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Discovery of small molecule agonists for the bombesin receptor subtype 3 (BRS-3) based on an omeprazole lead

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ABSTRACT

Starting from a weak omeprazole screening hit, replacement of the pyridine with a 1,3-benzodioxole moiety, modification of the thioether linkage, and substitution of the benzimidazole pharmacophore led to the discovery of nanomolar BRS-3 agonists.

Many drugs exercise their pharmacodynamic effects as ligands for the G-protein coupled seven transmembrane receptor (7-TM) protein superfamily.[1] In addition to pursuing the discovery of ligands for the receptors with known functions, the pharmaceutical industry has also studied the orphan 7-TM receptors identified through high homology to known 7-TMs in the human genome, because of the perceived drugability of this class of proteins. One such orphan is the 399 amino acid bombesin receptor subtype 3 (BRS-3, BB_3) located on the X chromosome (Xq25).[2] BRS-3 belongs to the subfamily of 7-TMs that were originally identified as the receptors for the bioactive peptide family ligands with high homology to bombesin (pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2), a tetradecapeptide originally isolated from the skin of the frog *Bombina bombina*.[3] The other human members of this receptor family are the gastrin-releasing peptide (GRP) preferring receptor (GRP-R, BB_2) and the neuromedin B (NMB) preferring receptor (NMB-R, BB_1).[4] BRS-3 has 51% identity to GRP-R and 47% identity to NMB-R, but the natural ligands for these receptors, GRP and NMB, as well as bombesin, have low affinity for BRS-3. To date, the natural ligand for BRS-3 is unknown and it remains an unadopted receptor.

BRS-3 is highly expressed in testes and widely expressed in the brain, including the hypothalamus, caudate nucleus, pituitary gland, amygdala, and hippocampus.[5] It is also expressed in liver, lung, intestine, and pancreas. Through interaction with $G\alpha_q$, BRS-3 is coupled to phospholipase C, causing the loss of phosphoinositides, and leading to calcium mobilization and activation of protein kinase C.[6] BRS-3 activation also stimulates phospholipase D activity, promoting diacylglycerol formation. BRS-3 (Y/−) mice are viable and fertile, but become hyperphagic and develop a late onset mild obesity with increased white adipose tissue mass,[7] despite high leptin levels.[8] These knockout mice also have a reduced met-

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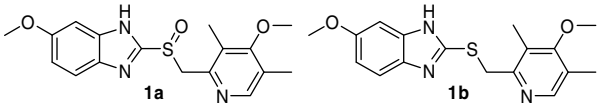
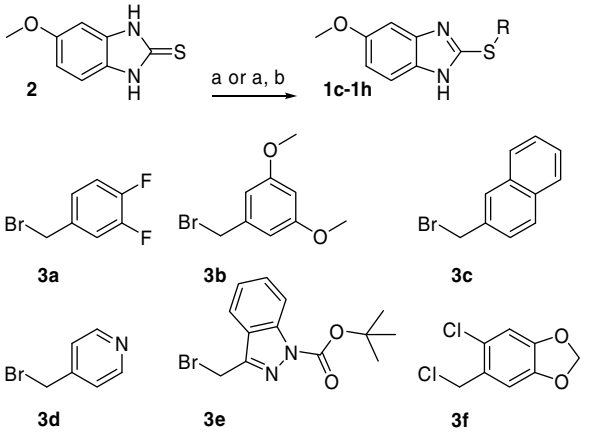
abolic rate and altered taste preference.[9] This obesity is accompanied by hypertension and impaired glucose metabolism[10,11] as well. Male BRS-3 (Y/−) mice also exhibit altered social interaction.[12,13] Furthermore, BRS-3 has been implicated in bronchial epithelial cell proliferation.[14] Thus, ligands that modulate BRS-3 signalling may have utility in diabetes, obesity, and cancer.[15]

A non-selective, high affinity peptide ligand for BRS-3, D-Phe-Gln-Trp-Ala-Val-β-Ala-His-Phe-Nle-NH_2 (BB_1 K_i = 8.9 nM, BB_2 K_i = 0.99 nM, BB_3 K_i = 0.36 nM), has been discovered and replacement of the D-phenylalanine residue with a ^{125}I-D-tyrosine provided a radioligand for BRS-3 binding studies.[16] In addition to this molecule and other peptide ligands,[17–20] a German group has also disclosed small molecule agonists of BRS-3.[21,22]

