Bioorganic & Medicinal Chemistry Letters 20 (2010) 1415–1419

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design, synthesis, and biological evaluation of potent thiosemicarbazone based cathepsin L inhibitors

G. D. Kishore Kumar [a], Gustavo E. Chavarria [a], Amanda K. Charlton-Sevcik [a], Wara M. Arispe [a], Matthew T. MacDonough [a], Tracy E. Strecker [a], Shen-En Chen [a], Bronwyn G. Siim [b], David J. Chaplin [b], Mary Lynn Trawick [a], Kevin G. Pinney [a,*]

[a] Department of Chemistry and Biochemistry, Baylor University, One Bear Place #97348, Waco, TX 76798-7348, USA
[b] OXiGENE Inc., Magdalen Center, Robert Robinson Avenue, The Oxford Science Park, OX4 4GA, UK

ARTICLE INFO

Article history:
Received 2 December 2009
Revised 23 December 2009
Accepted 24 December 2009
Available online 6 January 2010

Keywords:
Inhibitors of cathepsin L
Inhibitors of cathepsin B
Chemical synthesis
Molecular design
Molecular modeling

ABSTRACT

A small library of 36 functionalized benzophenone thiosemicarbazone analogs has been prepared by chemical synthesis and evaluated for their ability to inhibit the cysteine proteases cathepsin L and cathepsin B. Inhibitors of cathepsins L and B have the potential to limit or arrest cancer metastasis. The six most active inhibitors of cathepsin L (IC_{50} < 85 nM) in this series incorporate a *meta*-bromo substituent in one aryl ring along with a variety of functional groups in the second aryl ring. These six analogs are selective for their inhibition of cathepsin L versus cathepsin B (IC_{50} > 10,000 nM). The most active analog in the series, 3-bromophenyl-2′-fluorophenyl thiosemicarbazone **1**, also efficiently inhibits cell invasion of the DU-145 human prostate cancer cell line.

The papain family of proteases includes a subfamily of eleven human lysosomal cysteine peptidases known as the cathepsins (B, C, F, H, K, L, O, S, V, W and X).[1] Although the first cathepsin was discovered in the 1940s, cathepsin C, these cysteine proteases have only been more thoroughly characterized structurally and biologically in the last 10–15 years.[2] Several of these proteins are responsible for the degradation of the extracellular matrix (ECM) in addition to other physiological processes such as intracellular protein turnover. Recent studies have shown that specific cysteine proteases, such as cathepsins B and L, participate in tumor progression, hyperproliferation, apoptosis, angiogenesis and metastasis by malignant cells.[3] Cathepsins L and B have a broad tissue distribution and are overexpressed in several human tumors including breast, prostate, lung, gastrointestinal, and epidermal.[4] Therefore, cysteine proteases are of special interest as targets for the development of novel chemotherapeutic drugs.[5]

Cathepsin L is a globular endosomal cysteine protease that plays a vital role in biological and physiological processes such as bone remodeling, cancer metastasis, tumor cell invasion, rheumatoid and osteo-arthritis.[6] Cathepsin L also catalyzes the hydrolytic cleavage of specific peptide bonds involved in antigen processing, bone resorption, and turnover of proteins. Cathepsin L activity has also been reported to be involved in diseases such as Ebola hemorrhagic fever,[7] severe acute respiratory syndrome (SARS),[8] and Leishmania.[9] Inhibitors of cathepsin L-like cysteine proteases also have potential utility in the treatment of protozoan infections such as trypanosomiasis and malaria.[10]

Cathepsin B is characterized by a dual function as a lysosomal endopeptidase and as an exopeptidase.[11] In addition to facilitating ECM and basal membrane degradation, cathepsin B also plays an important role in promoting invasion of certain solid tumors.[12] Overexpression of cathepsins L and B contributes to the migration of cancer cells into surrounding tissue resulting in further metastasis.[13]

The thiosemicarbazone moiety has been recognized as a functional group that presumably interacts with the cysteine-25 residue in the active site of the cathepsin L-like cysteine protease cruzain from the protozoan *Trypanosoma cruzi*.[14] Recent studies by our group and others have demonstrated the effectiveness of the thiosemicarbazone moiety (I & II) for inhibition of cathepsin L (Fig. 1).[14f,15] In addition, a variety of other small molecules including peptidic: thiocarbazate (III),[16] aldehyde (IV),[17] epoxide (V),[18] nitrile (VI),[19] cyanopyrrolidine (VII)[20] and non-peptidic: azepanon (VIII)[21] and cyanamides (IX)[4] have been identified as inhibitors of cathepsin L (Fig. 1).

