Bioorganic & Medicinal Chemistry Letters 18 (2008) 4477–4481

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of dihydroquinoxalinone acetamides containing bicyclic amines as potent Bradykinin B1 receptor antagonists

Jian Jeffrey Chen [a,*], Wenyuan Qian [a], Kaustav Biswas [a], Vellarkad N. Viswanadhan [b], Benny C. Askew [d], Stephen Hitchcock [a], Randall W. Hungate [a], Leyla Arik [c], Eileen Johnson [c]

[a] Chemistry Research and Development, Amgen Inc., One Amgen Center Drive, MS-B29-4-1-B, Thousand Oaks, CA 91320, USA
[b] Molecular Structure and Design, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, USA
[c] Neuroscience, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, USA
[d] Department of Medicinal Chemistry, Serono Research Institute, Rockland, MA, USA

ARTICLE INFO

Article history:
Received 26 June 2008
Revised 9 July 2008
Accepted 14 July 2008
Available online 17 July 2008

Keywords:
Dihydroquinoxalinone acetamides
Bradykinin B1
Receptor antagonist
Pain
Inflammation
Sulfonamides

ABSTRACT

Replacement of the core β-amino acid in our previously reported piperidine acetic acid and β-phenylalanine-based Bradykinin B1 antagonists by dihydroquinoxalinone acetic acid increases the in vitro potency and metabolic stability. The most potent compounds from this series have IC_{50}s < 0.2 nM in a human B1 receptor functional assay. A molecular modeling study of the binding modes of key compounds, based on a B1 homology model, explains the structure–activity relationship (SAR) for these analogs.

Kinins are released at sites of tissue injury inducing pain and inflammation via activation of two G protein-coupled receptors termed Bradykinin B2 and B1. The B2 subtype is expressed in most cell types. On the other hand, the B1 receptor is absent or expressed at very low levels under normal conditions and is induced during tissue injury and inflammation. It has been implicated in the humoral, cellular, and neuronal mechanisms underlying chronic inflammatory pain.[1,2] Peripherally restricted B1 peptide antagonists are efficacious in reversing neurogenic pain induced by capsaicin as well as inflammatory pain induced by UV irradiation, carrageenan, CFA or LPS. B1 receptor knockout mice exhibit hypoalgesia to chemical and thermal noxious stimuli. They show attenuated inflammatory responses and reduced neutrophil accumulation following tissue injury. Therefore, B1 receptor antagonists are potentially useful in the treatment of chronic pain and inflammation.[3–5]

