Bioorganic & Medicinal Chemistry Letters 23 (2013) 1622–1625

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Substituted piperidinyl glycinyl 2-cyano-4,5-methano pyrrolidines as potent and stable dipeptidyl peptidase IV inhibitors

Guohua Zhao *, Chet Kwon, Aiying Wang, James G. Robertson, Jovita Marcinkeviciene, Rex A. Parker, Mark S. Kirby, Lawrence G. Hamann

Bristol-Myers Squibb Research and Development, PO Box 5400, Princeton, NJ 08543-5400, USA

ARTICLE INFO

Article history:
Received 24 August 2012
Revised 18 January 2013
Accepted 22 January 2013
Available online 31 January 2013

Keywords:
Piperidine
Pyrrolidine
Dipeptidyl peptidase IV inhibitor
Type 2 diabetes

ABSTRACT

Synthesis and structure–activity relationship of a series of substituted piperidinyl glycine 2-cyano-4,5-methano pyrroline DPP-IV inhibitors are described. Improvement of the inhibitory activity and chemical stability of this series of compounds was respectively achieved by the introduction of bulky groups at the 4-position and 1-position of the piperidinyl glycine, leading to a series of potent and stable DPP-IV inhibitors.

Type 2 diabetes mellitus is a chronic disorder currently affecting approximately 366 million people worldwide of which the prevalence continues to rise every year.[1] Current primary treatments, originally discovered serendipitously, rely on insulin secretagogues and insulin sensitizers. Unfortunately, since these treatments are often associated with undesired side effects such as hypoglycemia, edema, and weight gain significant unmet medical need remains for novel effective drugs to treat the underlying disease.[2] The clinically validated target, serine exopeptidase dipeptidyl peptidase IV (DPP-IV, EC 3.4.14.5, CD), has received much attention for the treatment of type 2 diabetes.[3] DPP-IV is widely expressed on epithelial or endothelial cells of a variety of different tissues including kidney and intestinal brush border membranes.[4]

Type 2 diabetes also contributes to the pathophysiology of cardiovascular diseases (CVD). Emerging data support the idea that potentiating the levels of some DPP-IV natural substrates may be associated with cardio-protective effects. In the saxaglitpin clinical program, no increased risk of CV death, myocardial infarction, or stroke was seen in saxagliptin treatment groups across the entire program, supporting a hypothesis that saxagliptin may reduce CV events.[5] While the possible role DPP-IV inhibitors in CVD treatment will require additional clinical study, it is notable that the receptor for GLP-1 is expressed in cardiac tissue, and that GLP-1 is cardio-protective in some experimental ischemia/reperfusion models.[6] Other potential DPP-IV substrates relevant to CVD include stromal cell-derived factor-1 (SDF-1), which improves cardiac function and may reduce apoptosis of cardiomyocytes, and increases angiogenesis after experimental coronary artery occlusion.[7] Another is the chemokine Regulated on Activation Normal T-cell Expressed and Secreted (RANTES). Low plasma RANTES levels are associated with cardiac mortality in angiography patients (including diabetic men),[8] and genetic polymorphisms that cause lower RANTES levels are associated with increased CV mortality in diabetics and end stage renal disease patients.[9]

Incretins, such as glucagon-like peptide 1 (GLP-1) and gastric-inhibitory polypeptide (GIP), promote the secretion of insulin in response to nutrient ingestion. DPP-IV mediated cleavage of a N-terminal dipeptide from incretins with proline or alanine at the penultimate position abrogates this signaling. Therefore, inhibition of DPP-IV mediated incretin degradation represents a mechanistic approach for the treatment of type 2 diabetes. DPP-IV inhibitors have demonstrated robust antidiabetic efficacy in the clinic.[10] Both dipeptidic inhibitors vildagliptin[11] and saxagliptin,[12] and the non-peptidic sitagliptin[13] and linagliptin[14] have received FDA and/or EMEA approval for treatment of type 2 diabetes (Fig. 1). Other DPP-IV inhibitors including alogliptin[15] have advanced into late stages of clinical development.[16]

