Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 5781–5784

Synthetic approach to condensed heterocyclic analogues from etoposide revisited. Synthesis of A-ring pyridazine etoposide

Emmanuel Bertounesque,* Philippe Meresse, Claude Monneret and Jean-Claude Florent

UMR 176 CNRS-Institut Curie, Centre de Recherche, 26 rue d'Ulm, 75248 Paris Cedex 05, France

Received 27 April 2007; revised 13 June 2007; accepted 18 June 2007
Available online 24 June 2007

Abstract—The synthetic approach to condensed heterocyclic analogues from etoposide was revisited. The described procedure allows the synthesis of A-ring pyridazine etoposide **13**, featuring the use of tetrakis(triphenylphosphine)palladium as catalyst under optimized conditions in the key Stille cross coupling between bistriflate **14** and the vinylstannane without epimerization at C-2. The TBDMS-protecting group was critical to cleanly obtain the pivotal intermediate **19**.
© 2007 Elsevier Ltd. All rights reserved.

Etoposide (VP-16, Vepesid®) **1** is a cancer chemotherapeutic agent[1] widely used in the treatment of small cell lung cancer (SCLC), testicular carcinoma, lymphoma and Kaposi's sarcoma. Topoisomerase II is the target for **1** that induces cell death by enhancing enzyme-mediated double-strand DNA scission.[2] To overcome drug resistance and poor water solubility observed in clinic with **1**, intensive research has been devoted to the synthesis of etoposide analogues[3] (Fig. 1).

In the absence of the 3D structure of the topoisomerase II active site for conducting rational drug design in this family, a composite pharmacophore model has been proposed by MacDonald et al.[4,5] for expression of topoisomerase II activity, defining three structural domains: the intercalation-like domain in ternary complex (i.e., the planar polycyclic region), the variable substituent domain (i.e., the C_4 molecular region) and the minor groove binding domain (i.e., the pendant E-ring region). The comparative molecular force field analysis (CoMFA), applied by Lee et al.[6] to develop QSAR models for epipodophyllotoxins, further specified by the nature of the C_4 molecular region for topoisomerase II inhibition (i.e., bulky substituents).

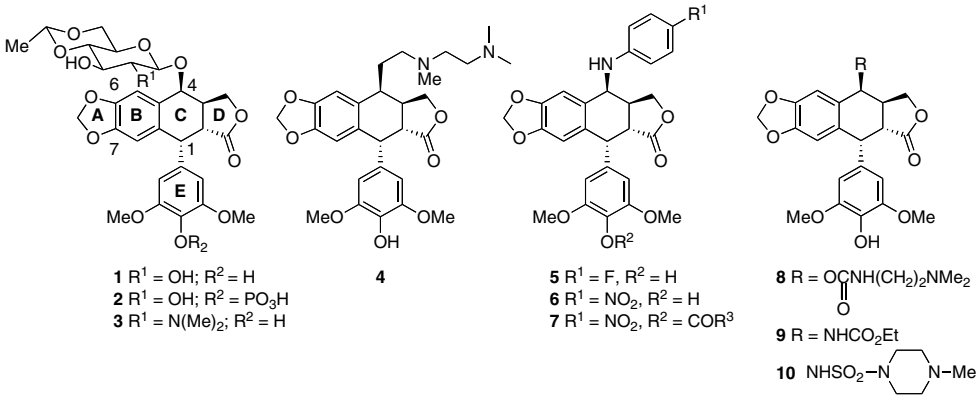


Figure 1. Structures of etoposide **1** and analogues **2**–**10**.