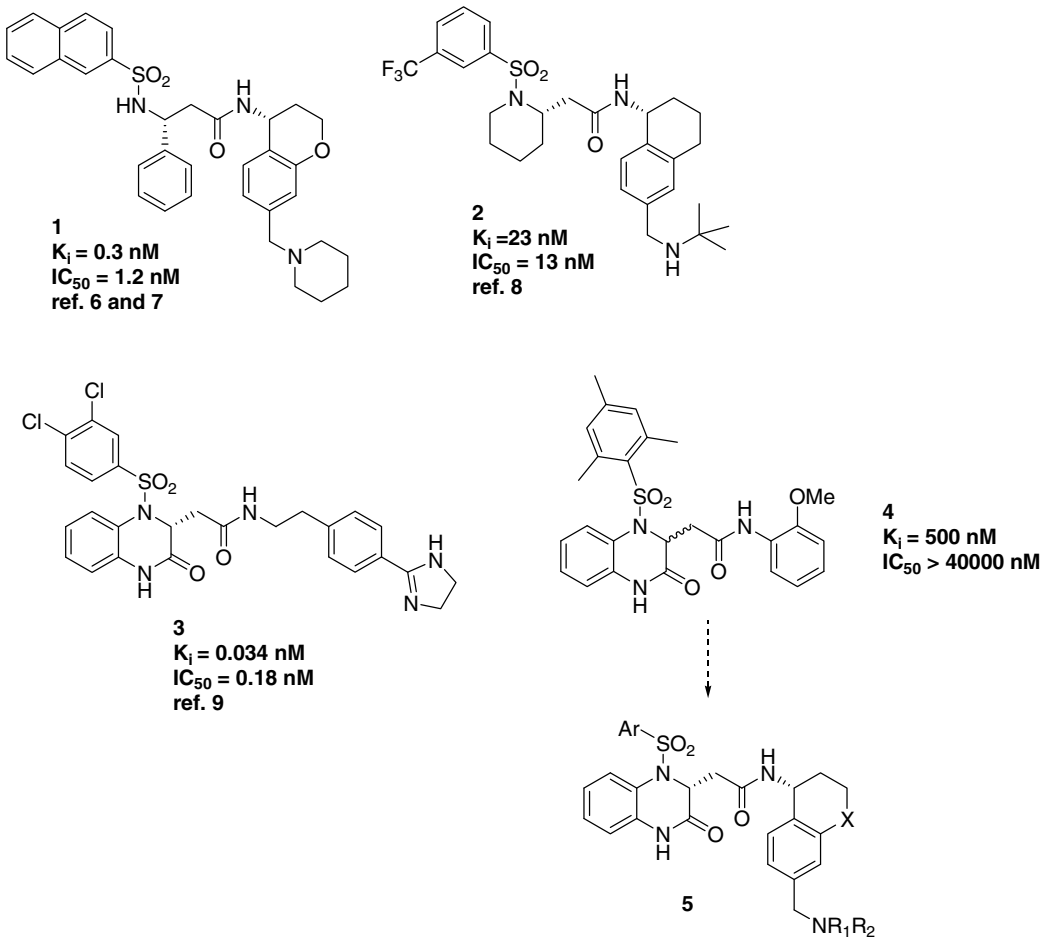
We recently disclosed a series of sulfonylated β-amino acid derivatives containing chiral chroman and tetralin diamine moieties, such as **1**[6,7] and **2**,[8] respectively, as novel B1 antagonists. These compounds were generated from conformational restriction of acyclic amine-based initial hits to improve in vitro potency. These first generation leads served as excellent proof of concept molecules that showed in vitro and in vivo efficacy in B1 antagonism assays. Subsequent studies focused on further improving the in vitro functional potency, the metabolic stability, and reducing the human plasma protein binding.

Recently, Su and co-workers reported that dihydroquinoxalinone-based compounds such as **3** and its analogs are highly potent B1 antagonists.[9] Molecular modeling studies of **3** and its analogs,[9–12] using a B1 homology model, revealed putative binding modes of these compounds. These models were supported by data from site-directed mutagenesis of B1 receptor.[11]

Through high throughput screening we identified a related compound, 2-(1-(mesitylsulfonyl)-3-oxo-1,2,3,4-tetrahydroquinoxalin-2-yl)-*N*-(2-methoxyphenyl)acetamide **4**, as a weak B1 antagonist in the receptor binding assay (K_i = 500 nM). Structurally these compounds are benzo-fused β-amino acid derivatives. It would be interesting to investigate whether the replacement of the right-hand amine in **4** with the chiral bicyclic amines from **1 to 2** might result in enhanced potency. Our efforts led to a number of highly potent (IC_{50}s < 0.2 nM) metabolically more stable compounds. Furthermore, they are less protein bound than previous compounds. Herein, we report the detailed synthesis, SAR, and molecular modeling studies of this new class of B1 antagonists.