A variety of small-molecule inhibitors of DPP-IV have been discovered in the last two decades.[17] The majority of known inhibitors resemble the N-terminal dipeptide residues of the enzymatic substrates: a penultimate N-terminal proline mimic at the P1 position joined to an additional L-amino acid or similar surrogate at the P2 position. Many potent reversible DPP-IV inhibitors contain cyanopyrrolidine P1 group as proline mimics.[18] It is generally believed

* Corresponding author. Tel.: +1 609 818 5553.
E-mail addresses: guohua.zhao@bms.com, zhaog@bms.com (G. Zhao).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.01.104

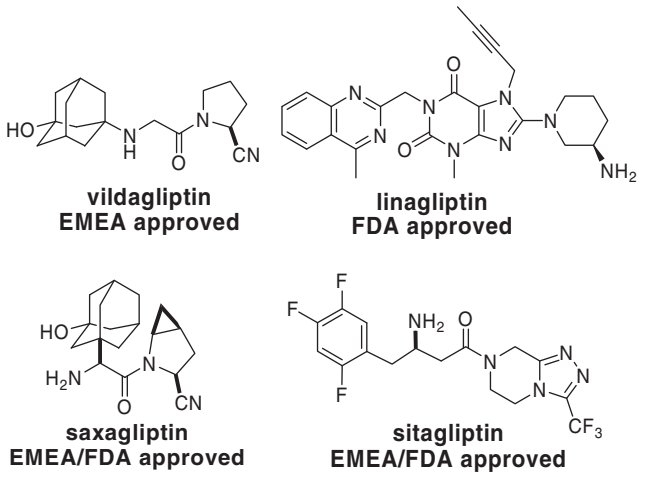


Figure 1. Approved DPP-IV inhibitors as type 2 diabetes medications.

that covalent binding of this nitrile group with the catalytically active site Ser630 hydroxyl contributed to the potency of these DPP-IV inhibitors (Fig. 2). However, the nitrile group was also responsible for chemical instability noted for the early nitrile-based inhibitors, whereby the free amine intramolecularly cyclized with the nitrile, forming inactive cyclic imidates and/or diketopiperizines after hydrolysis. This issue has been largely solved by further structure modifications employing various combinations of sterically hindered P1 and P2 residues, use of conformationally constrained amide bond mimics, and removal or replacement of the nitrile group, to avoid or block the cyclization reaction. Although these modifications significantly attenuated the potency of natural amino acid-based inhibitors, the potency was restored upon incorporation of an optimized unnatural amino acid at the P2 position.

In contrast to the strict steric limitation of the P1 position, a wide range of functionalities are tolerated at the P2 position. The preferred β-branching at this position not only improved chemical stability but also enhanced potency. Moreover, the X-ray crystallographic finding of the deep S2 pocket in the enzyme suggested that binding activity should be increased if a large elongated P2 substituent completely filled this pocket.[19] However, this goal was not realized by a large number of small molecule inhibitors that introduced steric bulk at the β-position.[17,18] Likewise an attempt to completely fill the S2 pocket by designing a flexible linearly extended P2 substituent failed due to the propensity of the assemblage to adopt a folded conformation.[20]

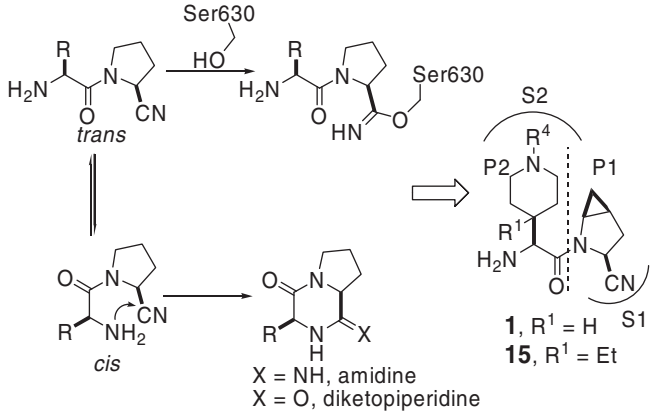


Figure 2. Potency and instability of cyanopyrrolidine dipeptidic inhibitors and opportunities for optimization.