Keywords: Anticancer drugs; Etoposide; Topoisomerase II; Stille reaction.
* Corresponding author. Tel.: +33 142346659; fax: +33 142346631; e-mail: emmanuel.bertounesque@curie.fr

0040-4039/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2007.06.105

More recently renewed interest in drugs belonging to this class has led to the clinical development of etopophos (Bristol Myers), a 4′-phosphate prodrug of etoposide **2**.[7] Several non-glycoside derivatives including NK611 (Nippon-Kayaku) **3**, a 2″-dimethylamino-etoposide,[8] TOP-53 (Taiho) **4**,[9] NPF **5**,[10] and GL-331 **6**[11] and its analogues **7**[12] emerged as promising drug candidates for cancer but led to some unsuccessful phase II clinical trials. However, new developments of these compounds are not excluded. Our continuous interest in the synthesis of etoposide analogues prompted us to extend the evaluation of the variable substituent domain.[4,5] Thus, incorporation of a 4-*O*-β-carbamate[13] (**8**) or a 4-*N*-β-carbamate[14] (**9**), or a 4-sulfonamide[15] (**10**) resulted in an increase in the cytotoxicity compared to etoposide **1**. SAR studies centered on A-ring modification of etoposide or non-glycoside analogues turn out to have been far less investigated. Kadow et al.[16] reported the synthesis and in vivo anti-P388 leukemia activity of compounds **11** wherein the 6,7-dimethoxy ($R^1 = R^2 = Me$) and 6,7-diacetoxy ($R^1 = R^2 = COMe$) analogues retained the most activity but inferior to etoposide (Fig. 2). These developments emphasize the importance of the methylenedioxy group of **1** for optimal antitumor activity. Lee et al.,[17] also investigated the DNA intercalating domain of the pharmacophore model by introducing various phenazine rings into 4′-*O*-demethyl-epipodophyllotoxin analogues. Among these compounds, 4′-*O*-demethyl-4β-(4‴-nitroanilino)-4-desoxy-podophenazine **12** displays an activity superior to etoposide when evaluated against KB and KB/7d (VP-16-Resistant cells) in vitro. It is interesting to note that extension of the phenazine ring to the benzophenazine ring led to a loss of activity. Unlike etoposide, podophenazine **12** was found to be a weak topoisomerase II inhibitor in vitro, but exhibits a novel mechanism of action.

Based on MacDonald's composite pharmacophore model, we designed condensed heterocyclic analogues from etoposide[18] with the aim to enhance π stacking interactions, intercalation-based pathway and improve the solubility in water. Herein we report the synthesis of A-ring pyridazine etoposide **13** from revisiting our synthetic approach based on the Stille coupling reaction.

The synthesis of pyridazine etoposide **13** began with bistriflate **14** obtained previously[18] in four steps from etoposide **1** (Scheme 1). After screening of the reaction conditions of the Stille reaction[19] in order to avoid the

