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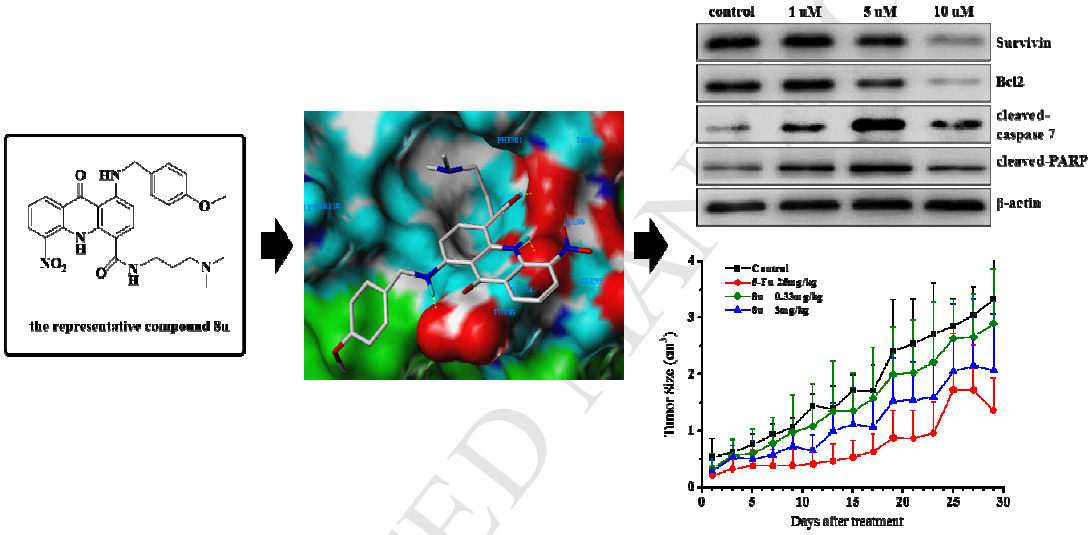

Novel multi-substituted benzyl acridone derivatives as survivin inhibitors for hepatocellular carcinoma treatment

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Abstract

Sorafenib was the only small-molecule drug approved by FDA for treatment of the advanced hepatocellular carcinoma (HCC). Recent study indicated that YM155 was a promising agent for HCC cells with high survivin expression, however, the antitumor activity needs to be further improved. Based on molecular docking and rational design method, a series of multi-substituted benzyl acridone derivatives were designed and synthesized. MTT assay indicated that some of the synthesized compounds displayed better antiproliferative activity against HepG2 cells than YM155. Later study indicated that the representive compound **8u** may directly interact with survivin protein and induce HepG2 cells apoptosis, which is different from YM155. In addition, ADME property was predicted *in silico*, and it performed well. Moreover, *in vivo* preliminary experiments showed that **8u** may be a good lead compound in the treatment of HCC.

Key words: Acridones, Survivin, YM155, Molecular docking, Antitumor

1. Introduction

Currently, hepatocellular carcinoma (HCC) is one of the most common potentially lethal human malignancies worldwide [1]. The only approved small-molecule drug for advanced HCC is the multikinase inhibitor Sorafenib, however, it has only little survival advantage in the presence of major side effects [2]. Therefore, the development of novel small molecule inhibitors against HCC is urgently required. Survivin is the smallest member of Inhibitor of Apoptosis Proteins (IAPs) [3, 4], which is highly expressed in most human solid tumors including HCC, but with little expression in most normal terminally differentiated adult tissues [5-8]. Therefore, survivin has become a putative drug target for cancer therapy [9-14]. Now, it has been regarded as an attractive therapeutic target for the treatment of HCC [15-19].

YM155 (Sepantronium Bromide), a small-molecule survivin suppressant, has entered clinical trials for the treatment of lymphoma, melanoma, prostate and non-small cell lung carcinoma [20, 21]. Additionally, preclinical studies have also shown that YM155 was a promising agent for HCC cells with high survivin expression [17], and it yielded significantly better therapeutic effect than sorafenib in an orthotopic mouse model [16]. Although the anti-cancer mechanisms of YM155 have not yet been fully elucidated [17, 22], a library of YM155 analogs have been designed and synthesized as anti-cancer small molecule inhibitors in recent years because of its excellent anti-cancer activities [23]. The United States National Cancer Institute also developed a novel dioxonaphthoimidazolium derivative NSC80467, the

chemical structure of which is similar to YM155, and it displayed similar antitumor activity against NCI-60 cell lines compared to YM155 [24]. Although series of YM155 derivatives have been developed, few compounds displayed better antitumor activity against HCC have been found. Therefore, it is important to develop new YM155 analogues to treat HCC.

Comparing the structures of YM155 and NSC80467, both of them contain a tricyclic conjugated system (**Figure 1a**, D, red ellipses) and two main side chains, one of which contains an aromatic ring group (**Figure 1a**, blue ellipses) and the other is an alkyl or alkoxy side chain (**Figure 1a**, green ellipses). We found that the D part of YM155 and NSC80467 is similar to acridone structure, which have been developed as good HCC inhibition agents by our group and other researches [23, 25-34]. We think that if two appropriate side chains were introduced to the acridone ring, acridone derivatives can be developed as survivin inhibitors similar to YM155 for the treatment of HCC. The 3D structural superposition of the representive compound **8u** and YM155 is shown in **Figure 1b,** which indicated that **8u** and YM155 might exhibit similar bioactivity. As the main targets of acridones are DNA and its related enzymes (such as topoisomerases, telomerase, etc.), it is very interesting and reasonable to found their new mechanisms of action.

In this paper, a library of novel multi-substituted benzyl acridone derivatives were designed and synthesized based on the chemical structural features of YM155 and NSC80467. The antiproliferative activity of the compounds were tested and their

SAR were established. The typical compound **8u** could inhibit the expression of survivin to display HCC inhibition activity *in vitro* and *in vivo*.

2. Results and discussion

2.1. Chemistry

The synthesis of targets compounds **5a-b, 7a-b** and **8a-w** was shown in **Scheme 1**. Compounds **3a-d** were obtained from the Ullmann reaction of anthranilic acid derivatives **1a-d** with 2,4-dichlorobenzoic acid **2** in DMF using Cu as the catalyst [35, 36]. Subsequent Friedel-Crafts acylation in concentrated sulfuric acid at 80 □ for 3-5 h gave acridone-4-carboxylic acid derivatives **4a-d** in high yields [37], which were then reacted with the corresponding primary aliphatic amines using N,N′-carbonyldiimidazole (CDI) as the condensation agent [38] at room temperature to afford the intermediates acridone-4-carboxamides **6a-h**. It is noteworthy that when the reaction temperature raised to 70 □, it will generate a small amount of **5a-b**. The desired compounds **7a-b** and **8a-v** were produced by the nucleophilic substitution between the corresponding acridone-4-carboxamides and hydrazine hydrate or various multi-substituted benzyl amines. Additionally, compound **8w** was obtained by the reduction reaction of **8u** under the iron and ammonium chloride conditions [39]. The structures of all target compounds were established *via* ^{1}H NMR, ^{13}C NMR and high resolution mass spectral data.

2.2. *In vitro* cytotoxicity

The *in vitro* cytotoxicity of 30 desired acridone derivatives against HepG2 cells were firstly assayed by MTT reduction method, and YM155 was used as the positive

control. In order to verify the inhibitory activity of the synthesized compounds on other solid tumor cells, human breast cancer MCF-7 cells were also tested. The structures and bioactivity results were shown in **Table 1** and **Table 2**. Most of these compounds had better antitumor activity than YM155. **Table 1** showed the MTT results for the benzylamino-substituted acridone compounds. It can be seen that the R_2 group on the 4-carboxamide side chain had a great effect on the antitumor activity. Acridones with N,N-dimethylamino group displayed better antitumor activity than those with methoxy group except **8n**, which can be seen from the IC_{50} values of **8e**, **8f**, **8g**, **8h** and **8i**. These compounds with methoxy group displayed no cytotoxicity (IC_{50} > 50 μM). Interestingly, when the R_3 substituent was 4-methoxy group, the activities were also outstanding. For example, **8c** had an IC_{50} value of 2.11 μM against HepG2 cells, and **8r** was 1.05 μM against MCF-7 cells. In addition, as shown in **Table 1**, four compounds (**8b**, **8c**, **8t**, and **8u**) had IC_{50} values of less than 4 μM against HepG2 cells, and these compounds also exhibited good inhibitory activity against MCF-7 cells (2.92 to 6.84 μM). These results suggested that benzylamino acridones had almost the same inhibitory effects on both two solid tumor cells. It should be noted that the difference in chemical structure between **8r** and **8u** was only the length of the alkyl chains between the N,N-dimethylamino group and 4-carboxamide group (the number of methylene units, n), and the IC_{50} values of **8r** and **8u** on HepG2 cells were almost the same. Thus, the length of the amide side chain had little effect on the antitumor activity. Additionally, the difference between **8w** and **8u** was only the substituent at the C-5 position (**8w**:5-NH_2, **8u: 5-NO_2**), but the IC_{50} values against HepG2 cells

were 5.68 μM and 3.56 μM, respectively. Moreover, the antiproliferative activity of **8w** against MCF-7 cells was about 3 times lower than that of **8u**.

The electronic effects of the 5-position substituent of acridone scaffold were also discussed, including different electron-donating and electron-withdrawing groups on C5 position, such as 5-methyl, 5-methoxy, 5-amino, 5-nitro and 5,7-dichloro substituents. From the biological data shown in **Table 1**, compounds with good activities generally had methyl or nitro groups at the C5 position (**8d** vs. **8u**). The results indicated that electron-negativity and steric effect on C5 position of acridone scaffold might have little change in the cytotoxic profile. However, when 5,7-dichloro substituent was introduced (e.g. **8o**), the antiproliferative activities of HepG2 cells and MCF-7 cells were significantly decreased compared to the other substituents at the 5-position.

Table 2 showed the structure-activity relationship of no benzyl substituted acridone derivatives (the benzylamino substituents are replaced by -Cl, $-NHNH_2$ and $-N(CH_3)_2$ substituents). From the data we can see, most of non-benzylamino acridinone compounds showed poor antiproliferative activities, for instance, **5b** had poor antitumor activity against HepG2 cells with an IC_{50} value of 36.82 μM. These results indicated that the introduction of the benzylamino group in the target compounds was important for the anticancer activity.

For the most active compounds (**8b**, **8c**, **8t** and **8u**) against HepG2 cells, we further tested their *in vitro* toxicity against human liver cells QGY-7701 [40, 41]. From **Table 3** we can see that only **8u** was less toxic than **8b**, **8c** and **8t** to normal

hepatocytes, and its IC_{50} value is 11 times lower than for HepG2 cells. However, the other three compounds are still toxic to QGY-7701. Therefore, **8u** was selected for subsequent biological research.

2.3. *In silico* predictions of druggability, pharmacokinetic properties and toxicity

As compound **8u** displayed good antiproliferative activity and low toxic side effect, the *in silico* predictions of drugability and pharmacokinetics of **8u** using ACD/Percepta Platform (**Table 4, 5**), and toxicity prediction using Osiris soft (**Table 6**) were performed. It is well-known that the Lipinski's rule of five [42] based on the pharmacokinetic properties of small molecule drugs, is important for the prediction of ligand druggability. It can be seen from **Table 4** that the number of hydrogen bond donors and acceptors of **8u** were 10 and 3, respectively; the calculated log P values was 4.77, the molecular weight was 503.55 (Slightly higher); the number of rotatable bonds was 10, the number of rings were 4 and the surface area were 131.53 $Å^2$, all of which except the slightly larger molecular weight were within the range specified by the rule. Therefore, the Lipinski's violations of **8u** was 1, which are accorded with the limit of Lipinski's rule ($\leqslant$1).

The evaluation of pharmacokinetic properties of **8u** was carried out using ACD/Percepta software 14.0.0 (**Table 5**). *In silico* prediction of the passive absorption of drugs based on Caco-2 cell permeability, provides a useful drug discovery tool by predicting human oral absorption of new compounds. Yazdanian et al. [43] reported that compounds with values greater than 7×10^{-6} cm/s had excellent oral absorption. The permeability coefficient of **8u** was greater than 7×10^{-6} cm/s, which indicated

good absorption characteristics. In the analysis of a new drug, it is very important to know the basic pharmacokinetic parameters such as protein binding. For the estimation of binding of compounds to human plasma proteins, e.g., albumin, lipoproteins and alpha-1-acid glycoprotein, we used the %PPB parameter as the cumulative percentage. An ability to cross the blood brain barrier (BBB) is an asset for compounds to be used for therapies of central nervous system (CNS) afflictions, which depends on the lipid-solubility and molecular weight of the drug itself. BBB transport parameters contain the rate of brain penetration (LogPS), extent of brain penetration (LogBB) and the brain/plasma equilibration rate (Log(PS*fu, brain)). These values were used to classify the compounds as CNS permeable or non-permeable. However, these results showed that **8u** had low brain penetration, which may indicated inactive to CNS.

P-glycoprotein (P-gp) is a well characterized transporter, which moves a variety of substrates across extra- and intracellular membranes. It is an important drug efflux pump, therefore, predicting P-gp transport is important aiming to develop anti-cancer drugs. However, the prediction result is inconclusive. Similarly, the first-pass metabolism also failed to give a prediction. In addition, the predicted solubility of **8u** was only 0.1mg/mL, apparently this result was not good. To this end, we synthesized **8u** hydrochloride, which its solubility in water had a large extent improved. Therefore, we used its hydrochloric acid salt to study *in vivo* experiments.

The toxicity prediction for **8u** was based on the online software Osiris Property Explorer ver. 2. The software was used to predict the toxicity from mutagenic,

tumorigenic, irritant and reproductive aspects. It should be noted that whether compounds are mutagenic, based on their ability to induce DNA damage. The toxicity prediction results were shown in **Table 6**，in mutagenic, tumorigenic, irritant and reproductive aspects, **8u** has shown low toxicity, which was in accordance to the MTT values.

2.4. DNA binding and Topo I/II inhibition experiments

Literature has proved that YM155 is a DNA damaging agent while suppression of survivin is a secondary event [21, 24]. As the structure of **8u** is very similar to YM155, therefore, we firstly studied the DNA binding ability of **8u**, which was shown in **Figure 1s**. The results showed that **8u** was not a DNA binding agent compared to our published DNA binders [25-27, 35, 44].

As most of acridine/acridone derivatives had the inhibitory activity of topoisomerases, it is of interest to evaluate whether our synthesized novel acridone derivative **8u** can also inhibit the activity of topoisomerases. **Figure 2a** showed the relative affinity of **8u** on the relaxation of plasmid pBR322 DNA mediated by Topo I, which indicated that **8u** displayed no Topo I inhibitory activity. **Figure 2b** showed the human Topo II decatenation assay, and two concentrations (1 and 10 μM) of Doxorubicin (DOX) were used as the positive controls. From the results we can see, **8u** (50 μM) seemed to be weakly bound to Topo II, however, the inhibition rate was only about 10.9% while Doxorubicin (10 μM) was 74.4%. Therefore, Topo II is not the main drug target of **8u**. The above results indicated that DNA and topoisomerases are not the main targets of **8u**, which is different from YM155.

2.5. Identification of YM155 and 8u targeting to survivin protein

2.5.1 Molecular modeling

As survivin small-molecule inhibitor, YM155 displays an obvious antiproliferative activity in a large panel of tumor cellular model [45], however, its molecular mechanisms of action needs to be further evaluated [17, 22]. Survivin protein exists as a homodimer in most tumor cells, and the presence of a homodimer is a necessary condition for its physiological function. Targeting of the small molecule into a domain that restricts the dimerization hydrophobic interface may cause destabilization and degradation of the protein, thereby further leading to cancer cells apoptosis [14]. To this end, we used molecular docking methods to verify whether **8u** can be directly binding to survivin hydrophobic surface.

There is a high hydrophobicity surface in survivin dimerization domain, its key residues comprised of Leu6, Pro7, Pro8, Ala9, Trp10, Phe93, Glu94, Glu95, Leu96, Thr97, Leu98, Gly99, Phe101, Leu102, and it is considered to be a good target site for drug discovery [14]. Therefore, molecular docking studies of the representative compound **8u** and YM155 with survivin protein model were conducted using SYBYL-X v1.3 program. As seen from **Figure 3**, **8u** can effectively target the dimerization hydrophobic domain of survivin, and the hydrophobic effect between the ligand and receptor might be due to π-conjugated planar acridone scaffold of **8u**. Moreover, three hydrogen bonds of **8u** were also formed in the hydrophobic domain (residues Phe93, Glu94 and Leu96). Therefore, it was confirmed that **8u** can effectively bind to the critical hydrophobic core, which suggested that survivin protein

may be the main drug target of **8u**. However, there was no interaction between YM155 and survivin, which can be seen in **Figure 2s**.

2.5.2 Fluorogenic titration assay

To verify that **8u** indeed binds to survivin, we performed a fluorogenic titration assay in the presence or absence of survivin. **Figure 4** showed that the fluorescence of **8u** dramatically increased in the presence of survivin, indicating that **8u** likely interacted with survivin protein. Meanwhile, we also carried out the fluorescence experiments of YM155 and survivin protein (**Figure 3s**) and the results showed that with the YM155 concentration increased, the fluorescence trend was not changed, indicating that YM155 may not target survivin protein directly.

2.6. 8u causes survivin degradation and induces cells apoptosis

We next determined if **8u** causes survivin degradation by western blot analysis. For this purpose, HepG2 cells were treated with **8u** with different concentration gradients. **Figure 5** showed that the content of survivin protein in HepG2 cells was significantly decreased with the increase of the concentration of **8u** from 1 μM to 10 μM.

In order to understand whether **8u** induced cancer cells apoptosis, the activity of cleaved caspase-7 was firstly tested. As shown in **Figure 5**, **8u** at 10 μM for 24 h induced significant activation of cleaved caspase-7, suggesting that **8u** induced HepG2 cells 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

revealed the generation of the cleaved PARP. Additionally, Bcl-2, an important member of Bcl-2 family proteins, plays an important role in extending cellular survival. **8u** at 10 μM effectively decreased the expression of Bcl-2 protein as shown in **Figure 5**, which further confirmed that **8u** could induce HepG2 cells apoptosis.

2.7. *In vivo* preliminary experiments

We finally evaluated the *in vivo* anti-tumor effect of compound **8u** (hydrochloride salt) on subcutaneously xenografts tumor models of human hepatocarcinoma cell line HepG2 in nude mice, and measured the body weight and tumor size of the nude mice once every two days after the first administration. **Figure 6a** showed the effect of **8u** on tumor size in the HepG2 implanted model with the days of administration increased. From the data we can see that treatment with **8u** (3 mg/kg) significantly reduced HepG2 tumor volume by 54% after 30 days of treatment, which was comparable to the positive control (64%, 25 mg/kg). In addition, little toxicity of **8u** was observed, as demonstrated by the absence of weight loss (**Figure 6b**) and by the normal growth and behavior of all mice tested. Moreover, **8u** had no significant effect on the organ weight of nude mice (**Table 7)**. **8u** could obviously increase the spleen weight in a dose-dependent manner, which suggested that **8u** might be able to enhance the body's immune capacity. All the results indicated that **8u** had efficacious antitumor effect and low adverse effects, which may constitute a novel lead in the search for anticancer drug candidates with new mechanisms of action.

3. Conclusion

Based on the structural characteristics of survivin inhibitors YM155 and NSC80467, 30 multi-benzylamino acridone derivatives were designed and

synthesized. The synthesized compounds were evaluated through MTT screening, most of them showed good *in vitro* anti-cancer activities against HepG2 cells and MCF-7 cells. Among them, compound **8u** with high activity showed low toxicity to hepatocyte QGY-7701 cells *in vitro*. Druggability and toxicity prediction showed that **8u** conformed to the rules of Lipinski and showed low toxicity in mutagenic, tumorigenic, irritant and reproductive aspects. In addition, the ADME property prediction indicated that **8u** has good absorption characteristics, but it was ineffective to CNS. Molecular docking showed that **8u** restricts the survivin dimerization hydrophobic domain, and the fluorescence experiments further confirmed that **8u** can binding with the survivin protein. Interestingly, DNA binding experiments and topoisomerase inhibition experiments (Topo I/II) found that **8u** did not cause direct or indirect DNA damage, which is different from YM155 and NSC80467. In addition, we found that **8u** can significantly decrease the content of survivin in HepG2 cells with the increase of drug concentration, which proved the possible mechanism proposed in the molecular docking. The apoptosis mechanism of hepatocellular carcinoma cells further showed that **8u** could induce the apoptosis of HepG2 cells. *In vivo* preliminary experiments exhibited that **8u** effectively suppressed the growth of HepG2 xenograft tumors, and the tumor inhibition rate of **8u** reached 54% after 30 days of administration. Moreover, **8u** had not affected the body weight, organ weight and organ index of nude mice, which was consistent with *in vitro* anticancer activity and toxicity assay. Therefore, **8u** may be a promising lead compound in the treatment of HCC, moreover, metabolomics and proteomics research of **8u** on HepG2 cells is in progress.

4. Experimental section

4.1. Synthesis and characterization

See supporting information for synthetic methods and the preparation of compounds **3a-d**, **4a-d**, **5a-b** and **6a-h**.

4.1.1. General procedure for compounds 7a-b and 8a-v.

9-oxo-9,10-dihydroacridine-4-carboxamide derivatives (**6a-h**, 0.28 mmol) and hydrazine hydrate or various multi-substituted benzyl amines (0.84 mmol) were dissolved in 2-ethoxyethanol, and the mixture was stirred at 90-100°C 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 **7a-b** and **8a-v**.

4.1.1.1. N-(2-(dimethylamino)ethyl)-1-hydrazinyl-5-methyl-9-oxo-9,10-dihydro acridine-4-carboxamide (7a) yellow solid powder, Yield 20.2%; mp 190-192℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.57 (s, 1H), 11.55 (s, 1H), 8.21 (d, *J* = 8.1 Hz, 1H), 7.72 (d, *J* = 9.0 Hz, 1H), 7.48 (d, *J* = 7.0 Hz, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 6.95 (s, 1H), 6.88 (d, *J* = 9.0 Hz, 1H), 3.78 (br, 2H), 3.55 (dd, *J* = 10.7, 5.2 Hz, 2H), 2.62 (s, 3H), 2.60-2.52 (m, 2H), 2.32 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.9, 169.4, 157.6, 144.5, 138.6, 133.6, 133.4, 124.9, 123.9, 121.7, 121.3, 105.8, 101.4, 99.1, 57.8, 45.2, 36.9, 17.2; HR-MS(ESI): Calcd for $[M+H]^+$ 354.1930; Found: 354.1928.

4.1.1.2.N-(3-(dimethylamino)propyl)-1-hydrazinyl-5-methoxy-9-oxo-9,10-dihydro acridine-4-carboxamide (7b) yellow solid powder, Yield 40.4%; mp 236-238℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.58 (s, 1H), 11.58 (s, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.74 (d, *J* = 9.0 Hz, 1H), 7.18 (t, *J* = 8.0 Hz, 1H), 7.08 (d, *J* = 7.7 Hz, 1H), 6.91 (d, *J* = 8.8

Hz, 2H), 4.09 (s, 3H), 3.78 (br, 2H), 3.62-3.51 (m, 2H), 2.58 (t, *J* = 5.8 Hz, 2H), 2.32 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.5, 169.1, 157.7, 148.0, 144.0, 133.5, 131.1, 122.4, 121.0, 117.2, 111.3, 106.3, 101.9, 99.0, 57.8, 56.3, 45.2, 36.9; HR-MS(ESI): Calcd for $[M+H]^+$ 370.1879; Found: 370.1870.

4.1.1.3. 1-(benzylamino)-N-(2-(dimethylamino)ethyl)-5-methyl-9-oxo-9,10-dihydroacridine-4-carboxamide (8a) yellow solid powder, Yield 60.9%; mp 144-146℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.58 (s, 1H), 11.41 (s, 1H), 8.22 (d, *J* = 8.1 Hz, 1H), 7.60 (d, *J* = 8.9 Hz, 1H), 7.46 (d, *J* = 6.9 Hz, 1H), 7.39 (d, *J* = 7.3 Hz, 2H), 7.34 (t, *J* = 7.5 Hz, 2H), 7.28-7.24 (m, 1H), 7.15 (t, *J* = 7.6 Hz, 1H), 6.89 (s, 1H), 6.14 (d, *J* = 8.7 Hz, 1H), 4.55 (d, *J* = 5.5 Hz, 2H), 3.50 (dd, *J* = 10.6, 5.2 Hz, 2H), 2.60 (s, 3H), 2.53 (t, *J* = 5.8 Hz, 2H), 2.27 (s, 6H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 180.4, 169.3, 154.6, 144.6, 138.9, 138.5, 135.1, 134.1, 129.1, 127.8, 127.6, 125.1, 123.7, 121.7, 121.5, 106.2, 101.5, 100.3, 58.6, 46.4, 45.7, 37.6, 17.0; HR-MS(ESI): Calcd for $[M+H]^+$ 429.2291; Found: 429.2300.

4.1.1.4. N-(2-(dimethylamino)ethyl)-1-((3-methoxybenzyl)amino)-5-methyl-9-oxo-9,10-dihydroacridine-4-carboxamide (8b) yellow solid powder, Yield 27.3%; mp 164-166℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.58 (s, 1H), 11.43 (s, 1H), 8.24 (d, *J* = 8.1 Hz, 1H), 7.63 (d, *J* = 8.9 Hz, 1H), 7.48 (d, *J* = 7.0 Hz, 1H), 7.28-7.24 (m, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 6.99 (d, *J* = 7.6 Hz, 1H), 6.93 (s, 2H), 6.81 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.17 (d, *J* = 8.9 Hz, 1H), 4.55 (d, *J* = 5.7 Hz, 2H), 3.80 (d, *J* = 13.0 Hz, 3H), 3.53 (dd, *J* = 10.8, 5.2 Hz, 2H), 2.62 (s, 3H), 2.57 (t, *J* = 5.8 Hz, 2H), 2.30 (s, 6H); ^{13}C NMR (100MHz, $CDCl_3$) δ 181.2, 169.4, 160.1, 155.3, 144.7, 139.8, 138.6, 133.6,

133.4, 129.8, 124.9, 123.9, 121.9, 121.4, 119.4, 112.8, 112.8, 106.8, 101.3, 100.0, 57.7, 55.2, 46.8, 45.1, 36.7, 17.1; HR-MS(ESI): Calcd for $[M+H]^+$ 459.2396; Found: 459.2408.

4.1.1.5. N-(2-(dimethylamino)ethyl)-1-((4-methoxybenzyl)amino)-5-methyl-9-oxo-9,10-dihydroacridine-4-carboxamide (8c) yellow solid powder, Yield 54.6%; mp 225-227℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.59 (s, 1H), 11.34 (s, 1H), 8.22 (d, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 9.0 Hz, 1H), 7.47 (d, *J* = 7.0 Hz, 1H), 7.31 (d, *J* = 8.5 Hz, 2H), 7.15 (t, *J* = 7.6 Hz, 1H), 6.98 (s, 1H), 6.88 (d, *J* = 8.5 Hz, 2H), 6.18 (d, *J* = 9.0 Hz, 1H), 4.48 (d, *J* = 5.5 Hz, 2H), 3.79 (s, 3H), 3.53 (dd, *J* = 10.7, 5.2 Hz, 2H), 2.61 (s, 3H), 2.56 (t, *J* = 5.7 Hz, 2H), 2.29 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.2, 169.4, 158.9, 155.1, 144.8, 138.5, 133.5, 133.5, 130.0, 128.4, 124.9, 123.8, 121.9, 121.4, 114.2, 106.7, 101.1, 99.9, 57.8, 55.3, 46.3, 45.1, 36.8, 17.1; HR-MS(ESI): Calcd for $[M+H]^+$ 459.2396; Found: 459.2401.

