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Conversion of an MMP-potent scaffold to an MMP-selective HER-2 sheddase inhibitor via scaffold hybridization and subtle P_1' permutations

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Abstract—A series of β-sulfonamide piperidine hydroxamates were prepared and shown to be potent inhibitors of the human epidermal growth factor receptor-2 (HER-2) sheddase with excellent selectivity against MMP-1, −2, −3, and −9. This was achieved by exploiting subtle differences within the otherwise highly conserved S_1' binding pocket of the active sites within the metalloprotease family. In addition, it was discovered that the introduction of polarity to the P_1 and P_1' groups reduced the projected human clearance.
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The human epidermal growth factor receptor-2 (HER-2 or ErbB-2) is a tyrosine kinase receptor that is activated upon homo- and heterodimerization with another member of the HER family or by proteolytic cleavage (shedding) of the extracellular domain (ECD).[1] Once activated, intracellular signal transduction pathways are initiated that mediate a diverse range of essential cellular activities such as cell proliferation, differentiation, motility, adhesion, and survival.[2] Overexpression of the oncogene HER-2/*neu* has been associated with aggressive pathogenesis, poor prognosis, and decreased responsiveness to conventional chemotherapeutic and hormonal treatment regimes in non-small cell lung cancer, ovarian cancer, and breast cancer patients.[1a] In addition, elevated plasma levels of HER-2 ECD have been associated with increased metastatic potential and a decrease in disease-free and overall survival in patients with breast cancer.[1b,3] Therefore, inhibition of the protease responsible for HER-2 ECD shedding may be therapeutically desirable for treating cancer patients that overexpress HER-2, particularly breast cancer patients.

Recently we described the design, synthesis, evaluation, and identification of a novel class of (6*S*,7*S*)-*N*-hydroxy-6-carboxamide-5-azaspiro[2.5] octane-7-carboxamides as the first potent and selective inhibitors of HER-2 sheddase.[4] From this class of compounds INCB3619 (**1**) was identified to possess excellent pharmacodynamic and pharmacokinetic properties and was shown to decrease cleaved HER-2 ECD plasma levels, tumor size, and potentiate the effects of the humanized anti-HER-2 monoclonal antibody (trastuzumab) in vivo in a HER-2 overexpressing cancer murine xenograft model. In an effort to expand upon these results we wanted to design an alternative novel scaffold that was chemically more accessible from a synthetic vantage point. Thus, we desired to eliminate the spiro-cyclopropyl ring as well as the chirality associated with scaffold **1** (see Fig. 1). Becker et al. described the facile synthesis of β- and α-piperidine sulfone hydroxamic acids **2** from readily available cheap starting materials as inhibitors of MMP-2, −9, and −13.[5] Despite our desire to be selective *against* these MMPs, we hypothesized that hybridization of this scaffold **2** with our scaffold **1** to produce

Keywords: HER-2 sheddase; ADAM-10; Beta-sulfonamide piperidine hydroxamates; Metalloprotease; MMP; P1′ substituent; Projected clearnace; Scaffold hybridization.
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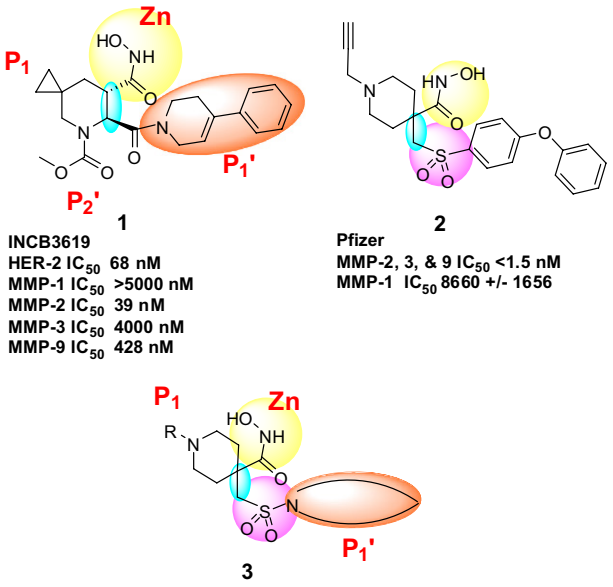


