Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 2731–2735

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Pharmacological Evaluation of Novel Non-lactone Analogues of Camptothecin†

Patrick Hautefaye,[a] Bernard Cimetière,[a] Alain Pierré,[a] Stéphane Léonce,[a] John Hickman,[a] William Laine,[b] Christian Bailly[b] and Gilbert Lavielle[a,*]

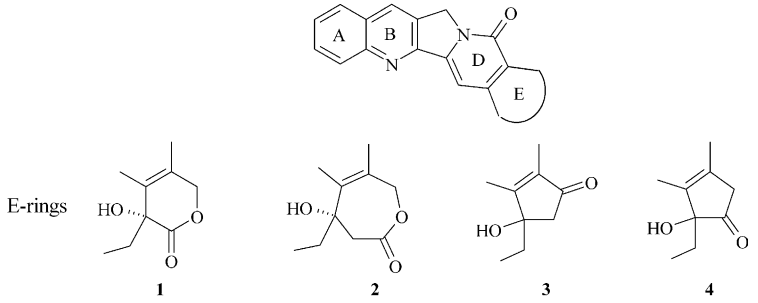
[a]*Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy sur Seine, France*
[b]*Laboratoire de Pharmacologie Antitumorale du Centre Oscar Lambert, INSERM U-524, IRCL, Place de Verdun, 59045 Lille, France*

Received 18 February 2003; revised 22 April 2003; accepted 19 May 2003

Abstract—Ten novel camptothecin (CPT) derivatives devoid of the lactone function in the E-ring were synthesized and evaluated as anticancer agents. Several of these CPT analogues bearing a five-membered E-ring are potent inhibitors of the DNA relaxation and cleavage reactions catalyzed by topoisomerase I and exhibit promising cytotoxic activities with IC_{50} values in the nM range. This is the first successful design of lactone-free CPT, providing thus a new avenue to the development of topoisomerase I targeted antitumor agents.

Introduction

Camptothecin (CPT) **1** a pentacyclic alkaloid isolated from *Camptotheca acuminata* by Wall and Wani in 1966, is one of the most important lead compounds in anticancer research.[2] The antitumoral activity of CPT is attributed to the selective poisoning of DNA topoisomerase I, an enzyme essential for the relaxation of DNA during important cellular processes.[3] It has been postulated that CPT, as a closed lactone E-ring, forms a ternary complex with topoisomerase I and DNA. Different molecular models have been proposed for the structure of this ternary complex taking into account the key role of the six-membered α-hydroxy lactone E-ring for the enzyme poisoning activity.[4,5] Recently, the 3D structure of a ternary complex formed between topoisomerase I, DNA and the clinically used CPT derivative topotecan has been solved by X-ray crystallography.[6] The E-ring of CPT is essential to the interaction with the enzyme. However, this lactone E-ring of CPT opens rapidly to an inactive hydroxy acid form in human plasma. Over the past 10 years, a number of E-ring modified CPT analogues have been prepared, most of them being inactive or less active than CPT.[7]



†See ref 1.
*Corresponding author. Fax: +33-1-5572-2470; e-mail: gilbert.lavielle@fr.netgrs.com

0960-894X/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00534-1

Consequently, the search for more stable analogues possessing the necessary structural requirements for the formation of stable ternary complexes is still very active.

In 1997, an E-ring modified CPT **2** with enhanced lactone stability was found to be a very potent topoisomerase I inhibitor and a powerful cytotoxic.[8,9] The seven-membered lactone ring characteristic of the homocamptothecin derivatives limits the conversion into an inactive carboxylate form.[10]

These observations raise the possibility that novel E-ring modified analogues of CPT with improved chemical stability might possess enhanced antitumor activity. To study this possibility, we have designed new compounds, **3** and **4**, lacking the oxygen of the lactone E-ring and therefore possessing a five-membered E-ring. Here, we report the synthesis of these compounds and we demonstrate that the anti-topoisomerase I activity is preserved.