As part of a protein systems-based research strategy, many G-protein coupled seven transmembrane receptors, including orphan receptors like BRS-3, were screened by GSK versus a compound set consisting of the commercially marketed drugs. Surprisingly, the gastric H^+/K^+-ATPase proton pump inhibitor omeprazole **1a** (Losec®), for peptic ulcer treatment, was identified as a weak partial BRS-3 agonist (BB_3 EC_{50} = 14,000 nM, %Max = 27%). Furthermore, its sulfide analog **1b** was slightly more potent (BB_3 EC_{50} = 3900 nM) with better efficacy (%Max = 64%). Moreover, a literature search revealed that eleven weeks of treatment with omeprazole suppressed body weight gain in rats.[23] Was this result dependent on agonism of BRS-3? With this lead, the intriguing biology of the knockout mice, and the therapeutic effect of omeprazole treatment on body weight in rats, a chemistry program was started to develop small molecule BRS-3 agonists for the treatment of obesity, based on the omeprazole lead.



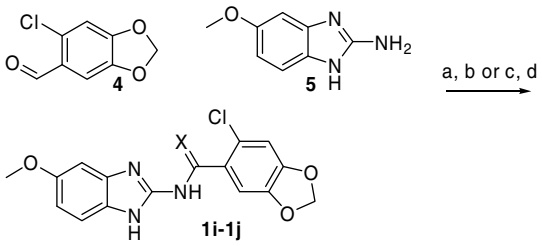
Omeprazole undergoes an acid catalyzed rearrangement at gastric fluid pH, involving the sulfoxide and the pyridine nitrogen, to irreversibly inhibit the parietal cell H^+/K^+-ATPase proton pump in the stomach.[24] Replacement of the sulfoxide or removal of the 2-pyridine nitrogen should prevent the rearrangement and eliminate proton pump inhibition, improving the off-target selectivity of the BRS-3 lead. Both strategies were explored. First, a small array of 5-methoxybenzimidazole thioether analogs was synthesized as depicted in Scheme 1. Alkylation of the commercially available thiourea **2** with a group of purchased arylmethyl halides **3a**–**3f**, followed by removal of the protecting group subsequent to the reaction of bromide **3e**, provided the thioethers **1c**–**1h**.



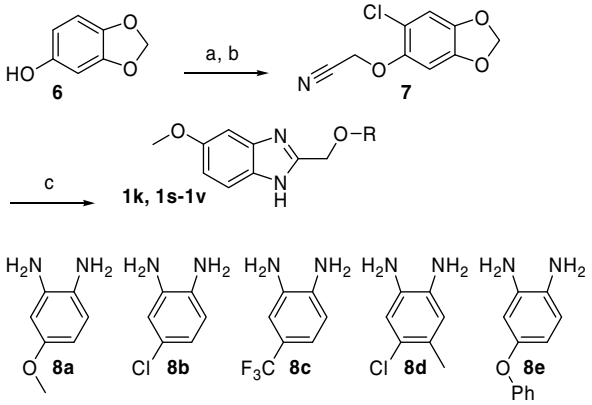
Scheme 1. Reagents and conditions: (a) RBr **3a**–**3e** or RCl **3f**, Cs_2CO_3, DMF, 0 °C–rt, 45–80%; (b) TFA, CH_2Cl_2, 0 °C–rt, 87%.

Most of these analogs, including the difluoride **1c**, the β-naphthalene **1e**, the pyridine **1f**, and the indazole **1g** did not activate BRS-3, as measured by intracellular calcium mobilization, at concentrations up to 32,000 nM. In contrast, the 3,5-dimethoxyphenyl derivative **1d** (EC_{50} = 1,600 nM, %Max = 68%) was a 2-fold more potent BRS-3 partial agonist than omeprazole sulfide **1b**. Furthermore, the 1,3-benzodioxole **1h** (EC_{50} = 380 nM, %Max = 67%) was a 10-fold more potent activator of intracellular calcium mobilization than the omeprazole sulfide lead **1b**.