Previous work[10,14a,d,15a] has explored the role of functionalized benzophenone thiosemicarbazone analogs as inhibitors of cruzain and rhodesain with potential application in the treatment of Chagas' disease, sleeping sickness, and malaria. In order to

* Corresponding author. Tel.: +1 254 710 4117; fax: +1 254 710 4272.
E-mail address: Kevin_Pinney@baylor.edu (K.G. Pinney).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.12.090

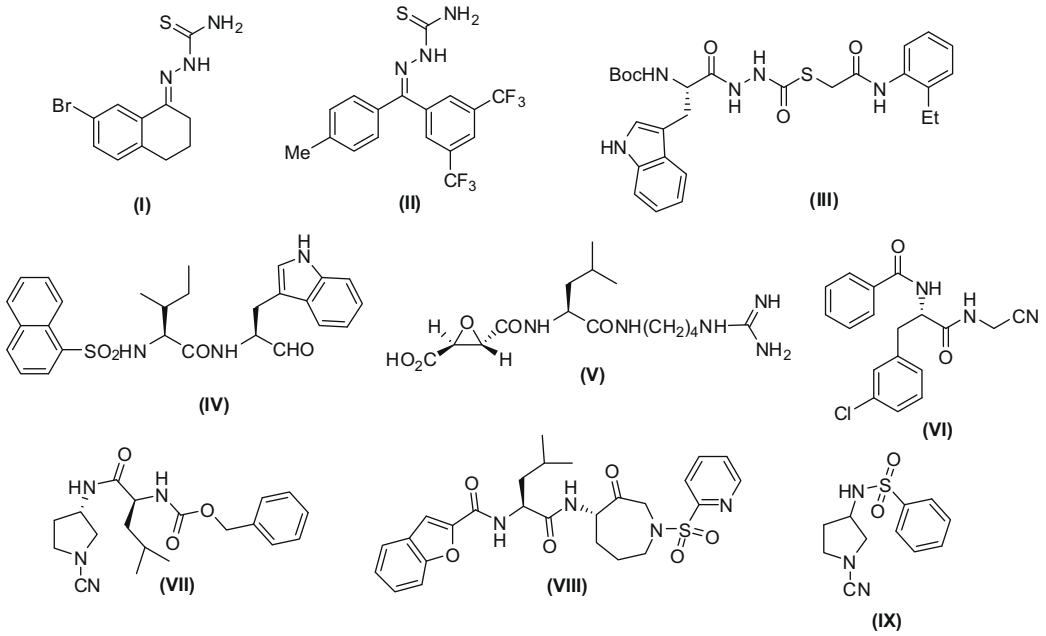
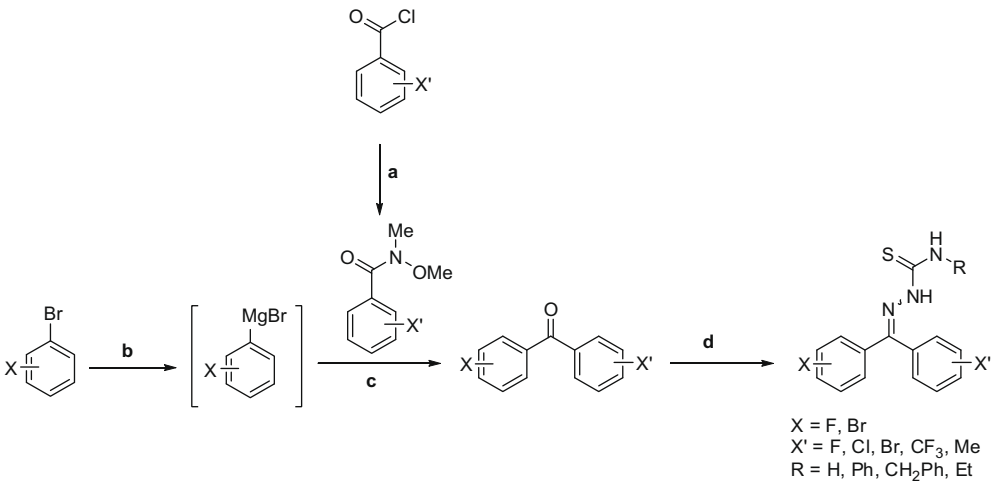


