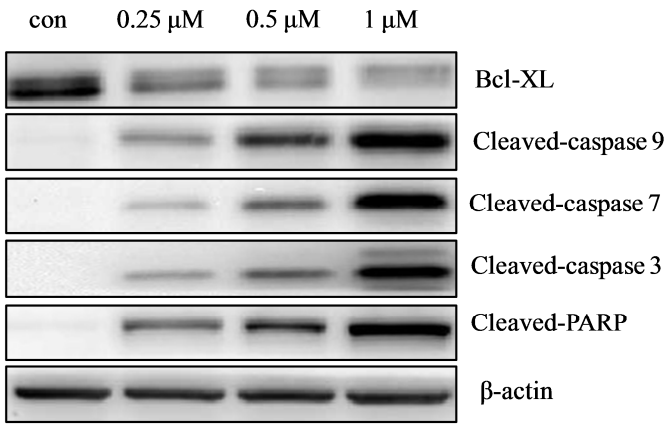


Figure 10

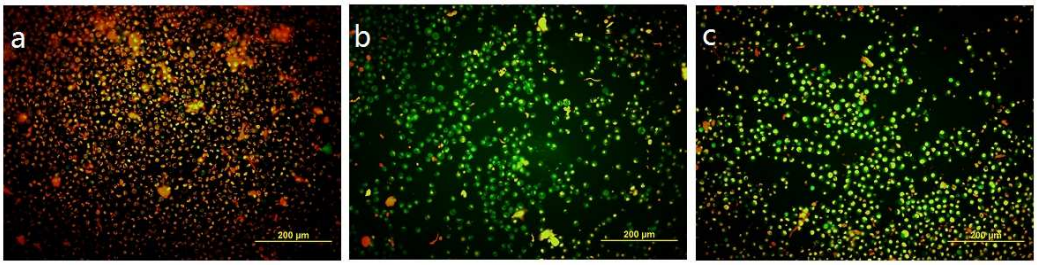


Figure 11

- 26 novel pyridyl acridone derivatives were designed and synthesized.
- Most synthesized compounds displayed excellent activity against K562 cells.
- The typical compound **6d** also showed potent activity against solid tumor cell lines.
- The antitumor mode of **6d** involved DNA intercalation and topoisomerase I inhibition.
- Compound **6d** induced apoptosis via a Mitochondria-Mediated Pathway.

Molecular design, synthesis and biological research of novel pyridyl acridones as DNA-binding and apoptosis-inducing agents

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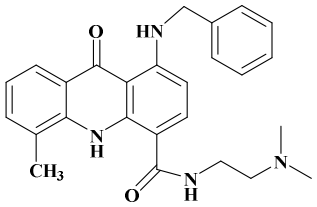
Email address: jiangyy@sz.tsinghua.edu.cn; chunmeigao@sz.tsinghua.edu.cn

1. Molecular modeling

a



compound **A**

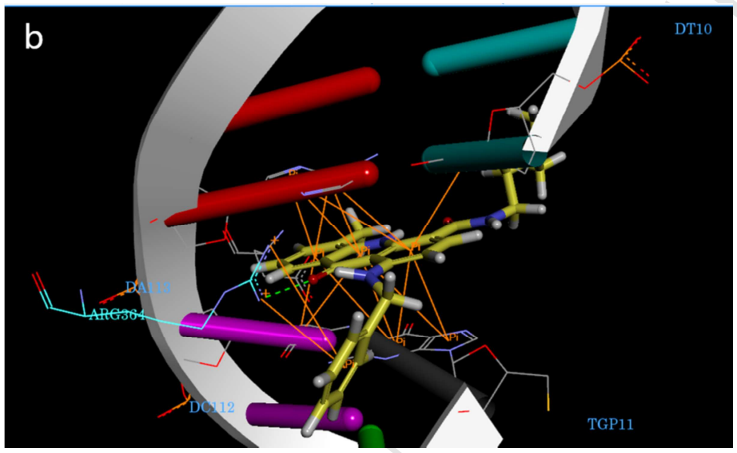
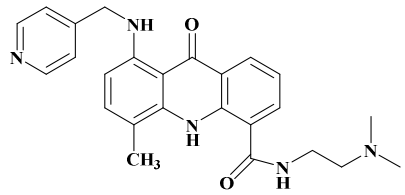


Figure 1s. (a) the structure of 1-anilinoalkylamino acridone derivative (compound **A**). (b) Molecular modeling of compound **A** binding to topo I-DNA complex. Specific interactions of **A** with topo I included: only one hydrogen bond formed between C=O at B-ring of acridone and NH_2 of Arg364 (C=O···H-NH); only one cation-pi interaction existed between the benzene ring and an ammonium cation of Arg364. Additionally, pi-pi interactions formed between the acridone ring of **A** and DNA base pairs (DT10, TGP11, DC112 and DA113). Compound **A** is represented by the yellow color and hydrogen bond was represented by green dotted line, while pi-pi interactions and cation-pi interaction were represented by orange lines.

a



compound **B**

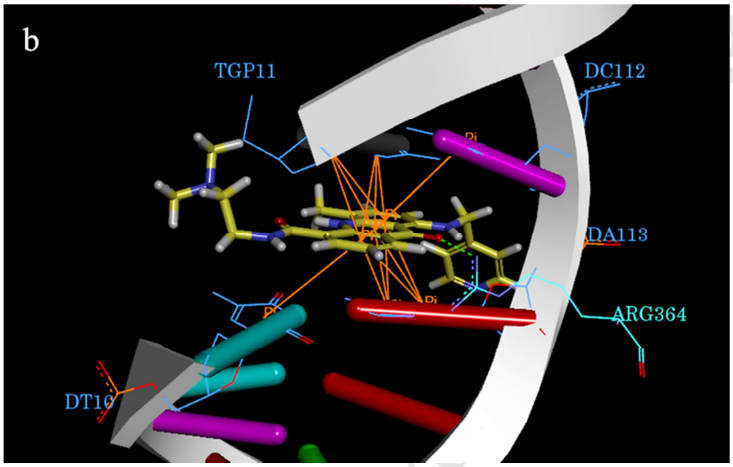


Figure 2s. (a) the structure of 8-pyridylalkylamino acridone derivative (compound **B**). (b) Molecular modeling of compound **B** binding to topo I-DNA complex. Specific interactions of **B** with topo I included: only one hydrogen bond formed between C=O at B-ring of acridone and NH_2 of Arg364 (C=O···H-NH). Additionally, pi-pi interactions existed between the acridone ring of **B** and DNA base pairs (DT10, TGP11, DC112 and DA113). Compound **B** is represented by the yellow color and hydrogen bond was represented by green dotted line, while pi-pi interactions were represented by orange lines.

2. General Notes

NMR spectra were recorded on a Bruker 400 (400 MHz) spectrometer at room temperature. Chemical shifts are given in ppm (δ) relative to SiMe4 as internal standard. Coupling constants (J) are in hertz (Hz), and signals are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br s, broad singlet, etc. The mass spectra were obtained on a Waters Micromass Q-TOF Premier Mass Spectrometer. Melting points were determined with a SGW X-4 digital apparatus and are uncorrected. Thin layer chromatography was carried out using plate silica gel F254. All chemical yields are unoptimized and generally represent the result of a single experiment.

3. General Synthetic Procedures

3.1.General procedure for compounds 3a-e.

2,4-dichlorobenzoic acid **2** (0.61 g, 4.05 mmol), anthranilic acid **1a** or its derivatives **1b-e** (5.26 mmol), potassium carbonate (1.12 g, 8.10 mmol) and copper powder (0.13 g, 2.03 mmol) was stirred in DMF (30 mL) and heated at 130℃ overnight. The suspension was cooled to room temperature and water (40 mL) was added. The mixture was filtered on cellite to remove the copper. The filter bed was washed with water and the resulting solution was acidified with concentrated hydrochloric acid to a pH of 3-4. The resulting suspension was stirred for 30 minutes, and the precipitate was filtered and washed with water and then dried to give yellow solid.

3.1.1. 2-((2-carboxyphenyl)amino)-4-chlorobenzoic acid (3a) Yield 58.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.20 (s, 2H), 10.93 (s, 1H), 7.92 (t, J = 7.8 Hz, 2H), 7.58-7.50 (m, 2H), 7.38 (s, 1H), 7.09-7.03 (m, 1H), 6.95 (d, J = 7.8 Hz, 1H).

3.1.2. 2-((2-carboxy-5-chlorophenyl)amino)-3-methylbenzoic acid (3b) Yield 91.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.10 (s, 2H), 9.90 (s, 1H), 7.86 (d, J = 8.2 Hz, 1H), 7.72 (d, J = 7.2 Hz, 1H), 7.56 (t, J = 7.5 Hz, 1H), 7.31 (t, J = 7.5 Hz, 1H),

6.74 (d, *J* = 7.5 Hz, 1H), 6.08 (s, 1H), 2.12 (s, 3H).

3.1.3. 2-((2-carboxy-5-chlorophenyl)amino)-3-methoxybenzoic acid (3c) Yield 98.9%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.12 (s, 2H), 10.07 (s, 1H), 7.85 (d, *J* = 5.4 Hz, 1H), 7.48 (d, *J* = 5.2 Hz, 1H), 7.41-7.21 (m, 2H), 6.76 (d, *J* = 5.4 Hz, 1H), 6.28 (s, 1H), 3.79 (s, 3H).

3.1.4. 2-((2-carboxy-5-chlorophenyl)amino)-3-(trifluoromethyl)benzoic acid (3d) Yield 67.1%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.30 (s, 2H), 9.85 (s, 1H), 8.09 (d, *J* = 7.3 Hz, 1H), 8.04 (d, *J* = 7.9 Hz, 1H), 7.87 (d, *J* = 8.5 Hz, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 6.78 (dd, *J* = 8.5, 1.9 Hz, 1H), 6.25 (d, *J* = 1.9 Hz, 1H).

3.1.5. 2-((2-carboxy-5-chlorophenyl)amino)-3,5-dichlorobenzoic acid (3e) Yield 45.6%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.36 (s, 2H), 10.10 (s, 1H), 7.99 (d, *J* = 2.4 Hz, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 7.86 (d, *J* = 2.4 Hz, 1H), 6.85 (dd, *J* = 8.5, 1.9 Hz, 1H), 6.34 (d, *J* = 1.8 Hz, 1H).

3.2. General procedure for compounds 4a-e.

Compound **3a-e** (1.19 mmol) was dissolved in concentrated sulfuric acid (10 mL) and heated to 80℃ for 5h. The reaction was added onto ice (50 mL) dropwise and then buffered to pH 5 with NaOH (a.q.). The resulting suspension was stirred for 30 minutes, and the precipitate was filtered and washed with water and then dried to give solid powder **4a-e**.

3.2.1. 1-chloro-9-oxo-9,10-dihydroacridine-4-carboxylic acid (4a) Yield 35.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.66 (s, 1H), 8.29 (d, *J* = 8.2 Hz, 1H), 8.18 (d, *J* = 8.5 Hz, 1H), 7.75 (t, *J* = 7.4 Hz, 1H), 7.66 (d, *J* = 8.2 Hz, 1H), 7.34-7.28 (m, 2H).

3.2.2. 1-chloro-5-methyl-9-oxo-9,10-dihydroacridine-4-carboxylic acid (4b) Yield

95.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.59 (s, 1H), 8.32 (d, *J* = 8.3 Hz, 1H), 8.05 (d, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 6.8 Hz, 1H), 7.32 (d, *J* = 8.3 Hz, 1H), 7.24 (dd, *J* = 7.9, 7.3 Hz, 1H), 2.54 (s, 3H).

3.2.3. 1-chloro-5-methoxy-9-oxo-9,10-dihydroacridine-4-carboxylic acid (4c) Yield 92.7%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.97 (s, 1H), 12.56 (s, 1H), 8.26 (d, *J* = 8.2 Hz, 1H), 7.70 (d, *J* = 8.1 Hz, 1H), 7.34 (d, *J* = 7.8 Hz, 1H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.22 (t, *J* = 7.9 Hz, 1H), 4.02 (s, 3H).

3.2.4. 1-chloro-9-oxo-5-(trifluoromethyl)-9,10-dihydroacridine-4-carboxylic acid (4d) Yield 85.6%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.24 (s, 1H), 8.36 (d, *J* = 7.5 Hz, 1H), 8.24 (d, *J* = 8.0 Hz, 1H), 8.06 (d, *J* = 7.0 Hz, 1H), 7.38 (t, *J* = 7.2 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 1H).

3.2.5. 1,5,7-trichloro-9-oxo-9,10-dihydroacridine-4-carboxylic acid (4e) Yield 90.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.60 (s, 1H), 8.30 (s, 1H), 8.13-8.04 (m, 2H), 8.02 (s, 1H), 7.35-7.33 (m, 1H).

3.3. Preparation of 1-chloro-N-(2-(dimethylamino)ethyl)-9-oxo-9,10–dihydro acridine-4-carboxamide (5a)

A solution of 1-chloro-9-oxo-9,10-dihydroacridine-4-carboxylic acid (**4a**) (2.0 g, 7.33 mmol) in DMF (25mL) was added dropwise, with stirring, to a solution of N,N′-carbonyldiimidazole (1.78 g, 10.99 mmol) in DMF (10 mL) at room temperature. After the addition was complete, the mixture was still stirred for 30 minutes. Then, N,N-dimethyl-1,2-ethanediamine (1.90 g, 21.99 mmol) was added. The reaction mixture was stirred at room temperature until the TLC showed the disappearance ofthe starting material. After the reaction was complete, the mixture was partitioned betweenCH_2Cl_2 (50 mL) and water (50 mL).The organic extract was washed with water (40 mL×4), dried ($MgSO_4$) and CH_2Cl_2 was removed under reduced pressure

to give a residue which was purified by column-chromatography eluted with EtOAC/EtOH (9:1 v/v) to yield pure **5a**. Yield 40.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.12 (s, 1H), 8.55 (s, 1H), 8.32 (d, *J* = 8.0 Hz, 1H), 8.24 (d, *J* = 9.0 Hz, 1H), 7.58 (t, *J* = 7.7 Hz, 1H), 7.32 (d, *J* = 8.0 Hz,1H), 7.21 (t, *J* = 7.5 Hz, 1H), 6.62 (d, *J* = 8.9 Hz, 1H), 3.95-3.85 (m, *J* = 4.5 Hz, 2H), 3.35-3.25 (m, *J* = 4.7 Hz, 2H), 2.89 (s, 6H).

3.4. Preparation of 1-chloro-N-(2-(dimethylamino)ethyl)-5-methyl-9-oxo-9,10-dihydroacridine-4-carboxamide (5b)

A solution of 1-chloro-5-methyl-9-oxo-9,10-dihydroacridine-4-carboxylic acid (**4b**) (1.0 g, 3.48 mmol) in DMF (20 mL) was added dropwise, with stirring, to a solution of N,N′-carbonyldiimidazole (0.85 g, 5.22 mmol) in DMF (10 mL) at room temperature. After the addition was complete, the mixture was still stirred for 30 minutes. Then, N,N-dimethyl-1,2-ethanediamine (0.92 g, 10.44 mmol) was added. The reaction mixture was stirred at room temperature until the TLC showed the disappearance of the starting material. After the reaction was complete, the mixture was partitioned betweenCH_2Cl_2 (50 mL) and water (50 mL).The organic extract was washed with water (40 mL×4), dried ($MgSO_4$) and CH_2Cl_2 was removed under reduced pressure to give a residue which was purified by column-chromatography eluted with EtOAC/EtOH (9:1 v/v) to yield pure **5b**. Yield 65.3%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.93 (s, 1H), 8.27 (d, *J* = 8.0 Hz, 1H), 7.72 (d, *J* = 8.3 Hz, 1H), 7.50 (d, *J* = 7.0 Hz, 1H), 7.19 (t, *J* = 7.7 Hz, 1H), 7.15 (d, *J* = 8.1 Hz, 1H), 3.57 (dd, *J* = 10.9, 5.3 Hz, 2H), 2.64-2.59 (m, 2H), 2.58 (s, 3H), 2.33 (s, 6H).

3.5. Preparation of 1-chloro-N-(3-(dimethylamino)propyl)-5-methyl-9-oxo-9,10-dihydroacridine-4-carboxamide (5c)

A solution of 1-chloro-5-methyl-9-oxo-9,10-dihydroacridine-4-carboxylic acid (**4b**) (0.5 g, 1.74 mmol) in DMF (10 mL) was added dropwise, with stirring, to a solution of N,N′-carbonyldiimidazole (0.42 g, 2.61 mmol) in DMF (10 mL) at room temperature. After the addition was complete, the mixture was still stirred for 30

minutes. Then, N,N-dimethyl-1,3-propanediamine (0.53 g, 5.22 mmol) was added. The reaction mixture was stirred at room temperature until the TLC showed the disappearance of the starting material. After the reaction was complete, the mixture was partitioned betweenCH_2Cl_2 (50 mL) and water (50 mL).The organic extract was washed with water (40 mL×4), dried ($MgSO_4$) and CH_2Cl_2 was removed under reduced pressure to give a residue which was purified by column-chromatography eluted with EtOAC/EtOH (9:1 v/v) to yield pure **5c**. Yield 61.9%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.34 (s, 1H), 9.65 (s, 1H), 8.29 (d, *J* = 8.1 Hz, 1H), 7.65 (d, *J* = 8.3 Hz, 1H), 7.51 (d, *J* = 7.1 Hz, 1H), 7.21-7.15 (m, 2H), 3.64 (dd, *J* = 10.2, 5.8 Hz, 2H), 2.67-2.62 (m, 2H), 2.61 (s, 3H), 2.39 (s, 6H), 1.85 (dt, *J* = 11.4, 5.8 Hz, 2H).

3.6. Preparation of 1-chloro-N-(2-methoxyethyl)-5-methyl-9-oxo-9,10-dihydroacridine-4-carboxamide (5d)

A solution of 1-chloro-5-methyl-9-oxo-9,10-dihydroacridine-4-carboxylic acid (**4b**) (0.5 g, 1.74 mmol) in DMF (10 mL) was added dropwise, with stirring, to a solution of N,N′-carbonyldiimidazole (0.42 g, 2.61 mmol) in DMF (10 mL) at room temperature. After the addition was complete, the mixture was still stirred for 30 minutes. Then, 2-methoxyethanamine (0.39 g, 5.22 mmol) was added. The reaction mixture was stirred at room temperature until the TLC showed the disappearance of the starting material. After the reaction was complete, the mixture was partitioned betweenCH_2Cl_2 (50 mL) and water (50 mL).The organic extract was washed with water (40 mL×4), dried ($MgSO_4$) and CH_2Cl_2 was removed under reduced pressure to give a residue which was purified by column-chromatography eluted with EtOAC/EtOH (9:1 v/v) to yield pure **5d**.Yield 59.6%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.79 (s, 1H), 8.27 (d, *J* = 8.1 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.51 (d, *J* = 7.0 Hz, 1H), 7.19 (t, *J* = 7.6 Hz, 1H), 7.14 (d, *J* = 8.2 Hz, 1H), 6.90 (s, 1H), 3.74 (dd, *J* = 9.7, 4.8 Hz, 2H), 3.65 (t, *J* = 4.8 Hz, 2H), 3.45 (s, 3H), 2.58 (s, 3H).

