Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3443–3447

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Trifluoromethyl Ketones as Inhibitors of Histone Deacetylase

Robin R. Frey,* Carol K. Wada, Robert B. Garland, Michael L. Curtin, Michael R. Michaelides, Junling Li, Lori J. Pease, Keith B. Glaser, Patrick A. Marcotte, Jennifer J. Bouska, Shannon S. Murphy and Steven K. Davidsen

Cancer Research, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064, USA

Received 27 June 2002; accepted 15 August 2002

Abstract—Trifluoromethyl ketones were found to be inhibitors of histone deacetylases (HDACs). Optimization of this series led to the identification of submicromolar inhibitors such as **20** that demonstrated antiproliferative effects against the HT1080 and MDA 435 cell lines.

Chemical modifications of histones, including acetylation of the lysine side chains of histone proteins, are known to influence gene transcription.[1] Inappropriate recruitment of histone deacetylases (HDACs) has been shown to lead to transcriptional silencing in cancer cell lines. Inhibition of HDACs results in transcriptional reactivation, cell-cycle arrest and terminal differentiation of transformed cells, and has been indicated as a novel mechanism for cancer chemotherapy.[2]

A number of structurally diverse HDAC inhibitors have been reported in the literature and include many hydroxamic acids,[3] typified by SAHA (lit. IC_{50} 10 nM)[4] and trichostatin A,[5] as well as non hydroxamate-based inhibitors such as phenylbutyric acid,[6] MS 27–275 (lit. IC_{50} 2.0 μM),[7] and the macrocyclic natural products trapoxin and apicidin[8] (Fig. 1). Hydroxamic acid **1**,[9] from the Abbott compound library, has been identified as a potent inhibitor of HDAC as well (IC_{50} 9.2 nM).[10] These compounds tend to fit a structural template consisting of a group that can interact with the active-site zinc attached to a 'cap group' by a hydrophobic tether.[11] Thus far, with the exception of the macrocyclic natural products, non-hydroxamate inhibitors have reduced potency (>1 μM) as compared to hydroxamate-based inhibitors, which typically exhibit nanomolar IC_{50} values. Because of the short half-life and poor bioavailability associated with the hydroxamic acid functional group,[12] it became desirable to find replacement groups that would possess nanomolar activity against HDACs.

In the search for a suitable hydroxamic acid replacement, electrophilic ketones, which have been shown to be inhibitors of various hydrolytic enzymes, appeared to be attractive targets. Trifluoromethyl ketones are known to be readily hydrated,[13] and have been reported as inhibitors of phospholipases,[14] aspartyl,[15] cysteine and serine proteases,[16] as well as zinc-dependent enzymes.[17] Published structural data for HDAC-like protein (HDLP), a bacterial enzyme sharing high homology to the HDACs in its active site, confirmed that this protein contains a zinc in the active site.[18] Because trifluoromethyl ketones have been reported as inhibitors of zinc-dependent proteases, it seemed reasonable that they might inhibit HDACs as well. A series of compounds modeled after SAHA and **1** was prepared, in which the hydroxamic acid was replaced by a trifluoromethyl ketone.

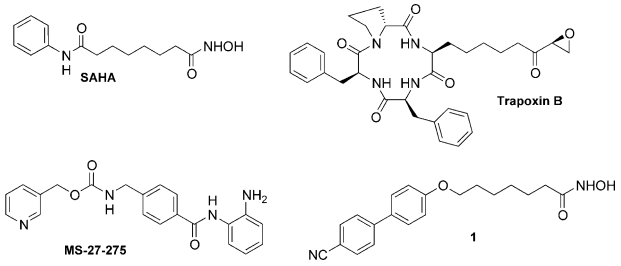


Figure 1. HDAC inhibitors.

