Bioorganic & Medicinal Chemistry Letters 19 (2009) 119–122

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Sulfonamido-aryl ethers as bradykinin B1 receptor antagonists

Andrew G. Cole [a,*], Axel Metzger [a], Marc-Raleigh Brescia [a], Lan-Ying Qin [a], Kenneth C. Appell [a], Christopher T. Brain [b], Allan Hallett [c], Pam Ganju [c], Alastair A. Denholm [d], James R. Wareing [b], Timothy J. Ritchie [c], Gillian M. Drake [c], Stuart J. Bevan [c], Aisling MacGloinn [c], Andrew McBryde [c], Viral Patel [c], Paul J. Oakley [c], Ximena Nunez [c], Hubert Gstach [e], Peter Schneider [f], John J. Baldwin [a], Roland E. Dolle [a], Edward McDonald [a], Ian Henderson [a]

[a] Pharmacopeia Inc., PO Box 5350, Princeton, NJ 08543, USA
[b] Novartis Institutes of Biomedical Research, 100 Technology Square, Cambridge, MA 02139, USA
[c] Novartis Institutes of Biomedical Research, 5 Gower Place, London WC1E 6BS, UK
[d] Novartis Institutes of Biomedical Research, Wimblehurst Road, Horsham, West Sussex, RH12 5AB, UK
[e] Novartis Institutes of Biomedical Research, Vienna, Austria
[f] Novartis Institutes of Biomedical Research, Basel, Switzerland

ARTICLE INFO

Article history:
Received 9 September 2008
Revised 28 October 2008
Accepted 3 November 2008
Available online 6 November 2008

Keywords:
Bradykinin
Sulfonamide
Combinatorial chemistry
ECLiPS™

ABSTRACT

The synthesis and identification of sulfonamido-aryl ethers as potent bradykinin B1 receptor antagonists from a ~60,000 member encoded combinatorial library are reported. Two distinct series of compounds exhibiting different structure–activity relationships were identified in a bradykinin B1 whole-cell receptor-binding assay. Specific examples exhibit K_i values of ~10 nM.

Bradykinin B1 and B2 receptors are G-protein coupled receptors that play a role in pain and inflammation pathways. The peptides, bradykinin and kallidin, are released following tissue injury and serve as physiological agonists for the constitutively expressed B2 receptor.[1,2] Further metabolism of bradykinin and kallidin result in the formation of [des-Arg9]-bradykinin and [des-Arg10]-kallidin, the natural agonists for the B1 receptor. While this receptor is not widely expressed in non-disease states, it is induced during conditions of inflammation and tissue trauma.[3] Subsequent activation of the B1 receptor is believed to contribute to chronic inflammatory diseases and inflammatory and neuropathic pain.[4] As such there is considerable interest in the identification of small molecule antagonists of the B1 receptor with therapeutic interest against chronic inflammation and pain.

Recent literature reports have detailed the discovery of non-peptide B1 receptor antagonists incorporating sulfamoylbenzamide,[5] benzodiazepine,[6] arylsulfone,[7] benzimidazole[8] and diaminopyridine[9] core structures, and multiple classes of non-peptide B2 antagonists.[10,11] We herein describe the results of our research resulting in the identification of novel, potent B1 receptor antagonists from high-throughput screening of a ~60,000 member sulfonamido-aryl ether combinatorial ECLiPS™ (Encoded Combinatorial Libraries on Polymeric Support)[12] library. The general structures of the compounds present within the library are consistent with a proposed B1 antagonist pharmacophore of an aryl sulfonamide incorporating a spacer and either a carboxamide or other hydrogen-bond acceptor[13] that has been observed in a number of B1 antagonists. This proposed common pharmacophore is exemplified by the reported non-peptide bradykinin B1 antagonists **SSR-240612**[4] and **I**[14] (Fig. 1).

The sulfonamido-aryl ether library was constructed on solid-phase in four combinatorial steps. The library was designed to provide two distinct spurs as defined by the synthetic strategy used to generate the sulfonamide-based aryl ether framework.