3.7. Preparation of 1-chloro-N-(2-(dimethylamino)ethyl)-5-methoxy-9-oxo-

9,10-dihydroacridine-4-carboxamide (5e)

A solution of 1-chloro-5-methoxy-9-oxo-9,10-dihydroacridine-4-carboxylic acid (**4c**) (0.5 g, 1.65 mmol) in DMF (10 mL) was added dropwise, with stirring, to a solution of N,N′-carbonyldiimidazole (0.40 g, 2.48 mmol) in DMF (10 mL) at room temperature. After the addition was complete, the mixture was still stirred for 30 minutes. Then, N,N-dimethyl-1,2-ethanediamine (0.44 g, 4.95 mmol) was added. The reaction mixture was stirred at room temperature until the TLC showed the disappearance of the starting material. After the reaction was complete, the mixture was partitioned betweenCH_2Cl_2 (50 mL) and water (50 mL).The organic extract was washed with water (40 mL×4), dried ($MgSO_4$) and CH_2Cl_2 was removed under reduced pressure to give a residue which was purified by column-chromatography eluted with EtOAC/EtOH (9:1 v/v) to yield pure **5e**. Yield 58.7%; mp 203-205℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.90 (s, 1H), 7.97 (dd, *J* = 8.2, 0.9 Hz, 1H), 7.75 (d, *J* = 8.2 Hz, 1H), 7.19 (dt, *J* = 8.1, 3.8 Hz, 2H), 7.11 (dd, *J* = 7.8, 1.1 Hz, 1H), 4.08 (s, 3H), 3.60 (dd, *J* = 11.2, 5.0 Hz, 2H), 2.65-2.56 (m, 2H), 2.32 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.02, 168.82, 155.70, 147.73, 144.89, 132.92, 130.22, 123.41, 121.16, 117.57, 112.37, 109.91, 105.48, 104.58, 58.68, 56.80, 45.76, 43.80, 37.73; HR-MS(ESI): Calcd for $[M+H]^+$ 374.1271; Found: 374.1283.

3.8. Preparation of 1-chloro-N-(3-(dimethylamino)propyl)-5-methoxy-9-oxo-9,10-dihydroacridine-4-carboxamide (5f)

A solution of 1-chloro-5-methoxy-9-oxo-9,10-dihydroacridine-4-carboxylic acid (**4c**) (0.9 g, 2.97 mmol) in DMF (20 mL) was added dropwise, with stirring, to a solution of N,N′-carbonyldiimidazole (0.72 g, 4.46 mmol) in DMF (10 mL) at room temperature. After the addition was complete, the mixture was still stirred for 30 minutes. Then, N,N-dimethyl-1,3-propanediamine (0.91 g, 8.91 mmol) was added. The reaction mixture was stirred at room temperature until the TLC showed the disappearance of the starting material. After the reaction was complete, the mixture was partitioned betweenCH_2Cl_2 (50 mL) and water (50 mL).The organic extract was

washed with water (40 mL×4), dried ($MgSO_4$) and CH_2Cl_2 was removed under reduced pressure to give a residue which was purified by column-chromatography eluted with EtOAC/EtOH (9:1 v/v) to yield pure **5f**. Yield 60.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.27 (s, 1H), 9.51 (s, 1H), 7.97 (d, *J* = 8.4 Hz, 1H), 7.63 (d, *J* = 8.3 Hz, 1H), 7.18 (t, *J* = 8.4 Hz, 1H), 7.17 (d, *J* = 8.0 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 4.08 (s, 3H), 3.65 (dd, *J* = 10.0, 5.7 Hz, 2H), 2.69 – 2.57 (m, 2H), 2.36 (s, 6H), 1.83 (dt, *J* = 11.3, 5.8 Hz, 2H).

3.9. Preparation of 1-chloro-5-methoxy-N-(2-methoxyethyl)-9-oxo-9,10-dihydroacridine-4-carboxamide (5g)

A solution of 1-chloro-5-methoxy-9-oxo-9,10-dihydroacridine-4-carboxylic acid (**4c**) (0.9 g, 2.97 mmol) in DMF (20 mL) was added dropwise, with stirring, to a solution of N,N′-carbonyldiimidazole (0.72 g, 4.46 mmol) in DMF (10 mL) at room temperature. After the addition was complete, the mixture was still stirred for 30 minutes. Then, 2-methoxyethanamine (0.67 g, 8.91 mmol) was added. The reaction mixture was stirred at room temperature until the TLC showed the disappearance of the starting material. After the reaction was complete, the mixture was partitioned betweenCH_2Cl_2 (50 mL) and water (50 mL).The organic extract was washed with water (40 mL×4), dried ($MgSO_4$) and CH_2Cl_2 was removed under reduced pressure to give a residue which was purified by column-chromatography eluted with EtOAC/EtOH (9:1 v/v) to yield pure **5g**.Yield 57.4%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.75 (s, 1H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.22-7.15 (m, 2H), 7.11 (dd, *J* = 7.8, 1.1 Hz, 1H), 6.82 (s, 1H), 4.08 (s, 3H), 3.74 (dd, *J* = 10.3, 5.1 Hz, 2H), 3.67-3.61 (m, 2H), 3.43 (s, 3H).

3.10. Preparation of 1-chloro-N-(4-(dimethylamino)butyl)-5-methoxy-9-oxo-9,10-dihydroacridine-4-carboxamide (5h)

A solution of 1-chloro-5-methoxy-9-oxo-9,10-dihydroacridine-4-carboxylic acid (**4c**) (0.5 g, 1.65 mmol) in DMF (15 mL) was added dropwise, with stirring, to a

solution of N,N′-carbonyldiimidazole (0.40 g, 2.48 mmol) in DMF (10 mL) at room temperature. After the addition was complete, the mixture was still stirred for 30 minutes. Then, N,N-dimethyl-1,4-butanediamine (0.58 g, 4.95 mmol) was added. The reaction mixture was stirred at room temperature until the TLC showed the disappearance of the starting material. After the reaction was complete, the mixture was partitioned betweenCH_2Cl_2 (50 mL) and water (50 mL).The organic extract was washed with water (40 mL×4), dried ($MgSO_4$) and CH_2Cl_2 was removed under reduced pressure to give a residue which was purified by column-chromatography eluted with EtOAC/EtOH (9:1 v/v) to yield pure **5h**. Yield 47.8%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.97 (s, 1H), 9.16 (s, 1H), 7.92 (d, *J* = 8.1 Hz, 1H), 7.73 (d, *J* = 8.2 Hz, 1H), 7.15 (t, *J* = 8.0 Hz, 1H), 7.07 (d, *J* = 8.0 Hz, 2H), 4.05 (s, 3H), 3.50-3.40 (m, 2H), 2.34 (t, *J* = 6.2 Hz, 2H), 2.19 (s, 6H), 1.78 (dt, *J* = 12.2, 6.2 Hz, 2H), 1.72-1.60 (m, 2H).

3.11. Preparation of 1-chloro-N-(2-(dimethylamino)ethyl)-9-oxo-5-(trifluoro methyl)-9,10-dihydroacridine-4-carboxamide (5i)

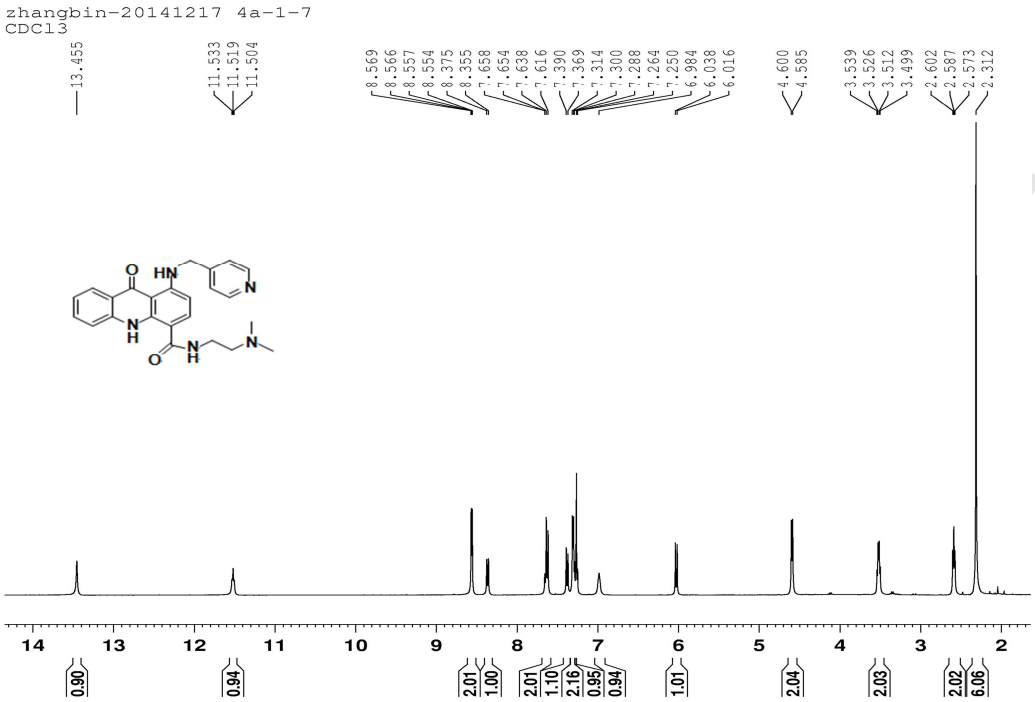
A solution of 1-chloro-9-oxo-5-(trifluoromethyl)-9,10-dihydroacridine-4-carboxylic acid (**4d**) (0.7 g, 2.05 mmol) in DMF (15 mL) was added dropwise, with stirring, to a solution of N,N′-carbonyldiimidazole (0.50 g, 3.08 mmol) in DMF (10 mL) at room temperature. After the addition was complete, the mixture was still stirred for 30 minutes. Then, N,N-dimethyl-1,2-ethanediamine (0.54 g, 6.16 mmol) was added. The reaction mixture was stirred at room temperature until the TLC showed the disappearance of the starting material. After the reaction was complete, the mixture was partitioned between CH_2Cl_2 (50 mL) and water (50 mL).The organic extract was washed with water (40 mL×4), dried ($MgSO_4$) and CH_2Cl_2 was removed under reduced pressure to give a residue which was purified by column-chromatography eluted with EtOAC/EtOH (9:1 v/v) to yield pure **5i**.Yield 43.9%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.58 (s, 1H), 8.62 (d, *J* = 8.0 Hz, 1H), 7.97 (d, *J* = 7.5 Hz, 1H), 7.80 (d, *J* = 8.3 Hz,

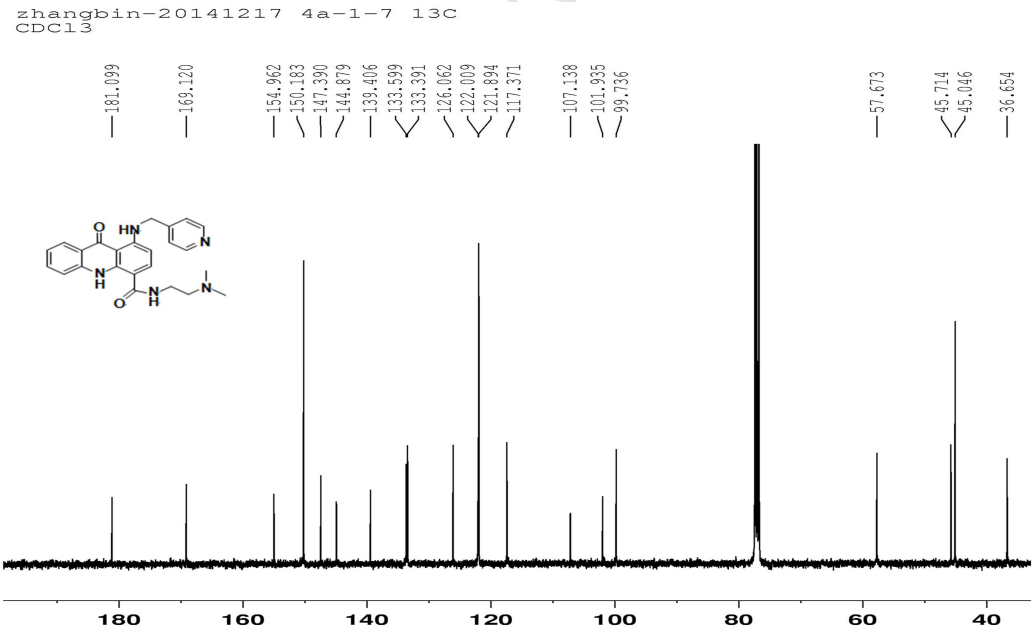
1H), 7.33 (t, *J* = 7.7 Hz, 2H), 7.24 (d, *J* = 8.0 Hz, 1H), 3.65-3.55 (m, 2H), 2.60 (t, *J* = 5.8 Hz, 2H), 2.32 (s, 6H).

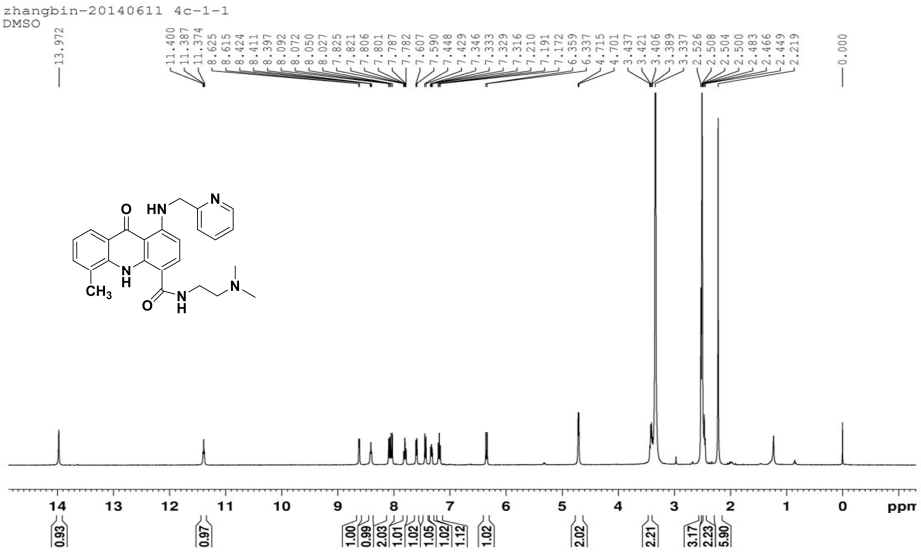
3.12. Preparation of 1,5,7-trichloro-N-(2-(dimethylamino)ethyl)-9-oxo-9,10-dihydroacridine-4-carboxamide (5j)

A solution of 1,5,7-trichloro-9-oxo-9,10-dihydroacridine-4-carboxylic acid (**4e**) (0.8 g, 2.35 mmol) in DMF (15 mL) was added dropwise, with stirring, to a solution of N,N′-carbonyldiimidazole (0.57 g, 3.52 mmol) in DMF (10 mL) at room temperature. After the addition was complete, the mixture was still stirred for 30 minutes. Then, N,N-dimethyl-1,2-ethanediamine (0.62 g, 7.04 mmol) was added. The reaction mixture was stirred at room temperature until the TLC showed the disappearance of the starting material. After the reaction was complete, the mixture was partitioned betweenCH_2Cl_2 (50 mL) and water (50 mL).The organic extract was washed with water (40 mL×4), dried ($MgSO_4$) and CH_2Cl_2 was removed under reduced pressure to give a residue which was purified by column-chromatography eluted with EtOAC/EtOH (9:1 v/v) to yield pure **5j**.Yield 49.2%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.43 (s, 1H), 8.28 (d, *J* = 2.3 Hz, 1H), 7.83 (d, *J* = 8.3 Hz, 1H), 7.72 (d, *J* = 2.3 Hz, 1H), 7.39 (d, *J* = 8.3 Hz, 1H), 7.25 (s, 1H), 3.60 (dd, *J* = 10.0, 5.2 Hz, 2H), 2.68-2.56 (m, 2H), 2.33 (s, 6H).

