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Novel synthetic 9-benzyloxyacridine analogue as both tyrosine kinase and topoisomerase I inhibitor

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ABSTRACT

Multi-target agents against tyrosine kinases and topoisomerases are potentially useful for the effective treatment of cancers. Discovery of new multi-target scaffolds are important for developing such agents. A series of five novel acridine analogues, **LXL 1–5**, were synthesized and their antiproliferative activity against HepG-2 cell lines were evaluated, among which the 9-benzyloxyacridine analogue, **LXL-5**, showed inhibitory activity against tyrosine kinases, VEGFR-2 and Src. The results of UV–visible absorption spectra and fluorescence emission spectra, as well as DNA topoisomerase I inhibition assay, indicated topoisomerase I inhibitory activity. Our study suggested that acridine scaffold, previously shown to have no multi-target kinase and topoisomerase inhibitory activity, might be potentially developed as a multi-target inhibitor of tyrosine kinases and topoisomerase I.

1. Introduction

Cancer has become the main cause of death and the development of new drugs with promising anticancer efficacies has attracted great attention [1]. As topoisomerases are highly expressed in cancer cells, they represent effective targets for cancer chemotherapy. Topoisomerase I (topo I), one of the topoisomerases which can break and reseal a single DNA strand, is the target of several approved anticancer drugs, such as camptosar, topotecan and their derivatives [2]. The involvement of topo I in various cancers and the clinical success of topo I inhibitors indicate the inhibition of topo I is an effective strategy for the treatment of cancers.

VEGFR-2 and Src kinases are two types of tyrosine kinases which play important roles in modulating multiple pathways in the progression of cancers. Development of novel anticancer compounds that can inhibit VEGFR-2 and Src kinases may hold considerable promise in cancer therapeutics [3]. Several VEGFR-2 inhibitors have been approved in advanced-stage clinical trials as anticancer therapeutics [4].

Acridine and its derivatives have been reported to display potent antitumor activity primarily due to their inhibition of topoisomerases [5]. The 9-anilinoacridine derivative, *m*-amsacrine (*m*-AMSA), was the typical analogue approved for clinical use in 1976. It can form DNA-topisomerase complex and the substitution pattern in the benzyl ring plays an important role in the antitumor activity. However, few of them have been found to inhibit the activity of tyrosine kinases [6]. In 2011, our group first reported a 9-benzylaminoacridine derivative which was a dual inhibitor of the tyrosine kinases, VEGFR-2 and Src, without inhibitory activity against topoisomerase [7]. In certain cancers, topo I inhibitors work synergistically with multi-tyrosine kinase inhibitors to kill cancer cells [8], however no compound with acridine scaffold has been found as inhibitors of both topoisomerases and tyrosine kinases. An interesting question is whether compounds of the acridine scaffold can be potentially developed into novel multi-target tyrosine kinases and topo I inhibitors.

This study is based on the structures of *m*-AMSA derivatives and our earlier work in the discovery of 9-benzylaminoacridine compound [7] and 9-aminoacridine derivatives with chloro and methoxy groups substituted at C-2 and C-6 positions of acridine

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ring [7,9]. In this work as part of our continuous efforts for developing anticancer compounds, we addressed this question by synthesizing a series of novel 9-benzylaminoacridine and the bioisostere, 9-benzyloxyacridine, with antitumor activity. Our new compound **LXL-5** showed inhibitory activity against topo I, Src and VEGFR-2 and showed cytotoxicity against HepG-2 cell lines *in vitro*, which represented, for the first time, the success of this scaffold as a multi-target inhibitor of both topoisomerases and tyrosine kinases.

2. Experimental

The synthetic methods and the preparation of compounds **3** and **4** can be found in the Supporting information. The synthesis of the acridine derivatives **LXL 1–5** is described in Scheme 1.

