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Synthesis and SAR of derivatives based on 2-biarylethylimidazole as bombesin receptor subtype-3 (BRS-3) agonists for the treatment of obesity

Jian Liu [a,*,†], Shuwen He [a,†], Tianying Jian [a], Peter H. Dobbelaar [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], Kevin Pattamana [b], Megan Ruenz [b], Bruce J Sargent [b], Brian Swenson [b], Larry Yet [b], Constantin Tamvakopoulos [e], Qianping Peng [e], Jie Pan [c], Yanqing Kan [c], Oksana Palyha [c], Theresa M. Kelly [c], Xiao-Ming Guan [c], Andrew D. Howard [c], Donald J. Marsh [c], Joseph M. Metzger [d], Marc L. Reitman [c], Matthew J. Wyvratt [a], Ravi P. Nargund [a]

[a] Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA
[b] AMRI, 26 Corporate Circle, Albany, NY12212, USA
[c] Department of Metabolic Disorders, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA
[d] Department of Pharmacology, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA
[e] Department of Drug Metabolism, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA

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ABSTRACT

This Letter describes a series of potent and selective BRS-3 agonists containing a biarylethylimidazole pharmacophore. Extensive SAR studies were carried out with different aryl substitutions. This work led to the identification of a compound 2-{2-[4-(pyridin-2-yl)phenyl]ethyl}-5-(2,2-dimethylbutyl)-*1H*-imidazole **9** with excellent binding affinity (IC_{50} = 18 nM, hBRS-3) and functional agonist activity (EC_{50} = 47 nM, 99% activation). After oral administration, compound **9** had sufficient exposure in diet induced obese mice to demonstrate efficacy in lowering food intake and body weight via BRS-3 activation.

Obesity is one of the most active therapeutic areas in the pharmaceutical industry. It is a serious and chronic medical condition which is growing rapidly through out the world. Moreover, obesity also causes complications such as diabetes, hypertension, cardiovascular disease, cancer and arthritis.[1] Currently, sibutramine[2] and orlistat[3] are the only two drugs approved for the chronic treatment of obesity, with suboptimal tolerability and limited efficacy. Hence, obesity remains an active area of research and thus far, a variety of molecular targets have been evaluated in the search of more effective obesity treatments.[4]

The bombesin receptor subtype 3 (BRS-3) is an orphan receptor which belongs to the bombesin receptor sub-family of G-protein coupled receptors because of its high sequence homology with the neuromedin B (NMB-R) (BB1, 47%) and gastrin-releasing peptide (GRP-R) (BB2, 51%) receptors.[5] Tissue distribution studies have shown that BRS-3 mRNA is present primarily in the central nervous system. BRS-3 mRNA has also been found within secondary spermatocytes, pregnant uterus, as well as various human lung, breast, and epidermal cancer cell lines.[3,6] BRS-3 is implicated in the regulation of energy homeostasis because mice lacking functional BRS-3 develop mild obesity, hypertension, and diabetes.[7] However, understanding of the role of BRS-3 in physiological or pathological processes has been hampered by the lack of identification of selective ligands, endogenous or exogenous. Although some progress has been recently made in developing potent and selective peptides and small molecules that are BRS-3 agonists,[8] few of these agents has been successfully used in pharmacological studies to explore biologic functions of BRS-3 pathways. In this Letter, we present the synthesis and SAR of a series of 2-biarylethylimidazole analogues as highly potent and selective BRS-3 agonists. We also discuss results from testing in rodent obesity models.

In the previous Letter, we described the discovery of a biphenyl acid analogue **2** as a potent and selective BRS-3 agonist, starting from our HTS lead compound **1**.[9] The optimized substituents on the 4-position of imidazole includes 2,2-dimethyl butyl, cyclopentylmethyl and cyclohexyl methyl. Despite the high potency, compound **2** demonstrated no efficacy on food intake reduction in rodent models after oral dosing, presumably due to its inability

* Corresponding author. Tel.: +1 732 594 9600; fax: +1 732 594 3007.
E-mail address: jian_liu@merck.com (J. Liu).
† Both authors contribute equally to this Letter as senior authors.

