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Synthesis and biological evaluation of benzo[*b*]furo[3,4-e][1,4]diazepin-1-one derivatives as anti-cancer agents

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ABSTRACT

A new series of novel Podophyllotoxin-like benzo[*b*]furo[3,4-e][1,4]diazepin-1-ones possessing structural elements of 4-aza-2,3-didehydropodophyllotoxins with central diazepine ring was designed and synthesized as anti-cancer agents. In initial assessment, the cytotoxic activity of the synthesized compounds was evaluated against three cancer cell lines including MCF-7, PC3 and B16-F10 employing the MTT assay. Some of compounds (**12h**, **13a**, **13c** and **14b**) showed significant cytotoxic activity. So, we investigated the cytotoxicity of compounds **12h**, **13a**, **13c** and **14b**, along with podophyllotoxin as the reference drug in different cancer cell lines including A549, A2780, DU145, HeLa, and normal Huvec cell line. Among these four compounds, **13c** showed promising antiproliferative activity against all cancer cells stronger than the other compounds and comparable to reference drug podophyllotoxin in some cancer cells. All these four compounds did not show significant cytotoxicity on normal Huvec cell line. The flow cytometry analysis of the MCF-7, PC3 and A2780 human cancer cell lines treated with **13c** showed that **13c**, induced apoptosis in the MCF-7, PC3 and A2780 human cancer cell lines, which is in good agreement to its cytotoxic activity as well. Compound **13c** did not show significant influence on tubulin assembly and exert its cytotoxic effects via induction of apoptosis and has potent and selective cytotoxic effects in cancer cells.

1. Introduction

Cancer is a major public health problem in the world and it is now the second leading cause of death in the United States, and is predicted to surpass heart diseases as the leading cause of death in the futures [1] Consequently; increasing interest has been devoted to the design and discovery of more effective anticancer agents in current medicinal chemistry [2–9]. Podophyllotoxin (**1**) is the main component of podophyllum resin, a naturally occurring antimitotic cyclolignan which displays strong anticancer activity against numerous cancer cell lines [10]. Although podophyllotoxin possesses notable in vitro antitumor effects, it is not used as an anticancer drug because of its various side effects such as nausea, vomiting and damage of normal tissues. Though it's unfavorable pharmacological profiles, podophyllotoxin can still be used as a lead compound for the discovering of possible anticancer drugs. Etoposide (**2**), teniposide (**3**), and etoposide phosphate (**4**) are semisynthetic derivatives of 4-epipodophyllotoxin. They have numerous limitations, such as poor water solubility, metabolic inactivation, development of drug resistance and toxic effects. To overcome such problems, extensive synthetic efforts have been carried out by a number of researchers. This has led to the development of TOP-53 (**5**) and F14512 (**6**). Recently novel podophyllotoxin derivatives have been reported by researchers [11–17]. 4-aza-2,3-didehydropodophyllotoxins (**7**) have been also reported as anticancer agents [18–21], which displayed cytotoxicity comparable to that of podophyllotoxin. In the present study we report the synthesis of novel podophyllotoxin-like benzo[*b*]furo[3,4-e][1,4]diazepin-1-ones possessing structural elements of 4-aza-2,3-didehydropodophyllotoxins with central dihydrodiazepine ring (Fig. 1). The synthesized compounds were initially evaluated for their cytotoxic activity towards three different cancer cell cancer cell lines including MCF-7, PC3 and B16-F10 cancer cells employing the MTT assay. Then we investigated the cytotoxicity of compounds showed the most antiproliferative activity in initial assessment, in different human cancer cell lines including A549, A2780, DU145, HeLa, and normal Huvec cell line. The effect of the most cytotoxic compounds to induce apoptosis was evaluated by apoptosis

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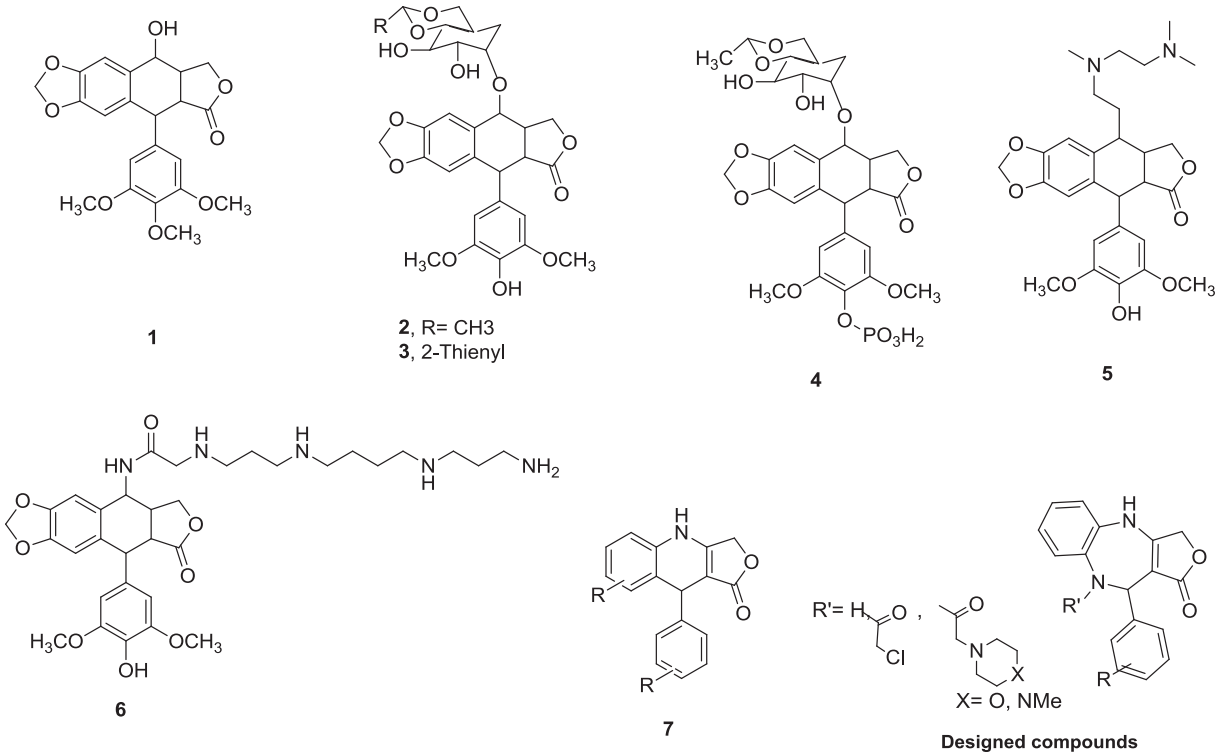