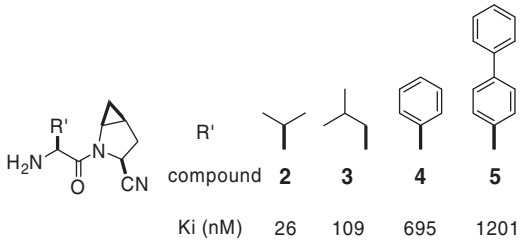


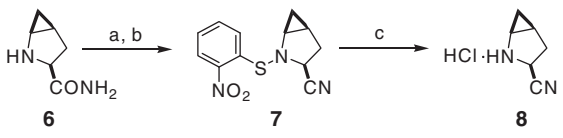
Figure 3. Some early 2-cyano-4,5-methano pyrrolidine DPP-IV inhibitors with a variety of P2 moieties.

Our prior investigation of acylated 2-cyano-4,5-methano pyrrolidines had revealed two important constraints regarding the P2 position: (1) the fourfold greater potency obtained following condensation with L-valine (**2**) rather than L-leucine (**3**) suggested the P2 site preferentially accommodated sterically less demanding substituents at the opening (Fig. 3); (2) the even greater 25- to 50-fold reduction in potency upon incorporation of L-phenylglycine (**4**) or its biphenyl analog (**5**) relative to L-valine (**2**) suggested that a nonplanar constrained elongated substituent would be preferable. Accordingly we envisioned that merging the valine and phenylglycine moieties to generate a 4-piperdinyl glycine moiety (**1**) would provide a constrained spacer amenable for attachment of progressive larger substituents that might fully occupy the deep S2 pocket. Our strategy involved sequential evaluation of the effect of substituents at the 1-position and 4-position of the piperdine to enhance inhibitory activity and improve chemical stability prior to identification of potent and chemically stable inhibitors produced by optimal additive effects from both positions.

Herein we describe the synthesis and structure-activity relationship of a series of DPP-IV inhibitors by conjugation of substituted piperidinyl glycines with 2-cyano-4,5-methano pyrrolidine. 2-Cyano-4,5-methano pyrrolidine **8** was prepared from amide **6**[21] using Ashworth's procedure[22] (Scheme 1). Protection of the proline nitrogen with 2-nitro-phenylsulfenyl group followed by dehydration with $POCl_3$ and imidazole gave nitrile **7**. Subsequent acidic deprotection afforded 2-cyano-4,5-methano pyrrolidine **8** in excellent yield.

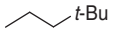
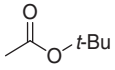
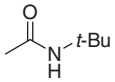
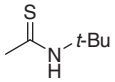
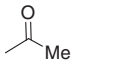
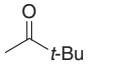
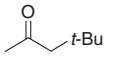
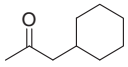
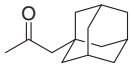
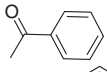
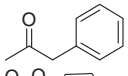
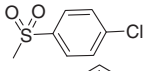
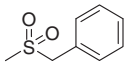
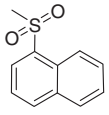
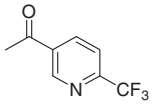
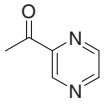
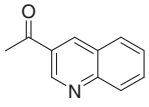
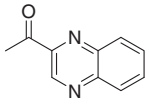
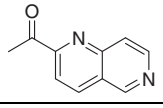
The 4-piperidinyl glycine derivatives **1a**–**1t** (Table 1) were prepared as described in Scheme 2. Coupling of commercially available Fmoc-N-4-Boc piperidinyl glycine **9** with 2-cyano-4,5-methano pyrrolidine **8** followed by de-protection of the Boc group gave key intermediate **10**. Introduction of different functional groups by respective reductive amination, acylation, thioacylation, or sulfonylation and subsequent removal of the Fmoc protecting group provided compounds **1b**–**1t**, whereas direct Fmoc group deprotection of intermediate **10** afforded compound **1a** in good yield.

