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Design and synthesis of the novel DNA topoisomerase II inhibitors: Esterification and amination substituted 4′-demethylepipodophyllotoxin derivates exhibiting anti-tumor activity by activating ATM/ATR signaling pathways

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ABSTRACT

According to the structure–activity relationship, drug combination principle and bioisosterism, a series of the novel esterification and amination 4′-demethylepipodophyllotoxin derivates were rationally designed in order to discover the potential antitumor prodrug. And then these compounds were tested by the drug-topoisomerase II docking models for virtual screening. Thus, twelve target compounds were screened out and synthesized. Most of compounds exhibited promising in vitro anti-tumor activity, particularly 4-*N*-tris(hydroxymethyl)metylaminomethane-4-deoxy-4′-demethylepipodophyllotoxin (Compound **1**). The anti-tumor activity of Compound **1** against the tumor cell lines BGC-823 (i.e., the IC_{50} value of 5.35 ± 0.77 μM), HeLa (i.e., the IC_{50} value of 160.48 ± 14.50 μM), and A549 (i.e., the IC_{50} value of 13.95 ± 5.41 μM) was significantly improved by 706%, 31% and 900% than that of etoposide (i.e., the IC_{50} values of 43.74 ± 5.13, 209.90 ± 13.42, and 139.54 ± 7.05 μM), respectively. Moreover, the IC_{50} value of Compound **1** against the normal human cell line HK-2 (i.e., 16.3 ± 3.77 μM) was 78% lower than that of etoposide (i.e., 9.17 ± 1.58 μM). Compound **1** could diminish the relaxation reaction topoisomerase II DNA decatenation at a concentration of 10 μM and induce BGC-823 apoptosis by breaking DNA double-strand and activating ATM/ATR signaling pathways.

1. Introduction

4′-demethylepipodophyllotoxin (DMEP) is one of the best-known naturally occurring aryltetralin lignans exhibiting anti-tumor activity [1], which can bind at the pocket between the link site of DNA and topoisomerase II (Topo II) and then diminishing the relaxation reaction of Topo II-DNA decatenation. The clinical success of etoposide and teniposide has triggered the search for compounds with a similar mechanism of action but without their inconveniences (anti-tumor activity need to be further improved) [2–4]. This has resulted in the discovery of many compounds with very different chemical structures. So, most of currently numerous studies focus on the structure modification of DMEP for generate derivatives with superior pharmacological profiles and broader therapeutic scope [5].

To discover a series of the novel 4′-demethylepipophyllum Topo II inhibitors, a rational prodrug design strategy should be developed to the structure modification of DMEP with the superior inhibition on Topo II, the higher antitumor activity and the lower cytotoxicity on the human normal cells. The steric clash and electrostatic contour plots of the comparative molecular field analysis models [6,7] shows that the carbon atom at the 4 position of cycloparaffin (C ring) of DMEP is more reactive by comparing other carbon atoms in the tetranap skeleton of DMEP, and is easier nucleophilic attacked by the molecule with the electronegativity. Thus, from the structure–activity relationship point of view, the structure modifications at the 4 position of cycloparaffin (C ring) of DMEP maybe a very effective pathway for improving the antitumor

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activity. According to drug combination principle, the combination of two kinds of drugs through the ester bond can gain some new compounds with better bioactivity. Many famous drugs in clinic use such as sultamicillin and benorilate were discovered through this way. So, DMEP could be combined with other compounds through ester bond for the better bioactivity and attenuated toxicity. From the bioisosterism point of view [8,9], bioisosteres are substituents with similar physical or chemical properties which produce broadly similar biological properties to a chemical compound for the activity improvement and the toxicity reduction of the lead compound. Both of the ester bond (−COO−) and the −NH− bond are the bioisosteres with the same numbers of the outermost electrons. So, the −NH− bond at the C-4 position of DMEP may be also a potential modification direction for improving the antitumor activity of DMEP. On the other hand, the prodrug approach is a very versatile strategy to increase the utility of pharmacologically active compounds. According to the prodrug principle [10,11], both of esters (−COOR) or amides (−NHCOR) are commonly used ionizable groups, which can be introduced into the hydroxyl, thiol, amine, or carboxylic acid functionalities of the parent drug molecule to compensate for poor aqueous solubility. A variety of esterases, amidases, and/or peptidases in plasma or in other tissues can bioconvert these prodrugs to their active counterparts. Quite often, esters and amides can also be used to enhance absorption and consequently oral drug delivery of parent drugs, because the brush-border membrane of intestinal epithelium possesses a considerable number of transporters for amino acids and peptides. Take these into consideration, many famous drugs in clinic were discovered by using esterification and amidation such as sultamicillin and benorilate. So, esterification and amidation of DMEP at the C-4 position were permitted for two useful DMEP structure modifications. Finally, from an antitumor mechanistic point of view, substituents with the high electron density at the C-4 position of DMEP derivatives (i.e., etoposide) has been demonstrated to be bound via the hydrogen bonding interactions with the tyrosine residue of Topo II and the bases of DNA breakage [12–14]. A series of heterocyclic with pharmacological activity and the high electron density were chosen as module at the C-4 position of DMEP for improving the stabilization and inhibition of the complex of Topo II-DNA cleavage complex. In order to identify molecular properties which have the largest affinity with DNA Topo II, dock the designed DMEP derivates in the active site of DNA Topo II for virtual screening.