Aminomethyl-terminated Tentagel® resin (**1**) was used as the polymeric support together with a 2-nitrobenzylic photo-cleavable linker.[15] Solid-phase synthesis was initiated by acylating **1** with *N*-α-*N*-ε-bis-Fmoc-lysine followed by Fmoc deprotection to generate **2** (Scheme 1). This increases the loading capacity of the resin by doubling the number of amino groups for further functionalization. This was followed by diisopropyl carbodiimide (DIC)-mediated acylation with 4-(bromomethyl)-3-nitrobenzoic to provide **3**. This

* Corresponding author. Tel.: +1 609 452 3653; fax: +1 609 655 4187.
E-mail addresses: acole@pcop.com, acole4@verizon.net (A.G. Cole).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.11.005

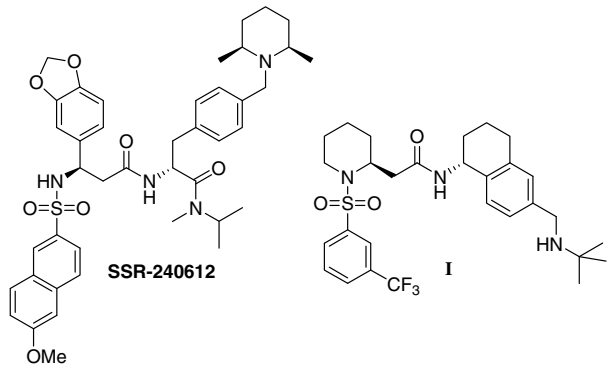


Figure 1. Reported non-peptide bradykinin B1 antagonists.[4,14]

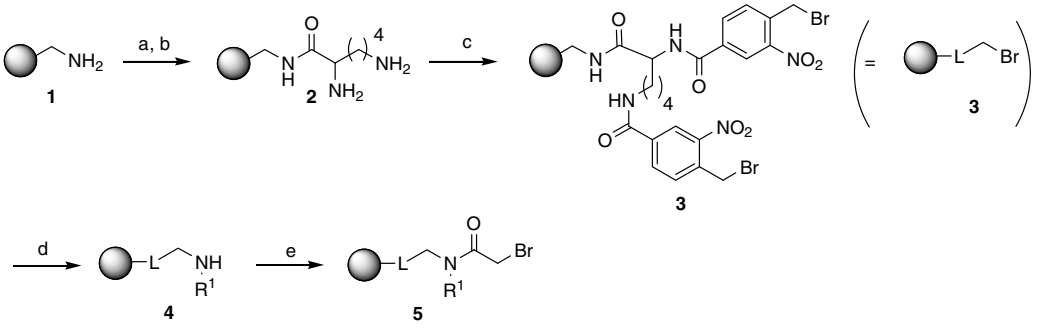
linker allows photo-mediated cleavage of the final compounds from solid-phase, and is compatible with the chemistry required to conduct the solid-phase synthesis. Generation of resin-bound secondary amines **4** was achieved by alkylation of a series of primary amines (R^1-NH_2) with **3** in THF. DIC-mediated bromoacetylation of **4** with bromoacetic acid provided the common intermediate **5**, which was used in the construction of the two library spurs.

The generation of library spur 1 involved alkylation of a series of *O*-TBS protected amino-, methylamino- and ethylaminophenols with **5** to provide **6** (Scheme 2). Sulfonylation of **6** with a diverse selection of sulfonyl chlorides followed by acid-mediated silyl deprotection provided the resin-bound phenols **8**. Mitsunobu reaction of **8** with a solution-phase alcoholic component mediated by diisopropylazodicarboxylate (DIAD) and triphenyl phosphine generated aryl ethers **10**. The generation of library spur 2 was conducted via alkylation of a series of *O*-TBS-protected aminoalcohols with **5** to provide **7** (Scheme 3). Sulfonylation of **7** with a diverse selection followed by silyl deprotection provided **9**. DIAD/triphenylphosphine-mediated Mitsunobu reaction of **9** employing a solution-phase phenolic component generated aryl ethers **11**.

