ACS Medicinal Chemistry Letters

Letter

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Semisynthesis, Cytotoxic Activity, and Oral Availability of New Lipophilic 9-Substituted Camptothecin Derivatives

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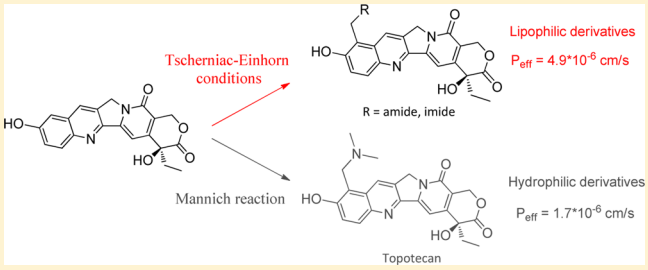
Supporting Information

ABSTRACT: Despite that 9-substituted camptothecins are promising candidates in cancer therapy, the limited accessibility to this position has reduced the studies of these derivatives to a few standard modifications. We report herein a novel semisynthetic route based on the Tscherniac–Einhorn reaction to synthesize new lipophilic camptothecin derivatives with amidomethyl and imidomethyl substitutions in position 9. Compounds were evaluated for their antiproliferative activity, topoisomerase I inhibition, and oral availability. Preliminary data demonstrated that bulky imidomethyl modification is an appropriate lipophilic substitution for an effective oral administration relative to topotecan. In addition, this general procedure paves the way for obtaining new camptothecin derivatives.



KEYWORDS: *Lipophilic camptothecin, Tscherniac–Einhorn derivatives, 9-substituted camptothecins, oral administration*

Antitumor compound camptothecin (CPT), a pentacyclic alkaloid isolated from *Camptothecin acuminata* by Wall and Wani in 1966,[1] is considered one of the most potent antineoplastic agents in a broad list of tumor cell lines.

Extremely poor solubility and severe toxicity[2,3] have been considered the main challenge in order to optimize new CPT derivatives to find more effective candidates. Extensive structure–activity relationship (SAR) studies have predicted well-known substitution rules where positions 7 and 9 are feasible for the development of new potent CPT analogues.[4,5] In particular, recent evidence indicates that hydrophobic modifications confer a rapid cellular accumulation, improved uptake, ternary complex stabilization, and lactone E ring stability over the water-soluble derivatives.[6−10]

As soon as the nature and favorable positions in the alkaloid were known, many investigations have been focused on highly efficient semisynthetic routes paving the way for new chemical possibilities and novel derivatives. It is unquestionable that Mannich reaction,[11] Claisen rearrangement,[12,13] or Minisci reaction,[14,15] for example, have been useful methodologies to obtain topotecan, chimmitecan, and most of 7-substituted CPTs, respectively (Figure 1). Furthermore, these strategies, have been applied to activate specific positions for further development on CPTs chemistry.[16] Despite intensive efforts, not many more alternative semisynthetic methodologies are known to work with acceptable yield on CPT substrate. In fact, Dallavalle and co-workers reported one of the most clear examples of the difficulty to obtain the formylation of 10-

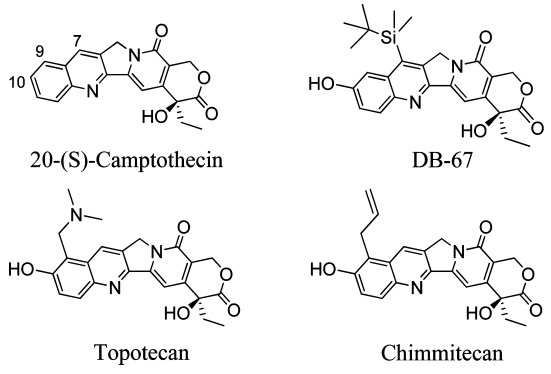


Figure 1. Structures of camptothecin and derivatives.

