Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 1707–1711

BIOORGANIC & MEDICINAL CHEMISTRY

A Series of Enediynes as Novel Inhibitors of Topoisomerase I

Chi-Fong Lin,[a] Pei-Chen Hsieh,[a] Wen-Der Lu,[a] Huey-Fen Chiu[b] and Ming-Jung Wu[a,*]

[a]*School of Chemistry, Kaohsiung Medical University, Kaohsiung, Taiwan*
[b]*Graduate Insititute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan*

Received 14 December 2000; accepted 26 February 2001

Abstract—A series of acyclic enediynes, 2-((6-substituted)-3-hexen-1,5-diynyl)benzonitriles (**8**–**11**), display potent inhibition against topoisomerase I without the formation of active biradical intermediates and show inhibitory activity against topoisomerase I at 10 μM, which is five times that of camptothecin from the results of agarose gel electrophoresis.

Introduction

A series of alkaloids containing enediyne cores which were isolated from *Streptomyces* have manifold biological activities[1] owing to the generation of radicals. Several biologically active synthetic enediynes are also observed in the formation of radicals, However, besides formation of biradical intermediates, there is little attention to other feasible reaction modes by which enediynes could act and their relative biological activities that enediynes could exhibit, though reports of novel biradical reactions have begun to surface.[2] There is no investigation describing that enediynes could deactivate topoisomerase I without the formation of radical intermediates. During a routine screening of cytotoxicity, it was surprising that a series of 2-(6-substituted-3(*Z*)-hexen-1,5-diynyl)benzonitriles showed potent cytotoxicity with a KB cell (see Table 1). The high activity which 2-(6-substituted-3(*Z*)-hexen-1,5-diynyl) benzonitriles possessed promoted our studies toward this unexpected biological behavior.

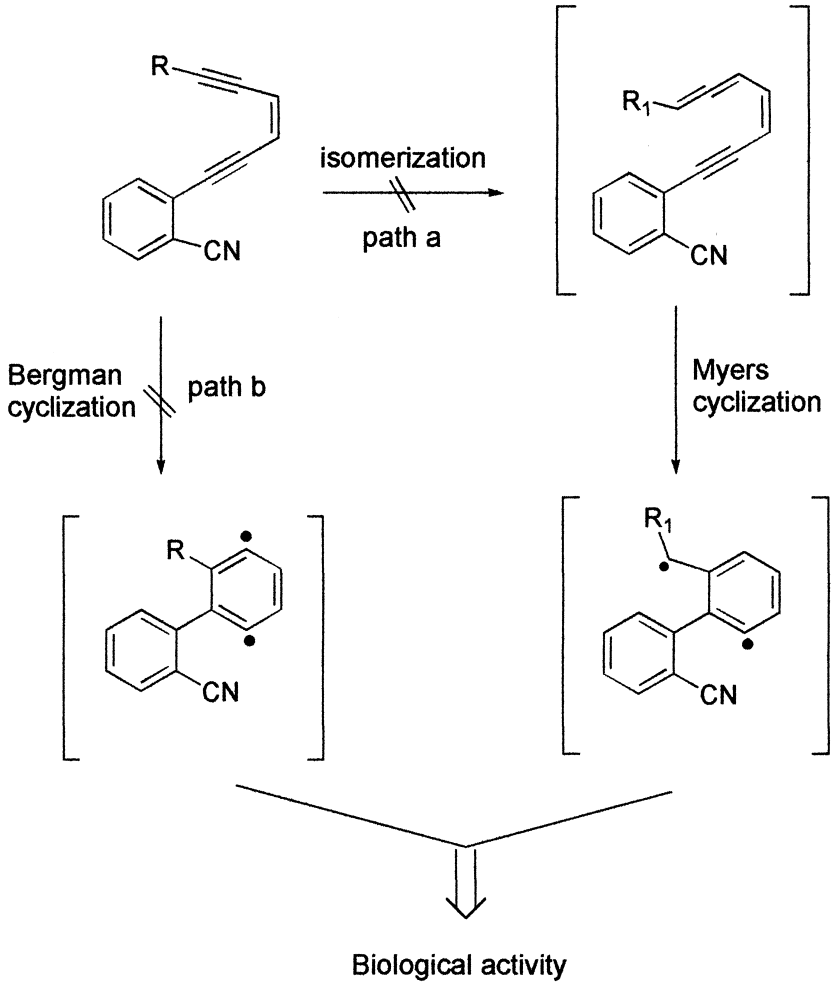
It is considered that these molecules comprise an enediyne structure but unlike the accustomed mode of enediynes[3] which generate biradicals and facilitate the decease of cells (Scheme 1), there is no initiating factor to promote the enediynes to form active biradicals via Myers cycloaromatization (path a). Similarly, according to Bergman's reports, the acyclic enediynes cannot cycloaromatize to produce biradical intermediates under 37 °C (path b). An overview of the possible mechanisms which anticancer drugs would proceed. The most probable pathway of 2-(6-substituted-3(*Z*)-hexen-1,5-diynyl)benzonitriles to induce the death of cancer cells is the physiological enzyme inhibitors, especially the topological enzymes; the topoisomerase, which was essential for DNA replication, transcription, repair, recombination, and chromosome segregation.[4] Human topoisomerase I (topo I) is a protein consisting of 765 amino acids, reconstituted from two fragments of the protein involving three subdomain cores, the COOH-terminal domains, and a positively charged surface, complexed either covalently or noncovalently with a 22-base pair DNA oligonucleotide. Several potent anticancer therapies achieve their effects by pharmacological inhibition of either topo I or II.[5] However, cleavage of duplex DNA by topo I is monomeric and transiently includes nucleophilic attack by a catalytic tyrosine residue on the scissile phosphodiester bond that culminates in the formation of a covalent bond between the enzyme and one end of the broken strand. Topo I is also the sole target of the camptothecin family of anticancer drugs which had two analogues of camptothecin, topotecan, and irinotecan, has been used successfully in the treatment of several human cancers.[6,7] Besides camptothecin