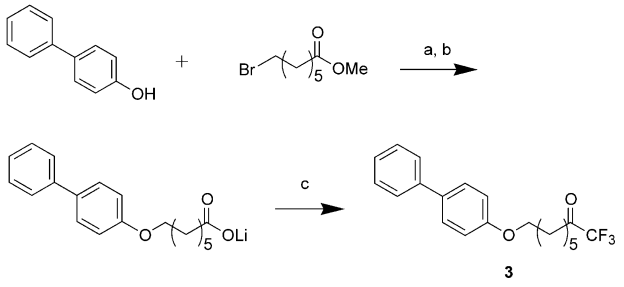
*Corresponding author. Fax: +1-847-935-5165; e-mail: robin.r.frey@abbott.com

0960-894X/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00754-0

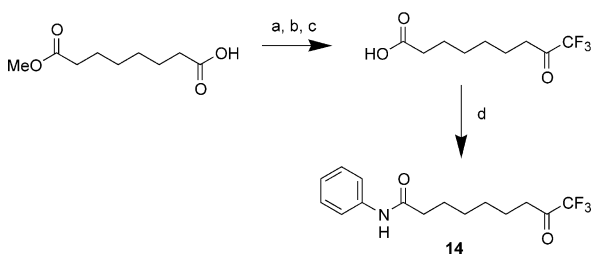
Preparation of the trifluoromethyl ketone ether analogues proceeded as shown for compound **3** (Scheme 1). 4-Phenylphenol was coupled with methyl 7-bromoheptanoate under basic conditions, and the ester was saponified using lithium hydroxide. The lithium salt was then treated with trifluoroacetic anhydride under modified Dakin–West conditions to provide the trifluoromethyl ketone in moderate but reproducible yield.[19] Synthesis of the analogous amide derivative **14** is shown in Scheme 2. Suberic acid monomethyl ester was converted to the acid chloride in refluxing thionyl chloride, then treated with trifluoroacetic anhydride and pyridine to provide the trifluoromethyl ketone. The methyl ester was cleaved by saponification to give the trifluoromethyl ketone acid, which was coupled with aniline in the presence EDCI and HOBt. Using solid-phase coupling reagents, this synthetic route also allowed for the synthesis of a large number of amide analogues in parallel. Analogous methods were used to prepare the corresponding pentafluoroethyl ketones[20] in comparable yields.

Along with these simple, straight-chain analogues a number of compounds containing varied linkages between the aryl moiety and the trifluoromethyl ketone were prepared. These included the unsaturated and *trans*-cyclopropyl compounds **5**, **6** and **7** (Scheme 3), as well as aryl-linked compounds. In addition, a few heteroatom-linked compounds were prepared as outlined in Schemes 4 and 5.

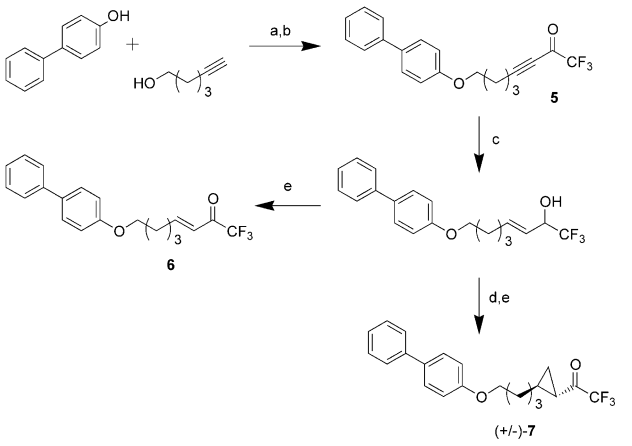
The compounds from this work were tested in an in vitro assay using a partially purified HDAC preparation consisting primarily of HDAC1 and HDAC2,[10] and promising compounds were further characterized in cellular proliferation assays in two human tumor cell lines.[21] In the ether-linked trifluoromethyl ketone series, the optimal chain length for the linker proved to be 5 or 6 atoms, as shown by comparison of **2** and **3** with **4**, which has a longer linker (Table 1). A variety of aryl substituents were tolerated at the terminal end of the inhibitors. Unsaturated compounds **5** and **6** and the cyclopropyl analogue **7** showed similar potencies to the aliphatic chain-linked **3**. Incorporation of oxygen or sulfur into the linker as in **8** and **9** also led to compounds with comparable potency to those containing alkyl tethers, with all of the compounds generally having IC_{50} values in the single digit micromolar range.



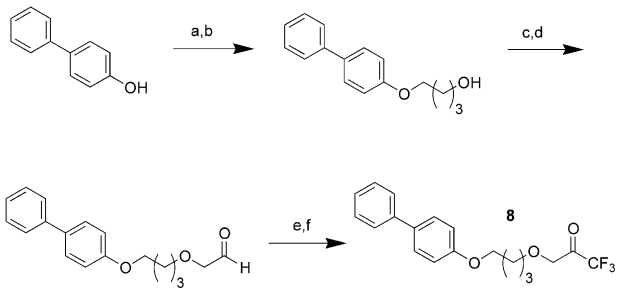
Scheme 1. (a) Cs_2CO_3, DMF, 98%; (b) LiOH, H_2O, THF, 95%; (c) TFAA, pyridine, 40%.