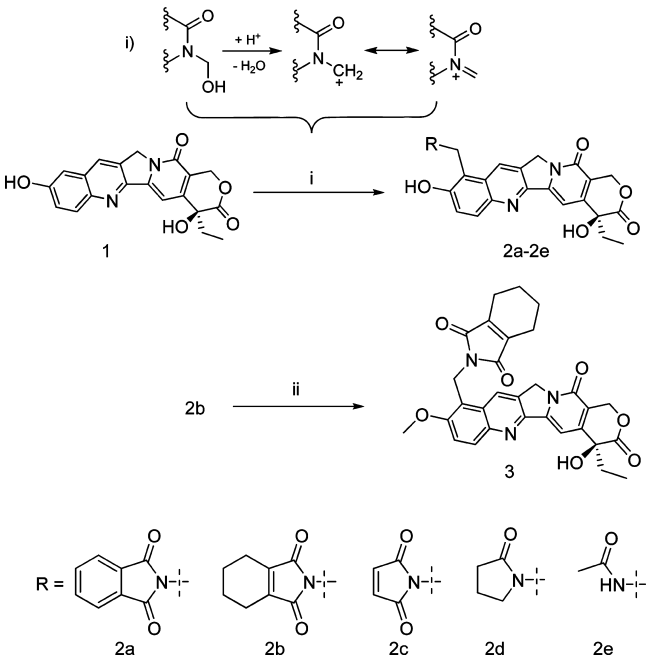
Received: April 2, 2013
Accepted: May 28, 2013
Published: May 28, 2013

hydroxyCPT (**1**) by an efficient pathway in order to evaluate new CPT derivatives.[17]

The high cytotoxic activity of 9-substituted CPTs reported in the literature[18,19] and the limited functionalization of this position, based on specific modifications,[20,21] encouraged us to investigate the reactivity of the Tscherniac−Einhorn reaction (T−E) to afford new lipophilic 9-amidomethyl-10HCPT and 9-imidomethyl-10HCPT derivatives as well as their potential for oral administration.

The T−E,[22−24] the condensation of methyleneimmonium ions with aromatic compounds under acidic conditions (expanded reaction, Scheme 1), has a broad application in organic chemistry to introduce functionalities, which are inaccessible or require multistep pathways. We envisioned a novel pharmacological behavior by the replacement of the charged aminomethyl moiety of Mannich reaction by a neutral and hydrophobic functionality. Furthermore, on the basis of reported molecular dynamic calculations of water participation in the topo I-DNA-CPT ternary complex,[25,26] insertion of amidomethyl or imidomethyl groups enables novel hydrogen bonding acceptors, which are susceptible to stabilize the cleavable complex.

Scheme 1. Synthesis of 9-Substituted Camptothecin Derivatives: General Formation and Stabilization of Tscherniac−Einhorn Intermediates (Expanded Reaction, i)[a]



[a]Reagents and conditions: (i) *N*-hydroxymethylamide or *N*-hydroxymethylimide, neat H_2SO_4, 0 °C to room temperature, 12 h. (ii) $(CH_3O)_2SO_2$, K_2CO_3, DMF, 80 °C, 4 h.

To the best of our knowledge, this is the first publication where the T−E is applied to obtain new CPT derivatives and its preliminary in vitro evaluation. In our hands, no extensive biological studies or synthetic methodologies have been reported for derivative **4**.[27,28] However, its chemical structure is clearly favorable to develop further CPT analogues, for example, derivative **5** was obtained with high yield in contrast with reported Duff reaction[11] and several other attempts.[17]

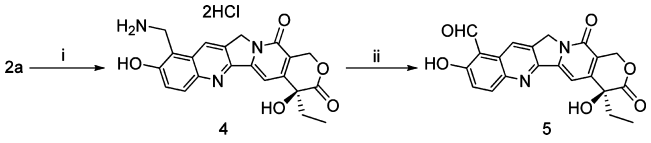
Oral administration of newer camptothecins is continuously evaluated for achieving prolonged drug exposure and optimal efficiency. Thus, intestinal absorption and secretion are specific parameters that are applied to provide a preliminary prediction of oral delivery convenience.[29,30]

In this work, an internally validated correlation between Caco-2 permeability values and oral fraction absorbed in rat has been used to predict the oral fraction absorbed of the new derivatives.