4.1.1.6. N-(3-(dimethylamino)propyl)-1-((4-methoxybenzyl)amino)-5-methyl-9-oxo-9,10-dihydroacridine-4-carboxamide (8d) yellow solid powder, Yield 22.4%; mp 185-187℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.82 (s, 1H), 11.32 (s, 1H), 8.68 (s, 1H), 8.22 (d, *J* = 7.8 Hz, 1H), 7.60 (d, *J* = 8.3 Hz, 1H), 7.47 (d, *J* = 6.1 Hz, 1H), 7.33 (d, *J* = 7.7 Hz, 2H), 7.16 (d, *J* = 7.2 Hz, 1H), 6.89 (d, *J* = 7.6 Hz, 2H), 6.20 (d, *J* = 8.8 Hz, 1H), 4.49 (s, 2H), 3.80 (s, 3H), 3.59 (br, 2H), 2.62 (br, 5H), 2.39 (s, 6H), 1.84 (br, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.2, 169.5, 159.0, 155.0, 144.9, 138.59, 133.5, 133.4, 130.0, 128.5, 124.9, 123.8, 121.8, 121.3, 114.2, 106.7, 101.4, 99.8, 59.1, 55.3, 46.3, 45.1, 40.1, 24.9, 17.2; HR-MS(ESI): Calcd for $[M+H]^+$ 473.2552; Found:

473.2566.

4.1.1.7. 1-(benzylamino)-N-(2-methoxyethyl)-5-methyl-9-oxo-9,10-dihydro acridine-4-carboxamide (8e) yellow solid powder, Yield 62.1%; mp 191-193℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.50 (s, 1H), 8.24 (d, *J* = 8.0 Hz, 1H), 7.60 (d, *J* = 8.8 Hz, 1H), 7.50 (d, *J* = 7.0 Hz, 1H), 7.41 (d, *J* = 7.3 Hz, 2H), 7.36 (t, *J* = 7.4 Hz, 2H), 7.30 (d, *J* = 7.2 Hz, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 6.49 (s, 1H), 6.18 (d, *J* = 8.8 Hz, 1H), 4.58 (s, 2H), 3.67 (br, 2H), 3.59 (t, *J* = 4.7 Hz, 2H), 3.42 (d, *J* = 8.1 Hz, 3H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.2, 169.3, 155.2, 144.7, 138.5, 137.9, 133.7, 133.3, 128.8, 127.4, 127.2, 124.9, 123.9, 121.9, 121.5, 106.8, 101.1, 100.0, 71.3, 58.9, 46.88, 39.4, 17.1; HR-MS(ESI): Calcd for $[M+H]^+$ 416.1974; Found: 416.1980.

4.1.1.8. 1-((3-methoxybenzyl)amino)-N-(2-methoxyethyl)-5-methyl-9-oxo-9,10-dihydroacridine-4-carboxamide (8f) yellow solid powder, Yield 61.8%; mp 138-140℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.50 (s, 1H), 11.46 (s, 1H), 8.24 (d, *J* = 8.0 Hz, 1H), 7.59 (d, *J* = 8.9 Hz, 1H), 7.50 (d, *J* = 6.9 Hz, 1H), 7.31-7.23 (m, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 7.00 (d, *J* = 7.6 Hz, 1H), 6.94 (s, 1H), 6.83 (d, *J* = 8.0 Hz, 1H), 6.48 (s, 1H), 6.17 (d, *J* = 8.9 Hz, 1H), 4.56 (d, *J* = 5.6 Hz, 2H), 3.80 (s, 3H), 3.72-3.64 (m, 2H), 3.59 (t, *J* = 4.7 Hz, 2H), 3.41 (s, 3H), 2.63 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.3, 169.3, 160.0, 155.3, 144.7, 139.7, 138.5, 133.7, 133.3, 129.9, 124.9, 123.8, 121.8, 121.5, 119.4, 112.8, 112.7, 106.8, 101.1, 100.0, 71.3, 58.9, 55.3, 46.8, 39.4, 17.2; HR-MS(ESI): Calcd for $[M+H]^+$ 446.2080; Found: 446.2082.

4.1.1.9. 1-((4-methoxybenzyl)amino)-N-(2-methoxyethyl)-5-methyl-9-oxo-9,10-dihydroacridine-4-carboxamide (8g) yellow solid powder, Yield 38.6%; mp

232-234℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.49 (s, 1H), 8.22 (d, *J* = 7.5 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.48 (d, *J* = 5.4 Hz, 1H), 7.32 (d, *J* = 7.6 Hz, 2H), 7.16 (s, 1H), 6.89 (d, *J* = 7.5 Hz, 2H), 6.50 (s, 1H), 6.20 (d, *J* = 8.1 Hz, 1H), 4.49 (s, 2H), 3.80 (s, 3H), 3.66 (br, 2H), 3.59 (br, 2H), 3.41 (s, 3H), 2.61 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.2, 169.3, 159.0, 155.1, 144.7, 138.5, 133.6, 133.2, 129.8, 128.5, 124.9, 123.8, 121.9, 121.5, 114.2, 106.7, 101.1, 100.0, 71.3, 58.9, 55.31, 46.4, 39.4, 17.1; HR-MS(ESI): Calcd for $[M+H]^+$ 446.2080; Found: 446.2078.

4.1.1.10. N-(2-methoxyethyl)-5-methyl-9-oxo-1-((4-(trifluoromethyl)benzyl) amino)-9,10-dihydroacridine-4-carboxamide (8h) yellow solid powder, Yield 35.6%; mp 260-262℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.51 (s, 1H), 8.24 (d, *J* = 8.1 Hz, 1H), 7.61-7.58 (m, 3H), 7.52-7.50 (m, 3H), 7.22-7.15 (m, 1H), 6.49 (s, 1H), 6.08 (d, *J* = 8.9 Hz, 1H), 4.64 (s, 2H), 3.66 (d, *J* = 4.6 Hz, 2H), 3.60-3.53 (m, 2H), 3.40 (s, 3H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.3, 169.2, 155.1, 144.7, 142.2, 138.5, 133.8, 133.3, 127.3, 125.8, 125.7, 125.0, 123.8, 121.8, 121.6, 106.9, 101.7, 99.7, 71.2, 58.9, 46.4, 39.4, 17.1; ^{19}F NMR (376 MHz, $CDCl_3$) δ -62.45; HR-MS(ESI): Calcd for $[M+H]^+$ 484.1848; Found: 484.1856.

4.1.1.11. N-(2-methoxyethyl)-5-methyl-9-oxo-1-((3,4,5-trimethoxybenzyl)amino)-9,10-dihydroacridine-4-carboxamide (8i) yellow solid powder, Yield 68.0%; mp 230-232℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.53 (s, 1H), 8.25 (d, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 8.6 Hz, 1H), 7.52 (d, *J* = 6.6 Hz, 1H), 7.20 (t, *J* = 7.4 Hz, 1H), 6.64 (s, 2H), 6.54 (s, 1H), 6.21 (d, *J* = 8.5 Hz, 1H), 4.52 (s, 2H), 3.86 (s, 9H), 3.69 (br, 2H), 3.61 (br, 2H), 3.43 (s, 3H), 2.64 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.2, 169.3,

155.1, 153.6, 144.7, 138.5, 137.2, 133.7, 133.7, 133.3, 125.0, 123.8, 121.8, 121.6, 106.8, 104.1, 101.5, 100.2, 71.3, 60.9, 58.9, 56.2, 47.4, 39.4, 17.1; HR-MS(ESI): Calcd for $[M+H]^+$ 506.2291; Found: 506.2293.

4.1.1.12. N-(2-(dimethylamino)ethyl)-5-methyl-9-oxo-1-((3,4,5-trimethoxybenzyl) amino)-9,10-dihydroacridine-4-carboxamide (8j) yellow solid powder, Yield 33.1%; mp 195-197℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.61 (s, 1H), 11.38 (s, 1H), 8.23 (d, *J* = 8.1 Hz, 1H), 7.71 (d, *J* = 8.9 Hz, 1H), 7.49 (d, *J* = 6.9 Hz, 1H), 7.23-7.13 (m, 1H), 7.06 (s, 1H), 6.62 (s, 2H), 6.18 (d, *J* = 8.9 Hz, 1H), 4.50 (d, *J* = 5.5 Hz, 2H), 3.84 (s, 9H), 3.56 (dd, *J* = 10.6, 5.1 Hz, 2H), 2.62 (br, 5H), 2.35 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.3, 169.4, 155.2, 153.6, 144.7, 138.5, 137.3, 133.8, 133.6, 133.6, 124.9, 123.8, 121.8, 121.4, 106.8, 104.1, 101.4, 100.1, 60.8, 57.8, 56.2, 47.2, 45.0, 36.6, 17.1; HR-MS(ESI): Calcd for $[M+H]^+$ 519.2607; Found: 519.2623.

4.1.1.13. 1-(benzylamino)-N-(2-(dimethylamino)ethyl)-5-methoxy-9-oxo-9,10-dihydroacridine-4-carboxamide (8k) yellow solid powder, Yield 57.1%; mp 230-231℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.61 (s, 1H), 11.44 (s, 1H), 7.94 (d, *J* = 8.1 Hz, 1H), 7.67 (d, *J* = 8.9 Hz, 1H), 7.42 (d, *J* = 7.2 Hz, 2H), 7.36 (t, *J* = 7.3 Hz, 2H), 7.30 (d, *J* = 7.2 Hz, 1H), 7.18 (t, *J* = 7.9 Hz, 1H), 7.09 (d, *J* = 7.6 Hz, 1H), 6.97 (s, 1H), 6.17 (d, *J* = 8.9 Hz, 1H), 4.57 (d, *J* = 5.4 Hz, 2H), 4.09 (s, 3H), 3.56 (d, *J* = 5.2 Hz, 2H), 2.56 (t, *J* = 5.5 Hz, 2H), 2.29 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.9, 169.1, 155.2, 148.0, 144.2, 138.1, 133.6, 131.0, 128.8, 127.3, 127.2, 122.5, 121.1, 117.1, 111.3, 107.2, 101.8, 99.8, 57.9, 56.3, 46.9, 45.1, 36.8; HR-MS(ESI): Calcd for $[M+H]^+$ 445.2240; Found: 445.2252.

4.1.1.14. N-(2-(dimethylamino)ethyl)-5-methoxy-1-((4-methoxybenzyl) amino)-9-oxo-9,10-dihydroacridine-4-carboxamide (8l) yellow solid powder, Yield 70.8%; mp 245-246℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.57 (s, 1H), 11.36 (s, 1H), 7.92 (d, *J* = 8.1 Hz, 1H), 7.67 (d, *J* = 8.9 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.17 (t, *J* = 8.0 Hz, 1H), 7.07 (d, *J* = 7.5 Hz, 1H), 6.96 (s, 1H), 6.89 (d, *J* = 8.5 Hz, 2H), 6.20 (d, *J* = 8.9 Hz, 1H), 4.49 (d, *J* = 5.4 Hz, 2H), 4.08 (s, 3H), 3.80 (s, 3H), 3.61-3.48 (m, 2H), 2.60 (t, *J* = 5.6 Hz, 2H), 2.33 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.8, 169.1, 158.9, 155.2, 147.9, 144.2, 133.6, 131.0, 130.0, 128.4, 122.5, 121.0, 117.1, 114.2, 111.3, 107.2, 101.6, 99.8, 57.9, 56.3, 55.3, 46.4, 45.0, 36.6; HR-MS(ESI): Calcd for $[M+H]^+$ 475.2345; Found: 475.2336.

4.1.1.15. N-(3-(dimethylamino)propyl)-5-methoxy-1-((4-methoxybenzyl)amino)-9-oxo-9,10-dihydroacridine-4-carboxamide (8m) Yellow solid powder, Yield 63.4%; mp 260-261℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.84 (s, 1H), 11.32 (s, 1H), 8.66 (s, 1H), 7.91 (d, *J* = 8.1 Hz, 1H), 7.53 (d, *J* = 8.9 Hz, 1H), 7.33 (d, *J* = 8.5 Hz, 2H), 7.16 (t, *J* = 8.0 Hz, 1H), 7.07 (d, *J* = 7.5 Hz, 1H), 6.89 (d, *J* = 8.5 Hz, 2H), 6.18 (d, *J* = 8.9 Hz, 1H), 4.49 (d, *J* = 5.4 Hz, 2H), 4.07 (s, 3H), 3.80 (s, 3H), 3.60 (dd, *J* = 10.4, 5.4 Hz, 2H), 2.59-2.50 (m, 2H), 2.33 (s, 6H), 1.84-1.72 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.8, 169.1, 158.9, 154.9, 148.0, 144.3, 133.3, 131.0, 130.0, 128.5, 122.4, 121.0, 117.0, 114.2, 111.1, 107.2, 101.9, 99.6, 59.7, 56.2, 55.3, 46.4, 45.5, 40.7, 25.0; HR-MS(ESI): Calcd for $[M+H]^+$ 489.2502; Found: 489.2493.

4.1.1.16. N-(2-(dimethylamino)ethyl)-5-methoxy-9-oxo-1-((3,4,5-trimethoxy benzyl)amino)-9,10-dihydroacridine-4-carboxamide (8n) yellow solid powder,

Yield 57.3%; mp 257-259℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.60 (s, 1H), 11.39 (t, *J* = 5.4 Hz, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.70 (d, *J* = 8.9 Hz, 1H), 7.19 (t, *J* = 8.0 Hz, 1H), 7.09 (d, *J* = 7.7 Hz, 1H), 6.98 (s, 1H), 6.63 (s, 2H), 6.19 (d, *J* = 8.9 Hz, 1H), 4.50 (d, *J* = 5.6 Hz, 2H), 4.09 (s, 3H), 3.85 (s, 9H), 3.57 (dd, *J* = 10.8, 5.2 Hz, 2H), 2.60 (t, *J* = 5.7 Hz, 2H), 2.33 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.9, 169.1, 155.2, 153.6, 148.0, 144.1, 137.1, 133.8, 133.7, 131.0, 122.4, 121.2, 117.0, 111.3, 107.2, 104.0, 101.8, 100.0, 60.9, 57.8, 56.3, 56.2, 47.3, 45.1, 36.7; HR-MS(ESI): Calcd for $[M+H]^+$ 535.2557; Found: 535.2556.

4.1.1.17. 5,7-dichloro-N-(2-(dimethylamino)ethyl)-1-((4-methoxybenzyl)amino)-9-oxo-9,10-dihydroacridine-4-carboxamide (8o) yellow solid powder, Yield 69.6%; mp 238-240℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.11 (d, *J* = 5.1 Hz, 1H), 8.23 (d, *J* = 2.3 Hz, 1H), 7.70-7.67 (m, 2H), 7.32 (d, *J* = 8.6 Hz, 2H), 7.02 (s, 1H), 6.91 (dd, *J* = 6.7, 4.8 Hz, 2H), 6.25 (d, *J* = 9.0 Hz, 1H), 4.50 (d, *J* = 5.5 Hz, 2H), 3.81 (s, 3H), 3.56 (dd, *J* = 10.7, 5.3 Hz, 2H), 2.62-2.55 (m, 2H), 2.32 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.2, 168.9, 159.1, 154.9, 144.6, 135.4, 134.0, 132.5, 129.6, 128.4, 126.7, 124.4, 123.4, 122.5, 114.3, 106.7, 101.5, 100.9, 57.7, 55.3, 46.4, 45.1, 36.7; HR-MS(ESI): Calcd for $[M+H]^+$ 513.1460; Found: 513.1467.

4.1.1.18. 1-(benzylamino)-N-(2-(dimethylamino)ethyl)-5-nitro-9-oxo-9,10-dihydroacridine-4-carboxamide (8p) Red solid powder, Yield 33.8%; mp 243-245℃; ^{1}H NMR (400 MHz, d_6-DMSO) δ 15.00 (s, 1H), 10.92 (s, 1H), 8.65 (d, *J* = 7.2 Hz, 1H), 8.57 (d, *J* = 7.2 Hz, 1H), 8.46 (s, 1H), 8.03 (d, *J* = 8.8 Hz, 1H), 4.40-7.37 (m, 5H), 7.29 (d, *J* = 6.5 Hz, 1H), 6.45 (d, *J* = 8.6 Hz, 1H), 4.61 (s, 2H), 2.53 (br, 2H), 2.27 (s,

6H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 178.8, 168.2, 154.3, 138.7, 135.9, 135.2, 134.4, 131.7, 129.2, 127.8, 127.7, 124.2, 120.7, 106.5, 103.2, 102.5, 58.4, 46.4, 45.4, 37.3; HR-MS(ESI): Calcd for $[M+H]^+$ 460.1985; Found: 460.2000.

4.1.1.19. N-(2-(dimethylamino)ethyl)-1-((3-methoxybenzyl)amino)-5-nitro-9-oxo-9,10-dihydroacridine-4-carboxamide (8q) Red solid powder, Yield 19.8%; mp 155-157℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.02 (s, 1H), 11.08 (s, 1H), 8.71 (d, *J* = 7.6 Hz, 1H), 8.66 (d, *J* = 8.0 Hz, 1H), 7.70 (d, *J* = 8.9 Hz, 1H), 7.30-7.26 (m, 2H), 6.98 (d, *J* = 7.4 Hz, 2H), 6.92 (s, 1H), 6.83 (d, *J* = 7.6 Hz, 1H), 6.29 (d, *J* = 8.9 Hz, 1H), 4.54 (d, *J* = 5.3 Hz, 2H), 3.80 (s, 3H), 3.59 (d, *J* = 4.6 Hz, 2H), 2.65-2.56 (m, 2H), 2.31 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.4, 168.2, 160.1, 154.7, 144.1, 139.3, 135.0, 134.9, 134.5, 134.4, 131.2, 130.0, 124.6, 119.8, 119.3, 112.9, 112.8, 107.1, 103.3, 102.2, 57.8, 55.3, 46.9, 45.1, 36.8; HR-MS(ESI): Calcd for $[M+H]^+$ 490.2090; Found: 490.2091.

4.1.1.20. N-(2-(dimethylamino)ethyl)-1-((4-methoxybenzyl)amino)-5-nitro-9-oxo-9,10-dihydroacridine-4-carboxamide (8r) Red solid powder, Yield 27.7%; mp 228-230℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.01 (s, 1H), 10.99 (s, 1H), 8.69 (d, *J* = 6.8 Hz, 1H), 8.65 (d, *J* = 7.5 Hz, 1H), 7.75 (d, *J* = 8.1 Hz, 1H), 7.31 (d, *J* = 7.5 Hz, 2H), 7.12 (s, 1H), 6.90 (d, *J* = 7.3 Hz, 2H), 6.31 (d, *J* = 8.2 Hz, 1H), 4.48 (s, 2H), 3.80 (s, 3H), 3.62 (br, 2H), 2.65 (br, 2H), 2.37 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.3, 168.2, 159.1, 154.6, 144.1, 135.0, 134.8, 134.5, 134.4, 131.1, 129.5, 128.5, 124.6, 119.7, 114.3, 106.9, 103.0, 102.1, 57.9, 55.3, 46.4, 45.0, 36.6; HR-MS(ESI): Calcd for $[M+H]^+$ 490.2090; Found: 490.2099.

4.1.1.21. N-(2-(dimethylamino)ethyl)-5-nitro-9-oxo-1-((3,4,5-trimethoxybenzyl) amino)-9,10-dihydroacridine-4-carboxamide (8s) Red solid powder, Yield 49.4%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.04 (s, 1H), 11.01 (s, 1H), 8.68 (dd, *J* = 15.5, 7.3 Hz, 2H), 7.71 (d, *J* = 8.7 Hz, 1H), 7.30-7.28(m, 1H), 6.95 (s, 1H), 6.61 (s, 2H), 6.30 (d, *J* = 8.7 Hz, 1H), 4.48 (d, *J* = 4.0 Hz, 2H), 3.85 (s, 9H), 3.59 (br, 2H), 2.57 (br, 2H), 2.31 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.4, 168.1, 154.6, 153.7, 144.0, 137.4, 135.0, 134.8, 134.4, 134.4, 133.2, 131.2, 124.6, 119.8, 107.0, 104.2, 103.4, 102.1, 60.9, 57.7, 56.2, 47.4, 45.1, 36.8; mp 243-244℃; HR-MS(ESI): Calcd for $[M+H]^+$ 550.2302; Found: 550.2308.

4.1.1.22. 1-(benzylamino)-N-(3-(dimethylamino)propyl)-5-nitro-9-oxo-9,10-dihydroacridine-4-carboxamide (8t) Red solid powder, Yield 73.7%; mp 222-224℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.25 (s, 1H), 11.06 (s, 1H), 8.71 (d, *J* = 7.6 Hz, 2H), 8.65 (d, *J* = 7.9 Hz, 1H), 7.61 (d, *J* = 9.0 Hz, 1H), 7.46-7.33 (m, 4H), 7.33-7.26 (m, 2H), 6.31 (d, *J* = 8.9 Hz, 1H), 4.57 (d, *J* = 5.3 Hz, 2H), 3.64 (br, 2H), 2.58 (br, 2H), 2.35 (s, 6H), 1.82 (br, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.4, 168.1, 154.5, 144.2, 137.6, 135.1, 134.9, 134.4, 134.1, 131.1, 128.9, 127.5, 127.2, 124.6, 119.6, 107.1, 103.6, 102.0, 59.4, 46.9, 45.3, 40.5, 24.9; HR-MS(ESI): Calcd for $[M+H]^+$ 474.2141; Found: 474.2126.

4.1.1.23. N-(3-(dimethylamino)propyl)-1-((4-methoxybenzyl)amino)-5-nitro-9-oxo-9,10-dihydroacridine-4-carboxamide (8u) Red solid powder, Yield 63.9%; mp 251-252℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.27 (s, 1H), 10.97 (s, 1H), 8.76 (s, 1H), 8.70 (d, *J* = 7.6 Hz, 1H), 8.65 (d, *J* = 7.9 Hz, 1H), 7.59 (d, *J* = 9.0 Hz, 1H), 7.33

(d, *J* = 8.2 Hz, 2H), 7.27-7,24 (m, 1H), 6.91 (d, *J* = 8.3 Hz, 2H), 6.32 (d, *J* = 8.9 Hz, 1H), 4.49 (d, *J* = 5.1 Hz, 2H), 3.81 (s, 3H), 3.64 (br, 2H), 2.56 (br, 2H), 2.35 (s, 6H), 1.80 (br, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.4, 168.1, 1591, 154.4, 144.2, 135.1, 134.85, 134.4, 134.1, 131.1, 129.5, 128.5, 124.6, 119.6, 114.3, 107.0, 103.4, 101.9, 59.6, 55.3, 46.5, 45.4, 40.7, 24.9; HR-MS(ESI): Calcd for $[M+H]^+$ 504.2247; Found: 504.2245.

4.1.1.24. N-(3-(dimethylamino)propyl)-1-((4-ethylbenzyl)amino)-5-nitro-9-oxo-9,10-dihydroacridine-4-carboxamide (8v) Red solid powder, Yield 24.0%; mp 266-267℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.27 (s, 1H), 11.03 (s, 1H), 8.79-8.71 (m, 1H), 8.66 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.69 (d, *J* = 9.0 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 2H), 7.28 (d, *J* = 8.1 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 2H), 6.35 (d, *J* = 9.1 Hz, 1H), 4.53 (d, *J* = 5.5 Hz, 2H), 3.66-3.65 (m, 2H), 2.68-2.66 (m, 2H), 2.65-2.63 (m, 2H), 2.41 (s, 6H), 1.86-1.85 (m, 2H), 1.24 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.4, 168.3, 154.6, 144.3, 143.6, 135.1, 134.9, 134.7, 134.4, 134.31, 131.1, 128.4, 127.2, 124.6, 119.6, 107.0, 103.3, 102.1, 59.0, 46.8, 45.0, 40.0, 28.5, 24.8, 15.5; HR-MS(ESI): Calcd for $[M+H]^+$ 502.2454; Found: 502.2467.

4.1.2. General procedure for compound 8w.

Compound **8u** (0.18 mmol) and iron powder (0.89 mmol) was stirred in anhydrous ethanol (20 mL) and heated at 50℃ for 20 minutes. Subsequently, an aqueous solution of ammonium chloride was added to the reaction system (0.014 g ammonium chloride in 3 mL water, 0.27 mmol), the temperature was raised to 80 ° C and stirring for 3 hours. The suspension was cooled to room temperature and the

mixture was filtered on cellite to remove the iron. The filtrate 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 **8w**.

5-amino-N-(3-(dimethylamino)propyl)-1-((4-methoxybenzyl)amino)-9-oxo-9,10-dihydroacridine-4-carboxamide (8w) yellow solid powder, Yield 47.3%; mp 220-222℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.91 (s, 1H), 11.31 (s, 1H), 8.68 (br, 1H), 7.87 (br, 1H), 7.58 (br, 1H), 7.33 (d, *J* = 8.0 Hz, 2H), 7.06 (br, 2H), 6.90 (br, 2H), 6.19 (d, *J* = 8.0 Hz, 1H), 4.49 (br, 2H), 3.80 (br, 5H), 3.58 (br, 2H), 2.59 (br, 2H), 2.38 (s, 6H), 1.82 (br, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.1, 169.5, 159.0, 155.0, 144.7, 134.0, 133.2, 130.9, 130.0, 128.5, 122.5, 121.6, 118.9, 117.1, 114.2, 106.8, 101.3, 99.7, 59.2, 55.3, 46.3, 45.2, 40.2, 24.9; HR-MS(ESI): Calcd for $[M+H]^+$ 474.2505; Found: 474.2514.

4.2. Molecular docking

The molecular modeling of small molecule compounds were performed with the molecular modeling package SYBYL-X 1.3 (Tripos associate Inc., St. Louis, MO, USA) according to the reported process [46]. Briefly, the three-dimensional coordinates of survivin protein were acquired from PDB (PDB ID: 1F3H). Only one of the two chains was kept and the protein chain was prepared for docking. 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) docking the test compounds; (d) analysis of results.

4.3. *In silico* physicochemical, pharmacokinetics and toxicity analysis

Physicochemical, pharmacokinetic and toxicity parameters for selected compound were predicted *in silico* using Advanced Chemistry Development, Inc. (ACD/Labs, ACD/Percepta Platform, version 14.0.0), and Osiris Property Explorer (http://www.organic-chemistry.org/prog/peo).

4.4. Bioassay

4.4.1. Cell culture

HepG-2, MCF-7 and QGY-7701 (adherent cell lines) were cultured in DMEM, with 10% fetal bovine serum (FBS) in humidified air at 37 ºC with 5% CO_2.