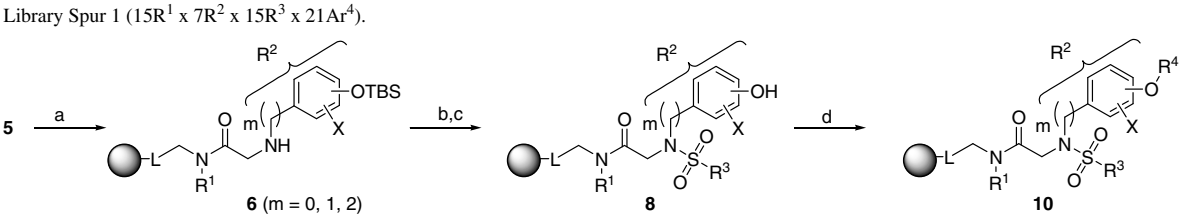
Combinatorial components R^1 and R^3 are common to both library spurs, whereas R^2 and R^4 differ in nature between the library spurs. Amino substituents included at the R^1 position incorporate aliphatic, aromatic, acidic and basic functionalities. Sulfonamide formation to install R^3 utilizes primarily aromatic and heteroaromatic sulfonyl chlorides, incorporating electron-withdrawing, electron-donating and basic functionalities. In library spur 1, R^2 is based on a series of substituted and unsubstituted anilino-, benzylic- and phenethyl-based fragments, whereas the R^2 component of library spur 2 is based on unsubstituted alkyl chains of varying length. In each spur, R^2 provides varying separation of the sulfonamide functionality and what may be a key hydrogen-bond acceptor in the form of the ether oxygen atom.[13] The alcohols employed in the final synthetic step of spur 1 install aliphatic, benzylic and basic functionalities and the phenolic components present within spur 2 provide varying substitutions of the aromatic system.

Compounds were photo-released from the polymeric support under previously optimized photolysis conditions[16] prior to high-throughput screening in a whole-cell binding assay. Structural assignment of the active compounds was performed by electron-capture gas-chromatographic analysis of the binary encoding sequence of electrophoric molecular tags incorporated during ECLiPS™ library synthesis.[12,17]

Screening of library spur 1 in a whole-cell binding assay using WI-38 cells[18] yielded a set of potent B1 binding ligands exhibiting distinct structural features. A strong preference was observed for the 2-chlorobenzyl functionality at the secondary carboxamide position in conjunction with a 4-functionalized aminomethyl benzenesulfonamide. This highlights the possible importance of a basic center at this position within this series. In addition, a benzylic spacer separating the sulfonamide from the aryl ether oxygen atom was exclusively selected. The isobutyl-substituted phenyl ether incorporated via the final Mitsunobu reaction was also shown to be desirable. Specific examples were resynthesized in multi-milligram quantities for accurate K_i determinations in the B1 receptor-binding assay[18] (Table 1). These K_i's revealed superior affinity for incorporation of the 4-(4-methylpiperazin-1-yl)-

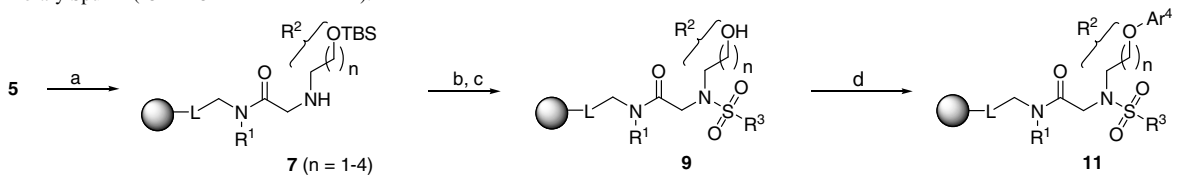


Scheme 1. Reagents and conditions: (a) *N*-α-*N*-ε-bis-Fmoc-Lys, HOBt monohydrate, DIC, CH_2Cl_2, DMF, 25 °C; (b) piperidine, DMF, 25 °C; (c) 4-(bromomethyl)-3-nitrobenzoic acid, HOBt monohydrate, DIC, CH_2Cl_2, DMF, 25 °C; (d) R^1-NH_2, THF, 25 °C; (e) bromoacetic acid DIC, CH_2Cl_2, DMF, 25 °C.