Table 1. Cytotoxic of 2-(6-substituted-3(*Z*)-hexen-1,5-diynyl) benzonitriles (IC_{50})

Compound/cell	KB (μM)	Hep2,2,15 (μM)
7	0.1	>10
8	$<10^{-2}$	>10
9	$<10^{-2}$	17
10	$<10^{-2}$	>10

*Corresponding author. Tel.: +886-7-312-1101, ext 2220; fax: +886-731-5339; e-mail: mijuwu@cc.kmu.edu.tw

0968-0896/01/$ - see front matter
PII: S0968-0896(01)00081-5



Scheme 1.

and its related derivatives, there are few detailed approaches on such burgeoning topo I inhibitors, although some natural products have also appeared to restrain topo I.[8] As a major drawback to the application of antibiotics in clinical therapies, many mutations are known to render topo I resistant to camptothecins, and the continued development of topoisomerase inhibitors is necessary.

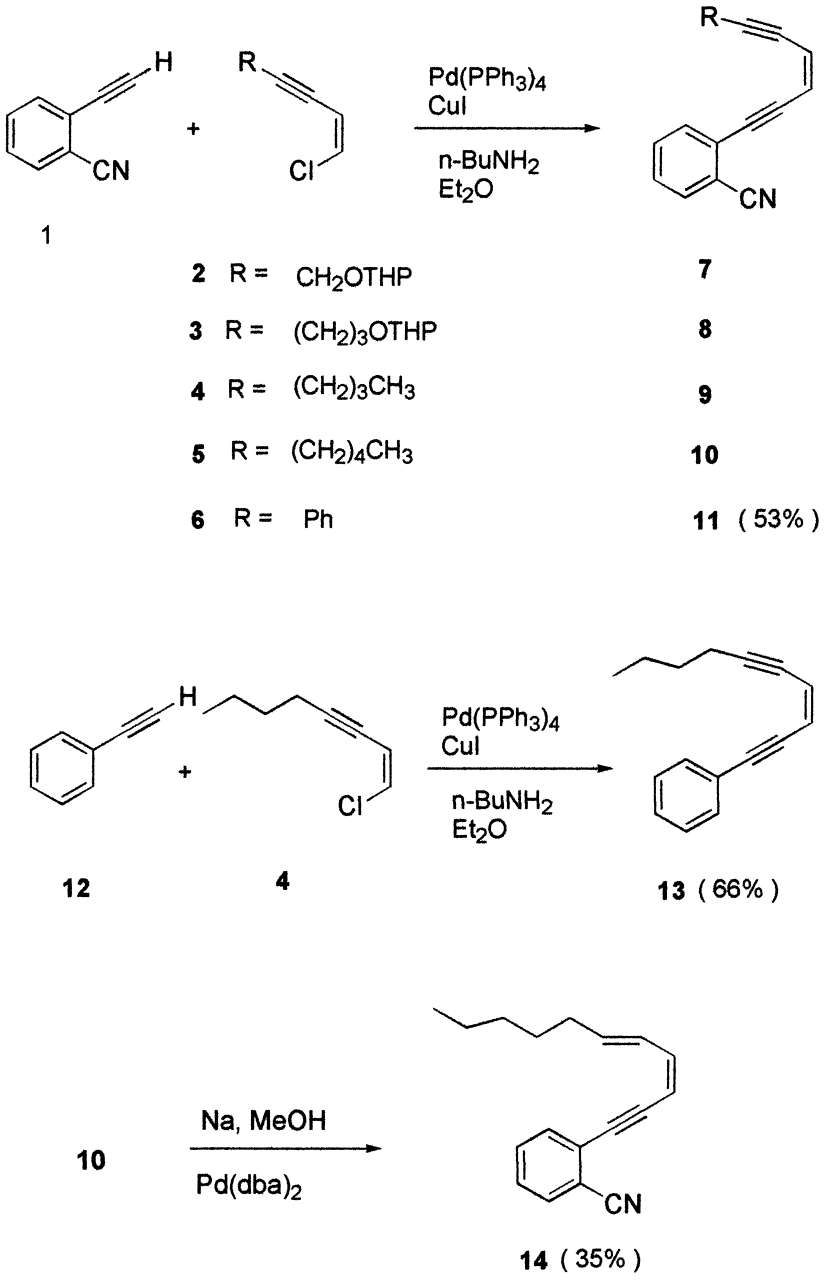
To explore how the requisite fundamental mainstay of these compounds deactivates topo I, several compounds were synthesized (**11**, **13**–**14**) and were regenerated (**7**–**10**).

Chemistry

The 2-(6-substituted-3(Z)-hexen-1,5-diynyl)benzonitriles (**7**–**11**) were prepared by the palladium-catalyzed coupling reaction of 2-ethynylbenzonitrile (**1**) with 1-substituted-4-chloro-3-buten-1-yne (**2**–**6**).[9] Compound **13** was generated by the same coupling reaction of phenylacetylene (**12**) and 1-butyl-4-chloro-3-buten-1-yne (**4**). Compound **14** was obtained by the reduction of **10** under the reaction of sodium methoxide and $Pd(dba)_2$ (Scheme 2).

Results and Discussion