Figure 1. Representative peptidic and non-peptidic inhibitors of cathepsin L.

evaluate the efficacy of selected benzophenone thiosemicarbazone derivatives as reversible inhibitors of cathepsins L and B, a series of analogs was prepared by chemical synthesis as detailed in Scheme 1. Briefly, the appropriate acid chloride was converted to its corresponding Weinreb amide and then reacted with a Grignard reagent (formed from dibromobenzene) to yield the requisite ketone. A condensation reaction with thiosemicarbazide in the presence of *para*-toluenesulfonic acid resulted in formation of the desired thiosemicarbazone analogs.

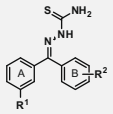
Most of the unsymmetrical benzophenone analogs gave two peaks (major and minor) in the HPLC trace consistent with either (i) formation of *E* and *Z* isomers[14d] around the carbon–nitrogen double bond or (ii) exchange of a bromine atom for hydrogen during the Grignard addition. For example, analog **1** showed two peaks in the HPLC trace suggesting the existence of *E*/*Z* isomers. However, LC–MS confirmed that the minor peak in the HPLC trace of analog **1** represents hydrogen replacing bromine. It is important to note that with other compounds distinct peaks in the HPLC trace correspond to both *E* and *Z* isomers as well as hydrogen replacing a bromine atom (see Tables 1 and 2 and Supplementary data).

In general, cysteine proteases are small monomeric proteins with an average mass of approximately 30 kDa. Structurally, the cathepsins consist of two domains (left and right) with a V-shaped active site cleft located along the domain interface.[22] This site contains two active residues, a Cys-25 located on the left domain and a His-159 located on the right domain which together, form a stable thiolate-imidazolium ion pair required for the enzyme's activity.[4] Molecular modeling of the most active analog **1** with cathepsin L showed that the conformation with the most favorable relative interaction energy places the bromophenyl ring deep in the S2 pocket with the thiosemicarbazone in close proximity to the active site Cys-25 (Fig. 2). The thiosemicarbazone is oriented at the active site by two hydrogen bonds between the



Scheme 1. Synthesis of benzophenone based thiosemicarbazone derivatives. Reagents and conditions: (a) HCl·NH(OMe)Me, Et_3N, CH_2Cl_2, rt, 3–4 h; (b) Mg, Et_2O, reflux, 1–2 h; (c) Et_2O, rt, 3–6 h; (d) $NH_2NHCSNHR$, *para*-toluenesulfonic acid, MeOH, reflux, 6–12 h.

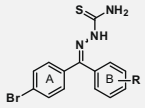
Table 1
Inhibition of human cathepsins L and B by substituted 3-bromobenzophenone thiosemicarbazone derivatives **1**–**23**



	R^1	R^2	Isomer ratio estimated by HPLC[a]	H replaced by Br (% determined by HPLC)	IC_{50} (nM) Cat L[b]	IC_{50} (nM) Cat B[b]
1	Br	2-F	ND[c]	9	30.5	>10,000
2	Br	2-Cl	ND	2.3	1610	>10,000
3	Br	2-Br	NA[d]	ND	2600	>10,000
4	Br	2-Me	ND	1.5	>10,000	>10,000
5	Br	3-F	ND	1.7	250	>10,000
6	Br	3-Cl	ND	1.2	131	>10,000
7	Br	3-CF_3	1:0.7	6	46.5	>10,000
8	Br	3-Me	1:0.4	8	224	>10,000
9	Br	4-F	1:0.9	1.3	79.6	>10,000
10	Br	4-Cl	ND	8.3	327	>10,000
11	Br	4-Br	ND	2.4	>10,000	>10,000
12	Br	4-CF_3	ND	2.2	521	>10,000
13	Br	4-Me	1:1	1.5	2160	5690
14	Br	2,3-F	1:0.1	ND	83.8	>10,000
15	Br	2,6-F	1:0.4	ND	610	>10,000
16	Br	3,5-F	1:0.5	1.1	59.4	>10,000
17	Br	3,5-Cl	1:0.8	1.4	415	>10,000
18	Br	3,5-CF_3	ND	2.2	96.0	1590
19	Br	3-Br,2-F	ND	10	233	>10,000
20	Br	3-Br,4-F	ND	7	114	>10,000
21	Br	3,4,5-F	1:0.5	4	118	>10,000
22	Br	2,3,4,5-F	ND	1.1	63.2	>10,000
23	H	2-F	NA	ND	>10,000	>10,000

