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Novel synthetic acridine-based derivatives as topoisomerase I inhibitors

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ABSTRACT

Novel DNA binding agents against topoisomerases are needed for effective treatment of cancers. A series of new acridine-based derivatives **7a**–**7d** were synthesized and their antiproliferative activity against K562 and HepG-2 cell lines were evaluated. Compound **7c** with pyridin-2-yl-methanamino group substituted at the C9 position of acridine showed good antitumor activity against both cell lines. The DNA-binding affinity of compound **7c** was evaluated by UV–vis absorption spectra and fluorescence emission spectra. DNA topoisomerase I mediated relaxation of plasmid pBR322 DNA was also tested. Our results suggested that compound **7c** with good antitumor activity and topoisomerase I inhibition activity can be developed as a prime candidate for further chemical optimization.

1. Introduction

Cancer is one of the most common malignant diseases obsessing mankind and new effective drugs are needed [1–6]. DNA topoisomerase, either in prokaryotes or eukaryotes, plays a very important role in cell proliferation, survival and apoptosis, which has been one of the most potential targets for the development of new anticancer agents [7–9].

Acridine analogs have been used for treatment of inflammation and cancer for many years. The unique planar ring structure makes its strong interaction with DNA base pairs [10,11]. A variety of acridine derivatives have been designed and synthesized, some of which have entered clinical studies, such as DACA [12–14], C-1305 [15] and *m*-AMSA, *etc.* (Fig. 1) [16]. Among them, *m*-AMSA was the first used in clinical treatment for several cancers as topoisomerase inhibitors and much attention had been paid to the modification of *m*-AMSA to improve its activity and bioavailability [17]. A variety of analogs of *m*-AMSA, such as AHMA and D3CLP (Fig. 1), have been developed [18,14]. By far, most of the modification of the *m*-AMSA was focused on the position and nature of substituents in the 9-aminobenzene moiety and acridine rings. A variety of bis-acridine derivatives have also been developed to increase the DNA binding affinity. However, little attention has been paid to the replacement of benzene ring by naphthalene or heterocycles, such as pyridine.

Previously, we have found that the linker between acridine and benzene ring played an important role in the antitumor activity and identified the $-NHCH_2-$ or $-NHCH_2CH_2-$ linker was appropriate [18] as part of our continuous efforts [18,16,19–23] in developing novel antitumor compounds. In this paper we synthesized a series of naphthalene, pyridine and indole substituted-acridine analogs with $-NHCH_2-$ or $-NHCH_2CH_2-$ linker as potent antitumor agents. Our results indicated that some of these 9-heteroaromatic substituted acridines displayed better antiproliferative activity than the corresponding 9-benzene substituted acridine **5b** [18]. The DNA binding capability and topoisomerase I (topo I) mediated relaxation of plasmid pBR322 DNA were also evaluated.

2. Experimental

The synthetic methods and the preparation of compounds **3** and **4** can be found in the Supporting information.