4. NMR Spectrum



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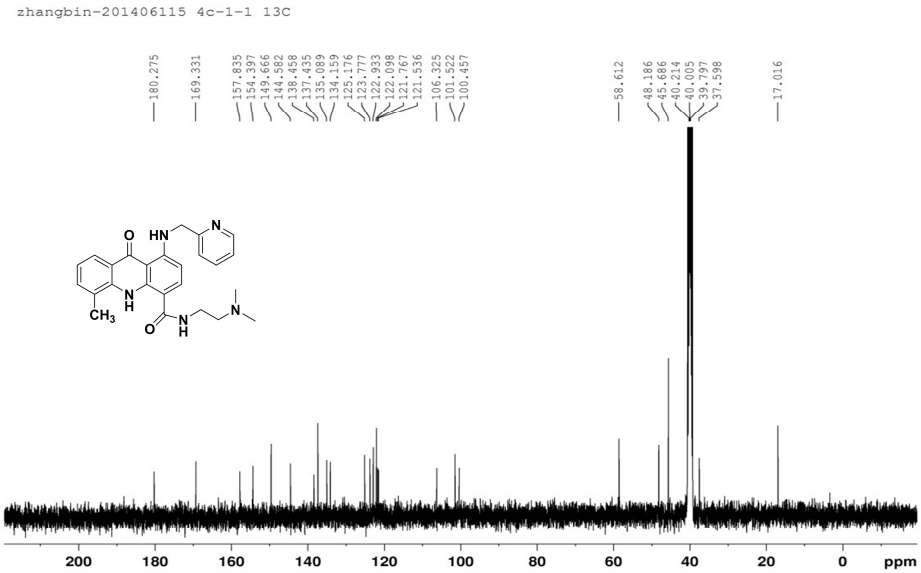
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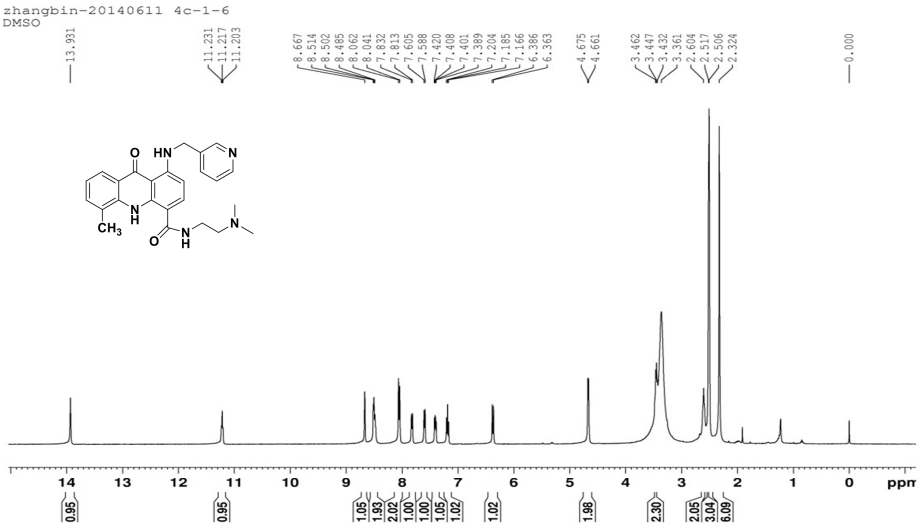
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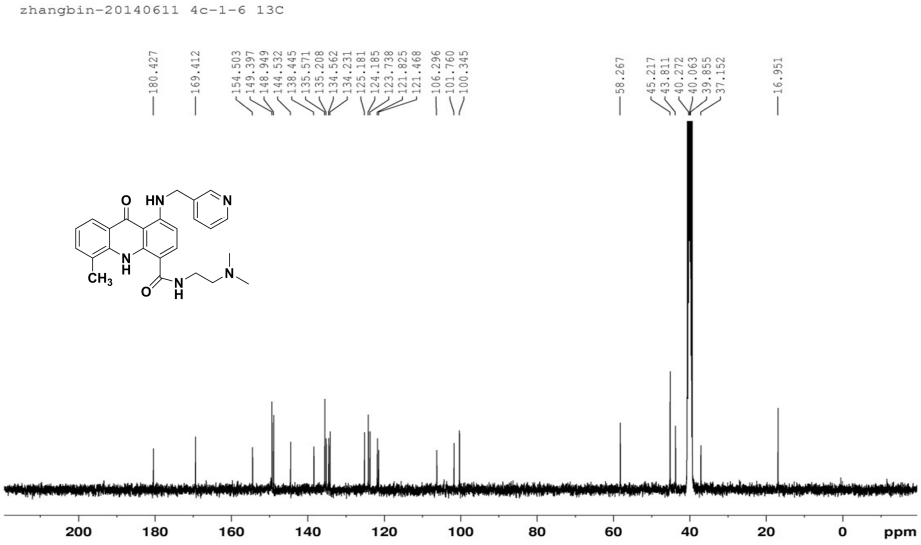
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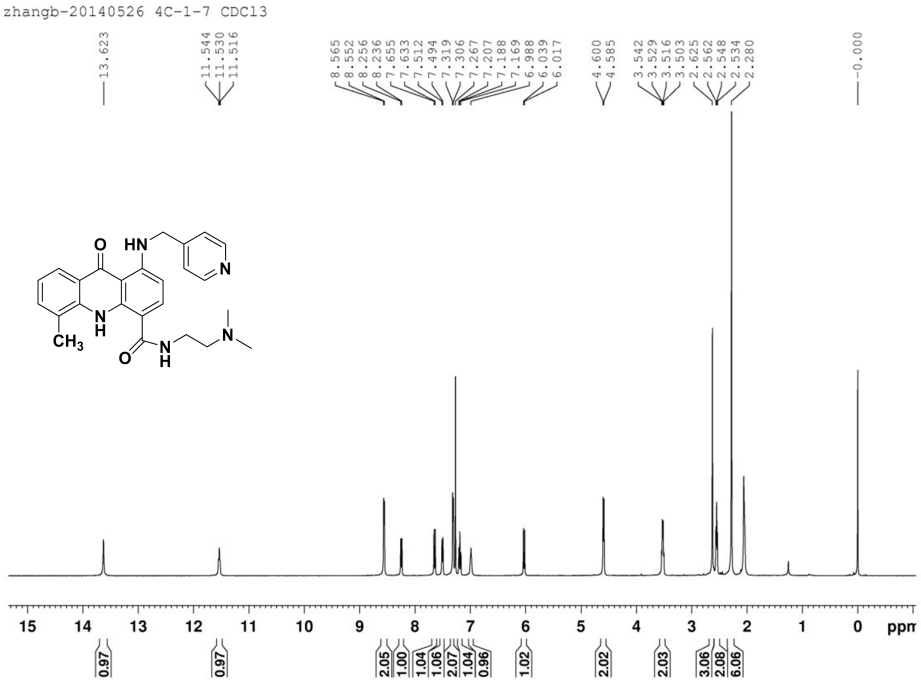
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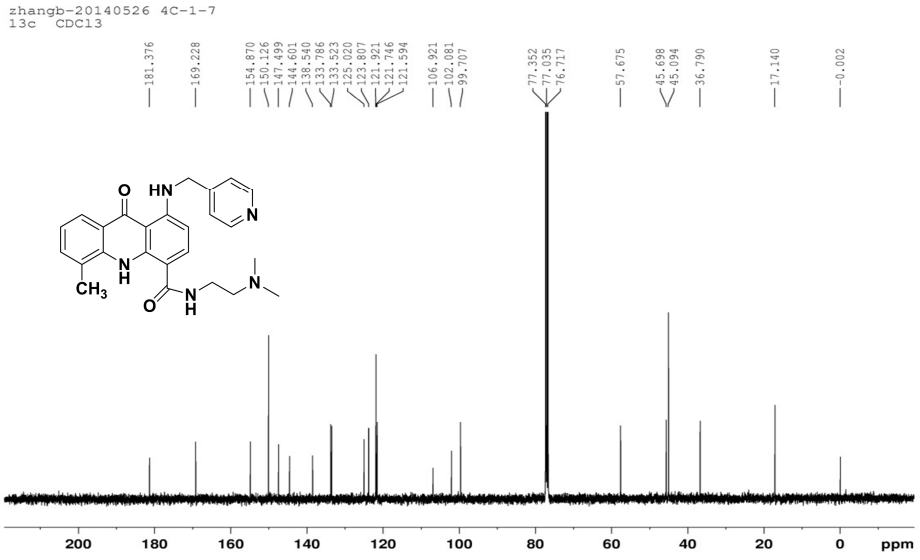
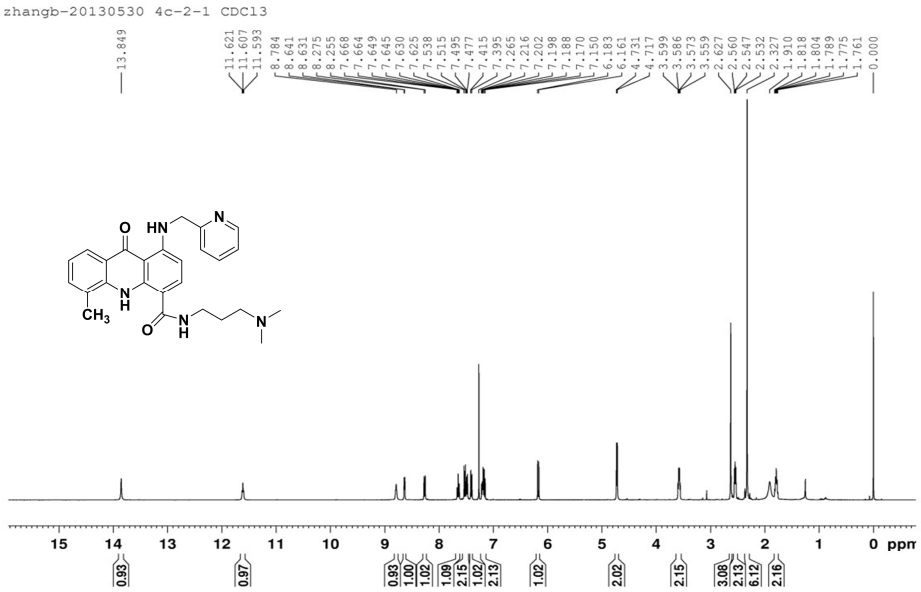
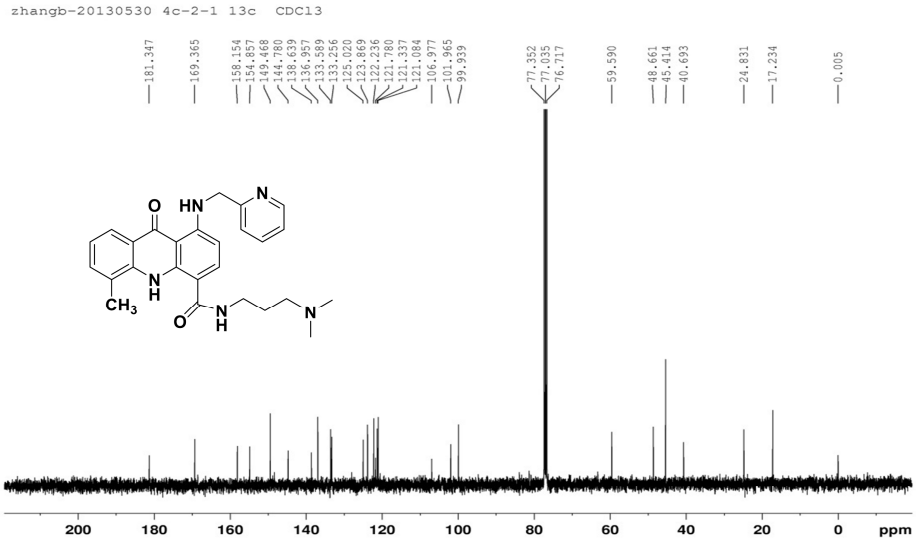
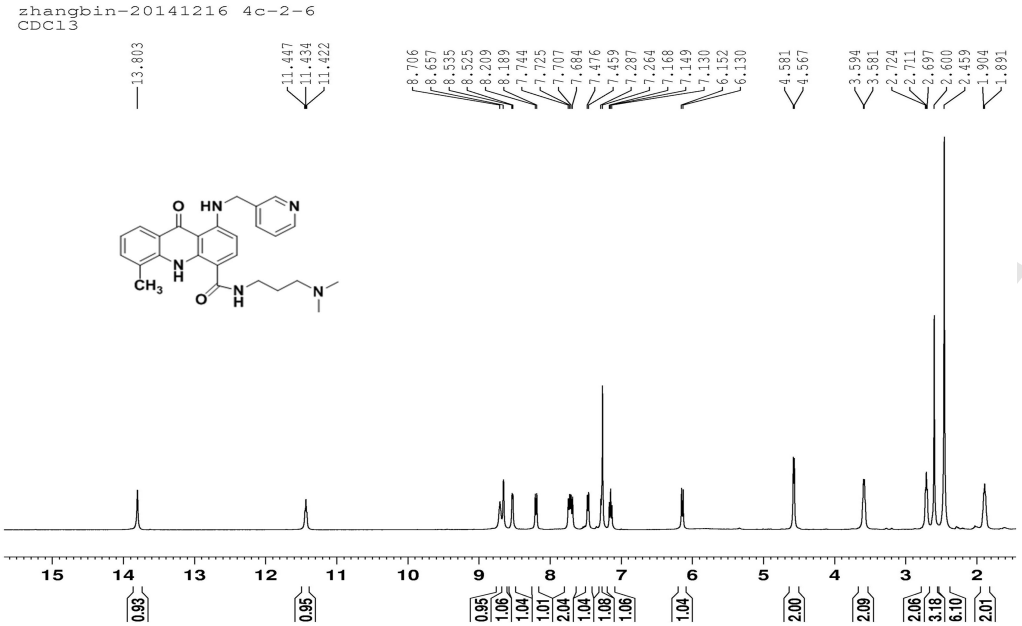
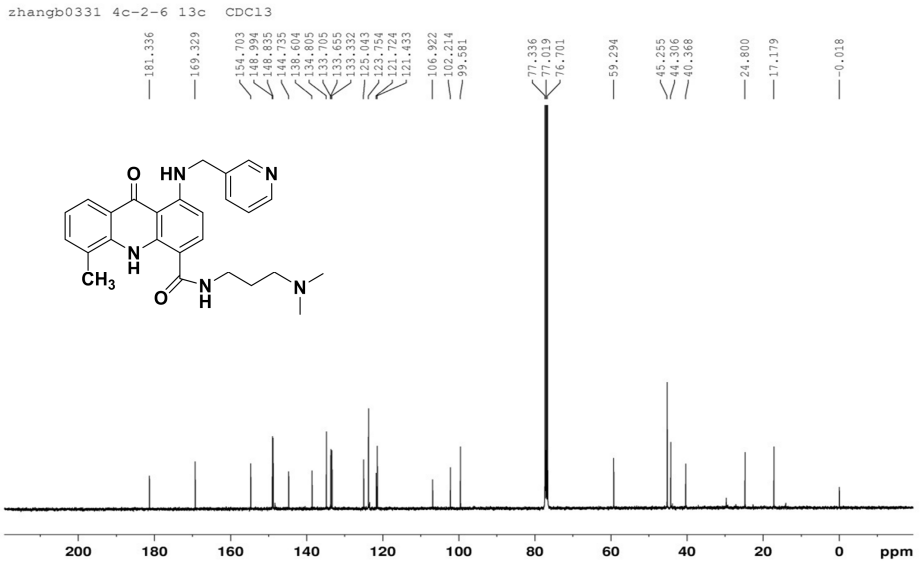
8.552
8.256
8.236
7.655
7.653
7.512
7.494
7.319
7.306
7.267
7.207
7.188
7.169
6.988
6.039
6.017
4.600
4.585
3.542
3.529
3.516
3.503
2.625
2.562
2.548
2.534
2.280
0.000
O
HN
N
N
H
CH3
O
N
H
N
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.97
0.97
2.05
1.00
1.04
1.06
2.07
1.04
0.96
1.02
2.02
2.03
3.06
2.08
6.06

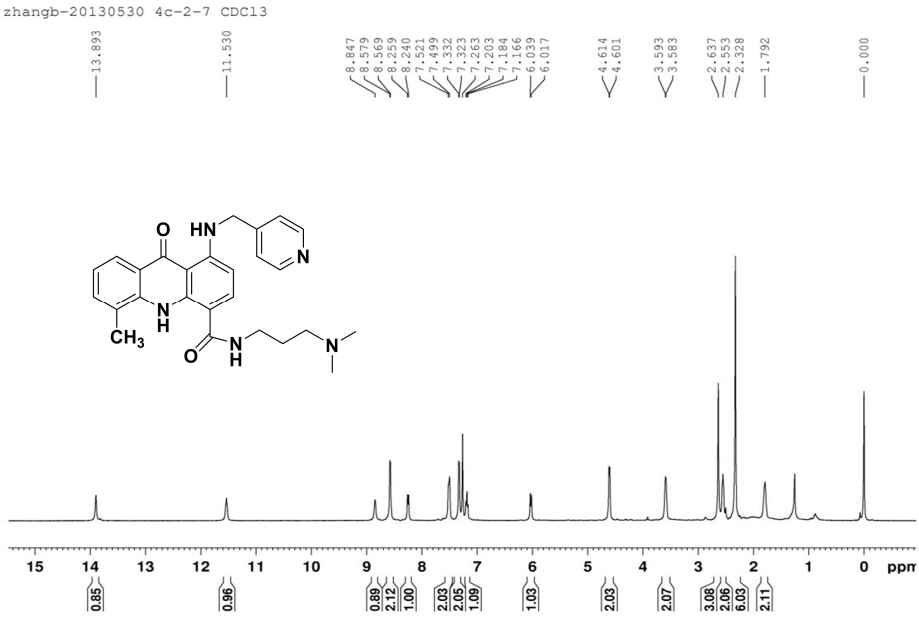

zhangb-20140526 4C-1-7
13c CDCl3
181.376
169.228
154.870
150.126
147.499
144.601
138.540
133.786
133.523
125.020
123.807
121.921
121.746
121.594
106.921
102.081
99.707
77.352
77.035
76.717
57.675
45.698
45.094
36.790
17.140
0.002
O
HN
N
N
H
CH3
O
N
H
N
200 180 160 140 120 100 80 60 40 20 0 ppm

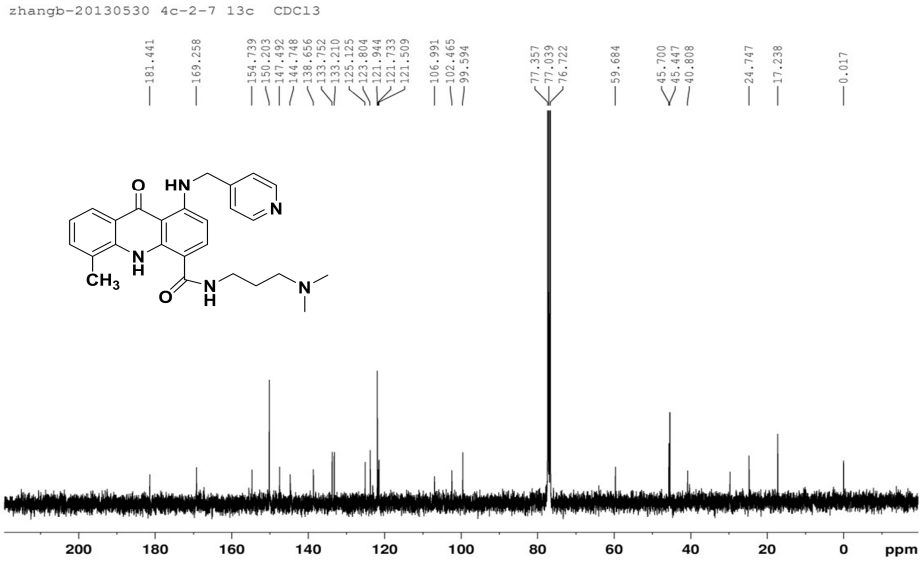

zhangb-20130530 4c-2-1 CDCl3
13.849
11.621
11.607
11.593
8.784
8.641
8.631
8.275
8.255
7.668
7.664
7.649
7.645
7.630
7.625
7.538
7.515
7.495
7.477
7.415
7.395
7.265
7.216
7.202
7.198
7.188
7.170
7.150
6.183
6.161
4.731
4.717
3.599
3.586
3.573
3.559
2.627
2.560
2.547
2.532
2.327
1.910
1.818
1.804
1.789
1.775
1.761
0.000
O
HN
N
N
H
CH3
O
N
H
N
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.93
0.97
0.93
1.00
1.02
1.08
2.15
1.02
2.13
1.02
2.02
2.15
3.08
2.13
6.12
2.16

