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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.

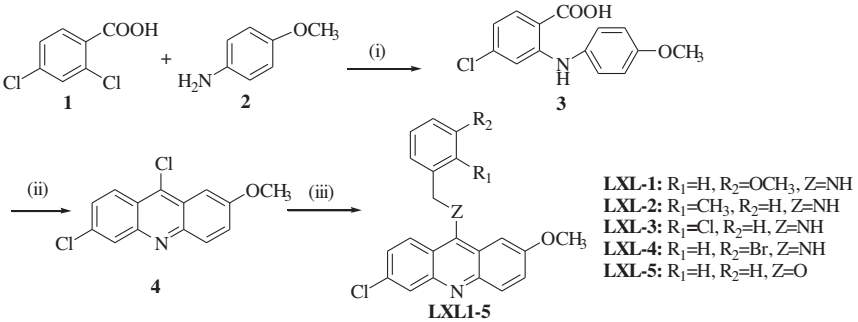
2.2. 6-Chloro-2-methoxy-9-(benzyloxy)acridine (*LXL-5*)

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.