The synthesis of compounds **15a**–**15d** utilized the Claisen rearrangement to introduce the β quaternary center (Scheme 3).[12] Beginning with commercially available ester **11**, DIBAL-mediated reduction followed by coupling with Cbz-glycine and subsequent Claisen rearrangement generated the vinyl intermediate acid **12** in good yield. Coupling of acid **12** with compound **8** yielded the key intermediate amide **13**. Amide **13** was separated by chiral



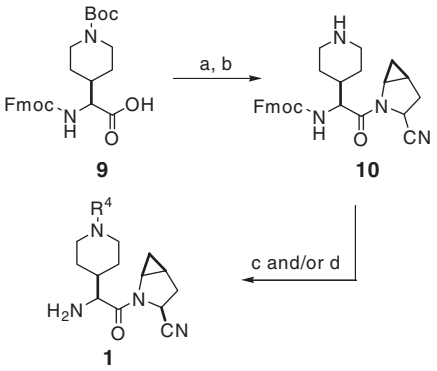
Scheme 1. Reagents and conditions: (a) 2-nitrophenylsulfenyl chloride, Et_3N, CH_2Cl_2 (64%); (b) $POCl_3$, imidazole, pyridine (94%); (c) 4 N HCl, dioxane, Et_2O (91%).

Table 1
DPP IV inhibitory activity for compound **1**

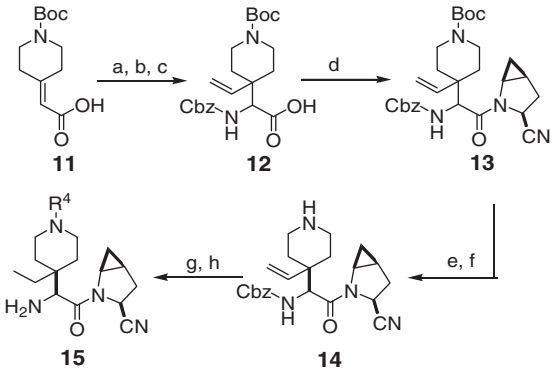
Compound	$-R^4$	DPP-IV K_i[a] (nM)
1a	–H	98 ± 7
1b		75 ± 5
1c		25 ± 1
1d		23 ± 2
1e		18 ± 1
1f		14 ± 1
1g		18 ± 3
1h		4 ± 0.2
1i		4 ± 1
1j		18 ± 1
1k		14 ± 2
1l		18 ± 2
1m		11 ± 1
1n		18 ± 2
1o		13 ± 2
1p		30 ± 2
1q		8 ± 0.4
1r		5 ± 0.1
1s		4 ± 1
1t		2 ± 0.5

[a] All K_i values are the mean ± s.d. of at least triplicate determinations.

HPLC to give the desired *S*-enantiomer that was subjected to Boc deprotection to give amine **14**.[12,21] The introduction of different functional groups at the 4-position as described above followed by simultaneous Cbz deprotection and vinyl reduction under standard condition (H_2, Pd/C) provided **15a**–**15d** in good yield.

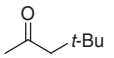
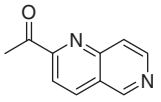
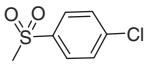
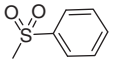


Scheme 2. Reagents and conditions: (a) compound **8**, PyBOP, DIEA, CH_2Cl_2 (78%); (b) 4 N HCl, dioxane (quantitative); (c) couple R^4: aldehyde, $NaBH(OAc)_3$, AcOH, CH_2Cl_2 or PyBOP, DIEA, CH_2Cl_2 or isocyanate, Et_3N, CH_2Cl_2 or thioisocyanate, Et_3N, CH_2Cl_2 or sulfonylchloride, DIEA, CH_2Cl_2; (d) piperidine, CH_2Cl_2.



Scheme 3. Reagents and conditions: (a) DIBAL, CH_2Cl_2, −40 °C to 0 °C (68%); (b) Cbz-Gly, DCC, DMAP, CH_2Cl_2 (91%); (c) $ZnCl_2$, LDA, THF, −78 °C to 0 °C (71%); (d) compound **8**, PyBOP, DIEA, CH_2Cl_2 (80%); (e) AD chiral HPLC separation; (f) 4 N HCl, dioxane (quantitative); (g) couple R^4: PyBOP, DIEA, CH_2Cl_2 or sulfonylchloride, Et_3N, CH_2Cl_2; (h) H_2, 10% Pd/C, MeOH.