2.1. General procedure for compounds (*LXL 1–4*)

Various amines (2.00 mmol) were dissolved in absolute alcohol (15 mL) and then potassium carbonate (2.00 mmol) was added. The mixture was stirred for 45 min at room temperature. Compound **4** (1.00 mmol) and potassium iodide (0.25 mmol) were added and the mixture stirred and refluxed overnight. Then the mixture was poured into water (50 mL), extracted with ethyl acetate to give the crude product. The crude product was purified by column chromatography using petroleum ether and ethyl acetate.

6-Chloro-2-methoxy-N-(3-methoxybenzyl)acridin-9-amine (***LXL-1***): Yield 77%; mp 101–104 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (d, 1H, J = 1.7 Hz), 8.04–7.91 (m, 2H), 7.39 (dd, 1H, J = 9.4, 2.5 Hz), 7.34–7.27 (m, 2H), 7.16 (d, 1H, J = 2.4 Hz), 6.98 (d, 1H, J = 7.5 Hz), 6.93 (s, 1H), 6.90 – 6.79 (m, 1H), 4.84 (s, 2H), 3.78 (s, 3H), 3.76 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 160.25, 156.26, 149.66, 148.07, 140.92, 135.02, 131.29, 130.90, 130.17, 128.14, 125.02, 124.91, 123.88, 119.59, 118.23, 116.19, 113.39, 113.05, 99.39, 55.42, 55.30, 54.57; HR-MS(ESI): calcd. for $C_{22}H_{19}ClN_2O_2$ $[M+H]^+$ 379.1213; found: 379.1220.

6-Chloro-2-methoxy-N-(2-methylbenzyl)acridin-9-amine (***LXL-2***): Yield 56%; mp 150–151 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (t, 1H, J = 2.2 Hz), 7.99 (dd, 1H, J = 9.4, 2.4 Hz), 7.92 (dd, 1H, J = 9.2, 5.3 Hz), 7.54 (dd, 1H, J = 5.9, 2.7 Hz), 7.39 (dt, 1H, J = 9.4, 2.8 Hz), 7.31–7.19 (m, 4H), 7.10 (t, 1H, J = 2.4 Hz), 4.90 (s, 1H), 4.79 (s, 2H), 3.70 (s, 3H), 2.23 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.12, 149.69, 148.34, 147.06, 137.30, 135.79, 134.83, 131.58, 130.81, 128.43, 128.14, 128.07, 126.64, 124.91, 124.74, 123.66, 118.02, 116.03, 99.30, 55.30, 52.65, 19.00; HR-MS(ESI): calcd. for $C_{22}H_{19}ClN_2O$ $[M+H]^+$ 363.1264; found: 363.1252.

6-Chloro-2-methoxy-N-(2-chlorobenzyl)acridin-9-amine (***LXL-3***): Yield 61%; mp 163–165 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (s, 1H), 7.99 (d, 1H, J = 9.2 Hz), 7.94 (d, 1H, J = 9.3 Hz), 7.43 (d, 1H, J = 7.3 Hz), 7.35 (d, 1H, J = 9.1 Hz), 7.30 (d, 2H, J = 6.1 Hz), 7.24 (dd, 1H, J = 7.8, 1.5 Hz), 7.16 (t, 2H, J = 7.0 Hz), 4.91 (s, 2H), 3.78 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.38, 149.70, 147.10, 136.33, 135.37, 133.18, 132.40, 130.89, 129.84, 129.56, 129.37, 128.87, 127.38, 125.26, 124.03, 99.35, 55.34, 52.04; HR-MS(ESI): calcd. for $C_{21}H_{16}Cl_2N_2O$ $[M+H]^+$ 383.0718; found: 383.0717.

