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Research paper

Synthesis, cytotoxicity and structure-activity relationship of indolizinoquinolinedione derivatives as DNA topoisomerase IB catalytic inhibitors

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ABSTRACT

Our previous studies reveal that indolizinoquinolinedione scaffold is a base to develop novel DNA topoisomerase IB (TOP1) catalytic inhibitors. In this work, twenty-three novel indolizinoquinolinedione derivatives were synthesized. TOP1-mediated relaxation, nicking and unwinding assays revealed that three fluorinated derivatives **26**, **28** and **29**, and one *N,N-trans* derivative **46** act as TOP1 catalytic inhibitors with higher TOP1 inhibition (++++) than camptothecin (+++) and without TOP1-mediated unwinding effect. MTT assay against five human cancer cell lines indicated that the highest cytotoxicity is **20** for CCRF-CEM cells, **25** for A549 and DU-145 cells, **26** for HCT116 cells, and **33** for Huh7 cells with GI_{50} values at nanomolar range. The drug-resistant cell assay indicated that compound **26** may mainly act to TOP1 in cells and are less of Pgp substrates. Flow cytometric analysis showed that compounds **26**, **28** and **29** can obviously induce apoptosis of HCT116 cells. Moreover, the structure-activity relationship (SAR) of indolizinoquinolinedione derivatives was analyzed.

1. Introduction

DNA topoisomerase IB (TOP1) is an essential enzyme that regulates DNA topology structure so that it may be replicated and transcribed [1–4]. To perform its functions, TOP1 breaks one strand of DNA by transesterification reactions using the active site tyrosine as the nucleophile that attacks the DNA phosphodiester backbone, and covalently attaches to the 3′-end of the broken DNA to form a transient enzyme-DNA covalent complex (TOP1cc) [2,5]. TOP1 is a validated molecular target for anticancer agents [6,7]. Inhibition of TOP1 or trapping of TOP1cc can result in DNA damage, which triggers cell death [8–10].

TOP1 inhibitors are classified as TOP1 “poisons” and “catalytic inhibitors” based on their molecular mechanism of action. TOP1 poisons are able to trap TOP1cc to prevent further relegation of the DNA single-strand breaks [6,7,9]. TOP1 catalytic inhibitors inhibit the catalytic DNA cleavage reaction of enzyme [11–14], which is different from “poisons”. The classical TOP1 poisons are camptothecin (CPT, Fig. 1) derivatives, of which the only known target in cells is TOP1 [7,15]. To date, two camptothecin derivatives, topotecan (**1**) and irinotecan (**2**) have been approved by the FDA for cancer treatment, and several derivatives are in clinical trials [7,9]. In addition to that there are several non-camptothecin TOP1 poisons in clinical trials, including indolocarbazole **3**, dibenzonaphthyridinone **4** and indenoisoquinolines **5** and **6** [16–18]. In spite of their effectiveness in solid tumors, camptothecin poisons suffer from many shortcomings, including bone marrow dose-limiting toxicity, severe gastrointestinal toxicity [19], poor solubility, chemical instability under physiological pH, and drug efflux-mediated resistance [7].

In previous publication, we reported a new class of TOP1 catalytic inhibitors, the indolizinoquinolinedione derivatives (Fig. 1, **7**–**9**) [11,13,20]. Derivative **8** can inhibit catalytic cleavage DNA reaction of TOP1, which prevent the formation of TOP1cc [11]. Further structural modification indicated that the ester functionalized derivatives at position 6 with alkylamino terminus exhibited significantly increased TOP1 inhibition and cytotoxicity, and provided a TOP1 catalytic inhibitor **9** with good cytotoxicity and higher TOP1 inhibition than CPT [13]. The previous structure-activity relationship (SAR) evaluation also indicated that: 1) the existence

