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A promising camptothecin derivative: Semisynthesis, antitumor activity and intestinal permeability

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ABSTRACT

Oral administration of camptothecin (CPT) derivatives and other antitumoral agents is being actively developed in order to improve the quality of life of patients with cancer. Though several lipophilic derivatives of CPT have shown interesting oral bioavailability in preclinical and clinical studies, only Topotecan has been approved for this route of administration. Semisynthesis, antitumor activity, biological inhibition mechanism, and *in situ* intestinal permeability of 9, 10-[1,3]-Dioxinocamptothecin (CDiox), an unexplored CPT derivative, have been studied in this paper. The hexacyclic analog was as effective as Topotecan and CPT in different tumor cell lines, showing an expected similar apoptosis cell mechanism and high ability to inhibit DNA synthesis in HeLa, Caco-2, A375 and MDA-MB-231 cell lines. Furthermore, *in vitro* and *in situ* pharmacokinetics transport values obtained for CDiox displayed more favorable absorption profile than CPT and Topotecan.

1. Introduction

Camptothecin (CPT), a natural alkaloid isolated from *Camptotheca acuminata*, was first reported by Wall et al. to have a potent antitumor activity against a broad spectrum of tumors [1,2]. As soon as the identification of the enzyme Topoisomerase I (TopoI) as the major cellular target of CPT was confirmed (with the discovery of overexpressed levels of TopoI in tumor cells relative to normal cells), the elucidation of the structure–activity relationship of the alkaloid and the molecular mechanism of inhibition were priority in many investigations in the area of medicinal chemistry [3].

It is well established that successful inhibition of TopoI requires an unmodified lactone E ring moiety in the structure, however, the pH-dependent reversible equilibrium of the α-hydroxy-δ-lactone ring is shifted toward the carboxylate open-ring form at physiological pH or above [4]. CPTs are S-phase-specific drugs and the stabilization of the covalent TopoI-DNA complex by CPT is a required step in its antitumor activity. Because of that, prolonged or repetitive exposure of this kind of drugs is necessary to increase cell killing, since the S phase is a short phase of the cell cycle [5].

Several CPT derivatives have been developed to improve the lactone stability and to limit the uncontrolled toxic effects. Among them, Topotecan (Hycamtin®) [6], Irinotecan (Camptosar®) [7] and Belotecan (Camtobell®) [8] are the only three analogs approved for clinical practice. While Irinotecan and Belotecan are used exclusively by i.v. infusion, Topotecan is also administered by oral route for the treatment of relapsed small cell lung cancer (SCLC) in patients with a prior complete or partial response who are ≥45 days from the end of first-line chemotherapy [9]. Fig. 1

Further studies focused on the nature of the CPT derivatives, argue that highly lipophilic analogs provide several pharmaceutical advantages relative to water-soluble such as lactone stability, lack of metabolic conversion, broad antitumor activity, oral bioavailability and optimized therapeutic efficiency [10–14].

Abbreviations: CPT, 20-(S)-camptothecin; CDiox, 9, 10-[1,3]-dioxinocamptothecin; SAz, sodium azide; Pgp, P-glycoprotein 1; Annexin V-FITC/PI, annexin V–fluorescein isothiocyanate/propidium iodide.

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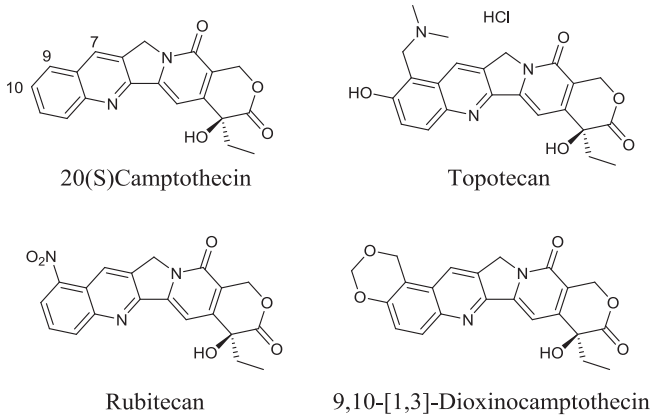

Fig. 1. Camptothecin, Dioxinocamptothecin (CDiox) and representative analogs.

According to previous modifications of CPT, additional ring combination in positions 10–11, 7–9 and 9–10 showed potent cytotoxicity, probably due to the extended planarity exerted by an additional hexacyclic-fused ring [15]. Oxazines, furan and dihydrofuran rings fused on CPT structure have been extensively studied exhibiting antitumor activity superior to those of the original pentacyclic ring system [16–18]. [1,3] Dioxino [5,4-f]camptothecin (CDiox), which can be regarded as a close structural variant of 9,10-(ethylenedioxy) CPT, has not been considered for any prior study in spite of being as active as CPT in enhancing topo I-mediated cleavage of the DNA duplex [19,20]. In addition, no violations of Lipinski rules, adequate polar surface area and a lipophilic cLogP value, complete a favorable theoretical calculation behavior for developing a more consistent study for this hexacyclic derivative [21].