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to cross the blood–brain barrier to activate the BRS-3 receptors in the brain, since significant food intake reduction was observed when a close analogue of **2** with *tert*-butylmethyl on imidazole was dosed intracerebroventricularly in rat.[9] We hypothesized that the carboxylic acid group in compound **2** is the major reason for its low brain penetrability. Thus, our effort was focused on identifying potent and selective BRS-3 agonists with a general structure as **3** which could penetrate the blood–brain barrier by replacing the benzoic acid moiety with a variety of aryl groups (Fig. 1).

To facilitate rapid synthesis of these novel analogues, a convergent synthetic route was designed as exemplified by the synthesis of compound **9** (Scheme 1).[10] The Heck reaction of 4-bromoiodobenzene **4** with allyl alcohol **5** catalyzed by palladium acetate provided 3-(4-bromophenyl)propanal **6**.[11] Condensation between aldehyde **6** and 1-hydroxy-4,4-dimethylhexan-2-one (**A**)[12] in the presence of ammonium acetate and copper(II) acetate in acetic acid afforded a moderate yield of the imidazole product **7**. After the imidazole was protected with a Boc group, **7** was converted to pinacol boronic ester **8**. The protection of the imidazole was shown to be necessary to effect the boronation reaction. Suzuki cross coupling of the boronic ester **8** with 2-bromopyridine and simultaneous removal of the Boc protecting group provided compound **9** in good yield. Starting with different terminal alkenes, and aryl halides or triflates, this sequence allowed rapid access to a series of 2-biarylethyl imidazole analogues.

Similarly, starting from key intermediate **7**, we were also able to generate the C–N linked biaryl analogues, exemplified by the preparation of **11** (Scheme 2). In this case, the imidazole was protected with a thermally more stable trityl group, since the instability of the Boc group under the condition required for C–N coupling failed the reaction. The coupling of aromatic bromide **10** with pyrazole was carried out in DMF catalyzed by copper powder, and removal of the trityl protection with trifluoroacetic acid provided excellent yields of compound **11**. These above described two synthetic routes enabled us to prepare significant numbers of this series of C–C and C–N linked biaryl imdazoles for the SAR study.[13]

All final compounds were evaluated in vitro for BRS-3 binding[14] and agonist functional potency.[15] Selected SAR are summarized in Table 1. The carboxylic acid was mapped around the biphenyl ring (structures not shown); however, all of these compounds lost potency (IC_{50} >10,000 nM) compared to the original compound **2**. Substitutions on the phenyl ring with halides (fluorine in **3a**) led to slight loss of potency with no improvements of other biological properties. Replacing phenyl ring with pyridine (**3b** and **3c**) caused significant loss of potency. Typical acid replacements were also investigated,[16] again no obvious advantages. A breakthrough was made when heterocyclic aromatic rings were used to replace the benzoic acid moiety (**9**, **11** and **3d**–**3s**). With an appropriately positioned nitrogen, the potency for BRS-3 was maintained. To our great delight, by switching the benzoic acid to basic aromatic heterocycles such as pyridine, isothiazole, triazole or pyrazole, resulted in compounds (**3d**, **3n**, **3p**, **9**, **11**) that crossed the blood–brain barrier and achieved acceptable brain/plasma ratios. As summarized in Table 2, all five compounds achieved respectable brain drug level in rats.