Chemistry

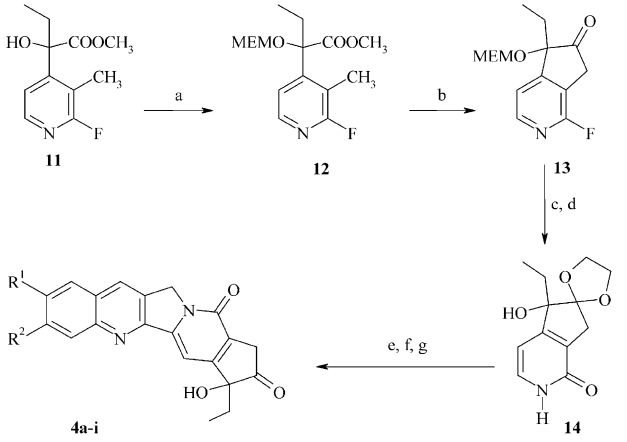
Our synthesis was based on the procedure used by Comins for the total synthesis of CPT.[11]

N-Alkylation of cyclopenta[*c*]pyridine derivatives **5** and **14** with different bromomethyl-3-quinolines, followed by an intramolecular Heck reaction gave the desired pentacyclic compounds.

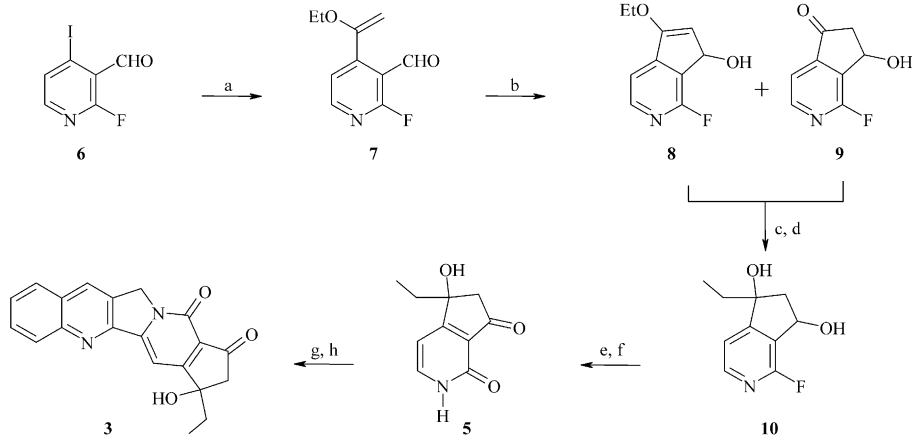
The cyclopenta[*c*]pyridine-1-one **5** needed to prepare compound **3** was obtained following the sequence depicted in Scheme 1.

[(Fluoro-2-iodo-4)pyrid-3-yl]-carboxaldehyde **6**[12,13] was reacted with 1-ethoxy-1-(tributyltin) ethylene under Stille conditions to give **7**. The crude product **7** underwent a spontaneous ring closure to give **8** and **9** during the purification on silica gel with CH_2Cl_2/MeOH (98/2) as eluent. Treatment of the mixture (**8** and **9**) in trifluoroacetic acid in water–acetonitrile at room temperature completed the hydrolysis of the cyclic enol. Addition of an excess of ethylmagnesiumbromide to **9** in diethylether at −30 °C afforded, after purification, a 50% yield of diol **10** as a mixture of diastereoisomers (75/25). Oxidation of **10** gave an intermediate hydroxyketone, the fluoro-2-pyridine function of which was hydrolyzed in acidic medium to lead to the targeted cyclopenta[*c*]pyridine-1-one **5**. Then **5** was alkylated with bromo-2-bromomethyl-3-quinoline following conditions described by Curran,[14] that provide selectively the *N*-alkylated product in yield over 80%. The final ring closure was accomplished by an intramolecular Heck reaction[11] to give **3** in moderate yield (25%).

Compounds **4** were prepared using a novel cyclopenta[*c*]pyridine-1-one possessing an oxo group at position 6 (Scheme 2). The various AB quinolines (substituted bromo-(chloro)-2-bromomethyl-3-quinolines) used in the last stage of the preparation of the



Scheme 2. (a) NaH, MEMCl, THF; (b) 5 equiv LDA/HMPA, THF; (c) 3 N HCl, reflux, 1 h; (d) $HO(CH_2)_2OH$, pTSA (cat.), toluene, reflux; (e) variously substituted bromo-2-bromomethyl-3-quinolines, NaH, LiBr, DME, DMF; (f) $Pd(OAc)_2$, NBu_4Br, KOAc CH_3CN, reflux; (g) CF_3COOH/H_2O/reflux.