Scheme 2. Library spur 1. Reagents and conditions: (a) R^2-NH_2, DMSO, 25 °C; (b) R^3SO_2Cl, 2,6-lutidine, CH_2Cl_2, 25 °C; (c) HCl, MeOH, 25 °C; (d) R^4-OH, DIAD, PPh_3, Et_3N, CH_2Cl_2, THF, 25 °C.

Library Spur 2 (15R^1 x 5R^2 x 22R^3 x 21Ar^4).



Scheme 3. Library spur 2. Reagents and conditions: (a) R^2-NH_2, DMSO, 25 °C; (b) R^3SO_2Cl, 2,6-lutidine, CH_2Cl_2, 25 °C; (c) HCl, MeOH, 25 °C; (d) Ar^4-OH, DIAD, PPh_3, Et_3N, CH_2Cl_2, 25 °C.

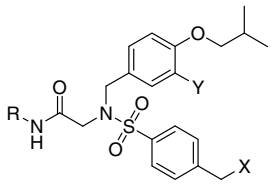
methyl substitution of the benzene sulfonamide (**10a**) in comparison to the corresponding 4-piperidinylmethyl (**10c**), 4-(dimethylamino)methyl (**10d**) and 4-morpholinomethyl substitutions (**10f**). Methoxylation (**10a**) or chlorination (**10b**) of the central aromatic ring was also shown to provide a 5- to 10-fold increase in affinity in comparison to the phenyl analog (**10e**), suggesting the potential contribution of a steric effect to increased binding.

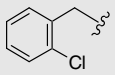
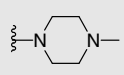
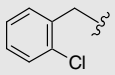
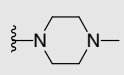
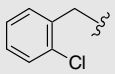
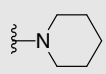
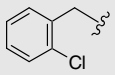
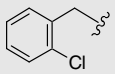
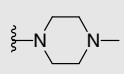
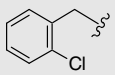
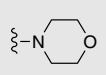
Screening of library spur 2 also yielded compounds with affinity for the B1 receptor. Similarly, specific examples were resynthesized in multi-milligram quantities for accurate K_i determinations in the B1 receptor-binding assay[18] (Table 2). Although compounds from this library spur exhibited weaker activity, distinct structural features were still observed. A different SAR to spur 1 was evident for both the secondary carboxamide and sulfonamide substitutions. In contrast to the hydrophobic 2-chlorobenzyl amide present in all of the B1 binding ligands from spur 1, a distinct preference was observed for a more hydrophilic amide in spur 2. Notably, compounds **11b**, **11c** and **11e**, incorporate an amido-substituent derived from β-alanine. Comparison of **11a** and **11e** reveals increased activity for the 2-methoxyphenethyl-based secondary carboxamide compared with the carboxylic acid-containing compound. However, the β-alanine-derived compound (**11b**) does show superior affinity to the corresponding methoxypropyl (**11d**) and methyl (**11f**) analogs. The R^2 component present within the active compounds consistently displayed a two carbon spacer between the sulfonamide nitrogen and the ether oxygen-acceptor atom. The 2,4-dichlorosubstituted benzene sulfonamide was also a common structural feature, being exclusively selected from the diverse set of sulfonamides in the active compounds identified from spur 2. A number of fragments could be incorporated in the final combinatorial variant (R^4), with library members based on sesamol (**11a** and **11e**), 2,3-dihydro-1*H*-inden-5-ol (**11b**, **11d** and **11f**) and 3-chlorophenol (**11c**) all demonstrating activity.

For comparison, the compound exhibiting R^4 as unsubstituted phenyl (**11g**) was synthesized and exhibited considerably weaker activity than the bicyclic or chlorinated examples.

The more potent compounds from library spur 1 were also evaluated in a functional assay to confirm their role as antagonists. Functional antagonism was demonstrated by evaluation in a calcium mobilization assay against cloned hBK1 expressed in Cos-7 cells also expressing aequorian.[19] Active compounds displayed K_i's of 37–500 nM in the functional assay (Table 3).