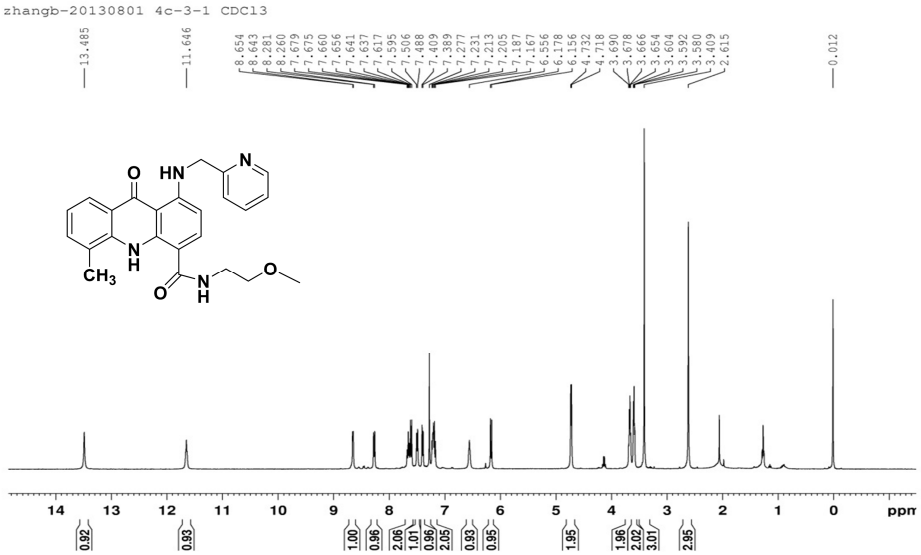

zhangb-20130530 4c-2-1 13c CDCl3
181.347
169.365
158.154
154.857
149.468
144.780
138.639
136.957
133.589
133.256
125.020
123.869
122.236
121.780
121.337
121.084
106.977
101.965
99.939
77.352
77.035
76.717
59.590
48.661
45.414
40.693
24.831
17.234
0.005
O
HN
N
N
H
CH3
O
N
H
N
200 180 160 140 120 100 80 60 40 20 0 ppm

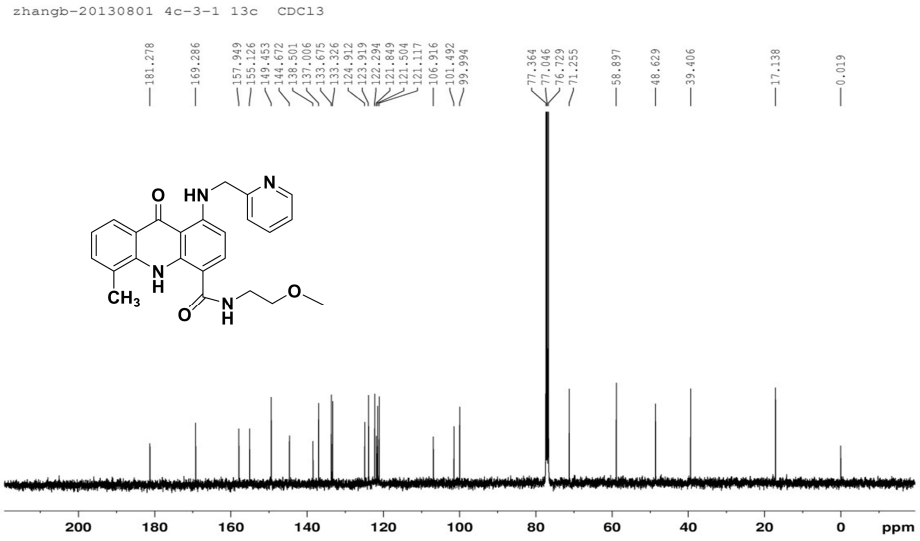

zhangbin-20141216 4c-2-6
CDCl3
13.803
11.447
11.434
11.422
8.706
8.657
8.535
8.525
8.209
8.189
7.744
7.725
7.707
7.684
7.476
7.459
7.287
7.264
7.168
7.149
7.130
6.152
6.130
4.581
4.567
3.594
3.581
2.724
2.711
2.697
2.600
2.459
1.904
1.891
O
HN
N
N
H
CH3
O
N
H
N
15
14
13
12
11
10
9
8
7
6
5
4
3
2
0.93
0.95
0.95
1.06
1.04
1.01
2.04
1.04
1.08
1.06
1.04
2.00
2.09
2.06
3.18
6.10
2.01

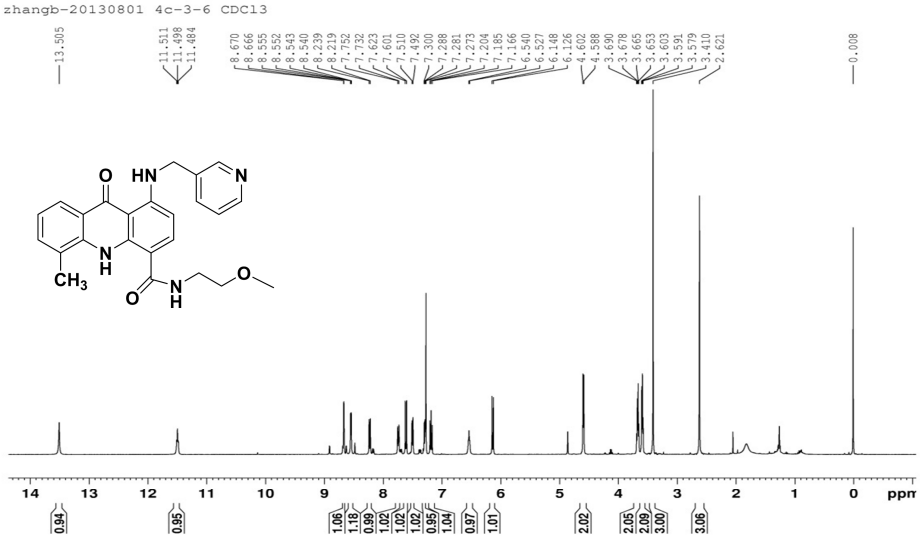

zhangb0331 4c-2-6 13c CDCl3
181.336
169.329
154.703
148.994
148.835
144.735
138.604
134.805
133.705
133.655
133.332
125.043
123.754
121.724
121.433
106.922
102.214
99.581
77.336
77.019
76.701
59.294
45.255
44.306
40.368
24.800
17.179
-0.018
O
HN
N
N
H
CH3
O
N
H
N
200
180
160
140
120
100
80
60
40
20
0
ppm

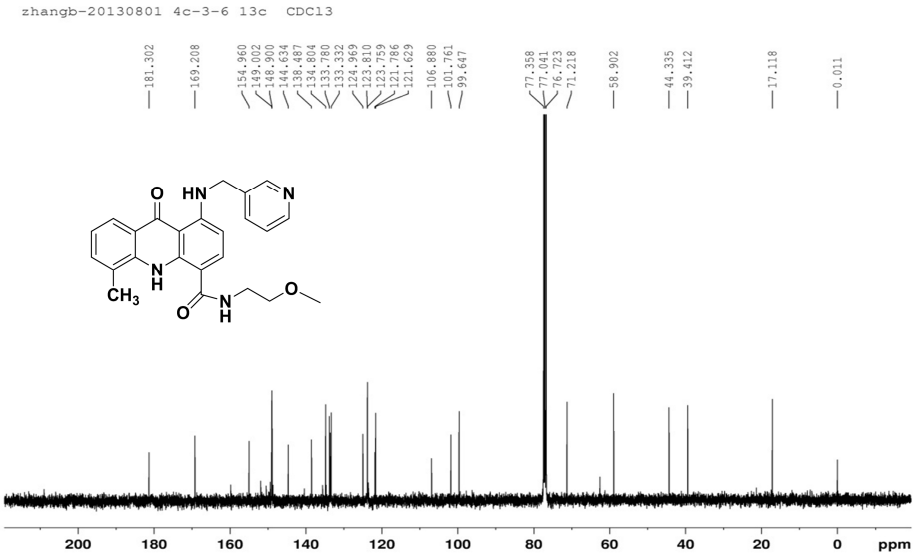

zhangb-20130530 4c-2-7 CDC13
13.893
11.530
8.847
8.579
8.569
8.259
8.240
7.521
7.499
7.332
7.323
7.263
7.203
7.184
7.166
6.039
6.017
4.614
4.601
3.593
3.583
2.637
2.553
2.328
1.792
0.000
O
HN
N
N
H
CH3
O
N
H
N
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.85
0.96
0.89
2.12
1.00
2.03
2.05
1.09
1.03
2.03
2.07
3.08
2.06
6.03
2.11

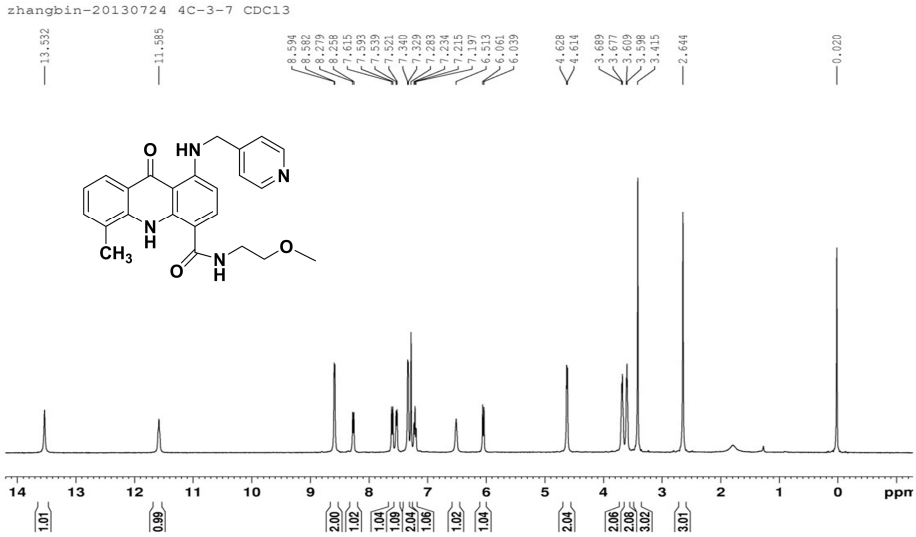

zhangb-20130530 4c-2-7 13c CDC13
181.441
169.258
154.739
150.203
147.492
144.748
138.656
133.752
133.210
125.125
123.804
121.944
121.733
121.509
106.991
102.465
99.594
77.357
77.039
76.722
59.684
45.700
45.447
40.808
24.747
17.238
0.017
O
HN
N
N
H
CH3
O
N
H
N
200 180 160 140 120 100 80 60 40 20 0 ppm

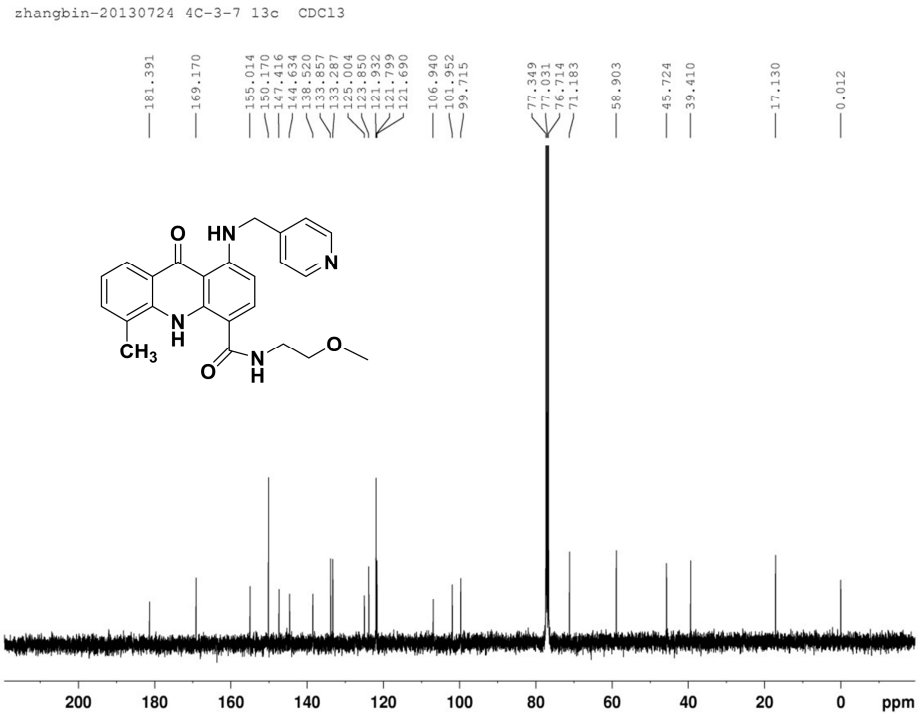

zhangb-20130801 4c-3-1 CDCl3
13.485
11.646
8.654
8.643
8.281
8.260
7.679
7.675
7.660
7.656
7.641
7.637
7.617
7.595
7.506
7.488
7.409
7.389
7.277
7.231
7.213
7.205
7.187
7.167
7.156
6.178
6.156
4.732
4.718
3.690
3.678
3.666
3.654
3.604
3.592
3.580
3.409
2.615
0.012
HN
N
O
N
H
CH3
O
N
H
O
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.92 0.93 1.00 0.96 2.06 1.01 0.96 2.05 0.93 0.95 1.95 1.96 2.02 3.01 2.95

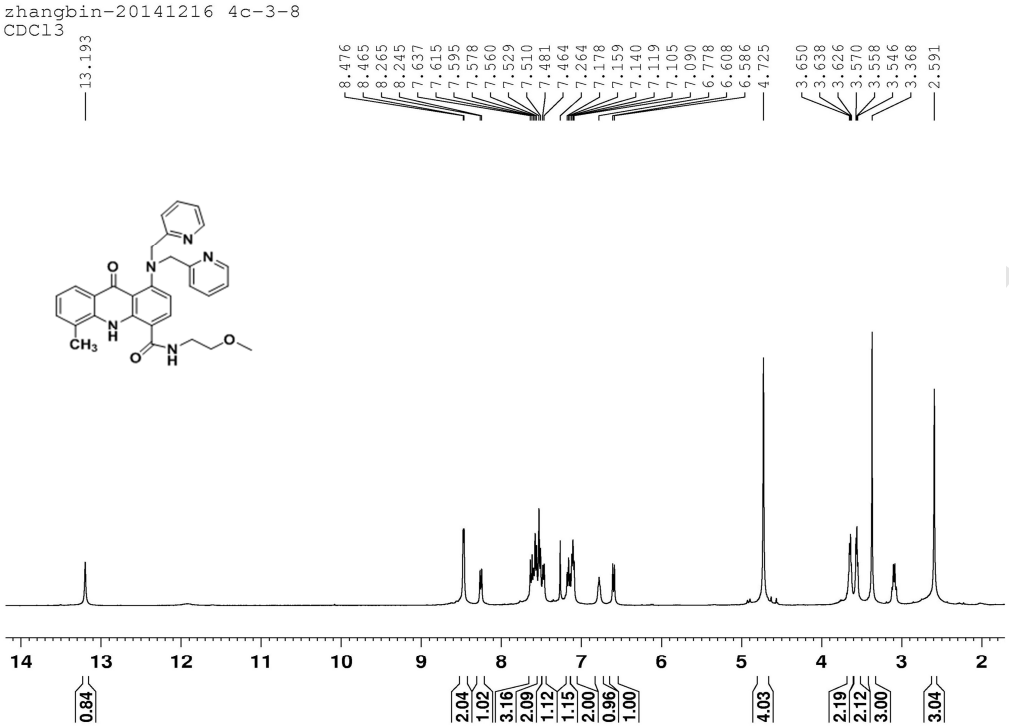

zhangb-20130801 4c-3-1 13c CDCl3
181.278
169.286
157.949
155.126
149.453
144.672
138.501
137.006
133.675
133.326
124.912
123.919
122.294
121.849
121.504
121.117
106.916
101.492
99.994
77.364
77.046
76.729
71.255
58.897
48.629
39.406
17.138
0.019
HN
N
O
N
H
CH3
O
N
H
O
200 180 160 140 120 100 80 60 40 20 0 ppm

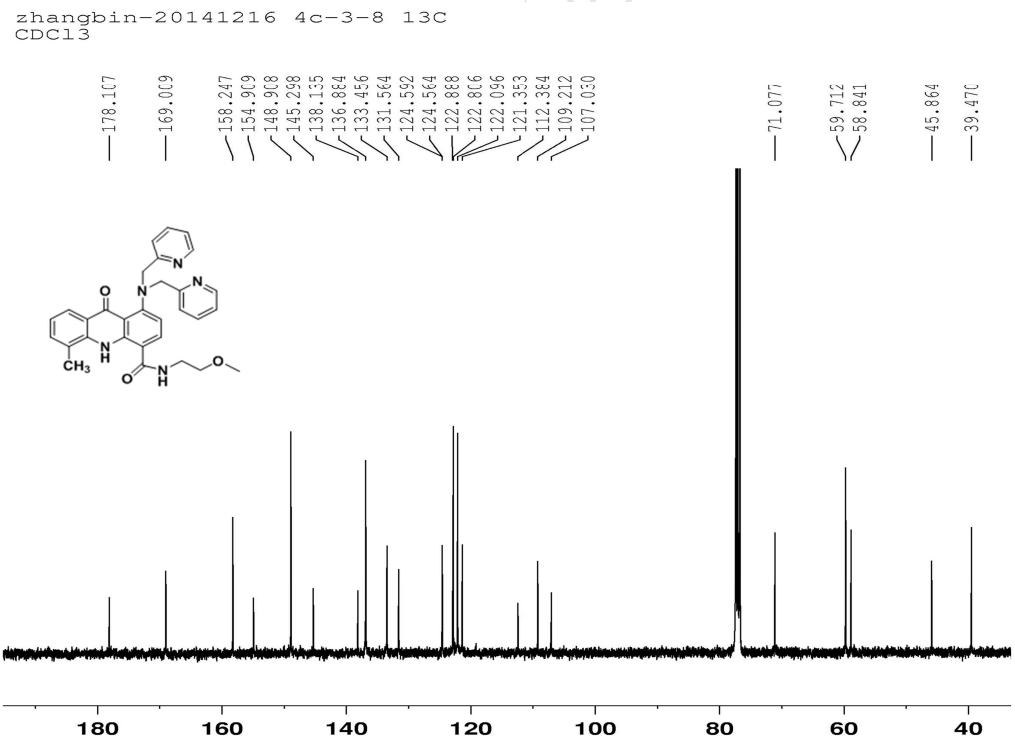

zhangb-20130801 4c-3-6 CDC13
13.505
11.511
11.498
11.484
8.670
8.666
8.555
8.552
8.543
8.540
8.239
8.219
7.752
7.732
7.623
7.601
7.510
7.492
7.300
7.288
7.281
7.273
7.204
7.185
7.166
7.540
6.527
6.148
6.126
4.602
4.588
3.690
3.678
3.665
3.653
3.603
3.591
3.579
3.410
2.621
0.008
O
HN
N
N
H
CH3
O
N
H
O
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.94
0.95
1.06
1.18
0.99
1.02
1.02
1.02
0.95
1.04
0.97
1.01
2.02
2.05
2.09
3.00
3.06

