Bioorganic & Medicinal Chemistry Letters 20 (2010) 1913–1917

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of substituted biphenyl imidazoles as potent, bioavailable bombesin receptor subtype-3 agonists

Shuwen He [a,*], Peter H. Dobbelaar [a], Jian Liu [a], Tianying Jian [a], Iyassu K. Sebhat [a], Linus S. Lin [a], Allan Goodman [b], Cheng Guo [b], Peter R. Guzzo [b], Mark Hadden [b], Alan J. Henderson [b], Megan Ruenz [b], Bruce J. Sargent [b], Larry Yet [b], Theresa M. Kelly [c], Oksana Palyha [c], Yanqing Kan [c], Jie Pan [c], Howard Chen [c], Donald J. Marsh [c], Lauren P. Shearman [d], Alison M. Strack [d], Joseph M. Metzger [d], Scott D. Feighner [c], Carina Tan [c], Andrew D. Howard [c], Constantin Tamvakopoulos [a], Qianping Peng [a], Xiao-Ming Guan [c], Marc L. Reitman [c], Arthur A. Patchett [a], Matthew J. Wyvratt Jr. [a], Ravi P. Nargund [a]

[a] Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA
[b] Albany Molecular Research Institute, 26 Corporate Circle, Albany, NY 12203, USA
[c] Department of Obesity Research, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA
[d] Department of Pharmacology, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA

ARTICLE INFO

Article history:
Received 5 January 2010
Revised 21 January 2010
Accepted 27 January 2010
Available online 4 February 2010

Keywords:
Bombesin receptor subtype-3
BRS3
Agonist
Biphenyl imidazole

ABSTRACT

We report SAR studies on a novel non-peptidic bombesin receptor subtype-3 (BRS-3) agonist lead series derived from high-throughput screening hit **RY-337**. This effort led to the discovery of compound **22e** with significantly improved potency at both rodent and human BRS-3.

© 2010 Elsevier Ltd. All rights reserved.

Obesity has become a major global health issue causing the World Health Organization (WHO) to officially declare obesity a disease.[1] Obesity causes or exacerbates many health problems, such as hypertension, type 2 diabetes mellitus, and cardiovascular disease. Current drugs approved for the chronic treatment of obesity have suboptimal tolerability and limited efficacy.[2] In order to address this unmet medical need, we are interested in developing therapies based on novel mechanisms.

Bombesin receptor subtype-3 (BRS-3 or BB3), a G-protein coupled receptor (GPCR), belongs to the bombesin receptor family.[3] Bombesin is a peptide (pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2), originally isolated from the skin of the European frog *Bombina bombina*. Two mammalian bombesin-related peptides have been identified, gastrin-releasing peptide (GRP) and neuromedin B (NMB). The biological effects of these peptides are mediated by the bombesin family receptors (GRPR or BB1 and NMBR or BB2). BRS-3 is primarily expressed in the central nervous system, particularly the hypothalamus.[4,5] The natural ligand for the BRS-3 is unknown and, despite its name, BRS-3 does not bind bombesin with high affinity.

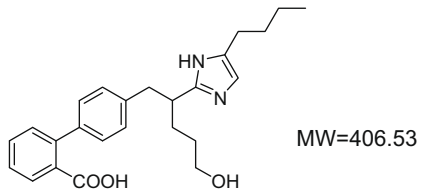
Validation of BRS-3 as a potential target for the treatment of obesity comes from rodent genetics and pharmacology. Mice lacking BRS-3 develop metabolic defects and obesity.[6,7] They are hyperphagic with reduced metabolic rate and reduced core temperature. Complementing the BRS-3 knock-out mouse data, Merck scientists demonstrated BRS-3 antagonist **Bantag-1**, when administered intracerebroventricularly, increases food intake and body weight in rats.[8] Peptides with functional activity at human BRS-3 have been reported.[9] More recently, small molecule agonists based on an omeprazole lead also appeared.[10] In this paper, we will describe Merck's early efforts at identifying small molecule BRS-3 agonists.