Fig. 1. Structures of podophyllotoxin, its semisynthetic analogs and our designed compounds.

assay and compounds **13c** was evaluated for tubulin polymerization inhibitory effect.

2. Results and discussion

2.1. Synthesis

As illustrated in Scheme 1, benzene-1,2-diamine **8** and tetronic acid **9** were stirred in ethanol in the presence of catalytic amount of acetic acid to afford 4-((2-aminophenyl)amino)furan-2(5H)-one **10**, then substituted benzaldehydes **11** and compound **10** were dissolved in chloroform and combined in the presence of catalytic amount of acetic acid under microwave irradiation [22] to obtain the target benzo[*b*]furo[3,4-e][1,4]diazepin-1-ones **12**, then chloroacetyl derivatives **13** were prepared using compounds **12** and chloroacetyl chloride in THF [23]. Reaction of **13** with morpholine or N-methylpiperazine in THF afforded the target compounds **14**. The compounds were characterized by nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR), infrared and mass spectrometry.

2.2. Biological evaluation

2.2.1. In vitro anticancer activity

The cytotoxic activity of the synthesized compounds was evaluated against MCF-7 (human breast cancer cells), PC3 (human prostate cancer cells), and B16-F10 (Melanoma cells) cancer cells employing the MTT assay. As depicted in Table 1, concentrations of 5 and 50 μM of compounds were used for evaluation of cytotoxicity of these compounds. Initial evaluation showed that most of the compounds were not potent cytotoxic agents at concentrations below 50 μM, among the compounds, **13c**, 2-chloroacetyl analog of **12j** possessing *p*-methyl phenyl showed significant cytotoxic activity in all three cancer cells. Compound **12h** possessing *p*-Hydroxy phenyl, **13a**, 2-chloroacetyl analog of **12a** possessing phenyl and **14b**, 2-(4-methylpiperazin-1-yl)acetyl analog of **12a**, also showed cytotoxic activity in cancer cells. As 2-chloroacetyl analogs showed more cytotoxicity in cancer cells compared to 2-morpholinoacetyl and 2-(4-methylpiperazin-1-yl)acetyl analogs, it can be concluded that the higher cytotoxic activity of 2-chloroacetyl analogs could be attributed to their higher lipophilicity. Among the compounds **12a-12k**, Compound **12h** possessing *p*-Hydroxy phenyl was the most cytotoxic agent in MCF-7 and B16-F10 cancer cells. Among the chloroacetyl derivatives **13a-13c**, **13c**, possessing *p*-tolyl, was the most cytotoxic agents. Surprisingly, compound **13b** possessing 3,4,5-trimethoxyphenyl similar to podophyllotoxin, showed the least cytotoxic activity among the series, it might be because of more steric hindrance occurred by three methoxy groups. So it can be concluded that in 2-chloroacetyl analogs, small lipophilic group in *para* position of phenyl ring can increase the cytotoxic activity in cancer cells. Since researchers reported 4-aza-2,3-didehydropodophyllotoxins (**7**) as anticancer agents [18–21], which displayed cytotoxicity comparable to that of podophyllotoxin and more than those of our compounds **12a-12k**, we can conclude that expansion of the central ring of 4-aza-2,3-didehydropodophyllotoxins from six-membered ring (dihydroquinoline) to seven-membered ring (dihydrodiazepine) can decrease the cytotoxic activity of these compounds. Among the 2-morpholinoacetyl and 2-(4-methylpiperazin-1-yl)acetyl derivatives **14a-14e**, **14b**, 2-(4-methylpiperazin-1-yl)acetyl analog of **12a**, showed the highest cytotoxicity. So we investigated the cytotoxicity of compounds **12h**, **13a**, **13c** and **14b** and podophyllotoxin as the reference drug in six cancer cell lines including A549 (adenocarcinoma human alveolar basal epithelial cells), A2780 (human ovarian cancer cells), DU145 (human prostate cancer cells), HeLa (cervical cancer cells), MCF-7 (human breast cancer cells), PC3 (human prostate cancer cells), and normal Huvec cell line (Human Umbilical Vein Endothelial Cells) (Table 2). Among these four compounds, chloroacetyl derivatives **13a** showed significant cytotoxic activity in all cancer cells. Compound **13c** showed promising antiproliferative activity against all cancer cells stronger than the other compounds and comparable to reference drug podophyllotoxin in some cancer cells. All these four compounds did not show significant cytotoxicity on normal Huvec cell line. Compounds **13a** and **13c** showed potent and selective cytotoxic effects in cancer cells.