Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 5547–5550
Bioorganic & Medicinal Chemistry Letters

Structurally simplified macrolactone analogues of halichondrin B

Boris M. Seletsky,[a] Yuan Wang,[a] Lynn D. Hawkins,[a] Monica H. Palme,[a] Gregory J. Habgood,[a] Lucian V. DiPietro,[a] Murray J. Towle,[b] Kathleen A. Salvato,[b] Bruce F. Wels,[b] Kimberley K. Aalfs,[b] Yoshito Kishi,[c] Bruce A. Littlefield[b] and Melvin J. Yu[a,*]

[a]*Department of Medicinal Chemistry, Eisai Research Institute, 4 Corporate Drive, Andover, MA 01810, USA*
[b]*Department of Anticancer Research, Eisai Research Institute, 4 Corporate Drive, Andover, MA 01810, USA*
[c]*Advisory Board, Eisai Research Institute, 4 Corporate Drive, Andover, MA 01810, USA*

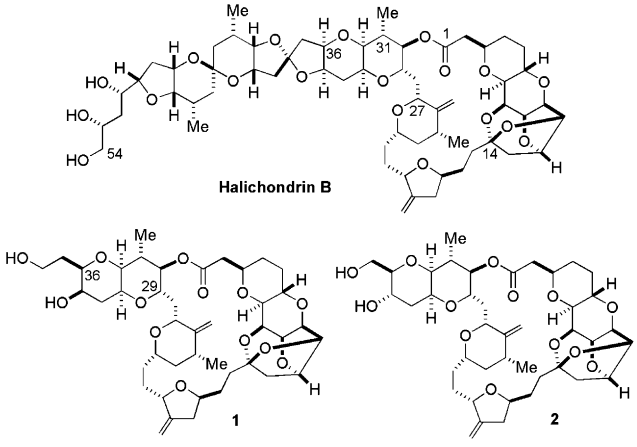
Received 30 July 2004; accepted 31 August 2004
Available online 21 September 2004

Abstract—A structurally simplified macrolactone analogue of halichondrin B was identified that retains the potent cell growth inhibitory activity of the natural product in vitro.

1. Introduction

Halichondrin B (HB) is a structurally complex marine natural product that exhibits extraordinary cytotoxic activity in vitro[1,2] and antitumor efficacy in vivo.[3] Preclinical development as a new anticancer chemotherapeutic, however, was hampered by extremely limited supply of this agent isolated from natural sources. Despite synthetic studies from a number of laboratories, the only successful total synthesis of HB and norhalichondrin B was reported by Kishi and co-workers in 1992.[4]

The discovery that macrolactone right half (RH) synthetic intermediates **1** and **2** exhibited potent cell growth inhibitory activity in vitro[5,6] provided an exciting and compelling starting point for a drug discovery effort around this important class of antitumor agents. Our initial structure–activity relationship study explored modifications to the C.29–C.36 octahydropyrano[3,2-*b*]pyran ring system.[7] Herein, we report our efforts to further simplify the structure of these macrolactones while maintaining potent cell growth inhibitory activity.



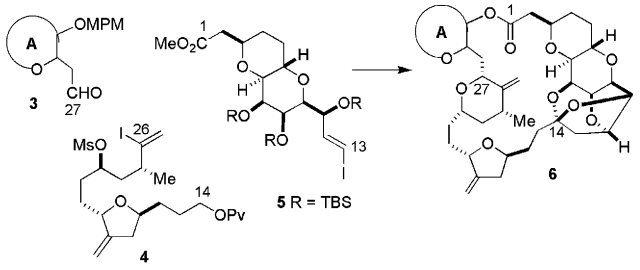
2. Synthesis

Macrolactone analogues represented generically by **6** were assembled from three key fragments **3**, **4**,[4a,8] and **5**[4a,9] in a manner identical to that described by Kishi and co-workers in their total synthesis of HB (Scheme 1). Preparation of aldehyde **3** generically represented below is outlined in Schemes 2–4.