9-Substituted CPTs were prepared by straightforward T−E, using **1** as starting material and readily available *N*-hydroxymethylamides and *N*-hydroxymethylimides in one step as shown in Scheme 1. First, methyleneimmonium ions were generated in excess of neat sulfuric acid at 0 °C; subsequently, solid **1** was slowly added, and the reaction was monitored by TLC. It should be noted that solvents are critical in the reaction, whereas neat triflic acid proved to be as efficient as sulfuric acid, and the presence of DMF, MeOH, dioxane, acetic acid, or nitromethane afforded a substantially lower yield or no reaction. Similarly, a one pot reaction of **1**, phthalimide, and different aldehydes was unsuccessful. In order to screen the modifications at C-9, we introduced small amide moiety, cyclic amide, imide group, and bulky bicyclics imides.

We take advantage of the efficient synthesis of **2a** to obtain the water-soluble derivative **4** (78% yield) and the analogue **5** (60% yield), by a simple two-step route, removing the phthalimide moiety followed Sommelet standard conditions[31] (Scheme 2).

Scheme 2. Synthesis of 9-Formyl-10HCPT[a]



[a]Reagents: (i) conc. HCl reflux 12 h.; (ii) HOAc/H_2O, HMTA reflux 4 h; H_3O^+ reflux 1 h.

As expected in well-known lactone-form CPT derivatives,[32] new substituted analogues showed antitumor activity by topoisomerase I inhibition (see Supporting Information).

The compounds **2a−2e** were screened for antiproliferative activity against human cervical cancer cell line (HeLa). As showed in Table 1, all new derivatives, except **2b**, are less active than topotecan, showing a preferential substitution for aminomethylated moieties.

However, modifications with bulky methylimide groups (**2a** and **2b**) instead of cyclic or secondary methylamides (**2e** and **2d**) are clearly favorable, suggesting an improvement to cross plasma membrane due to the stronger lipophilicity and no steric restriction at molecular level.

On the basis of preceding publications,[33,34] we hypothesized an improvement of the cytotoxic activity by methoxylation of the position 10.

The most active compound, **2b**, was selected for a broad comparative test with its 10-methoxylated form, **3**, in seven human cancer cell lines: MOLT-4 (leukemia cancer), K562 (chronic myelogenous leukemia), A2780 (ovarian cancer), A549 (lung cancer), MDA-MB231 (breast cancer hormone insensitive), PC3 (prostate cancer), and MCF-7 (breast cancer hormone sensitive) (Table 1).

Cytotoxicity data showed better activity of **3** compared to **2b** across all the cell lines profiled and more potent that topotecan in all tumor cell lines except MCF-7. Particularly, **3** was 5-fold

dx.doi.org/10.1021/ml400125z | ACS Med. Chem. Lett. 2013, 4, 651−655

Table 1. Cytotoxicity of CPT Derivatives against Different Human Tumor Cell Lines and IC_{50} (nM)[a]

compd	HeLa	MOLT4	A2780	MCF-7	K562	PC3	A549	MDA-MB231
CPT	24	0.04	13	26	31	78	99	162
topotecan	376	19	33	63	500	62	354	>500
2a	1274							
2b	214	15	41	159	>500	103	346	>500
2c	>5000							
2d	>5000							
2e	>5000							
3	173	14	28	78	109	51	226	500

[a]Antitumor activity was measured by the MTT assay after 72 h of drug exposure. CPT and topotecan were used as reference compounds. Data correspond to IC_{50} values of a minimum of three independent experiments. Blank cells: not tested.

more potent than topotecan in K562 cell line and showed similar cytotoxicity in PC3 cell line compared with CPT. Methoxyl at C-10 displays to improve the activity when the group at position 9 is large. With our experimental possibilities, we cannot suggest an enhancement due to a favorable molecular conformation of the bulky substitution determining by the methoxylation or a simple better cellular drug accumulation. However, we have taken into account the higher lipophilicity and cytotoxicity of **3** compared with topotecan for testing its availability in oral administration.