* Corresponding author.
E-mail address: jianc@amgen.com (J.J. Chen).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.07.055



Scheme 1 outlines the general synthetic routes for the dihydroquinoxalinone acetamides **5**. The dihydroquinoxalinone acetate was prepared according to modified literature.[13] 2-Fluoronitrobenzene **6** was condensed with commercially available (D)-aspartic acid dimethyl ester **7** to give the SN_{Ar} adduct. Hydrogenation of the nitro group followed by intramolecular cyclization gave methyl dihydroquinoxalinone acetate **8**. Sulfonylation of **8** with different aryl sulfonyl chlorides in neat pyridine yielded the sulfonamides **9**. Under normal basic ester hydrolysis conditions, the sulfonamides in **9** are prone to elimination and racemization. Acid-catalyzed hydrolysis avoided these side reactions and gave the desired acids **10**, although the reaction was often slow and did not go to completion. The crude acids were used directly in the next coupling step without any trouble.

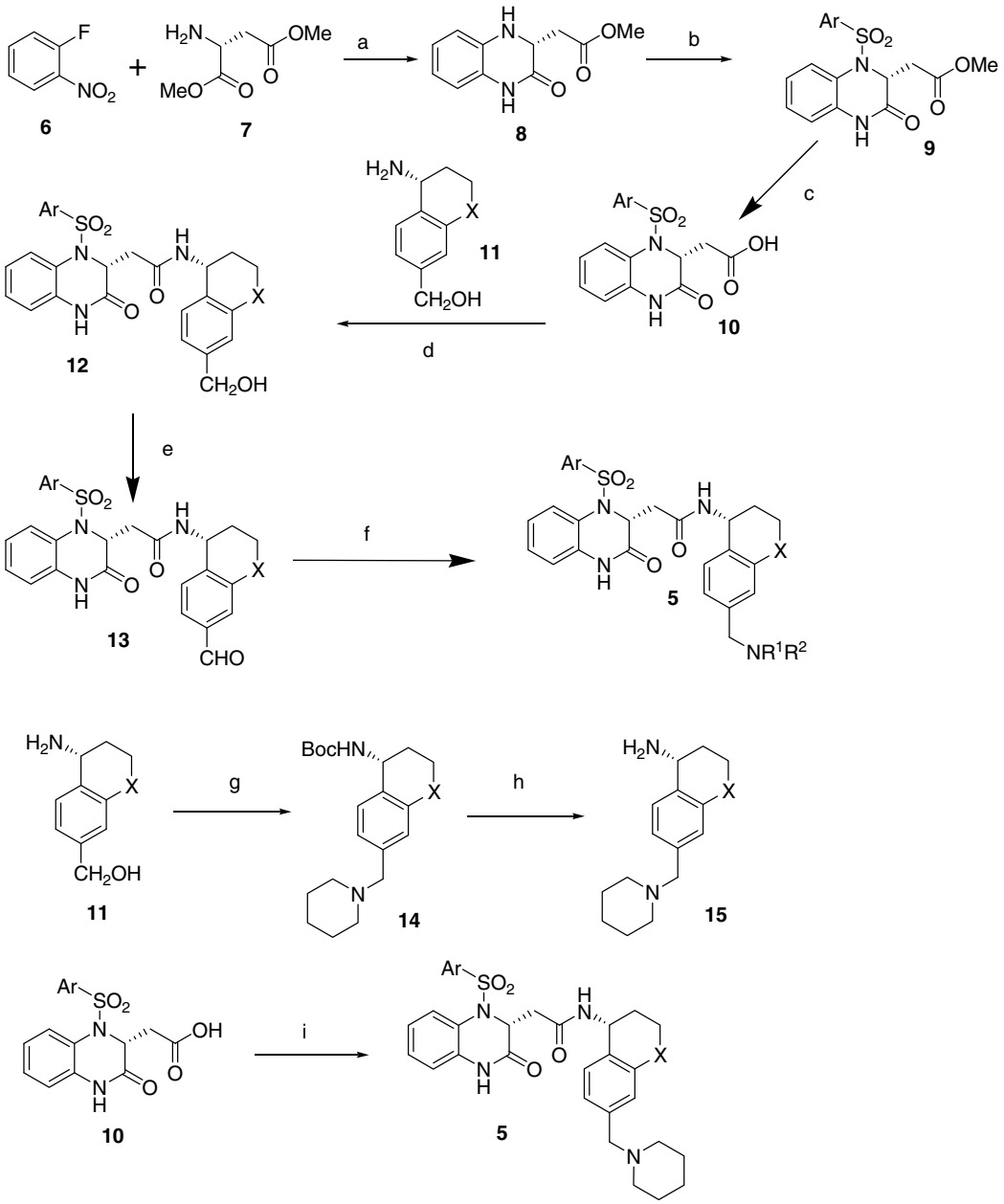
The amino bicyclic chroman and tetralin alcohol **11**[6–8] were coupled with the acid **10** to give the amide **12**. The oxidation of the benzylic alcohol **12** with MnO_2 yielded the aldehyde **13**. Reductive amination of **13** with a variety of primary and secondary amines gave the desired product **5**. The piperidine derivatives **5g–h** and **5j–k** were prepared from the coupling of the acid **10** and bicyclic piperidine **15**.