Scheme 1. (a) 1-Ethoxy-1-(tributyltin)ethylene, $Pd(PPh_3)_4$; (b) silica gel, CH_2Cl_2/MeOH (98/2); (c) CF_3COOH, H_2O/CH_3CN, rt; (d) EtMgBr, Et_2O, −30 °C; (e) PCC, CH_2Cl_2; (f) 2 N HCl, reflux, 1 h; (g) bromo-2-bromomethyl-3-quinoline, NaH, LiBr, DME, DMF; (h) $Pd(OAc)_2$, NBu_4Br, KOAc, CH_3CN, reflux.

different compounds **4a–i** were obtained following methods described in the litterature.[8,15]

Intermediate **11**, previously prepared by Comins,[13] was used as starting material. Treatment of **11** with MEMCl in the presence of sodium hydride in THF gave MEM ether **12** in high yield. Then, conditions of the intramolecular ring closure of **12** were investigated. Reaction of **12** with one equivalent of LDA/HMPA in THF at 0° C during several hours afforded only small quantities of the bicyclic ketone **13**.[16] The best results were observed after treatment of **12** with a 5-fold excess of LDA/HMPA in THF at 0 °C for 1 h. Following these conditions **13** was obtained in 73% yield. Then the fluoro-1-cyclopenta[*c*]pyridine **13** was hydrolyzed to the corresponding pyridine-1-one after treatment with 3 N HCl at reflux. To prevent an enolization reaction in basic medium and the formation of side products during the following step, the ketone was converted to the cyclic ketal **14**. Then, the cyclopenta[*c*]pyridine-1-one **14** was selectively *N*-alkylated with the variously substituted bromo-2-bromomethyl-3-quinolines in high yields. The final ring closure was performed using the conditions described in Scheme 1 and gave the pentacyclic compounds only in moderate yields. Then the deprotection of the ketone proceeded upon treatment with CF_3COOH/H_2O at reflux and gave the final products **4a–i** quantitatively.

Biological Results

Topoisomerase I inhibition

The topoisomerase I inhibitory properties of the tested compounds were examined using a DNA relaxation assay.[17] Negatively supercoiled plasmid pKMp27 was incubated with topoisomerase I in the presence of each drug at 1 or 10 μM. Parallel experiments were performed with CPT and SN-38, the active metabolite of the anticancer drug irinotecan. DNA samples were treated with SDS and proteinase K to remove any covalently bound protein and resolved in 1% agarose gels containing ethidium bromide. As shown in Figure 1, supercoiled DNA is fully relaxed by topoisomerase I in the absence of drug (compare lanes DNA and Topo I). In the presence of the test drugs, the intensity of the slowest-migrating band, corresponding to the nicked form of DNA, has increased significantly. This effect reflects the stabilization of topoisomerase I–DNA cleavable complexes. Band intensities were quantified and the extent of cleavage detected with each compound was expressed relative to that detected with SN38 at the same concentration (1 μM) (Table 1). With compound **4b**, the level of nicked DNA molecules was only slightly lower than that of the clinically relevant CPT. With the other compounds, the amount of DNA cleavage was less pronounced compared to SN38; however, there is no doubt that these lactone-free compounds behave as conventional topoisomerase I poison. These observations were fully confirmed by complementary experiments using a 117-mer ^{32}P-labeled DNA restriction fragments (Fig. 2). Compounds **4b**, **4c** and **4i** strongly promote the cleavage of DNA by topoisomerase I.

Cell-growth inhibition

The cytotoxicity of the newly designed CPT derivatives was measured using a conventional microculture tetrazolium assay as described.[18] L1210 leukemia cells were exposed to graded concentrations of drug for 48 h, and the results are expressed as IC_{50}. Except for **3** and **4e**, all the compounds were potent inhibitors of L1210 cell proliferation, **4i** being the most potent with an IC_{50} value of 60 nM, which is in the same range as topotecan IC_{50} (Table 1). The cytotoxic potential of **4i** was evaluated further on two human solid tumor cell lines, DU145 (prostate) and HT29 (colon), which were slightly more sensitive than L1210 with an average IC_{50} of 29 nM, versus 30 nM for SN38 or 40 nM for topotecan. Cell-cycle analyses[19] revealed that the CPT derivatives induce arrest in the G2 + M phases of the cell cycle (Table 1). These results suggest that all of these camptothecin derivatives share a similar mechanism of action in whole cells.