Figure 1. Hybridization of HER-2 and MMP scaffolds.

compounds of the general structure **3** may produce a viable novel scaffold that could be attenuated to selectively target the HER-2 sheddase (ADAM-10) by modifying the P_1' substituent. Thus, exploiting potential differences within the S_1' sub-site of the otherwise highly conserved active sites within the metalloprotease family.[4,6]

A series of tetrahydrofuran-3-yl 4-[(amino-sulfonyl)methyl]-4-[(hydroxyamino)carbonyl] piperidine-1-carboxylates were prepared and the inhibition of HER-2 sheddase was evaluated in both a cellular and an enzymatic assay, denoted as HER-2 and ADAM-10 in Tables 1 and 2, respectively. Selectivity was determined by comparison of the enzymatic binding to ADAM-10 to that of MMP-1, −2, −3, and −9.

It was found that simple 3-phenyl-2,5-dihydro-1*H*-pyrrol-1-yl **4** and 3-phenyl-pyrrolidin-1-yl **5** sulfonamides showed promising inhibition of HER-2 sheddase (Table 1). Expanding the heterocycle from a five-membered to a six-membered ring increased the potency by 5- to 50-fold. The dramatic increase in potency observed for compounds **6**, **7**, and **8** can be explained by examining the space occupied by the phenyl ring that is attached to the heterocycle. The six-membered heterocycles orient the phenyl ring in a linear manner in contrast to the five-membered heterocycles, which orient the phenyl ring askew approximately 60 °. Since the S_1' pocket of the HER-2 sheddase is believed to be narrow, a linear or tubular P_1' substituent would be preferred.[4] The high degree of selectivity of these three compounds against MMP-1, which has a shallow S_1' pocket, and overall lack of selectivity against MMP-2 and −9, which has a narrow and deep S_1' pocket, suggests that the S_1' pocket of the HER-2 sheddase might be similar to that of MMP-2 and −9, in agreement with our previous results.[4]

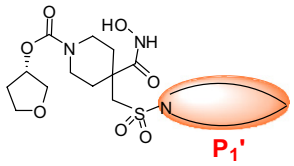
Based on our earlier work, we speculated that selectivity against MMP-2, -3, and -9 may be accomplished by exploiting the difference at residue 223 within the loop 3 region of the S_1' pocket, which is Tyr for MMP-2, -3, and -9 and Ala for ADAM-10.[4]

We postulated that the addition of a small substituent in the 2-position of the phenyl ring may be able to fill the vacancy left by the sterically less demanding Ala residue of ADAM-10 and have unfavorable steric interactions with the Tyr residue of MMP-2, −3, and −9. Therefore, a variety of substituents were placed in the 2-position of the phenyl ring of the 4-phenyl- piperidinyl sulfonamide analog **8**. Introduction of a 2- fluoro- substituent to produce **9** resulted in a 5-fold increase in selectivity against MMP-2 and a 2-fold gain in selectivity against MMP-3 and -9 in comparison to the parent phenyl analog **8**; albeit with a concomitant loss of potency. Exchanging the fluoro substituent of **9** with a cyano group resulted in a complete loss of potency for both ADAM-10 and MMP-2, which is in agreement with our hypothesis that the 2-substituent of the phenyl ring extends into a lipophilic region occupied by residue 223, that is, Ala or Tyr. Replacement of the small fluoro substituent of **9** with a slightly larger methyl group to afford **11** resulted in a 37-, 13-, and 139-fold increase in selectivity toward MMP-2, −3, and −9. Comparison of compound **11** to the parent phenyl compound **8** revealed an impressive 166-, 21-, and 278-fold gain in selectivity over MMP-2, −3, and −9, respectively. A minuscule increase in size of the 2-aryl substituent of **11** from the methyl to the trifluoromethyl group resulted in a significant loss in potency, indicating that there is limited space available within this region. Overall, these results insinuate that the 2-methyl group of compound **11** may be accommodating the space created by the Ala223 of ADAM-10 with optimal lipophilic interactions, while being impeded by the Tyr223 of MMP-2, -3, and -9 resulting in the desired selectivity toward these MMPs, as originally proposed.