6-Chloro-2-methoxy-N-(3-bromobenzyl)acridin-9-amine (***LXL-4***): Yield 69%; mp 156–159 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (d, 1H, J = 1.8 Hz), 7.99 (d, 1H, J = 9.4 Hz), 7.91 (d, 1H, J = 9.3 Hz), 7.61 (s, 1H), 7.45 (d, 1H, J = 7.8 Hz), 7.39 (dd, 1H, J = 9.4, 2.6 Hz), 7.28 (dd, 2H, J = 6.6, 2.7 Hz), 7.22 (t, 1H, J = 7.7 Hz), 7.09 (d, 1H, J = 2.5 Hz), 4.76 (s, 2H), 3.76 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.43, 149.09, 148.15, 146.93, 141.72, 134.92, 131.49, 130.99, 130.55, 130.38, 128.33, 125.88, 125.25, 124.99, 123.62, 123.13, 118.57, 116.52, 99.04, 55.39, 53.87; HR-MS(ESI): calcd. for $C_{21}H_{16}BrClN_2O$ $[M+H]^+$ 427.0213; found: 427.0224.

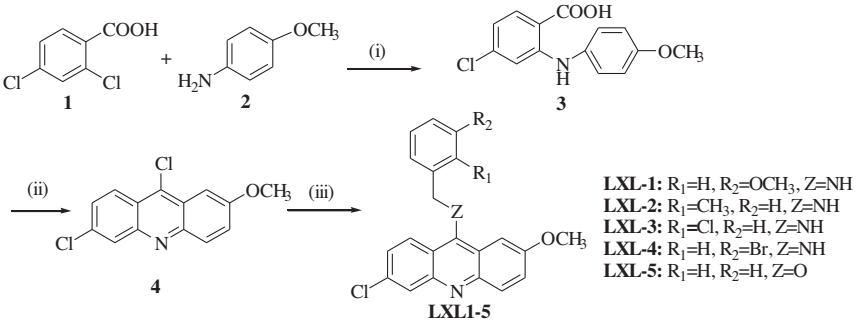
2.2. 6-Chloro-2-methoxy-9-(benzyloxy)acridine (*LXL-5*)

Benzyl alcohol (3.00 mmol) was dissolved in dry THF (15 mL) and then sodium hydride (3.00 mmol) was added. The mixture was stirred for 45 min at room temperature. Compound **4** (1.00 mmol) and potassium iodide (0.25 mmol) were added and the mixture stirred and refluxed overnight. Then the solution was evaporated. The solid was poured into water (50 mL), and extracted with ethyl acetate to give the crude product. The crude product was purified by column chromatography using petroleum ether and ethyl acetate (20:1, v/v). Yield 17%; mp 128–130 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (d, 1H, J = 1.9 Hz), 8.14 (d, 1H, J = 9.2 Hz), 8.07 (d, 1H, J = 9.2 Hz), 7.53–7.37 (m, 7H), 7.25 (d, 1H, J = 2.8 Hz), 5.32 (s, 2H), 3.83 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.92, 157.20, 148.79, 148.50, 136.56, 135.29, 131.25, 128.88, 128.79, 128.28, 127.23, 126.72, 126.12, 123.69, 121.45, 119.15, 98.08, 55.47; HR-MS(ESI): calcd. for $C_{21}H_{16}ClNO_2$ $[M+H]^+$ 350.0948; found: 350.0951.

DNA topo I inhibition assay, kinase assays, experiments on absorption and fluorescence emission; ^{1}H NMR and ^{13}C NMR spectra, and High resolution mass spectrometry can be found in Supporting information.

3. Results and discussion

Utilizing commercial materials, the Ullmann reaction of 2, 4-dichloro-benzoic acid **1** with 4-methoxyaniline **2** in DMF using Cu as the catalyst and under basic condition gave anthranilic acid **3**, which was stirred in $POCl_3$ to afford the 9-chloroacridine derivative **4**. The compounds **LXL 1–4** were obtained by the reaction of substituted benzylamines and compound **4** using KI and K_2CO_3 in absolute ethanol. Compound **LXL-5** was achieved by the etherification of compound **4** with benzyl alcohol in the presence of NaH and catalytic amounts of potassium iodide in THF.