4.4.2. Cell growth inhibition assay

HepG2 cells, MCF-7 cells and QGY-7701 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).

4.5. Biophysical evaluation

4.5.1. DNA binding experiment

Concentrated stock solutions of compounds were prepared by dissolving them in DMSO. Calf thymus DNA (ct DNA) was obtained from Sigma Chemical Co. All the measurements involving the interactions of tested compound **8u** 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.

The emission spectra were carried out on Fluorolog spectrometer. 16 µL of tested compound **8u** (hydrochloride salt, 5 mM in DMSO) was incubated in Tris-HCl buffer solution (the final concentration of the tested compound was 40 µM), and then ct DNA solution (2 mM in Tris-HCl buffer) was also added with the final concentration from 0 to 120 µM. The excitation wavelength was set at 280 nm. The incubating time before testing was 5 min.

4.5.2. 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.

4.5.3. DNA Topo II inhibition assay

DNA Topo II inhibition assay were performed by HD Biosciences Corporation, Shanghai. The main steps as follows: (a) Transfer 1 µl of compound or 50% DMSO into 96-well plates; (b) Add 19 µl reaction mix; (c) Add 5 µl of diluted enzyme in the

1X assay buffer; (d) Mix by pipeting up-and-down; (e) Cover the assay plates and incubate at 35 ± 2 ºC for 30 min; (f) Add 5 µl of 6X stop buffer; (g) Perform DNA electrophoresis in 1% agarose gel according to the gel layout; (h) Stain the gel with 2.3 µg/ml ethidium bromide for 10 min and destain in water for 20 min; (i) Take gel picture with Tanon GIS 2010 system; (j) Quantify DNA bands using Image J 1.47.

4.5.4. Survivin protein binding assay

The emission spectra were carried out on Fluorolog spectrometer. 10 mg purified survivin protein (obtained from Sino Biological Inc.) was incubated in 10 mM PBS buffer solution (pH 7.4), and then **8u** (100 µM in PBS buffer) was also added with the final concentration from 0 to 1.6 µM. The excitation wavelength was set at 280 nm. The ambient temperature of this experiment was maintained at 37 °C, and the incubating time before testing was 20 min.

4.5.5. Western blot analysis

HepG-2 cells were cultured in 6 cm dishes, followed by treatment with **8u** for different concentration-periods for 24 h. Protein concentrations in the supernatant were determined using bicinchonininc 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.

4.5.6. *In vivo* antitumor effect analysis in xenograft tumor model

In vivo experiments were performed by Fuwai Hospital, Beijing. For efficacy study, human hepatocarcinoma cell line HepG2 were injected subcutaneously in the flanks of null mice, and the mice were randomized into four different groups (5/group) with one group used as blank control, one group treated by 5-Fluorouracil (5-FU) control and the others by tested drug **8u** at 0.33 mg/kg and 3 mg/kg, respectively. These groups via i.p. injection once every 2 days. It should be noted that the body weight of mice in the 5-FU control group showed a significant 30% reduction at 10th day, and then we stopped administration of the 5-FU group. Tumor volume and body weight were measured every 2 days. On the 30th day after the initial treatment, mice were euthanized and the tumor tissues were harvested and weighed. We also weighed the weights of the kidneys and spleen and calculated the corresponding organ index. Tumor Growth Inhibition (TGI) was calculated in this experiment and the antitumor effect of the tested drug was evaluated.

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Supplementary data

Supplementary data (the molecular docking of YM155-survivin, the results of YM155-survivin binding assay, the fluorescence spectroscopy of **8u**-DNA binding

experiments, general methods and the preparation of compounds **3a-d**, **4a-d**, **5a-b** and **6a-h**, ^{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 (a) The chemical structures of YM155, NSC80467 and novel benzylaminoacridone derivative **8u**; (b) The 3D structural superposition of designed

compound **8u** and YM155.

Figure 2 Human Topo I/II inhibition. (a) Effect of the test compound on the relaxation of plasmid DNA by human topo I. Lanes 1-2, DNA pBR322 relaxation by topo I and **8u**; Lane 3, DNA pBR322; Lane 4, topo I + DNA pBR322 + DMSO; Lanes 5-6, Camptothecin (CPT) + Topo I + pBR322 DNA; (b) Gel images of **8u** (50 μM) from human topo II decatenation assay. Two concentrations (1 and 10 μM) of Doxorubicin (DOX) were used as the positive controls, and no compound (NC) were used as the negative controls. All assays were performed in duplicate.

Figure 3 Docking mode of compound **8u** (stick and ball) and survivin using SYBYL-X v1.3 program. Molecules are colored by atom type and hydrogen bonds are represented by yellow dotted lines. (a) Survivin protein was presented as ribbon; (b) the protein added hydrophobic surface.

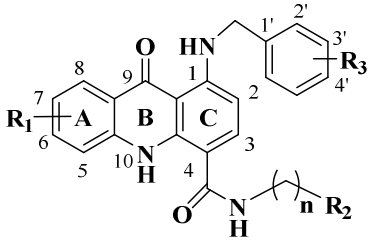
Figure 4 Dose-dependent binding of **8u** to survivin. The fluorescence was measured in the absence or presence of survivin, λ_{ex} = 280 nm. The concentration of survivin was 0.3 μM.

Figure 5 HepG2 cells were treated with different concentrations of compound **8u**, and western blot analysis was used to evaluate the levels of Survivin, Bcl-2, C-caspase-7 and C-PARP.

Figure 6 (a) Effect of **8u** on ectopic xenograft tumor growth in nude mice. The tumor size of nude mice changed with the increase of the days after administration; (b) Effect of **8u** on body weight in nude mice.

Scheme 1 Reagents and conditions: (i) K_2CO_3, Cu, DMF, 130 ºC, overnight; (ii) Concentrated H_2SO_4, 80 ºC, 5 h; (iii) CDI, various N,N-dimethyldiamines or 2-methoxyethanamine, DMF; (iv) hydrazine hydrate, sodium methoxide, ethanol, reflux; (v) various multi-substituted benzyl amines, 2-eyhoxyethanol, 90 ºC; (vi) Fe, NH_4Cl, ethanol.

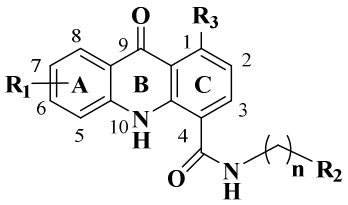
Table 1 Antiproliferative activities of benzyl acridone derivatives against HepG2 and MCF-7 cells.



I

Compd	R_1	R_2	R_3	n	IC_{50} (μM)	
					HepG2	MCF7
YM155					15.26	4.13
8a	5-CH_3	-$N(CH_3)_2$	-H	2	7.99	5.48
8b	5-CH_3	-$N(CH_3)_2$	3′-OCH_3	2	2.82	3.65
8c	5-CH_3	-$N(CH_3)_2$	4′-OCH_3	2	2.11	6.84
8d	5-CH_3	-$N(CH_3)_2$	4′-OCH_3	3	5.94	4.12
8e	5-CH_3	-OCH_3	-H	2	17.17	>50
8f	5-CH_3	-OCH_3	3′-OCH_3	2	>50	>50
8g	5-CH_3	-OCH_3	4′-OCH_3	2	>50	>50
8h	5-CH_3	-OCH_3	4′-CF_3	2	>50	>50
8i	5-CH_3	-OCH_3	3′,4′,5′-Trimethoxy	2	>50	>50
8j	5-CH_3	-$N(CH_3)_2$	3′,4′,5′-Trimethoxy	2	4.96	5.06
8k	5-OCH_3	-$N(CH_3)_2$	-H	2	5.50	13.66
8l	5-OCH_3	-$N(CH_3)_2$	4′-OCH_3	2	5.15	6.13
8m	5-OCH_3	-$N(CH_3)_2$	4′-OCH_3	3	10.80	9.97
8n	5-OCH_3	-$N(CH_3)_2$	3′,4′,5′-Trimethoxy	2	>50	>50
8o	5,7-dichloro	-$N(CH_3)_2$	4′-OCH_3	2	17.46	17.49
8p	5-NO_2	-$N(CH_3)_2$	-H	2	9.59	5.61
8q	5-NO_2	-$N(CH_3)_2$	3′-OCH_3	2	8.61	5.09
8r	5-NO_2	-$N(CH_3)_2$	4′-OCH_3	2	4.50	1.05
8s	5-NO_2	-$N(CH_3)_2$	3′,4′,5′-Trimethoxy	2	7.00	5.73
8t	5-NO_2	-$N(CH_3)_2$	-H	3	2.43	2.92
8u	5-NO_2	-$N(CH_3)_2$	4′-OCH_3	3	3.56	4.40
8v	5-NO_2	-$N(CH_3)_2$	4′-C_2H_5	3	5.78	4.69
8w	5-NH_2	-$N(CH_3)_2$	4′-OCH_3	3	5.68	13.39

Table 2 Antiproliferative activities of no benzyl substituted acridone derivatives against HepG2 and MCF-7 cells.



II

Compd	R_1	R_2	R_3	n	IC_{50} (μM)	
					HepG2	MCF7
YM155					15.26	4.13
5a	5-NO_2	-$N(CH_3)_2$	-$N(CH_3)_2$	2	28.57	3.54
5b	5-NO_2	-$N(CH_3)_2$	-$N(CH_3)_2$	3	36.82	9.29
6b	5-CH_3	-OCH_3	-Cl	2	>50	>50
6e	5-OCH_3	-$N(CH_3)_2$	-Cl	2	24.70	18.53
6g	5-NO_2	-$N(CH_3)_2$	-Cl	2	14.49	2.28
7a	5-CH_3	-$N(CH_3)_2$	-$NHNH_2$	2	8.85	12.98
7b	5-OCH_3	-$N(CH_3)_2$	-$NHNH_2$	2	12.31	14.82

Table 3 *In vitro* toxicity test of compounds against normal hepatocytes cells QGY-7701.

Compd	IC_{50} (μM)	
	QGY-7701	HepG2
8b	3.12	2.82
8c	6.91	2.11
8t	3.97	2.43
8u	40.67	3.56

Table 4 *In silico* prediction of physicochemical and drugability properties of **8u** using ACD/Percepta 14.0.0

Lipinski's violations	HBA[a]	HBD[b]	logP[c]	MW[d]	NROTB[e]	Numbers of rings	TPSA[f]
1	10	3	4.77	503.55	10	4	131.53

[a] Number of hydrogen-bond acceptors; [b] Number of hydrogen-bond donors; [c] Octanol/water partition coefficient; [d] Molecular weight; [e] Number of rotatable bonds; [f] Topological polar surface area.

Table 5 *In silico* prediction of pharmacokinetic properties of **8u** using ACD/Percepta 14.0.0.

Caco-2 Permeability (cm/s)	%PPB [a]	LogPS [c]	LogBB [d]	Log (PS*fu, brain)[e]	P-gp efflux [f]	First-pass metabolism	Solubility (mg/ml)
$18*10^{-6}$	99%	-2.3	-0.21	-4.2	-[g]	-[g]	0.1

[a] Plasma protein binding rate; [b] Human intestinal absorption; [c] Rate of brain penetration; [d] Extent of brain penetration; [e] Brain/plasma equilibration rate; [f] P-glycoprotein mediated cellular efflux; [g] inconclusive.

Table 6 Toxicity Prediction of **8u** using Osiris predictions.

Mutagenic	Tumorigenic	Irritant	Reproductive
low	low	low	low

Table 7 Organ weight and organ index (liver and spleen) of nude mice.

Group	Statistic	Liver		Spleen	
		Organ Weight (g)	Organ Index	Organ Weight (g)	Organ Index
Blank	$\bar{x}$	0.434	0.0187	0.248	0.0106
	s	0.069	0.0009	0.071	0.0020
5-FU	$\bar{x}$	0.419	0.0231	0.260	0.0144
	s	0.062	0.0034	0.091	0.0050
8u 3 mg/kg	$\bar{x}$	0.421	0.0198	0.344	0.0162
	s	0.032	0.0025	0.084	0.0042
8u 0.33 mg/kg	$\bar{x}$	0.441	0.0217	0.231	0.0113
	s	0.040	0.0016	0.044	0.0016

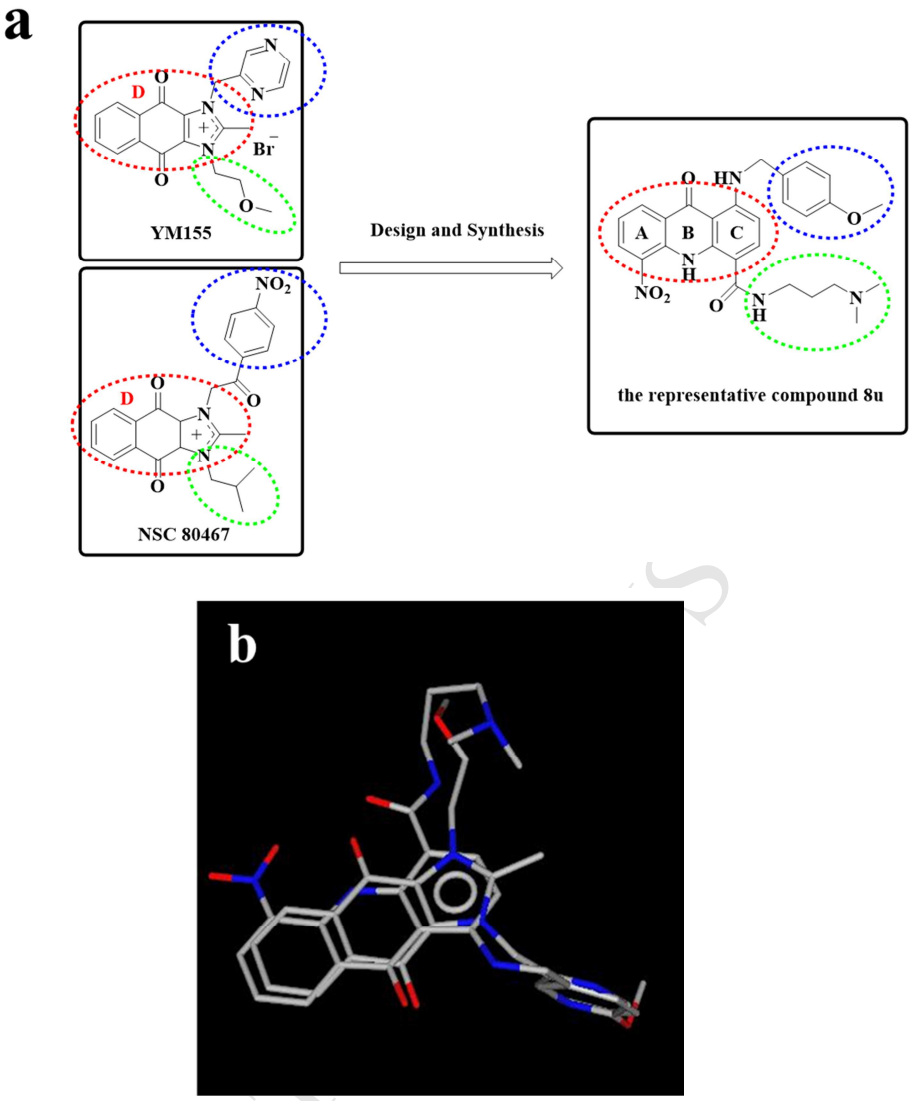
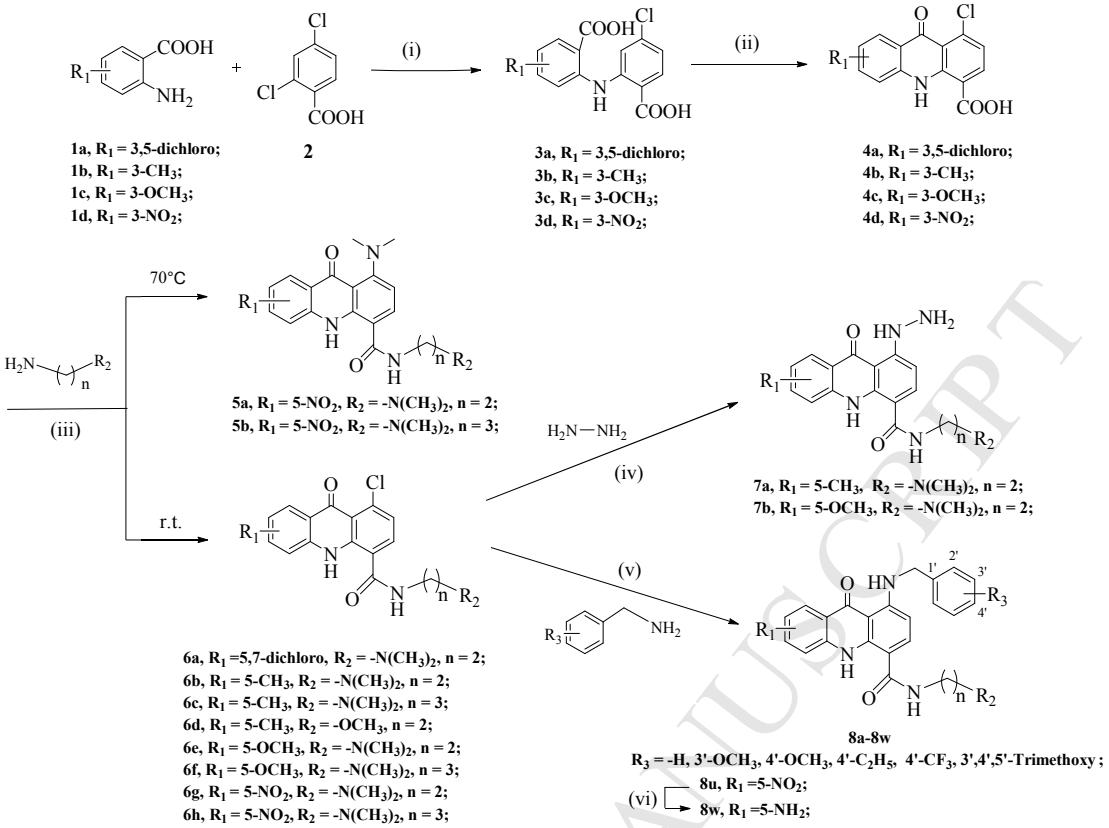

a
YM155
NSC 80467
Design and Synthesis
the representative compound 8u
b

Figure 1



Scheme 1

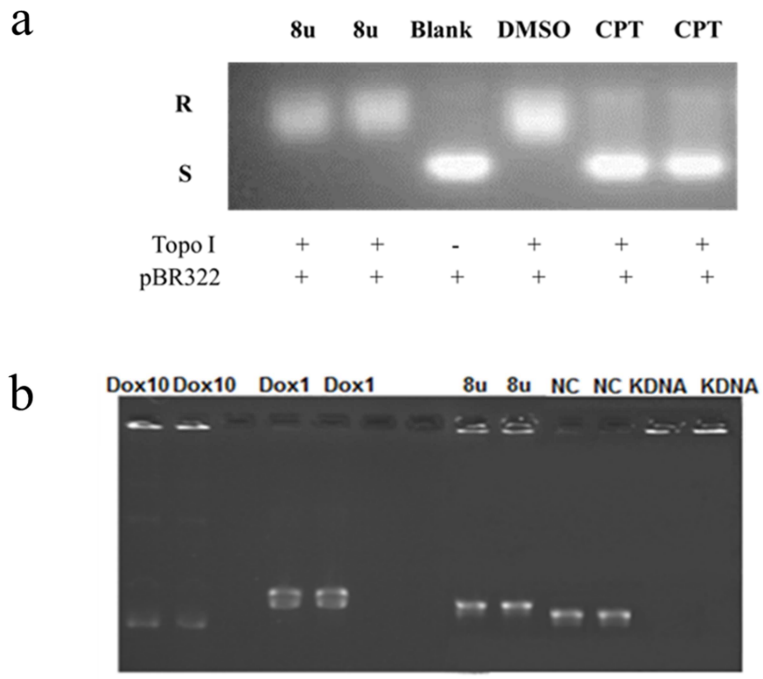


Figure 2

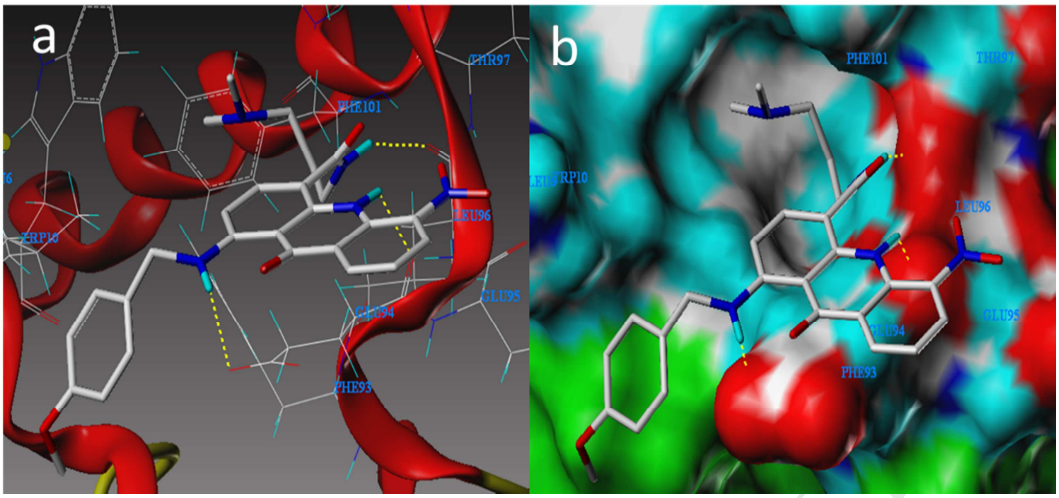


Figure 3

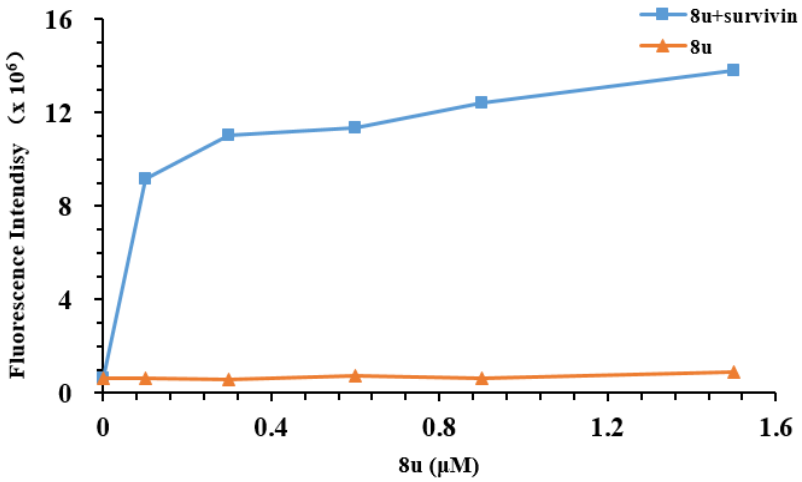


Figure 4

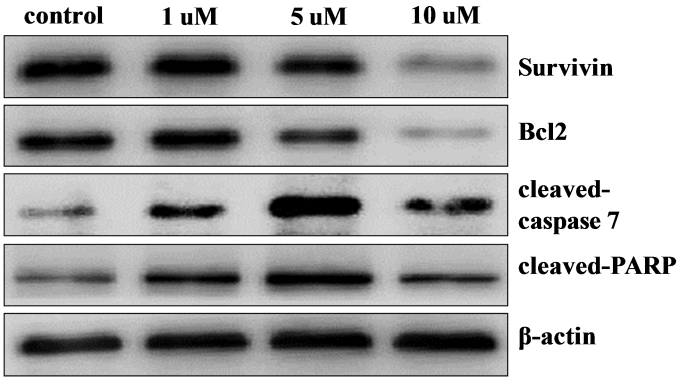


Figure 5

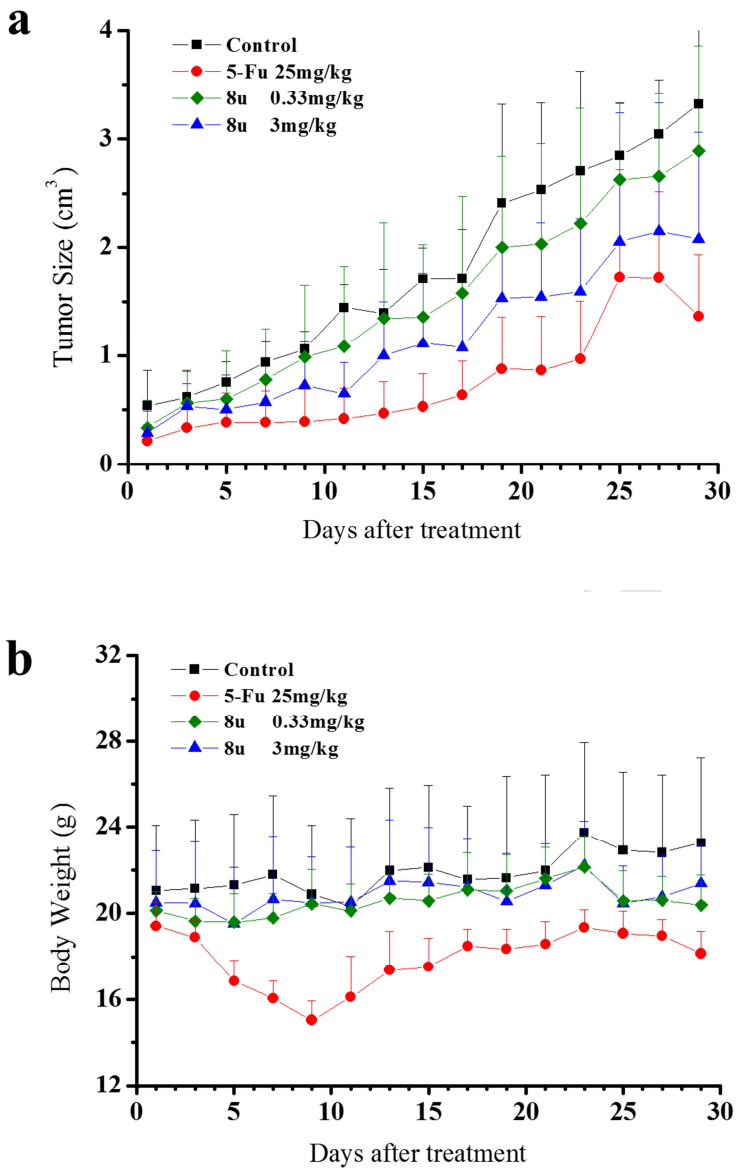

a
Control
5-Fu 25mg/kg
8u 0.33mg/kg
8u 3mg/kg
Tumor Size (cm3)
Days after treatment
b
Control
5-Fu 25mg/kg
8u 0.33mg/kg
8u 3mg/kg
Body Weight (g)
Days after treatment

Figure 6

- 30 multi-benzylamine acridone derivatives were designed and synthesized.
- Compound 8u showed high activity against HepG2 cells and low toxicity *in vitro*.
- *In silico* predictions revealed that 8u had good druggability.
- Molecular docking and biological assays verified the mechanism of action of 8u.
- 8u induced HepG2 cells apoptosis and it also had a good *in vivo* anti-tumor effect.