Table 2
DPP IV inhibitory activity for compound **15**

Compound	$-R^4$	DPP-IV K_i[a] (nM)
15a		35 ± 1
15b		38 ± 0.4
15c		15 ± 2
15d		10 ± 4

[a] All K_i values are the mean ± s.d. of at least triplicate determinations.

All compounds were tested in vitro against purified human DPP-IV under steady state conditions with gly-pro-*p*-nitroanilide as substrate as previously described.[21]

Our SAR study was guided by our prior findings that hydrophobic and/or aromatic substituents are preferred at the P2 position of diprolyl DPP-IV inhibitors.[23] Initially, we sought to extend a lipophilic bulky *t*-butyl group into the S2 pocket by employing a variety of two atom spacers to attach the *t*-butyl group to the piperdine nitrogen while keeping the 1-position of the piperidine

Table 3
Solution stability of the selected compounds at pH 7.2 and 37 °C

		Degradation half lives $t_{1/2}$ (days)		
Concenteration (mM)		10	30	50
Compound	**1h**	2.0	1.6	1.6
	15a	19.7	10.2	8.0

ring unsubstituted (Table 1). The near equivalent potency of **1a** (K_i = 98 nM) and **1b** suggested that the flexible pendent *t*-butyl group did not interact strongly with the S2 pocket. However, the ~fourfold increase in potency upon conversion of the tertiary amine **1b** to a carbamate **1c**, urea **1d**, or thiourea **1e** suggested that the acyl moiety acting as a hydrogen bond acceptor engaged in a favorable interaction with some residue in the S2 pocket. This conclusion is underscored by the near equivalence in potency for the acetamide **1f** and pivalamide **1g**. The ~20-fold increase with the amide **1h** and **1i** may suggest that due to the conformational mobility arising from the intervening methylene the t-butyl group of **1h** or cyclohexyl of **1i** can engage in additional favorable non-polar interactions with the pocket which is not available to the sterically more demanding adamantyl analog **1j** or the more rigid aryl amides **1k** and **1l**. The twofold loss in potency of **1p** relative to **1k** may reflect attenuation in H-bonding acceptor capability due to the electron deficient pyridinyl ring. Sulfonamides **1m**–**1o** can also serve as H-bonding acceptors thereby accounting for comparable potency with benzamide **1k**. The enhanced potency of the nitrogen containing heterocyclic amides **1q**–**1t** relative to benzamide **1k** may be due to additional H-bonding of S2 residues with the ring nitrogens or favorable dipolar interactions with the heteroaryl rings.

The representative examples with β-quaternary center (**15**, R^4 = Et) are listed in Table 2. Although it has been previously demonstrated that introduction of a β-quaternary center confers stability and potency to closely related analogues,[12,21] an ethyl substituent at the 1-position of the piperidine ring attenuated potency. Analogues **15a** and **15b** with substituents *t*-butylacetyl and 1-(1,6-naphthyridin-2-yl)-carbonyl exhibited a 9- and 19-fold loss of potency respectively to their unsubstitued counterpart **1h** and **1t**. This reduction of potency may be in part due to the conformational change of the α,β-carbon bond from predominantly equatorial to partially axial by the introduction of the ethyl group, thus altering the spatial orientation of the 4-position substituents. Interestingly, only the aryl sulfonamides (**15c** and **15d**) maintained comparable potency with the non-β-quaternary series, presumably due to the flexible sulfonyl spacer enabling the 4-position substituents to better interact with the distal S2 pocket than rigid coplanar acyl spacer. Of these, analogue **15d** was the most potent compound prepared in the β-quaternary 4-substituted piperidinyl glycine series, exhibiting a K_i value of 10 nM.