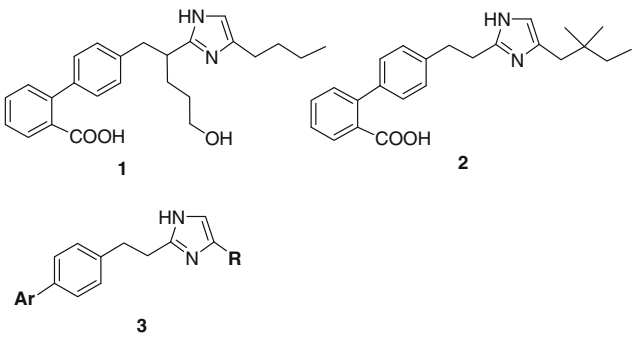
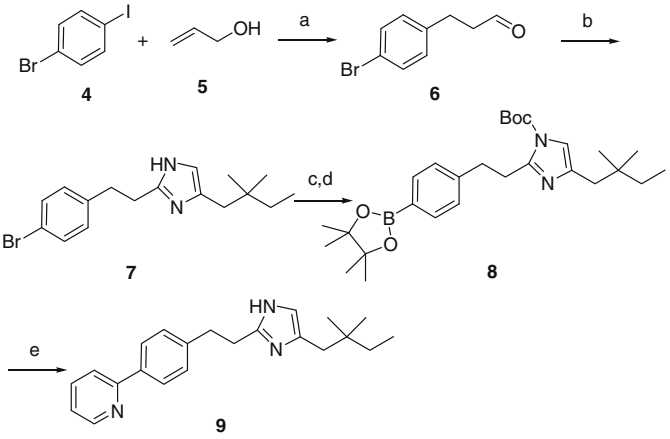
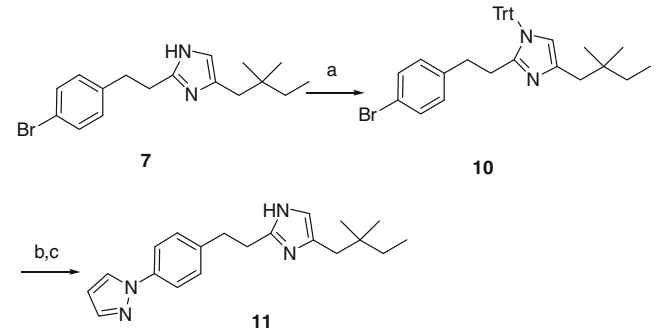


Figure 1.



Scheme 1. Synthesis of **9**. Reagents and conditions: (a) $Pd(OAc)_2$, Bu_4NCl, $NaHCO_3$, DMF, 40 °C, 85%; (b) **A**, NH_4OAc, $Cu(OAc)_2$ in AcOH, 100 °C, 40 min, 45%; (c) Boc_2O, TEA, DMAP in CH_2Cl_2, 1 h at rt, 80%; (d) bis(pinacolate)diboron, Pd(dppf), KOAc, DMF, 2 h, 85%; (e) 2-bromopyridine, Pd(dppf), Na_2CO_3 (2 N), DMF, 85 °C, overnight, 72%.



Scheme 2. Synthesis of **11**. Reagents and conditions: (a) TrtCl, TEA, CH_2Cl_2, 72%; (b) pyrazole, NaH, Cu, DMF; (c) TFA, 95% (two steps).

Compound **9**, 2-{2-[4-(pyridin-2-yl)phenyl] ethyl}-5-(2,2-dimethylbutyl)-1*H*-imidazole, was selected for further profiling. It is a potent BRS-3 agonist with excellent binding affinity (human IC_{50} = 18 nM) and high selectivity over NMB-R (human IC_{50} = 7000 nM) and GRP-R (human IC_{50} = 6400 nM).[16,17] It also binds potently to rodent BRS-3 (IC_{50} = 6.9 and 2.4 nM for mouse and rat, respectively). Although Compound **9** has limited oral bioavailability and short half life in mice,[18] when dosed at 50 mg/kg, a brain/plasma ratio of 0.84 was observed at 1 h, with drug levels of 0.49 μM and 0.41 μM in the plasma and brain, respectively. With oral dosing of compound **9**, we have demonstrated reduction of food intake by a single dose and reduction of body weight with 14-day treatment of diet induced obese mice.[18]