A high-throughput screening campaign of the Merck sample collection identified racemic **RY-337**. The compound was particularly appealing due to its low molecular weight and non-peptidic structure. In vitro binding and functional assay data for **RY-337** are summarized in Table 1.[11] **RY-337** is completely inactive on human BB1 and BB2 receptors and is 10-fold more potent on rodent BRS-3 compared to the human receptor.

* Corresponding author. Tel.: +1 732 594 0881; fax: +1 732 594 3007.
E-mail address: shuwen_he@merck.com (S. He).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.01.154

Table 1
Binding affinity and functional activity[a] of **RY-337** at human, rat and mouse bombesin family receptors



RY-337

Receptor	Binding[a] IC_{50} (nM)	Functional EC_{50} (nM)	Activation[c] at 10 μM (%)
hBB1	>10,000	ND[b]	ND
hBB2	>10,000	ND	ND
hBRS-3	3676	1897	74
rBRS-3	ND	174.3	101
mBRS-3	ND	237.5	98

[a] Data are averages of at least three repeated measurements.
[b] Not determined.
[c] The percent activation is the maxim activation of tested compound relative to that of dY-peptide.

Synthesis of **RY-337** is outlined in Scheme 1. The key step is the formation of imidazole **11** from the reaction of aldehyde **10** and an α-hydroxylketone **6** in the presence of copper (II) acetate and ammonium acetate in acetic acid[12] with concomitant loss of the TBS protecting group. α-Hydroxylketone **6** was conveniently prepared from commercially available diol **5**.[13a] Alkylation of the imine derived from aldehyde **9** with benzylic bromide **4** followed by mild acidic hydrolysis produced aldehyde **10**.[14] α-Hydroxylketones can conveniently be prepared in one step from a variety of commercially available starting materials, such as alkenes, 1,2-diols, carboxylic acids, α-halo-ketones, and alkynes.[13] This convergent strategy enabled rapid synthesis of new analogs.

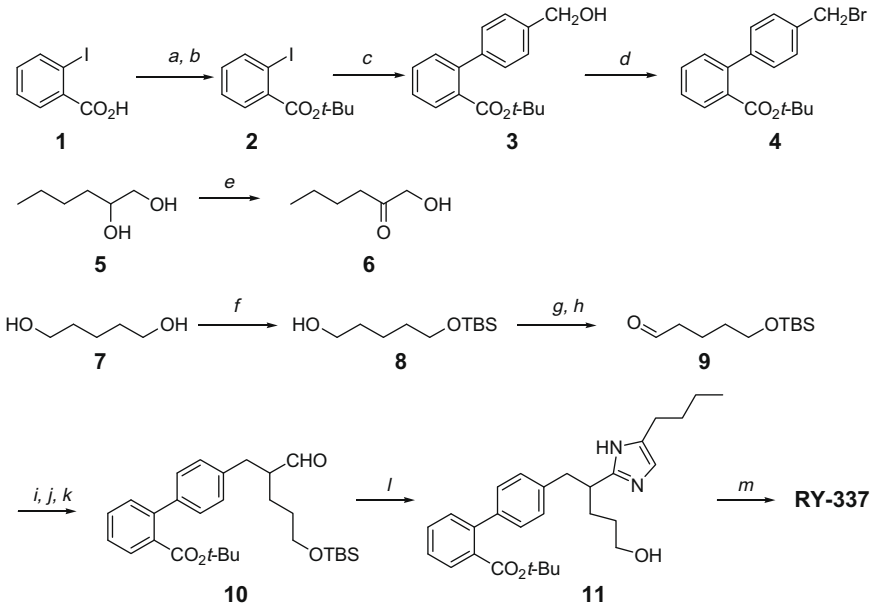
Guided by functional activity assays on human and mouse receptors, we embarked on an SAR study to improve the human potency of this structural series.