Novel multi-substituted benzyl acridone derivatives as survivin inhibitors for hepatocellular carcinoma treatment

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† Authors with equal contributions.

1. DNA binding experiment

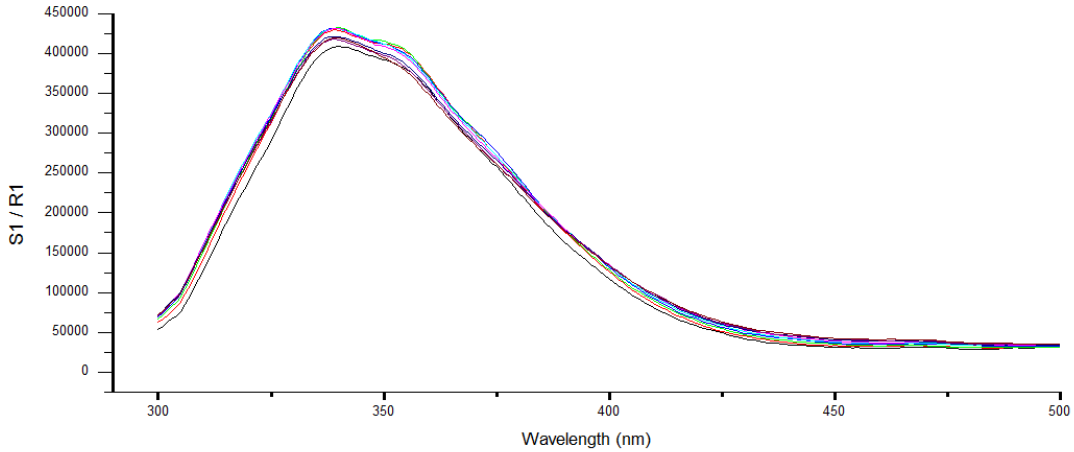


Figure 1s Spectrofluorimetric titration of **8u** (40 mM) in 5 mM Tris-HCl buffer containing 50 mM NaCl (pH 7.2) by increasing the concentrations of ct DNA; [DNA] = 0, 8, 16, 24, 32, 40, 60, 80, 120 μM; λ_{ex} = 280 nm. The different colors represent the fluorescence intensity at different DNA concentrations.

2. Molecular docking

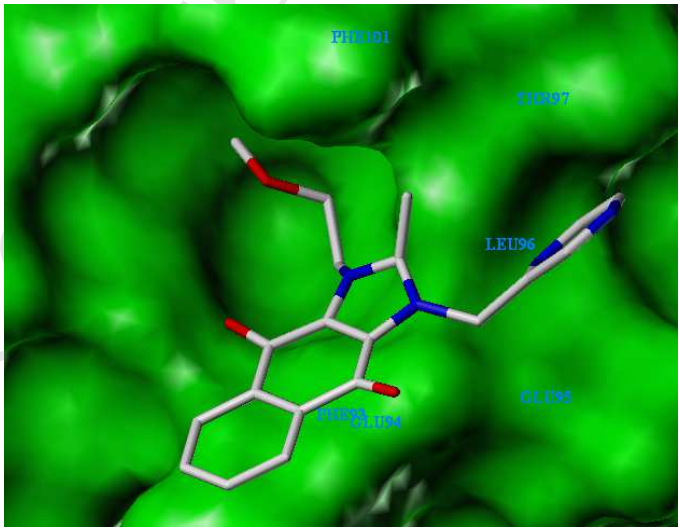


Figure 2s Docking mode of compound YM155 (stick and ball) and survivin using SYBYL-X v1.3 program.

3. Spectrofluorimetric titration

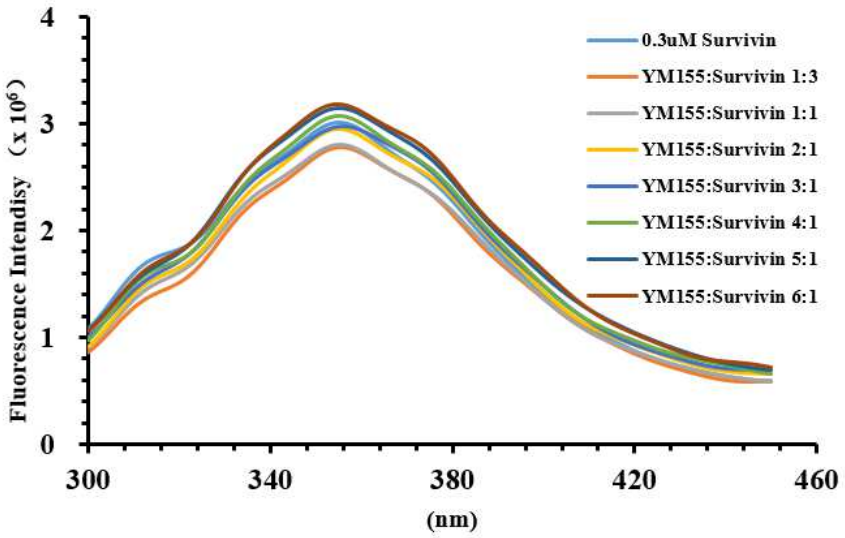


Figure 3s Spectrofluorimetric titration of survivin (0.3μM) in 10 mM PBS buffer (pH 7.4) by increasing the concentrations of YM155; [YM155] = 0, 0.1, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8 μM; λ_{ex} = 280 nm. The different colors represent the fluorescence intensity at different YM155 concentrations.

4. General notes and synthetic procedures

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.

4.1. General procedure for compounds 3a-d.

2,4-dichlorobenzoic acid **2** (0.61 g, 4.05 mmol), anthranilic acid derivatives **1a-d** (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 solid powder.

4.1.1. 2-((2-carboxy-5-chlorophenyl)amino)-3,5-dichlorobenzoic acid (3a) 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).

4.1.2. 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).

4.1.3. 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).

4.1.4. 2-((2-carboxy-5-chlorophenyl)amino)-3-nitrobenzoic acid (3d) Yellow solid powder, Yield 59.6%; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.19-8.14 (m, 2H), 7.89 (d, *J* = 8.5 Hz, 1H), 7.37 (t, *J* = 8.0 Hz, 1H), 6.95 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.71 (d, *J* = 2.0 Hz, 1H).

4.2. General procedure for compounds 4a-d.

Compound **3a-d** (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-d**.

4.2.1. 1,5,7-trichloro-9-oxo-9,10-dihydroacridine-4-carboxylic acid (4a) 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).

4.2.2. 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).

4.2.3. 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).

4.2.4. 1-chloro-5-nitro-9-oxo-9,10-dihydroacridine-4-carboxylic acid (4d)

Red solid powder, Yield 73.9%; ^{1}H NMR (400 MHz, d_6-DMSO) δ 14.62 (s, 1H), 8.72 (dd, *J* = 8.1, 1.4 Hz, 1H), 8.60 (d, *J* = 7.8 Hz, 1H), 8.35 (d, *J* = 8.3 Hz, 1H), 7.46 (t, *J* = 8.0 Hz, 1H), 7.42 (d, *J* = 8.3 Hz, 1H).

4.3. General procedure for compounds 6a-h.

A solution of 9-oxo-9,10-dihydroacridine-4-carboxylic acid (**4**) (2.35 mmol) in DMF (15 mL) was added dropwise, with stirring, to a solution of N,N′-carbonyldiimidazole (3.52 mmol) in DMF (10 mL) at room temperature. After the addition was complete, the mixture was still stirred for 30 minutes. Then, the corresponding primary aliphatic amine (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 **6**.

4.3.1. Preparation of 1,5,7-trichloro-N-(2-(dimethylamino)ethyl)-9-oxo-9,10-dihydroacridine-4-carboxamide (6a)

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).

4.3.2. Preparation of 1-chloro-N-(2-(dimethylamino)ethyl)-5-methyl-9-oxo-9,10-dihydroacridine-4-carboxamide (6b)

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).

4.3.3. Preparation of 1-chloro-N-(3-(dimethylamino)propyl)-5-methyl-9-oxo-9,10-dihydroacridine-4-carboxamide (6c)

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).

4.3.4. Preparation of 1-chloro-N-(2-methoxyethyl)-5-methyl-9-oxo-9,10-dihydro acridine-4-carboxamide (6d)

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).

4.3.5. Preparation of 1-chloro-N-(2-(dimethylamino)ethyl)-5-methoxy-9-oxo-9,10-dihydroacridine-4-carboxamide (6e)

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.

4.3.6. Preparation of 1-chloro-N-(3-(dimethylamino)propyl)-5-methoxy-9-oxo-9,10-dihydroacridine-4-carboxamide (6f)

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).

4.3.7. 1-chloro-N-(2-(dimethylamino)ethyl)-5-nitro-9-oxo-9,10-dihydroacridine-4-carboxamide (6g)

Red solid powder, Yield 61.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (dd, *J* = 13.7, 7.9 Hz, 2H), 7.83 (d, *J* = 8.2 Hz, 1H), 7.32 (t, *J* = 7.9 Hz, 1H), 7.27-7.25 (m, 1H), 3.63-3.61 (m, 2H), 2.61 (t, *J* = 5.5 Hz, 2H), 2.32 (s, 6H).

4.3.8. 1-chloro-N-(3-(dimethylamino)propyl)-5-nitro-9-oxo-9,10-dihydroacridine-4-carboxamide (6h)

Red solid powder, Yield 28.6%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.78 (s, 1H), 9.53 (s, 1H), 8.77 (d, *J* = 7.6 Hz, 1H), 8.70 (d, *J* = 7.9 Hz, 1H), 7.72 (d, *J* = 8.1 Hz, 1H), 7.33 (dd, *J* = 14.3, 7.9 Hz, 2H), 3.69 (br, 2H), 2.63 (br, 2H), 2.37 (s, 6H), 1.85 (br, 2H).

4.4. General procedure for compounds 5a-b.

In the previous reaction to produce intermediate **6**, we also found it will generate a small amount of byproducts **5a-b** when the reaction temperature was raised to 70℃.

4.4.1. 1-(dimethylamino)-N-(2-(dimethylamino)ethyl)-5-nitro-9-oxo-9,10-dihydr

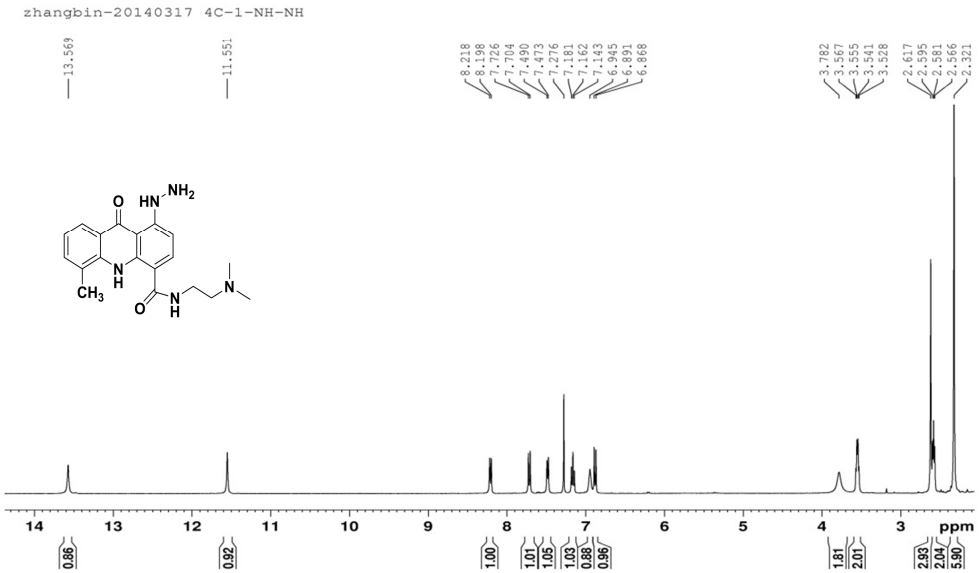
oacridine-4-carboxamide (5a)

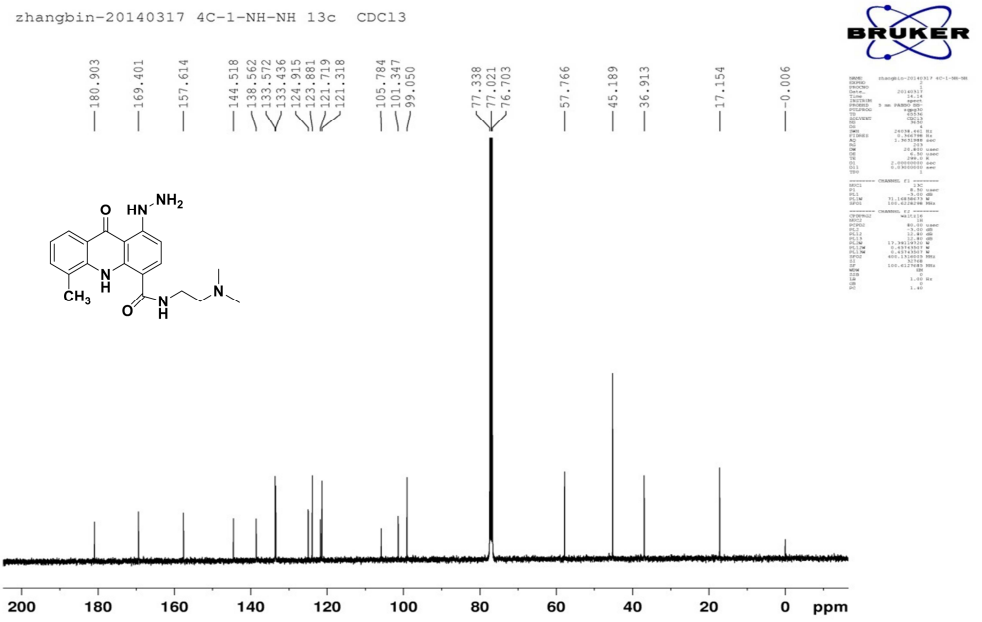
Red solid powder, Yield 24.4%; mp 168-170℃; HR-MS(ESI): Calcd for $[M+H]^+$ 398.1828; Found: 398.1833. ^{1}H NMR (400 MHz, d_6-DMSO) δ 14.75 (s, 1H), 8.60 (d, *J* = 7.5 Hz, 1H), 8.50 (br, 1H), 8.48 (s, 1H), 8.02 (d, *J* = 8.8 Hz, 1H), 7.37-7.28 (m, 1H), 6.72 (d, *J* = 8.9 Hz, 1H), 3.44-3.43 (m, 2H), 2.96 (s, 6H), 2.54 (br, 2H), 2.28 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.32, 168.30, 155.59, 144.71, 134.96, 134.67, 134.51, 132.76, 130.43, 125.85, 119.41, 109.89, 107.19, 105.56, 58.06, 44.78, 43.69, 36.38.

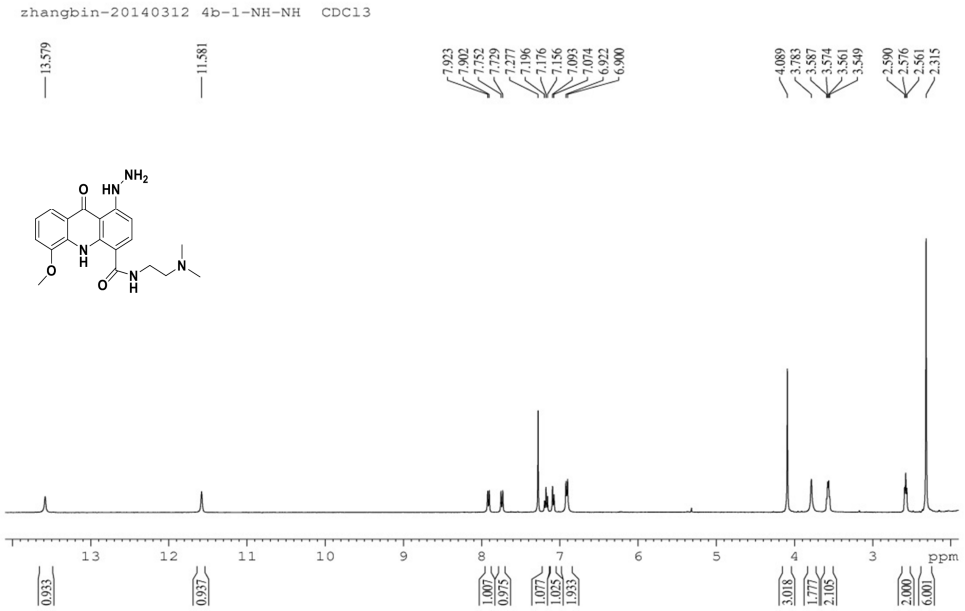
4.4.2. 1-(dimethylamino)-N-(3-(dimethylamino)propyl)-5-nitro-9-oxo-9,10-dihyd roacridine-4-carboxamide (5b)

Red solid powder, Yield 34.2%; mp 216-218℃; HR-MS(ESI): Calcd for $[M+H]^+$ 412.1985; Found: 412.1983. ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.96 (s, 1H), 8.83 (br, 1H), 8.65-8.60 (m, 2H), 7.72 (s, 1H), 7.26-7.22 (m, 1H), 6.62 (br, 1H), 3.65 (br, 2H), 3.05 (s, 6H), 2.67 (br, 2H), 2.43 (s, 6H), 1.88 (br, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.44, 168.16, 155.50, 144.85, 134.91, 134.74, 134.66, 132.23, 130.40, 125.89, 119.29, 110.14, 107.22, 106.10, 58.93, 45.01, 43.70, 39.99, 24.78.

5. NMR Spectra



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7.902
7.752
7.729
7.277
7.196
7.176
7.156
7.093
7.074
6.922
6.900
4.089
3.783
3.587
3.574
3.561
3.549
2.590
2.576
2.561
2.315
ppm
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0.975
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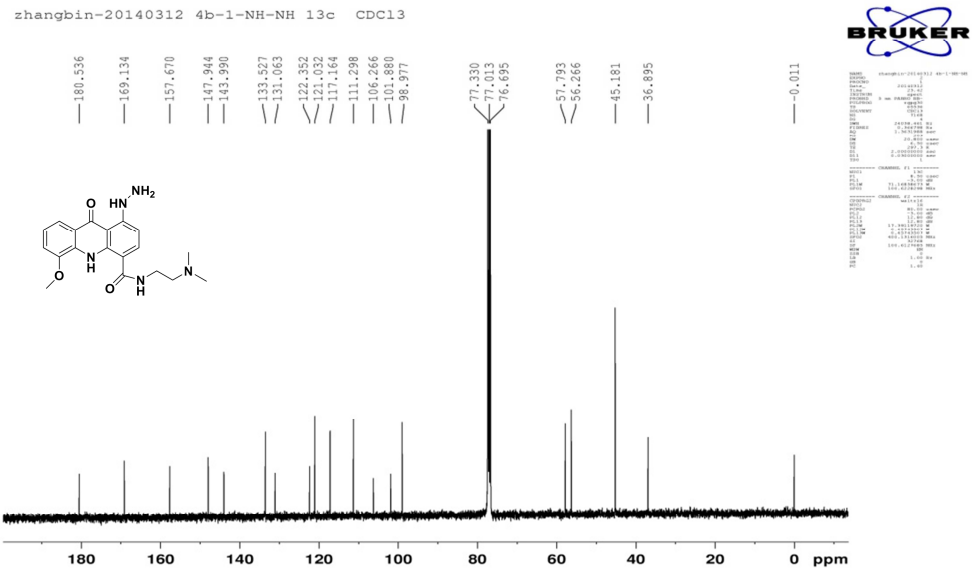
2.000
6.001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BRUKER
180.536
169.134
157.670
147.944
143.990
133.527
131.063
122.352
121.032
117.164
111.298
106.266
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76.695
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56.266
45.181
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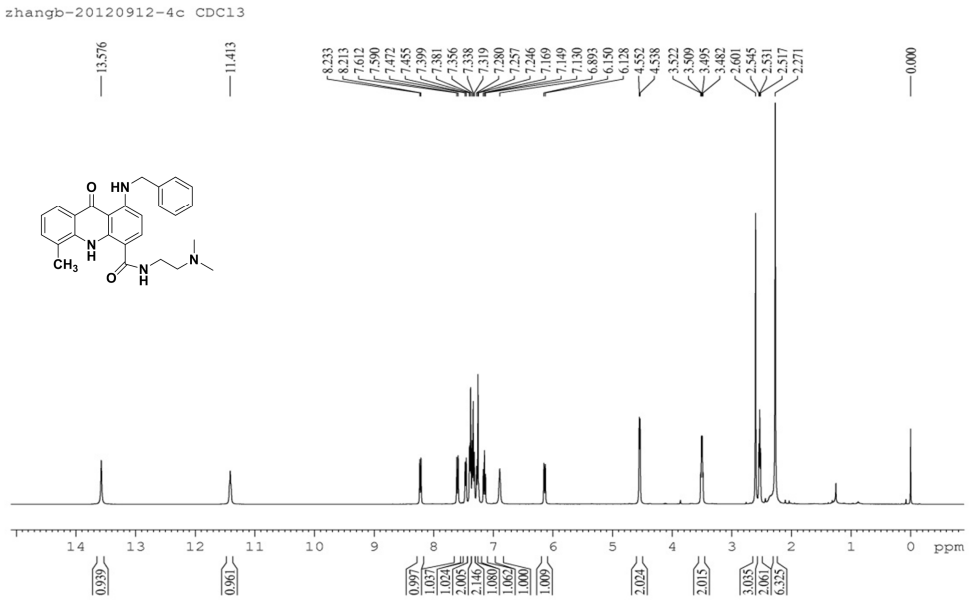
-0.011
ppm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8.233
8.213
7.612
7.590
7.472
7.455
7.399
7.381
7.356
7.338
7.319
7.280
7.257
7.246
7.169
7.149
7.130
6.893
6.150
6.128
4.552
4.538
3.522
3.509
3.495
3.482
2.601
2.545
2.531
2.517
2.271
0.000
HN
O
N
H
CH3
N
O
N
H
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0.961
0.997
1.037
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1.009
2.024
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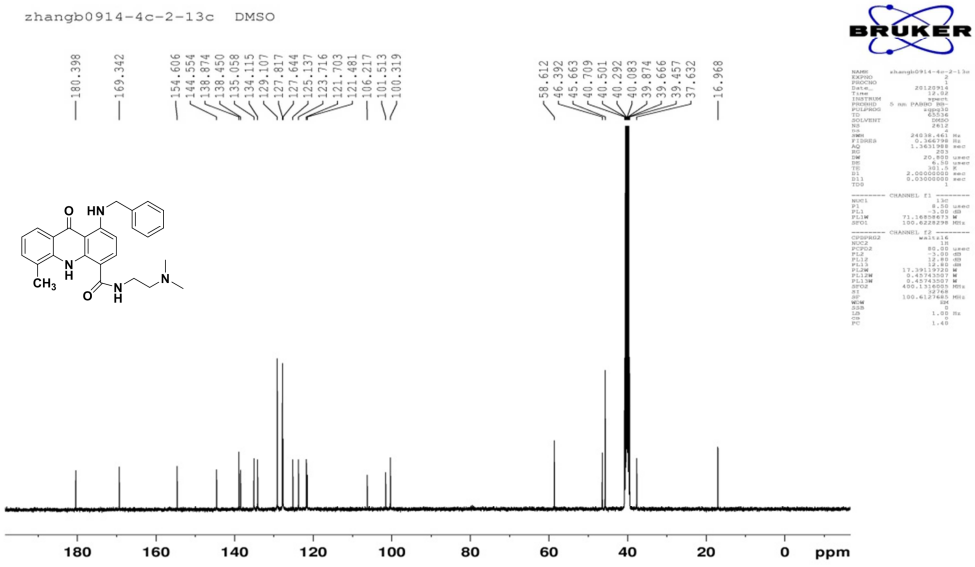
6.325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138.874
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135.058
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127.817
127.644
125.137
123.716
121.703
121.481
106.217
101.513
100.319
58.612
46.392
45.663
40.709
40.501
40.292
40.083
39.874
39.666
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HN
O
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N
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N
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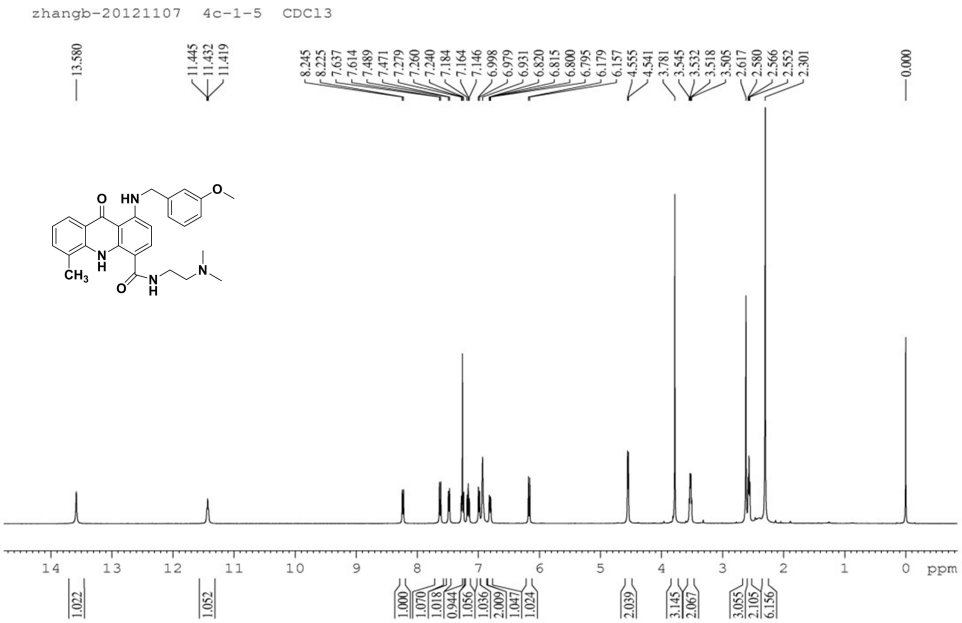
BRUKER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7.489
7.471
7.279
7.260
7.240
7.184
7.164
7.146
6.998
6.979
6.931
6.820
6.815
6.800
6.795
6.179
6.157
4.555
4.541
3.781
3.545
3.532
3.518
3.505
2.617
2.580
2.566
2.552
2.301
0.000
O
HN
O
N
H
CH3
O
N
H
N
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
1.022
1.052
1.000
1.070
1.018
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1.036
2.009
1.047
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2.039
3.145
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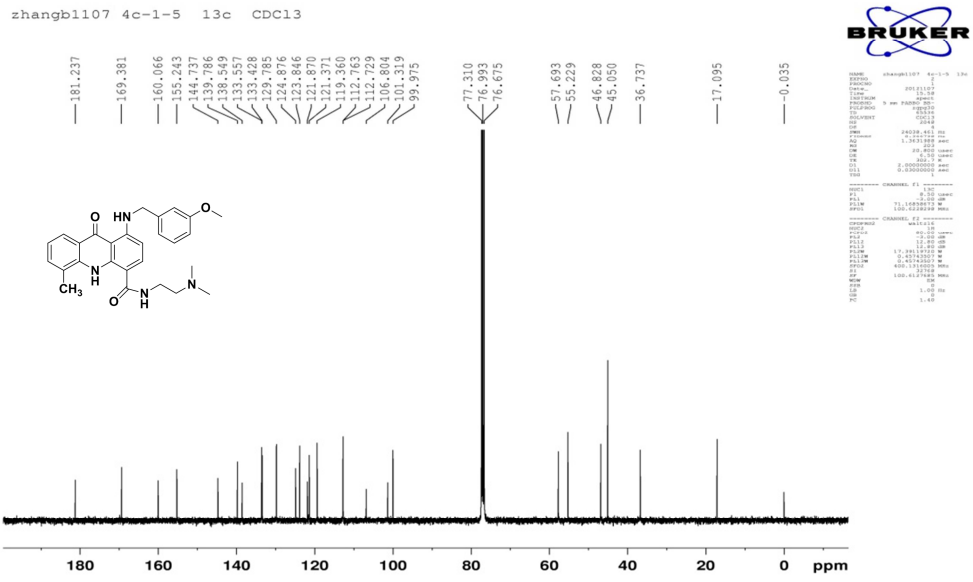
6.156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133.428
129.785
124.876
123.846
121.870
121.371
119.360
112.763
112.729
106.804
101.319
99.975
77.310
76.993
76.675
57.693
55.229
46.828
45.050
36.737
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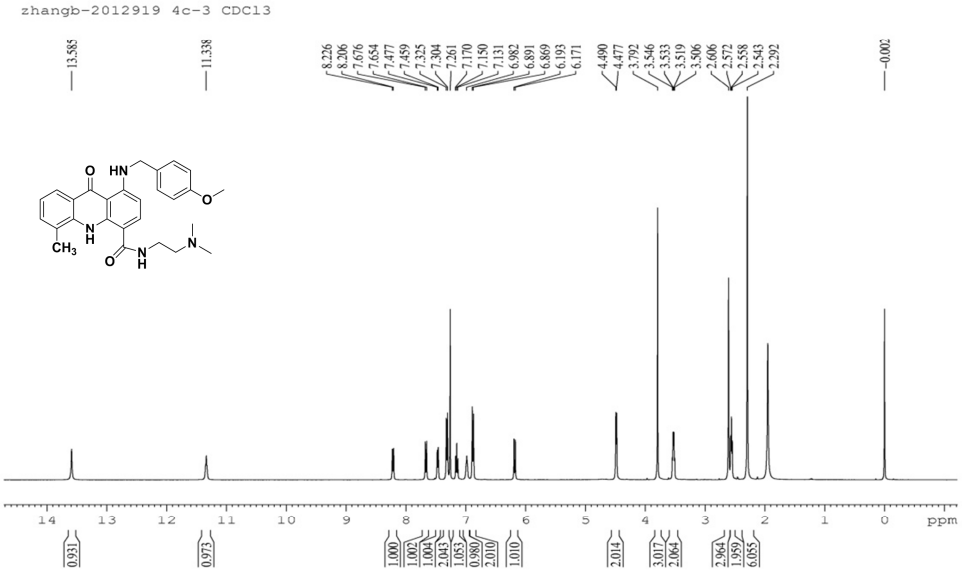
0.035
O
HN
O
N
H
CH3
O
N
H
N
180 160 140 120 100 80 60 40 20 0 ppm