[a] *E*/*Z* isomer not assigned.
[b] 2% DMSO.
[c] ND = not detected.
[d] NA = not applicable.

Table 2
Inhibition of human cathepsins L and B by substituted 4-bromobenzophenone thiosemicarbazone derivatives **24**–**30**



	R	Isomer ratio estimated by HPLC[a]	H replaced by Br (% determined by HPLC)	IC_{50} (nM) Cat L[b]	IC_{50} (nM) Cat B[b]
24	2-F	ND[c]	1.2	2220	>10,000
25	2-Br	ND	ND	>10,000	>10,000
26	4-F	1:0.8	2.1	3320	>10,000
27	4-Cl	ND	8	>10,000	>10,000
28	4-Br	ND	ND	>10,000	>10,000
29	4-CF_3	1:0.6	6	>10,000	>10,000
30	4-Me	1:0.6	1.9	4570	>10,000

[a] *E*/*Z* isomer not assigned.
[b] 2% DMSO.
[c] ND = not detected.

NH and NH_2 groups and the enzyme Asp-162 (Fig. 2). Details regarding the molecular modeling studies can be found in the Supplementary data.

All 36 thiosemicarbazone analogs were evaluated based on their ability to inhibit both cathepsin L and cathepsin B in separate assays (Tables 1–3). The most active inhibitors of cathepsin L all contain a 3-bromo functionality in one of the aryl rings (Table 1). Activity against cathepsin L decreases dramatically when the A-ring bromide is located at the 4-position (Table 2), as observed in a comparison of analog **1** (IC_{50} = 30.5 nM) with **24** (IC_{50} = 2220 nM). Within the 3-bromo A-ring series, as the functional group at position 2 in the B-ring is varied (F, Cl, Br, and Me) the activity decreases substantially. A fluorine substituent at positions 2 or 4 in the B-ring leads to compounds that are more active inhibitors of cathepsin L compared to analogs containing a fluorine substituent at the 3-position. Additional fluorine substituents in the B-ring tend to provide compounds that are potent inhibitors of cathepsin L (analogs **14**, **16** and **22**), although certain substituent patterns are less desirable in terms of cathepsin activity (analogs **15** and **21**). Analog **23** (nor-3-bromo, 2′-fluoro) is an important control compound verifying that the strong inhibitory activity of analog **1** (3-bromo, 2′-fluoro) against cathepsin L is due to this compound itself and not the inseparable by-product (H replacing Br).

In an effort to extend the binding of the inhibitors from the S2 to the S1′ pockets of the enzyme, three analogs were prepared, which are functionalized with phenyl, benzyl, and ethyl at the terminal nitrogen of the thiosemicarbazone moiety. Unfortunately, these analogs were not effective inhibitors of cathepsin L (Table 3).

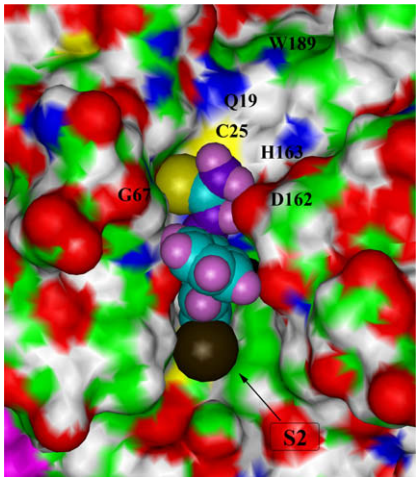


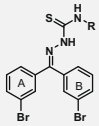
Figure 2. Analog **1** modeled at active site of cathepsin L [enzyme: oxygen (red), carbon (green), nitrogen (blue), hydrogen (white); analog **1**: carbon (cyan), nitrogen (purple), sulfur (yellow), hydrogen (lavender)].