The SAR study began with the branch side chain. The compounds and data are listed in Table 2. The synthesis of the analogs was similar to Scheme 1, except **12f**, which required a slightly different synthetic route for its key aldehyde intermediate **17** (Scheme 2). From Table 2, it was clear that the length of the side chain had minimal effect on activity at human BRS-3. The bulky iso-propyl group drastically reduced the potency at the mouse receptor. The discovery that removal of the side chain (**12f**) maintained good potency and that the compound had a reasonable PK profile in rats (Table 3) led to the decision to continue the SAR study without the branch side chain.

We next examined effects of the linkage between the imidazole and biphenyl moiety (Table 4). Shortening the linkage to methylene (**18a**) completely abolished activity at both human and mouse receptors. Extension to a three carbon linkage (**18b**) had a smaller effect on potency. Finally, rigidifying with a *trans* C–C double bond (**18c**) significantly reduced the potency. Therefore two carbon linkage appeared to be optimal.

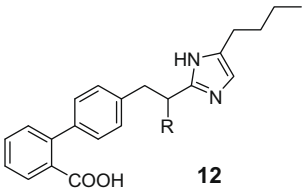
We next turned our attention to replacement of the *n*-butyl chain on the imidazole ring (Table 5). While there was little improvement with a phenyl substituent (**19a**), we were excited to find that a benzyl group (**19b**) tremendously improved potency at the mouse receptor. More importantly, the change also improved potency at the human receptor. Extending the length of the linker (**19c**) reduced the potency.

Encouraged by these results, we synthesized a series of compounds with different substitutions on the phenyl ring (Table 6, **20a–p**). Introduction of an electron-donating methoxy group reduced the potency. The same is largely true for the electron-withdrawing nitrile, although *meta*-CN substitution maintained most of the activity. Among analogs bearing a Me group at different positions, *ortho*-Me substitution (**20g**) maintains similar potency to the un-substituted analog (**19b**), while other positional isomers diminished activity at the human receptor. Chloro substitution re-



Scheme 1. Reagents and conditions: (a) $(COCl)_2$, DMF (cat.), CH_2Cl_2, 0 °C; (b) *t*-BuOH, pyridine, 0 °C to rt; (c) 4-(hydroxylmethyl) phenyl-boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME–H_2O, reflux; (d) NBS, PPh_3, CH_2Cl_2, −78 °C to rt; (e) $NaBrO_3$, $NaHSO_3$, CH_3CN–H_2O; (f) NaH, TBSCl, THF; (g) DMSO, $(COCl)_2$ −78 °C; (h) Et_3N, −78 °C to rt; (i) cyclohexylamine, C_6H_6; (j) LDA, **4**, THF; (k) 0.5 N HCl (aq); (l) $Cu(OAc)_2$, **6**, NH_4OAc, HOAc, reflux 0.5 h; (m) TFA.

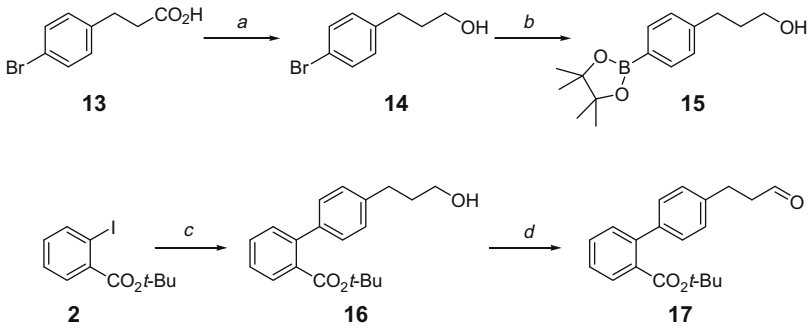
Table 2
Activity of **12** at human and mouse BRS-3[a]



12

	R	Human functional EC_{50} (nM) (%activation)	Mouse functional EC_{50} (nM) (%activation)
12a	*n*-Butyl	2656 (66%)	527 (96%)
12b	*n*-Propyl	1183 (96%)	165 (102%)
12c	Ethyl	2722 (76%)	340 (104%)
12d	Methyl	2760 (79%)	184 (110%)
12e	*iso*-Propyl	4618 (60%)	2240 (87%)
12f	H	2707 (78%)	93 (116%)

[a] Data are averages of at least three repeated measurements.