In an effort to increase the cellular potency of compound **11** a variety of substituents were placed in the 4-position.[4] Introduction of a 4-fluoro substituent to **11** resulted in a 3-fold loss in HER-2 potency. Replacement of the 4-fluoro substituent of **13** with the polar *N*-dimethyl amide group resulted in complete loss of potency. Installation of a 4-cyano substituent to compound **11**, to afford analog **15**, increased the HER-2 cellular potency by 3-fold while maintaining the excellent selectivity against MMP-1, −2, −3, and −9. Removal of the 2- methyl substituent of **15** resulted in a compound that had a higher binding affinity for MMP-2 over ADAM-10, which demonstrates the importance of the 2-methyl substituent in achieving selectivity. The 3,5-dimethyl analogs **17** and **18** exemplify the importance of both the 2-methyl and the 4-cyano substituents with regard to both selectivity and potency.

The piperazine and tetrahydropiperidine analogs of the two lead compounds **11** and **15** were prepared and were shown to have inferior cellular and enzymatic potency in comparison to the piperidine leads. Comparison of the

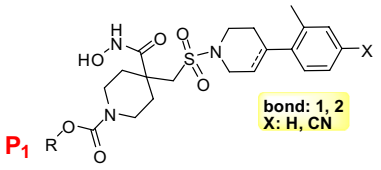
Table 1. In vitro data for P_1' substituents



Compound	P_1'	IC_{50} (nM)	Enzymatic binding IC_{50}[b] (nM)				
		HER-2[a]	ADAM-10	MMP-1	MMP-2	MMP-3	MMP-9
4	3-Ph-2,5-dihydro-1*H*-pyrrol-1-yl	102	332	—	52	—	—
5	3-Ph-pyrrolidin-1-yl	358	261	—	549	—	—
6	4-Ph-piperazin-1-yl	13	15	774	13	925	78
7	4-Ph-1,2,3,6-tetrahydropyridin-1yl	7.1	10	259	4	237	25
8	4-Ph-piperidin-1-yl	22	28	753	18	366	22
9	4-(2-F-Ph)-piperidin-1-yl	76	44	>5000	135	942	100
10	4-(2-CN-Ph)-piperidin-1-yl	>1000	>2000	—	>2000	—	—
11	4-(2-Me-Ph)-piperidin-1-yl	41	18	>5000	>2000	>5000	>5000
12	4-(2-CF_3-Ph)-piperidin-1-yl	>1000	1036	—	>2000	—	—
13	4-(2-Me-4-F-Ph)-piperidin-1-yl	141	92	—	>2000	—	—
14	4-(2-Me-4-C(O)NMe_2-Ph)-piperidin-1-yl	>1000	>2000	—	655	—	—
15	4-(2-Me-4-CN-Ph)-piperidin-1-yl	14	12	>5000	1999	>5000	>5000
16	4-(4-CN-Ph)-piperidin-1-yl	77	39	—	9	—	—
17	4-(3,5-di-Me-4-CN-Ph)-piperidin-1-yl	96	57	>5000	126	756	535
18	4-(3,5-di-Me-Ph)-piperidin-1-yl	833	286	>5000	315	2157	955
19	4-(2-Me-Ph)-piperazin-1-yl	217	107	—	>2000	—	—
20	4-(2-Me-Ph)-1,2,3,6-tetrahydropyridin-1yl	133	18	—	1999	—	—
21	4-(2-Me-4-CN-Ph)-piperazin-1-yl	25	26	>5000	>5000	>5000	>5000
22	4-(2-Me-4-CN-Ph)-1,2,3,6-tetrahydropyridin-1yl	45	107	—	1563	—	—

[a] BT-474 cellular proliferation assay, see Ref. 4.
[b] See Ref. 4.