zhangb-2012919 4c-3 CDCl3
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7.477
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7.325
7.304
7.261
7.170
7.150
7.131
6.982
6.891
6.869
6.193
6.171
4.490
4.477
3.792
3.546
3.533
3.519
3.506
2.606
2.572
2.558
2.543
2.292
0.002
ppm
0.931
0.973
1.000
1.002
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0.980
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3.017
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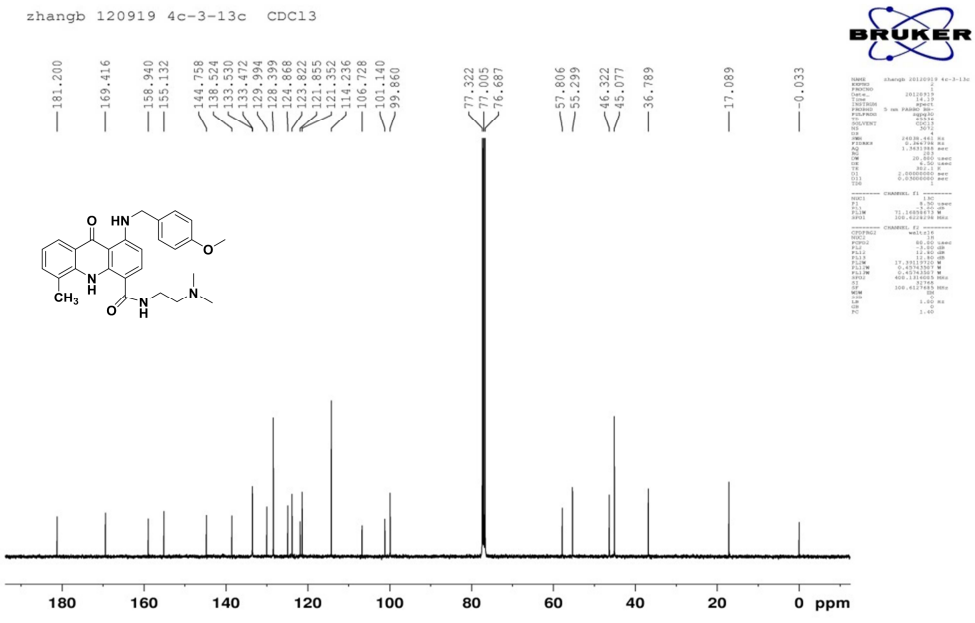
6.055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138.524
133.530
133.472
129.994
128.399
124.868
123.822
121.855
121.352
114.236
106.728
101.140
99.860
77.322
77.005
76.687
57.806
55.299
46.322
45.077
36.789
17.089
0.033
BRUKER
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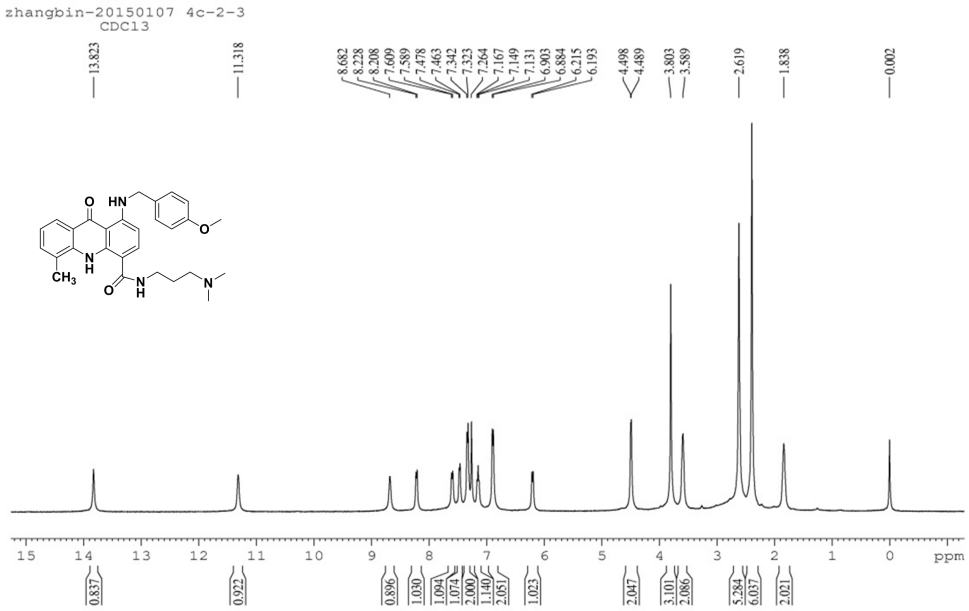
160
140
120
100
80
60
40
20
0 ppm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CDCl3
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8.228
8.208
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7.478
7.463
7.342
7.323
7.264
7.167
7.149
7.131
6.903
6.884
6.215
6.193
4.498
4.489
3.803
3.589
2.619
1.838
0.002
ppm
0.837
0.922
0.896
1.030
1.094
1.074
2.000
1.140
2.051
1.023
2.047
3.101
2.086
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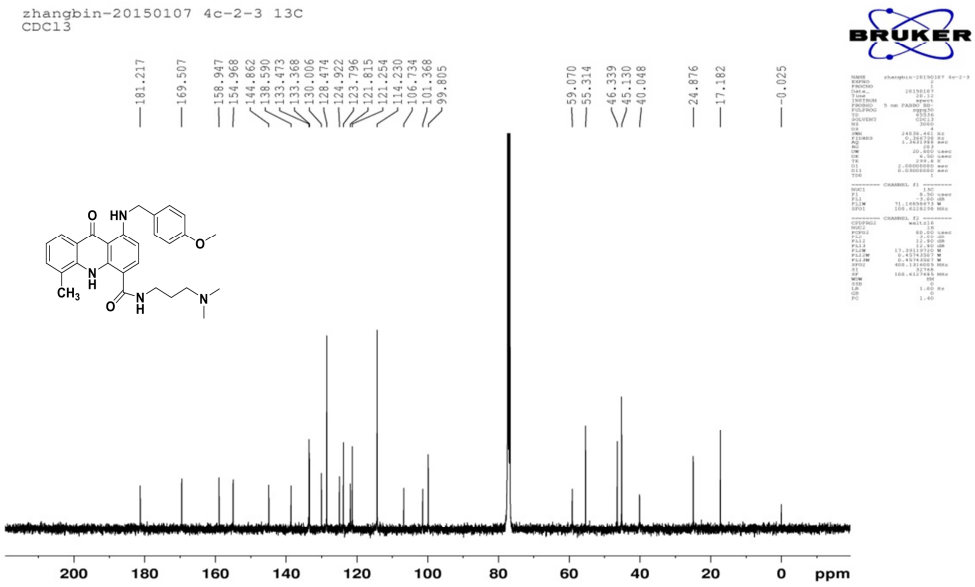
6.037
2.021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CDCl3
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154.968
144.862
138.590
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133.368
130.006
128.474
124.922
123.796
121.815
121.254
114.230
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99.805
59.070
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17.182
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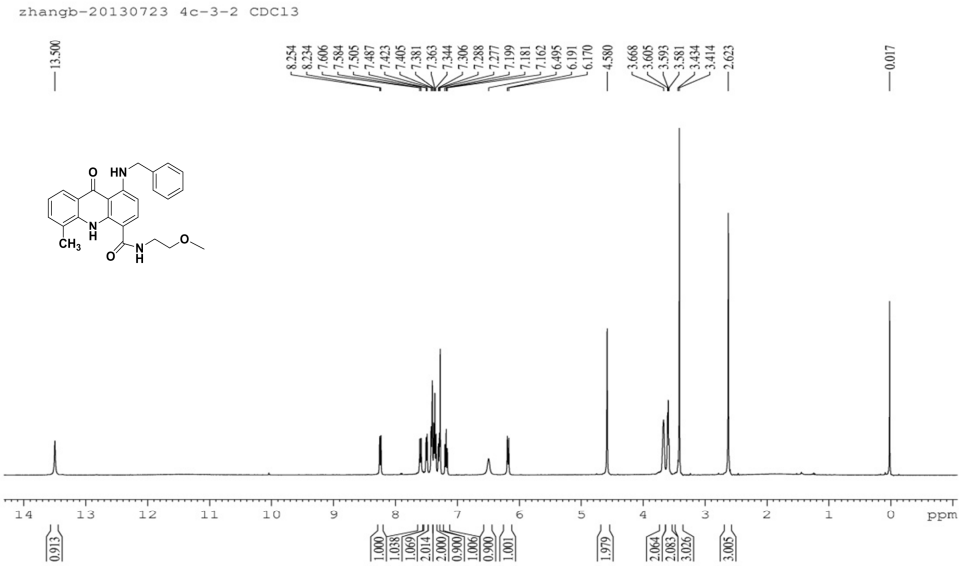
zhangb-20130723 4c-3-2 CDCl3
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8.234
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7.505
7.487
7.423
7.405
7.381
7.363
7.344
7.306
7.288
7.277
7.199
7.181
7.162
6.495
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3.668
3.605
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3.581
3.434
3.414
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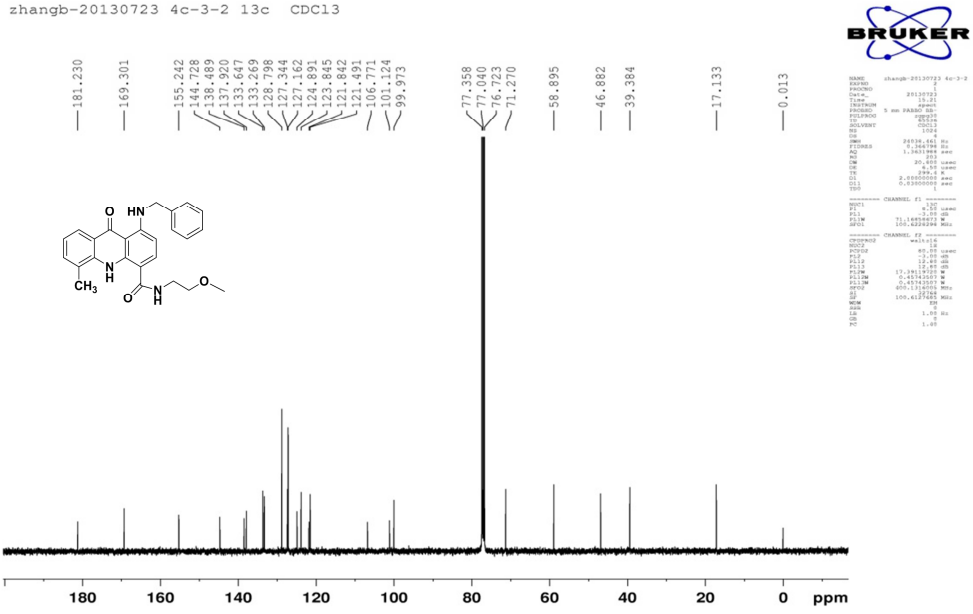
0.017
ppm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169.301
155.242
144.728
138.489
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133.647
133.269
128.798
127.344
127.162
124.891
123.845
121.842
121.491
106.771
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39.384
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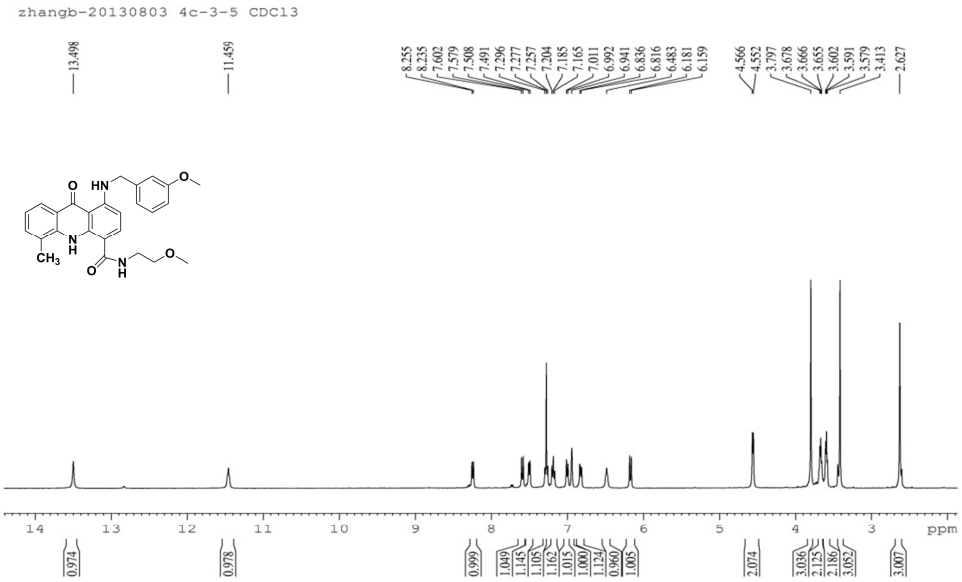
ppm


zhangb-20130803 4c-3-5 CDCl3
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8.235
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7.579
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7.277
7.257
7.204
7.185
7.165
7.011
6.992
6.941
6.836
6.816
6.483
6.181
6.159
4.566
4.552
3.797
3.678
3.666
3.655
3.602
3.591
3.579
3.413
2.627
HN
O
O
N
H
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O
N
H
O
14
13
12
11
10
9
8
7
6
5
4
3
ppm
0.974
0.978
0.999
1.049
1.145
1.105
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1.000
1.124
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3.036
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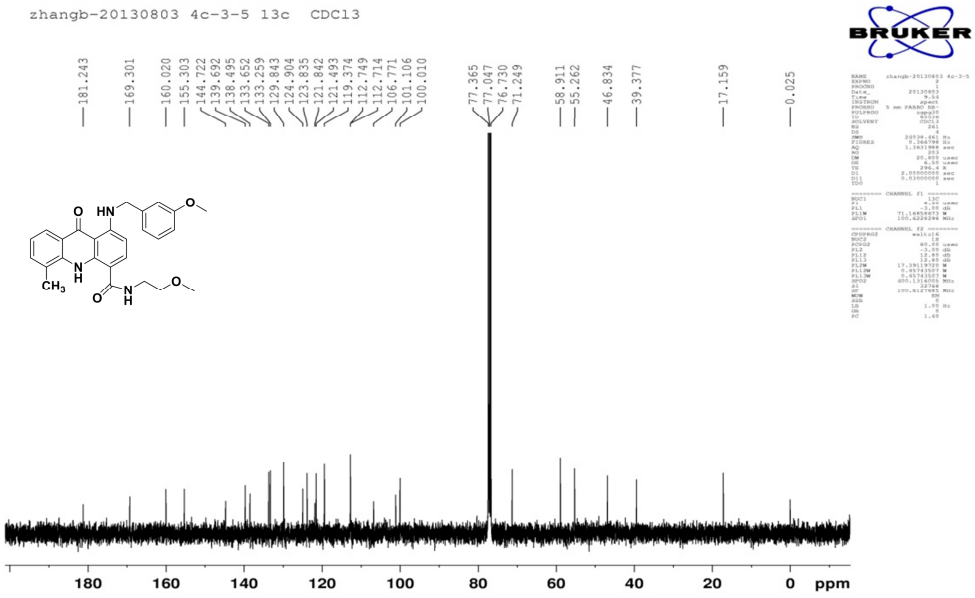
3.007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133.259
129.843
124.904
123.835
121.842
121.493
119.374
112.749
112.714
106.771
101.106
100.010
77.365
77.047
76.730
71.249
58.911
55.262
46.834
39.377
17.159
0.025
HN
O
O
N
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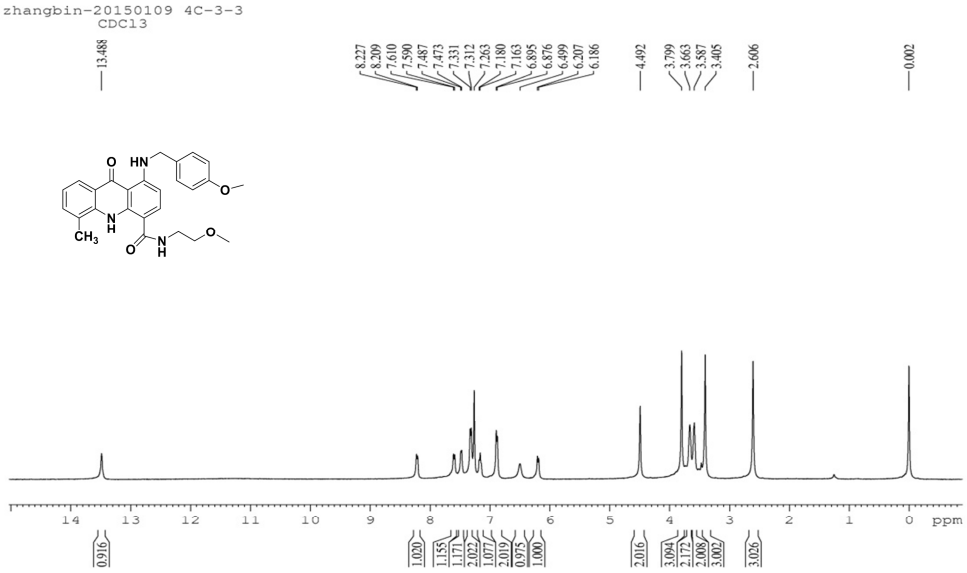
CH3
O
N
H
O
180
160
140
120
100
80
60
40
20
0
ppm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7.487
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7.312
7.263
7.180
7.163
6.895
6.876
6.499
6.207
6.186
4.492
3.799
3.663
3.587
3.405
2.606
0.002
HN
O
N
H
CH3
O
N
H
O
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.916
1.020
1.155
1.171
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2.019
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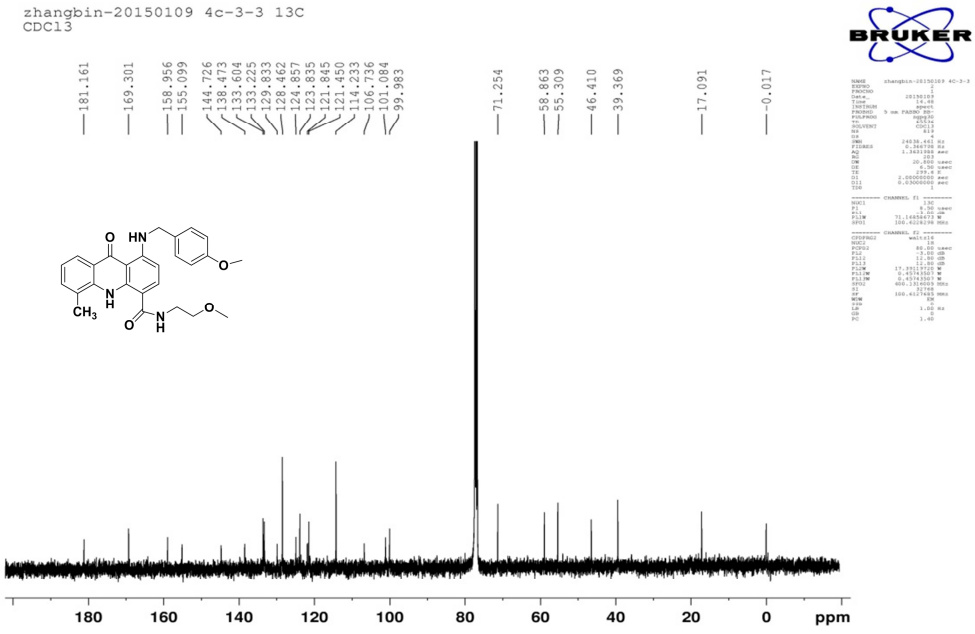
2.008
3.002
3.026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CDCl3
BRUKER
181.161
169.301
158.956
155.099
144.726
138.473
133.604
133.225
129.833
128.462
124.857
123.835
121.845
121.450
114.233
106.736
101.084
99.983
71.254
58.863
55.309
46.410
39.369
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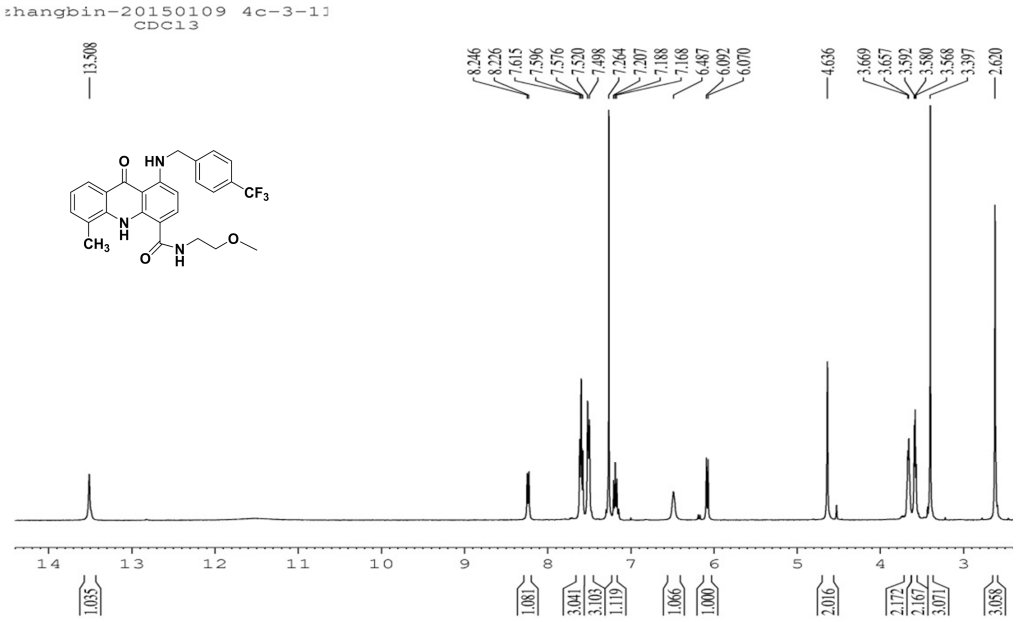
-0.017
O
HN
O
N
H
CH3
O
N
H
O
180 160 140 120 100 80 60 40 20 0 ppm


zhangbin-20150109 4c-3-11
CDCl3
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8.246
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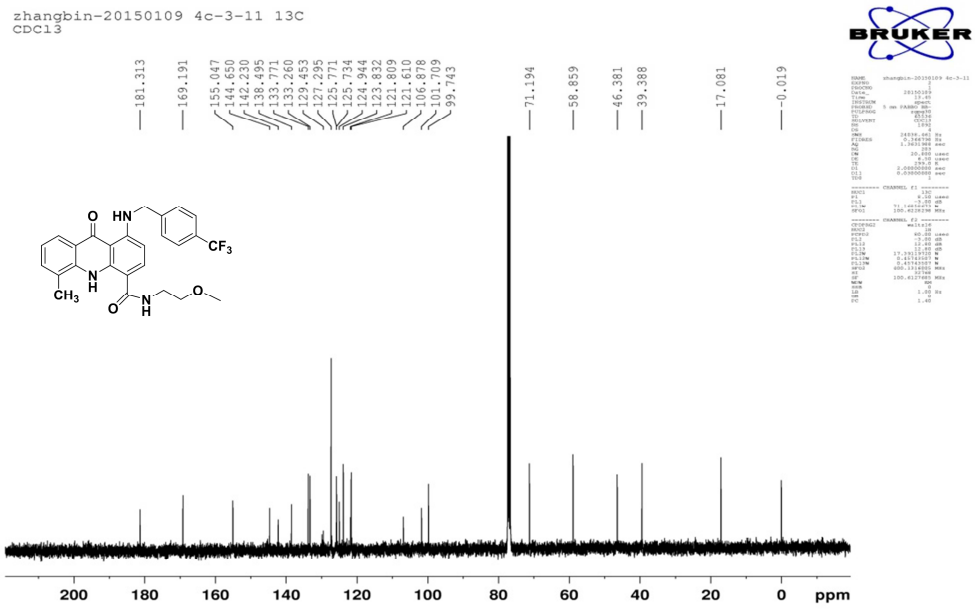
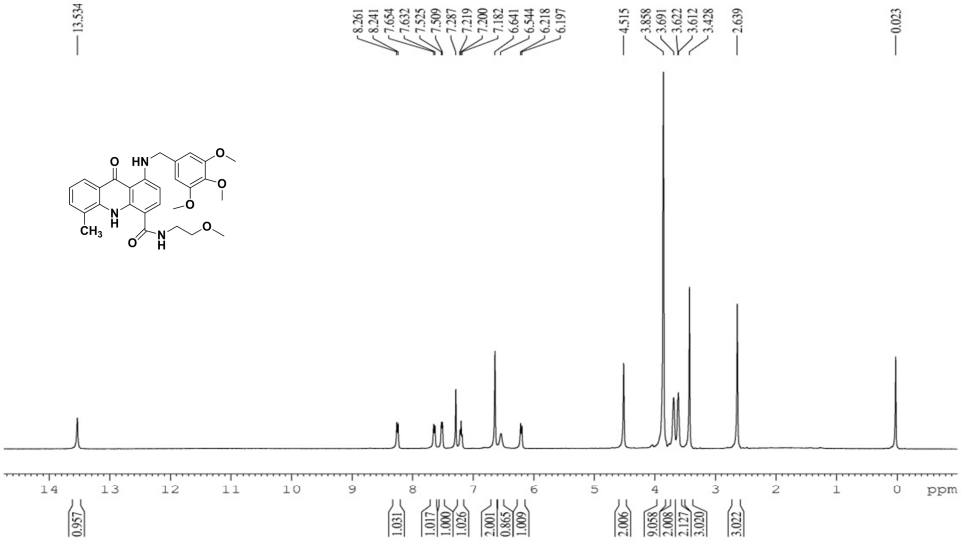
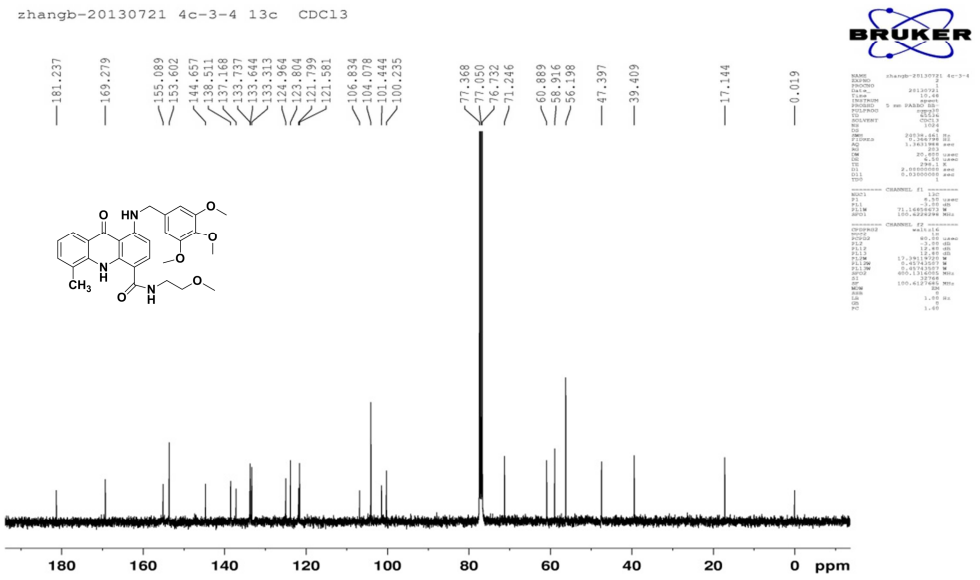
7.596
7.576
7.520
7.498
7.264
7.207
7.188
7.168
6.487
6.092
6.070
4.636
3.669
3.657
3.592
3.580
3.568
3.397
2.620
HN
CF3
O
N
H
CH3
O
N
H
O
14
13
12
11
10
9
8
7
6
5
4
3
1.035
1.081
3.041
3.103
1.119
1.066
1.000
2.016
2.172
2.167
3.071
3.058

