Bioorganic & Medicinal Chemistry Letters 19 (2009) 6364–6367

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and bioevaluation of novel maleamic amino acid ester conjugates of 3,5-bisarylmethylene-4-piperidones as cytostatic agents

Dani Youssef [a,b], Elizabeth Potter [b], Mamta Jha [b], Erik De Clercq [c], Jan Balzarini [c], James P. Stables [d], Amitabh Jha [b,*]

[a] Département des Sciences, Université Sainte-Anne, Pointe-de-l'Église, NÉ, Canada BOW 1MO
[b] Department of Chemistry, Acadia University, Wolfville, NS, Canada B4P 2R6
[c] Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium
[d] National Institute of Neurological Disorders and Stroke, Rockville, MD 20852, USA

ARTICLE INFO

Article history:
Received 25 August 2009
Revised 15 September 2009
Accepted 17 September 2009
Available online 22 September 2009

Keywords:
3,5-Bisarylmethylene-4-piperidones
Cytostatic activity
Anticancer
Topoisomerase inhibitions
QSAR

ABSTRACT

A novel series of maleamic amino acid ester conjugates of 3,5-bisarylmethylene-4-piperidones were prepared to investigate the efficacy of micronutrient conjugation in enhancing cytotoxic potency by improving selectivity and delivery. These compounds, prepared as anticancer agents, were expected to demonstrate enhanced selectivity towards malignant cells through the inhibition of topoisomerase IIα via protein thiolation. The cytostatic effects of these compounds were evaluated against three cell lines, namely murine L1210 leukemia cells, human Molt 4/C8 and CEM T-lymphocyte cells. All compounds were found to have greater potency than the reference drug melphalan. Several compounds were found to potently inhibit topoisomerase IIα and displayed cytostatic activity in the nanomolar range.

Since the discovery of chemotherapeutic agents capable of treating cancer, chemists have sought to develop novel compounds with the ability to selectively induce apoptosis in cancerous tissues, while leaving healthy normal tissues unharmed. A number of 3,5-bisarylmethylene-4-piperidone derivatives have been investigated over recent years, which have demonstrated promising cytotoxic and anticancer properties.[1–16] As α,β-unsaturated ketones, these compounds are capable of undergoing Michael addition, resulting in alkylation of cellular nucleophiles. These compounds possess a greater affinity towards cellular thiols than towards amino and hydroxyl groups, thus compounds of this nature may be devoid of the genotoxic side effects associated with many alkylating agents.[2,4]

Topoisomerase IIα, an enzyme rich in cysteine residues and essential for cell proliferation,[17–19] has been identified through preliminary work in our laboratory as a potential target of 3,5-bisarylmethylene-4-piperidone derivatives.[1,20,21] This enzyme creates transient double stranded nicks in the DNA helix, releasing torsional strain and facilitating separation of the DNA template, allowing replication and transcription processes to occur.[17,18,22–24] Interruption of these processes via catalytic inhibition causes replication and transcription to stall, impeding cell growth. If topoisomerase poisoning occurs, the double stranded nicks created by the enzyme cannot religate, resulting in potentially lethal lesions in the DNA frame work, which may then lead to apoptosis.[17,18] In rapidly proliferating cancerous cells, topoisomerase IIα expression is enhanced, thus thiol-alkylators targeting this enzyme offer a means of selectivity targeting these malignant cells to inhibit tumour growth.

The interest in 3,5-bisarylmethylene-4-piperidones stems from the high antiproliferative activity which was shown by the parent molecule **1a** (Fig. 1) towards leukemia and colon cancer cell lines.[7,14] Not only was compound **1a** highly selective, but the hydrochloride salt was found to be well-tolerated in mice, as no mortalities were noted after five consecutive daily doses of 240 mg/kg.[1,14] Various substitutions in the aryl rings led to the synthesis of **1b–f**. A National Cancer Institute (NCI) screen of **1a–c** and **1f** against 54 human tumour cell lines from eight types of cancers, including leukemia, melanoma, colon, non-small-cell lung, small-cell lung, central nervous system, ovarian and renal cancers, indicated both selectivity and potency were affected by the nature of the aryl substituent. In general, members of series **1**, were found to have greater or similar potency to melphalan (Alkeran®), an established alkylating agent for cancer treatment.[1,7]