Table 2. In vitro data for P_1 substituents



Compound	R	Bond	X	IC_{50} (nM)	Enzymatic binding IC_{50} (nM)[b]			Proj. h-Clr[c]
				HER-2[a]	ADAM-10	MMP-1	MMP-2	L/h/kg (%free)
23	3-Me-tetrahydrofuran-3-yl	1	H	36	11	>5000	564	1.1 (15%)
24	4,4-di-Me-tetrahydrofuran-3-yl	1	H	14	22	>5000	574	1.1 (10%)
25	*trans*-2-OH-cyclopent-1-yl	1	H	6.2	16	4999	537	1.0 (20%)
26	pyrrolidin-3-yl	1	H	16	18	>5000	546	<0.5 (>60%)
27	(2*S*)-pyrrolidin-2-ylmethyl	1	H	23	34	>5000	>2000	1.1 (10%)
28	(2*R*)-pyrrolidin-2-ylmethyl	1	H	30	19	>5000	>2000	0.7 (40%)
29	(2*S*)-pyrrolidin-2-ylmethyl	1	CN	6.5	9.1	>5000	1272	0.6 (50%)
30	(2*R*)-pyrrolidin-2-ylmethyl	1	CN	23	11	>5000	1380	<0.5 (>60%)
31	pyrrolidin-3-yl	1	CN	13	7.8	>5000	>2000	0.6 (50%)
32	(2*S*)-pyrrolidin-2-ylmethyl	2	CN	27	34	>5000	>2000	0.7 (40%)
33	(2*R*)-pyrrolidin-2-ylmethyl	2	CN	19	24	>5000	>2000	<0.5 (>60%)

[a] BT-474 cellular proliferation assay, see Ref. 4.
[b] See Ref. 4.
[c] See Ref. 7.

2-methyl analogs **11**, **19**, and **20** to their 2-methyl-4-cyano counterparts **15**, **21**, and **22** reveals the importance of the 4-cyano group in boosting the cellular inhibition of HER-2 sheddase. For the analog pairs **17/18** and **19/21** there was a pronounced 9-fold gain in HER-2 inhibition, which coincided with a 5- and 4-fold gain in ADAM-10 binding. In contrast, analog pairs **11/15** and **20/22** had a modest 3-fold gain in HER-2 inhibition

that was accompanied by a negligible change and a 6-fold *loss* in ADAM-10 binding. These results suggest that the observed gain in HER-2 cellular potency by the introduction of a 4-cyano substituent to the P_1' phenyl group is a net result of a positive permutation in physiochemical properties, such as an increase in polar surface area, taken in combination with either a positive or negative variance in the enzymatic binding affinity (ADAM-10).