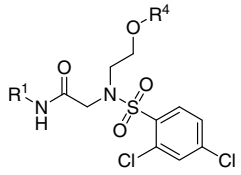
Table 1
B1 antagonists from library spur 1

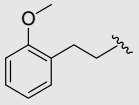
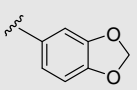
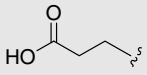
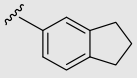
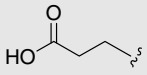
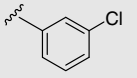
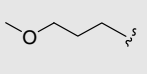
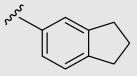
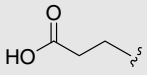
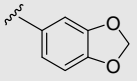
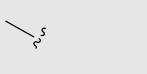
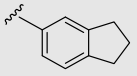
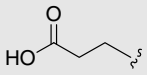
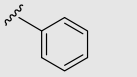


Compound	R	X	Y	K_i[a] (μM)
10a				0.011 ± 0.002
10b				0.021 ± 0.003
10c				0.034 ± 0.008
10d				0.093 ± 0.022
10e				0.113 ± 0.024
10f				0.149 ± 0.05

[a] Binding K_i ± SEM based on 4 independent determinations.

Table 2
B1 antagonists from library spur 2



Compound	R^1	R^4	K_i[a] (μM)
11a			0.21 ± 0.19
11b			0.41 ± 0.30
11c			1.09 ± 0.31
11d			1.21 ± 0.29
11e			1.57 ± 0.72
11f			2.20 ± 1.10
11g			13.01 ± 11.30

[a] Binding K_i ± SEM based on ⩾2 independent determinations.

Table 3
Binding[18] and functional[19] activities of B1 antagonists from library spur 1

Compound	K_i binding[a] (μM)	K_i functional[a] (μM)
10a	0.011 ± 0.002	0.037 ± 0.009
10b	0.021 ± 0.003	0.188 ± 0.057
10c	0.034 ± 0.008	0.267 ± 0.050
10d	0.093 ± 0.022	0.497 ± 0.196
10f	0.149 ± 0.05	0.463 ± 0.080

[a] K_i ± SEM based on $\geqslant$3 independent determinations.

In summary, evaluation of a ~60,000-member sulfonamide-aryl ether combinatorial library in a high-throughput whole-cell binding screen vs bradykinin B1 has resulted in the successful discovery of two series of antagonists with differing SAR. Although all library compounds are consistent with the proposed common pharmacophore of an aryl sulfonamide incorporating a spacer and either a carboxamide or other hydrogen-bond acceptor,[13] specific combinatorial components were selected at the diversity positions in each of the active series. The selection of key fragments highlights the benefits of screening large compound collections to obtain valuable SAR information that extends beyond the core structure.