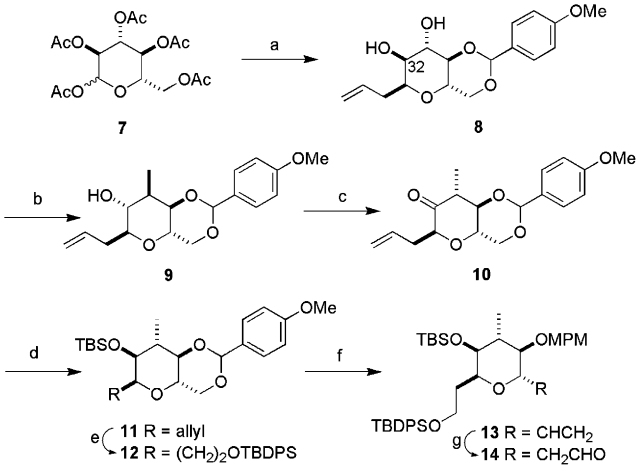
L-Glucose acetate **7** was converted to aldehyde **14** as summarized in Scheme 2. Stereoselective C-glycosidation[10] and adjustment of protecting groups furnished diol **8**. Tosylation at C.32 and opening of the intermediate epoxide with methyl Grignard afforded intermediate **9**.

* Corresponding author. Tel.: +1 978 661 7202; fax: +1 978 657 7715; e-mail: melvin_yu@eri.eisai.com

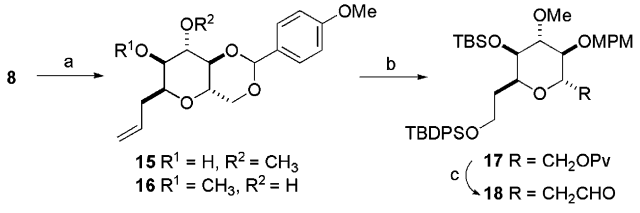
0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.08.068



Scheme 1. Final assembly from key fragments.

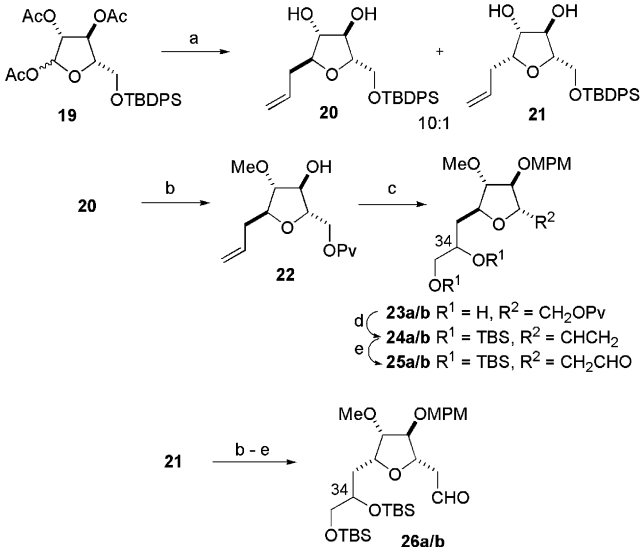


Scheme 2. Synthesis of aldehyde **14**. Reagents and conditions: (a) (i) allylTMS, $BF_3{\cdot}Et_2O$, MeCN, 80 °C, 67%;[10] (ii) NaOMe, MeOH, rt, 84%; (iii) *p*-$MeOC_6H_4CH(OMe)_2$, *p*-TsOH, DMF, rt, 90%; (b) (i) TsCl, Pyr, rt, 71%; (ii) NaOMe, MeOH, rt, 79%; (iii) MeMgCl, Et_2O, reflux, 75%; (c) (i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C to 0 °C; (ii) Et_3N, DMF, rt, 74%; (d) (i) LAH, Et_2O, 0 °C, 88%; (ii) TBSOTf, DIEA, CH_2Cl_2, 0 °C; (e) (i) O_3, CH_2Cl_2, −78 °C; (ii) $NaBH_4$, EtOH, rt, 80%; (iii) TBDPSCl, imidazole, DMAP, DMF, rt, 73%; (f) (i) DIBAL-H, CH_2Cl_2, −78 °C, 59%; (ii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C to 0 °C; (iii) $(MePPh_3)^+Br^-$, *n*-BuLi, THF–DMSO, 96%; (g) (i) $BH_3{\cdot}DMS$, THF, −10 °C to rt; (ii) $NaBO_3$, H_2O, rt, 46%; (iii) Dess–Martin oxidation,[11] 65%.