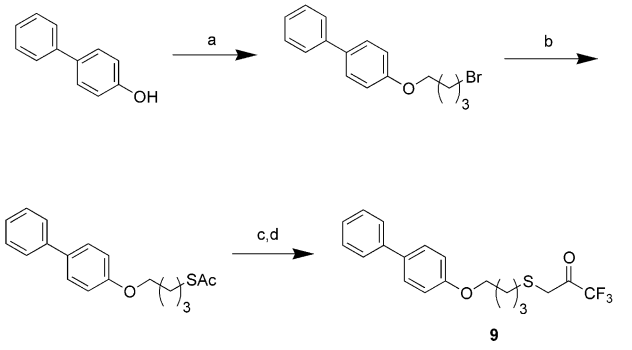
Scheme 2. (a) $SOCl_2$, reflux; (b) TFAA, pyridine, 44% (two steps); (c) LiOH, THF, H_2O, 98%; (d) aniline, EDCl, HOBt, *N*-methylmorpholine, DMF, 82%.



Scheme 3. (a) PPh_3, DEAD, THF (67%); (b) *n*-BuLi, THF, CF_3COOEt, BF_3OEt, −78 °C to rt (46%); (c) $LiAlH_4$, THF, 0 °C to reflux, 78%; (d) Sm°, CH_2I_2, THF, 0 °C to rt, 63%; (e) Dess–Martin periodinane, CH_2Cl_2, 55–90%.



Scheme 4. (a) $Br(CH_2)_3COOEt$, Cs_2CO_3, DMF, 88%; (b) DIBAL, CH_2Cl_2, −78 °C, 90%; (c) $BrCH_2CH(OEt)_2$, NaH, DMF, rt, 2 h, then 90 °C, 4 h; (d) H_2SO_4, acetone, water, reflux, 82%; (e) TMS–CF_3, TBAF, THF, 0 °C 94%; (f) Dess–Martin periodinane, CH_2Cl_2, 28%.

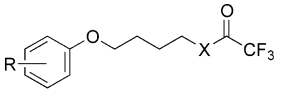


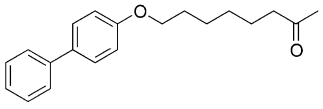
Scheme 5. (a) $Br(CH_2)_4Br$, Cs_2CO_3, DMF, 71%; (b) KSAc, DMF, 100%; (c) NaOH, H_2O, acetone, 100%; (d) $BrCH_2COCF_3$, Et_3N, DMSO, 24%.

Little difference in potency was seen between *para*- and *meta*-substitution on the aromatic ring in the ether series, although biaryl compounds such as **3** tended to be slightly more potent than monoaryls such as **10**. Overall, the ether-linked trifluoromethyl ketones possessed micromolar potency against HDACs and weak activity in the cellular anti-proliferation assays (Table 3).

In the amide-linked trifluoromethyl ketone series, a more defined SAR was observed upon alteration of the left-hand portion of the molecule (Table 2). Similar potencies were observed for monoaryl amide **14**, homologated aryl and amino acid derivatives. *para*-Substituted biaryl **15** showed similar potency to monoaryl compound **14**, but *meta*-biaryl amide **16** (IC_{50} 380 nM) showed a 10-fold increase in potency. The incorporation of an *ortho*-substituted biaryl amide as in **17** was not well tolerated. This preference for the *meta*-regioisomer did not carry over to phenoxyphenyl amide analogues, as the *para*-linked analogue **18** is about 10-fold more potent than the *meta* compound **19**. The 4-substituted phenylthiazole **20** (IC_{50} 310 nM) showed enhanced potency similar to that of the *meta*-substituted biaryl **16**. A phenyl group in the tether attached directly to the trifluoromethyl ketone in **21** resulted in a complete loss of activity, while the styrenyl-linked phenyl analogue **22** retained the potency of related compounds **6** and **14**. The importance of the trifluoromethyl ketone moiety to the HDAC inhibitory properties of these compounds is underscored by the inactivity of the methyl ketone and trifluoromethyl alcohol analogues **13** and **23**. Pentafluoroethyl ketones proved inactive in both the ether and amide series. Several of the trifluoromethyl ketones demonstrated significant cellular anti-proliferative activity (Table 3), with thiazole **20** standing out as one of the most potent against both cell lines.