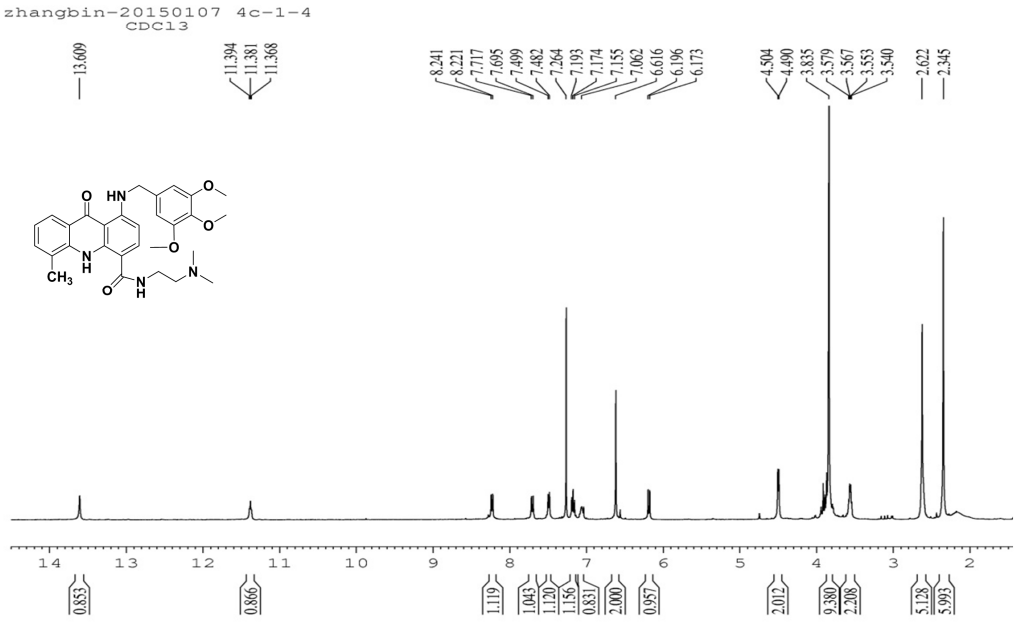

zhangbin-20150109 4c-3-11 13C
CDCl3
181.313
169.191
155.047
144.650
142.230
138.495
133.771
133.260
129.453
127.295
125.771
125.734
124.944
123.832
121.809
121.610
106.878
101.709
99.743
71.194
58.859
46.381
39.388
17.081
-0.019
HN
CF3
O
N
H
CH3
O
N
H
O
200
180
160
140
120
100
80
60
40
20
0
ppm

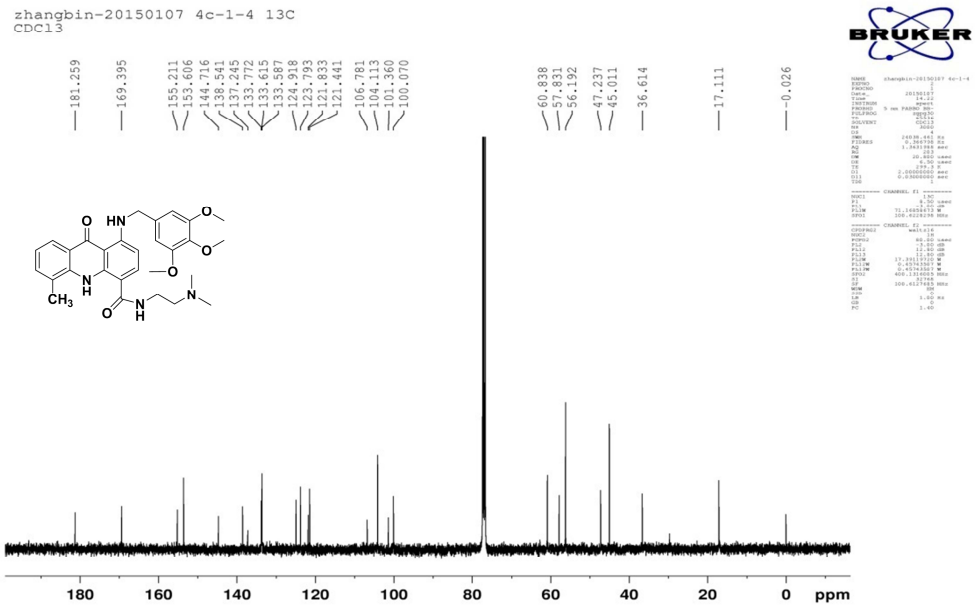
zhangbin-20150109 4c-3-11_CDCl3(F)

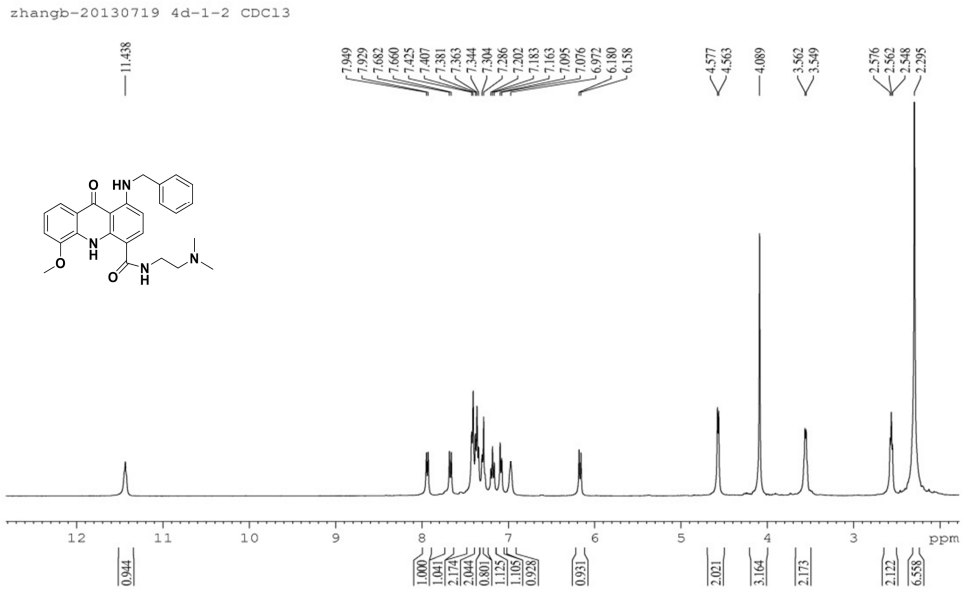
zhangb-20130721 4c-3-4 CDCl3

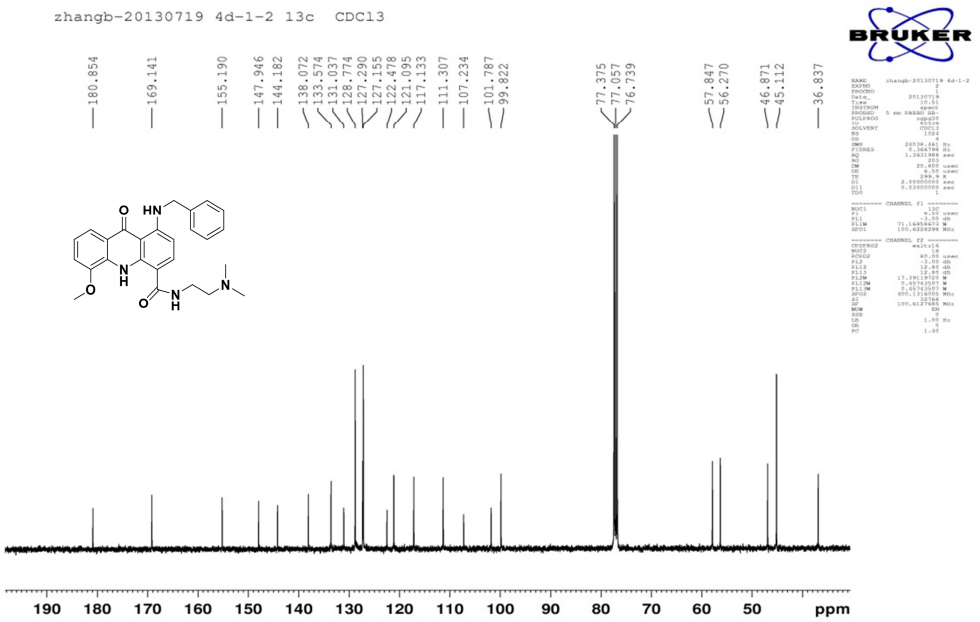


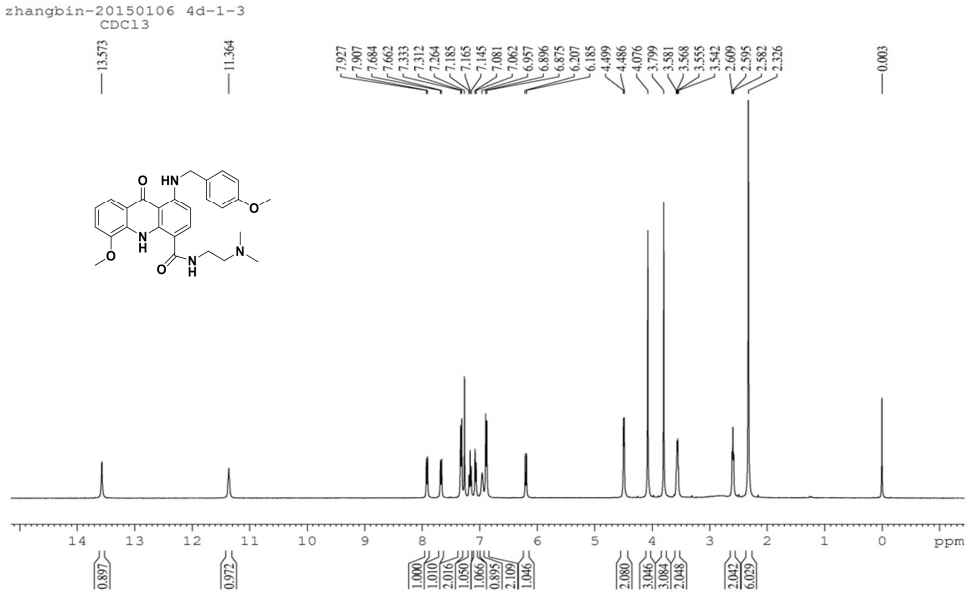

zhangb-20130721 4c-3-4 13c CDCl3
BRUKER
181.237
169.279
155.089
153.602
144.657
138.511
137.168
133.737
133.644
133.313
124.964
123.804
121.799
121.581
106.834
104.078
101.444
100.235
77.368
77.050
76.732
71.246
60.889
58.916
56.198
47.397
39.409
17.144
0.019
O
HN
O
O
O
N
H
CH3
O
N
H
180
160
140
120
100
80
60
40
20
0 ppm

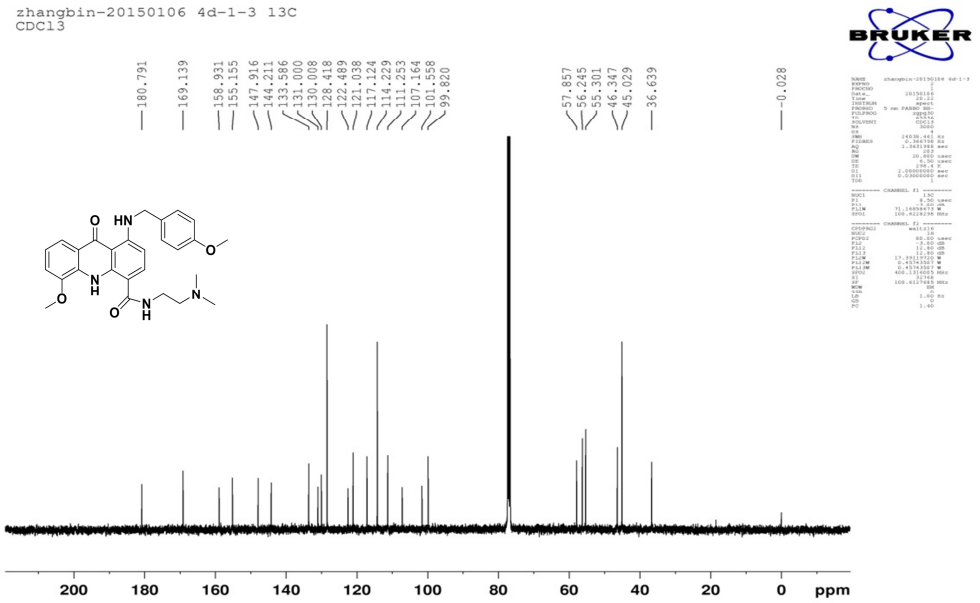

zhangbin-20150107 4c-1-4
CDCl3
13.609
11.394
11.381
11.368
8.241
8.221
7.717
7.695
7.499
7.482
7.264
7.193
7.174
7.155
7.062
6.616
6.196
6.173
4.504
4.490
3.835
3.579
3.567
3.553
3.540
2.622
2.345
O
HN
O
O
O
N
H
CH3
O
N
H
N
14
13
12
11
10
9
8
7
6
5
4
3
2
0.853
0.866
1.119
1.043
1.120
1.156
0.831
2.000
0.957
2.012
9.380
2.208
5.128
5.993

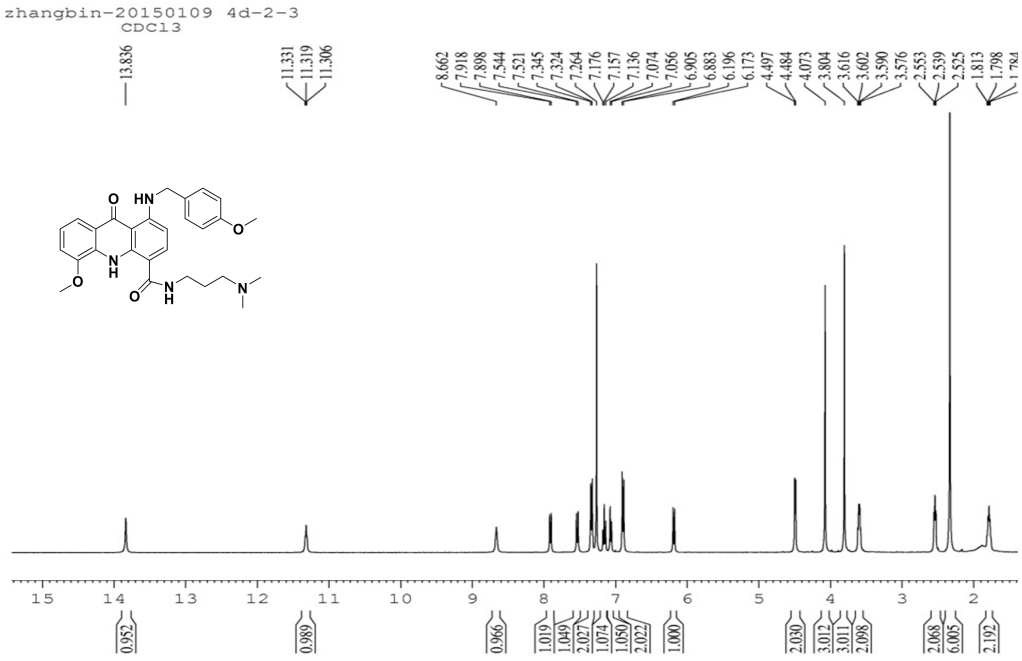

zhangbin-20150107 4c-1-4 13C
CDCl3
181.259
169.395
155.211
153.606
144.716
138.541
137.245
133.772
133.615
133.587
124.918
123.793
121.833
121.441
106.781
104.113
101.360
100.070
60.838
57.831
56.192
47.237
45.011
36.614
17.111
0.026
BRUKER
180
160
140
120
100
80
60
40
20
0
ppm

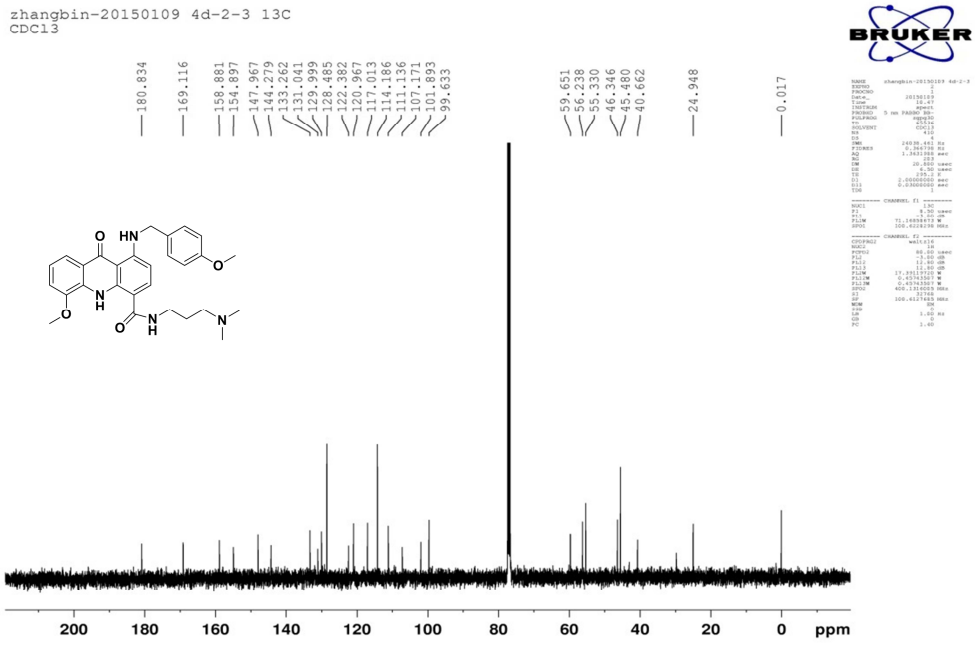

zhangb-20130719 4d-1-2 CDCl3
11.438
7.949
7.929
7.682
7.660
7.425
7.407
7.381
7.363
7.344
7.304
7.286
7.202
7.183
7.163
7.095
7.076
6.972
6.180
6.158
4.577
4.563
4.089
3.562
3.549
2.576
2.562
2.548
2.295
12
11
10
9
8
7
6
5
4
3
ppm
0.944
1.000
1.041
2.174
2.044
0.801
1.125
1.105
0.928
0.931
2.021
3.164
2.173
2.122
6.558

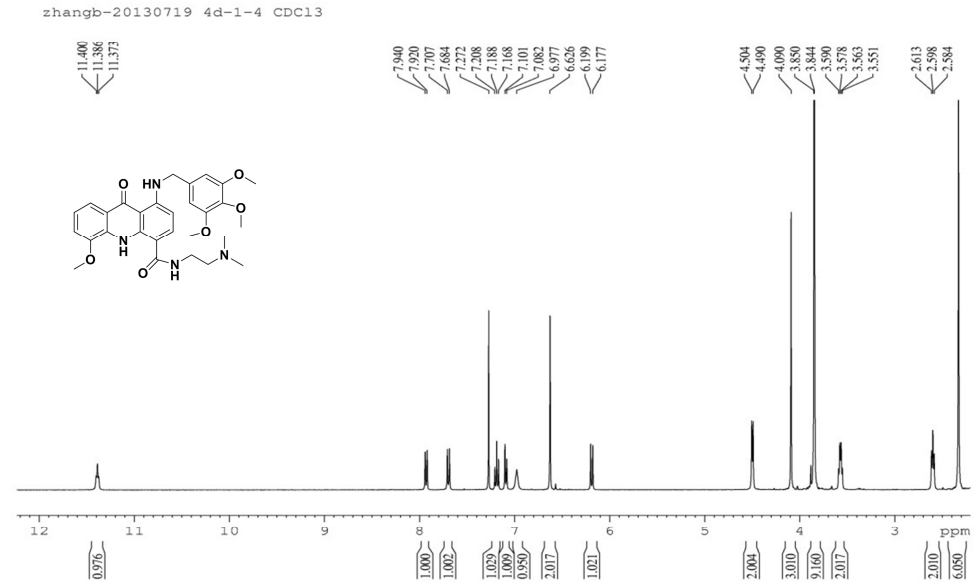

zhangb-20130719 4d-1-2 13c CDCl3
180.854
169.141
155.190
147.946
144.182
138.072
133.574
131.037
128.774
127.290
127.155
122.478
121.095
117.133
111.307
107.234
101.787
99.822
77.375
77.057
76.739
57.847
56.270
46.871
45.112
36.837
BRUKER
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 ppm

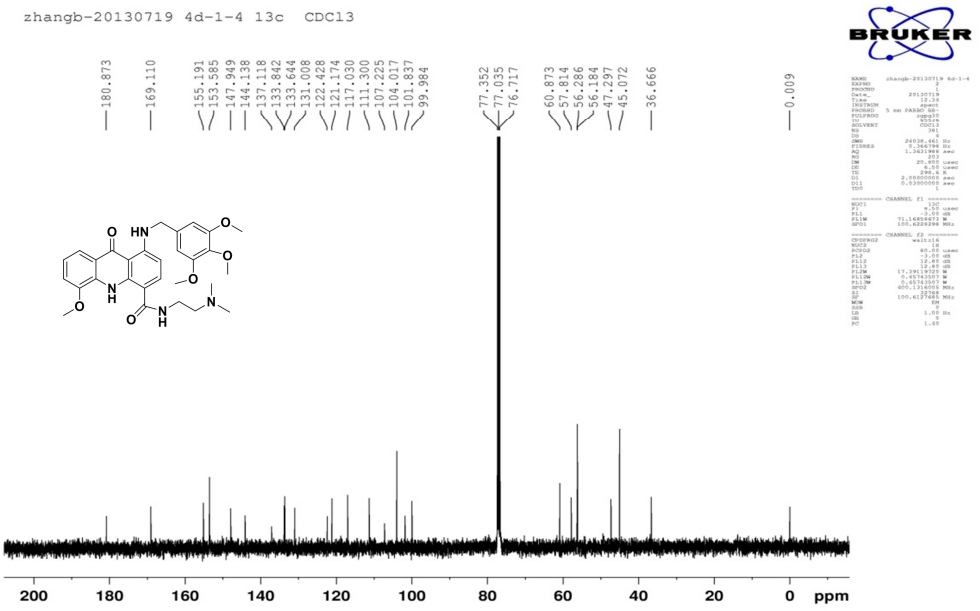

zhangbin-20150106 4d-1-3
CDCl3
13.573
11.364
7.927
7.907
7.684
7.662
7.333
7.312
7.264
7.185
7.165
7.145
7.081
7.062
6.957
6.896
6.875
6.207
6.185
4.499
4.486
4.076
3.799
3.581
3.568
3.555
3.542
2.609
2.595
2.582
2.326
0.003
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.897
0.972
1.000
1.010
2.016
1.050
1.066
0.895
2.109
1.046
2.080
3.046
3.084
2.048
2.042
6.029