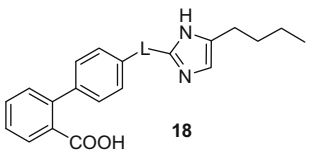
Scheme 2. Reagents and conditions: (a) LAH, ether, 0 °C to rt; (b) bis(pinacolate)diboron, Pd(dppf), KOAc, DMSO; (c) **15**, Pd(dppf), Na_2CO_3 (2 N),DMF, 85 °C; (d) TEMPO (cat.), trichloroisocyanuric acid, CH_2Cl_2, 0 °C to rt.

Table 3
Pharmacokinetic data for **12f**[a]

PK parameter	Rat[a]
F (%)	16
Cl (mL min^{-1} kg^{-1})	1.3
V_{dss} (L kg^{-1})	0.1
$t_{1/2}$ (h)	1.56
AUCn (μM h/mpk)	6.48

[a] Compound dosed in Sprague–Dawley rats as a solution in EtOH/PEG400/water (10:40:50) at 1 mg/kg, iv and 4 mg/kg, po.

Table 4
Activity of **18** at human and mouse BRS-3[a]

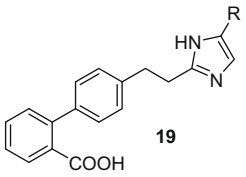


18

	L	Human functional EC_{50} (nM) (%activation)	Mouse functional EC_{50} (nM) (%activation)
18a	CH_2	>10,000 (0%)	>10,000 (0%)
18b	$(CH_2)_3$	5598 (54%)	382 (101%)
18c	CH=CH (trans)	4376 (8%)	10,000 (39%)

[a] Data are averages of at least three repeated measurements.

Table 5
Activity of **19** at human and mouse BRS-3[a]



19

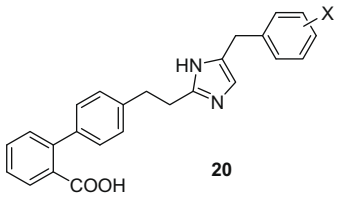
	R	Human functional EC_{50} (nM) (%activation)	Mouse functional EC_{50} (nM) (%activation)
19a	Ph	4949 (30%)	872 (101%)
19b	$-CH_2Ph$	205 (109%)	11 (111%)
19c	$-CH_2CH_2Ph$	1323 (86%)	174 (89%)

[a] Data are averages of at least three repeated measurements.

sults in a loss of potency. Finally, compounds with mono-F substitution maintain potency at both *ortho* (**20m**) and *meta* (**20n**) positions. The *ortho*, *meta* di-F substituted benzyl analog (**20p**) appeared optimal in this limited series.

In order to further improve the potency at the human receptor, we continued the SAR study with aliphatic substituents (Table 7). Since the benzyl group gave better potency than the *n*-butyl substituent, we decided to synthesize analogs with $-CH_2$-cycloalkyl substitution. A small cyclopropyl ring (**21a**) gave poor activity while larger sized rings (**21b–e**) offered single digit nanomolar potency at the mouse receptor and significantly improved human potency.

Table 6
Comparison of activity of **20** with **19b** on human and mouse BRS-3[a]

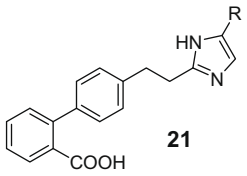


20

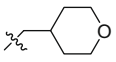
	X	Human functional EC_{50} (nM) (%activation)	Mouse functional EC_{50} (nM) (%activation)
19b	H	205 (109%)	11 (111%)
20a	*o*-OMe	1724 (88%)	163 (101%)
20b	*m*-OMe	4124 (73%)	79 (80%)
20c	*p*-OMe	5360 (44%)	513 (88%)
20d	*o*-CN	5479 (51%)	642 (139%)
20e	*m*-CN	812 (95%)	20 (102%)
20f	*p*-CN	5887 (25%)	723 (85%)
20g	*o*-Me	127 (93%)	9.3 (106%)
20h	*m*-Me	4307 (87%)	75 (89%)
20i	*p*-Me	2663 (88%)	162 (120%)
20j	*o*-Cl	292 (100%)	22 (109%)
20k	*m*-Cl	825 (91%)	31 (98%)
20l	*p*-Cl	2288 (89%)	102 (105%)
20m	*o*-F	177 (108%)	9.5 (121%)
20n	*m*-F	159 (107%)	7.9 (108%)
20o	*p*-F	1601 (84%)	50 (104%)
20p	*o*, *m*-di-F	87 (104%)	8 (110%)