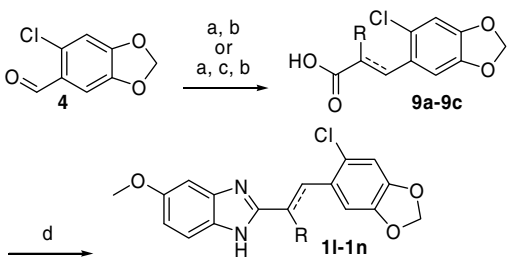
With a submicromolar BRS-3 agonist in hand, attention turned to replacing the thioether linkage with a more metabolically stable moiety. The synthesis of the amide and methylene amine linked analogs **1i** and **1j** are shown in Scheme 2. The purchased aldehyde **4** was oxidized to the carboxylic acid with barium permanganate, then coupled to the commercially available 2-amino-benzimidazole **5** to give the amide **1i** (X = O). Whereas, condensation of aldehyde **4** with the guanidine **5** to provide an imine, followed by



Scheme 2. Reagents and conditions: (a) **4**, $Ba(MnO_4)_2$, CH_2Cl_2, rt, 6%; (b) **5**, DCC, HOBt, THF, rt, 23%; (c) **4**, **5**, piperidine, EtOH, rt to ↑↓, 57%; (d) $NaBH_4$, EtOH, 0 °C to ↑↓, 63%.



Scheme 3. Reagents and conditions: (a) SO_2Cl_2, Et_2O, rt, 54%; (b) $BrCH_2CN$, K_2CO_3, acetone, ↑↓, 89%; (c) **8a–8e**, NaOMe, MeOH, rt, 13–76%.



Scheme 4. Reagents and conditions: (a) $EtO_2CCH_2P(=O)(OEt)_2$ or $EtO_2CCH(CH_3)P(=O)(OEt)_2$, *t*-$BuO^-K^+$, THF, 0 °C to ↑↓, 29–94%, (b) NaOH, THF, H_2O, rt, 73–99%; (c) H_2/10% Pd-C or PtO_2, EtOH, EtOAc, rt, 81–87%; (d) HBTU, *i*-Pr_2NEt, DMF; **8a**, rt; AcOH, 100 °C, 40–62%.

Table 1
Activation of human BB_3

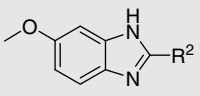
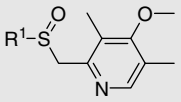
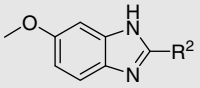
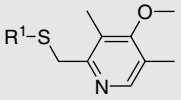
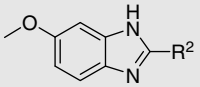
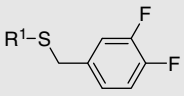
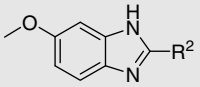
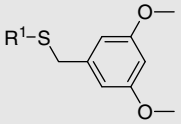
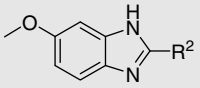
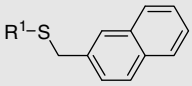
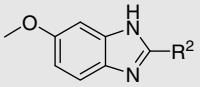
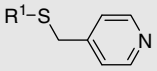
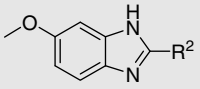
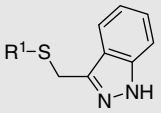
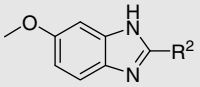
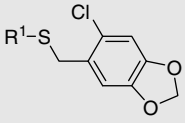
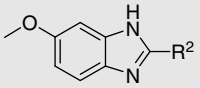
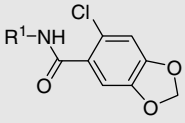
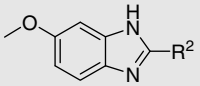
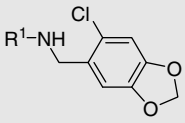
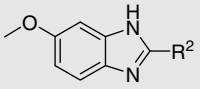
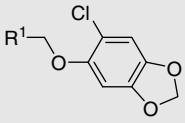
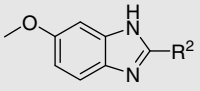
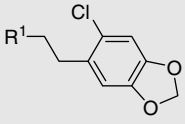
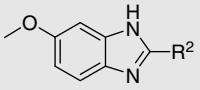
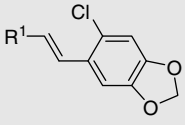
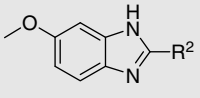
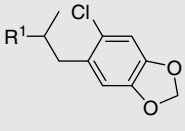
#	R^1	R^2	BB_3 EC_{50}[a] (nM)	BB_3 %[b] (Max %)
1a			14,000	27
1b			3900	64
1c			>32,000	–
1d			1600	68
1e			>32,000	–
1f			>32,000	–
1g			>32,000	–
1h			380	67
1i			>32,000	–
1j			>32,000	–
1k			510	71
1l			210	82
1m			>32,000	–
1n			350	83