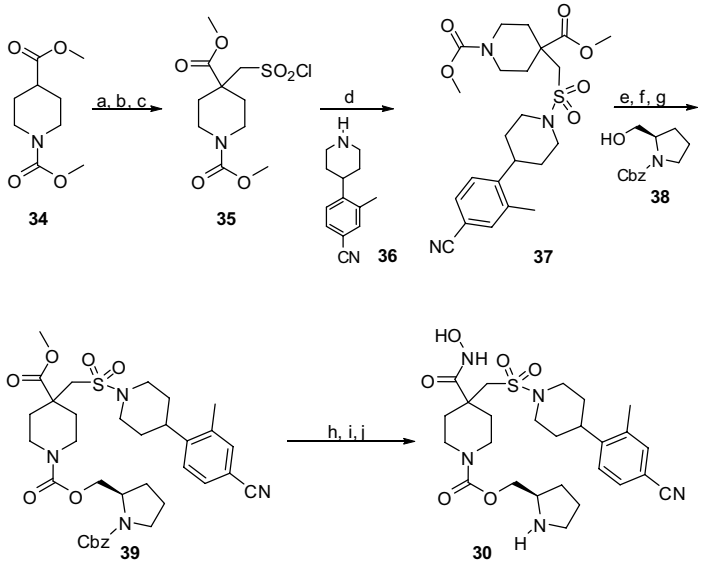
Despite the excellent potency and selectivity that compounds **11**, **15**, and **21** possessed, these compounds suffered from high projected human clearance (proj. h-Clr) and thus low projected human maximal bioavailability. Metabolic studies suggested that the carbamate moiety was being cleaved. It was hypothesized that this cleavage could be prevented by either sterically blocking the carbamate moiety from cleavage or by introducing a polar group to the P_1 substituent, which could reduce the binding affinity to cytochrome P450 and thus decrease the clearance. We speculated that modification of the P_1 group could be conducted with minimum disruption to the binding profile since it was believed that the P_1 group was primarily solvent exposed.

Attempts to sterically block the tetrahydrofuran carbamate of **11** by installation of a 3-methyl group **23** or a 4,4-dimethyl group **24** were both unsuccessful at lowering the proj. h-Clr (Table 2). Exchanging the tetrahydrofuran carbamate of **11** with the more polar *trans*-2-hydroxyl-cyclopent-1-yl carbamate **25** also did not improve the proj. h-Clr. Replacement of the tetrahydrofuran oxygen of **11** with nitrogen to form the corresponding pyrrolidine **26** resulted in a significant decrease in proj. h-Clr that may be attributed to a decrease in lipophilicity. Despite the encouraging observed decrease in proj. h-Clr the selectivity against MMP-2, while still good, diminished almost 4-fold in comparison to **11**. Switching the P_1 group to the pyrrolidin-2-ylmethyl carbamates **27** and **28** resulted in a minor improvement for only the (2*R*)-enantiomer **28**. Installation of the 4-cyano group to the P_1' phenyl ring of compounds **27** and **28** to afford analogs **29** and **30** ameliorated the proj. h-Clr while maintaining the excellent selectivity toward MMP-2. Installation of the 4-cyano group to the P_1' phenyl ring of compound **26** restored the selectivity against MMP-2 while maintaining the adequate proj. h-Clr. The corresponding 4-(2-methyl-4-cyano-phenyl)-1,2,3,6-tetrahydropyridin-1yl analogs **32** and **33** displayed comparable improvements in the proj. h-Clr in comparison to the (3*S*)-tetrahydrofuran-3-yl parent compound **22**. These results suggest that increasing the polarity at the P_1 position of the molecule by the introduction of a pyrrolidine N–H may reduce the affinity toward cytochrome P450. However, it is also reasonable that these observed improvements in Proj. h-Clr may be a manifestation of favorable changes in physiochemical properties resulting in a reduction in cytochrome P450 binding.