The results of inhibition of compound (**7**–**11**, **13**–**14**) towards topo I were evaluated by gel electrophoresis as shown in Figure 1. In panel (a), compounds **8**–**10** displayed inhibitory activity against topo I at 10 μM concentration. In panel (b), compound **11** induced a similar amount of untwisting DNA compared with compounds **8**–**10** at 10 μM concn, though it showed slight activity at 1 μM, and no inhibition at 0.1 μM. Compound **13**, which has the same structure as compound **9**, besides the cyano group displayed weak inhibition of topo I at 10 μM. Moreover, there was not any supercoiled DNA revealed at both 1 and 0.1 μM. The lack of activity of **13** related to **9** is not unexpected. It has been suggested that there is little possibility for a hydrocarbon compound to restrain topo I under such a low concentration without any binding between target compound and receptor, which is usually provided by heteroatoms. Furthermore, compound **7** showed inactivity at 10 μM and, when raised to 50 μM, complete fragmentation of DNA was observed in panel (c). This might be due to the side chain not being long enough; even if the essential length of the side chain which restrained topo I required must be more than one carbon, elementarily. To verify whether the enediyne core was necessary, compound **14** was synthesized (from **10**); while there was full unwinding



Scheme 2.

DNA demonstrated, no matter the concentration of 100, 10, or 1 μM, in panel (d). This result revealed that to exhibit restraint of topo I, the enediyne core was imperative, even with a minor change of the acetylenes.