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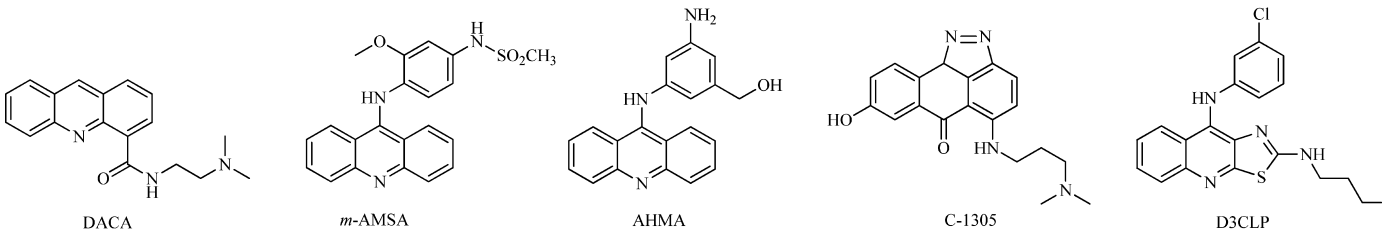
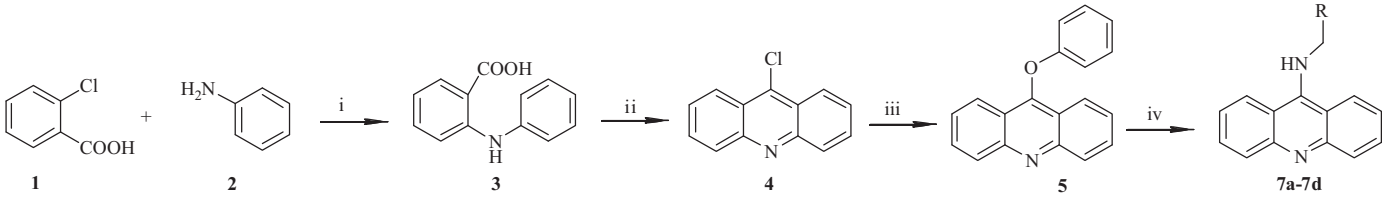


Fig. 1. The structures of some acridine derivatives.



Scheme 1. Synthesis of acridine derivatives **7a**–**7d**. Reagents and conditions: (i) K_2CO_3, Cu, DMF, 130 °C; (ii) $POCl_3$, 105 °C; (iii) phenol, 60 °C. (iv) the corresponding amines **7a**–**7d**, 120 °C.

General procedure for compounds (**7a-7d**): Initially, compound **4** (1.0 mmol) and phenol (10 mmol) were added to a 100 mL dried round-bottom. The mixture was incubated at 60 °C for 1 h under argon atmosphere to give the intermediate **5**. Then, the corresponding amine **6** (1.1 mmol) was added and the mixture was heated to 130 °C for 2 h. The mixture was then poured into a mixture of *N*-methyl morpholine (1 mL) and ethyl acetate (100 mL). The precipitation was separated by vacuum filtration to give the crude products (**7a**–**7d**) (Scheme 1).

N-(2-(1*H*-indol-3-yl)ethyl)acridin-9-amine (**7a**). Compound **7a** was purified by column chromatography (methanol/ethyl acetate = 1/4, v/v). Yield 57%; mp 245–247 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.92 (s, 1H), 8.60 (d, 2H, J = 8.3 Hz), 8.00–7.86 (m, 4H), 7.58 (d, 1H, J = 7.8 Hz), 7.50 (t, *2H*, J = 6.7 Hz), 7.33 (d, 1H, J = 8.1 Hz), 7.25 (d, 1H, J = 2.2 Hz), 7.06 (t, 1H, J = 7.2 Hz), 6.95 (t, 1H, J = 7.2 Hz), 5.30–5.29 (m, 1H), 4.37 (t, 2H, J = 7.4 Hz), 4.35 (t, 2H, J = 7.4 Hz). HR-MS (ESI): Calcd. for $[M+H]^+$: m/z 338.1657; found: 338.1670.

N-((Pyridin-4-yl)methyl)acridin-9-amine (**7b**). Compound **7b** was purified by recrystallization from ethyl acetate. Yield 27%; mp 225–228 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.75 (s, 1H), 8.67–8.48 (m, 3H), 8.03–7.95 (m, 4H), 7.92 (d, 1H, J = 7.8 Hz), 7.51 (s, 2H), 7.43 (dd, 1H, J = 7.7, 4.8 Hz), 5.41 (s, 2H). HR-MS (ESI): Calcd. for $[M+H]^+$: m/z 286.1344; found: 286.1345.

N-((Pyridin-2-yl)methyl)acridin-9-amine (**7c**). Compound **7c** was purified by recrystallization from ethyl acetate. Yield 25%; mp 228–229 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.77 (s, 1H), 8.66–8.46 (m, 3H), 8.08–7.97 (m, 2H), 7.92–7.74 (m, 3H), 7.52–7.45 (m, 3H), 5.41 (s, 2H). HR-MS (ESI): Calcd. for $[M+H]^+$: m/z 286.1344; found: 286.1338.