The conversion of series **1** to *N*-acryloyl amides (series **2**, Fig. 1) provided an additional site for thiolation. In general, these compounds were found to be more potent than **1**, and on average, a 26-fold increase was observed in activity against P388 screens,

* Corresponding author.
E-mail address: ajha@acadiau.ca (A. Jha).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.09.069

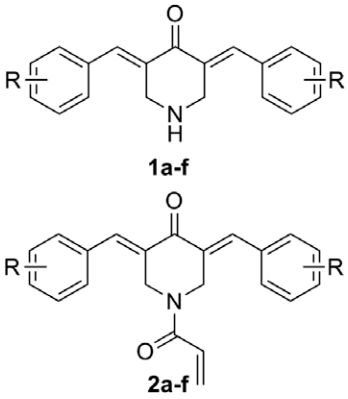


Figure 1. Structures of series **1** and **2**. The substituents in the aryl rings were as follows: **a**, R = H; **b**, R = 4-Cl; **c**, R = 3,4-Cl; **d**, 4-F; **e**, 4-NO_2; **f**, 4-OCH_3.

when compared to the parent molecule.[1,2,5] Placement of electron-withdrawing substituents on the molecule improved potency, due to an increase in the electrophilicity of the olefinic bond where thiolation is believed to occur;[7] compound **2e** was found to have the greatest potency.[1,15]

The promising activity displayed by *N*-acryloyl derivatives of 3,5-bisarylmethylene-4-piperidones made the development of series **3**–**6** (Fig. 2) a logical progression in the investigation of the activity of these molecules. Replacement of a terminal acryloyl hydrogen with an aryl ring or an arylcarbamoyl moiety enabled the electrophilicity of the acryloyl moiety to be investigated, as it relates to thiol alkylation and cytotoxicity, by modification of the aryl substituents.[1,2,4–6] In general, compounds of series **3** were less potent than their precursor **2a** and were found to display greater selectivity than melphalan, the reference drug.[3,4]

The arylcarbamoyl containing *Z* congeners, **4**, were generally found to have greater potency than the parent molecule, **1a** and were significantly more potent than melphalan.[2] Both **5** and **6** were found to have comparable potencies to melphalan.[5]

Malignant tumours, which are marked by rapid cell division, have a constant requirement for amino acids for protein biosynthesis. Thus, drugs conjugated with amino acids have the potential to improve both site specificity and absorption, as the drug is delivered to areas where the amino acid requirement is high. It is commonly known that polar amino acids and their corresponding esters are moieties capable of enhancing aqueous solubility[25a] facilitating bioavailability.[25b] Conjugation of acyclovir, an antiviral indicated against infections caused by the herpes virus, with amino acid esters was found to significantly enhance bioavailability.[25b] The chirality of the amino acid moiety was found to influence activity, as the L-isomers were found to be more active than the corresponding D-isomers.[25b] This indicates transporters must be

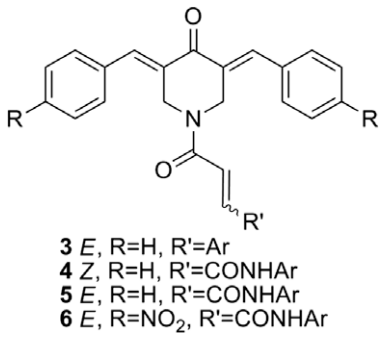


Figure 2. Investigated analogs of series **2**.

involved which are capable of differentiating between the L- and D-isomers of the amino acid.[25b] The bioavailability of the L-valyl ester of acyclovir was found to be 3–4 times greater than the parent molecule.[25b] Tamoxifen, an anticancer agent, was also found to have increased water solubility when conjugated to amino acid esters.[26] Doxorubicin derivatives incorporating an amino acid functionality have also been found to express enhanced bioavailability, as well as reduced toxicity.[27] The L-leucine conjugate of doxorubicin resulted in a fourfold decrease in toxicity, while maintaining equal potency against L1210 murine leukemia.[26,27] It was also found to be active in three of nine doxorubicin-resistant malignancies.[27] L-Leucine-doxorubicin is slowly hydrolyzed to the parent molecule in vivo, and is found to remain in the tumour for a longer period of time.[27]

The 3,5-bisarylmethylene-4-piperidone derivatives reported in this investigation have been designed to improve drug delivery via conjugation of amino acid derivatives which may engage amino acid transporters, facilitating entry to the cell. The amino acid conjugates are expected to display enhanced selectivity towards rapidly dividing cells, as these are essential micronutrients for biosynthesis.