Conclusion

Several preliminary structural elements of a series of 2-(6-substituted-3(*Z*)-hexen-1,5-diynyl)benzonitriles are indispensable for ceasing fragmentation of supercoiled DNA promoted by topo I (shown in Scheme 3). Studies on a series of analogues **7**–**11**, **13**–**14** confirmed the necessity for an enediyne core, the cyano group on the *o*-substituted position of benzene and a long alkyl chain or aryl group on position-6, by virtue of compounds **7**, **13,** and **14** displaying inactivity. As is well known for camptothecin, deactivation of topo I to untwist supercoiled DNA is due to a stable complex that is formed between DNA–topo I and camptothecin. It is speculated that a possible mode by which 2-(6-substituted-3(*Z*)-hexen-1,5-diynyl) benzonitriles proceeds is in the complexing of DNA–topo I by the enediyne core, while the *o*-cyano group of benzene provides hydrogen bonding with the COOH-terminal domain of topo I, although more evidence is clearly required. These investigations open the door to a novel acting mode of enediynes that observe biological activity without the formation of active radical intermediates. In short, this study has provided some elementary insight into drug–topoisomerase interactions.

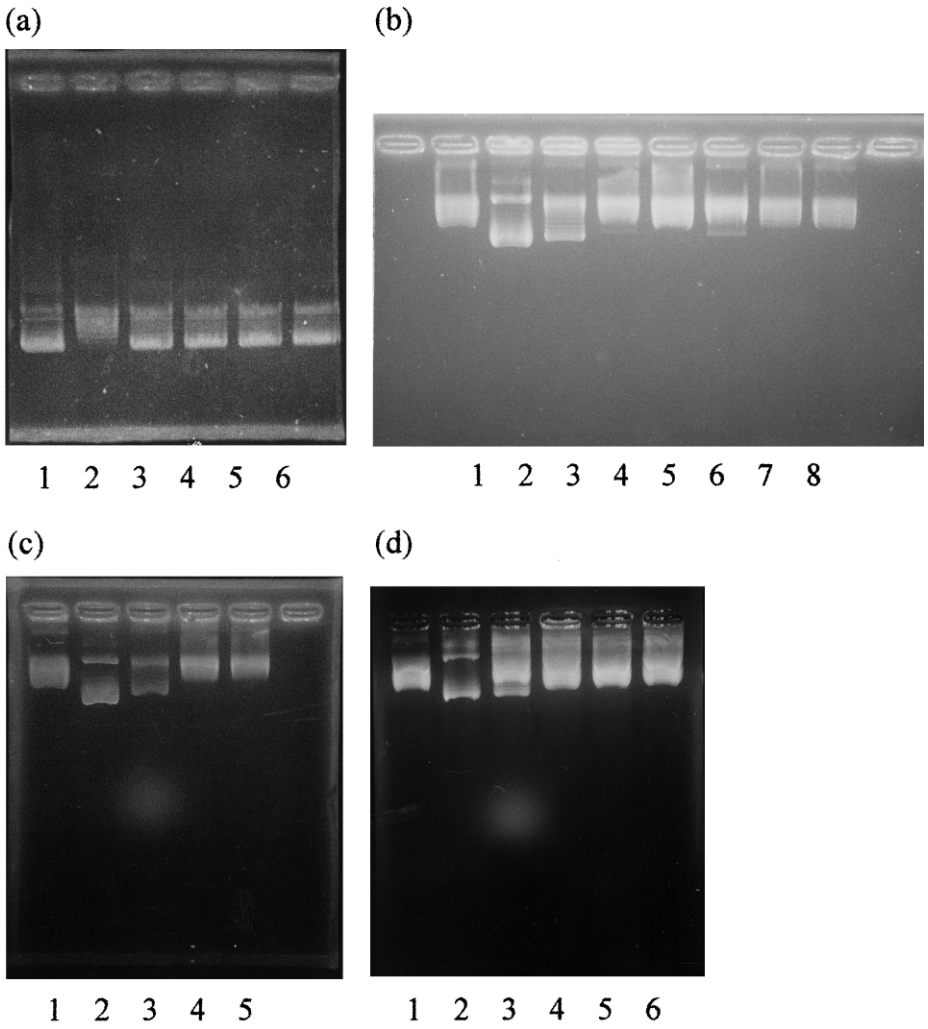


Figure 1. Cleavage of supercoiled pGEM9zf(−) DNA by topoisomerase I in the presence of 2-(6-substituted-3(*Z*)-hexen-1,5-diynyl)benzonitriles (**7**--**11**, **13**–**14**). Supercoiled DNA was treated with 0.025 μg/μL topoisomerase I and compounds (**7**–**11**, **13**), then analyzed on a 2% agarose gel. In panel (a), lane 1: DNA only; lane 2: DNA + topo I; lane 3: DNA + topo I + 50 μM camptothecin; lanes 4–6: DNA + topo I + 10 μM compound **8**, **9**, **10.