The in vitro binding (K_i) and functional (IC_{50}) assay data with the new antagonists are shown in Table 1. Earlier work in the β-phenylalanine series such as **1** indicated that the basic amino group is required for potency.[7] Interestingly, the intermediate alcohol **12** and aldehyde **13** were moderately active in both binding and functional assays, with **13** (IC_{50} = 23 nM) about 3-fold more potent than **12** (IC_{50} = 62 nM). These results suggest that dihydroquinoxalinone acetamides could provide superior potency over the β-phenylalanine core.

Encouraged by these findings, we investigated the effect of introducing various amino groups. The primary amine **5a** had an IC_{50} = 1.2 nM and is about 52-fold more potent than the corresponding alcohol **12** in the functional assay. Interestingly, the introduction of a single NH_2 group not only improves the potency dramatically but also has the beneficial effect of increasing the metabolic stability (e.g., compare HLM value of 264 vs 127 μL/min/mg for alcohol **12** and amine **5a**). Mono-methylation of NH_2 improves the binding affinity slightly but has minimal effect on functional potency (compare **5a** and **5b**, Table 1). Further methylation of the amino group to give **5c** did not yield any significant improvement in potency. The metabolic stability was, however, compromised.

Replacement of the NHMe of **5b** with a more lipophilic amine moiety such as those in **5d–g** improved the binding potency only slightly. For example, the piperidine analog **5g** is only 8-fold more potent than **5b** (compare **5b**, **5d–g**, Table 1). Interestingly, in the β-phenylalanine series such as **1**, the potency increases with lipophilicity on the right-hand side amino group. For example, replacement of NHMe by piperidine improves the binding affinity by 460-fold.[7] In order to gain better understanding of the interactions between these antagonists and the receptor, we carried out the molecular modeling of these compounds using a B1 homology model.

The recently proposed homology models of the B1 receptor by Ha and co-workers,[10,11] based on site-directed mutagenesis experiments and SAR data, were employed to assess the binding modes of compounds discussed. Conformational analyses of several compounds reported were performed, each of which followed a procedure described recently.[14] As an example, the molecular modeling



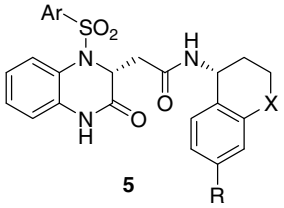
Scheme 1. Reagents and conditions: (a) 1—*i*Pr$_2$NEt, DMSO, 60–70 °C, 60–70%; 2—Pd/C, HCO_2NH_4, EtOAc/EtOH, 88%; (b) $ArSO_2Cl$, pyridine, 32–76%; (c) 10% HCl, MeOH, dioxane (1/1/1), reflux; (d) EDCI, HOBt, **11**, DMF, 52%; (e) MnO_2, CH_2Cl_2, 100%; (f) 1—R^1R^2NH, HOAc, $CHCl_3$, then $NaBH_4$, MeOH, 40–70% (for **5b**, **5d–f**, **5i**); or 2—Me_2NH, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, 64% (for **5c**); or 3—NH_4OAc, $Ti(OiPr)_4$, $ClCH_2CH_2Cl$/DMF, then $NaBH_4$, MeOH, 59% (for **5a**); (g) 1—Boc_2O, NEt_3, THF; 2—MnO_2, CH_2Cl_2; 3—piperidine, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$; (h) 1.4 N HCl in dioxane and then NaOH, 40–81% yield from **11** to **15**; (i) EDCI, HOBt, 15, DMF, 23–40%. *Note:* In this scheme, X = O or CH_2.