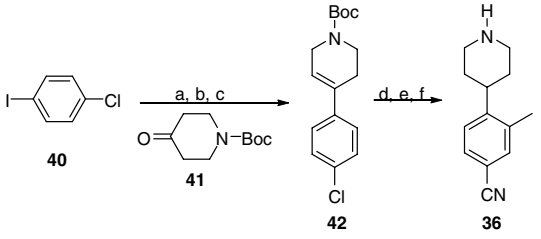
The syntheses of the analogs discussed herein are exemplified by the synthesis of compound **30** depicted in Schemes 1 and 2.[8] The enolate of dimethyl piperidine-1,4-dicarboxylate **34** was quenched with diiodomethane followed by S_{N^2} displacement of the primary iodide by potassium thioacetate. The thioacetate was oxidized to the corresponding sulfonyl chloride by treatment with H_2O_2 and acetic acid followed by reaction with sodium acetate to form the sodium sulfonate. Reaction with thionyl chloride afforded **35**. Alternatively, the sulfonyl chloride **35** can be prepared in one step by bubbling Cl_2 (g) into a solution of the thioacetate in methylene chloride and water. The sulfonamide **37** was easily prepared by treatment of **35** with the appropriate amine, such as **36**, in the presence of a base, such as DIEA. Removal of the methyl carbamate was achieved by reaction with iodotrimethylsilane in refluxing dichloromethane. The piperidine N–H was acylated with CDI or *p*-nitrophenyl chloroformate followed by reaction with the appropriate alcohol, such as **38**, in the presence of a strong base, such as NaH, to afford the desired carbamate **39**. Basic hydrolysis of the ester **39** followed by BOP reagent-mediated amide coupling afforded the hydroxamic acid. Deprotection of the Cbz group with H_2 and 5% $Pd/BaSO_4$ afforded the desired product **30**.

3-Methyl-4-piperidine-4-yl-benzonitrile **36** was prepared from 4-chloro-2-methyl-iodobenzene **40** by mono-lithium halogen exchange and subsequent reaction with Boc-piperid-4-one **41**. The tertiary alcohol is dehydrated with concomitant loss of the Boc group, which is susbequently reapplied. The tetrahydropiperidine double bond is reduced in the presence of the aryl chloride using either a homogeneous catalyst or 5% Pt/C under an atmosphere of H_2 (g). Cyanation of the aryl chloride was conducted using zinc cyanide and $Pd(PBu_3)_2$ and Zn as catalysts in NMP at 150 °C.[9] Removal of the Boc protecting group with 4 N HCl in 1,4-dioxane afforded the free amine **36**.

In conclusion, it has been demonstrated that the β-piperidine sulfonamide hydroxamic acid core can serve as a viable scaffold for the selective inhibition of the HER-2 sheddase, ADAM-10. Selectivity can be achieved by the installation of a small substituent in the *ortho*- position of the P_1' phenyl ring, particularly a methyl group. The cellular inhibition of HER-2 sheddase can be enhanced by the installation of a cyano group in the *para*- position. Subtle permutations to this substitution pattern or deviations from these substituents resulted in a cogent fluctuation in the enzymatic binding profile. Thus highlighting the high sensitivity within the S_1' pocket of the metalloprotease family. Conversely, the P_1 substituent can be modified to attenuate the pharmacokinetic properties of the molecule with only minor repercussions to the metalloprotease binding profile. It was found that the introduction of a polar –NH group to the P_1 carbamate substituent decreased the projected human clearance presumably by diminishing the affinity of the molecule to the active site of the cytochrome P450. In comparison to the azaspiro-hydroxamic acid INCB3619, the new lead compounds **29**–**31** exhibit a 3- to 10-fold gain in cellular inhibition of HER-2 sheddase with a 125- to 250-fold increase in selectivity against MMP-2 and a 35- to 125-fold increase



Scheme 1. Reagents and conditions: (a) (i) LDA, THF −78 °C; (ii) CH_2I_2 (99%); (b) KSAc, DMF (74%); (c) Cl_2, H_2O, DCM (92%); (d) DIEA, **36** (88%); (e) TMSI, DCM, reflux (100%); (f) CDI or *p*-NO_2-Ph-OC(O)Cl, DIEA, ACN (95%); (g) NaH, **38**, THF (90%); (h) LiOH, THF, MeOH, H_2O, rt 2d (99%); (i) BOP, NMM, NH_2OH, DMF (84%); (j) H_2, 5% $Pd/BaSO_4$ (100%).