N-((Naphthalen-1-yl)methyl)acridin-9-amine (**7d**). Compound **7d** was purified by recrystallization from DMSO/ethyl acetate (1/1, v/v). Yield 46%; mp 249–252 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.13–8.09 (m, 2H), 8.09–8.04 (m, 1H), 8.01–7.93 (m, 5H), 7.73 (d, 1H, J = 7.0 Hz), 7.65 (dd, 3H, J = 6.3, 3.3 Hz), 7.59–7.51 (m, 1H), 7.43 (s, 2H), 5.77 (s, 2H). HR-MS (ESI): Calcd. for $[M+H]^+$: m/z 335.1548; found: 335.1533.

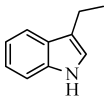
The experiments of UV–vis absorption spectroscopy and fluorescence emission; DNA topo I inhibition assay; ^{1}H NMR and high resolution mass spectra can be found in Supporting information.

3. Results and discussion

Synthesis of the acridine-based derivatives **7a**–**7d** was accomplished as described in Scheme 1. First, an Ullmann coupling reaction of 2-chloro-benzoic acid **1** with aniline **2** in DMF using Cu as the catalyst gave anthranilic acid **3**, which was then stirred in $POCl_3$ to afford the 9-chloroacridine **4**. The reaction of **4** with phenol gave the intermediate **5**, which was then reacted with the corresponding amines to afford the desired acridines **7a**–**7d**.

MTT assay was used to test whether compounds **7a**–**7d** displayed antiproliferative activity. The cytotoxicity of compounds **7a**–**7d** against K562 and HepG-2 cells was evaluated for comparison with that of the 9-benzylamino acridine (**5b**) reported in our earlier paper [18]. Colchicine and podophyllotoxin were used as the positive controls. The results can be reported in Table 1. The newly synthesized four acridines displayed moderate to good antiproliferative activity against the two tumor cell lines. The 4-pyridine substituted acridine **7b** showed about 3-fold less activity against K562 cells than **5b**. The other three compounds demonstrated similar, or more cytotoxicity compared to **5b**. The low micromolar IC_{50} values of the compounds indicate that the replacement of benzene ring by naphthalene or other heterocycles may improve the antitumor activity. As the mode of toxic action of

Table 1
Antiproliferative activity of compounds against K562 and HepG-2 cells.

Compound	R	IC_{50} (μmol/L)	
		K562 IC_{50} (μmol/L)	HepG-2 IC_{50} (μmol/L)
7a		2.92	6.187
7b		15.163	8.592
7c		2.517	10.73
7d		4.673	7.849
5b [18]		5.24	6.07
Colchicine		ND[a]	1.8
Imatinib		0.47	ND[a]

[a] Not detected.

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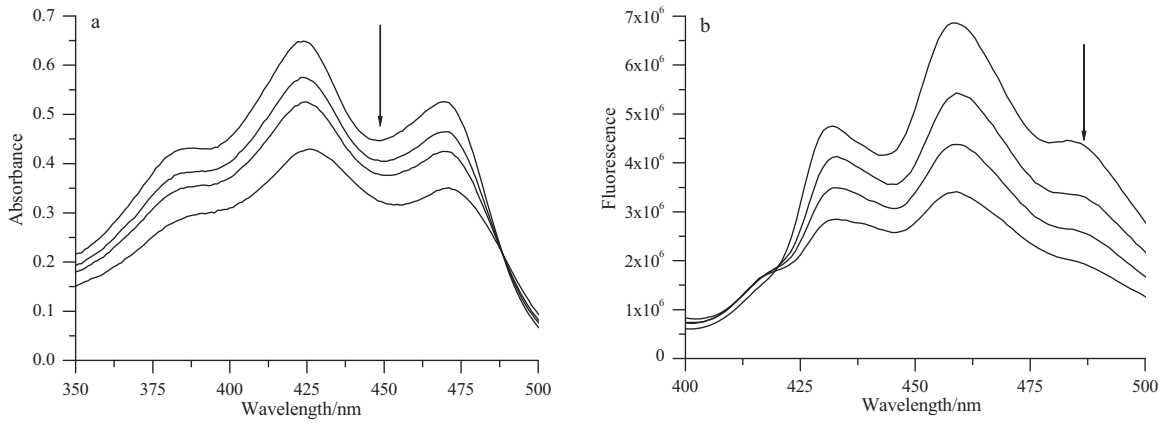