In the present investigation, we decided to develop series **8** and **9**, in which the side chain aryl moiety of **5** and **6**, respectively, has been replaced with an amino acid ethyl ester. It was hypothesized that this exercise would lead to molecules with improved bioavailability and site-specific delivery to cancer cells resulting in enhanced efficacy. While such conjugations may be deemed as prodrug formation, it should be noted that the molecules incorporate the 3 enone pharmacophore and therefore may act as drugs on their own right. Compounds **1a** and **1e** were chosen as precursors as their *N*-acryloyl derivatives, viz. **2a** and **2e**, were found to be highly potent cytotoxics.[15]

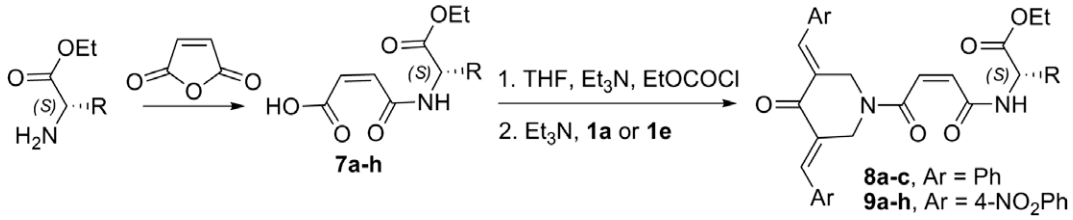
The target molecules **8a–c** and **9a–h** were prepared as shown in Scheme 1. The 3,5-bisarylmethylene-4-piperidones, **1a**,**e**, were prepared via condensation of 4-piperidone monohydrate hydrochloride with the appropriate aromatic aldehyde, as previously described.[7] Ethyl esters of eight different natural L-amino acids reacted with maleic anhydride, following a procedure reported in the literature,[28] to yield the corresponding maleamic acids, **7a–h**. The final step of the synthesis, condensing **7a–h** to the appropriate 3,5-bisarylmethylene-4-piperidone, was performed following chemistry reported previously by our group.[6] All reaction products were purified by column chromatography and fully characterized based on their spectroscopic data.[29]

To evaluate the cytotoxicity of compounds **8a–c** and **9a–h** against malignant cell lines, the compounds were evaluated for cytostatic activity against murine L1210 leukemia cells, as well as human Molt4/C8 and CEM T-lymphocyte cells, following literature procedure.[30] The results are presented in Table 1.

The high cytotoxicity of all of the compounds tested provides sufficient evidence that these molecules are indeed potential potent antitumour agents as well as promising lead molecules for further development. Compared to melphalan,[4] an established anticancer agent, the compounds generally display significantly greater potency, with the exception of **8a–c** which show lower cytotoxicity than melphalan in the L1210 screen.

Previous literature indicates the placement of a nitro group at the para position of the arylidene ring results in enhanced cytotoxicity due to the electronic effects of the substituent.[1,5,11,16] In accordance with this observation, **9a–h** were all found to have greater potency than compounds **8a–c** and melphalan against all cell lines, displaying activity at nanomolar concentrations.

The cytostatic activity of compounds **8a–c** and **9a–h** were all found to have greater potency than the parent pharmacophores **1a** and **1e**, respectively (Table 1). Thus, the antiproliferative activity of these compounds is likely due to the intact molecule rather



Scheme 1. Synthesis of compounds **7a–c** and **8a–h**. The substituents were as follows: **a**, R = H; **b**, R = $CH_2CH(CH_3)_2$; **c**, R = CH_2(4-OH-C_6H_4), **d**, R = CH_3; **e**, R = $CH(CH_3)_2$; **f**, R = $CH_2CH_2SCH_3$; **g**, R = CH_2Ph;**h**, R = CH_2(3-indolyl).