In summary, SAR studies were carried out on a series of 2-biarylethyl imidazole analogues as BRS-3 agonists. A highly selective and potent BRS-3 agonist **9** was discovered. Compound **9** (**Bag-1** in Ref. 18) has acceptable pharmacokinetic properties and brain penetrability to enable testing of the effects of a BRS-3 agonist on food intake and body weight. The discovery of compound **Bag-1**, with its suitability for in vivo studies, will propel a better understanding of the biology and physiology of BRS-3.[18]

Table 1
The potency of BRS-3 agonists in human and mice BRS-3 receptors

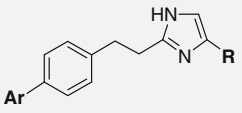
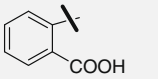
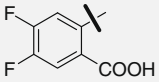
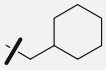
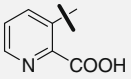
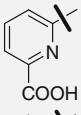
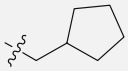
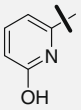
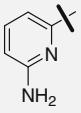
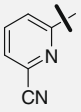
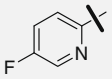
Compound 	Ar	R	hBRS-3 binding IC_{50}[a] (nM)	hBRS-3 function EC_{50}[a] (nM) (activation%)[b]	mBRS-3 Function EC_{50}[a] (nM) (activation%)[b]
2			11	25 (101)	9.6 (94)
3a			36	63 (103)	4.7 (96)
3b			560	1263 (95)	14 (97)
3c			2821	5234 (15)	305 (90)
3d			177	425 (98	22 (103)
9			18	47 (99)	2.8 (78)
3e			7.5	131 (98)	4.3 (97)
3f			101	305 (95)	15 (103)
3g			6.3	105 (102)	19 (106)
3h			51	169 (100)	11 (91)
3i			7.3	37 (97)	3.9 (119)
3j			140	193 (95)	26 (115)
3k			19	66 (97)	16 (105)
3l			11	63 (102	3.1 (103)
3m			45	538 (104)	11 (109)
3n			10	44(100)	6.9 (119)
3o			51	431 (100)	11 (107)
11			33	135 (106)	9.0 (113)
3p			340	570 (89)	12 (121)

Table 1 (*continued*)

Compound	Ar	R	hBRS-3 binding IC_{50}[a] (nM)	hBRS-3 function EC_{50}[a] (nM) (activation%)[b]	mBRS-3 Function EC_{50}[a] (nM) (activation%)[b]
3q			390	641 (93)	57 (111)
3r			205	422 (89)	25 (99)
3s[c]			28	171 (108)	28 (133)

[a] The reported data are the average of at least three repeated experiments.
[b] The percentages of activation are the maxim activation of tested compounds relative to that of [D-Tyr^6,β-Ala^{11},Phe^{13},Nle^{14}]-bombesin (**6**–**14**).
[c] Compound with piperidine as a saturated ring maintains most potency.

Table 2
Plasma and brain levels[a] of BRS-3 agonists 1 h following IV or PO dose in rats

Compd	Dose	Plasma (μM)	Brain (μM)	b/p ratio
3d	1 mg/kg IV	0.039	0.030	0.77
9	50 mg/kg PO	0.89	0.15	0.17
3n	1 mg/kg IV	0.54	0.23	0.42
11	1 mg/kg IV	0.03	0.02	0.67
3p	1 mg/kg IV	0.20	0.08	0.40

[a] Data are averages of three repeated experiments.