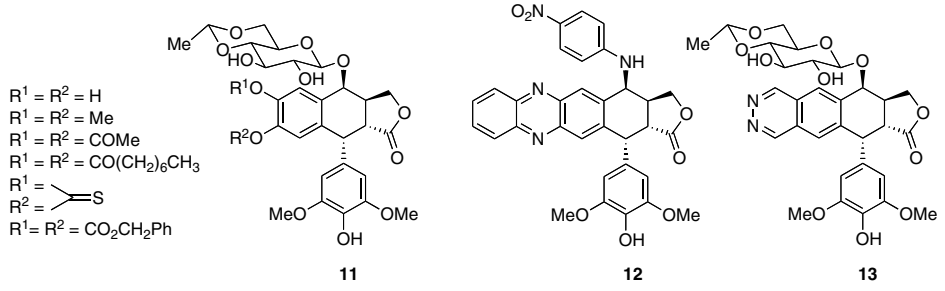
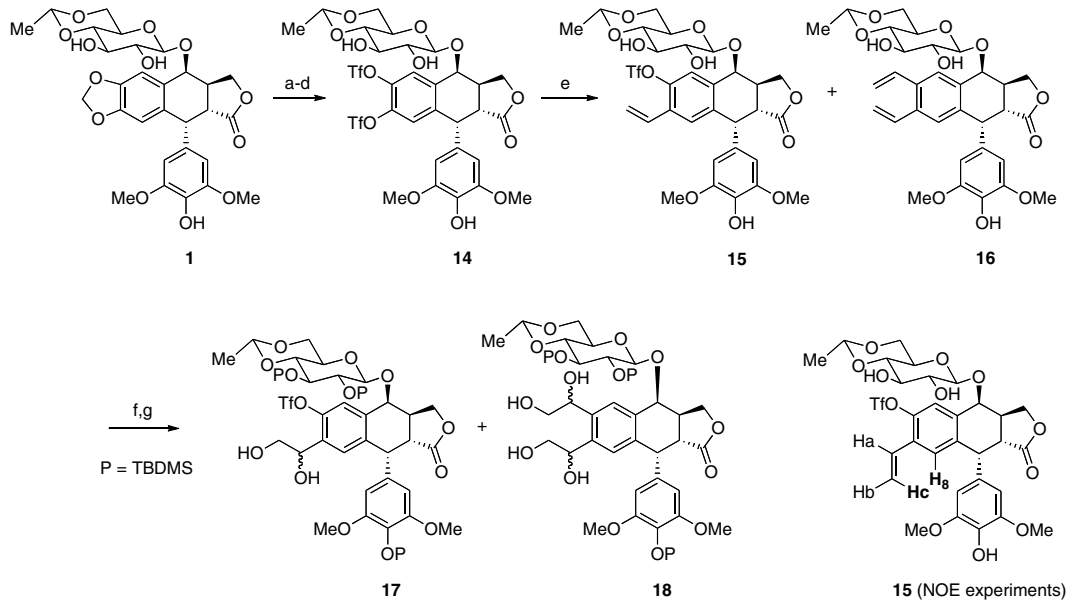
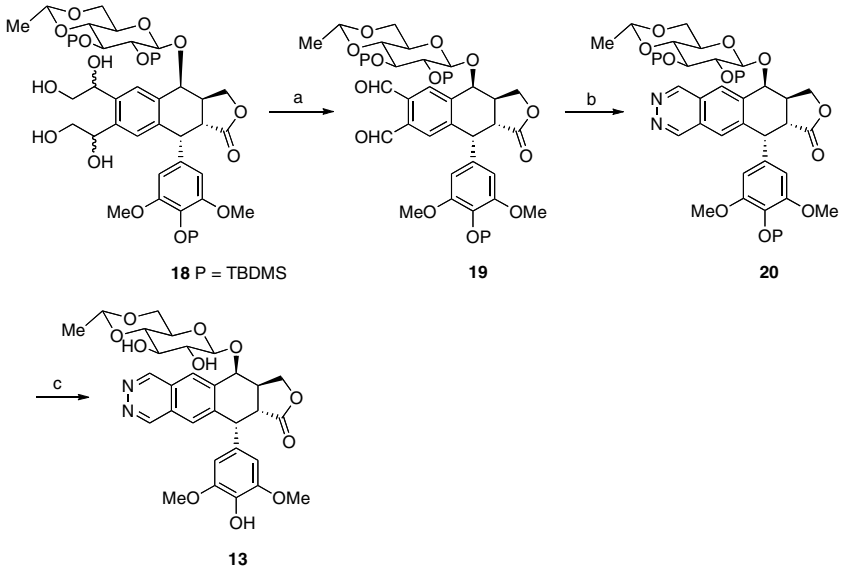


Figure 2. A-ring etoposide analogues.



Scheme 1. Reagents and conditions: (a–d) See Ref. 18; (e) $Pd(PPh_3)_4$ (0.2 equiv), $Bu_3Sn(C_2H_3)$ (10 equiv), LiCl (6 equiv), dioxane, 100 °C, 1.5 h, **15** (18%) and **16** (48%); (f) TBDMSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt, 88% yield of triprotected monovinyl relative to **15** and 88% yield of triprotected bisvinyl relative to **16**; (g) OsO_4 cat., NMO, acetone:H_2O (8/1), rt, 5 h.