Scheme 3. Synthesis of aldehyde **18**. Reagents and conditions: (a) (i) Bu_2SnO, toluene, reflux;[12] (ii) KF, MeI, DMF; (iii) separate **15**, 70%; (b) (i) TBSCl, imidazole, DMF, rt; (ii) DIBAL-H, CH_2Cl_2–toluene, −40 °C to −5 °C; (iii) PvCl, Pyr, CH_2Cl_2, 0 °C to rt, 80%; (iv) OsO_4, NMO, acetone–H_2O, rt; (v) $NaIO_4$, MeOH–H_2O, rt; (vi) $NaBH_4$, MeOH–Et_2O, 0 °C; (vii) TBDPSCl, imidazole, DMF, rt, 96%; (c) (i) LAH, Et_2O, 0 °C; (ii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C to 0 °C; (iii) $(MePPh_3)^+Br^-$, *n*-BuLi. THF–DMSO, 0 °C to rt, 66%; (iv) $BH_3{\cdot}THF$, THF, 0 °C; (v) $NaBO_3$, H_2O, rt, 60%; (vi) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C to 0 °C, 99%.

Inversion of this center was accomplished by an oxidation, epimerization, and reduction sequence. The desired aldehyde **14** was subsequently obtained following a series of standard transformations.



Scheme 4. Synthesis of aldehydes **25a/b** and **26a/b**. Reagents and conditions: (a) (i) allylTMS, $BF_3{\cdot}Et_2O$, hexane, 0 °C;[10] (ii) K_2CO_3, MeOH; (iii) separate isomers (10:1), 87% for **20**, 9% for **21**; (b) (i) TBSCl, imidazole, CH_2Cl_2, rt; (ii) separate isomers (4:1); (iii) MeI, NaH, THF–DMF, 0 °C, 54%; (iv) TBAF, THF, rt; (v) PvCl, pyridine, CH_2Cl_2, 0 °C to rt, 88%; (c) (i) MPMOTCI, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C, 85%; (ii) OsO_4, NMO, acetone–H_2O, rt (89%); (iii) separate isomers (1:1); (d) (i) TBSOTf, Et_3N, CH_2Cl_2, 0 °C; (ii) LAH, Et_2O, 0 °C, 91%; (iii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C to 0 °C; (iv) $(MePPh_3)^+Br^-$, *n*-BuLi, THF–DMSO, 0 °C to rt, 76%; (e) (i) $BH_3{\cdot}THF$, THF, 0 °C; (ii) $NaBO_3$, H_2O, rt, 63%; (iii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C to 0 °C, 100%.

Our earlier SAR studies with RH demonstrated a degree of steric tolerance at the C.31 center.[7] If the methyl group could be replaced with a methoxyl substituent, then the corresponding aldehyde fragment could be prepared in a more straightforward manner. Toward that end, diol **8** was selectively methylated[12] and converted to aldehyde **18** (Scheme 3).

Aldehydes **25a/b** and **26a/b** were prepared from L-arabinose derivative **19**[13] as outlined in Scheme 4. The two C.34-diastereomeric diols **23a/b** were chromatographically separated and carried forward separately to final products **25a** and **25b**. C.32α-isomer **21** was converted to C.34-epimers **26a** and **26b**.

Aldehydes **14**, **18**, **25a/b**, and **26a/b** were coupled with fragments **4** and **5** (Scheme 1) and carried forward to final compounds **27**, **28**, **41**, **42**, **46**, and **47**, respectively. The other macrolactone analogues were prepared in a similar manner.