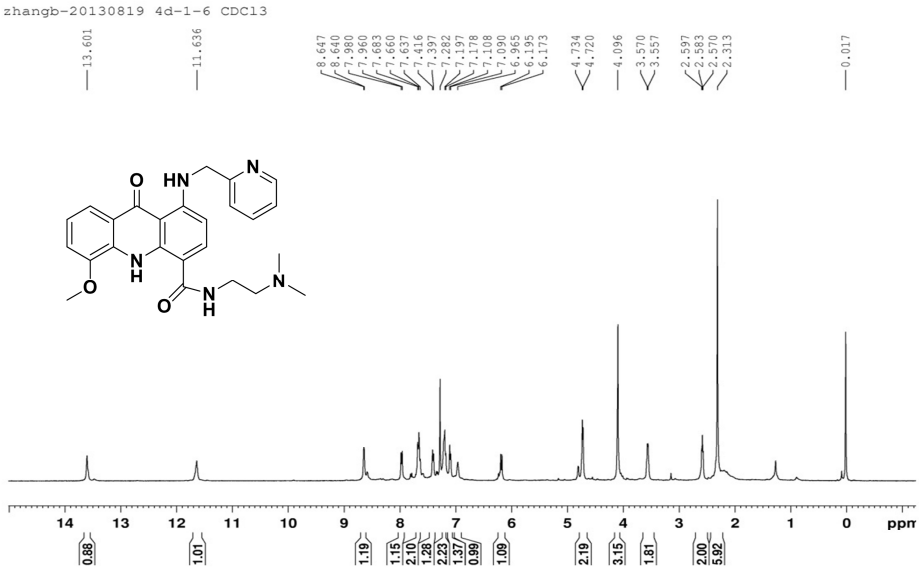

zhangb-20130801 4c-3-6 13c CDC13
181.302
169.208
154.960
149.002
148.900
144.634
138.487
134.804
133.780
133.332
124.969
123.810
123.759
121.786
121.629
106.880
101.761
99.647
77.358
77.041
76.723
71.218
58.902
44.335
39.412
17.118
0.011
O
HN
N
N
H
CH3
O
N
H
O
200 180 160 140 120 100 80 60 40 20 0 ppm

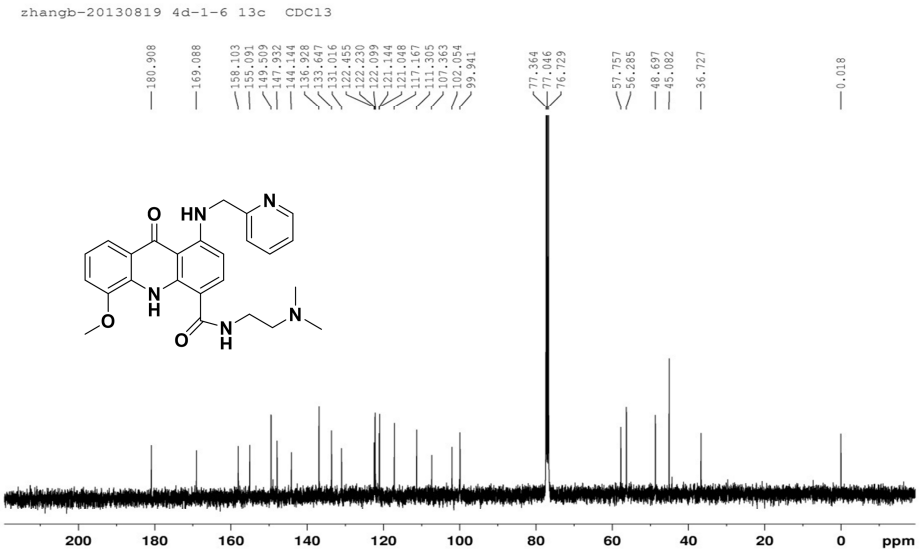

zhangbin-20130724 4C-3-7 CDCl3
13.532
11.585
8.594
8.582
8.279
8.258
7.615
7.593
7.539
7.521
7.340
7.329
7.283
7.234
7.215
7.197
6.513
6.061
6.039
4.628
4.614
3.689
3.677
3.609
3.598
3.415
2.644
0.020
O
HN
N
N
H
CH3
O
N
H
O
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.01
0.99
2.00
1.02
1.04
1.09
2.04
1.06
1.02
1.04
2.04
2.06
2.08
3.02
3.01

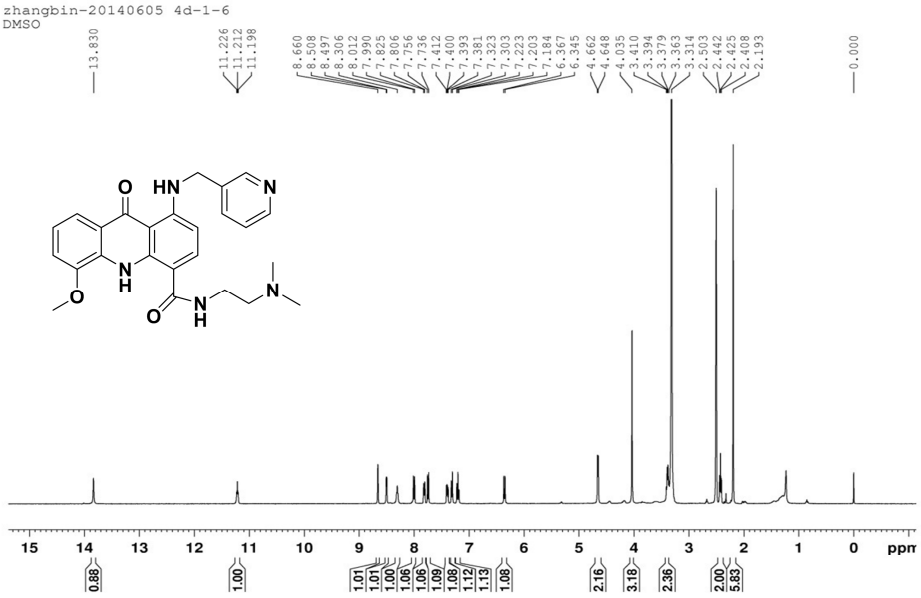

zhangbin-20130724 4C-3-7 13c CDCl3
181.391
169.170
155.014
150.170
147.416
144.634
138.520
133.857
133.287
125.004
123.850
121.932
121.799
121.690
106.940
101.952
99.715
77.349
77.031
76.714
71.183
58.903
45.724
39.410
17.130
0.012
O
HN
N
N
H
CH3
O
N
H
O
200
180
160
140
120
100
80
60
40
20
0
ppm

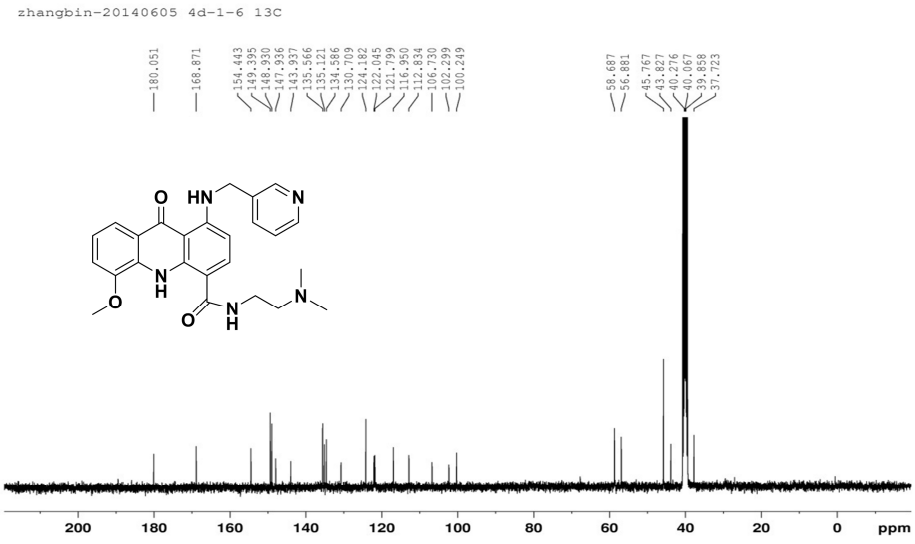

zhangbin-20141216 4c-3-8
CDCl3
13.193
8.476
8.465
8.265
8.245
7.637
7.615
7.595
7.578
7.560
7.529
7.510
7.481
7.464
7.264
7.178
7.159
7.140
7.119
7.105
7.090
6.778
6.608
6.586
4.725
3.650
3.638
3.626
3.570
3.558
3.546
3.368
2.591
0.84
2.04
1.02
3.16
2.09
1.12
1.15
2.00
0.96
1.00
4.03
2.19
2.12
3.00
3.04

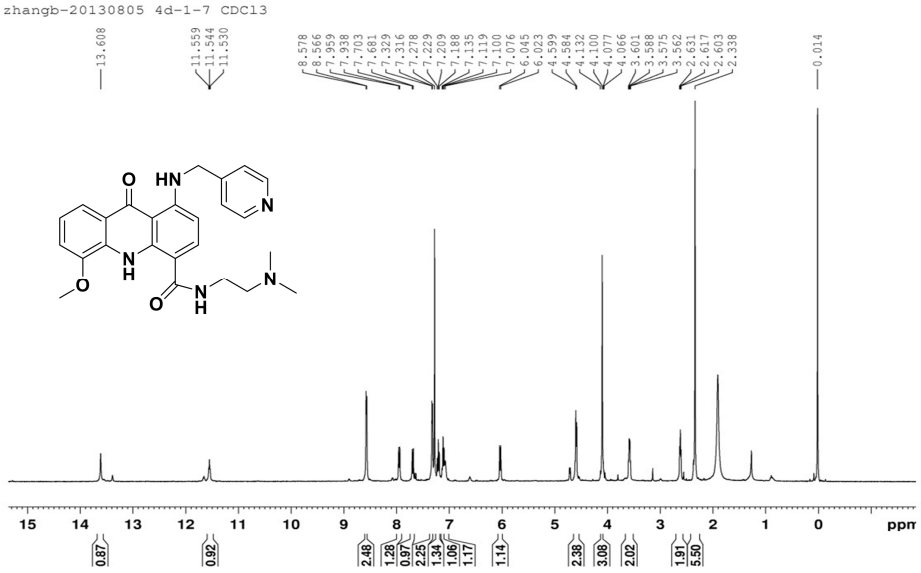

zhangbin-20141216 4c-3-8 13C
CDCl3
178.107
169.009
158.247
154.909
148.908
145.298
138.135
136.864
133.456
131.564
124.592
124.564
122.868
122.806
122.096
121.353
112.364
109.212
107.030
71.077
59.712
58.841
45.864
39.470

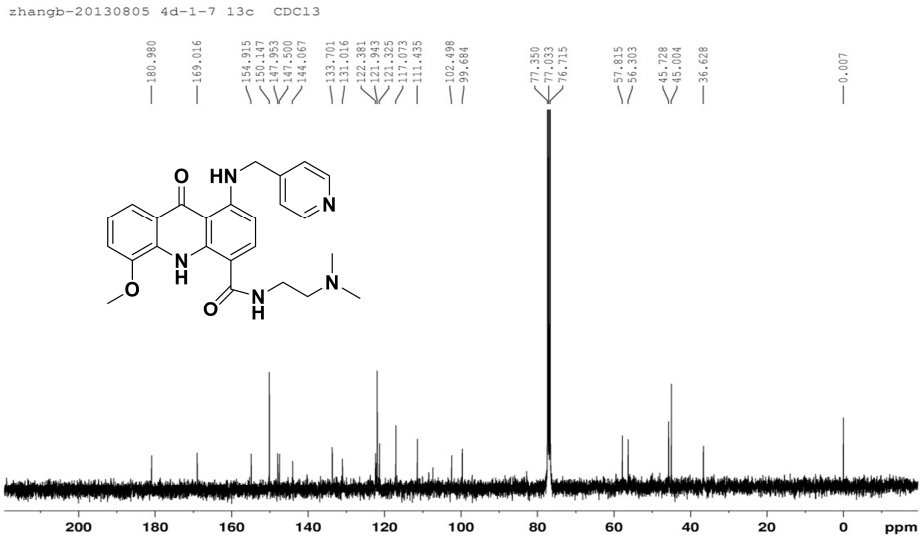

zhangb-20130819 4d-1-6 CDCl3
13.601
11.636
8.647
8.640
7.980
7.960
7.683
7.660
7.637
7.416
7.397
7.282
7.197
7.178
7.108
7.090
6.965
6.195
6.173
4.734
4.720
4.096
3.570
3.557
2.597
2.583
2.570
2.313
0.017
O
HN
N
N
H
O
O
N
H
N
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.88
1.01
1.19
1.15
2.10
1.28
2.23
1.37
0.99
1.09
2.19
3.15
1.81
2.00
5.92

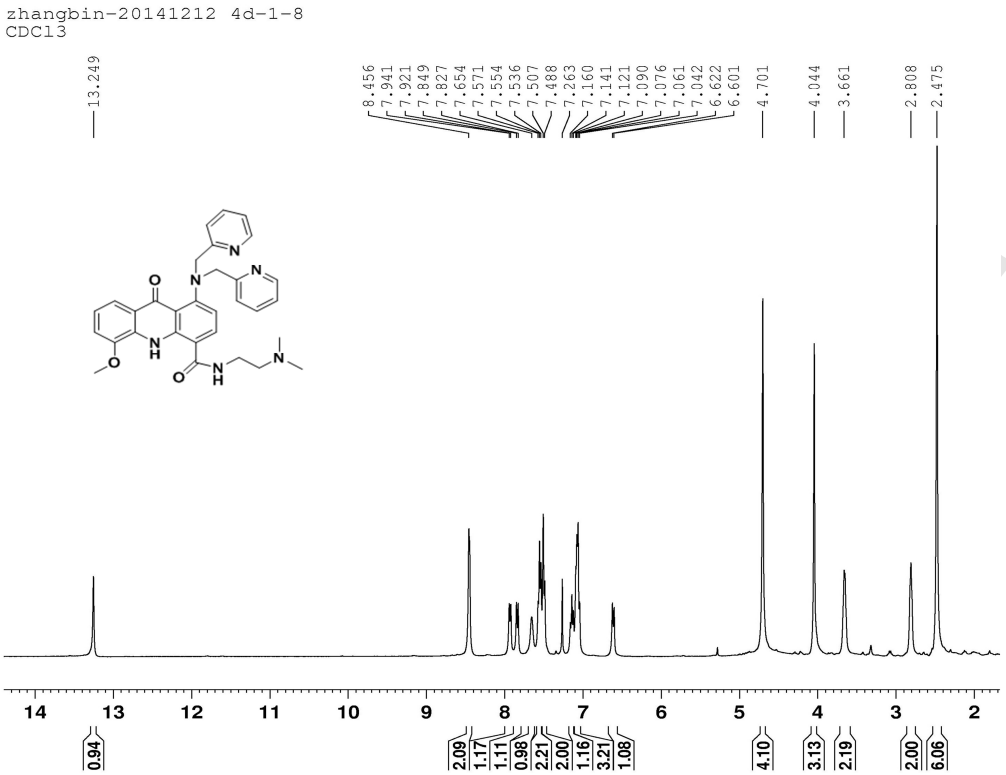

zhangb-20130819 4d-1-6 13c CDCl3
180.908
169.088
158.103
155.091
149.509
147.932
144.144
136.928
133.647
131.016
122.455
122.230
122.099
121.144
121.048
117.167
111.365
107.363
102.054
99.941
77.364
77.046
76.729
57.757
56.285
48.697
45.082
36.727
0.018
O
HN
N
N
H
O
O
N
H
N
200 180 160 140 120 100 80 60 40 20 0 ppm

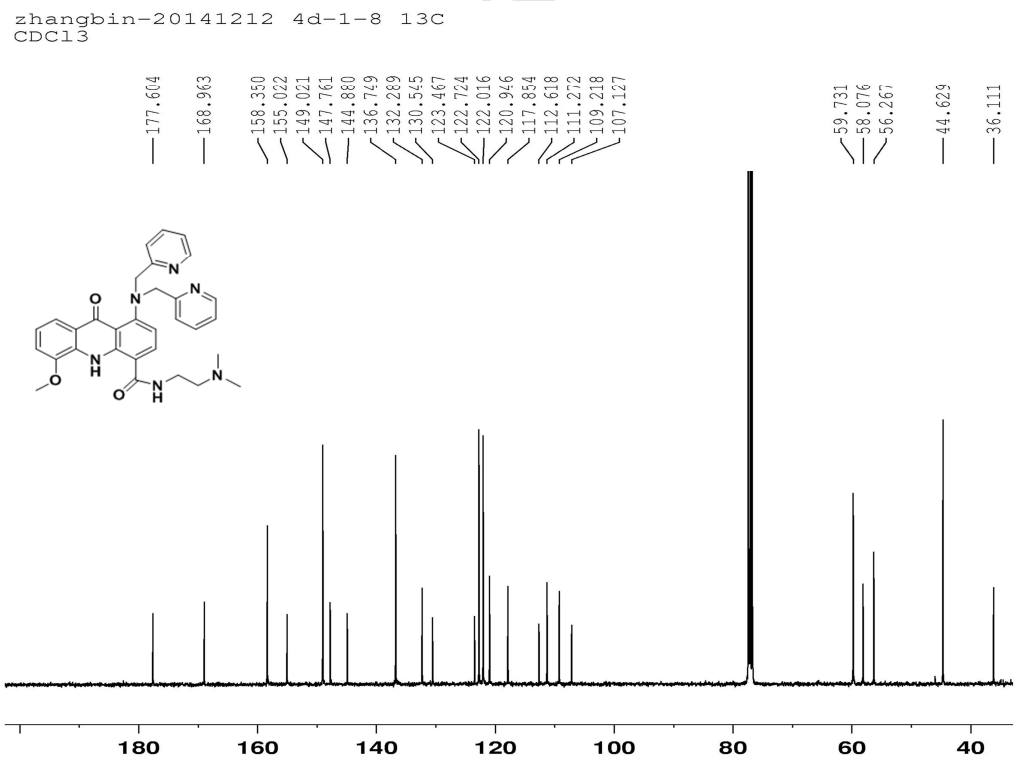

zhangbin-20140605 4d-1-6
DMSO
13.830
11.226
11.212
11.198
8.660
8.508
8.497
8.306
8.012
7.990
7.825
7.806
7.756
7.736
7.412
7.400
7.393
7.381
7.323
7.303
7.223
7.203
7.184
6.367
6.345
4.662
4.648
4.035
3.410
3.394
3.379
3.363
3.314
2.503
2.442
2.425
2.408
2.193
0.000
O
HN
N
N
H
O
N
H
O
N
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.88
1.00
1.01
1.01
1.00
1.06
1.06
1.09
1.08
1.12
1.13
1.08
2.16
3.18
2.36
2.00
5.83