Scheme 2. Reagents and conditions: (a) $Pb(OAc)_4$, benzene, rt, 2 h, 69%; (b) $NH_2NH_2.H_2O$, CH_2Cl_2:EtOH (1/1), −55 °C for 1 h, 84%; (c) $HF{\cdot}NEt_3$, acetonitrile, rt, 6 days, 84%.

undesired epimerization of the D-ring[18] deleterious to the cytotoxic activity of this class of compounds,[1d,e] we found that $Pd(PPh_3)_4$, under optimized conditions, gave a mixture of *trans*-monovinyl **15** and *trans*-bisvinyl **16** in a 27:73 ratio (18% and 48% yields, respectively, based on NMR analysis), which were very difficult to separate. The C-7 site of monovinylation has been determined on the basis of NOE experiments: irradiation of the H_c signal caused the enhancement of H_8 and H_b signals. Complete silylation of this mixture as TBDMS ethers followed by osmylation led to a separable mixture of diols **17** (83%) and tetrols **18** (92%).[20]

Oxidation of this latter with $Pb(OAc)_4$ gave dialdehyde **19** (2,3-*trans* relationship: $J_{2,3} = 14.1$ Hz). Formation of the A-ring pyridazine occurred when **19** was treated with hydrazine hydrate. Since, as expected, the basic TBAF led to pyridazine picroetoposide[18] (TBAF, CH_2Cl_2:THF (10/2), rt, 24 h, 72%), deprotection of **20** was carried out using $HF{\cdot}NEt_3$[21] to furnish the target pyridazine etoposide **13**[22] (Scheme 2). The TBDMS protecting group was critical for obtaining **13**. Unlike the synthesis of pyridazine picroetoposide,[18] protection with the 2,2,2-trichloroethyl chloroformate group failed to cleanly deliver the key intermediate dialdehyde **19**. Synthesis of **13** using phenoxyacetyl chloride for the triprotection or benzyl chloroformate for the monoprotection at C-4′ was also unsuccessful.

Pyridazine etoposide is a weak inhibitor of topoisomerase II (18% of inhibition at 50 μM for **13** versus 50% of inhibition at 50 μM for etoposide **1**). It was found inactive in vitro against the A549 human lung carcinoma epithelial cell line ($IC_{50} > 10^{-4}$ M for **13** versus $IC_{50} = 4.4$ μM for etoposide **1**), and in vivo against P388 lymphocytic leukemia in mice (no activity at a dose of 40 mg/kg for **13** versus *T*/*C* 189% at a dose of 50 mg/kg for etoposide **1**).

In conclusion, these results confirm the importance of the methylenedioxy A-ring of etoposide for optimal activity. The use of the pivotal intermediate **19** should give access to novel analogues of **1**, in particular those which have, fused to C-ring, either three rings (e.g., phthalazine) as in the case of **12** or methylenedioxyphenyl-containing four rings, to pursue the investigation of the unexplored intercalating domain of McDonald's composite pharmacophore model.

Acknowledgment

We thank CNRS and Institut Curie for financial support. Laboratoires Servier are gratefully acknowledged for a doctoral grant to Philippe Meresse and for biological evaluation on A549 cancer cell line and P388 lymphocytic leukemia in mice. We also thank Dr. Paola Arimondo (CNRS UMR 5153, INSERM U565, Muséum National d'Histoire Naturelle USM 0503, Paris) for inhibition assay on toposiomerase II.