References and notes

1. Regoli, D.; Barabe, J. *Pharmacol. Rev.* **1980**, *32*, 1.
2. Regoli, D.; Nsa Allogho, S.; Gobeil, F. J. *Eur. J. Pharmacol.* **1998**, *348*, 1.
3. Marceau, F.; Hess, J. F.; Bachvarov, B. R. *Pharmacol. Rev.* **1998**, *50*, 357.
4. (a) Gougat, J.; Ferrari, B.; Sarran, L.; Planchenault, C.; Poncelet, M.; Maruani, J.; Alonso, R.; Cudennec, A.; Croci, T.; Guagnini, F.; Urban-Szabo, K.; Martinolle, J.; Soubrie, P.; Finance, O.; Le Fur, G. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 661; (b) Hara, D. B.; Leite, D. F. P.; Fernandes, E. S.; Passos, G. F.; Guimarães, A. O.; Pesquero, J. B.; Campos, M. M.; Calixto, J. B. *Br. J. Pharmacol.* **2008**, *154*, 1163.
5. Ritchie, T. J.; Dziadulewicz, E. K.; Culshaw, A. J.; Mueller, W.; Burgess, G. M.; Bloomfield, G. C.; Drake, G. S.; Dunstan, A. R.; Beattie, D.; Hughes, G. A.; Ganju, P.; McIntyre, P.; Bevan, S. J.; Davis, C.; Yaqoob, M. *J. Med. Chem.* **2004**, *47*, 4642.
6. Wood, M. R.; Kim, J. J.; Han, W.; Dorsey, B. D.; Homnick, C. F.; DiPardo, R. M.; Kuduk, S. D.; MacNeil, T.; Murphy, K. L.; Lis, E. V.; Ransom, R. W.; Stump, G. L.; Lynch, J. J.; O'Malley, S. S.; Miller, P. J.; Chen, T.-B.; Harrell, C. M.; Chang, R. S. L.; Sandhu, P.; Ellis, J. D.; Bondiskey, P. J.; Pettibone, D. J.; Freidinger, R. M.; Bock, M. G. *J. Med. Chem.* **2003**, *46*, 1803.
7. Biswas, K.; Aya, T.; Qian, W.; Peterkin, T. A. N.; Chen, J. J.; Human, J.; Hungate, R. W.; Kumar, G.; Arik, L.; Lester-Zeiner, D.; Biddlecome, G.; Manning, B. H.; Sun, H.; Dong, H.; Huang, M.; Loeloff, R.; Johnson, E. J.; Askew, B. C. *Bioorg. Med. Chem. Lett.* **2008**, *17*, 4764.
8. Guo, Q.; Chandrasekhar, J.; Ihle, D.; Wustrow, D. J.; Chenard, B. L.; Krause, J. E.; Hutchison, A.; Alderman, D.; Cheng, C.; Cortright, D.; Broom, D.; Kershaw, M. T.; Simmermacher-Mayer, J.; Peng, Y.; Hodgetts, K. J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5027.
9. Kuduk, S. D.; Chang, R. K.; Ng, C.; Murphy, K. L.; Ransom, R. W.; Tang, C.; Prueksaritanont, T.; Freidinger, R. M.; Pettibone, D. J.; Bock, M. G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3925.
10. Heitsch, H. *Curr. Med. Chem.* **2002**, *9*, 913.
11. Gurrath, M. *Curr. Med. Chem.* **2001**, *8*, 1605.
12. (a) Ohlmeyer, M. H. J.; Swanson, R. N.; Dillard, L.; Reader, J. C.; Asouline, G.; Kobayishi, R.; Wigler, M.; Still, W. C. *Proc. Natl. Acad. Sci U.S.A.* **1993**, *90*, 10922; (b) Nestler, H. P.; Bartlett, P. A.; Still, W. C. *J. Org. Chem.* **1994**, *59*, 4723.
13. Marceau, F. *Trends Pharmacol. Sci.* **2005**, *26*, 116.
14. Fotsch, C.; Biddlecome, G.; Biswas, K.; Chen, J. J.; D'Amico, D. C.; Groneberg, R. D.; Han, N. B.; Hsieh, F.-Y.; Kamassah, A.; Kumar, G.; Lester-Zeiner, D.; Liu, Q.; Mareska, D. A.; Riahi, B. B.; Wang, Y. J.; Yang, K.; Zhan, J.; Zhu, J.; Johnson, E.; Ng, G.; Askew, B. C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2071.
15. (a) Appell, K. C.; Chung, T. D. Y.; Ohlmeyer, M. H. J.; Sigal, N. H.; Baldwin, J. J.; Chelsky, D. *J. Biomol. Screen.* **1996**, *1*, 27; (b) Cole, A. G.; Stroke, I. L.; Qin, L.-Y.; Hussain, Z.; Simhadri, S.; Brescia, M.-R.; Waksmunski, F. S.; Strohl, B.; Tellew, J. E.; Williams, J. P.; Saunders, J.; Appell, K. C.; Henderson, I.; Webb, M. L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5420.
16. Compounds were photoreleased from the polymeric support by irradiation of individual polymeric beads in 100 μL of 2% TFA/*i*-PrOH at 365 nm over 1 h (intensity of lamp is equal to 4.5 mW measured at 365 nm using a 365 nm bandpass filter with a bandwidth of ±10 nm)
17. Dolle, R. E.; Guo, J.; Li, W.; Zhoa, N.; Connelley, J. A. *Mol. Divers.* **2000**, *5*, 35.