[a] Data are averages of at least three repeated measurements.

Table 7
Activity of **21** at human and mouse BRS-3[a]



21

21h : R= 

	R	Human functional EC_{50} (nM) (%activation)	Mouse functional EC_{50} (nM) (%activation)
21a	$-CH_2$-cyclopropyl	1004 (96%)	72 (144%)
21b	$-CH_2$-cyclobutyl	588 (105%)	7.1 (99%)
21c	$-CH_2$-cyclopentyl	133 (102%)	7.9 (94%)
21d	$-CH_2$-cyclohexyl	41 (104%)	7.3 (102%)
21e	$-CH_2$-cycloheptyl	88 (106%)	5.7 (102%)
21f	$-CH_2-CH_2$-cyclohexyl	642 (94%)	33 (101%)
21g	–Cyclohexyl	375 (102%)	14.7 (116%)
21h	See Scheme above	338 (96%)	23 (106%)

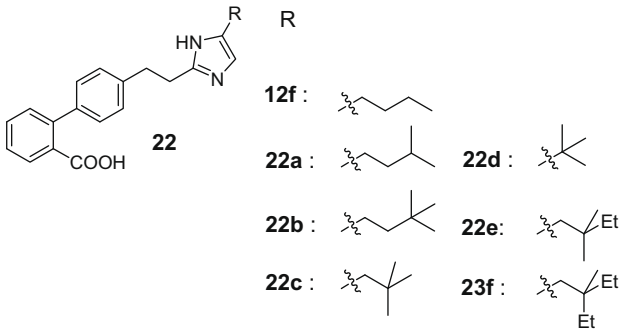
[a] Data are averages of at least three repeated measurements.

In particular, compound **21d** with $-CH_2$-cyclohexyl substitution maintained 41 nM EC_{50} at hBRS-3. We also tried changing the length of the linkage between the imidazole ring and the cyclohexyl ring. Both extending and shortening (**21f–g**) the linkage reduced potency. Introduction of an oxygen in the cyclohexyl ring (**21h**) did not offer much advantage in terms of potency.

Finally we studied uncyclized alkyl substituents (Table 8). Simple addition of a Me group on the *n*-butyl chain (**22a**) significantly improved potency on human and mouse receptors. Adding a second Me group further increased the potency (**22b**). Truncating one methylene (**22c**) had no effect on activity at mouse BRS-3 but resulted in reduced activity at the human receptor. Further shortening of the linkage (**22d**) resulted in a large loss of potency. Further homologation of **22c** recovered potency and compound **22e** stood out as the most potent compound at human BRS-3 in this series. Further alterations (e.g., **22f**) reduced potency. As outlined in Table 9, **22c** demonstrated good rat PK properties.

We tested several potent compounds in animal models to demonstrate efficacy on food intake. When dosed intracerebroventricularly in rat at 25 μg, compound **22c** reduced food intake by 29% compared to animals treated with vehicle (20% PG-water). However, none of the compounds exhibited efficacy in diet induced obese (DIO) mice when dosed orally. Although potent with good plasma drug levels after oral dosing, further investigation suggested that the compounds do not reach sufficient brain levels to interact with the target BRS-3 receptors in the hypothalamus.