References and notes

1. (a) Stahelin, H. F.; von Wartburg, A *Cancer Res.* **1991**, *51*, 5–15; (b) Imbert, T. F. *Biochimie* **1998**, *80*, 207–222; (c) Hande, K. R. *Eur. J. Cancer* **1998**, *34*, 1514–1521; (d) Damayanthi, Y.; Lown, J. W. *Curr. Med. Chem.* **1998**, *5*, 205–252; (e) Meresse, P.; Bertounesque, E.; Dechaux, E.; Monneret, C. *Curr. Med. Chem.* **2004**, *11*, 2443–2466; (f) Baldwin, E. L.; Osheroff, N. *Curr. Med. Chem. Anti-Cancer Agents* **2005**, *5*, 363–372.
2. (a) Burden, D. A.; Osheroff, N. *Biochim. Biophys. Acta* **1998**, *1400*, 139–154; (b) Wilstermann, A. M.; Osheroff, N. *Curr. Top. Med. Chem.* **2002**, *3*, 1349–1364.
3. Zhang, Y.; Lee, K.-H. *Chin. Pharm. J.* **1994**, *46*, 319–369.
4. MacDonald, T. L.; Lehnert, E. K.; Loper, J. T.; Chow, K. C.; Ross, W. E. *On the Mechanism of Interaction of DNA*

Topoisomerase II with Chemotherapeutics Agents. In *DNA Topoisomerase in Cancer*; Potmesil, M., Kohn, K. W., Eds.; Oxford University Press: New York, 1991; pp 199–214.