Table 1. HDAC inhibitory data for ether-linked compounds **2**–**13**[10]

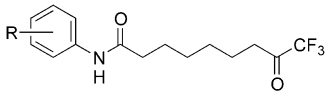


Compd	R	X	HDAC IC_{50} (μM)
2	4-Ph	$-CH_2-$	2.6
3	4-Ph	$-CH_2CH_2-$	2.9
4	4-Ph	$-CH_2CH_2CH_2-$	72% @ 50
5	4-Ph		8.6
6	4-Ph		5.0
7	4-Ph		2.9
8	4-Ph	$-OCH_2-$	11
9	4-Ph	$-SCH_2-$	4.6
10	H	$-CH_2CH_2-$	8.0
11	3-Ph	$-CH_2CH_2-$	2.8
12	4-OPh	$-CH_2CH_2-$	3.4
13			> 50

In order to verify that the antiproliferative activity observed with these novel non-hydroxamate compounds was indeed a result of HDAC inhibition, Western blots were run to determine whether biochemical markers characteristic of HDAC inhibition[22] were modulated in these cells. It was found that treatment of MDA-435 cells with compound **16** at 30 μM produced hyperacetylation of histone H4, similar to the effect produced by SAHA, whereas the methyl ketone **13**, which lacked HDAC activity, did not (Fig. 2). In addition, treatment with **16** resulted in induction of $p21^{Waf1/Cip1}$ in these cells.

Unfortunately, these trifluoromethyl ketone HDAC inhibitors exhibited a half-life of only ~0.5 h and poor intravenous exposure in mice at a dose of 10 mg/kg. Along with low aqueous solubilities, these compounds also have significant metabolic liabilities. Trifluoromethyl ketone **16** is rapidly metabolized to the

Table 2. HDAC Inhibitory data for amide-linked compounds **14**–**23**[10]



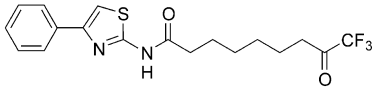
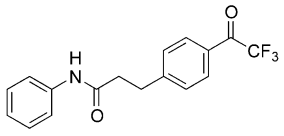
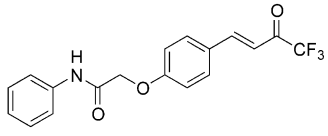
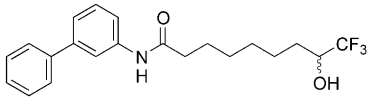
Compd	R	HDAC IC_{50} (μM)
14	H	6.7
15	4-Ph	3.0
16	3-Ph	0.38
17	2-Ph	16
18	4-OPh	0.30
19	3-OPh	3.2
20		0.31
21		> 50
22		2.2
23		> 50

Table 3. Anti-proliferative values for selected compounds[21]

Compd	MDA 435[a]	HT 1080[a]	Compd	MDA 435[a]	HT 1080[a]
2	—	13	**14**	15	2.2
3	30	20	**15**	12	7.0
4	—	21	**16**	23	16
5	> 50	> 50	**18**	15	12
9	9.8	—	**20**	3.4	4.2

[a]IC_{50} (μM).

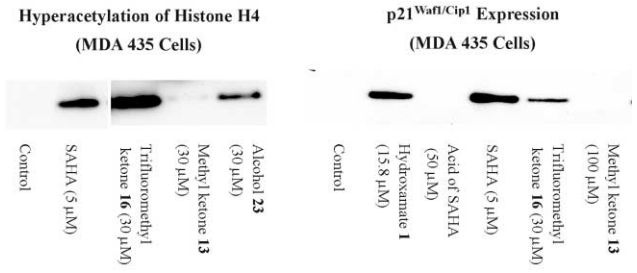


Figure 2. Western blot analysis of histone hyperacetylation and p21 induction in MDA 435 cells produced by **16** and by reference compounds including SAHA and **1**.

alcohol **23** in vivo, and upon further investigation the same transformation was found to occur upon incubation with cells or whole blood. This phenomenon has been previously documented for other trifluoromethyl ketones,[23] and seems to be especially pronounced in straight-chain alkyl-linked compounds. Modifications to the linker such as the introduction of heteroatoms beta- to the ketone or the incorporation of unsaturation or a cyclopropane ring adjacent to the ketone carbonyl did not improve the metabolic stability of these compounds, nor did changing the trifluoromethyl group to pentafluoroethyl.