Fig. 2. (a) UV–vis absorption spectra of **7c** (62.5 μmol/L) in 10 mmol/L Tris–HCl buffer containing 10 mmol/L NaCl (pH 7.0) by increasing the concentrations of ctDNA ([DNA]/[**7c**] = 0, 0.125, 0.25, 0.5). The arrow indicates the absorbance changes upon increasing DNA concentrations. (b) Spectrofluorimetric titration of **7c** (62.5 μmol/L) in the presence of increasing amounts of ctDNA ([DNA]/[**7c**] = 0, 1, 2, 4). The arrow indicates the fluorescence emission changes upon increasing DNA concentrations.

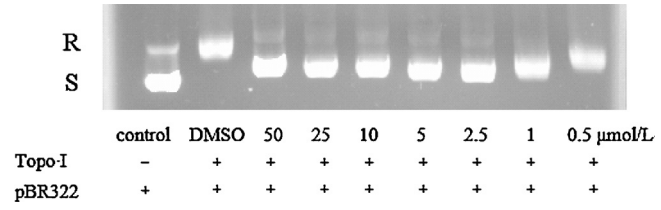

Fig. 3. Effect of the compound **7c** on the relaxation of plasmid DNA by human topo I (S: superhelix; R: relaxation).

acridines is mainly attributed to DNA and its related enzymes, compound **7c** with the best antiproliferative activity against K562 cells was selected to study the DNA binding property.

UV–vis absorption spectroscopy is extensively utilized to detecting the interaction between DNA and compounds. The interaction of compound **7c** with ctDNA was evaluated and the result shown in Fig. 2a. The major peaks in the spectrum of **7c** were observed at about 426 nm and 470 nm, while DNA did not absorb light in this region. The absorption spectra of **7c** decreased obviously by the addition of increasing amounts of ctDNA. In addition, a slight bathochromic effect was observed when ctDNA was added and a clear isosbestic point appeared. All the result suggested that compound **7c** can interact with DNA.

In addition to UV–vis absorption spectroscopy, fluorescence emission spectroscopy is also widely used to evaluate the binding ability of drugs and their targets. As shown in Fig. 2b, the fluorescence of **7c** was gradually decreased when the concentration of ct DNA increased, which suggested that there are interactions between **7c** and ct DNA.

Compound **7c** can interact with DNA, which will cause distortion of the DNA structure. Therefore, by the activity of topo I, the DNA related enzyme will be inhibited. To identify whether compound **7c** could inhibit topo I, the assay of compound **7c** on the relaxation of plasmid pBR322 DNA mediated by topo I was performed. As seen from Fig. 3, compound **7c** displayed excellent topo I inhibitory activity at about 1 μmol/L, whereas had undetectable activities at 0.5 μmol/L, which was in accordance with its antiproliferative activity. These data suggested that the antitumor activity of **7c** was attributed to its DNA binding and topo I inhibition, and might be a potential lead compound to be developed into novel topo I inhibitors.

4. Conclusion

In conclusion, a series of novel 9-aminoacridine derivatives **7a**–**7d** had been designed and synthesized, most of which showed good antiproliferative activity against both K562 and HepG-2 cells. The identified compound **7c** exhibited its antitumor activity through binding with DNA and inhibiting topo I activity, which can be developed into novel topoisomerase inhibitors. Further optimizations of the structure to improve the bioavailability and solubility are ongoing.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2014.03.028.

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