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Design, synthesis and biological evaluation of 3-substituted indenoisoquinoline derivatives as topoisomerase I inhibitors

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ABSTRACT

A new series of indenoisoquinoline derivatives was designed and synthesized. The in vitro anti-proliferative activity of these novel compounds was evaluated in HepG2, A549 and HCT-116 cell lines. Compounds **9a**, **9b**, **10a**, **10c**, **10e**, **18a** and **18b** manifested potent inhibitory activity against the three tested cancer cell lines. Nineteen compounds were also tested for Top I inhibition at 50 μM. Almost all the tested compounds showed potent Top I inhibition activity at this concentration. The most potent compounds **9a** and **10a** demonstrated more cytotoxicity than HCPT and TPT and was comparable to CPT in inhibitory activities on Top I in our biological assay.

DNA topoisomerase I (Top I) is an important nuclear enzyme to control replication, transcription, mitosis, recombination of DNA, as well as chromatin remodeling.[1–5] Since Top I involved in pivotal steps of cell viability, Top I is proven to be a potential therapeutic target for anticancer drugs.[6]

Irinotecan and topotecan (Fig. 1), two camptothecin (CPT) derivatives, are the only current Top I inhibitors approved by the Food and Drug Administration (FDA) as anticancer drugs.[7,8] Unfortunately, their clinic utility is hampered by several disadvantages such as dose-limiting bone marrow suppression and rapid inactivation when the lactone ring is hydrolyzed at physiological pH.[9,10] Furthermore, some cancer cells developed resistance to them.[11] As a result, these problems promoted further development of other noncamptothecin Top I inhibitors with preferable potency, selectivity and pharmacological profiles.

NSC 314622 (Fig. 2) was the first indenoisoquinoline compound reported to have anticancer activity and was synthesized in 1978.[11,12] Twenty years later, its cytotoxicity profile was found to reveal a strong resemblance with irinotecan and topotecan through Top I inhibition.[13]

Indenoisoquinolines are not confined within the same pharmacokinetical problems as camptothecins such as overcome the inherent chemical instability and blood plasma protein binding.[9] However, they still have a problem of intrinsically poor biological activity. Therefore, studies were focused on the optimization of indenoisoquinoline compounds to improve their biological activity.

Beck worked on optimizing 3-substituted indenoisoquinoline and found that the 3-nitro substituted compounds appended with linear sugar side chains outperformed the 2,3-dimethoxy substituted series in both Top I inhibition and growth inhibition.[14] Indimitecan (LMP 776), with the imidazole appended to the lactam side chain, was comparable to 1 μM camptothecin with an MGM of 0.079 μM and more potent than CPT in the Top I inhibition assay.[15] The work of Zhang demonstrated that modification of a methoxy group at the 9-position to the electron-withdrawing fluorine atom led to enhancement of Top I inhibition.[13]

Indotecan (LMP 400) and indimitecan (LMP 776), two indenoisoquinoline Top I inhibitors, are currently in phase I clinical trials at the National Cancer Institute (Fig. 3).[16,17]

Previous works demonstrated that nitration of the isoquinoline ring could significantly enhance the biological activity of indenoisoquinoline Top I inhibitors.[18] In addition, it was evident that heteroatom substituents appended to the lactam side chain could further improve biological activity with regard to the compounds lacking these nitrogenous substituents.[19–21]

Accordingly, we designed and synthesized some compounds with a nitro group on the isoquinoline ring and different nitrogenous substituents appended to the lactam side chain (series **A**). To further explore the importance of the nitro group, Zhang replaced the strong electron-withdrawing nitro substituent with amino substituent and found that could improve their Top I inhibiton.[13] Then we changed the nitro substituent to amino

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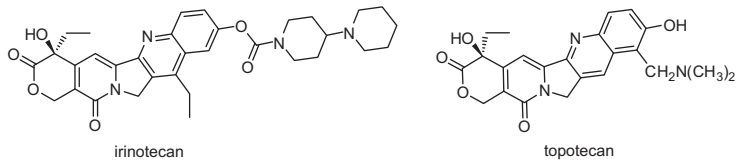

Figure 1. Structure of irinotecan and topotecan.

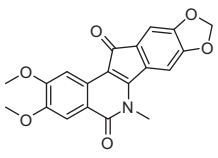

Figure 2. Structure of NSC 314622.

substituent (series **B**). In order to probe the influence of electronic effect to the isoquinoline ring, we also synthesized two series compounds with an acetamido or a methoxyl group instead of the nitro group (series **C** and **D**).