18. WI-38 cells are grown in Dulbecco's Modified Eagle Medium supplemented with 1% non-essential amino acids, 2 mM L-Glutamine, 100 IU/mL penicillin, 100 mg/mL streptomycin and 10% fetal calf serum. The cells were grown in 175 cm^2 tissue culture flasks and split approximately 1–2 times a week at a ratio of 1:2 using trypsin to detach the cells. WI-38 cells were plated onto 24-well plates at approximately 50,000 cells/well and grown overnight. To upregulate expression of the receptor the cells are treated with100 units/mL of IL-1b for 3 h prior to the assay. The binding buffer is 10 nM HEPES in Hank's Balanced Salt Solution (HBSS) at pH 7.4 plus 1 mM phenanthrinone and 0.14 mg/mL bacitracin. The cells were incubated for 1 h at 4 °C in binding buffer containing the radioligand [^{3}H]-desArg^{10}kallidin in a volume of 500 μL. Non-specific binding is determined with 3 μM desArg^{10}kallidin. At the end of the incubation, the cells were washed three times with 50 mM Tris–HCl, pH 7.4, containing 300 mM sucrose. The cells were solubilized with 0.2% SDS and the amount of radioactivity in the samples determined by liquid scintillation counting. The affinity constant (K_d) was obtained by incubating the cells with a range of [^{3}H]-desArg^{10}kallidin concentrations. For displacement experiments, the cells are incubated with approximately 1 nM [^{3}H]-desArg^{10}kallidin and various concentrations of test compound. Compounds were made up in DMSO and diluted into binding buffer to give a final DMSO concentration of 0.5%. Results are calculated by subtracting the value for non-specific binding from all values and calculating the amount of binding for each concentration of compound as a percentage of the specific binding with no compound. The IC_{50} values were calculated in ORIGIN using a logistical fit. K_i values were calculated from the IC_{50} values using the Cheng–Prusoff equation.
19. Cos-7 cells were grown in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and maintained at 37 °C in 6% carbon dioxide. The cells were split in a ratio of 1:10 twice a week using trypsin. On the day prior to infection confluent cells were split by a factor of 1 in 3 giving 75–85% confluency by time of transfection. The cells were detached by trypsination, washed twice by resuspension in phosphate buffered saline and finally resuspended at 5×10^6 cells/mL in the same buffer. 4 Million cells in a volume of 0.8 mL were added to a 0.4 cm gap Biorad electroporation cuvette containing the 16 μg human B1 cDNA plus 50 μg aequorian cDNA. The cells and cDNA were mixed several times with a 1 mL pipette and left to stand for 5–10 min at room temperature. The cells were electroporated using a Biorad Gene Pulsar. Following transfection, the cells were transferred to 30 mL of warmed media and mixed and pipetted into three 96-well microtiter plates (Packard ViewPlates) at a density of 12,000 cells/well in 100 μL media. Cells were used for compound screening 2 and 3 days after transient transfection. On the day of the experiment the media was removed from the cells and replaced with 50 μL of 20 μM coelenterzine made up in growth medium containing 30 μM glutathione for at least 2 h. Compounds were dissolved in DMSO to give a 2 mM solution and transferred to 1 mL Eppendorf tubes. A Biomek 2000 was used to dilute stocks serially, transfer diluted compounds to preplates and to plates of Cos-7 cells. The 2 mM stocks were serially diluted with DMSO in half log steps. Six concentrations of diluted test compound were transferred (15 μL of each concentration in triplicate) into 96-well deep well plates(preplates) and mixed with 485 μL of assay buffer (10 mM HEPES in Hanks Balanced Salts Solution pH 7.4). In addition 200 mL of the standards used as controls were also transferred to the preplate. The standards were: assay buffer + 3% DMSO to give the control response; assay buffer + 3% DMSO + 80 nM desArg^{10}HOE140, to give a partly inhibited response; assay buffer + 3% DMSO + 2 μM desArg^{10}HOE140, to give the basal value. At this stage the concentrations of the compounds and standards in the preplates were twice the final concentration. The coelenterzine solution was removed from the cells and replaced with 50 μL assay buffer. Fifty microliters from each well of the preplate was then transferred to the cells to give the final concentration. A cover was placed on the base of the plate, which was then placed in a Luminoskan luminometer. A 20 μL aliquot of 180 nM desArg^{10}kallidin was injected onto each well, thus giving a final concentration of 30 nM and the luminescence signal was integrated over 20 s.