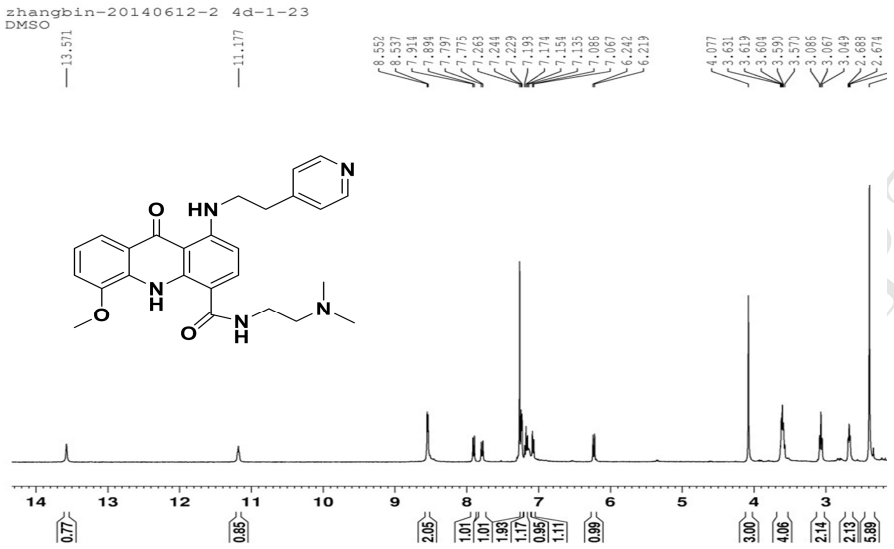

zhangbin-20140605 4d-1-6 13C
180.051
168.871
154.443
149.395
148.930
147.936
143.937
135.566
135.121
134.586
130.709
124.182
122.045
121.799
116.950
112.834
106.730
102.299
100.249
58.687
56.881
45.767
43.827
40.276
40.067
39.858
37.723
O
HN
N
N
H
O
N
H
O
N
200 180 160 140 120 100 80 60 40 20 0 ppm

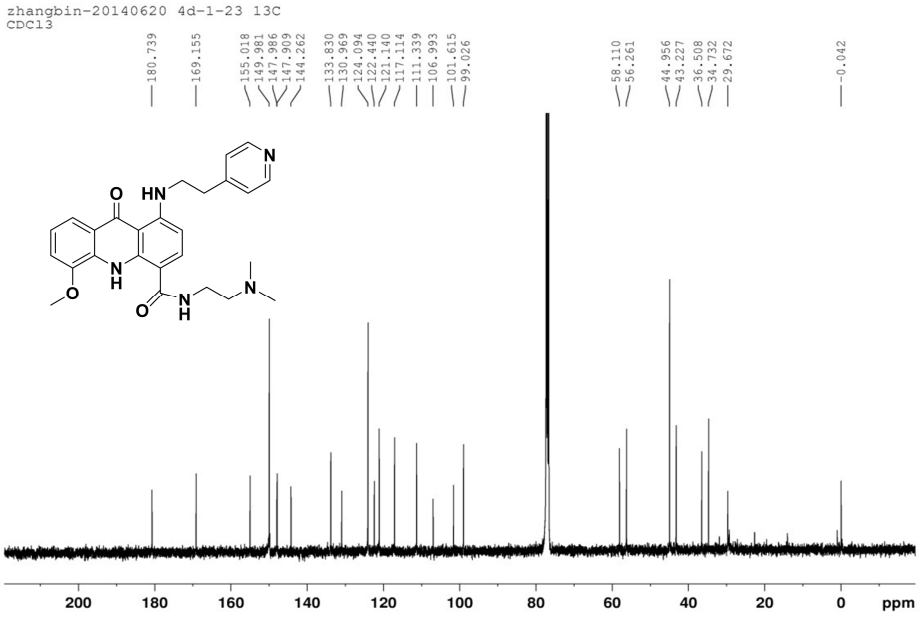

zhangb-20130805 4d-1-7 CDCl3
13.608
11.559
11.544
11.530
8.578
8.566
7.959
7.938
7.703
7.681
7.329
7.316
7.278
7.229
7.209
7.188
7.135
7.119
7.100
7.076
6.045
6.023
4.599
4.584
4.132
4.100
4.077
4.066
3.601
3.588
3.575
3.562
2.631
2.617
2.603
2.338
0.014
O
HN
N
N
H
O
O
N
H
N
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.87
0.92
2.48
1.28
0.97
2.25
1.34
1.06
1.17
1.14
2.38
3.08
2.02
1.91
5.50

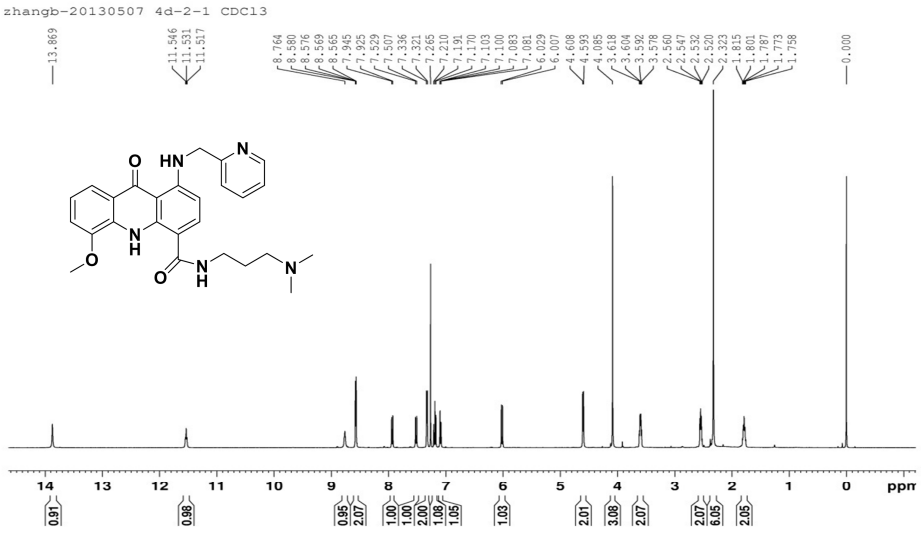

zhangb-20130805 4d-1-7 13c CDCl3
180.980
169.016
154.915
150.147
147.953
147.500
144.067
133.701
131.016
122.381
121.943
121.325
117.073
111.435
102.498
99.684
77.350
77.033
76.715
57.815
56.303
45.728
45.004
36.628
0.007
O
HN
N
N
H
O
O
N
H
N
200 180 160 140 120 100 80 60 40 20 0 ppm

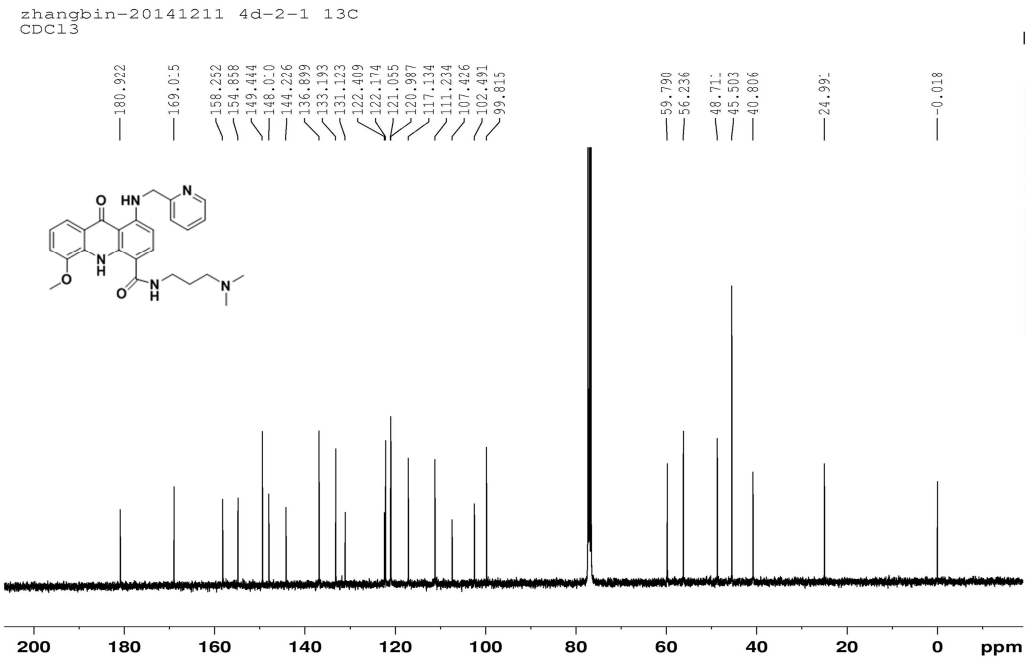

zhangbin-20141212 4d-1-8
CDCl3
13.249
8.456
7.941
7.921
7.849
7.827
7.654
7.571
7.554
7.536
7.507
7.488
7.263
7.160
7.141
7.121
7.090
7.076
7.061
7.042
6.622
6.601
4.701
4.044
3.661
2.808
2.475
14
13
12
11
10
9
8
7
6
5
4
3
2
0.94
2.09
1.17
1.11
0.98
2.21
2.00
1.16
3.21
1.08
4.10
3.13
2.19
2.00
6.06

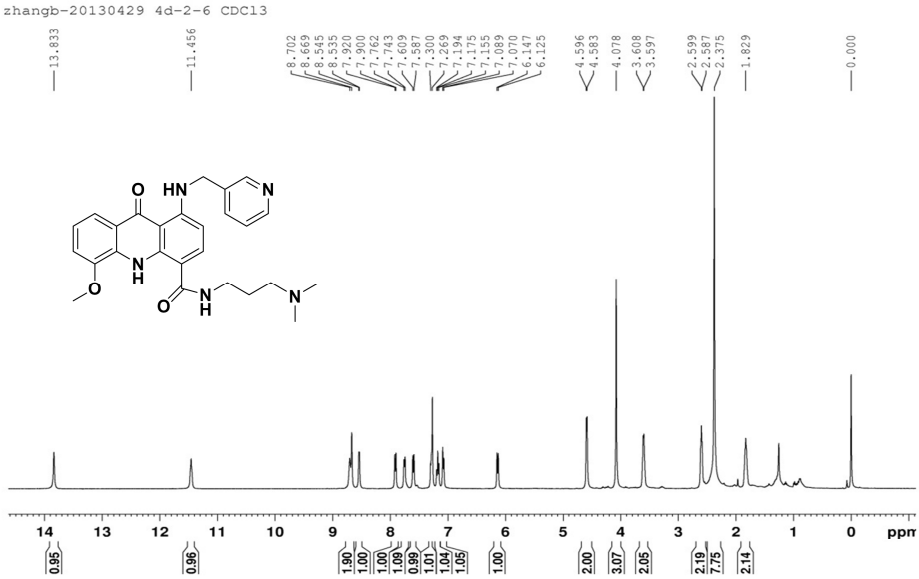

zhangbin-20141212 4d-1-8 13C
CDCl3
177.604
168.963
158.350
155.022
149.021
147.761
144.880
136.749
132.289
130.545
123.467
122.724
122.016
120.946
117.854
112.618
111.272
109.218
107.127
59.731
58.076
56.267
44.629
36.111
180
160
140
120
100
80
60
40

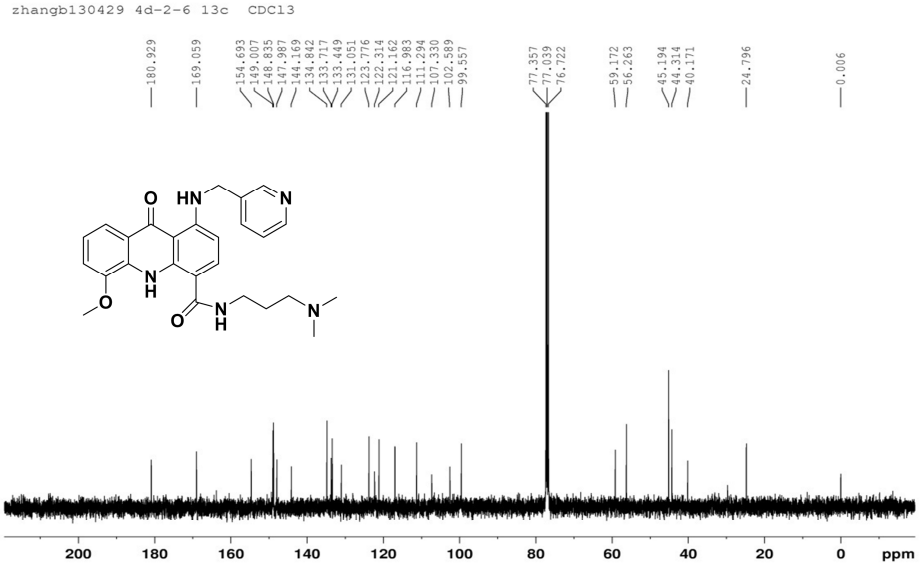

zhangbin-20140612-2 4d-1-23
DMSO
13.571
11.177
8.552
8.537
7.914
7.894
7.797
7.775
7.263
7.244
7.229
7.193
7.174
7.154
7.135
7.086
7.067
6.242
6.219
4.077
3.631
3.619
3.604
3.599
3.570
3.086
3.067
3.049
2.688
2.674
O
HN
N
N
H
O
N
O
N
H
14
13
12
11
10
9
8
7
6
5
4
3
0.77
0.85
2.05
1.01
1.01
1.93
1.17
0.95
1.11
0.99
3.00
4.06
2.14
2.13
5.69

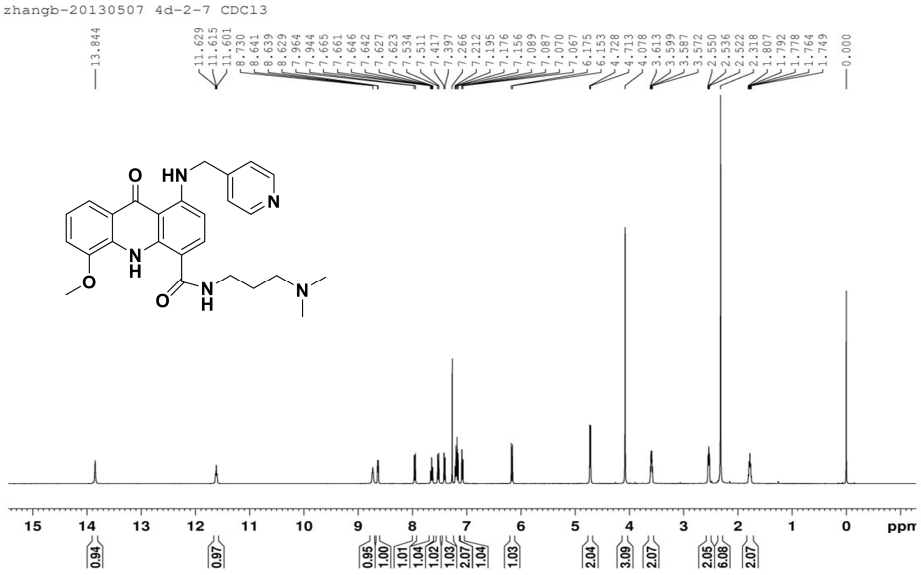

zhangbin-20140620 4d-1-23 13C
CDCl3
180.739
169.155
155.018
149.981
147.986
147.909
144.262
133.830
130.969
124.094
122.440
121.140
117.114
111.339
106.993
101.615
99.026
58.110
56.261
44.956
43.227
36.508
34.732
29.672
-0.042
O
HN
N
N
H
O
N
O
N
H
200
180
160
140
120
100
80
60
40
20
0
ppm

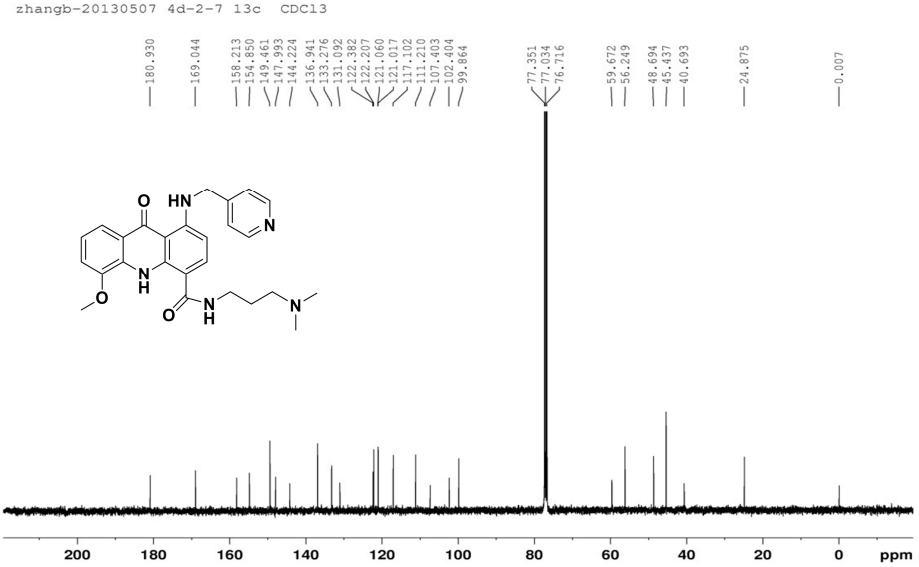

zhangb-20130507 4d-2-1 CDCl3
13.869
11.546
11.531
11.517
8.764
8.580
8.576
8.569
8.565
7.945
7.925
7.529
7.507
7.336
7.321
7.265
7.210
7.191
7.170
7.103
7.100
7.083
7.081
6.029
6.007
4.608
4.593
4.085
3.618
3.604
3.592
3.578
2.560
2.547
2.532
2.520
2.323
1.815
1.801
1.787
1.773
1.758
0.000
O
HN
N
N
H
O
N
O
N
H
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.91
0.98
0.95
2.07
1.00
1.00
2.00
1.08
1.05
1.03
2.01
3.08
2.07
2.07
6.05
2.05