The synthesis of the four series of indenoisoquinoline derivatives were demonstrated in Schemes 1 and 2. 2-Chlorobenzoic acid was nitrated, substituted, hydrolyzed, decarboxylated and cyclized to provide compound **5**. Condensation of compound **5** with Schiff base (**19**) yielded carboxylic acid **6**. Compound **6** was cyclized to provide **7**, which was treated with the corresponding amines to give the series **A** compounds (**8a**–**8e**). Compounds **8a**–**8e** were hydrogenated to give the series **B** compounds (**9a**–**9e**). Compounds **9a**–**9e** were acetylated to yield the series **C** compounds (**10a**–**10e**). Compound **4** was hydrogenated, substituted, esterified, substituted, hydrolyzed and cyclized to provide compound **16**. Condensation of compound **16** with Schiff base (**19**) yielded carboxylic acid **17**. Carboxylic acid **17** was cyclized to yield compound **18**, and then treated with appropriate amines to give the series **D** compounds (**18a**–**18e**). Synthesis and chemical data (including ^{1}H NMR, MS) in details were provided in supporting information. The chemical data including ^{1}H NMR, ^{13}C NMR, MS, IR, and elemental analysis of compound **9a** was provided in Ref. 22

To explore the in vitro anti-proliferative activity of these indenoisoquinoline derivatives, we used the 1-*N*-methyl-5-thiotetrazole (MTT)-based test in there human cancer cell lines HepG2 (human hepatocellular liver carcinoma), A549 (nonsmall cell lung carcinoma) and HCT-116 (human colon cancer cell line) with HCPT and TPT as positive control. We plated $6 * 10^4$/ml to $8 * 10^4$/ml cells per well in 96-well plates and incubated for 24 h. Over a range of concentrations from 0.01 to 100 μM, all compounds were added and treated for 48 h. Twenty microliters of MTT solution were added to each well, and plates were incubated for 4 h. DMSO (150 μl) was added to each well. Then shake the plate ten minutes in shaking table to dissolve the Formazan crystals. The absorbance (at tested wavelength of 570 nm) was measured on an enzyme-linked immunosorbent detector. Then the cytotoxicity

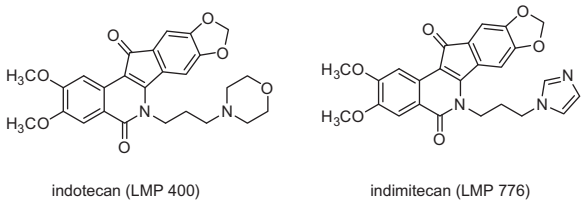

Figure 3. Structure of indotecan (LMP 400) and indimitecan (LMP 776).

(IC_{50}) values, which are the concentrations leading to 50% cell death in vitro, were obtained. In addition, nineteen compounds with the best anti-proliferative activity were tested at 50 μM for their capability to inhibit Top I-mediated DNA relaxation.

Table 1 presents cytotoxic activities of indenoisoquinoline derivatives and manifests that substitution pattern at the indenoisoquinoline 3-position and the lactam side chain has a significant effect on the in vitro cytotoxic activities of the molecules. From the listed data, we can conclude that the biological activity is greatly improved with the utilization of the nitro, amino, acetamido and methoxyl groups at 3-position of indenoisoquinolines and that follows this trends: amino and acetamido > methoxyl > nitro. Obviously, the effects of electron-donate groups on enhancing the biological activity of indenoisoquinoline Top I inhibitors are better than electron-withdrawing groups. Comparing series **A**, B, **C** and **D** compounds, we can see that series **B**, **C** and **D** compounds had nearly 2–100-fold preferable cytotoxicity than series **A** compounds (nitro group) with an IC_{50} of 0.048–29.91 μM range. Series **B** and **C** compounds with amino and acetamido groups at 3-position was the most potent compounds we synthesized. Comparing the different substituents appended to the lactam side chain, we can also conclude that the activity of nitrogenous substituted compounds was significantly better than the chloro ones. For instant, compounds **10a**–**e** and **18a**–**e** were more potent than compounds **10** and **18** in cell killing activity. Therefore, introducing nitrogenous group at lactam side chain resulted in a rapid increase in biological activity. The data manifests that the properties of the substituents at lactam side chain is a crucial factor for the potency and that follows this trends: 4-methyl piperazinyl > pyrrolidyl, piperidyl, diethylamino ≫ morpholinyl. Compounds **9a** and **10a** were discovered to be the best of all compounds we synthesized, with IC_{50} values of 0.08, 0.12 μM (HepG2), 0.048, 0.062 μM (A549) and 0.117, 0.233 μM (HCT-116), respectively; approximately 2–100-fold more potent than HCPT and TPT in this biological assay.

Figure 4 shows the effects of indenoisoquinoline derivatives on Top I-mediated DNA relaxation activity. We compared the inhibitory activities of these compounds with a famous DNA-Top I inhibitor, CPT. From the picture, we can see that all the tested compounds have moderate inhibitory activities on Top I which was right to the anti-proliferative activities in MTT assay. Compounds **9a** and **10a** show best activity, which is as potent as CPT.

In summary, 22 novel indenoisoquinoline derivatives were designed, synthesized, and evaluated for their in vitro antitumor activity in cellular proliferation assay. We also identified them as novel Top I inhibitors by Top I Inhibition Assay. In our research, we had found seven compounds more potent than HCPT and TPT by in vitro antitumor activity and as potent as CPT in inhibitory activities on Top I. For instance, compounds **9a** and **10a** were found to be the most potent among all the synthesized compounds, with IC_{50} values of 0.08, 0.12 μM (HepG2), 0.048, 0.062 μM (A549) and 0.117, 0.233 μM (HCT-116), respectively; approximately 2–100-fold more potent than HCPT and TPT in this biological assay. Compounds **9a** and **10a** was also as potent as CPT in Top I inhibition assay. Further research about the biological activities and antitumor mechanism of these potent compounds are currently going on in our laboratory.