Table 8
Comparison of activity of **22** with **12f** on human and mouse BRS-3[a]



	Human functional EC_{50} (nM) (%activation)	Mouse functional EC_{50} (nM) (%activation)
12f	2707 (78%)	93 (117%)
22a	119 (112%)	10 (111%)
22b	39 (102%)	6.2 (99%)
22c	106 (103%)	6.3 (105%)
22d	4334 (45%)	2745 (108%)
22e	25 (101%)	9.6 (94%)
22f	76 (97%)	30 (116%)

[a] Data are averages of at least three repeated measurements.

Table 9
Pharmacokinetic data for **22c**

PK parameter	Rat[a]
F (%)	19
Cl (mL min^{-1} kg^{-1})	1.02
V_{dss} (L kg^{-1})	0.128
$t_{1/2}$ (h)	1.54
AUCn (μM h/mpk)	9.41

[a] Compound dosed in Sprague–Dawley rats as a solution in EtOH/PEG400/water (10:40:50) at 1 mg/kg, iv and 4 mg/kg, po.

In summary, we report the SAR of a non-peptidic BRS3 agonist lead series. This work culminated in compounds with much improved potency on both rodent and human receptors (e.g., **22e**) and with good rodent PK profiles. Efforts at addressing the low brain penetration will be reported in the subsequent paper.