Conclusion

There is no doubt that these CPT analogues bearing a five-membered ketone E-ring are potent Topo I poisons, in spite of the absence of a lactone moiety. For the first time, a non-lactone CPT derivative active against Topo I has been successfully designed. The most potent derivatives were as cytotoxic for tumor cells as clinically relevant drugs such as SN38 and topotecan. Similar

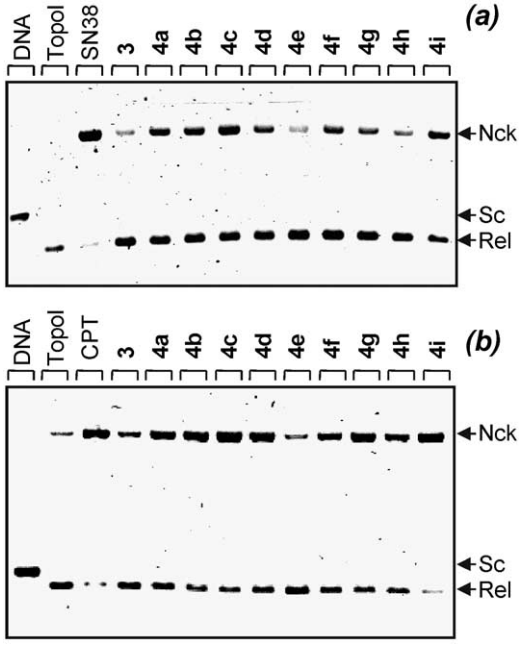


Figure 1. Effect of the camptothecin derivatives at (a) 1 μM and (b) 10 μM on the relaxation of plasmid DNA by human topoisomerase I. Native supercoiled pKMp27 DNA (lane DNA) was incubated with topoisomerase I in the absence (lane TopoI) or presence of the indicated compound. Lanes marked CPT and SN38 refer to camptothecin and the active metabolite of the antitumor drug irinotecan, respectively. DNA samples were separated by electrophoresis on agarose gels containing ethidium bromide (1 μg/mL). Gel were photographed under UV light. Nck, nicked; Rel, relaxed; Sc, supercoiled.

Table 1. Biological results for compounds **3** and **4a–i**

Compd	R_1	R_2	L1210 IC_{50} (μM)[a]	Cell cycle % of L1210 cells in G2 + M (μM)[b]	Topoisomerase I–DNA cleavage[c]
3	H	H	26.7	N.T.	0.27
4a	H	H	1.0	78 (5)	0.68
4b	OCH_3	H	0.6	79 (2.5)	0.72
4c	OH	H	1.7	77 (5)	0.88
4d	Cl	H	0.6	80 (2.5)	0.61
4e	CH_3	CH_3	> 50	N.T.	0.31
4f	F	F	0.2	78 (1)	0.64
4g	F	Cl	0.6	83 (1)	0.49
4h	CH_3	Cl	0.4	79 (2)	0.30
4i			0.06	82 (0.05)	0.64
Topotecan			0.10	78 (0.20)	0.88
SN38			0.04	77 (0.20)	1
CPT			0.03	81 (0.25)	0.77

N.T., not tested.
[a]Results are expressed as IC_{50}, the concentration (μM) which reduced by 50% the optical density of treated cells with respect to the optical density of untreated controls.
[b]Cells were exposed to graded concentrations of the compounds for 21 h and analyzed on a flow cytometer. Results are expressed as the higher % of cells arrested in the G2 + M phases of the cell cycle by the concentration (μM) given in parentheses.
[c]Extent of DNA cleavage measured with each compound at 1 μM relative to SN38.

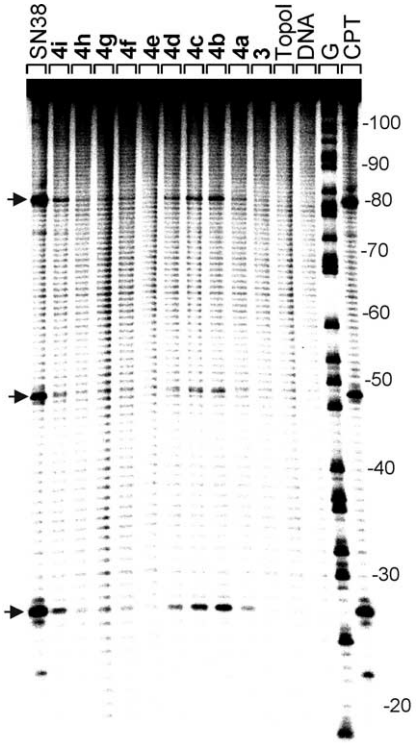


Figure 2. Cleavage of the 117 bp DNA fragment by topoisomerase I in the presence of the camptothecin derivatives. The 3′-end labeled fragment was incubated in the absence (lane TopoI) or presence of the test drug at 10 μM. Topoisomerase I cleavage reactions were analyzed on a 8% denaturing polyacrylamide gel. Numbers at the right side of the gel show the nucleotide positions, determined with reference to the guanine tracks labeled G. Arrows point to the three main cleavage sites.

perturbations of the cell cycle were induced by the novel derivatives and the reference drugs, suggesting that poisoning of topo I is the molecular mechanism underlying their cytotoxic properties.