** In panel (b), lane 1: DNA + topo I; lane 2: DNA only; lane 3: DNA + topo I + 50 μM camptothecin, lanes 4–6: DNA + topo I + compound **11**; lanes 7–9: DNA + topo I + compound **13**. Each group of three lanes contained 10, 1, 0.1 μM of enediyne analogue, respectively. In panel (c), lane 1: DNA only; lane 2: DNA + topo I; lane 3: DNA + topo I + camptothecin (50 μM); lanes 4–5: DNA + topo I + 10 μM, 50 μM of compound **7**. Panel (d), lane 1: DNA + topo I; lane 2: DNA only; lane 3: DNA + topo I + 50 μM camptothecin; lanes 4–6: DNA + topo I + 100 μM, 10 μM, 1 μM of compound **14**, respectively.

Experimental

Evalution of inhibitory concentration of camptothecin for gel electrophoresis

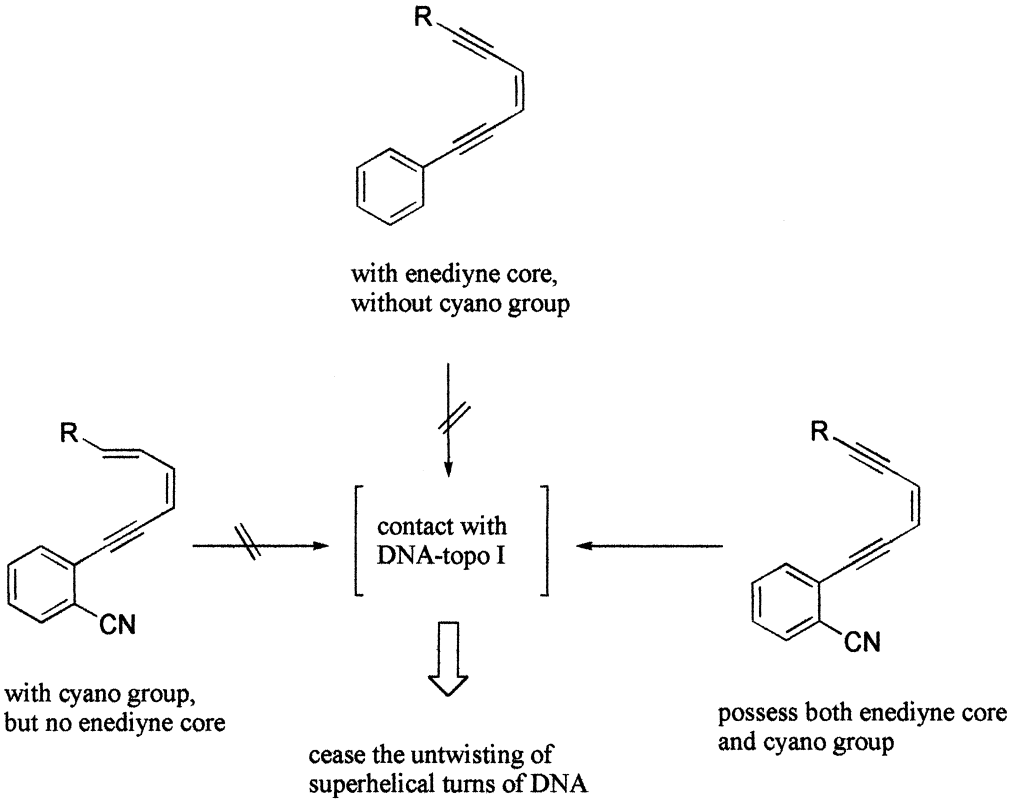
Camptothecin, which showed significant topo I inhibition, was widely used as the standard for the comparison of activity to the respective compounds. On behalf of affirming the modest concn of camptothecin that would still appear, the explicit suppression of topo I and used in the following experimental section, various concns were prepared, and the results were obtained by agarose gel electrophresis. It was suggested camptothecin showed less topo I inhibitory activity, when the concn was 30 μM. On the other hand, 50 μM of camptothecin seemed to be the fit observable inhibition of topo I. Therefore, the 50 μM was used as the standard concn of camptothecin, when the inhibitory of topo I of 2-(6-substituted-3(*Z*)-hexen-1,5-diynyl)benzonitriles were proceed.