Scheme 1. Synthesis of **LXL 1–5**. Reagents and conditions: (i) K_2CO_3, Cu, DMF, 130 °C; (ii) $POCl_3$, 140 °C; (iii) **LXL 1–4**: benzylamines, K_2CO_3, KI, ethanol, reflux; **LXL-5**: benzyl alcohol, NaH, KI, THF, reflux.

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

Compound	R_1	R_2	Z	IC_{50} (μmol/L)[a]
LXL-1	H	OCH_3	NH	13.70 (±1.21)
LXL-2	CH_3	H	NH	11.43 (±0.25)
LXL-3	Cl	H	NH	20.21 (±1.64)
LXL-4	H	Br	NH	9.12 (±1.08)
LXL-5	H	H	O	8.78 (±0.32)
Colchicin				1.80 (±0.23)

[a] All values are means of three experiments (±SD).

Table 2
Percent inhibition effect of compounds selected at 20 μmol/L on the activity of two kinases.[a]

Compound	Src	VEGFR-2
LXL-4	(12.3 ± 6.1)%	(9.2 ± 0.9)%
LXL-5	(63.8 ± 2.4)%	(12.6 ± 3.7)%
Staurosporine	(99.7 ± 0.1)%	(99.4 ± 2.5)%

[a] All values are means of two experiments (±SD).

The ability of compounds **LXL 1–5** to inhibit cell growth was evaluated against hepatoma HepG-2 cells by using the MTT assay. The results are depicted in Table 1. Colchicin was used as the positive control. Most of the compounds had low micromolar IC_{50} values, among which compounds **LXL-4** containing the bromobenzylamino group and **LXL-5** comprising the benzyloxy group showed good activity against HepG-2 cells with IC_{50} values at 9.12 and 8.78 μmol/L, respectively.

In order to identify whether compounds can inhibit tyrosine kinases Src and VEGFR-2, we conducted *in vitro* kinase inhibition assay studies with compounds **LXL-4** and **LXL-5** which displayed good antiproliferative activity. The kinase assay results were shown in Table 2. Staurosporine was used as a positive reference compound. It was determined that **LXL-4** displayed weak activity against the two kinases, while **LXL-5** exhibited moderate activity with Src and VEGFR-2 inhibition rates of 63.8% and 12.6% at 20 μmol/L, respectively. The results suggested that **LXL-5** may inhibit the antiproliferative activity partly by inhibiting the activity of Src and VEGFR-2, while **LXL-4** might inhibit cancer cell proliferation by another mechanism. Therefore, the 9-benzyloxyacridine analogue **LXL-5** was selected to confirm whether it could interact with ct DNA and inhibit the activity of topo I.

Absorption spectroscopy has been widely applied for investigating the interaction between drugs and DNA. To detect the binding properties of compound **LXL-5** with ct DNA, **LXL-5** was investigated using UV-visible spectral absorbance analysis in the absence and presence of ct DNA. In Fig. 1, the spectrum of **LXL-5** presented two signals at 385 nm and 410 nm, while DNA did not absorb light in this region. With the increase of the concentration of ct DNA, the intensity of the spectrum of **LXL-5** was obviously decreased, which suggested that the compound **LXL-5** might interact with DNA.

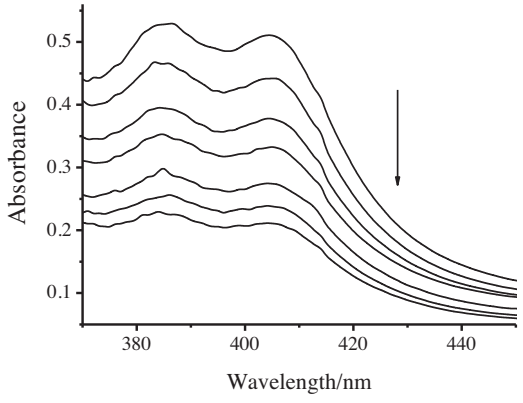


Fig. 1. UV–vis absorption spectra of **LXL-5** (50 μmol/L) in 10 mmol/L Tris-HCl buffer containing 10 mmol/L NaCl (pH 7.0) by increasing the concentrations of ct DNA ([DNA] = 0, 5, 10, 15, 20, 25, 30 μmol/L).