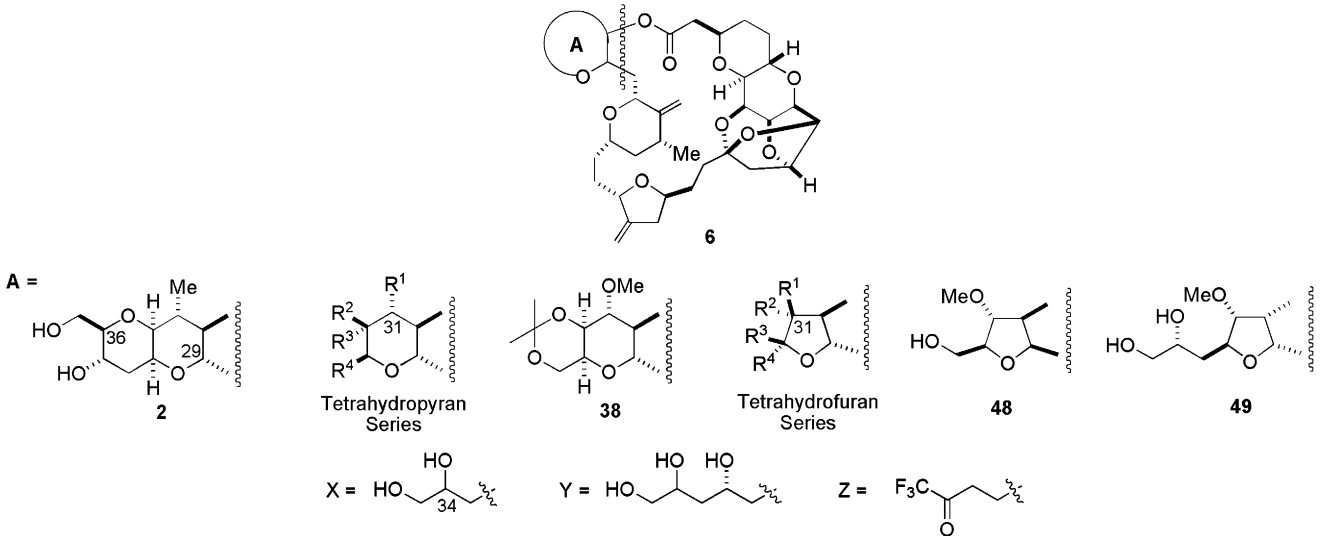
3. Results

The compounds were evaluated for cell growth inhibitory activity against DLD-1 human colon cancer cells under continuous exposure conditions (intrinsic potency), and for the ability to maintain a complete mitotic

block (CMB) 10 h after drug washout using flow cytometric analysis of U937 human histiocytic lymphoma cells (reversibility assay).[14] In addition, susceptibility to P-glycoprotein (PgP) mediated drug efflux using murine P388/VMDRC.04 cells, a multidrug resistant (MDR) subline of murine P388/S leukemia cells,[15] was determined (Table 1).

Tetrahydropyran derivative **27** exhibited biological profile similar to that of C.29–C.36 octahydropyrano[3,2-*b*]pyran analogue **2**. As expected, replacement of the C.31-methyl group with a methoxyl substituent (e.g., **28**) was well tolerated. Replacement with an ethoxyl group, however, led to a 30-fold loss in intrinsic potency (**35** vs **36** or **37**). Inverting the C.32 stereocenter as in **31** and **33** resulted in a three- to five-fold improvement over **28** and **32**, respectively, in ability to maintain a CMB. Shortening the C.33-side chain had no effect (**32** vs **28**), but extending the side chain to a propanediol (e.g., **36** and **37**) led to improved activity in the