General topoisomerase I course assay

All samples were kept in 20 μL total volume, which contained Tris (pH 7.9) 50 mM HCl, 100 mM KCl, 10 mM $MgCl_2$, 3 μg/mL BSA, 0.5 mM EDTA, 0.5 mM dithioreitol and 0.5 μg pGEM9zf(−) DNA, 10 units of topo I, were reacted with or without compounds **7**–**11**, **13**–**14** (dissolved in DMSO, final concn of DMSO was 10% v/v), were well mixed before incubation. The tubes were incubated in a 37 °C water bath for 30 min.

Gel electrophoresis

The reaction mixtures were electrophoresed by using 2% agarose gel in standard TBE buffer (1×, 0.06 M Tris, 0.06 M boric acid, 0.5 M EDTA), to which had previously been added 2 μL of loading buffer containing 0.25% bromophenol blue, 0.25% xylene cyanol, 5% SDS, and 0.25% sucrose. The gels were electrophoresed at 50 volt for 1.5 h, and stained with ethidium bromide



Scheme 3.

for 20 min. Then, it was placed on a UV box, and photographic images were made of the gels, using Polaroid 665 films.

Acknowledgements

The authors would like to thank the National Science Council of the Republic of China for financial support.

References and Notes

1. (a) Walkers, S.; Valentine, K. G.; Kahne, D. *J. Am. Chem. Soc.* **1990**, *112*, 6428. (b) Dark, L.; Iwasawa, N.; Danishefsky, S.; Crother, D. M. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 7464. (c) Povirk, L. F.; Goldberg, I. H. *Biochemistry*. **1980**, *19*, 4773. (d) Kappen, L. S.; Goldberg, I. H. *Nucleic Acid. Res.* **1978**, *5*, 2959.
2. (a) Wendi, D. M.; Kerwin, S. M. *J. Am. Chem. Soc.* **1997**, *119*, 1464. (b) Tarli, A.; Wang, K. K. *J. Org. Chem.* **1997**, *62*, 8841. (c) Xu, S. L.; Moore, H. W. *J. Org. Chem.* **1992**, *57*, 326.
3. (a) Jones, R. R.; Bergman, R. G. *J. Am. Chem. Soc.* **1972**, *94*, 660. (b) Bergman, R. G. *Acc. Chem. Res.* **1973**, *6*, 25. (c) Lockhart, T. P.; Comita, P. B.; Bergman, R. G. *J. Am. Chem. Soc.* **1981**, *103*, 4082. (d) Myers, A. G.; Kuo, E. Y.; Finny, N. S. *J. Am. Chem. Soc.* **1989**, *111*, 8057. (e) Myers, A. G. *Tetrahedron Lett.* **1987**, *28*, 4493.
4. (a) Champoux, J. J. In *DNA Topology and its Biological Effects*: Wang, J. C., Cozzarelli, N. R., Eds.; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1990; pp 217–242. (b) Wang, J. C. *Annu. Rev. Biochem.* **1996**, *65*, 635. (c) Champoux, J. J. Prog. Nucleic Acid Res. Mol. Biol. **1998**, *60*, 111.
5. Liu, L. F. Advances in Pharmacology, DNA Topoisomerases: Biochemistry and Molecular Biology, vol. 29A, Academic Press, San Diego, CA, 1994.
6. (a) Wang, H. K.; Morris-Natschke, S. L.; Lee., K. H. *Med. Res. Rev.* **1997**, *17*, 367. (b) Hsiang, Y. H.; Hertzberg, R.; Hecht, S.; Liu, L. F. *J. Biol. Chem.* **1985**, *260*, 14873. (c) Bjornsti, M. A.; Benedetti, P.; Viglianti, G. A.; Wang, J. C. *Cancer Res.* **1989**, *49*, 6318.
7. Seigo, S.; Shun-ichi, M.; Ken-ichiro, N.; Hiroshi, N.; Tomio, F.; Teruo, Y.; Tadashi *Chem. Pharmacol. Bull.* **1991**, *39*, 3183.
8. Lackey, K.; Besterman, J. M.; Fletcher, W.; Leitner, P.; Morton, B.; Sternbach, D. D. *J. Med. Chem.* **1995**, *38*, 906.
9. Wu, M. J.; Lin, C. F.; Chen, S. H. *Organic Lett.* **1999**, *1*, 767.