study of **1** is described here, which was carried out using molecular dynamics/mechanics. A conformational search for the small molecule was carried out using a genetic algorithm. Simulations were conducted using the program, FLAME,[14] which employs the MMFF forcefield.[15] Only low energy conformations lacking intramolecular H-bonding with the basic nitrogen (consistent with the earlier proposed binding modes of **3** and its analogs) were considered as putative binding modes and the 10 lowest energy conformations were considered for docking, visualization and optimization in the kinin binding pocket of the B1 homology model. These low energy conformations are also consistent with a conformational analysis of representative model structures reported recently.[6] These low energy conformations included the two different puckers of the chroman or tetralin on the right-hand side and different conformations of the aliphatic amine side chain on the right-hand side.

Energy minimization of the B1 homology model with each of the starting structures was performed using the AMBER forcefield[16] for proteins and the GAFF forcefield[17] for small molecules, to optimize the complex and estimate MMGB binding energies.[18] A mild positional restraint was placed on the ligand during the minimization/dynamics run. Protein residues within 2.0 Å of any atom of the docked ligand (including hydrogens) were treated as flexible, along with other residues in the close proximity of the ligand. These include Ile97, Trp98, Ile113, Asn114, Ile117, Lys118, Tyr266, His267, Glu273, Gln295, Ile290, Asn298, and Phe302. Site-directed mutagenesis studies of several kinins and small molecule B1 antagonists[9,10,12] showed that most of these residues are involved in the interactions with ligands.

Figure 1a and b shows the putative binding modes for **1** and **5i**. These models suggest that the phenyl group of the β-phenylalanine

Table 1
In vitro data for the B1 antagonists



5

Compound #	Ar	X	R	Binding K_i ± SEM[a] (nM)	Functional IC_{50} ± SEM[a] (nM)	HLM[b] (μL/min/mg)
12	3,4-Cl_2Ph	O	CH_2OH	86 ± 43	62 ± 15	264
13	3,4-Cl_2Ph	O	CHO	57 ± 18	23 ± 4	407
5a	3,4-Cl_2Ph	O	CH_2NH_2	2.6 ± 0.6	1.2 ± 0.2	127
5b	3,4-Cl_2Ph	O	CH_2NHMe	1.2 ± 0.2	0.8 ± 0.2	107
5c	3,4-Cl_2Ph	O	CH_2NMe_2	1.7 ± 1	1.0 ± 0.3	208
5d	3,4-Cl_2Ph	O	CH_2NH*i*Pr	0.7 ± 0.3	0.86 ± 0.2	156
5e	3,4-Cl_2Ph	O	CH_2NH*i*Bu	0.44 ± 0.16	1.4 ± 0.2	264
5f	3,4-Cl_2Ph	O	CH_2NH*t*Bu	1.1 ± 0.6	1.7 ± 0.3	174
5g	3,4-Cl_2Ph	O	CH_2piperidine	0.15 ± 0.02	0.48 ± 0.09	389
5h	3,4-Cl_2Ph	CH_2	CH_2piperidine	0.10 ± 0.01	0.55 ± 0.08	417
5i	Ph	O	CH_2NH*t*Bu	1.8 ± 0.8	0.37 ± 0.12	26
5j	Ph	O	CH_2piperidine	0.19 ± 0.05	0.12 ± 0.1	76
5k	4-MePh	O	CH_2piperidine	0.24 ± 0.01	0.14 ± 0.2	128

[a] The average of two or more independent experiments with duplicates at each concentration. For assays see Refs. 6–8.
[b] The experiments are performed with two replicates for each compound. The percent difference between replicates must be less than or equal to 20% in order for the result to be considered valid. The average value of the two replicates is then reported as the percent turnover.