Table 1
Evaluation of compounds **8a–c** and **9a–h** against murine L1210 leukemic cells and human Molt 4/C8 and CEM T-lymphocyte cells

Compound	IC_{50} (μM)		
	L1210	Molt 4C/8	CEM
1a[a]	7.96 ± 0.11	1.67 ± 0.15	1.70 ± 0.02
1e[a]	32.09 ± 4.2	8.28 ± 0.75	4.47 ± 2.28
2a[b]	8.69 ± 0.73	1.42 ± 0.27	1.48 ± 0.32
2e[b]	0.42 ± 0.07	0.15 ± 0.06	0.26 ± 0.02
8a	6.1 ± 1.2	1.4 ± 0.1	1.3 ± 0.1
8b	4.7 ± 1.1	1.1 ± 0.2	1.2 ± 0.0
8c	4.6 ± 1.3	0.57 ± 0.11	0.35 ± 0.02
9a	0.84 ± 0.07	0.23 ± 0.01	0.11 ± 0.05
9b	0.24 ± 0.08	0.21 ± 0.02	0.17 ± 0.03
9c	0.60 ± 0.09	0.28 ± 0.06	0.17 ± 0.05
9d	0.58 ± 0.20	0.24 ± 0.02	0.083 ± 0.015
9e	0.44 ± 0.07	0.22 ± 0.01	0.17 ± 0.06
9f	0.58 ± 0.08	0.25 ± 0.01	0.19 ± 0.07
9g	0.20 ± 0.03	0.24 ± 0.02	0.13 ± 0.06
9h	0.62 ± 0.31	0.27 ± 0.08	0.19 ± 0.06
Melphalan[c]	2.13 ± 0.03	3.24 ± 0.79	2.47 ± 0.03

[a] The data for **1a**,**e** are reproduced from Ref. 14.
[b] The data for **2a**,**e** are reproduced Ref. 15.
[c] The data for melphalan are reproduced from *Eur. J. Med. Chem.* **2001**, *35*, 970.

than the products of hydrolysis, which would yield the parent pharmacophore and the corresponding maleamic acid.

In an attempt to comprehend the effect of structural and electronic parameters on the cytostatic activity of compounds **9a–h**, a quantitative structure–activity relationship (QSAR) study was undertaken using certain physicochemical parameters as descriptors of bioactivity. The extent to which the lipophilicity of the amino acid side chain affected the bioactivity was evaluated by comparing log *P* of the side chain (calculated using commercial software)[31] with the IC_{50} values of the three cell lines. Likewise, molar refractivity (MR) values of the amino acid side chain were also obtained using commercial software[31] and utilized as a means of accessing the effect of the steric properties of the side chain with respect to bioactivity.

A positive correlation (p <0.05) was observed between log *P* and antiproliferative activity against L1210 cells. This indicates that the exploration of derivatives with increasingly lipophilic substituents is of value to fine tune the anticancer potential of these molecules. While no significant correlations (p >0.05) were observed between MR and activity, a positive correlation approaching significance was observed against Molt4/C8 and CEM cell lines (p = 0.094 and 0.056, respectively). This would indicate that the inclusion of more complex and bulky amino acid side chains (or possibly conjugation with di/tri-peptides) would enhance the inhibitory ability of these agents against human cell lines.

As compounds of this nature have been proposed as TOPO IIα inhibitors,[1,21] appropriate assays were undertaken to identify if these derivatives did indeed result in inhibition of this vital enzyme. TOPO IIα inhibition assays were performed using purified human DNA TOPO IIα and supercoiled pRYG DNA.[32] The results are presented in Table 2. A representative gel picture is presented in Figure 3.

Compounds **8a–c** were found to be inactive at 50 μM towards TOPO IIα. As these molecules were shown to be cytotoxic agents, their interaction with topoisomerase is likely not their primary mode of action. In contrast, compounds **9a–h** were all found to be catalytic inhibitors active towards TOPO IIα at 100 μM, with several derivatives, namely **9a**,**f–h** retaining their inhibitory activity at 10 μM. Further study of these compounds at concentrations below 10 μM may be valuable to determine the limit of their activity. Comparison of activity towards TOPO IIα and the potency of these molecules yielded no significant trends.

Two representative compounds **9a** and **9b** were evaluated for murine toxicity in vivo following a literature procedure.[33] The compounds were injected intraperitoneally into mice at doses of 30, 100 and 300 mg/kg. The animals were then monitored for 0.5 and 4 h. The compounds where found to be well tolerated, with **9b** showing no neurotoxicity. Neurological deficit was displayed at a dose of 100 mg/kg after 0.5 h in one out of eight animals studied in the case of compound **9a** (1/8), no neurotoxicity was observed at 300 mg/kg for the same duration, or after 4 h at all concentrations studied. These results indicate that compounds of series **9** are well tolerated and are not general biocidal agents.