After the discovery that 4-substituted piperidinyl glycinyl 2-cyano-4,5-methano pyrrolidines were potent DPP-IV inhibitors, the aqueous solution stability was compared for selected examples from both the non- and β-quaternary series. At pH 7.2 the β-ethyl analogue **15a** at concentration of 10, 30, 50 mM exhibited a half-life which was consistently 5- to 10-fold longer than the 1.6–2 day half-life measured for the non-β-quaternary analogue **1h** (Table 3) reflecting the expected enhanced chemical stability due to the sterically encumbered β-quaternary center.

In conclusion, systematic variation of the angle and size of moieties projecting into the large S2 binding pocket identified a series of compounds as potent and stable DPP-IV Inhibitors. Significant potency increases against DPP-IV enzyme were achieved with the introduction of sterically bulky substituents at the 4-position of piperidinylglycinyl-4,5-methanoprolinenitriles by employing a variety of spacers as H-bonding acceptors. Substituents such as the sterically less demanding alkyl and nitrogen-containing heteroaryl groups confer potency in the single-digit nM range, presumably by engaging in an additional favorable non-polar interactions and H-bonding or dipolar interactions with the S2 pocket.

Acknowledgment

We thank Dr. William N. Washburn for his suggestions during the manuscript preparation.

References and notes

1. IDF Diabetes Atlas 5th Ed., Nov 14, 2011 http://www.idf.org/diabetesatlas/news.
2. (a) Campbell, R. K. *J. Am. Pharm. Assoc. (2003)* **2009**, *49*, S3; (b) Sebokova, E.; Christ, A. D.; Boehringer, M.; Mizrahi, J. *Curr. Top. Med. Chem.* **2007**, *7*, 547; (c) McIntosh, C. H. S.; Demuth, H.-U.; Pospisilik, J. A.; Pederson, R. *Regul. Pept.* **2005**, *128*, 159.
3. (a) Ahrén, B. *Best Pract. Res. Clin. Endocrinol. Metab.* **2009**, *23*, 487; (b) Doupis, J.; Veves, A. *Adv. Ther.* **2008**, *25*, 627; (c) Langley, A. K.; Suffoletta, T. J.; Jennings, H. R. *Pharmacotherapy* **2007**, *27*, 1163; (d) Vilsboll, T.; Knop, F. K. *Br. J. Diab Vasc. Dis.* **2007**, *7*, 69; (e) Green, B. D.; Flatt, P. R.; Bailey, C. J. *Exp. Opin. Emerg. Drugs* **2006**, *11*, 525.
4. (a) Macnair, D. C.; Kenny, A. J. *Biochem. J.* **1979**, *1791*, 379; (b) Svensson, B.; Danielsen, M.; Staun, M.; Jeppesen, L.; Noren, O.; Sjostrom, H. *Eur. J. Biochem.* **1978**, *90*, 489.
5. Frederich, R.; Alexander, J. H.; Fiedorek, F. T.; Donovan, M.; Berglind, N.; Harris, S.; Chen, R.; Wolf, R.; Mahaffey, K. W. *Postgrad. Med.* **2010**, *122*, 16.
6. Ban, K.; Noyan-Ashraf, M. H.; Hoefer, J.; Bolz, S. S.; Drucker, D. J.; Husain, M. *Circulation* **2008**, *117*, 2340.
7. Saxena, A.; Fish, J. E.; White, M. D.; Yu, S.; Smyth, J. W.; Shaw, R. M.; DiMaio, J. M.; Srivastava, D. *Circulation* **2008**, *117*, 2224.
8. Cavusoglu, E.; Eng, C.; Chopra, V.; Clark, L. T.; Pinsky, D. J.; Marmur, J. D. *Arterioscler. Thromb. Vasc. Biol.* **2007**, *27*, 929.