The intestinal transport of CPT, topotecan, and derivative compounds have been characterized using the Caco-2 cell culture model, which has been proven to be a good model to predict human absorption.[35,36] The permeability values were determinate in both directions, apical to basal (Pab) and from basal to apical (Pba), in the presence and absence of sodium azide (SAz).

As can be observed in Figure 2, CPT and **1** do not show any difference in permeability values. In the case of topotecan, the

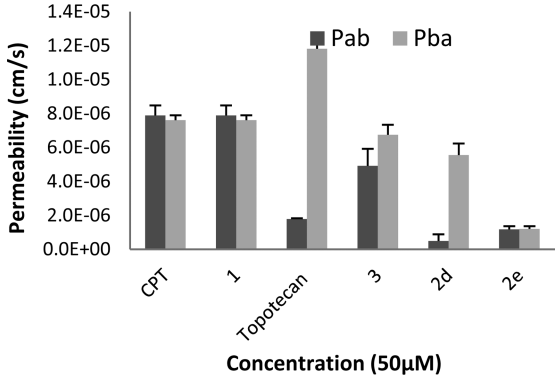


Figure 2. Permeability values obtained from apical to basal (Pab) and from basal to apical (Pba) at 50 μM in Caco-2 cell line. Data correspond to values of three independent experiments.

Pba/Pab ratio is clearly higher than the one in accordance with the common pattern of efflux substrates. Two of the new derivatives, **3** and **2d**, showed a Pba/Pab ratio statistically higher than one indicating they are substrates of an efflux transporter. The Pba to Pab ratio of **2e** is close to one, but a secretion process cannot be ruled out as the efflux process could be saturated.

The results in the presence of SAz are represented in Figure 3. SAz is supposed to cause a complete inhibition of any active carrier mediated processes, so the ratios Pba to Pab should be close to one and the permeability value would represent the diffusion component.

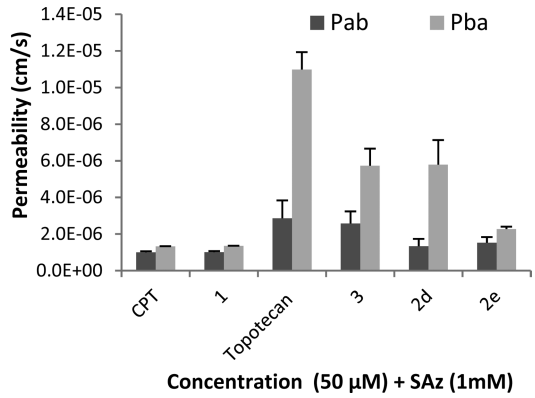


Figure 3. Permeability values obtained from apical to basal (Pab) and from basal to apical (Pba) at 50 μM in the presence of SAz in Caco-2 cell line. Data correspond to values of three independent experiments.

Results indicate that permeabilities of CPT and **1** in the presence of SAz have decreased, are similar in magnitude, and represent the passive diffusion component of the transport. In the case of topotecan, in the presence of SAz, a ratio of Pba/Pab clearly lower than that in the absence of metabolic inhibitor is observed, but a higher concentration of inhibitor would be necessary to nullify the active transport. The new derivatives **3** and **2d** showed a Pba/Pab ratio statistically higher than that in the absence of SAz indicating a more complicated transport mechanism with more than one transporter involved apart from Pgp.

Pab of all the compounds have been used to predict the oral fraction absorbed. Table 2 summarizes the oral fraction absorbed predicted based on the permeability values and on the correlation P_{eff}–F_a graphically represented in Figure 4.

In order to be a potential oral drug candidate, a good oral fraction absorbed (higher than 50%) would be desirable. Lower values would inhibit the development of an oral product and lead to the selection of other extravasal (intramuscular) or intravenous route. Preliminary results indicate that **3** could be a good candidate for oral administration instead of topotecan (Table 2).