Future studies on this novel series of potential anticancer molecules will be carried out to establish the effectiveness of micronutrient conjugation in improving selectivity and delivery in the appropriate models.

In conclusion, the present investigation reveals amino acid conjugates of 3,5-bisarylmethylene-4-piperidones to be promising leads for the development of novel cytostatic agents. Series **9** was found to be significantly more potent than the reference drug melphalan against L1210, Molt C4/8 and CEM cell proliferation. QSAR results indicate conjugation with more complex and increasingly lipophilic amino acids may further accentuate bioactivity. As these compounds have been shown to inhibit topoisomerase II, and as they are structurally divergent from known topoisomerase II inhibitors, they may be of value in treating drug-resistant tumours.

Table 2
Topoisomerase IIα inhibitory activity displayed by compounds **4a–c** and **5a–f**; + = active; – = inactive

Compound	Concentration employed in assay (μM)			
	100	50	25	10
8a	–	–	–	–
8b	–	–	–	–
8c	+	–	–	–
9a	+	+	+	+
9b	+	–	–	–
9c	+	+	+	–
9d	+	+	–	–
9e	+	+	+	–
9f	+	+	+	+
9g	+	+	+	+
9h	+	+	+	+

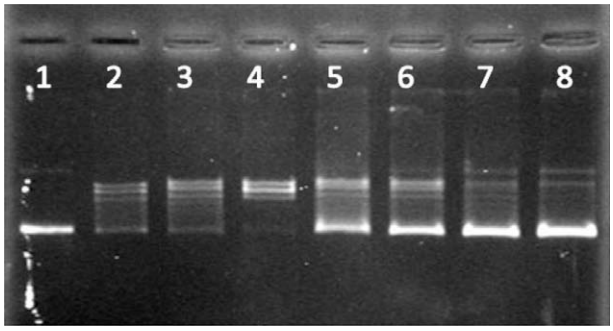


Figure 3. TOPO IIα inhibition by compound **9h**. Lane 1, supercoiled pRYG DNA; Lane 2, DNA with TOPO II; Lane 3, DNA with TOPO II in presence of DMSO control; Lane 4, DNA with TOPO II in presence of 100 μM VP-16, an established TOPO II poison; Lanes 5–8; DNA with TOPO II in presence of **9h** at 10, 25, 50, 100 μM, respectively.

In vivo neurotoxicity experiments reveal the compounds to be well-tolerated by mice at concentrations up to 300 mg/kg body weight. These results indicate that the investigation of 3,5-bisarylmethylene derivatives conjugated to amino acids is a promising area of study to fine tune the anticancer potential of these molecules.

Acknowledgements

The authors thank the following agencies for financial support, the Nova Scotia Health Research Foundation (A.J., E.P.) and the Concerted Research Actions (GOA 05/19) (J.B.). Appreciation is extended to Mrs. Lisette van Berckelaer for conducting the Molt 4/C8, CEM, and L1210 assays, and the National Institute of Neurological Disorders and Stroke, USA, for undertaking the short term toxicity studies in mice. Dr. Sherri McFarland (Acadia University) is thanked for demonstrating the topoisomerase assay protocol.