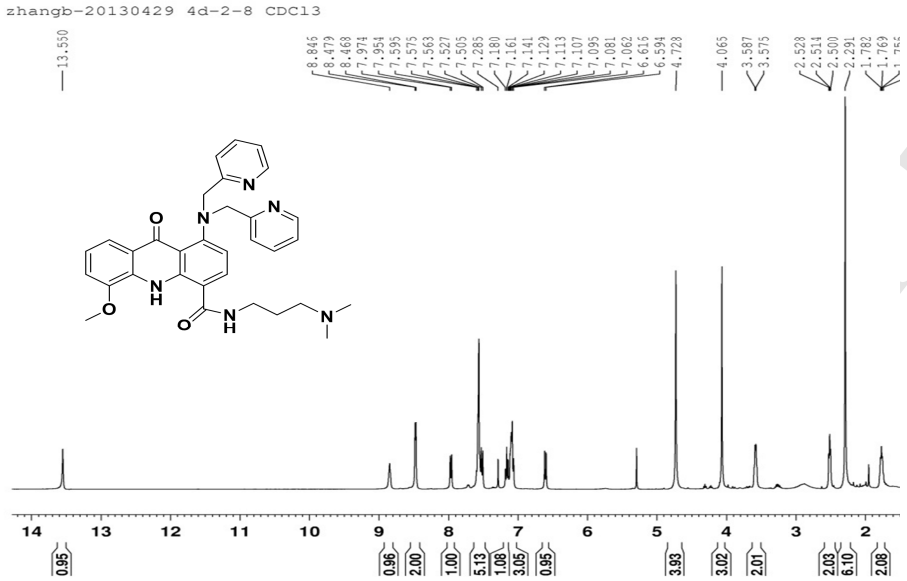

zhangbin-20141211 4d-2-1 13C
CDCl3
180.922
169.0:5
158.252
154.658
149.444
148.0:0
144.226
136.899
133.193
131.123
122.409
122.174
121.055
120.987
117.134
111.234
107.426
102.491
99.815
59.790
56.236
48.71:
45.503
40.806
24.99-
-0.018
O
HN
N
N
H
O
N
O
N
H
200
180
160
140
120
100
80
60
40
20
0
ppm

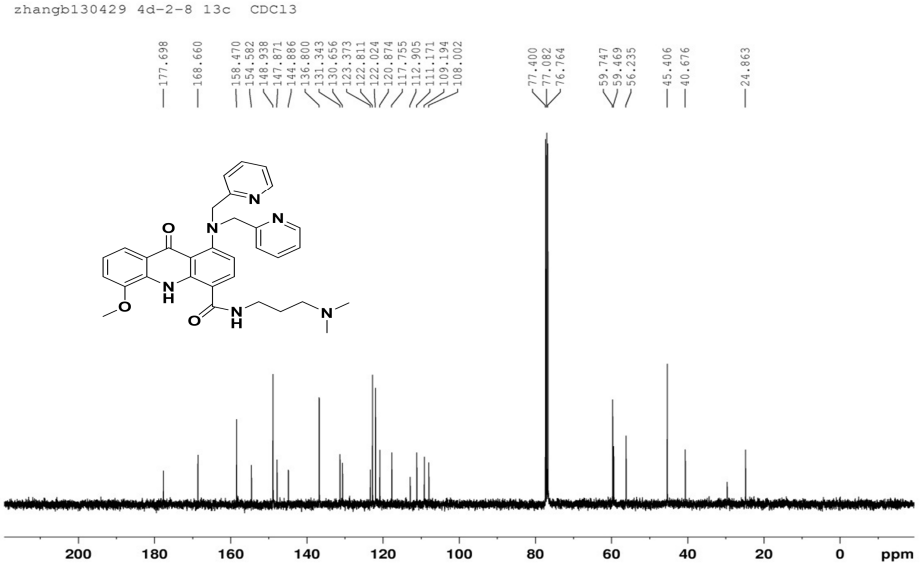

zhangb-20130429 4d-2-6 CDCl3
13.833
11.456
8.702
8.669
8.545
8.535
7.920
7.900
7.762
7.743
7.609
7.587
7.300
7.269
7.194
7.175
7.155
7.089
7.070
6.147
6.125
4.596
4.583
4.078
3.608
3.597
2.599
2.587
2.375
1.829
0.000
O
HN
N
N
H
O
O
N
H
N
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.95 0.96 1.90 1.00 1.00 1.09 0.99 1.01 1.04 1.05 1.00 2.00 3.07 2.05 2.19 7.75 2.14

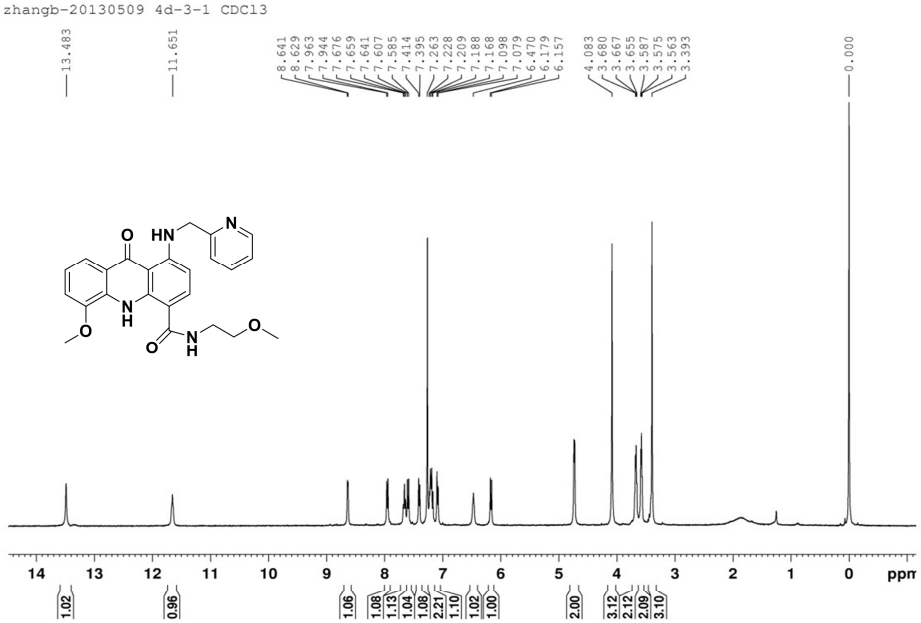

zhangb130429 4d-2-6 13c CDCl3
180.929
169.059
154.693
149.007
148.835
147.987
144.169
134.842
133.717
133.449
131.051
123.776
122.314
121.162
116.983
111.294
107.330
102.589
99.557
77.357
77.039
76.722
59.172
56.263
45.194
44.314
40.171
24.796
0.006
O
HN
N
N
H
O
O
N
H
N
200 180 160 140 120 100 80 60 40 20 0 ppm

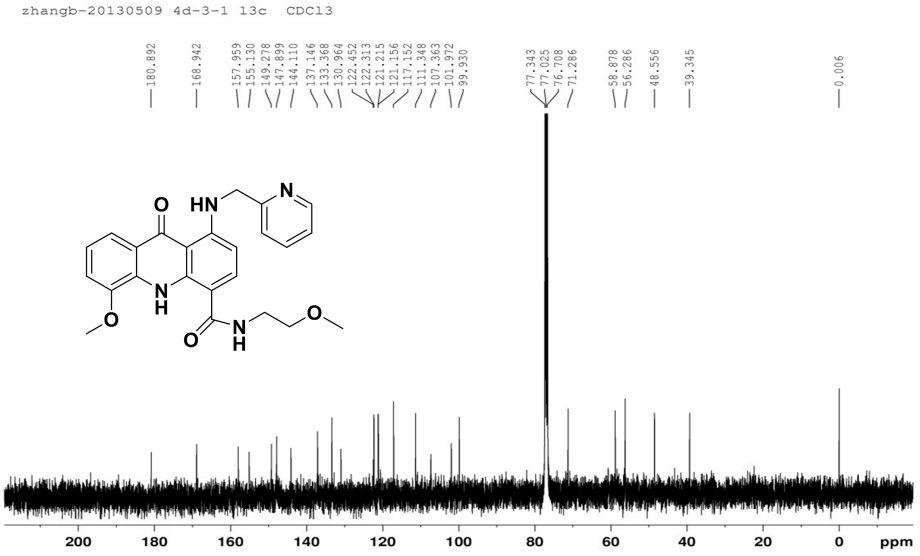

zhangb-20130507 4d-2-7 CDCl3
13.844
11.629
11.615
11.601
8.730
8.641
8.639
8.629
7.964
7.944
7.665
7.661
7.646
7.642
7.627
7.623
7.534
7.511
7.417
7.397
7.266
7.212
7.195
7.176
7.156
7.089
7.087
7.070
7.067
6.175
6.153
4.728
4.713
4.618
4.613
3.599
3.587
3.572
2.550
2.536
2.522
2.318
1.807
1.792
1.778
1.764
1.749
0.000
O
HN
N
N
H
O
O
N
H
N
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.94 0.97 0.95 1.00 1.01 1.04 1.02 1.03 2.07 1.04 1.03 2.04 3.09 2.07 2.05 6.08 2.07

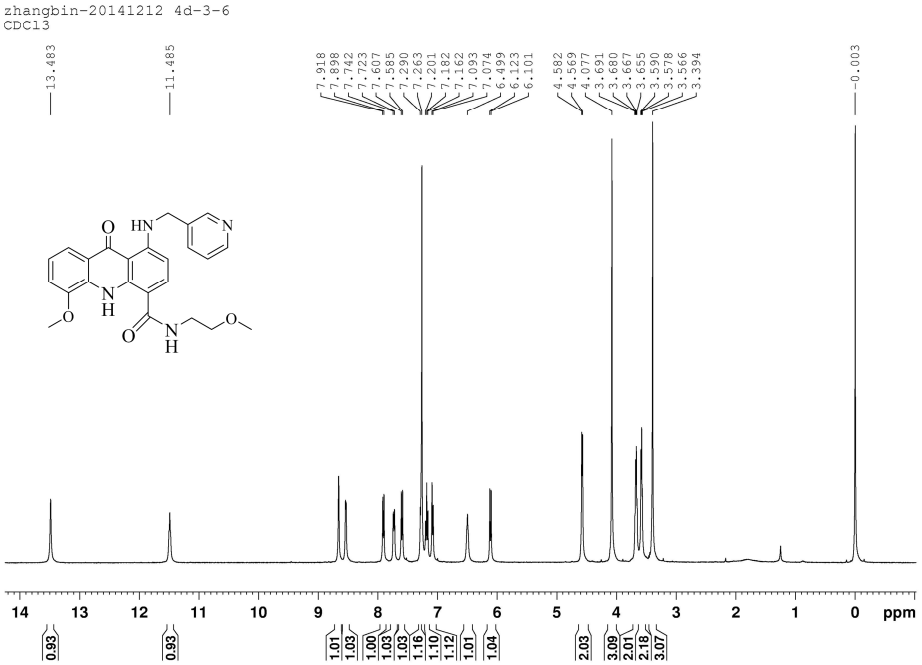

zhangb-20130507 4d-2-7 13c CDCl3
180.930
169.044
158.213
154.850
149.461
147.993
144.224
136.941
133.276
131.092
122.382
122.207
121.060
121.017
117.102
111.210
107.403
102.404
99.864
77.351
77.034
76.716
59.672
56.249
48.694
45.437
40.693
24.875
0.007
O
HN
N
N
H
O
O
N
H
N
200 180 160 140 120 100 80 60 40 20 0 ppm

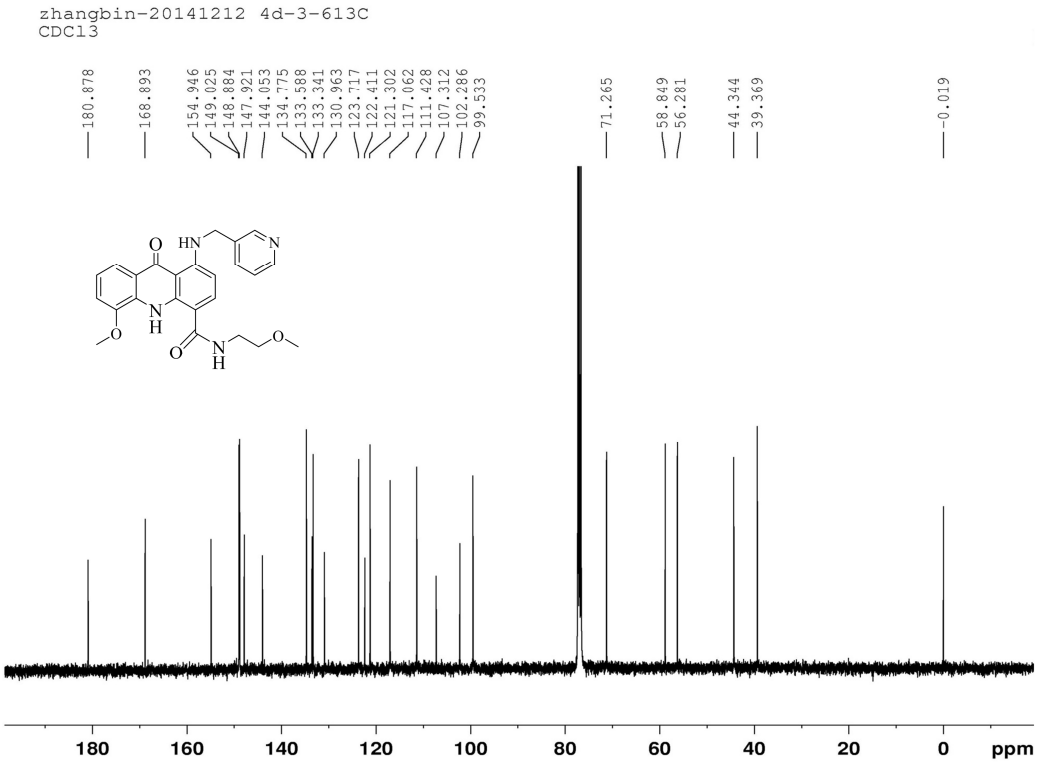

zhangb-20130429 4d-2-8 CDCl3
13.550
8.845
8.479
8.468
7.974
7.954
7.595
7.575
7.563
7.527
7.505
7.285
7.180
7.161
7.141
7.129
7.113
7.107
7.095
7.081
7.062
6.615
6.594
4.728
4.065
3.587
3.575
2.528
2.514
2.500
2.291
1.782
1.769
14 13 12 11 10 9 8 7 6 5 4 3 2
0.95 0.96 2.00 1.00 5.13 1.08 3.05 0.95 3.93 3.02 2.01 2.03 6.10 2.08

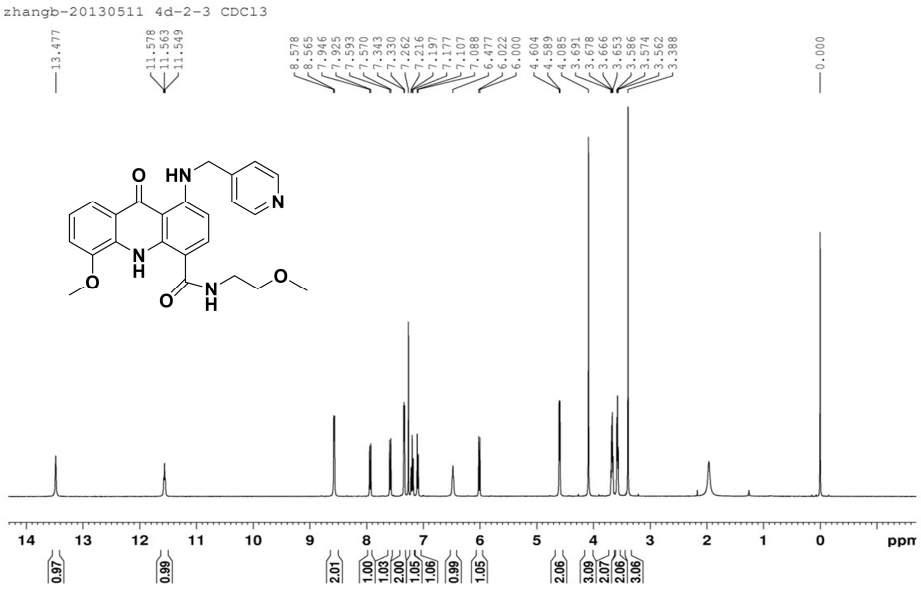

zhangb130429 4d-2-8 13c CDCl3
177.698
168.660
158.470
154.582
148.938
147.871
144.886
136.800
131.343
130.656
123.373
122.811
122.024
120.874
117.755
112.905
111.171
109.194
108.002
77.400
77.082
76.764
59.747
59.469
56.235
45.406
40.676
24.863
200 180 160 140 120 100 80 60 40 20 0 ppm