Table 1. In vitro activity profile for compounds of generic structure **6**



Compound	R^1	R^2	R^3	R^4	DLD-1[a] IC_{50}, nM (*n*)	U937[b] IC_{99}, nM (*n*)	FR[c]
HB					0.74 ± 0.03 (6)	25 (4)	2.8
2					3.4 ± 0.04 (4)	220 (6)	12
Tetrahydropyran series							
27	Me	OH	H	CH_2CH_2OH	2.5 ± 0.20 (3)	170 (3)	5.6
28	OMe	OH	H	CH_2CH_2OH	1.8 ± 0.00 (3)	300 (3)	10
29	OMe	OH	H	CH_2CH_2OMe	1.8 ± 0.10 (3)	>1000 (2)	1.6
30	OMe	OMe	H	CH_2CH_2OH	2.1 ± 0.06 (3)	>1000 (2)	2.6
31	OMe	H	OH	CH_2CH_2OH	1.8 ± 0.30 (3)	100 (2)	12
32	OMe	OH	H	CH_2OH	2.6 ± 0.40 (3)	300 (2)	8.5
33	OMe	H	OH	CH_2OH	2.0 ± 0.30 (3)	65 (2)	13
34	OMe	H	OH	H	>1000 (2)	ND	ND
35	OEt	H	OH	X	490 ± 90.0 (3)	ND	ND
36	OMe	H	OH	X	19.0 ± 1.70 (4)	53 (3)	23
37	OMe	H	OH	X (C.34-*epi*)	12.0 ± 1.60 (3)	65 (2)	20
38					11.0 ± 0.90 (3)	>1000	1.7
Tetrahydrofuran series							
39	H	OMe	CH_2OH	H	1.0 ± 0.20 (3)	100	2.8
40	H	OMe	CH_2CH_2OH	H	0.97 ± 0.07 (3)	100	2.8
41	H	OMe	X (C.34-*R*)	H	1.4 ± 0.10 (3)	30 (2)	8.8
42	H	OMe	X (C.34-*S*)	H	6.4 ± 0.80 (3)	100 (1)	8.2
43	OMe	H	X (C.34-*R*)	H	220 ± 18.0 (3)	ND	ND
44	H	OMe	Y	H	1.9 ± 0.10 (3)	30 (2)	15
45	H	OMe	Z	H	0.67 ± 0.09 (3)	30 (2)	4.5
46	H	OMe	H	X (C.34-*R*)	0.67 ± 0.07 (3)	20 (2)	9.3
47	H	OMe	H	X (C.34-*S*)	0.67 ± 0.09 (3)	15 (4)	6.2
48					>1000 (2)	ND	ND
49					>1000 (2)	ND	ND

[a] Cell growth inhibition under continuous exposure conditions for 3–4 days, IC_{50} ± SEM (*n* = number of experiments).
[b] Mitotic block reversibility assay; ND—not determined.
[c] Fold resistance calculated as the IC_{50} ratio between retrovirally transformed P388/VMDRC.04 and parental P388 cells; ND—not determined.

reversibility assay, albeit with increased susceptibility to PgP-mediated drug efflux. This inverse relationship was also observed when the hydroxyl groups were converted to methyl ethers **29** and **30** or to an acetonide **38**, resulting in diminished activity in the reversibility assay, and decreased susceptibility to PgP-mediated drug efflux. Removal of the C.33-side chain (i.e., **34**) led to complete loss of activity.

Since the C.31 substituent appeared to play a critical role, we hypothesized that alternative ring systems that preserve the spatial relationship of this group relative to the macrolactone ring may stabilize the bioactive conformation of the molecule and lead to analogues with improved biological activity. In the X-ray crystal structure of norhalichondrin A *p*-bromophenacyl ester,[1] the C.29–C.33 tetrahydropyran ring adopted a twist boat conformation that placed the C.31 methyl group in a pseudoequatorial position. Since a tetrahydrofuran ring could similarly orient the C.31 methyl group in a pseudoequatorial position, we prepared a series of tetrahydrofuran derivatives to determine if the biological activity limitations observed with the tetrahydropyran analogues could be circumvented.

Gratifyingly, tetrahydrofuran **41** exhibited a superior potency and reversibility profile to that found for the tetrahydropyran series (cf. **41** and **27**–**38**). Inversion of stereochemistry at C.31 led to a 150-fold loss of potency (**43** vs **41**), confirming the importance of this group in both the tetrahydropyran and tetrahydrofuran series. As expected based on conformational arguments, activity was also critically dependent on the configuration at C.29 and C.30 of the macrolactone ring (e.g., **48** and **49**, respectively). The trifluoromethyl ketone analogue **45** exhibited an in vitro biological profile similar to **41** suggesting the bioisosteric equivalence of a hydrated trifluoromethyl ketone acting as a formal geminal diol with a vicinal diol moiety.[16] The influence of stereochemistry at C.32 was further investigated. C.34-epimeric diols **46** and **47** showed superior activity in the reversibility assay compared with the corresponding C.32 diastereomers **41** and **42**. From these studies, tetrahydrofuran **47** emerged as the most promising analogue based on intrinsic potency, ability to maintain a CMB, and susceptibility to PgP-mediated drug efflux.

In summary, structurally simplified macrolactone analogues of HB were identified that retain the potent cell growth inhibitory activity of the natural product in vitro, and may represent the minimum pharmacophore for the halichondrin class of antimitotic agents.