References and notes

1. Jha, A.; Duffield, K. M. *Indian J. Chem., Sect. B* **2006**, *45*, 2313.
2. Dimmock, J. R.; Jha, A.; Zello, G. A.; Sharma, R. K.; Shrivastav, A.; Selvakumar, P.; Allen, T. M.; Santos, C. L.; Balzarini, J.; De Clercq, E.; Manavathu, E. K.; Stables, J. P. *J. Enzyme Inhib. Med. Chem.* **2003**, *18*, 325.
3. Dimmock, J. R.; Jha, A.; Kumar, P.; Zello, G. A.; Quail, J. W.; Oloo, E. O.; Oucharek, J. J.; Pasha, M. K.; Seitz, D.; Sharma, R. K.; Allen, T. M.; Santos, C. L.; Manavathu, E. K.; De Clercq, E.; Balzarini, J.; Stables, J. P. *Eur. J. Med. Chem.* **2002**, *37*, 35.
4. Dimmock, J. R.; Jha, A.; Zello, G. A.; Quail, J. W.; Oloo, E. O.; Nienaber, K. H.; Kowalczyk, E. S.; Allen, T. M.; Santos, C. L.; De Clercq, E.; Balzarini, J.; Manavathu, E. K.; Stables, J. P. *Eur. J. Med. Chem.* **2002**, *37*, 961.
5. Jha, A.; Mukherjee, C.; Prasad, A. K.; Parmar, V. S.; Clercq, E. D.; Balzarini, J.; Stables, J. P.; Manavathu, E. K.; Shrivastav, A.; Sharma, R. K.; Nienaber, K. H.; Zello, G. A.; Dimmock, J. R. *Bioorg. Med. Chem.* **2007**, *15*, 5854.
6. Jha, A.; Dimmock, J. R. *Synth. Commun.* **2003**, *33*, 1211.
7. Dimmock, J. R.; Arora, V. K.; Quail, J. W.; Pugazhenthi, U.; Allen, T. M.; Kao, G. Y.; De Clercq, E. *J. Pharm. Sci.* **1994**, *83*, 1124.
8. Makarov, M. V.; Odinets, I. L.; Lyssenko, K. A.; Rybalkina, E. Y. B. A.; Kosilkin, I. V.; Antipin, M. Y.; Timofeeva, T. V. *J. Heterocycl. Chem.* **2008**, *45*, 729.
9. Pati, H. N.; Das, U.; Quail, J. W.; Kawase, M.; Sakagami, H.; Dimmock, J. R. *Eur. J. Med. Chem.* **2008**, *43*, 1.
10. Pati, H. N.; Das, U.; Das, S.; Bandy, B.; De Clercq, E.; Balzarini, J.; Kawase, M.; Sakagami, H.; Quail, J. W.; Stables, J. P.; Dimmock, J. R. *Eur. J. Med. Chem.* **2009**, *44*, 54.
11. Das, U.; Alcorn, J.; Shrivastav, A.; Sharma, R. K.; De Clercq, E.; Balzarini, J.; Dimmock, J. R. *Eur. J. Med. Chem.* **2007**, *42*, 71.
12. Das, U.; Selvakumar, P.; Sharma, R. K.; Haas, T. A.; Dimmock, J. R. *J. Enzyme Inhib. Med. Chem.* **2007**, *22*, 451.
13. Das, U.; Das, S.; Bandy, B.; Stables, J. P.; Dimmock, J. R. *Bioorg. Med. Chem.* **2008**, *16*, 3602.
14. Dimmock, J. R.; Arora, V. K.; Wonko, S. L.; Hamon, N. W.; Quail, J. W.; Jia, Z.; Warrington, R. C.; Fang, W. D.; Lee, J. S. *Drug Des. Deliv.* **1990**, *6*, 183.
15. Dimmock, J. R.; Padmanilayam, M. P.; Puthucode, R. N.; Nazarali, A. J.; Motaganahalli, N. L.; Zello, G. A.; Quail, J. W.; Oloo, E. O.; Kraatz, H. B.; Prisciak, J. S.; Allen, T. M.; Santos, C. L.; Balzarini, J.; De Clercq, E.; Manavathu, E. K. *J. Med. Chem.* **2001**, *44*, 586.
16. Makarov, M. V.; Rybalkina, E. Y.; Roschenthaler, G. V.; Short, K. W.; Timofeeva, T. V.; Odinets, I. L. *Eur. J. Med. Chem.* **2009**, *44*, 2135.
17. McClendon, A. K.; Osheroff, N. *Mutat. Res.* **2007**, *623*, 83.
18. Deweese, J. E.; Osheroff, N. *Nucleic Acids Res.* **2009**, *37*, 738.
19. Burden, D. A.; Osheroff, N. *Biochim. Biophys. Acta* **1998**, *1400*, 139.
20. Chen, G. L.; Yang, L.; Rowe, T. C.; Halligan, B. D.; Tewey, K. M.; Liu, L. F. *J. Biol. Chem.* **1984**, *259*, 13560.
21. Duffield, K., Honours Thesis, Acadia University, 2004.
22. Wang, J. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 430.
23. Champoux, J. J. *Annu. Rev. Biochem.* **2001**, *70*, 369.