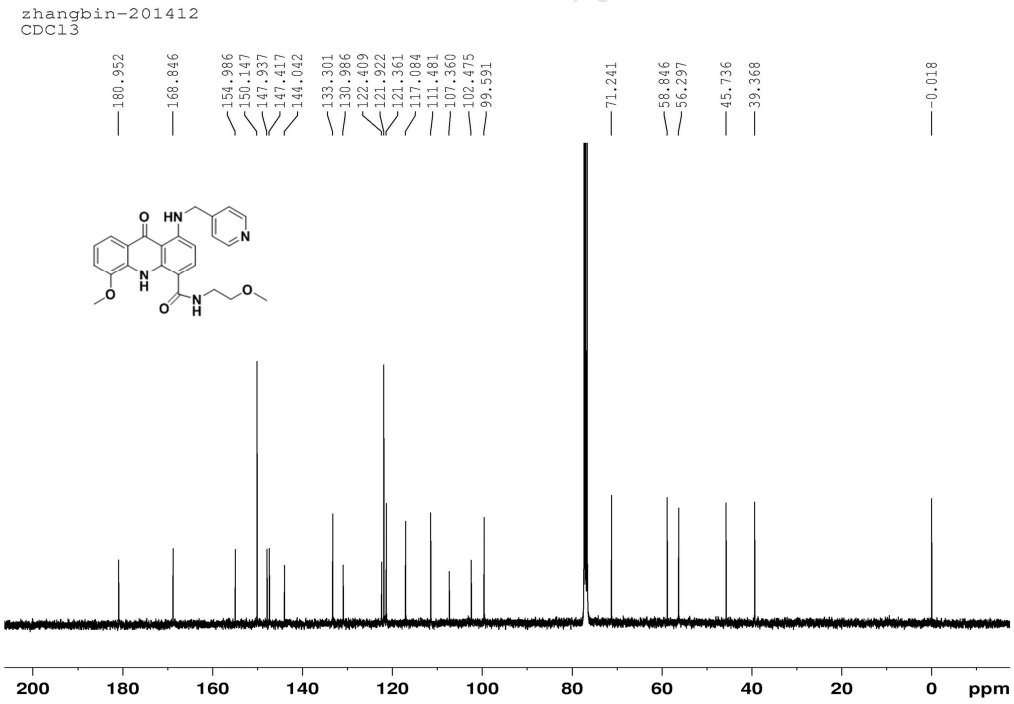

zhangb-20130509 4d-3-1 CDC13
13.483
11.651
8.641
8.629
7.963
7.944
7.676
7.659
7.641
7.607
7.585
7.414
7.395
7.263
7.228
7.209
7.188
7.168
7.098
7.079
6.470
6.179
6.157
4.083
3.680
3.667
3.655
3.587
3.575
3.563
3.393
0.000
O
HN
N
N
H
O
O
N
H
O
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
1.02 0.96 1.06 1.08 1.13 1.04 1.08 2.21 1.10 1.02 1.00 2.00 3.12 2.12 2.09 3.10

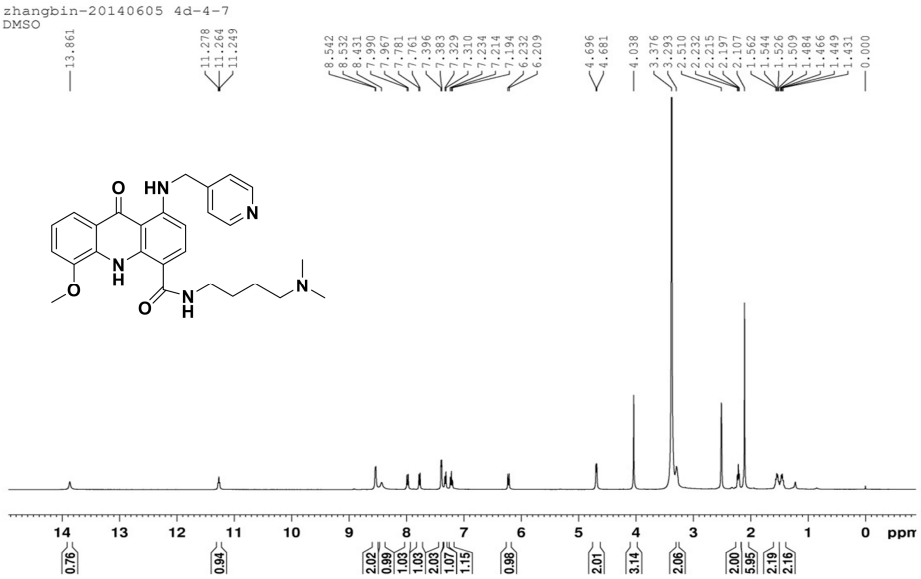

zhangb-20130509 4d-3-1 13c CDC13
180.892
168.942
157.959
155.130
149.278
147.899
144.110
137.146
133.368
130.964
122.452
122.313
121.215
121.156
117.152
111.348
107.363
101.972
99.930
77.343
77.025
76.708
71.286
58.878
56.286
48.556
39.345
0.006
O
HN
N
N
H
O
O
N
H
O
200 180 160 140 120 100 80 60 40 20 0 ppm

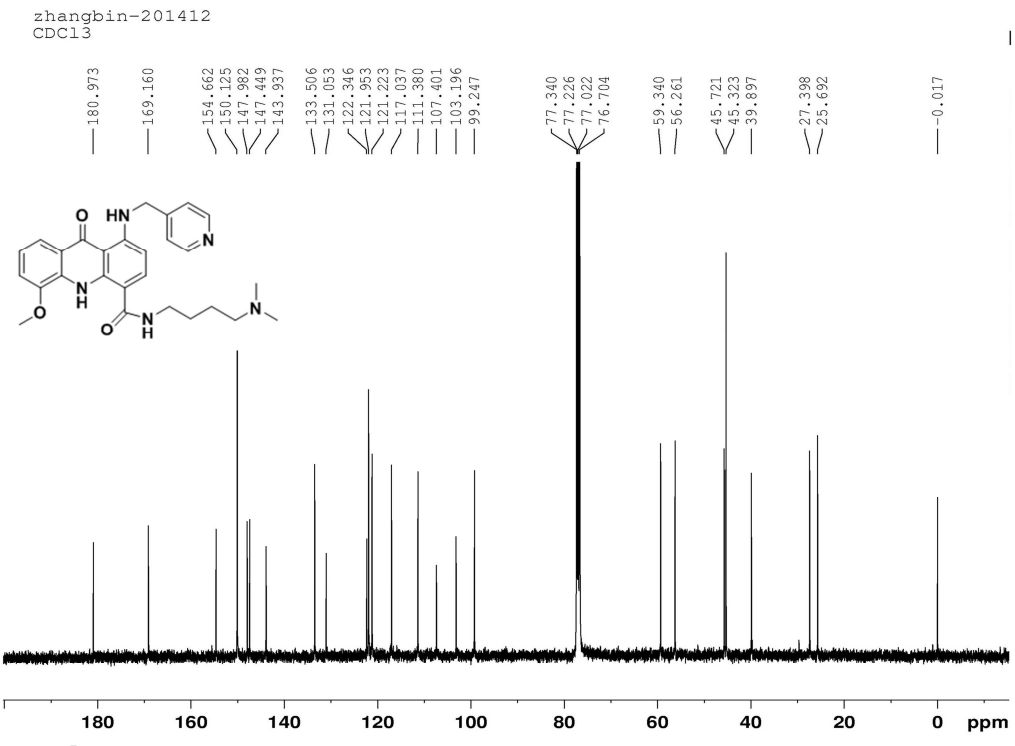

zhangbin-20141212 4d-3-6
CDCl3
13.483
11.485
7.918
7.898
7.742
7.723
7.607
7.585
7.290
7.263
7.201
7.182
7.162
7.093
7.074
6.499
6.123
6.101
4.582
4.569
4.077
3.691
3.680
3.667
3.655
3.590
3.578
3.566
3.394
0.003
O
HN
N
N
H
O
O
N
H
O
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.93
0.93
1.01
1.03
1.00
1.03
1.03
1.16
1.10
1.12
1.01
1.04
2.03
3.09
2.01
2.18
3.07

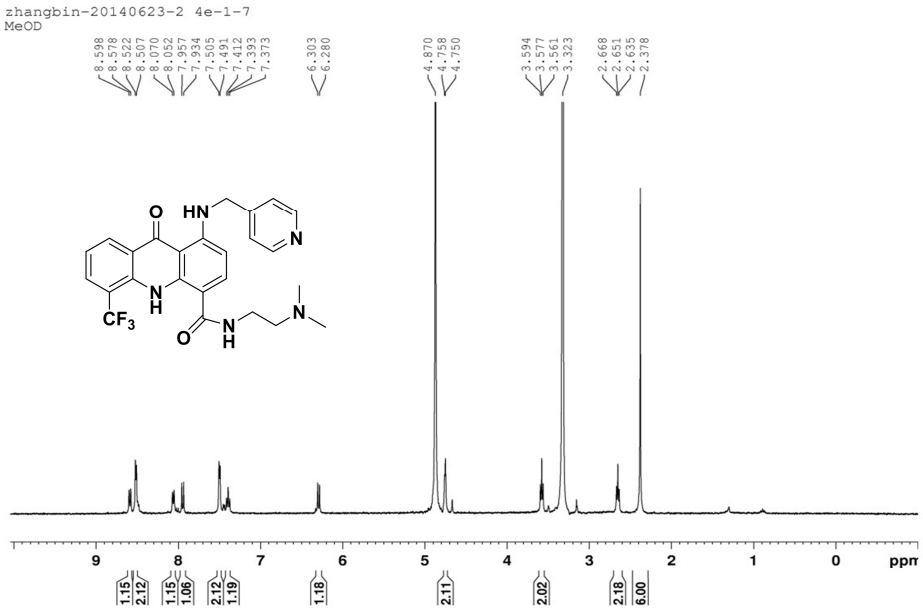

zhangbin-20141212 4d-3-613C
CDCl3
180.878
168.893
154.946
149.025
148.884
147.921
144.053
134.775
133.588
133.341
130.963
123.717
122.411
121.302
117.062
111.428
107.312
102.286
99.533
71.265
58.849
56.281
44.344
39.369
0.019
O
HN
N
N
H
O
O
N
H
O
180 160 140 120 100 80 60 40 20 0 ppm

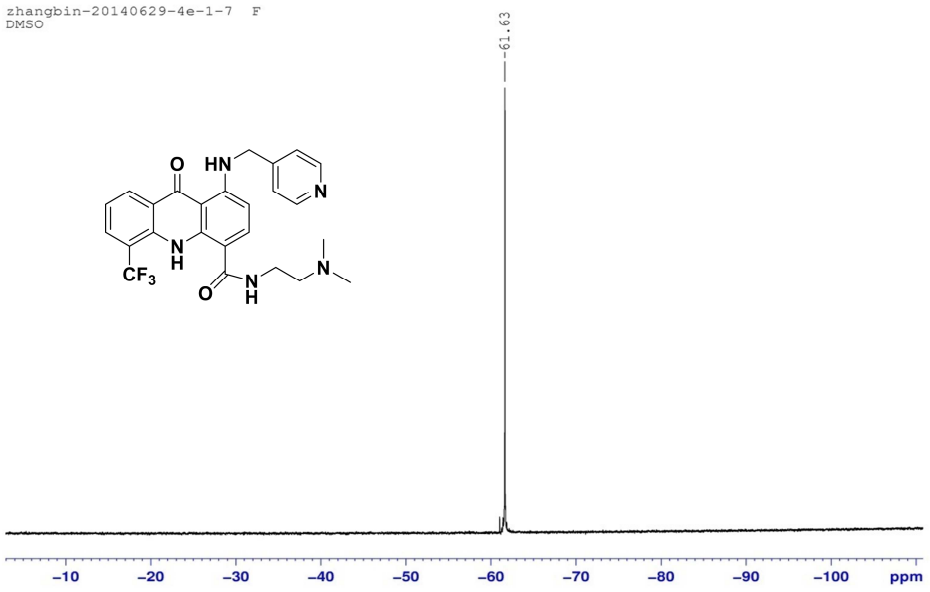

zhangb-20130511 4d-2-3 CDCl3
13.477
11.578
11.563
11.549
8.578
8.565
7.946
7.925
7.593
7.570
7.343
7.330
7.262
7.246
7.197
7.177
7.107
7.088
6.477
6.022
6.000
4.604
4.589
4.085
3.691
3.678
3.666
3.653
3.586
3.574
3.562
3.388
0.000
O
HN
N
N
H
O
O
N
H
O
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.97
0.99
2.01
1.00
1.03
2.00
1.05
1.06
0.99
1.05
2.06
3.09
2.07
2.06
3.06

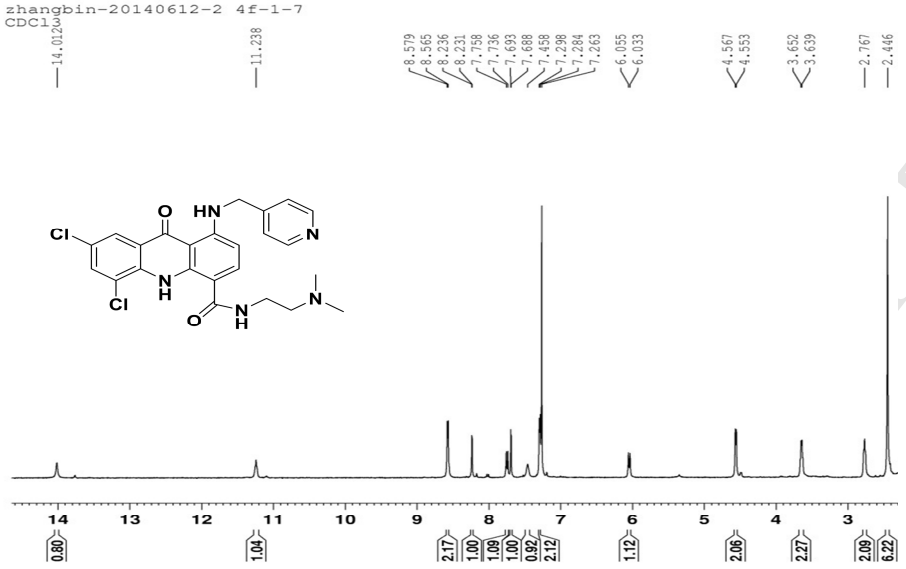

zhangbin-201412
CDCl3
180.952
168.846
154.986
150.147
147.937
147.417
144.042
133.301
130.986
122.409
121.922
121.361
117.084
111.481
107.360
102.475
99.591
71.241
58.846
56.297
45.736
39.368
0.018
O
HN
N
N
H
O
O
N
H
O
200
180
160
140
120
100
80
60
40
20
0
ppm

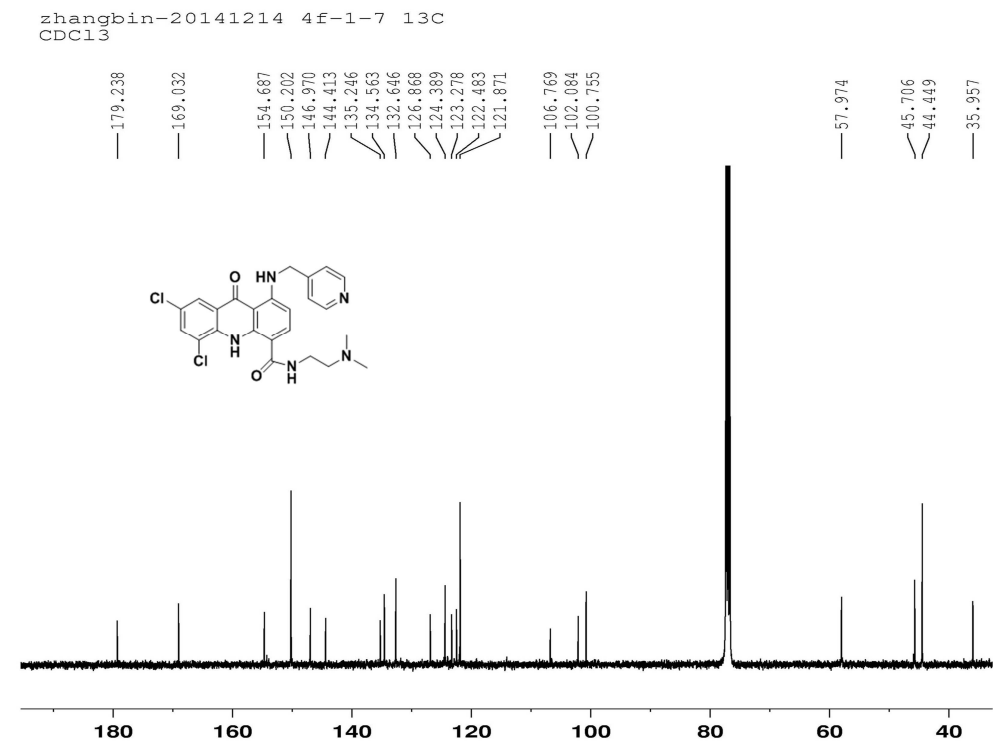

zhangbin-20140605 4d-4-7
DMSO
13.861
11.278
11.264
11.249
8.542
8.532
8.431
7.990
7.967
7.781
7.761
7.396
7.383
7.329
7.310
7.234
7.214
7.194
6.232
6.209
4.696
4.681
4.038
3.376
3.293
2.510
2.232
2.215
2.197
2.107
1.562
1.544
1.526
1.509
1.484
1.466
1.449
1.431
0.000
O
HN
N
N
H
O
N
O
N
H
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.76 0.94 2.02 0.99 1.03 1.03 2.03 1.07 1.15 0.98 2.01 3.14 2.06 2.00 5.95 2.19 2.16


zhangbin-201412
CDCl3
180.973
169.160
154.662
150.125
147.982
147.449
143.937
133.506
131.053
122.346
121.953
121.223
117.037
111.380
107.401
103.196
99.247
77.340
77.226
77.022
76.704
59.340
56.261
45.721
45.323
39.897
27.398
25.692
-0.017
O
HN
N
N
H
O
N
O
N
H
180 160 140 120 100 80 60 40 20 0 ppm


zhangbin-20140623-2 4e-1-7
MeOD
8.598
8.578
8.522
8.507
8.070
8.052
7.952
7.934
7.505
7.491
7.412
7.393
7.373
6.303
6.280
4.870
4.758
4.750
3.594
3.577
3.561
3.323
2.668
2.651
2.635
2.378
O
HN
N
N
H
CF3
O
N
H
N
9
8
7
6
5
4
3
2
1
0
ppm
1.15
2.12
1.15
1.06
2.12
1.19
1.18
2.11
2.02
2.18
6.00


zhangbin-20140629-4e-1-7 F
DMSO
-61.63
O
HN
N
N
H
CF3
O
N
H
N
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
ppm


zhangbin-20140612-2 4f-1-7
CDCl3
14.012
11.238
8.579
8.565
8.236
8.231
7.758
7.736
7.693
7.688
7.458
7.298
7.284
7.263
6.055
6.033
4.567
4.553
3.652
3.639
2.767
2.446
Cl
O
HN
N
N
H
Cl
O
N
H
N
14
13
12
11
10
9
8
7
6
5
4
3
0.80
1.04
2.17
1.00
1.09
1.00
0.92
2.12
1.12
2.06
2.27
2.09
6.22


zhangbin-20141214 4f-1-7 13C
CDCl3
179.238
169.032
154.687
150.202
146.970
144.413
135.246
134.563
132.646
126.868
124.389
123.278
122.483
121.871
106.769
102.084
100.755
57.974
45.706
44.449
35.957
Cl
O
HN
N
N
H
Cl
O
N
H
N
180
160
140
120
100
80
60
40

5. High resolution mass spectrometry