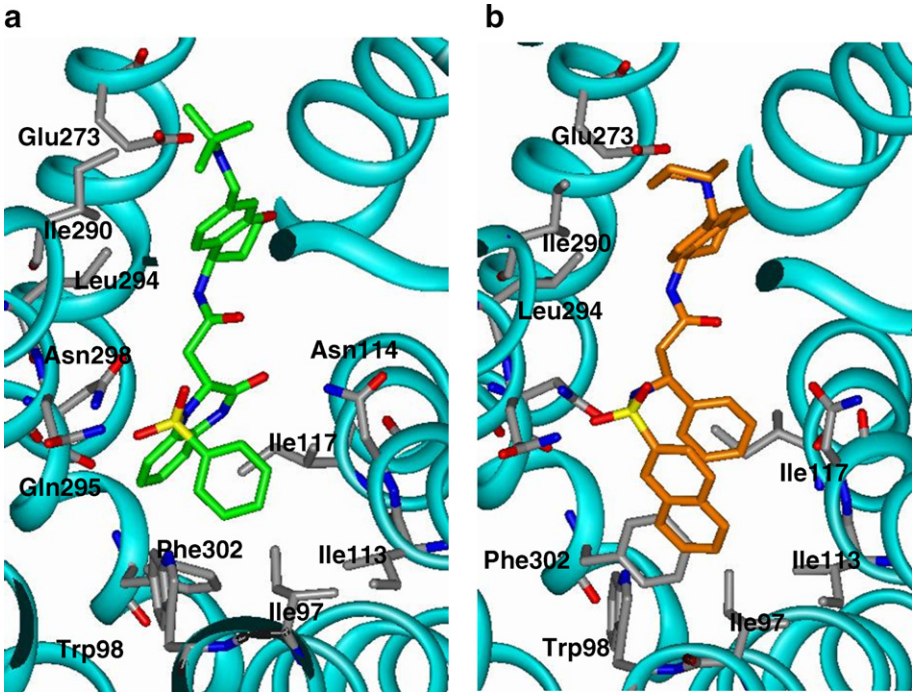


Figure 1. Predicted binding modes of **5i** (a) and **1** (b) in the putative binding site of the B1 receptor. For the protein, the carbons are colored gray. For the ligand **5i**, the carbons are colored green and for **1**, they are colored orange. The protein ribbon is shown in cyan color. Protein residues interacting with the ligand are identified.

series or the fused aryl ring of dihydroquinoxalinones interact with Phe302 and Ile117, whereas the aryl group of aryl sulfonamide interacts with Trp98, Ile97, and Ile113. The $-SO_2$ oxygens are seen to interact with Asn298 and Gln278 side chain amides. In the case of **5i**, an additional interaction of the endocyclic amide group of dihydroquinoxalinone ring with Asn114 is also observed in the model, leading to enhanced in vitro potency for these compounds and making them less sensitive to the changes in the other portion of the molecule (e.g., lipophilicity of the aliphatic amino group). Not surprisingly, these interactions are similar to those proposed in the models by Ha and co-workers[10,11] for **3** and its analogs.

Consistent with the observation that the basic amino group in the amide portion increases the in vitro potency, modeling shows that the amino group in **1** and **5i** interacts with the Glu273 acid

functionality. Furthermore, the alkyl part of the amino group can form hydrophobic contacts with Ile290 and the aliphatic part of Glu273 side chain.

Similar to the observation made in the case of 1,[6] replacement of the chroman unit by a tetralin has minimal effect on the in vitro potency (compare **5g–h**, Table 1).