24. Chene, P.; Rudloff, J.; Schoepfer, J.; Furet, P.; Meier, P.; Qian, Z.; Schlaeppi, J. M.; Schmitz, R.; Radimerski, T. *BMC Chem. Biol.* **2009**, *9*, 1.
25. (a) Colla, L.; De Clercq, E.; Busson, R.; Vanderhaeghe, H. *J. Med. Chem.* **1983**, *26*, 602; (b) Beauchamp, L. M.; Orr, G. F.; Demiranda, P.; Burnette, T.; Krenitsky, T. A. *Antiviral Chem. Chemother.* **1992**, *3*, 157.
26. Sinhababu, A. K.; Thakker, D. R. *Adv. Drug. Delivery Rev.* **1996**, *19*, 241.
27. Breistøl, K.; Hendriks, H. R.; Berger, D. P.; Langdon, S. P.; Fiebig, H. H.; Fodstad, O. *Eur. J. Cancer* **1998**, *34*, 1602.
28. Liwschitz, Y.; Zilkha, A. *J. Am. Chem. Soc.* **1955**, *77*, 1265.
29. *General procedure for the synthesis of compounds* **8a–c** *and* **9a–h**: To dry THF under anhydrous conditions, the appropriate maleamic amino acid ester (**7a-f**) was added. The reaction flask was cooled on ice and TEA (1.1 equiv) was added dropwise over 10 min, while maintaining the reaction mixture on ice. Ethyl chloroformate (1.1 equiv) was then added dropwise over 10 min. The reaction mixture was stirred for 30 min. The appropriate 3,5-bisarylmethylene-4-piperidone was added and the reaction was allowed to stir at room temperature for 24 h. Solvent was removed by rotary evaporation and the product purified in 0–5% DCM/methanol. Analytical data for **9f**: Yellow powder; yield: 38%; mp 97–100 °C. ^{1}H NMR (300 MHz; $CDCl_3$): δ 1.31 (t, J = 6.9 Hz, 3H, CH_3), 2.00–2.182 (m, 5H, SCH_3, CH_2), 2.50 (t, J = 7.3 Hz, 2H, SCH_2), 4.24 (q, J = 6.9 Hz, 2H, OCH_2), 4.56–4.74 (m, 3H, COCH, 2 × NCH), 4.89–5.11 (AB quartet, J = 16.7 Hz, 2H, 2 × NCH), 5.97 and 6.25 (d, J = 11.9 Hz, 1H each, 2 × Z-vinylic-H), 6.97 (br s, 1H, NH), 7.55 (d, J = 8.1 Hz, 2H, ArH), 7.67 (d, J = 8.4 Hz, 2H, ArH), 7.84 and 7.88 (s, 1H each, 2 × vinylic-H), 8.30–8.34 (m, 4H, ArH). ^{13}C NMR (75 MHz; $CDCl_3$): δ 14.18, 15.48, 29.94. 31.32, 42.66, 47.08, 51.85, 61.88, 123.21, 124.09, 127.79, 130.78, 130.86, 131.08, 132.29, 133.65, 133.81, 135.14, 136.13, 140.52, 140.63, 147.89, 163.47, 166.67, 171.50, 185.29. IR (KBr; ν_{max}): 1734, 1623, 1517, 1437, 1384, 1273, 1173, 1108, 1004, 984, 854, 805, 681 cm^{-1}. UV ($CHCl_3$; λ_{max}): 217, 329 nm. ESI-MS (amu): 645 [M+Na]$^+$.
30. Balzarini, J.; De Clerq, E.; Mertes, M.; Shugar, D.; Torrence, P. F. *Biochem. Pharmacol.* **1982**, *31*, 3673.
31. *ChemBioDraw*, 11.0.1; CambridgeSoft: 2007.
32. Assays investigating the inhibition of TOPO IIα were carried out as per the protocol supplied by TopoGEN Inc., the source for purified human DNA TOPO IIα (p170 form) and pRYG DNA. The enzyme was incubated with the compound for 30 min at 37 °C. Subsequently SDS and proteinase K were added. The reaction was stopped with the appropriate TopoGEN stop buffer after 15 min of incubation. The assay and all appropriate controls were loaded on a 1% agarose gel in 1× TAE and run at 60 V for 2 h. The gel was then visualized using ethidium bromide staining. Compounds were assayed at 100 μM, decreasing the concentrations to 50 μM, 25 μM and 10 μM if compounds still exhibited inhibitory activity. All assays were completed in duplicate.
33. *Molecular and Cellular Targets for Antiepileptic Drugs*; Stables, J. P., Kupferberg, H. J., Eds.; John Libbey: London, 1997.