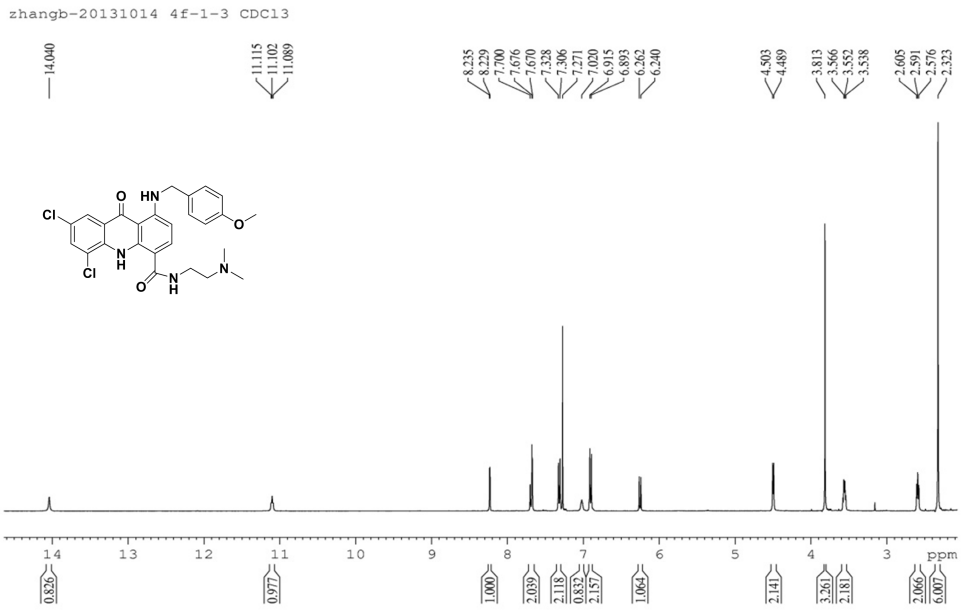

zhangbin-20150106 4d-1-3 13C
CDCl3
BRUKER
180.791
169.139
158.931
155.155
147.916
144.211
133.586
131.000
130.008
128.418
122.489
121.038
117.124
114.229
111.253
107.164
101.558
99.820
57.857
56.245
55.301
46.347
45.029
36.639
0.028
200
180
160
140
120
100
80
60
40
20
0
ppm

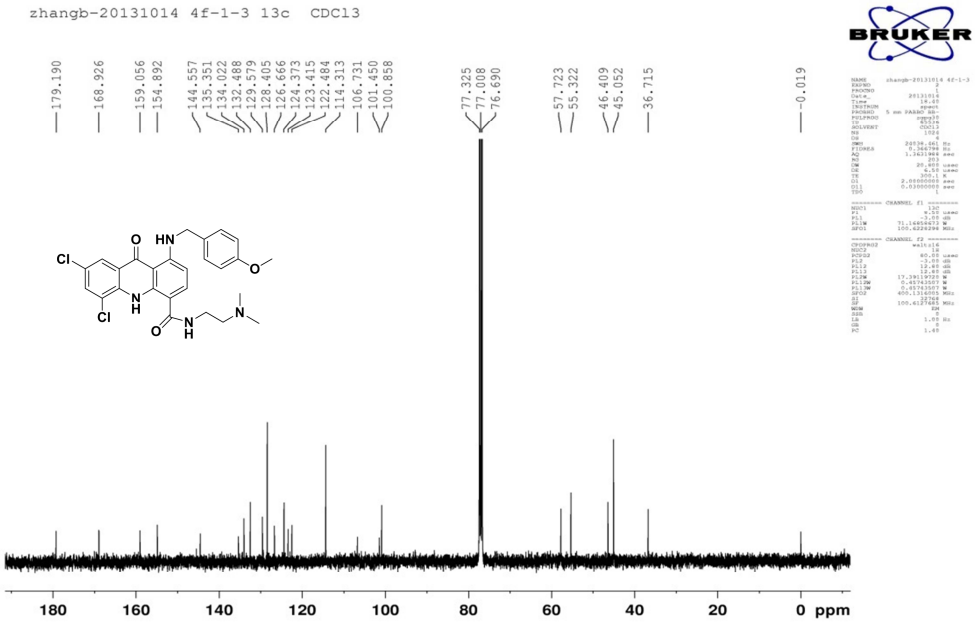

zhangbin-20150109 4d-2-3
CDCl3
13.836
11.331
11.319
11.306
8.662
7.918
7.898
7.544
7.521
7.345
7.324
7.264
7.176
7.157
7.136
7.074
7.056
6.905
6.883
6.196
6.173
4.497
4.484
4.073
3.804
3.616
3.602
3.590
3.576
2.553
2.539
2.525
1.813
1.798
15
14
13
12
11
10
9
8
7
6
5
4
3
2
0.952
0.989
0.966
1.019
1.049
2.027
1.074
1.050
2.022
1.000
2.030
3.012
3.011
2.098
2.068
6.005
2.192

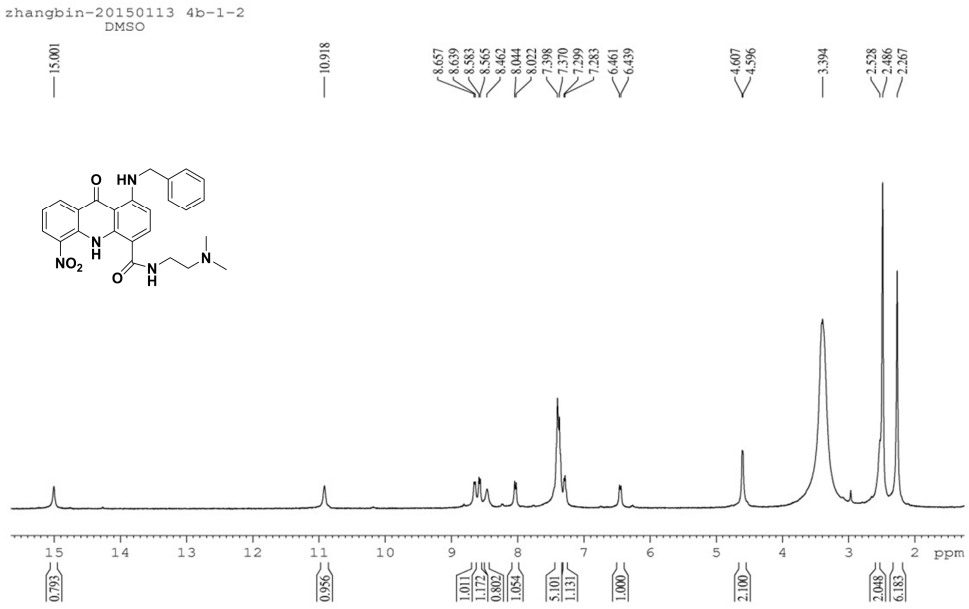

zhangbin-20150109 4d-2-3 13C
CDCl3
BRUKER
180.834
169.116
158.881
154.897
147.967
144.279
133.262
131.041
129.999
128.485
122.382
120.967
117.013
114.186
111.136
107.171
101.893
99.633
59.651
56.238
55.330
46.346
45.480
40.662
24.948
0.017
HN
O
N
H
O
O
N
O
N
200
180
160
140
120
100
80
60
40
20
0
ppm

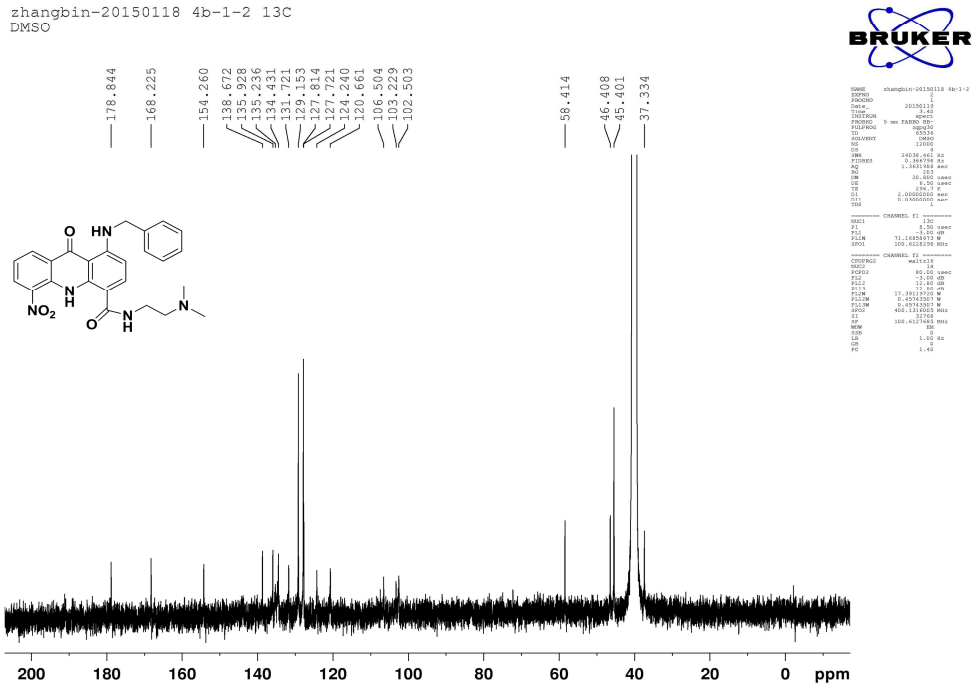

zhangb-20130719 4d-1-4 CDCl3
11.400
11.386
11.373
7.940
7.920
7.707
7.684
7.272
7.208
7.188
7.168
7.101
7.082
6.977
6.626
6.199
6.177
4.504
4.490
4.090
3.850
3.844
3.590
3.578
3.563
3.551
2.613
2.598
2.584
HN
O
N
H
O
O
O
O
N
12
11
10
9
8
7
6
5
4
3
ppm
0.976
1.000
1.002
1.029
1.009
0.950
2.017
1.021
2.004
3.010
9.160
2.017
2.010
6.050

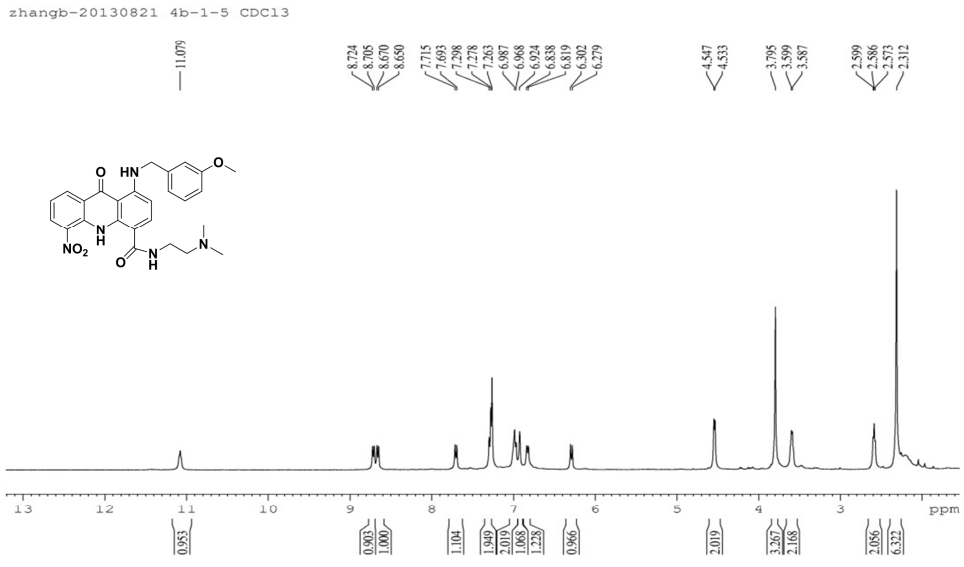

zhangb-20130719 4d-1-4 13c CDCl3
BRUKER
180.873
169.110
155.191
153.585
147.949
144.138
137.118
133.842
133.644
131.008
122.428
121.174
117.030
111.300
107.225
104.017
101.837
99.984
77.352
77.035
76.717
60.873
57.814
56.286
56.184
47.297
45.072
36.666
0.009
200
180
160
140
120
100
80
60
40
20
0
ppm

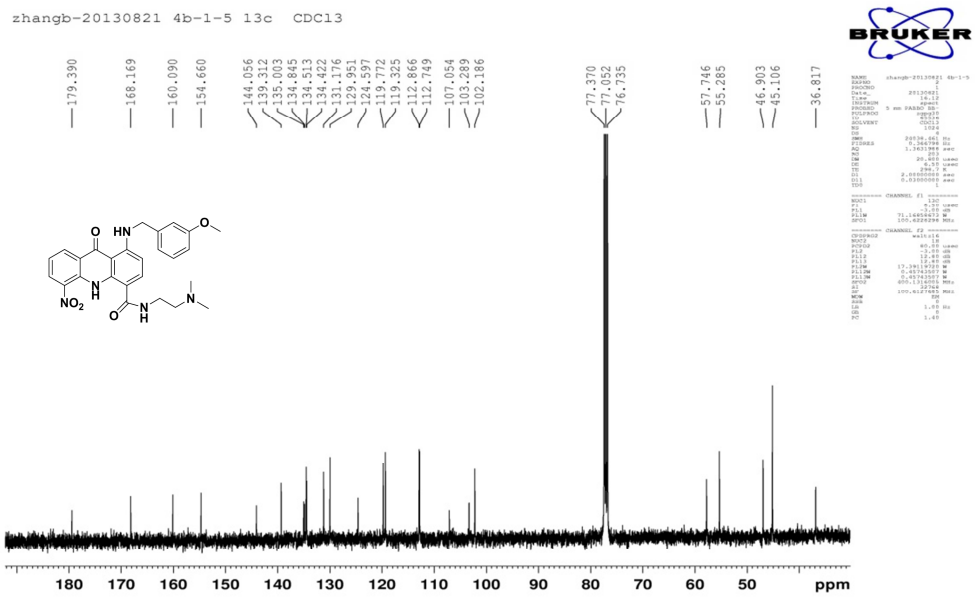

zhangb-20131014 4f-1-3 CDCl3
14.040
11.115
11.102
11.089
8.235
8.229
7.700
7.676
7.670
7.328
7.306
7.271
7.020
6.915
6.893
6.262
6.240
4.503
4.489
3.813
3.566
3.552
3.538
2.605
2.591
2.576
2.323
14
13
12
11
10
9
8
7
6
5
4
3
ppm
0.826
0.977
1.000
2.039
2.118
0.832
2.157
1.064
2.141
3.261
2.181
2.066
6.007

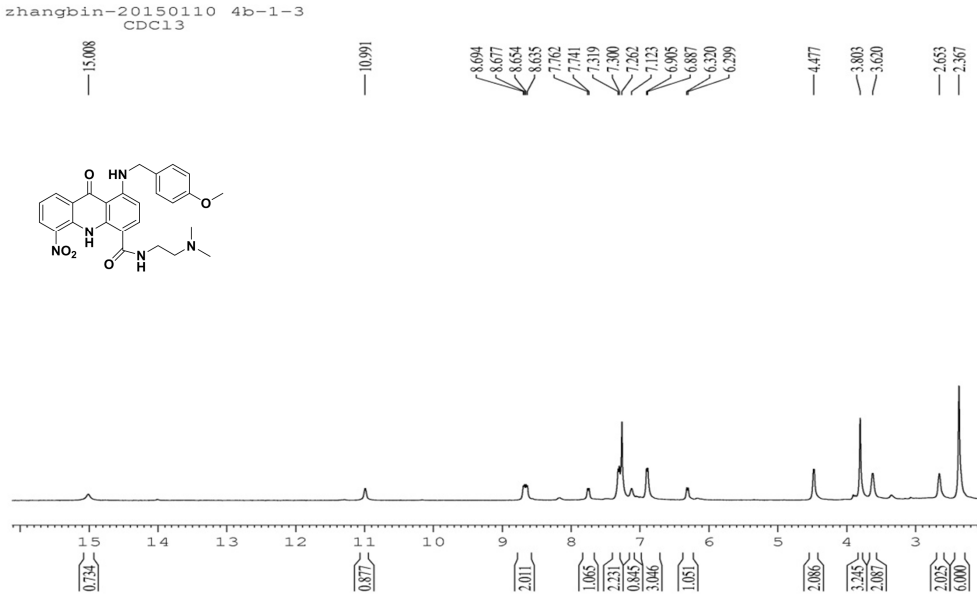

zhangb-20131014 4f-1-3 13c CDCl3
BRUKER
179.190
168.926
159.056
154.892
144.557
135.351
134.022
132.488
129.579
128.405
126.666
124.373
123.415
122.484
114.313
106.731
101.450
100.858
77.325
77.008
76.690
57.723
55.322
46.409
45.052
36.715
0.019
Cl
O
HN
O
N
H
Cl
N
H
O
N
H
180
160
140
120
100
80
60
40
20
0 ppm

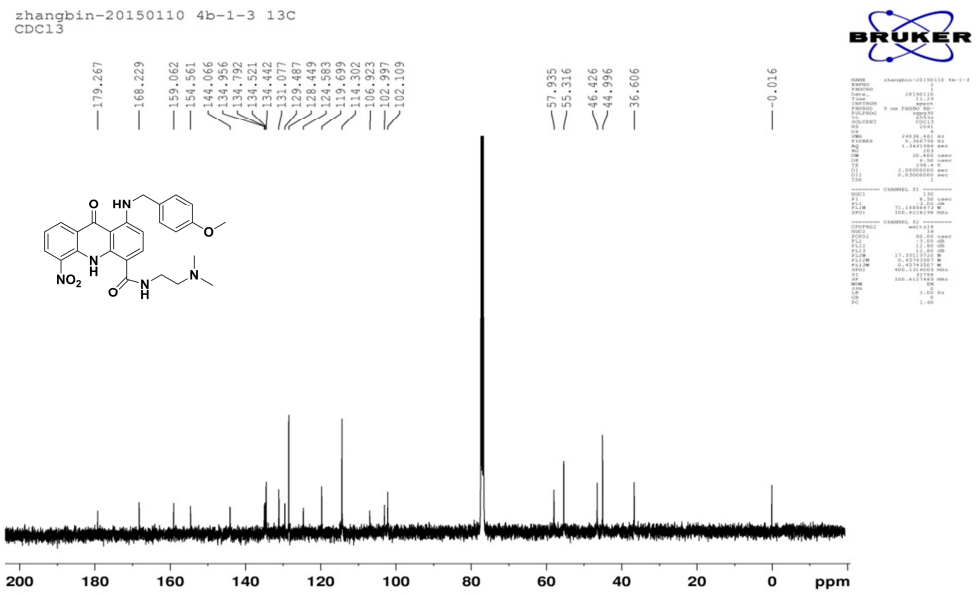

zhangbin-20150113 4b-1-2
DMSO
15.001
10.918
8.657
8.639
8.583
8.565
8.462
8.044
8.022
7.398
7.370
7.299
7.283
6.461
6.439
4.607
4.596
3.394
2.528
2.486
2.267
O
HN
N
H
NO2
O
N
H
N
15
14
13
12
11
10
9
8
7
6
5
4
3
2 ppm
0.793
0.956
1.011
1.172
0.802
1.054
5.101
1.131
1.000
2.100
2.048
6.183

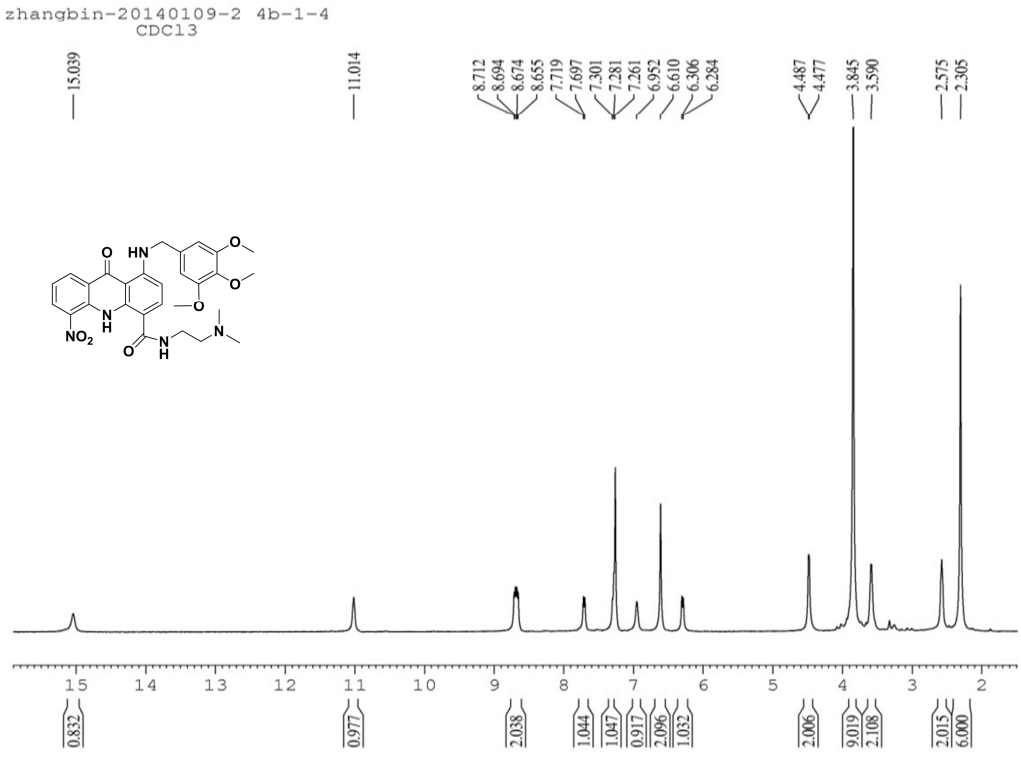

zhangbin-20150118 4b-1-2 13C
DMSO
BRUKER
178.844
168.225
154.260
138.672
135.928
135.236
134.431
131.721
129.153
127.814
127.721
124.240
120.661
106.504
103.229
102.503
58.414
46.408
45.401
37.334
O
HN
N
H
NO2
O
N
H
N
200
180
160
140
120
100
80
60
40
20
0
ppm

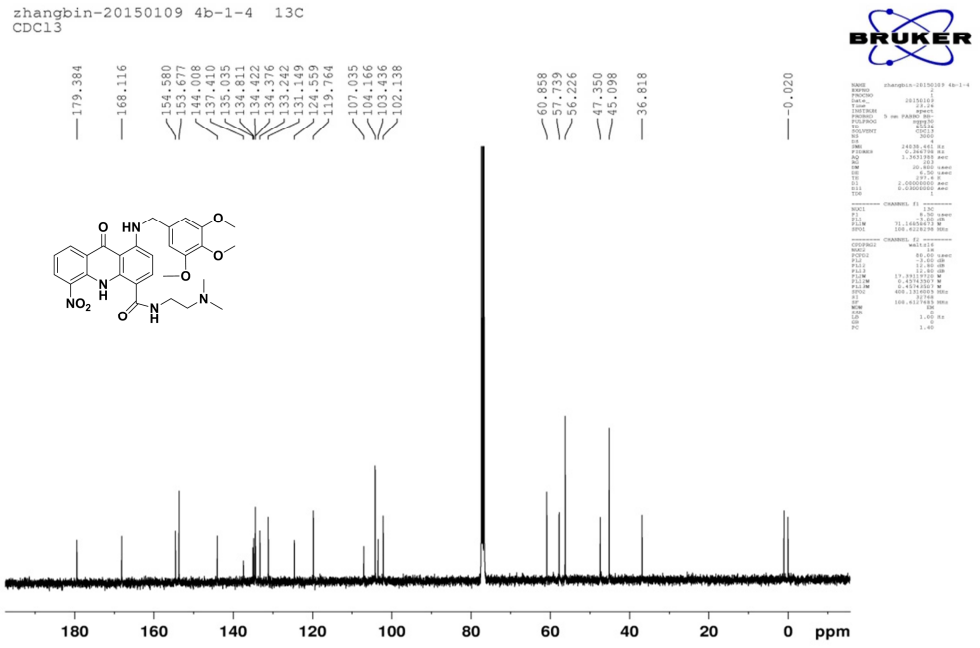

zhangb-20130821 4b-1-5 CDCl3
11.079
8.724
8.705
8.670
8.650
7.715
7.693
7.298
7.278
7.263
6.987
6.968
6.924
6.838
6.819
6.302
6.279
4.547
4.533
3.795
3.599
3.587
2.599
2.586
2.573
2.312
O
HN
O
N
H
NO2
O
N
13
12
11
10
9
8
7
6
5
4
3
ppm
0.953
0.903
1.000
1.104
1.949
2.019
1.068
1.228
0.966
2.019
3.267
2.168
2.056
6.322


zhangb-20130821 4b-1-5 13c CDCl3
BRUKER
179.390
168.169
160.090
154.660
144.056
139.312
135.003
134.845
134.513
134.422
131.176
129.951
124.597
119.772
119.325
112.866
112.749
107.054
103.289
102.186
77.370
77.052
76.735
57.746
55.285
46.903
45.106
36.817
180 170 160 150 140 130 120 110 100 90 80 70 60 50 ppm


zhangbin-20150110 4b-1-3
CDCl3
15.008
10.991
8.694
8.677
8.654
8.635
7.762
7.741
7.319
7.300
7.262
7.123
6.905
6.887
6.320
6.299
4.477
3.803
3.620
2.653
2.367
15 14 13 12 11 10 9 8 7 6 5 4 3 2
0.734
0.877
2.011
1.065
2.231
0.845
3.046
1.051
2.086
3.245
2.087
2.025
6.000


zhangbin-20150110 4b-1-3 13C
CDCl3
BRUKER
179.267
168.229
159.062
154.561
144.066
134.956
134.792
134.521
134.442
131.077
129.487
128.449
124.583
119.699
114.302
106.923
102.997
102.109
57.935
55.316
46.426
44.996
36.606
0.016
200
180
160
140
120
100
80
60
40
20
0
ppm


zhangbin-20140109-2 4b-1-4
CDCl3
15.039
11.014
8.712
8.694
8.674
8.655
7.719
7.697
7.301
7.281
7.261
6.952
6.610
6.306
6.284
4.487
4.477
3.845
3.590
2.575
2.305
15
14
13
12
11
10
9
8
7
6
5
4
3
2
0.832
0.977
2.038
1.044
1.047
0.917
2.096
1.032
2.006
9.019
2.108
2.015
6.000