In the piperidine acetic acid series such as **2**, the lipophilic sulfonamides are required for potency. For example, substitution of 3-chloro-4-methyl-phenylsulfonamide with simple unsubtituted phenyl sulfonamide decreases both binding and functional potency by 100-fold.[8] Initially 3,4-dichlorophenyl sulfonamide was also used in the current series to maximize potency. Since compounds in this series are at least 10-fold more potent than the corresponding analogs in the piperidine acetic acid series, it is reasonable to expect that the in vitro potency in the current series would be less sensitive to the lipophilicity change of the sulfonyl group. The simple phenyl sulfonamides **5i–j** are indeed highly potent, both in binding and in functional assays. For example, the *tert*-butyl amine **5i** is basically equally potent with the corresponding 3,4-dichlorophenyl sulfonamide analog **5f** in the binding assay, but it is 3-fold more potent than **5f** in functional assays. Likewise the piperidine **5j** has binding affinity similar to **5g** but its functional potency improves by 2-fold compared to **5g**. These results suggested that a reduction of the lipophilicity of the substituted phenyl sulfonamide has beneficial effect on functional potency in the current series. Furthermore, due to the reduced lipophilicity, the metabolic stability improved significantly. Compounds **5j** represents one of the most functionally potent and metabolically stable B1 antagonists. As expected, switching from phenylsulfonamide to *p*-toluenesulfonamide has miminal effect on the binding and functional potency (compare **5j** and **5k**).

The additional binding interactions provided by the endocyclic amide in the case of **5i** gave us more flexibility in optimizing the other portion of the molecule for pharmacokinetic properties while still maintaining good overall potency. For example, we were able to reduce the lipophilicity of both sulfonamide and terminal amine portions to improve metabolic stability such as in **5j–k**. The presence of the ring amide bond in the dihydroquinoxalinone moiety also decreases the protein binding, which could be an important factor in determining the in vivo efficacy. For example, **5f** is 79.8% human serum protein bound versus 94.1% for the corresponding β-phenylalanine analog.

In summary, replacement of the β-amino acid in **1–2** by dihydroquinoxalinone acetic acid afforded compounds with much improved potency, metabolic stability, and reduced protein binding. We have identified a number of highly potent and selective B1 antagonists that will serve as the basis for further studies that will be reported in due course.