Although trifluoromethyl ketone HDAC inhibitors did not possess metabolic stability superior to the hydroxamates, compounds such as **20** do show that non-hydroxamate inhibitors can exhibit submicromolar potency. Additional classes of electrophilic ketones are being studied in order to identify compounds with improved in vivo and in vitro potencies, and those results will be reported in due course.

Acknowledgements

The authors wish to thank Kent Stewart for molecular modeling studies and Carole Goodfellow for assisting in pharmacokinetic characterization of these compounds.

References and Notes

1. Jenuwein, T.; Allis, C. D. *Science* **2001**, *293*, 1074.
2. (a) Reviews: Krämer, O. H.; Göttlicher, M.; Heinzel, T. *Trends Endocrinol. Metab.* **2001**, *12*, 294. (b) Marks, P. A.; Richon, V. M.; Rifkind, R. A. *J. Natl. Cancer Inst.* **2000**, *92*, 1210.
3. (a) Kim, Y. B.; Lee, K.-H.; Sugita, K.; Yoshida, M.; Horinouchi, S. *Oncogene* **1999**, *18*, 2461. (b) Jung, M.; Hoffman, K.; Brosch, G.; Loidl, P. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1655.
4. The given IC_{50} was determined using immunoprecipitated HDAC1. Richon, V. M.; Emiliani, S.; Verdin, E.; Webb, Y.; Breslow, R.; Rifkind, R. A.; Marks, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3003.
5. (a) Tsuji, N.; Kobayashi, M.; Nagashima, K.; Wakisaka, Y.; Koizumi, K. *J. Antibiotics* **1976**, *29*, 1. (b) Yoshida, M.; Nomura, S.; Beppu, T. *Cancer Res.* **1987**, *47*, 3688.
6. Warrell, R. P., Jr.; He, L.-Z.; Richon, V.; Calleja, E.; Pandolfi, P. P. *J. Natl. Cancer Inst.* **1998**, *90*, 1621.
7. (a) The given IC_{50} was determined using a mixture of HDAC1 and HDAC2 prepared from nuclear extraction of K562 erythroleukemia cells.[10] (b) Saito, A.; Yamashita, T.; Mariko, Y.; Nosaka, Y.; Tsuchiya, K.; Ando, T.; Suzuki, T.; Tsuruo, T.; Nakanishi, O. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4592.
8. (a) Kijima, M.; Yoshida, M.; Sugita, K.; Horinouchi, S.; Beppu, T. *J. Biol. Chem.* **1993**, *268*, 22429. (b) Singh, S. B.; Zink, D. L.; Polishook, J. D.; Dombrowski, A. W.; Darkin-Rattray, S. J.; Schmatz, D. M.; Goetz, M. A. *Tetrahedron Lett.* **1996**, *37*, 8077.
9. Hajduk, P. J.; Sheppard, G.; Nettesheim, D. G.; Olejniczak, E. T.; Shuker, S. B.; Meadows, R. P.; Steinman, D. H.; Carrera, G. M., Jr.; Marcotte, P. A.; Severin, J.; Walter, K.; Smith, H.; Gubbins, E.; Simmer, R.; Holzman, T. F.; Morgan, D. W.; Davidsen, S. K.; Summers, J. B.; Fesik, S. W. *J. Am. Chem. Soc.* **1997**, *119*, 5818.
10. The given IC_{50} was determined using a mixture of HDAC1 and HDAC2 prepared from nuclear extraction of K562 erythroleukemia cells. This protocol is described in Yoshida, M.; Kijima, M.; Akita, M.; Beppu, T. *J. Biol. Chem.* **1990**, *265*, 17174.
11. Jung, M.; Brosch, G.; Kölle, D.; Scherf, H.; Gerhäuser, C.; Loidl, P. *J. Med. Chem.* **1999**, *42*, 4669.