In summary, a general semisynthetic methodology based on the T–E allows the synthesis of new lipophilic 9-substituted CPT derivatives in contrast with hydrophilic Mannich-type derivatives. Our preliminary data showed that methylamide or methylimide modifications are less active than aminomethyl substitutions. However, when position 10 is methoxylated, bulky methylimide substitution is available to provide a highly lipophilic derivative with appropriate behavior for oral administration. Furthermore, present methodology is highly

dx.doi.org/10.1021/ml400125z | ACS Med. Chem. Lett. 2013, 4, 651–655

Table 2. Predicted Values of Oral Fraction Absorbed

	in absence of sodium azide		in presence of sodium azide	
compd	P_{eff} (cm/s)[a]	F_a[b]	P_{eff} (cm/s)[a]	F_a[b]
CPT	7.88×10^{-06}	0.80	1.00×10^{-06}	0.18
topotecan	1.77×10^{-06}	0.30	2.88×10^{-06}	0.44
1	7.88×10^{-06}	0.80	1.01×10^{-06}	0.18
2d	0.48×10^{-06}	0.09	1.33×10^{-06}	0.24
2e	1.17×10^{-06}	0.21	1.52×10^{-06}	0.27
3	4.91×10^{-06}	0.63	2.58×10^{-06}	0.41

[a]Permeability coefficient of the compounds through Caco-2 monolayer calculated according to equations for sink or nonsink conditions (mentioned in Supporting Information). Values are the means of three independent experiments. [b]Oral fraction absorbed predicted based on the permeability values by interpolation on a validated correlation P_{eff}–F_a.

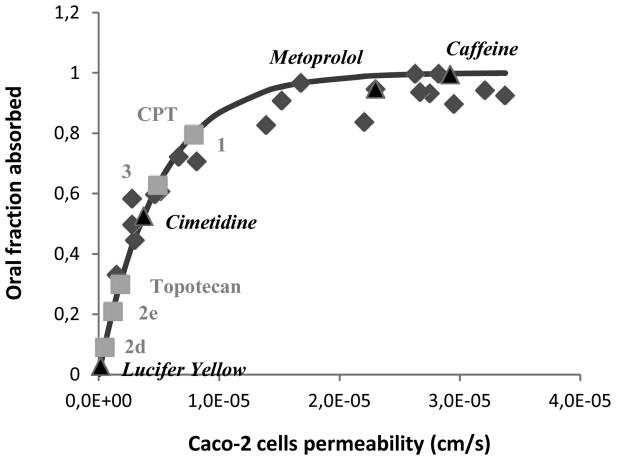


Figure 4. Oral fraction absorbed vs permeability values obtained from apical to basal (Pab) at 50 μM Caco-2 cell line. Gray squares correspond to CPT derivative compounds. Diamond data correspond to the internally validated correlation between Caco-2 permeability and oral fraction absorbed (unpublished data). Triangles correspond to standards of high (caffeine and metoprolol), medium (cimetidine), and low (lucifer yellow) permeability.

effective to obtain compounds **4** and **5** as potential starting materials for further development.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

General information, topoisomerase I inhibition, experimental procedures, and ^{1}H NMR and ^{13}C NMR spectra of CPT derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

Supported by Instituto de Tecnologia Quimica postgraduate scholarship (to G.R.-B.); Consolider-Ingenio 2010 (proyecto MULTICAT), Subprograma de Apoyo a Centros de Excelencia Severo Ochoa, and Agencia de Gestió d'Ajuts Universiteris i de Recerca (2009 SGR 758, to S.S.). I.G.-A, V.M.-S., M.G.-A., and M.B. acknowledge financial support from the European Financial Commission (Red bioFarma DCI-ALA/19.09.01/10/21526/245-297/ALFA III (2010).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We will like to thank Laura Garcia Latorre for her technical assistance in MTT assays.

■ ABBREVIATIONS

T–E, Tscherniac–Einhorn reaction; CPT, 20-(*S*)-camptothecin; IC_{50}, half maximal inhibitory concentration; HOAc, acetic acid; HMTA, hexamethylenetetramine; Pab, permeability form apical to basal; Pba, permeability form basal to apical; SAz, sodium azide

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