Reference and notes

1. Olshansky, S. J.; Passaro, D. J.; Hershow, R. C.; Layden, J.; Carnes, B. A.; Brody, J.; Hayflick, L.; Butler, R. N.; Allison, D. B.; Ludwig, D. S. *New Engl. J. Med.* **2005**, *352*, 1138–1145.
2. Luque, C. A.; Rey, J. A.; Fernandez, A. *Formulary* **1997**, *32*, 1025.
3. McNeely, W.; Benfield, P. *Drugs* **1998**, *56*, 241.
4. For reviews on obesity targets: (a) Kordik, C. P.; Reitz, A. B. *J. Med. Chem.* **1999**, *42*, 181; (b) Aronne, L. J.; Thornton-Jones, Z. D. *Clin. Pharm. Therap.* **2007**, *81*, 748.
5. Fathi, Z.; Corjay, M. H.; Wada, E.; Benya, R.; Jensen, R.; Viallet, J.; Sausville, E. A.; Battey, H. F. *J. Biol. Chem.* **1993**, *268*, 5979.
6. (a) Sano, H.; Feighner, S. D.; Hreniuk, D. L.; Iwaasa, H.; Sailer, A. W.; Pan, J.; Reitman, M. L.; Kanatani, A.; Howard, A. D.; Tan, C. P. *Genomics* **2004**, *84*, 139; (b) Jennings, C. A.; Harrison, D. C.; Maycox, P. R.; Crook, B.; Smart, D.; Hervieu, G. J. *Neuroscience* **2003**, *120*, 309; (c) Vigne, S. R.; Feolde, E.; Van Renterghem, C.; Breittmayer, J. P.; Frelin, C. *Eur. J. Biochem.* **1997**, *272*, R433.
7. 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.
8. (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; (e) Carton, D. L.; Collin-Smith, L. J.; Alejandro, J. D.; Neaton, 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.
9. He, S.; Dobbelaar, P. H.; Liu, J.; Jian, T.; Sebhat, I. K.; Lin, L. S.; Goodman, A.; Guo, C.; Guzzo, P. R.; Hadden, M.; Henderson, A. J.; Ruenz, M.; Sargent, B. J.; Yet, L.; Kelly, T. M.; Palyha, O.; Kan, Y.; Pan, J.; Chen, H.; Marsh, D. J.; Shearman, L. P.; Strack, A. M.; Metzger, J. M.; Feighner, S. D.; Tan, C.; Howard, A. D.; Tamvakopoulos, C.; Peng, Q.; Guan, X.; Reitman, M. L.; Patchett, A. A.; Wyvratt, M. J.; Nargund, N. P. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1913.
10. Dobbelaar, P. H.; Franklin, C. L.; Goodman, A.; Guo, C.; Guzzo, P. R.; Hadden, M.; He, S.; Henderson, A. J.; Jian, T.; Lin, L. S.; Liu, J.; Nargund, R. P.; Ruenz, M.; Sargent, B. J.; Sebhat, I. K.; Yet, L. PCT Int. Appl. WO 2008051405, 2008.
11. (a) Gibson, S. E.; Jones, J. O.; McCague, R.; Tozer, M. J.; Whitcombe, N. J. *Synlett* **1999**, 954; (b) Taylor, E. C.; Liu, B. *Tetrahedron Lett.* **1999**, *40*, 4023.
12. 1-Hydroxy-4,4-dimethylhexan-2-one (**A**) was prepared from commercially available 4,4-dimethylhex-1-ene by treatment with potassium permanganate in a mixture of acetic acid, acetone and water.
13. The synthesis of some compounds needed additional transformation of functional groups, such as ester to acid (**2**, **3a**, **3b** and **3c**).
14. For human BRS-3 binding assays, 1 to 4 μg of membrane protein obtained from NFAT-CHO cells expressing the receptor were incubated with 0.3 pM [^{125}I]-[D-Tyr^6,β-Ala^{11},Phe^{13},Nle^{14}]-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 $MgCl_2$, 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 measured by liquid scintillation counting of the radioactivity on the filter. The nonspecific binding was defined as the binding in the presence of 100 nM unlabeled dY-bombesin. The data, as %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.
15. The functional assay is an aequorin bioluminescence assay. It was performed in a 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 two times 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 BRS3 (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 vs. 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 percentages of activation are the maxim activations of tested compounds relative to that of dY-peptide.
16. Extensive SAR with various acid isosteres were carried out and the results will be published separately.
17. The binding protocols for human NMB-R and GRP-R 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.
18. For more detailed pharmacological profiling of **9** (referred to therein as **Bag-1**), see: 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.