12. Michaelides, M. R.; Dellaria, J. F.; Gong, J.; Holms, J. H.; Bouska, J. J.; Stacey, J.; Wada, C. K.; Heyman, H. R.; Curtin, M. L.; Guo, Y.; Goodfellow, C. L.; Elmore, I. B.; Albert, D. H.; Magoc, T. J.; Marcotte, P. A.; Morgan, D. W.; Davidsen, S. K. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1553.
13. Gelb, M. H.; Svaren, J. P.; Abeles, R. H. *Biochemistry* **1985**, *24*, 1813.
14. Trimble, L. A.; Street, I. P.; Perrier, H.; Tremblay, N. M.; Weech, P. K.; Bernstein, M. A. *Biochemistry* **1993**, *32*, 12560.
15. Patel, D. V.; Rielly-Gauvin, K.; Ryono, D. E.; Free, C. A.; Smith, S. A.; Petrillo, E. W., Jr. *J. Med. Chem.* **1993**, *36*, 2431.
16. (a) Peet, N. P.; Burkhart, J. P.; Angelastro, M. R.; Giroux, E. L.; Mehdi, S.; Bey, P.; Kolb, M.; Neises, B.; Schirlin, D. *J. Med. Chem.* **1990**, *33*, 394. (b) Boger, D. L.; Sato, H.; Lerner, A. E.; Austin, B. J.; Patterson, J. E.; Patricelli, M. P.; Cravatt, B. F. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 265. (c) Edwards, P. D.; Andisik, D. W.; Strimpler, A. M.; Gomes, B.; Tuthill, P. A. *J. Med. Chem.* **1996**, *39*, 1112.
17. (a) Walter, M. W.; Felici, A.; Galleni, M.; Soto, R. P.; Adlington, R. M.; Baldwin, J. E.; Frère, J.-M.; Gololobov, M.; Schofield, C. J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2455. (b) Christianson, D. W.; Lipscomb, W. N. *J. Am. Chem. Soc.* **1986**, *108*, 4998.
18. Finnin, M. S.; Donigan, J. R.; Cohen, A.; Richon, V. M.; Rifkind, R. A.; Marks, P. A.; Breslow, R.; Pavletich, N. P. *Nature* **1999**, *401*, 188.
19. Boivin, J.; El Kaim, L.; Zard, S. Z. *Tetrahedron* **1995**, *51*, 2573.
20. Angelastro, M. R.; Baugh, L. E.; Bey, P.; Burkhart, J. P.; Chen, T.-M.; Durham, S. L.; Hare, C. M.; Huber, E. W.; Janusz, M. J.; Koehl, J. R.; Marquart, A. L.; Mehdi, S.; Peet, N. P. *J. Med. Chem.* **1994**, *37*, 4538.
21. Procedure for cell growth inhibition assays: HT1080 or MDA-435 cells were seeded at 5×10^3 cells/well in a 96-well tissue culture 18 h prior to addition of compound or vehicle (DMSO) in complete growth medium (10% serum). After 72 h exposure to compound, the cells were washed with PBS. AlamarBlue (modified MTT) was added in serum free medium or PBS (10% AlamarBlue in 100 μL) and allowed to incubate for 4 h at 37 °C. Cell number was measured by determining the

fluorescence at 488/520 nm in an f_{max} fluorescence plate reader (Molecular Devices, San Diego, CA, USA). Treated cells were compared to vehicle treated cells to determine growth inhibition.

22. Han, J.-W.; Ahn, S. H.; Park, S. H.; Wang, S. Y.; Bae, G.-U.; Seo, D.-W.; Kwon, H.-K.; Hong, S.; Lee, H. Y.; Lee, Y.-W.; Lee, H.-W. *Cancer Res*. **2000**, *60*, 6068.

23. Veale, C. A.; Bernstein, P. R.; Bohnert, C. M.; Brown, F. J.; Bryant, C.; Damewood, J. R., Jr.; Early, R.; Feeney, S. W.; Edwards, P. D.; Gomes, B.; Hulsizer, J. M.; Kosmider, B. J.; Krell, R. D.; Moore, G.; Salcedo, T. W.; Shaw, A.; Silberstein, D. S.; Steelman, G. B.; Stein, M.; Strimpler, A.; Thomas, R. M.; Vacek, E. P.; Williams, J. C.; Wolanin, D. J.; Woolson, S. *J. Med. Chem*. **1997**, *40*, 3173.