References and notes

1. Obesity: Preventing and Managing the Global Epidemic, Report of a WHO Consultation, World Health Organization, Geneva, 2004.
2. Bays, H. E. *Obes. Res.* **2004**, *12*, 1197.
3. For a general review: Jensen, R. T.; Battey, J. F.; Spindel, E. R.; Benya, R. V. *Pharmacol. Rev.* **2008**, *60*, 1.
4. Ohki-Hamazaki, H.; Wada, E.; Matsui, K.; Wada, K. *Brain Res.* **1997**, *762*, 165.
5. Liu, J.; Lao, Z. J.; Zhang, J.; Schaeffer, M. T.; Jiang, M. M.; Guan, X. M.; Van der Ploeg, L. H.; Fong, T. M. *Biochemistry* **2002**, *41*, 8954.
6. Ohki-Hamazaki, H.; Watase, K.; Yamamoto, K.; Ogura, H.; Yamano, M.; Yamada, K.; Maeno, H.; Imaki, J.; Kikuyama, S.; Wada, E.; Wada, K. *Nature* **1997**, *390*, 165.
7. Ladenheim, E. E.; Hamilton, N. L.; Behles, R. R.; Bi, S.; Hampton, L. L.; Battey, J. F.; Moran, T. H. *Endocrinology* **2008**, *149*, 971.
8. Guan, X.-M.; Chen, H.; Dobbelaar, P. H.; Dong, Y.; Fong, T. M.; Gagen, K.; Gorski, J.; He, S.; Howard, A. D.; Jian, T.; Jiang, M.; Kan, Y.; Kelly, T. M.; Kosinski, J.; Lin, L. S.; Liu, J.; Marsh, D. J.; Metzger, J. M.; Miller, R.; Nargund, R. P.; Palyha, O.; Shearman, L.; Shen, Z.; Stearns, R.; Strack, A. M.; Stribling, S.; Tang, Y. S.; Wang, S.-P.; White, A.; Yu, H.; Reitman, M. L. *Cell Metab.* **2010**, *11*, 101.
9. (a) Mantey, S. A.; Weber, H. C.; Sainz, E.; Akeson, M.; Ryan, R. R.; Pradhan, T. K.; Searles, R. P.; Spindel, E. R.; Battey, J. F.; Coy, D. H.; Jensen, R. T. *J. Biol. Chem.* **1997**, *272*, 26062; (b) Mantey, S. A.; Coy, D. H.; Pradhan, T. K.; Igarashi, H.; Rizo, I. M.; Shen, L.; Hou, W.; Hocart, S. J.; Jensen, R. T. *J. Biol. Chem.* **2001**, *276*, 9219; (c) Weber, D.; Berger, C.; Heinrich, T.; Eickelmann, P.; Antel, J. *J. Pept. Sci.* **2002**, *8*, 461; (d) Boyle, R. G.; Humphries, J.; Mitchell, T.; Showell, G. A.; Apaya, R.; Iijima, H.; Shimada, H.; Arai, T.; Ueno, H.; Usui, Y.; Sakaki, T.; Wada, E.; Wada, K. *J. Pept. Sci.* **2005**, *11*, 136.
10. Carlton, D. L.; Collin-Smith, L. J.; Daniels, A. J.; Deaton, D. N.; Goetz, A. S.; Laudeman, C. P.; Littleton, T. R.; Musso, D. L.; Morgan, R. J. O.; Szewczyk, J. R.; Zhang, C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5451.
11. (a) For human BRS-3 binding assays, 1–4 μg of membrane protein obtained from NFAT-CHO cells expressing the receptor were incubated with 0.3 pM [^{125}I]-[D-Tyr6,β-Ala11,Phe13,Nle14]-Bombesin (6–14) (^{125}I-dY-peptide) and various concentrations of test compounds in 200 μL of binding buffer (50 mM Tris, pH 7.2, 5 mM MgCl2, 0.1% BSA). After a 2 h incubation at room temperature, the binding reaction was terminated by filtering through a GF/c filter and washing the filter with PBS using a Packard 96-well Harvester. The amount of radioligand bound to the receptor was determined by measurement of the radioactivities on the filter through liquid scintillation counting. The nonspecific binding was defined as the binding in the presence of 100 nM unlabeled dY-bombesin. The data in % inhibition of binding was plotted versus the log molar concentration of receptor ligand (compound). The IC_{50} was reported as the inflection point of the resulting sigmoidal curve.
(b) The functional assay is an aequorin bioluminescence assay. It was performed in 96-well format using a Wallac Microbeta luminometer equipped with microinjector module. Compounds in DMSO (0.5% final concentration) were titrated in the plates at 2× concentration in a volume of 0.1 mL ECB buffer (20 mM HEPES, pH 7.4, 140 mM NaCl, 20 mM KCl, 1 mM $MgCl_2$, 1 mM CaCl, 5 mM glucose, 0.1 mg/ml BSA). The HEK293AEQ cells from lines expressing either human, rat or mouse BRS-3 (20,000 per well) were charged with coelenterazine (Molecular Probes) and then injected in 0.1 mL ECB buffer into the compound containing wells. The bioluminescence was monitored for 30 s, or alternatively, total bioluminescence was determined over 10 min. The bioluminescent readings were plotted versus the log molar concentration of receptor ligands (compounds). The EC_{50} for activation was reported as the inflection point of the resulting sigmoidal curve. The percent activation is the maxim activation of tested compound relative to that of dY-peptide.
(c) The binding protocols for human BB1 & BB2 are the same as for BRS-3 except that less protein (membrane) is needed for these two receptors. Both used 0.5 μg per well instead of the 2 μg typically used for BRS-3.
12. Liverton, N. J.; Butcher, J. W.; Claiborne, C. F.; Claremon, D. A.; Libby, B. E.; Nguyen, K. T.; Pitzenberger, S. M.; Selnick, H. G.; Smith, G. R.; Tebben, A.; Vacca, J. P.; Varga, S. L.; Agarwal, A.; Dancheck, K.; Forsyth, A. J.; Fletcher, D. S.; Frantz, B.; Hanlon, W. A.; Harper, C. F.; Hofsess, S. J.; Kostura, M.; Jiunn Lin, J.; Luell, S.; O'Neill, E. A.; Orevillo, C. J.; Pang, M.; Parsons, J.; Rolando, A.; Sahly, Y.; Visco, D. M.; O'Keefe, S. J. *J. Med. Chem.* **1999**, *42*, 2180.
13. (a) Sakaguchi, S.; Kikuchi, D.; Ishii, Y. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 2561; (b) Srinivasan, N. S.; Lee, D. G. *Synthesis* **1979**, 520; (c) Wissner, A. *J. Org. Chem.* **1979**, *44*, 4617; (d) Zhu, G.; Casalnuovo, A. L.; Zhang, X. *J. Org. Chem.* **1998**, *63*, 8100.
14. Stevens, C.; De Kimpe, N. *Synlett* **1991**, 351.