zhangbin-20150109 4b-1-4 13C
CDCl3
BRUKER
179.384
168.116
154.580
153.677
144.008
137.410
135.035
134.811
134.422
134.376
133.242
131.149
124.559
119.764
107.035
104.166
103.436
102.138
60.858
57.739
56.226
47.350
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36.818
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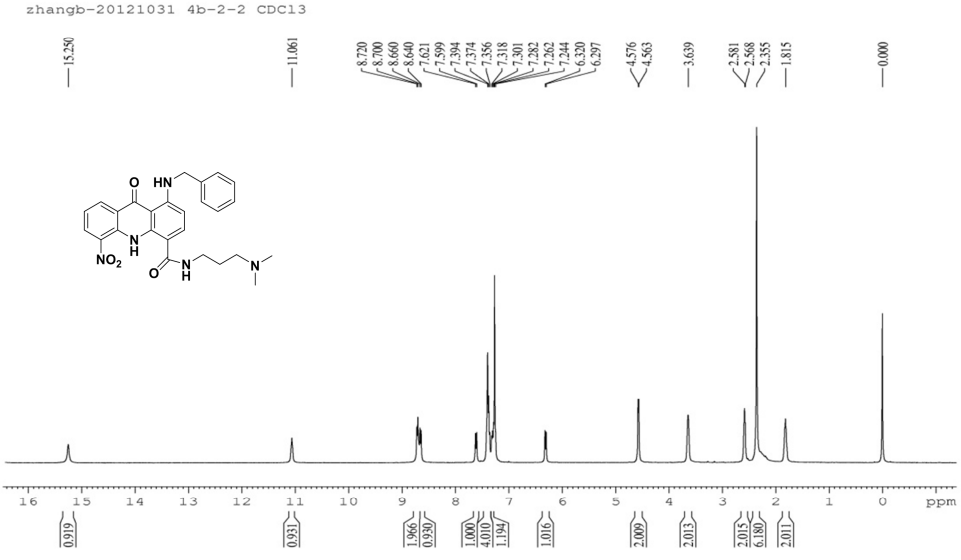
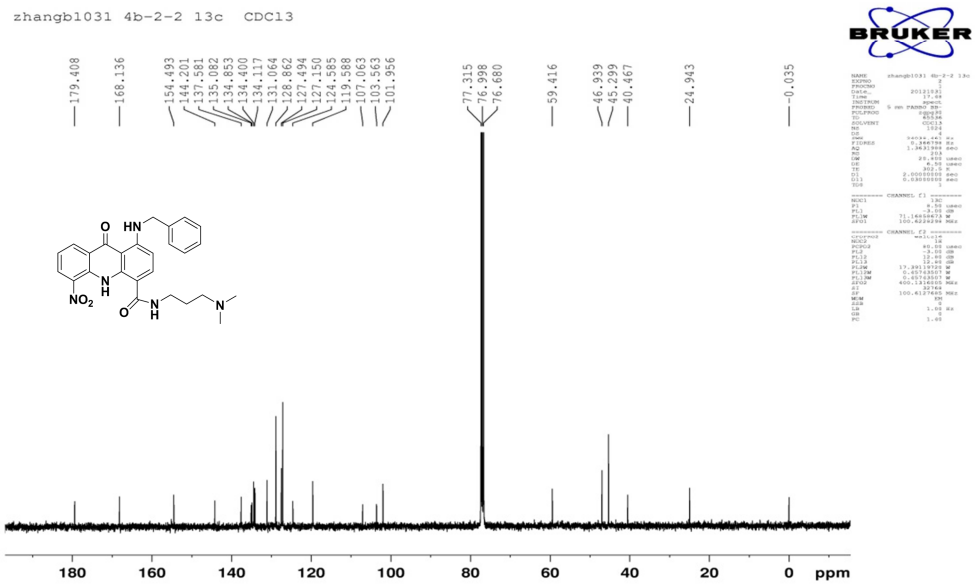
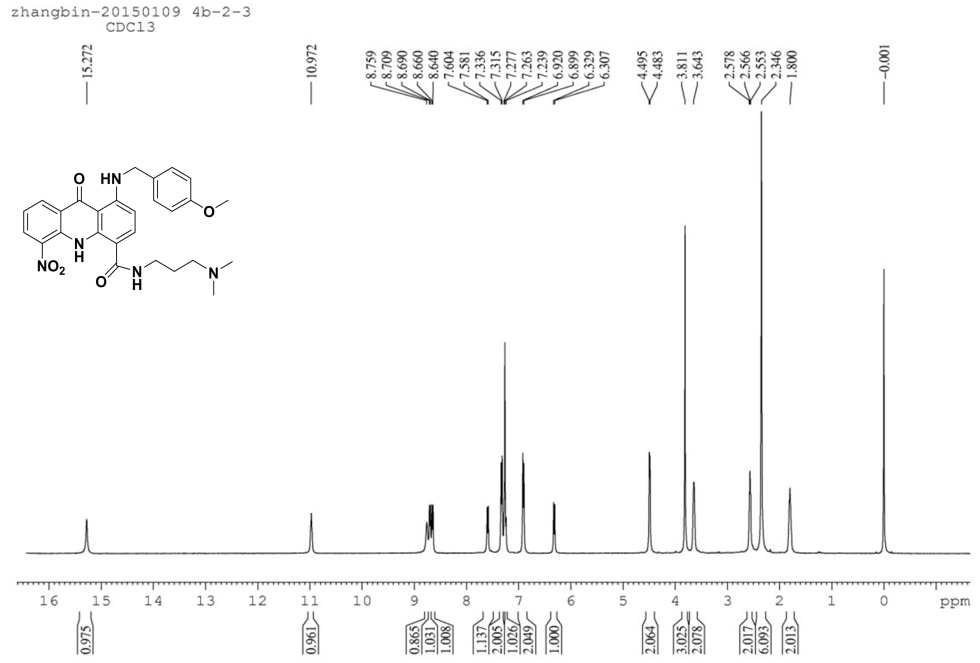
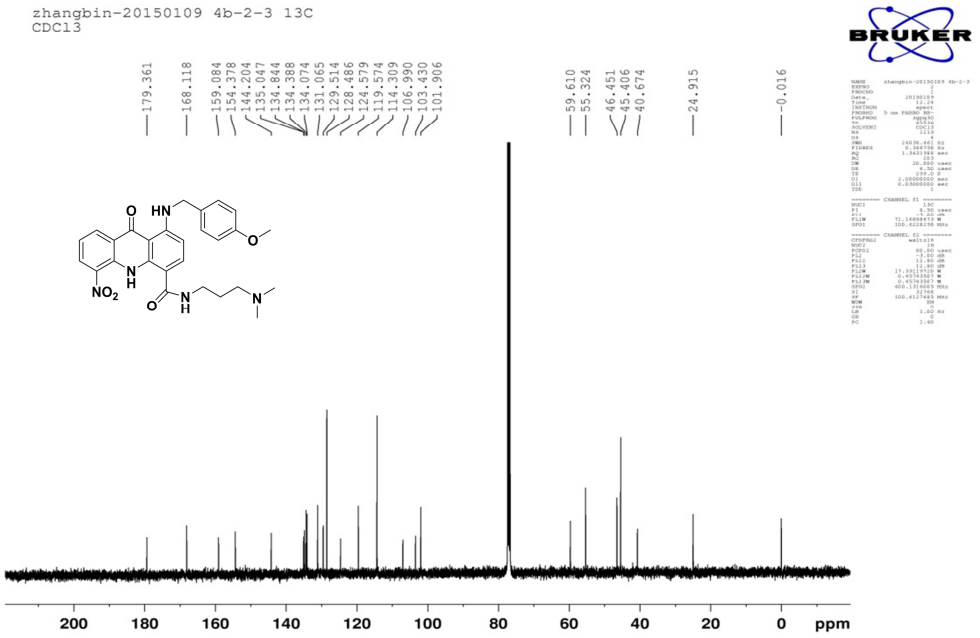
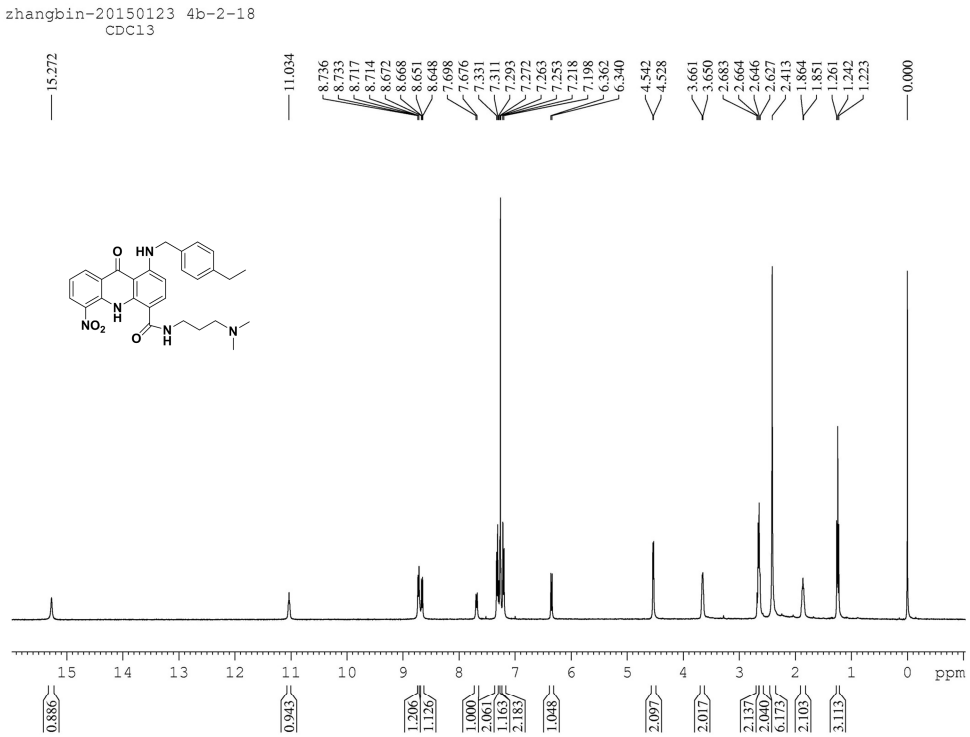
120
100
80
60
40
20
0
ppm

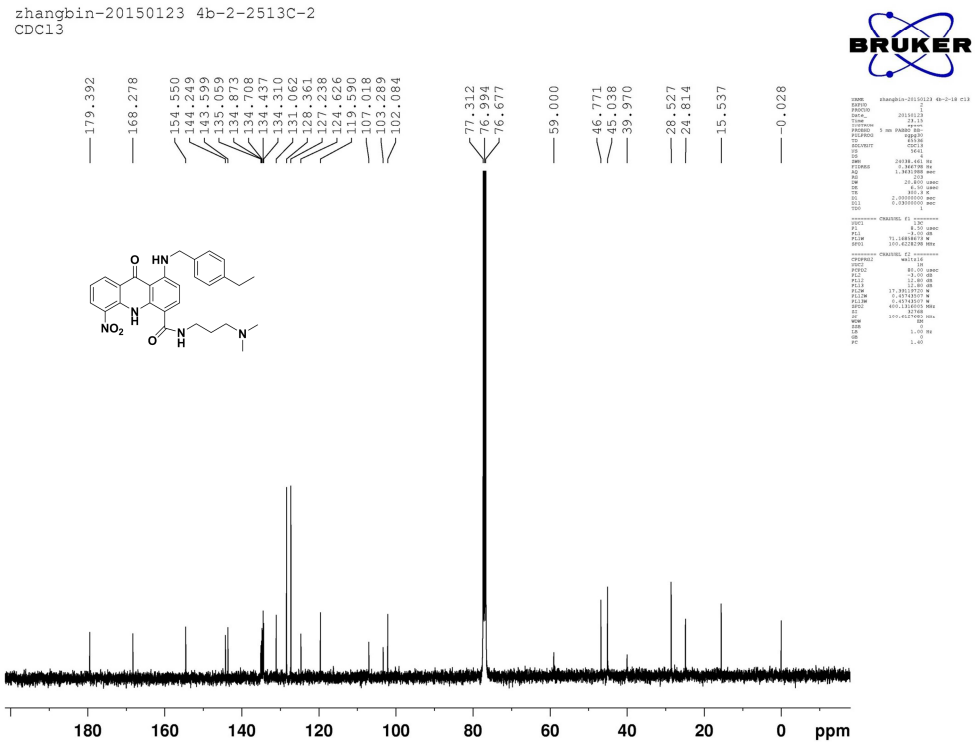

zhangb-20121031 4b-2-2 CDCl3
15.250
11.061
8.720
8.700
8.660
8.640
7.621
7.599
7.394
7.374
7.356
7.318
7.301
7.282
7.262
7.244
6.320
6.297
4.576
4.563
3.639
2.581
2.568
2.355
1.815
0.000
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.919
0.931
1.966
0.930
1.000
4.010
1.194
1.016
2.009
2.013
2.015
6.180
2.011

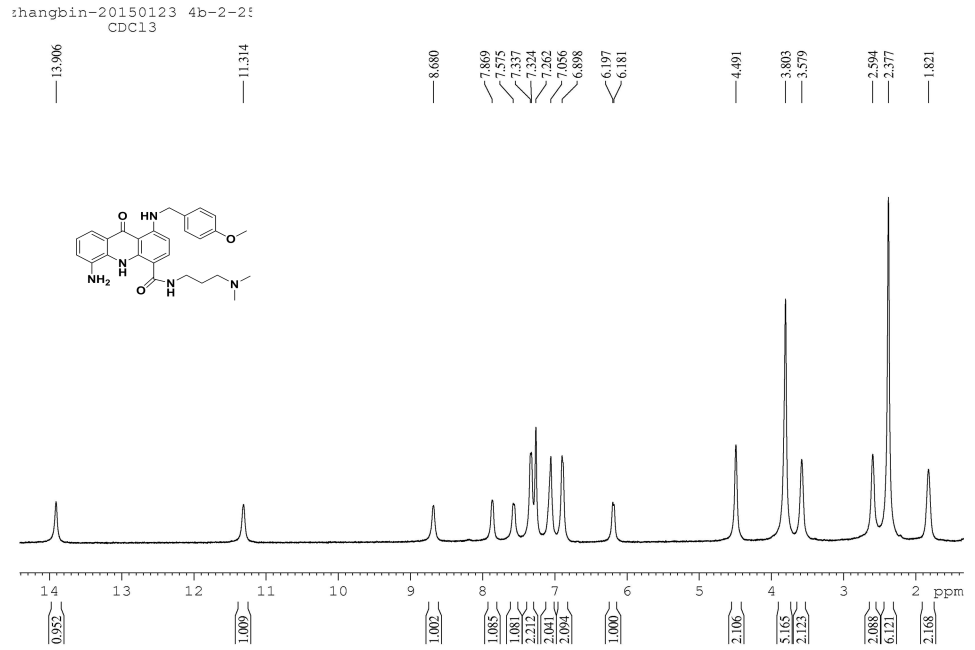

zhangb1031 4b-2-2 13c CDCl3
BRUKER
179.408
168.136
154.493
144.201
137.581
135.082
134.853
134.400
134.117
131.064
128.862
127.494
127.150
124.585
119.588
107.063
103.563
101.956
77.315
76.998
76.680
59.416
46.939
45.299
40.467
24.943
-0.035
O
HN
N
H
NO2
O
N
H
N
180
160
140
120
100
80
60
40
20
0
ppm

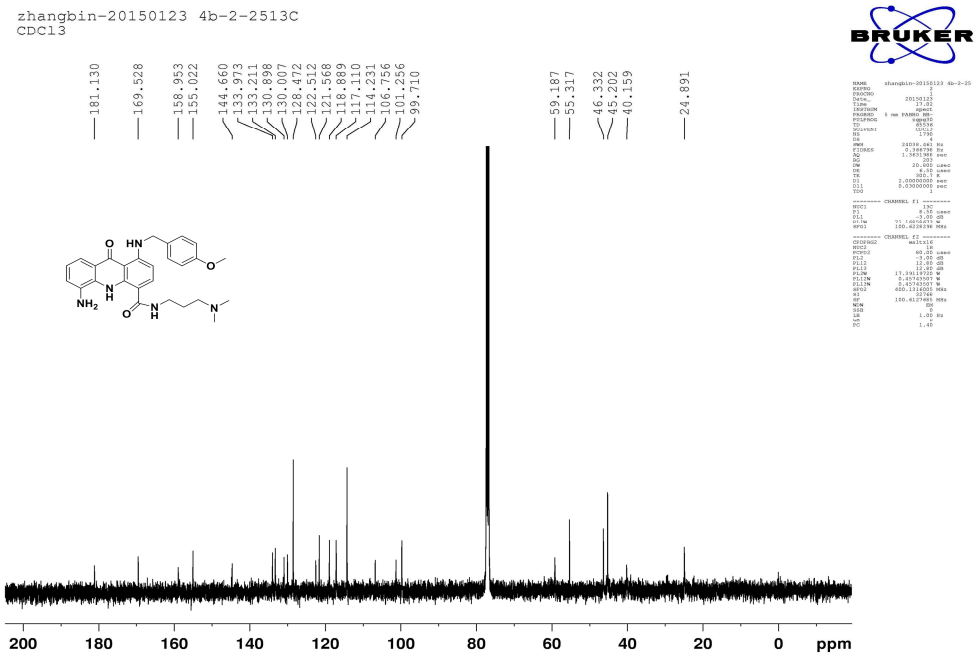

zhangbin-20150109 4b-2-3
CDCl3
15.272
10.972
8.759
8.709
8.690
8.660
8.640
7.604
7.581
7.336
7.315
7.277
7.263
7.239
6.920
6.899
6.329
6.307
4.495
4.483
3.811
3.643
2.578
2.566
2.553
2.346
1.800
-0.001
O
HN
O
N
H
NO2
O
N
H
N
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.975
0.961
0.865
1.031
1.008
1.137
2.005
1.026
2.049
1.000
2.064
3.025
2.078
2.017
6.093
2.013

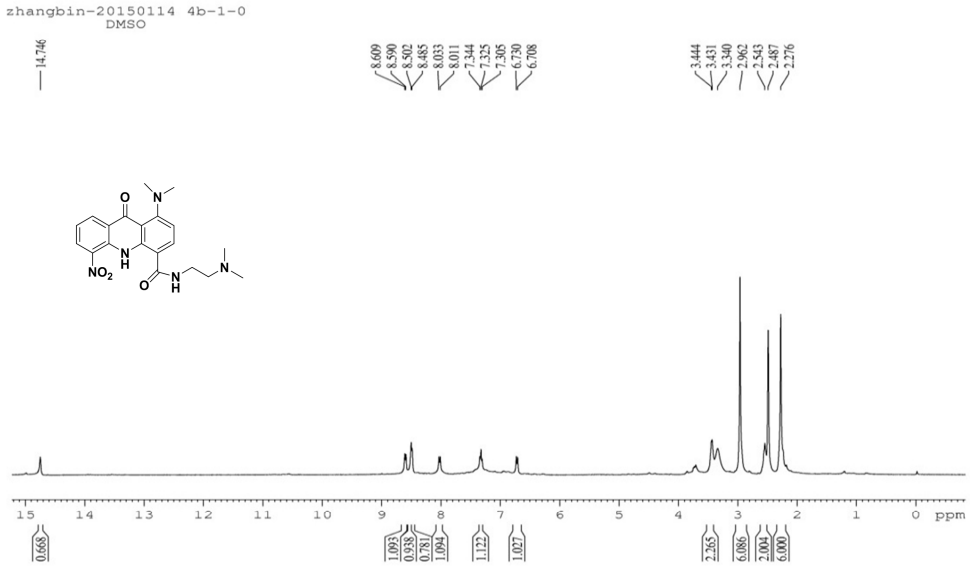

zhangbin-20150109 4b-2-3 13C
CDCl3
BRUKER
179.361
168.118
159.084
154.378
144.204
135.047
134.844
134.388
134.074
131.065
129.514
128.486
124.579
119.574
114.309
106.990
103.430
101.906
59.610
55.324
46.451
45.406
40.674
24.915
-0.016
200
180
160
140
120
100
80
60
40
20
0
ppm

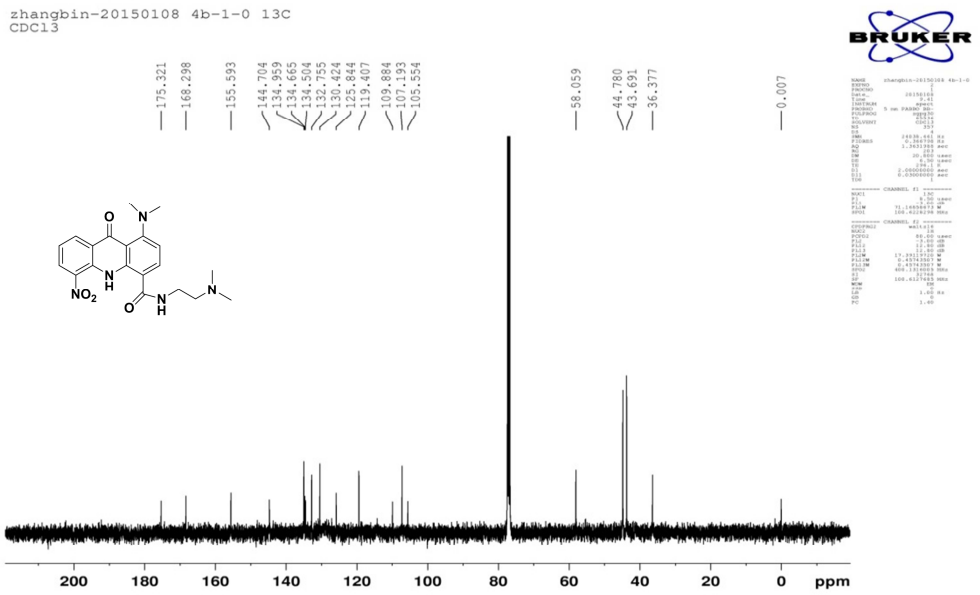

zhangbin-20150123 4b-2-18
CDCl3
15.272
11.034
8.736
8.733
8.717
8.714
8.672
8.668
8.651
8.648
7.698
7.676
7.331
7.311
7.293
7.272
7.263
7.253
7.218
7.198
6.362
6.340
4.542
4.528
3.661
3.650
2.683
2.664
2.646
2.627
2.413
1.864
1.851
1.261
1.242
1.223
0.000
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.886
0.943
1.206
1.126
1.000
2.061
1.163
2.183
1.048
2.097
2.017
2.137
2.040
6.173
2.103
3.113

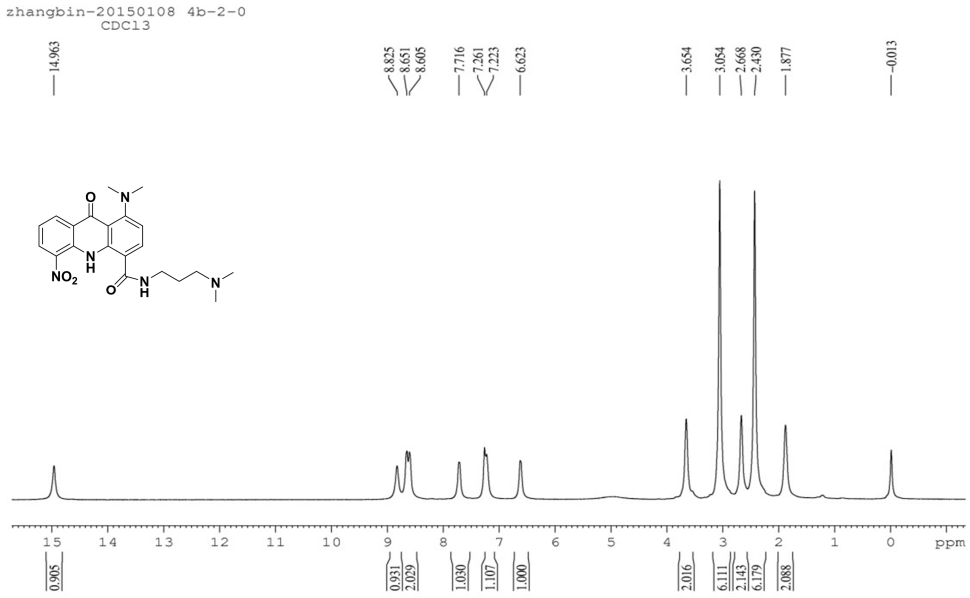

zhangbin-20150123 4b-2-2513C-2
CDCl3
BRUKER
179.392
168.278
154.550
144.249
143.599
135.059
134.873
134.708
134.437
134.310
131.062
128.361
127.238
124.626
119.590
107.018
103.289
102.084
77.312
76.994
76.677
59.000
46.771
45.038
39.970
28.527
24.814
15.537
-0.028
O
HN
N
H
NO2
O
N
H
N
180
160
140
120
100
80
60
40
20
0
ppm

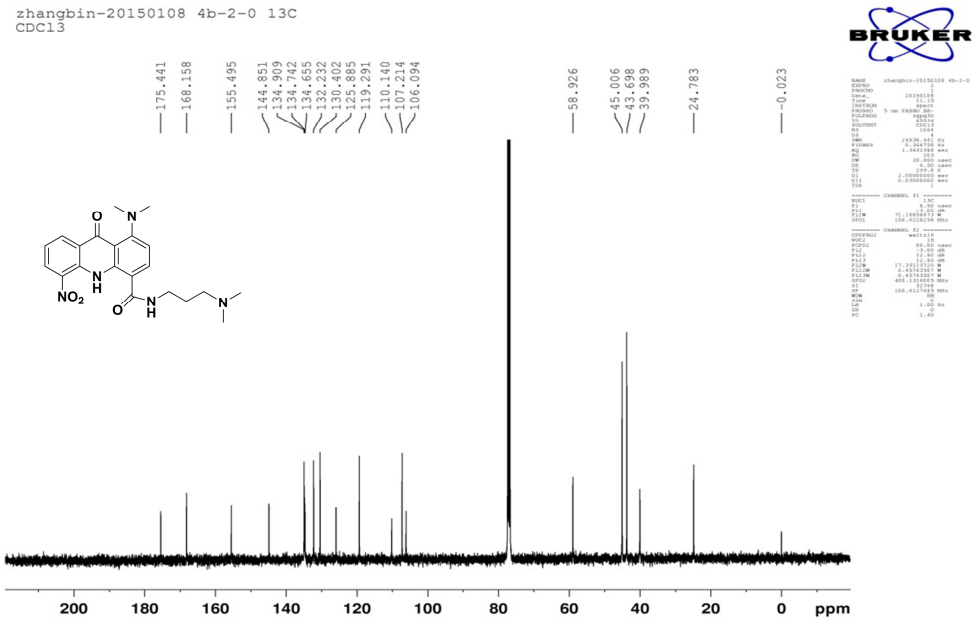

zhangbin-20150123 4b-2-2
CDCl3
13.906
11.314
8.680
7.869
7.575
7.337
7.324
7.262
7.056
6.898
6.197
6.181
4.491
3.803
3.579
2.594
2.377
1.821
O
HN
O
N
H
NH2
O
N
H
N
14
13
12
11
10
9
8
7
6
5
4
3
2
ppm
0.952
1.009
1.002
1.085
1.081
2.212
2.041
2.094
1.000
2.106
5.165
2.123
2.088
6.121
2.168


zhangbin-20150123 4b-2-2513C
CDCl3
181.130
169.528
158.953
155.022
144.660
133.973
133.211
130.898
130.007
128.472
122.512
121.568
118.889
117.110
114.231
106.756
101.256
99.710
59.187
55.317
46.332
45.202
40.159
24.891
200
180
160
140
120
100
80
60
40
20
0
ppm


zhangbin-20150114 4b-1-0
DMSO
14.746
8.609
8.590
8.502
8.485
8.033
8.011
7.344
7.325
7.305
6.730
6.708
3.444
3.431
3.340
2.962
2.543
2.487
2.276
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.668
1.093
0.938
0.781
1.094
1.122
1.027
2.265
6.086
2.004
6.000


zhangbin-20150108 4b-1-0 13C
CDCl3
BRUKER
175.321
168.298
155.593
144.704
134.959
134.665
134.564
132.755
130.424
125.844
119.407
109.884
107.193
105.554
58.059
44.780
43.691
36.377
0.007
200
180
160
140
120
100
80
60
40
20
0
ppm


zhangbin-20150108 4b-2-0
CDCl3
14.963
8.825
8.651
8.605
7.716
7.261
7.223
6.623
3.654
3.054
2.668
2.430
1.877
0.013
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.905
0.931
2.029
1.030
1.107
1.000
2.016
6.111
2.143
6.179
2.088



6. High resolution mass spectrometry