References and notes

1. Uemura, D.; Takahashi, K.; Yamamoto, T.; Katayama, C.; Tanaka, J.; Okumura, Y.; Hirata, Y. *J. Am. Chem. Soc.* **1985**, *107*, 4796–4798.
2. Hirata, Y.; Uemura, D. *Pure Appl. Chem.* **1986**, *58*, 701–710.
3. (a) Fodstad, Ø.; Breistøl, K.; Pettit, G. R.; Shoemaker, R. H.; Boyd, M. R. *J. Exp. Therap. Oncol.* **1996**, *1*, 119–125; (b) Towle, M. J.; Aalfs, K. K.; Budrow, J. A.; Kishi, Y.; Littlefield, B. A. Annual Meeting of the American Association for Cancer Research, Toronto, Ontario, March 18–22, 1995, 2342.
4. (a) Aicher, T. D.; Buszek, K. R.; Fang, F. G.; Forsyth, C. J.; Jung, S. H.; Kishi, Y.; Matelich, M. C.; Scola, P. M.; Spero, D. M.; Yoon, S. K. *J. Am. Chem. Soc.* **1992**, *114*, 3162–3164; (b) Aicher, T. D.; Buszek, K. R.; Fang, F. G.; Forsyth, C. J.; Jung, S. H.; Kishi, Y.; Scola, P. M. *Tetrahedron Lett.* **1992**, *33*, 1549–1552; (c) Buszek, K. R.; Fang, F. G.; Forsyth, C. J.; Jung, S. H.; Kishi, Y.; Scola, P. M.; Yoon, S. K. *Tetrahedron Lett.* **1992**, *33*, 1553–1556; (d) Fang, F. G.; Kishi, Y.; Matelich, M. C.; Scola, P. M. *Tetrahedron Lett.* **1992**, *33*, 1557–1560.
5. (a) Towle, M. J.; Kishi, Y.; Littlefield, B. A., unpublished observations, 1992; (b) Kishi, Y.; Fang, F. G.; Forsyth, C. J.; Scola, P. M.; Yoon, S. K. U.S. Patent 5,436,238.
6. Stamos, D. P.; Sean, S. C.; Kishi, Y. *J. Org. Chem.* **1997**, *62*, 7552–7553.
7. Wang, Y.; Habgood, G. J.; Christ, W. J.; Kishi, Y.; Littlefield, B. A.; Yu, M. *J. Bioorg. Med. Chem. Lett.* **2000**, *10*, 1029–1032.
8. (a) Xie, C.; Nowak, P.; Kishi, Y. *Org. Lett.* **2002**, *4*, 4427–4429; (b) Choi, H.; Nakajima, K.; Demeke, D.; Kang, F.-A.; Jun, H.-S.; Wan, Z.-K.; Kishi, Y. *Org. Lett.* **2002**, *4*, 4435–4438.
9. Stamos, D. P.; Kishi, Y. *Tetrahedron Lett.* **1996**, *37*, 8643–8646.
10. Lewis, M. D.; Cha, J. K.; Kishi, Y. *J. Am. Chem. Soc.* **1982**, *104*, 4976–4978.
11. Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155–4156.
12. Nashed, M. A.; Anderson, L. *Tetrahedron Lett.* **1976**, 3503–3506.
13. L-Arabinose derivative **19** was prepared in three steps from L-arabinose dithioacetal: (a) TBDPSCl, Et_3N; (b) I_2, $NaHCO_3$; (c) Ac_2O, pyridine.
14. Towle, M. J.; Salvato, K. A.; Budrow, J.; Wels, B. F.; Aalfs, K. K.; Zheng, W.; Seletsky, B. M.; Palme, M. H.; Habgood, G. J.; Singer, L. A.; DiPietro, L. V.; Wang, Y.; Chen, J. J.; Lydon, P. J.; Quincy, D. A.; Kishi, Y.; Yoshimatsu, K.; Yu, M. J.; Littlefield, B. A. Annual Meeting of the American Association for Cancer Research, New Orleans, LA, March 24–28, 2001, 1976.
15. Yang, J.-M.; Goldenberg, S.; Gottesman, M. M.; Hait, W. N. *Cancer Res.* **1994**, *54*, 730–737.
16. Patel, D. V.; Rielly-Gauvin, K.; Ryono, D. E.; Free, C. A.; Smith, S. A.; Petrillo, E. W. *J. Med. Chem.* **1993**, *36*, 2431–2447.