5. (a) Leteurtre, F.; Madalengoitia, J.; Orr, A.; Guzi, T. J.; Lehnert, E.; MacDonald, T. L.; Pommier, Y. *Cancer Res.* **1992**, *52*, 4478–4483; (b) Lehnert, E. K.; Miller, K. E.; Madalengoitia, J. S.; Guzi, T. J. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2411–2416; (c) Tepe, J. J.; Madalengoitia, J. S.; Slunt, K. M.; Werbovetz, K. W.; Spoors, P. G.; MacDonald, T. L. *J. Med. Chem.* **1996**, *39*, 2188–2196; (d) Madalengoitia, J. S.; Tepe, J. J.; Werbovetz, K. W.; Lehnert, E. K.; MacDonald, T. L. *Bioorg. Med. Chem. Lett.* **1997**, *5*, 1807–1815.
6. (a) Cho, S. J.; Tropsha, A.; Suffness, M.; Cheng, Y. C.; Lee, K.-H. *J. Med. Chem.* **1996**, *39*, 1383–1395; (b) Xiao, Z.; Xiao, Y. D.; Feng, J.; Golbraikh, A.; Tropsha, A.; Lee, K.-H. *J. Med. Chem.* **2002**, *45*, 2294–2309.
7. de Jong, R. S.; Slijf, E. A. M.; Uges, D. R. A.; Mulder, N. H.; de Vries, E. G. E. *Br. J. Cancer* **1997**, *76*, 1480–1483.
8. Rassmann, I.; Thodtmann, R.; Mross, M.; Huttmann, A.; Berdel, W. E.; Manegold, C.; Fiebig, H. H.; Kaeserfrolich, A.; Burk, K.; Hanauske, A. R. *Invest. New Drugs* **1998**, *16*, 319–324.
9. Utsugi, T.; Shibata, J.; Sugimoto, Y.; Aoyagi, K.; Wierzba, K.; Kobunai, T.; Terada, T.; Oh-hara, T.; Tsuruo, T.; Yamada, Y. *Cancer Res.* **1996**, *56*, 2809–2814.
10. Zhang, Y.-L.; Tropsa, A.; McPhail, A. T.; Lee, K.-H. *J. Med. Chem.* **1994**, *37*, 1460–1464.
11. Huang, T. S.; Lee, C.-C.; Chao, Y.; Shu, C.-H.; Chen, L.-T.; Chen, L.-L.; Chen, M.-H.; Yuan, C.-C.; Whang-Peng, J. *Pharm. Res.* **1999**, *16*, 997–1002.
12. (a) Han, S.; Xiao, Z.; Bastow, K. F.; Lee, K.-H. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2979–2982; (b) Xiao, Z.; Vance, J. R.; Bastow, K. F.; Brossi, A.; Wang, H.-K.; Lee, K.-H. *Bioorg. Med. Chem.* **2004**, *12*, 3363–3369.
13. Duca, M.; Guianvarc'h, D.; Meresse, P.; Bertounesque, E.; Dauzonne, D.; Kraus-Berthier, L.; Thirot, S.; Léonce, S.; Pierre, A.; Pfeiffer, B.; Renard, P.; Arimondo, P. B.; Monneret, C. *J. Med. Chem.* **2005**, *48*, 593–603.
14. Duca, M.; Arimondo, B. P.; Léonce, S.; Pierré, A.; Pfeiffer, B.; Monneret, C.; Dauzonne, D. *Org. Biomol. Chem.* **2005**, *3*, 1074–1080.
15. Guianvarc'h, D.; Duca, M.; Boukarim, C.; Kraus-Berthier, L.; Léonce, S.; Pierré, A.; Pfeiffer, B.; Renard, P.; Arimondo, P. B.; Monneret, C.; Dauzonne, D. *J. Med. Chem.* **2004**, *47*, 2365–2374.
16. Kadow, J. F.; Tun, M. M.; Crosswell, A. R.; Rose, W. C.; Vyas, D. M.; Doyle, T. W. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 17–22.
17. Cho, S. J.; Kashiwada, Y.; Bastow, K. F.; Cheng, Y.-C.; Lee, K.-H. *J. Med. Chem.* **1996**, *39*, 1396–1402.
18. Meresse, P.; Bertounesque, E.; Imbert, T.; Monneret, C. *Tetrahedron* **1999**, *55*, 12805–12818.
19. Echavarren, A. M.; Stille, J. K. *J. Am. Chem. Soc.* **1987**, *109*, 5478–5486.
20. Compounds **17** and **18** isolated as mixtures of diastereomers.
21. Hünig, S.; Wehner, G. *Synthesis* **1975**, 180–182.
22. All newly-synthesized compounds gave satisfactory analytical and spectroscopic data. Pyridazine etoposide **13**. Mp 210 °C; $[\alpha]_D^{20}$ −37 (*c* 0.1, $CHCl_3$); IR ($CDCl_3$) 3580, 3538, 2928 1776, 1620, 1519 cm^{-1}; ^1H NMR: (300 MHz, $CDCl_3$) δ 9.47 (br s, 1H, CH=N); 9.21 (br s, 1H, CH=N); 8.09 (s, 1H, H-5), 7.76 (s, 1H, H-8), 6.14 (s, 2H, H-2′, H-6′), 5.48 (br s, 1H, OH-4′), 5.27 (d, 1H, $J_{4,3} = 3{,}5$ Hz, H-4), 5.09 (d, 1H, $J_{1,2} = 5$ Hz, H-1), 4.83 (d, 1H, $J_{1'',2''} = 7.5$ Hz, H-1″), 4.79 (q, 1H, $J_{7'',Me} = 5$ Hz, H-7″), 4.55 (bt, 1H, $J_{11a,11b} = J_{11a,3} = 9$ Hz, H-11a), 4.34 (bt, 1H, $J_{11b,11a} = J_{11b,3} = 9$Hz, H-11b), 4.22 (dd, 1H, $J_{6''eq,6''ax} = 9.9$ Hz, $J_{6''eq,5''} = 3.8$ Hz, H-6″eq), 3.81 (t, 1 H, $J_{3'',4''} = J_{3'',2''} = 8.7$ Hz, H-6″ax), 3.70 (s, 6H, OMe-3′,5′), 3.63 (t, 1H, $J_{6''ax,6''eq} = J_{6''ax,5''} = 9.9$ Hz, H-3″), 3.54–3.38 (m, 4H, H-2, H-2″, H-5″, H-4″), 3.11 (m, 1H, H-3), 1.43 (d, $J_{Me,7''} = 5$ Hz, Me); HRMS (DIC/CH_4) *m*/*z* Calcd for $C_{30}H_{32}N_2O_{11}$: 597.2084 $[M+H]^+$; found, 597.2081.