Acknowledgements

Supported by a research grant (to C. B.) from the Ligue Nationale Contre le Cancer (Equipe labellisée LA LIGUE). The authors thank Solange Huet for excellent secretarial assistance.

References and Notes

1. Presented in part at the XVIIth International Symposium on Medicinal Chemistry, Sept. 1–5, 2002, Barcelona, Spain; P279.
2. Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. *J. Am. Chem. Soc.* **1966**, *88*, 3888.
3. Hsiang, Y. H.; Hertzberg, R. P.; Hecht, S.; Liu, L. F. *J. Biol. Chem.* **1985**, *260*, 14873.
4. Fan, Y.; Weinstein, J. N.; Kohn, K. W.; Shi, L. M.; Pommier, Y. *J. Med. Chem.* **1998**, *41*, 2216.
5. Redinbo, M. R.; Stewart, L.; Kuhn, P.; Champoux, J. J.; Hol, W. G. J. *Science* **1998**, *279*, 1504.
6. Staker, B. L.; Hjerrild, K.; Feese, M. D.; Behnke, C. A.; Burgin, A. B., Jr; Stewart, L. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15387.
7. For a recent review, see: Du, W.; Curran, D. P.; Bevins, R. L.; Zimmer, S. G.; Zhang, J.; Burke, T. G. *Bioorg. Med. Chem.* **2002**, *10*, 103.
8. Lavergne, O.; Lesueur-Ginot, L.; Pla Rodas, F.; Kasprzyk, P. G.; Pommier, J.; Demarquay, D.; Prevost, G.; Ulibarri, G.; Rolland, A.; Schiano-Liberatore, A.-M.; Harnett, J.; Pons, D.; Camara, J.; Bigg, D. C. H. *J. Med. Chem.* **1998**, *41*, 5410.
9. Lavergne, O.; Lesueur-Ginot, L.; Pla Rodas, F.; Bigg, D. C. H. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2235.
10. Bailly, C. *Crit. Rev. Oncol. Hematol.* **2003**, *45*, 91.
11. Comins, D. L.; Baevsky, M. F.; Hong, H. *J. Am. Chem. Soc.* **1992**, *114*, 10971.
12. Rocca, P.; Cochennec, C.; Marsais, F.; Thomas-dit-Dumont, L.; Mallet, M.; Godard, A.; Queguiner, G. *J. Org. Chem.* **1993**, *58*, 7832.

13. Comins, D. L.; Saha, J. K. *Tetrahedron Lett*. **1995**, *36*, 7995.
14. Liu, H.; Ko, S. B.; Josien, H.; Curran, D. P. *Tetrahedron Lett*. **1995**, *36*, 8917.
15. Depending on the nature of R^1 or R^2, the different quinolines were obtained from: Meth-Cohn, O.; Narine, B.; Tarnoruski, B. *J. Chem. Soc., Perkin Trans 1* **1981**, 1520. Meth-Cohn, O.; Narine, B. *Tetrahedron Lett*. **1978**, *23*, 2045. Bigg, D. C. H.; Lavergne, O.; Pla Rodas, F.; Pommier, J.; Ulibarri, G. WO Patent 9700876; *Chem. Abstr*. **1997**, *126*, 144433.
16. Kaiser, E. M.; Petty, J. D. *Synthesis* **1975**, 705.
17. Bailly, C. *Methods Enzymol*. **2001**, *340*, 610.
18. L1210 cells were cultivated in RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES buffer (pH = 7.4). For details: Costes, N.; LeDeit, H.; Michel, S.; Tillequin, F.; Koch, M.; Pfeiffer, B.; Renard, P.; Léonce, S.; Guilbaud, N.; Kraus-Berthier, L.; Pierré, A.; Atassi, G. *J. Med. Chem*. **2000**, *43*, 2395.
19. For the cell-cycle analysis, L1210 cells (5×10^5 cells/mL) were incubated for 21 h with various concentrations of the drugs. Cells were then fixed by 70% ethanol, and analyzed on a XL/MCL flow cytometer (Beckman Coulter, France).