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Novel Synthetic Azaacridine Analogues as Topoisomerase 1 Inhibitors

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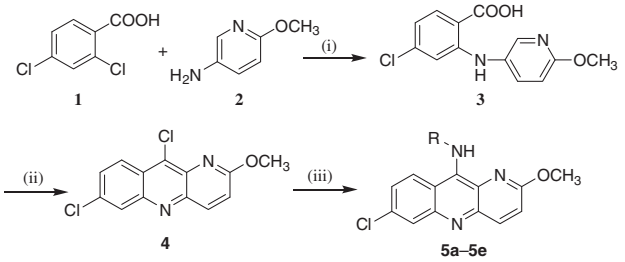
Novel azaacridine analogues were synthesized and their antiproliferative activities against K562 and HepG-2 cell lines were evaluated, among which compound **5a** was found to display good cytotoxicity. UV–visible spectral absorbance measurements showed that **5a** can bind with calf thymus DNA (ct DNA). A relaxation assay indicated that **5a** inhibits topoisomerase 1 activity.

Cancer can significantly affect people's health and development of anticancer agents, such as DNA-interacting agents which can distort the helix and affect the activity of topoisomerases, has attracted great attention.[1] Topoisomerase 1 (topo 1), one of the topoisomerases is essential for cell proliferation and it has been regarded as an important target for cancer.[2] However, topo 1 inhibitors are relatively rare and some of them have severe side effects. The success of camptosar and topotecan as topo 1 inhibitors, which have been approved and extensively used for anticancer therapy, has inspired a new search for additional agents as topo 1 inhibitors for cancer treatment.[3]

Azaacridine derivatives have been studied extensively in organic chemistry and pharmaceutical fields, most of which have been used as antibacterial,[4] antimalarial,[5] and antiparasitic,[6] while little attention has been paid to the exploration of azaacridines as antitumor[7] and particularly topo 1 inhibitory agents. As part of our efforts in the design and synthesis of compounds with potent antitumor activities, especially heterocyclic compounds,[8] a series of acridine derivatives with antitumor activity have been developed, some of which showed topo 1 inhibitory activity.[9] Herein, we report the preparation and evaluation of the antitumor activity of an additional series of azaacridine analogs with substituent(s) at the anilino ring. The inhibition effect of the compounds on topo 1 activity was also detected.

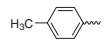
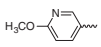
The synthetic route to the target compounds **5a**–**5e** is shown in Scheme 1. An Ullmann reaction was carried out by heating 2,4-dichlorobenzoic acid (**1**) with 5-amino-2-methoxypyridine (**2**) in the presence of Cu to afford the corresponding anthranilic acid **3**. Then **3** was stirred with $POCl_3$ giving the 9-chloroacridine derivative **4**. The reaction of **4** with aniline derivatives in the presence of a few drops of concentrated hydrogen chloride afforded the desired compounds **5a**–**5e**, the structures of which were confirmed by ^{1}H NMR and high-resolution MS.[13]

The ability of compounds to inhibit cell growth was evaluated against K562 leukemia cells and hepatoma HepG-2 cells by MTT assay. The cells were suspended at a concentration of 1.5×10^5 cells/mL and seeded in 96-well microtiter plates at 37 °C in a humidified atmosphere with 5% CO_2. The cells were then treated with various concentrations of compound dissolved in DMSO in quintuplet for 48 h. After treatment, the cells were incubated with 15 µL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide from Sigma] solution (5 mg mL^{-1}) for 4 h. The formazan precipitate was dissolved in 100 µL DMSO and the absorbance at 490 nm was measured by a Benchmark microplate reader (Molecular Devices Corporation). IC_{50} values are the concentration at which cell growth was inhibited by 50%. The results are depicted in Table 1. Imatinib and colchicin were used as the positives control. Compound **5a** with methyl substitute at the *para*-position on the aniline ring displayed good antiproliferative activity with IC_{50} values of 15.9 and 18.9 µM against K562 and HepG-2 cells respectively in vitro, while the other four compounds **5b**–**5e** exhibited weaker or no activity against these two cell lines.



Scheme 1. Synthesis of azaacridine derivatives **5**. Reagents and conditions: (i) K_2CO_3, Cu, DMF, 130 °C; (ii) $POCl_3$, 140 °C; (iii) various anilines, $CHCl_3$, EtOH, concd HCl.