Compared with analog **1**, the activity against cathepsin L decreases when the A-ring aryl bromide is replaced with aryl fluoride (Table 4).

With the exception of compounds **13** and **18**, none of the synthetic analogs showed appreciable activity against cathepsin B, thus demonstrating the selectivity of this series of analogs against cathepsin L. The best cathepsin L inhibitor in this new library, analog **1**, was also an effective inhibitor of DU-145 cell invasion and migration indicating that this compound may find application as a therapeutic agent against cancer metastasis. Since chronic dosing would likely be required of such an anti-metastatic treatment, it is important that such compounds demonstrate low cytotoxicity. Three of the analogs were tested against selected human cancer lines and found to exhibit low cytotoxicity (Table 5).[23]

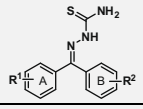
In summary, a small library of 36 functionalized thiosemicarbazone analogs was prepared by chemical synthesis and each compound was evaluated for its inhibitory activity against cathepsins L and B. The activity of six of these analogs (**1**, **7**, **9**, **14**, **16**, and **22**) against cathepsin L is especially noteworthy, and suggests that

Table 3
Inhibition of human cathepsins L and B by *N*-substituted 3,3′-dibromobenzophenone thiosemicarbazone derivatives **31**–**33**

	R	Isomer ratio estimated by HPLC[a]	H replaced by Br (% determined by HPLC)	IC_{50} (nM) Cat L[b]	IC_{50} (nM) Cat B[b]
31	Ph	NA	NA	>10,000	>10,000
32	CH_2Ph	NA	NA	>10,000	>10,000
33	CH_2CH_3	NA	NA	>10,000	>10,000

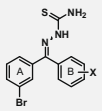
NA = Not applicable.
[a] *E*/*Z* isomer not assigned.
[b] 2% DMSO.

Table 4
Inhibition of human cathepsins L and B by difluoro-substituted benzophenone thiosemicarbazone derivatives **34**–**36**

	R^1	R^2	Isomer ratio estimated by HPLC[a]	H replaced by Br (% determined by HPLC)	IC_{50} (nM) Cat L[b]	IC_{50} (nM) Cat B[b]
34	2-F	4′-F	NA	NA	2460	>10,000
35	3-F	3′-F	NA	NA	4870	>10,000
36	4-F	4′-F	NA	NA	>10,000	>10,000

NA = not applicable.
[a] *E*/*Z* isomer not assigned.
[b] 2% DMSO.

Table 5
Cytotoxicity, invasion and migration for analogs **1**, **5,** and **6**

	X	Cytotoxicity GI_{50} (μM)			% Invasion DU-145[a]	% Migration DU-145[a]
		NCI-H460	DU-145	SK-OV-3		
1	2-F	23.5	13.8	ND	61.5 ± 3.5	40.5 ± 3.5
5	3-F	26.3	29.9	17.7	70.5 ± 7.8	43.2 ± 1.13
6	3-Cl	23.3	15.6	20.7	86.0 ± 1.4	41.9 ± 1.6

ND = not determined.
[a] Relative to normalized control.

these analogs are excellent candidates for future in vivo preclinical studies.

Acknowledgments

The authors are grateful to Oxigene Inc. (Waltham, MA, grants to M.L.T. and K.G.P.), The Welch Foundation (Grant AA-1278 to K.G.P.), and the Department of Chemistry and Biochemistry at Baylor University for generous financial support of this research, and the National Science Foundation for funding both the Varian 500 MHz NMR spectrometer (Award CHE-0420802) and the Bruker X8 APEX diffractometer (Grant CHE-0321214). The authors thank Dr. Alejandro Ramirez (Mass Spectrometry Core Facility, Baylor University) for HRMS analysis, and Dr. James Karban and Dr. Michelle Nemec (Director) for use of the shared Molecular Biosciences Center.