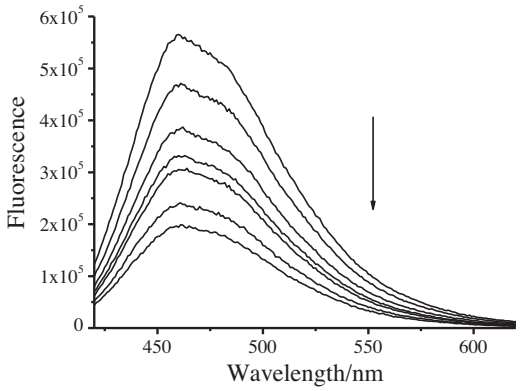


Fig. 2. Spectrofluorimetric titration of **LXL-5** (10 μmol/L) in the presence of increasing amounts of ct DNA ([DNA] = 0, 1, 2, 4, 5, 7.5, 10 μmol/L).

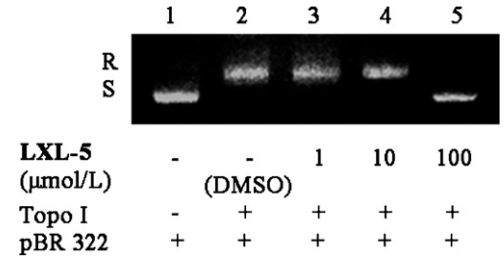


Fig. 3. Effect of the compound **LXL-5** on the relaxation of plasmid DNA by human topoisomerase I (S: superhelix; R: relaxation). Lane 1, DNA pBR322; Lane 2, topo I + DNA pBR322 + DMSO; Lanes 3–5, DNA pBR322 relaxation by topo I and **LXL-5** at concentrations of 1, 10, 100 μmol/L, respectively.

In addition to the electronic absorption spectroscopy, the binding capacity was also tested by the fluorescence emission spectroscopy. The compound **LXL-5** was gradually mixed with increasing equivalents of ct DNA in Tris-HCl at pH 7.0. As shown in Fig. 2, the fluorescence of **LXL-5** was gradually quenched with the increasing amounts of ct DNA, suggesting the interaction between **LXL-5** and ct DNA.

To test whether the novel tyrosine kinases inhibitor **LXL-5** also possesses activity to inhibit topo I activity, the assay of compound **LXL-5** on the relaxtion of plasmid pBR322 DNA mediated by topo I was performed. As shown in Fig. 3, the compound **LXL-5** displayed moderate topo I inhibitory activity at 100 μmol/L, whereas there were no detectable activities at low concentration. These data indicated that **LXL-5** could also inhibit topo I, and might be a potential lead compound for the development of topo I inhibitors.

4. Conclusion

In conclusion, a series of novel 9-benzylaminoacridine derivatives and 9-benzyloxyacridine, **LXL 1–5**, had been synthesized and showed good antiproliferative activity against HepG-2 cells *in vitro*, among which the 9-benzyloxyacridine analogue, **LXL-5**, could inhibit the activity of topoisomerase I, VEGFR-2 and Src. Our study for the first time suggested that the acridine scaffold, which had been historically used as topoisomerase inhibitors, had the potential to be developed as multi-target topoisomerase and tyrosine kinase inhibitors. Further optimizations of the structure to improve topo I, VEGFR-2 and Src activity are ongoing.

Acknowledgments

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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.2013.05.018.

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