On the basis of the lipophilic behavior of CDiox and the promising results published for other water-insoluble CPT derivatives [22], a preliminary study to establish the oral availability of this analog is presented in this work. To elucidate permeability and transport mechanism, experiments were carried out using Caco-2 cells and *in situ* perfusion studies in rats.

2. Results and discussion

2.1. Chemistry

CDiox was prepared by straightforward condensation of 10-hydroxycamptothecin with an excess of formaldehyde in neat trifluoromethanesulfonic acid (TfOH). Although the reaction is effective in solvents such as nitromethane, acetic acid and acetonitrile with catalytic amounts sulfuric acid or TfOH at 50 °C, optimal conditions were obtained by performing the synthesis in neat TfOH from 0 °C to room temperature overnight. The derivative was characterized by means of ^{1}H NMR, ^{13}C NMR, elemental analysis and exact mass. To the best of our knowledge no synthesis has been previously reported for this drug.

2.2. Biological studies

2.2.1. Cytotoxicity

The *in vitro* antitumor activity of CDiox was assayed by MTT method against five human cancer cell lines, including cervical cancer (HeLa), colon adenocarcinoma (Caco-2), malignant melanoma (A375), T cell lymphoblast (Jurkat), and breast cancer hormone sensitive cell line (MDA-MB-231). CPT as parent compound and Rubitecan and Topotecan as well known effective oral CPT derivatives were used as reference drugs. IC50 (μM) values are expressed in Table 1. The results indicate than CDiox is as potent as standard compounds across all the cell lines, revealing that the 1, 3-dioxine ring fused on the CPT structure retains the antiproliferative activity. Results indicate that CDiox and Rubitecan are slightly more active against HeLa and Caco-2 cells than CPT and Topotecan, while, unmodified CPT is more effective against MDA-MB-231.

2.2.2. Flow cytometry assay. Apoptosis induction

Cell death mechanism was investigated using Annexin V-FITC/PI flow cytometry assay. Experiments allow analyzing whether CDiox, CPT, Rubitecan and Topotecan were able to induce apoptosis in Caco-2 cells at concentrations of 0.05 μM and 0.5 μM after 24 h of incubation. In order to determine if the apoptosis induced by the compounds is time dependent, assays were also carried out after 48 h of incubation.

As shown in Table 2, all tested compounds induced cell death mainly through apoptosis. Interestingly, the exposition of cell cultures to increased concentrations of the compounds significantly increased the number of apoptotic cells (early + late apoptosis), while no important changes in the number of necrotic cells are observed (especially after 48 h of incubation). CDiox activity is similar or even better than the reference compounds, suggesting a promising anticancer candidate. The ability of compounds to induce cellular apoptosis was in agreement with the results of the MTT assay.

Fig. 2 shows an example of flow cytometry scheme for untreated cells and cells exposed to 0.05 μM or 0.5 μM of CDiox for 48 h.

2.2.3. Measurement of DNA synthesis

Since TopoI inhibition has been shown to be a feasible strategy to block cancer cell proliferation, the effect of the compounds on DNA synthesis was evaluated by measuring the incorporation of BrdU into the DNA of HeLa, A375, Caco-2 and MDA-MB-231 cancer cell lines. Results, summarized in Fig. 3, showed that all CPT derivatives, including CDiox, exhibited high ability to inhibit DNA synthesis in HeLa, A375, Caco-2 and MDA-MB-231 cell lines. Reduction values of BrdU incorporation can be considered similar for CDiox and the reference compounds when compared the effect at 0.8 μM. At higher concentration (1.6 μM) the activity of reference drugs is higher, showing a concentration-dependent effect. These results indicate that CDiox is a potent analog with similar activity to CPT, Topotecan and Rubitecan. Moreover, it is remarkable the strong inhibition of DNA synthesis observed for 1.6 μM of CDiox in HeLa and Caco-2 cell lines.

Table 1
In vitro antitumor activity of CDiox analog and reference compounds against five human tumor cell lines, were measured by the MTT assay after 24 h of incubation and expressed as IC50 (μM).

	HeLa	Caco-2	A375	Jurkat	MDA-MB231
CPT	0.420 ± 0.025	0.131 ± 0.011	0.187 ± 0.005	0.110 ± 0.007	0.342 ± 0.015
Topotecan	0.380 ± 0.011	0.119 ± 0.008	0.162 ± 0.012	0.127 ± 0.009	0.473 ± 0.023
Rubitecan	0.343 ± 0.012	0.112 ± 0.005	0.138 ± 0.014	0.128 ± 0.008	0.479 ± 0.013
CDiox	0.345 ± 0.013	0.104 ± 0.004	0.186 ± 0.007	0.124 ± 0.010	0.476 ± 0.018