Scheme 2. Reagents and conditions: (a) (i) *n*-BuLi, THF −78 °C (ii) **41** (68%); (b) TFA; (c) Boc_2O, DIEA, DCM (92%); (d) 5% Pt/C, H_2, MeOH (68%); (e) $ZnCN_2$, Zn, $Pd(Bu_3P)_2$, NMP 150 °C (76%); (f) 4 N HCl in 1,4-dioxane, EtOAc (100%).

in selectivity against MMP-9. Further pharmacodynamic and pharmacokinetic studies are impending to evaluate the potential leads **29**–**33**.

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References and notes

1. (a) Mass, R. D. *Int. J. Radiat. Oncol. Biol. Phys.* **2004**, *58*, 932; (b) Carney, W. P.; Neumann, R.; Lipton, A.; Leitzel, K.; Ali, S.; Price, C. P. *Clin. Chem.* **2003**, *49*, 1579; (c) Arribas, J.; Borroto, A. *Chem. Rev.* **2002**, *102*, 4627.
2. Marmor, M. D.; Skaria, K. B.; Yarden, Y. *Int. J. Radiat. Oncol. Biol. Phys.* **2004**, *58*, 903.
3. Molina, M. A.; Saez, R.; Ramsey, E. E.; Garcia-Barchino, M.-J.; Rojo, F.; Evans, A. J.; Albanell, J.; Keenan, E. J.; Lluch, A.; Garcia-Conde, J.; Baselga, J.; Clinton, G. M. *Clin. Cancer Res.* **2002**, *8*, 347.
4. Yao, W.; Zhou, J.; Burns, D. M.; Xu, M.; Zhang, C.; Li, Y-L.; Qian, D-Q.; He, C.; Weng, L.; Shi, E.; Lin, Q.; Agrios, C.; Burn, T. C.; Caulder, E.; Covington, M. B.; Fridman, J. S.; Friedman, S.; Katiyar, K.; Hollis, G.; Li, Y.; Liu, C.; Liu, X.; Marando, C. A.; Newton, R.; Pan, M.; Scherle, P.; Taylor, N.; Vaddi, K.; Wasserman, Z. R.; Wynn, R.; Yeleswaram, S.; Jalluri, R.; Bower, M.; Zhou, B-B.; Metcalf, B. *J. Med. Chem.* **2007**, *50*, 603.
5. Becker, D. P.; Villamil, C. I.; Barta, T. E.; Bedell, L. J.; Boehm, T. L.; DeCrescenzo, G. A.; Freskos, J. N.; Getman, D. P.; Hockerman, S.; Heintz, R.; Howard, S. C.; Li, M. H.; McDonald, J. J.; Carron, C. P.; Funckes-Shippy, C. L.; Mehta, P. P.; Munie, G. E.; Swearingen, C. A. *J. Med. Chem.* **2005**, *48*, 6713.
6. Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359.
7. Pooled human liver microsomes were purchased from XenoTech LLC. (Lenexa, KS, USA) and NADPH was purchased from Sigma–Aldrich (St. Louis, MO, USA). All other reagents and solvents used in this study were of the highest quality commercially available. A 1 μM solution of compound was preincubated for 3 min at 37 °C with 0.5 mg/mL of human liver microsome protein in 100 mM potassium phosphate buffer (pH 7.4). Incubations were initiated with the addition of NADPH (final concentration, 2 mM). Incubations were terminated at 0, 10, 20, and 30 min by the addition of two volumes of acetonitrile. Following sample centrifugation at 1500*g* for 10 min (Allegra 6 Centrifuge, Beckman Coulter, Inc., Fullerton, CA, USA) the supernatants were analyzed via LC/MS in positive ion ESI mode on a Thermo Finnigan LCQ Deca XP Plus.
8. Smith, I. H.; Alorati, A.; Frampton, G.; Jones, S.; O'Rourke, J.; Woods, M. W. *Org. Proc. Res. Dev.* **2003**, *7*, 1034; Burns, D. M.; Yao, W.; He, C. U.S. Patent 250,789, 2005.
9. Jin, F.; Confalone, P. N. *Tetrahedron Lett.* **2000**, *41*, 3721.