Table 1. Antiproliferative activity of compounds against tumor cell

Compound	R	IC_{50}/µM	
		K562	HepG-2
5a		15.9	18.9
5b		>50	>50
5c		38.5	>50
5d		>50	>50
5e		>50	>50
Imatinib		5.4	ND[a]
Colchicin		ND[a]	1.9

[a]ND: not detected.

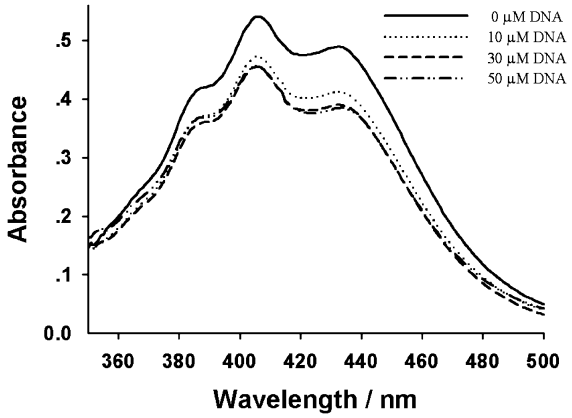


Figure 1. UV–visible absorption spectra of azaacridine analogue **5a** (50 μM) in the absence (solid line) or presence (dashed line) of increasing amounts of ct DNA.

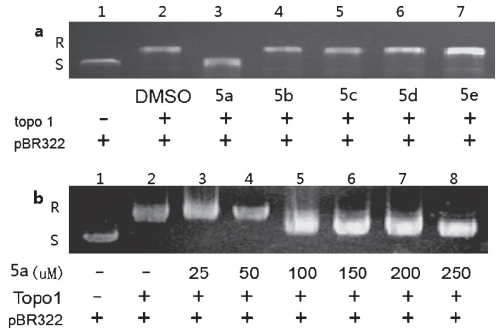


Figure 2. Effect of the compounds on the relaxation of plasmid DNA by human topoisomerase 1. (a) Lane 1, DNA pBR322; Lane 2, topo 1 + DNA pBR322 + DMSO; Lanes 3–7, DNA pBR322 relaxation by topo 1 and **5a**, **5b**, **5c**, **5d**, and **5e** at concentrations of 200 μM, respectively. (b) Lane 1, DNA pBR322; Lane 2, topo 1 + DNA pBR322; Lanes 3–8, DNA pBR322 relaxation by topo 1 and **5a** at concentrations of 25, 50, 100, 150, 200, and 250 μM, respectively.

The binding properties of compound **5a** with ct DNA, the most active in inducing the antiproliferative effect, were investigated by using UV–visible spectral absorbance analysis, which has been used as a convenient tool to detect the interaction between drugs and DNA.[10] The spectra of **5a** solution in the absence and presence of ct DNA are presented in Figure 1. The spectrum of **5a** presented three signals at 380, 405, and 430 nm, while DNA did not absorb light in this region. In the presence of increasing DNA concentration, the reduced intensity of these signals occurred. The hypochromic effect suggested that **5a** might interact with DNA.

The ability of compound **5a** to interact with ct DNA indicated that nuclear enzymes involved in DNA processing such as topo 1 might be inhibited. Figure 2 shows the relative affinity of compounds **5a**–**5e** on the relaxation of plasmid pBR322 DNA mediated by topo 1. Compound **5a**, which displayed the highest activity in vitro activity against K562 and HepG-2 cells, showed good topo 1 inhibitory activity at 100 μM, whereas other analogs have very low or undetectable activities at 250 μM. The activity of compound **5a** against K562 and HepG-2 is substantially higher than its activity against topo 1. Some topo inhibitors have been reported to show substantially better IC_{50} values against cell lines than their activities against topo 1. For instance, camptothecin shows cytotoxicity against A549, SK-OV-3, HepG-2, and HT-29d cell lines with IC_{50} values of 1–10.3 μM while it inhibits topo 1 at an IC_{50} value of 46 μM.[11] Maslinic acid and its diacetyl derivative show growth inhibition against various human solid tumor cell-lines with IC_{50} values of 5–18 μM, while they inhibit topo 1 at IC_{50} values of 76–80 μM.[12] These data suggest that **5a** might exert antiproliferative activity through topo 1 inhibition, and it may be a potential lead compound for the development of azaacridines as topo 1 inhibitors.

In conclusion, a series of azaacridines were synthesized and the biological activity was investigated, among which compound **5a** displayed good cytotoxicity against K562 and HepG-2 cells. The present results indicate that **5a** interact with ct DNA and inhibit topo 1 activity. Further modification of the structure of **5a** may produce a novel type of antitumor agent, which is in progress.

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