This work demonstrated that a series of DMEP derivates were rationally designed, and then the interactions between DMEP derivates and DNA Topo II were investigated by molecular docking for screening target compounds. The biologic activity evaluation indicates that the anti-tumor activities of them were stronger than those of etoposide. Moreover, the DNA Topo II inhibition of 4-*N*-(tris(hydroxymethyl)methylaminomethane)-4-deoxy-4′-demethylepipodophyllotoxin (Compound **1**) was significantly higher than that of etoposide. These results provided the determinants of DNA Topo II binding affinity for this important class of anti-tumor agents and pave the way for further rational structural modification.

2. Results and discussion

2.1. Drug molecular design

The process of drug molecular design was performed in four steps. Firstly, numerous studies on 4′-demethylepipodophyllotoxin (DMEP) currently focus on its structure modification of the cyclo-paraffin (C-ring) in the tetranap skeleton and the steric clash and electrostatic contour plots of the comparative molecular field analysis models shows that the electron-withdrawing substituents at the C-4 position would be a great modification direction to improve antitumor activity of DMEP [15]. Meanwhile, in our previous work, the DMEP derivate 4β-*S*-(1,2,4-triazole-3)-4-deoxy-podophyllotoxin that structurally modified at C-4 position of DMEP against tumor cell line BGC-823 (IC_{50} values of 0.28 μM), A549 (IC_{50} values of 0.76 μM) and HepG2 (IC_{50} values of 0.42 μM) were significantly improved by 91, 221 and 2.73 times than those of etoposide (IC_{50} values of 25.72, 167.97 and 1.15 μM), respectively [16]. Thus, the C-4 position of DMEP was ensured for an effective modification site. Secondly, both of the chemical group NH and the oxygen atom have the same numbers of the outermost electrons, but the electron cloud of the NH with the greater electronegativity was denser than that of the oxygen atom. According to bioisosterism, the O-linked (ethers, glycoside) compounds could be less active in comparison to the NH-linked congeners. So, the sulfur atom preferentially integrated with the large biological protein molecule with the hydrogen bond in tumor cell, and the sulfur-substituted is an effective pathway to improve anti-tumor activity and reduce toxic side-effects of the leading compounds. Moreover, the esterification was one of the most effectual reactions in drug combine principle, which was an effectual strategy for designing new compounds with absolute priority over than the substitution or substrate [17]. So, esterification was ensured another direction of modification. In this way, we ensured alkane amination and esterification for two modification mode. Thirdly, structure of the Topo II-DNA cleavage complex finding that electrophilic group in the C-4 position of DMEP rests in a spacious binding pocket with relatively few interactions, so it can be either modified or replaced to produce derivatives with enhanced Top-poisoning activities, it appears that those non-C4 substitutions would cause steric conflicts and impair the drug-binding site. Therefore, we favor the use of C-4 substitutions for generating new bioactive DMEP derivatives. Because the substituent with high electron density in favor of stabilization the complex of Topo II-DNA cleavage complex, a series of heterocyclic contain electronegative atom (i.e., oxygen, nitrogen or sulfur) were chosen as module. Such as benzimidazole [18,19], imidazole [20,21], thiophene [22] and Tris(hydroxymethyl)aminomethane, whose derivatives possessed anti-tumor activity. Taking these factors into account, nine new alkane amination podophyllum derivates were preliminary designed. Furthermore, according to drug combine principle some of groups and compounds with pharmacological activity were chosen as function module. Such as adamantane nucleus [23,24], phenylalanine nucleus, benzene ring and pyrimidine complexes which exhibited a high index of anti-tumor activity against a broad spectrum of cancers and a low associated nephrotoxicity [25]. So, eight esterification podophyllum derivates was preliminary designed. In conclusion, according to the bioisosterism and combine principle, seventeen novel esterification and alkane amination DMEP derivates were preliminary designed. At last, these compounds were tested by the interplay of the topoisomerase II docking models for virtual screening. In this study, a Topo II-DNA cleavage complex was obtained from the Brookhaven Protein Data Bank (PDB code: 3QX3) [14] and used as the target protein in molecular docking for virtual screening. As shown in Fig. 1, all of the designed DMEP derivates showed good interaction with DNA Topo II and shared a conformation similar to etoposide in the active site. The binding energy of seventeen virtual compounds with DNA Topo II was shown in Table 1. The result indicated that those new DMEP derivates affinity with Topo II as better as etoposide (interaction energy −9.07 kcal/mol). The binding energy of twelve compounds from −8.87 to −13.20 kcal/mol, E-4′ hydroxyl oxygen atom forming hydrogen bond with Asp 479, B ring formed π−π bond interaction with DG-13 or DT-9; in addition the oxygen and nitrogen atom from substituent group can form hydrogen bond with amino acid residue or