Supplementary data

Supplementary data (detailed experimental syntheses, ^{1}H NMR, ^{19}F NMR, HRMS, HPLC, full spectral characterization of analog **1**, and details regarding biological assays) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.090.

References and notes

1. Mohamed, M. M.; Sloane, B. F. *Nat. Rev. Cancer* **2006**, *6*, 764.
2. Turk, V.; Turk, B.; Turk, D. *EMBO J.* **2001**, *20*, 4629.
3. Im, E.; Venkatakrishnan, A.; Kazlauskas, A. *Mol. Biol. Cell* **2005**, *16*, 3488.
4. Falgueyret, J.-P.; Oballa, R. M.; Okamoto, O.; Wesolowski, G.; Aubin, Y.; Rydzewski, R. M.; Prasit, P.; Riendeau, D.; Rodan, S.; David Percival, M. *J. Med. Chem.* **2001**, *44*, 94.
5. Jedeszko, C.; Sloane, B. F. *Biol. Chem.* **2004**, *385*, 1017.
6. Lecaille, F.; Kaleta, J.; Bromme, D. *Chem. Rev.* **2002**, *102*, 4459.
7. Chandran, K.; Sullivan, N. J.; Felbor, U.; Whelan, S. P.; Cunningham, J. M. *Science* **2005**, *308*, 1643.
8. Simmons, G.; Gosalia, D. N.; Rennekamp, A. J.; Reeves, J. D.; Diamond, S. L.; Bates, P. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 11876.
9. Rafati, S.; Salmanian, A.-H.; Hashemi, K.; Schaff, C.; Belli, S.; Fasel, N. *Mol. Biochem. Parasitol.* **2001**, *113*, 35.
10. Fujii, N.; Mallari, J. P.; Hansell, J. E.; Mackey, H. Z.; Doyle, P.; Zhou, Y. M.; Gut, J.; Rosenthal, P. J.; McKerrow, J. H.; Guy, R. K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 121.
11. Janko, K.; Andreja, S.; Ales, P.; Valentina, Z. B.; Tomaz, L.; Boris, T.; Bernd, W.; Rastko, G. *Exp. Cell Res.* **2005**, *306*, 103.
12. Haeryoung, K.; Ji, K. R.; Yong, B. Y.; Yong-Tae, K.; Jin-Hyeok, H.; Sang, H. L.; Kwang, H. L.; Keung, Y. L. *Cancer Lett.* **2009**, *273*, 266.
13. Joyce, J. A.; Baruch, A.; Chehade, K.; Meyer-Morse, N.; Giraudo, E.; Tsai, F. Y. *Cancer Cell* **2004**, *5*, 443.
14. (a) Portions of this work have been reported in the following dissertation: Siles, R. E. Design, synthesis, and biological evaluation of new anti-cancer nitrogen-containing combrastatins and novel cysteine protease inhibitors for the treatment of chagas. Ph.D. Dissertation, Baylor University, Waco, TX, 2006.; (b) Du, X.; Guo, C.; Hansell, R.; Doyle, P. S.; Caffrey, C. R.; Holler, T. P.; McKerrow, J. H.; Cohen, F. E. *J. Med. Chem.* **2002**, *45*, 2695; (c) Greenbaum, D. C.; Mackey, Z.; Hansell, E.; Doyle, P.; Gut, J.; Caffrey, C. R.; Lehrman, J.; Rosenthal, P. J.; McKerrow, J. H.; Chibale, K. J. *J. Med. Chem.* **2004**, *47*, 3212; (d) Siles, R.; Chen, S.-E.; Zhou, M.; Pinney, K. G.; Trawick, M. L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4405; (e) Mallari, J. P.; Guiguemde, W. A.; Guy, R. K. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3546; (f) Trawick, M. L.; Chen, S.-E.; Arispe, W. M.; Siles, R. E.; Zhou, M., Pinney, K. G. American Society for Biochemistry and Molecular Biology (ASBMB), Experimental Biology Meeting, San Francisco, USA, 2006 (poster presentation).