9. Böger, C. A.; Fischereder, M.; Deinzer, M.; Aslanidis, C.; Schmitz, G.; Stubanus, M.; Banas, B.; Krüger, B.; Riegger, G. A.; Krämer, B. K. *Atherosclerosis* **2005**, *183*, 121.
10. (a) Banerjee, M.; Younis, N.; Soran, H. *Expert Opin. Pharmacother.* **2009**, *10*, 2745; (b) Deacon, C. F.; Holst, J. J. *Adv. Ther.* **2009**, *26*, 488; (c) Deacon, C. F. *Curr. Opin. Investig. Drugs* **2008**, *9*, 402; (d) Thornberry, N. A.; Weber, A. E. *Curr. Top. Med. Chem.* **2007**, *7*, 557.
11. Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Burkey, B. F.; Dunning, B. E.; Prasad, K.; Mangold, B. L.; Russell, M. E.; Hughes, T. E. *J. Med. Chem.* **2003**, *46*, 2774.
12. Augeri, D. J.; Robl, J. A.; Betebenner, D. A.; Magnin, D. R.; Khanna, A.; Robertson, J. G.; Simpkins, L. M.; Taunk, P. C.; Huang, Q.; Han, S.-P.; Abboa-Offei, B.; Wang, A.; Cap, M.; Xin, L.; Tao, L.; Tozzo, E.; Welzel, G. E.; Egan, D. M.; Marcinkeviciene, J.; Chang, S. Y.; Biller, S. A.; Kirby, M. S.; Parker, R. A.; Hamann, L. G. *J. Med. Chem.* **2005**, *48*, 5025.
13. Kim, D.; Wang, L.; Beconi, M.; Eiermann, G. J.; Fisher, M. H.; He, H.; Hickey, G. J.; Kowalchik, J. E.; Leiting, B.; Lyons, K. A.; Marsilio, F.; McCann, M. E.; Patel, R. A.; Petrov, A.; Scapin, G.; Patel, S. B.; Roy, R. S.; Wu, J. K.; Wyvratt, M. J.; Zhang, B. B.; Zhu, L.; Thornberry, N. A.; Weber, A. E. *J. Med. Chem.* **2005**, *48*, 141.
14. Scott, L. J. *Drugs* **2011**, *71*, 611.
15. Feng, J.; Zhang, Z.; Wallace, M. B.; Stafford, J. A.; Kaldor, S. W.; Kassel, D. B.; Navre, M.; Shi, L.; Skene, R. J.; Asakawa, T.; Takeuchi, K.; Xu, R.; Webb, D. R.; Gwaltney, S. L. *J. Med. Chem.* **2007**, *50*, 2297.
16. Gupta, R.; Walunj, S. S.; Tokala, R. K.; Parsa, K. V. L.; Singh, S. K.; Pal, M. *Curr. Drug Targets* **2009**, *10*, 71.
17. (a) Havale, S. H.; Pal, M. *Bioorg. Med. Chem.* **2009**, *17*, 1783; (b) Weber, A. E. *J. Med. Chem.* **2004**, *47*, 4135.
18. Peters, J.-U. *Curr. Top. Med. Chem.* **2007**, *7*, 579.
19. Rasmussen, H. B.; Branner, S.; Wiberg, F. C.; Nicolai, Wagtmann *Nat. Struct. Biol.* **2003**, *10*, 19.
20. Sakashita, H.; Akahoshi, F.; Yoshida, T.; Kitalima, H.; Hayashi, Y.; Ishii, S.; Takashina, Y.; Tsutsumiuchi, R.; Ono, S. *Bioorg. Med. Chem. Lett.* **2007**, *6*, 641.
21. Magnin, D. R.; Robl, J. A.; Sulsky, R. B.; Augeri, D. J.; Huang, Y.; Simpkins, L. M.; Taunk, P. C.; Betebenner, D. A.; Robertson, J. G.; Abboa-Offei, B.; Wang, A.; Cap, M.; Xin, L.; Tao, L.; Sitkoff, D. F.; Malley, M. F.; Gougoutas, J. Z.; Khanna, A.; Huang, Q.; Han, S.-P.; Parker, R. A.; Hamann, L. G. *J. Med. Chem.* **2004**, *47*, 2587.
22. Ashworth, D.; Atrash, B.; Baker, G. R.; Baxter, A. J.; Jenkins, P. D.; Jones, D. M.; Szelke, M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1163.
23. Zhao, G.; Taunk, P. C.; Magnin, D. R.; Simpkins, L. M.; Robl, J. A.; Wang, A.; Robertson, J. G.; Marcinkeviciene, J.; Sitkoff, D. F.; Parker, R. A.; Kirby, M. S.; Hamann, L. G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3992.