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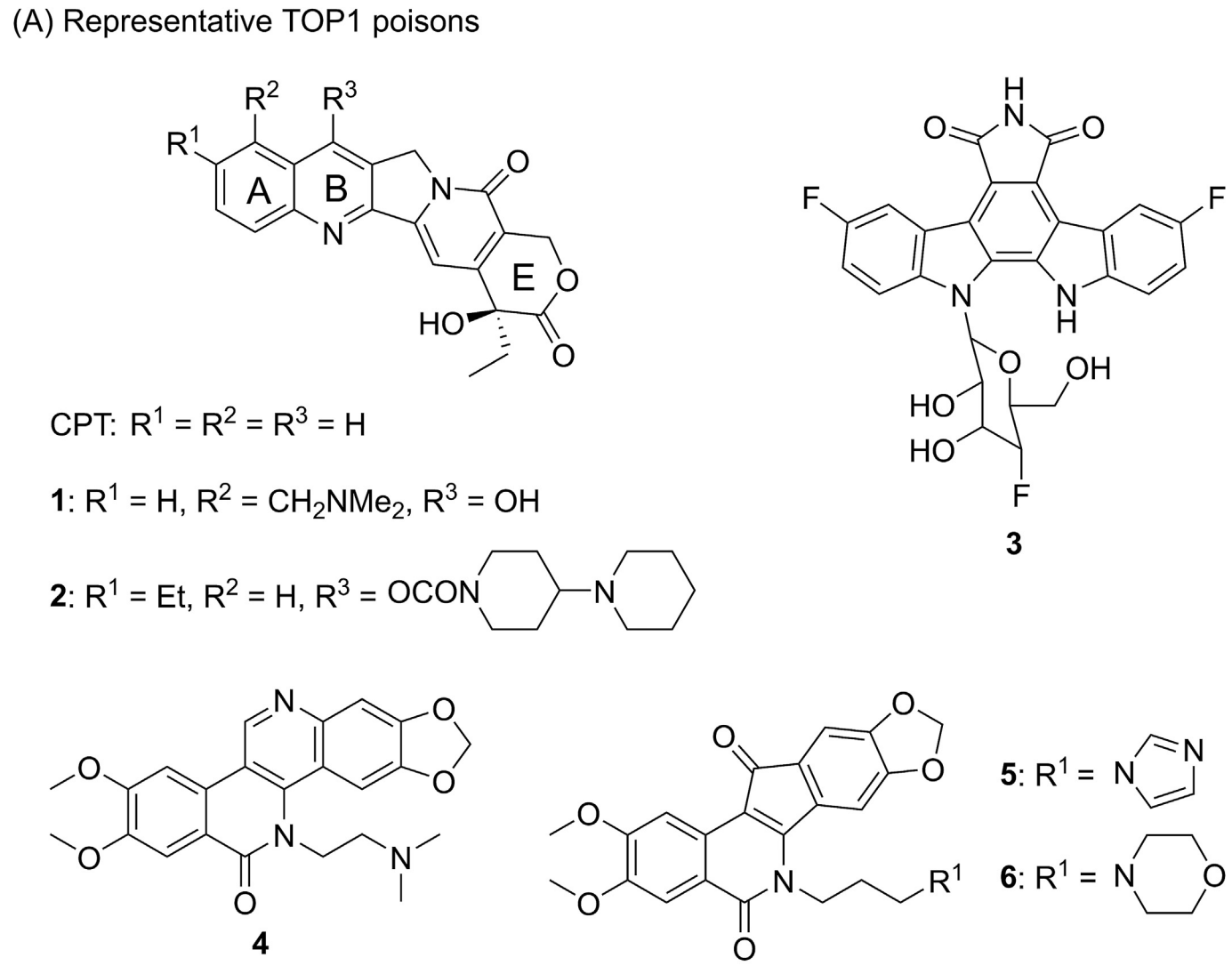

Fig. 1. Chemical structures of the representative TOP1 poisons (A) and our reported TOP1 catalytic inhibitors (B).

of a nitrogen atom in the A-ring is important for the cytotoxicity; 2) *N,N-syn* isomers have higher TOP1 inhibitory activity and cytotoxicity than the corresponding *N,N-trans* isomers; 3) the derivatives with electron-donating substituent at position 7 show poor cytotoxicity [20]. To investigate the effect of introduction of an electron-withdrawing group at D-ring, and the position and number of nitrogen atom in the A-ring, three kinds of novel indolizinoquinolinedione derivatives were designed and synthesized based on the previous SAR. TOP1 inhibition and cytotoxicity were evaluated and reported here.