References and notes

1. Calixto, J. B.; Medeiros, R.; Fernandes, E. S.; Ferreira, J.; Cabrini, D. A.; Campos, M. M. *Br. J. Pharmacol.* **2004**, *143*, 803.
2. Marceau, F.; Regoli, D. *Nat. Rev.* **2004**, *3*, 845.
3. Campos, M. M.; Leal, P. C.; Yunes, R. A.; Calixto, J. B. *Trends Pharmacol. Sci.* **2006**, *27*, 646.
4. Chen, J. J.; Johnson, E. J. *Expert Opin. Ther. Targets* **2007**, *11*, 21.
5. Kuduk, S. D.; DiMarco, C. N.; Chang, R. K.; Wood, M. R.; Schirripa, K. M.; Kim, J. J.; Wai, J. M. C.; DiPardo, R. M.; Murphy, K. L.; Ransom, R. W.; Harrell, C. M.; Reiss, D. R.; Holahan, M. A.; Cook, J.; Hess, J. F.; Sain, N.; Urban, M. O.; Tang, C.; Prueksaritanont, T.; Pettibone, D. J.; Bock, M. G. *J. Med. Chem.* **2007**, *50*, 272.
6. D'Amico, D. C.; Aya, T.; Human, J.; Fotsch, C.; Chen, J. J.; Biswas, K.; Riahi, B.; Norman, M. H.; Willoughby, C. A.; Hungate, R.; Reider, P. J.; Biddlecome, G.; Lester-Zeiner, D.; VanStaden, C.; Johnson, E.; Kamassah, A.; Arik, L.; Wang, J.; Viswanadhan, V. N.; Groneberg, R. D.; Zhan, J.; Suzuki, H.; Toro, A.; Mareska, D. A.; Clarke, D. E.; Harvey, D. M.; Burgess, L. E.; Laird, E. R.; Askew, B.; Ng, G. *J. Med. Chem.* **2007**, *50*, 607.
7. Biswas, K.; Li, A.; Chen, J. J.; D'Amico, D. C.; Fotsch, C.; Han, N.; Human, J.; Liu, Q.; Norman, M. H.; Riahi, B.; Yuan, C.; Suzuki, H.; Mareska, D. A.; Zhan, J.; Clarke, D. E.; Toro, A.; Groneberg, R. D.; Burgess, L. E.; Lester-Zeiner, D.; Biddlecome, G.; Manning, B. H.; Arik, L.; Dong, H.; Huang, M.; Kamassah, A.; Loeloff, R.; Sun, H.; Hsieh, F. Y.; Kumar, G.; Ng, G. Y.; Hungate, R. W.; Askew, B. C.; Johnson, E. *J. Med. Chem.* **2007**, *50*, 2200.
8. Fotsch, C.; Biddlecome, G.; Biswas, K.; Chen Jian, J.; D'Amico Derin, C.; Groneberg Robert, D.; Han Nianhe, B.; Hsieh, F.-Y.; Kamassah, A.; Kumar, G.; Lester-Zeiner, D.; Liu, Q.; Mareska David, A.; Riahi Babak, B.; Wang Yueh-Ju, J.; Yang, K.; Zhan, J.; Zhu, J.; Johnson, E.; Ng, G.; Askew Benny, C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2071.
9. Su, D.-S.; Markowitz, M. K.; DiPardo, R. M.; Murphy, K. L.; Harrell, C. M.; O'Malley, S. S.; Ransom, R. W.; Chang, R. S. L.; Ha, S.; Hess, F. J.; Pettibone, D. J.; Mason, G. S.; Boyce, S.; Freidinger, R. M.; Bock, M. G.*J. Am. Chem. Soc.* **2003**, *125*, 7516.
10. Ha, S. N.; Hey, P. J.; Ransom, R. W.; Bock, M. G.; Su, D.-S.; Murphy, K. L.; Chang, R.; Chen, T.-B.; Pettibone, D.; Hess, J. F. *Biochemistry* **2006**, *45*, 14355.
11. Ha, S. N.; Hey, P. J.; Ransom, R. W.; Harrell, C. M.; Murphy, K. L.; Chang, R.; Chen, T.-B.; Su, D.-S.; Markowitz, M. K.; Bock, M. G.; Freidinger, R. M.; Hess, F. J. *Biochem. Biophys. Res. Commun.* **2005**, *331*, 159.
12. Kuduk, S. D.; Di Marco, C. N.; Chang, R. K.; Wood, M. R.; Kim, J. J.; Schirripa, K. M.; Murphy, K. L.; Ransom, R. W.; Tang, C.; Torrent, M.; Ha, S.; Prueksaritanont, T.; Pettibone, D. J.; Bock, M. G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2791.
13. Su, D.-S.; Markowitz, M. K.; Murphy, K. L.; Wan, B.-L.; Zrada, M. M.; Harrell, C. M.; O'Malley, S. S.; Hess, J. F.; Ransom, R. W.; Chang, R. S.; Wallace, M. A.; Raab, C. E.; Dean, D. C.; Pettibone, D. J.; Freidinger, R. M.; Bock, M. G. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 6045.
14. Cho, S.-J.; Sun, Y. *J. Chem. Info. Model.* **2006**, *46*, 298.
15. Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490.
16. Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 5179.
17. Wang, J.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A. . *J. Comput. Chem.* **2004**, *25*, 1157.
18. Lee, M.; Sun, Y. *J. Chem. Theor. Comput.* **2007**, *3*, 1106.