15. (a) Portions of this work have been reported in the following dissertation: Arispe, W. M. Inhibitors of human cathepsin L and cruzain as therapeutic agents. Ph.D. Dissertation, Baylor University, Waco, TX, 2008.; (b) Kishore, G. D. K.; Lindsay, J. M.; Song, J.; Matthew, M.; Elizabeth, C.; Akash, D.; Grace, Y.; Conner, E.; Arispe, W. M.; Chavarria, G. E.; Charlton-Sevcik, A. K.; Strecker, T. E.; Chen, S.-E.; Trawick, M. L.; Pinney, K. G. 41st National Organic Symposium, Boulder, Colorado, USA, 2009 (poster presentation).; (c) Mallari, J. P.; Shelat, A.; Konsinki, A.; Caffrey, C. R.; Connelly, M.; Zhu, F.; McKerrow, J. H.; Guy, R. K. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2883.
16. (a) Myers, M. C.; Shah, P. P.; Diamond, S. L.; Huryn, D. M.; Smith, A. B., III *Bioorg. Med. Chem. Lett.* **2008**, *18*, 210; (b) Myers, M. C.; Shah, P. P.; Beavers, M. P.; Napper, A. D.; Diamond, S. L.; Smith, A. B., III; Huryn, D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3646.
17. (a) Yasuma, T.; Oi, S.; Choh, N.; Nomura, T.; Furuyama, N.; Nishimura, A.; Fujisawa, Y.; Sohda, T. *J. Med. Chem.* **1998**, *41*, 4301; (b) Sasaki, T.; Kishi, M.; Tanaka, T.; Higuchi, N.; Kominami, E.; Katunuma, N.; Murachi, T. *J. Enzyme Inhib.* **1990**, *3*, 195.
18. Towatari, T.; Tanaka, T.; Yoshikawa, D.; Katunuma, N. *J. Biochem.* **1978**, *84*, 659.
19. Asaad, N.; Bethel, P. A.; Coulson, M. D.; Dawson, J. E.; Ford, S. J.; Gerhardt, S.; Grist, M.; Hamlin, G. A.; James, M. J.; Jones, E. V.; Karoutchi, G. I.; Kenny, P. W.; Morley, A. D.; Oldham, K.; Rankine, N.; Ryan, D.; Wells, S. L.; Wood, L.; Augustin, M.; Krapp, S.; Simader, H.; Steinbacher, S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4280.
20. (a) Yadav, R.; Shinde, A. K.; Chouhan, B. S.; Giridhar, R.; Menard, R. *J. Enzyme Inhib. Med. Chem.* **2008**, *23*, 190; (b) Rydzewski, R. M.; Bryant, C.; Oballa, R.; Wesolowski, G.; Rodan, S. B.; Bass, K. E.; Wong, D. H. *Bioorg. Med. Chem.* **2002**, *10*, 3277; (c) Chowdhury, S. F.; Sivaraman, J.; Wang, J.; Devanathan, G.; Lachance, P.; Qi, H.; Ménard, R.; Lefebvre, J.; Konishi, Y.; Cygler, M.; Sulea, T.; Purisima, E. O. *J. Med. Chem.* **2002**, *45*, 5321.
21. Marquis, R. W.; James, I.; Zeng, J.; Lee Trout, R. E.; Thompson, S.; Rahman, A.; Yamashita, D. S.; Xie, R.; Ru, Y.; Gress, C. J.; Blake, S.; Lark, M. M.; Hwang, S.-M.; Tomaszek, T.; Offen, P.; Head, M. S.; Cummings, M. D.; Verber, D. F. *J. Med. Chem.* **2005**, *48*, 6870.
22. Vasilijeva, O.; Reinheckel, T.; Peters, C.; Turk, V.; Turk, B. *Curr. Pharm. Des.* **2007**, *13*, 387.
23. Ross, D. T.; Scherf, W.; Eisen, M. B.; Perou, C. M.; Rees, C.; Spellman, P.; Iyer, V.; Jeffrey, S. S.; Rijn, M. V.; Waltham, M.; Pregamenschikov, A.; Lee, J. C. F.; Lashkari, D.; Shalon, D.; Myer, T. G.; Weinstein, J. N.; Botstein, D.; Brown, P. O. *Nat. Genet.* **2004**, *24*, 227.