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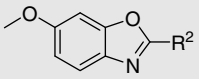
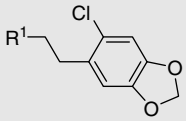
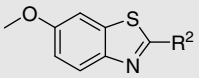
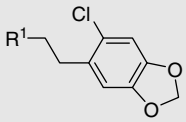
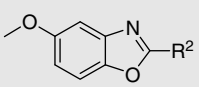
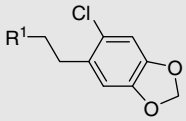
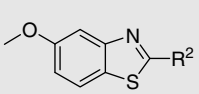
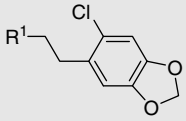
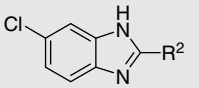
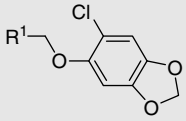
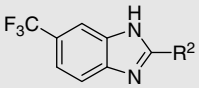
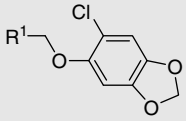
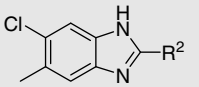
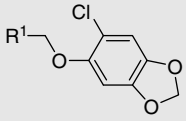
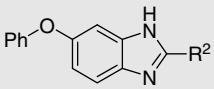
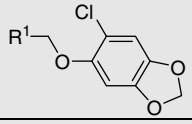
Table 1 (*continued*)

#	R^1	R^2	BB_3 EC_{50}[a] (nM)	BB_3 %[b] (Max %)
1o			>32,000	–
1p			>32,000	–
1q			>32,000	–
1r			>32,000	–
1s			570	59
1t			240	69
1u			>32,000	–
1v			>32,000	–

[a] Activation of BB_3 in recombinant BacMam Baculovirus transduced HEK 293 cells grown in EMEM media supplemented with 10% fetal calf serum, 2 nM L-glutamine, and 1% non-essential amino acids at 37 °C, 5% CO_2, and 95% humidity for 24 h. Growth media is removed via aspiration and replaced with HBSS media containing 2.5 mM probenicid and 0.1% bovine serum albumin (w/v) and FLIPR dye. The assay is initiated by the measurement of a background reading, followed by the addition of the test compound. Activation induced by the test compound is recorded for 2 min. The data points are fitted to a curve using non-linear regression analysis. The EC_{50} values are the mean of at least two assays. D-Phe-Gln-Trp-Ala-Val- β-Ala-His-Phe-Nle-NH_2 had an EC_{50} = 3.9 nM in this assay.

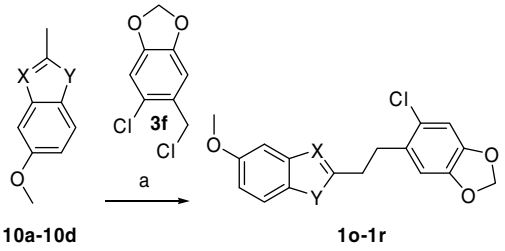
[b] Maximum percent efficacy of the test compound relative to BB_3 activation via D-Phe-Gln-Trp-Ala-Val- β-Ala-His-Phe-Nle-NH_2.

reduction with sodium borohydride, afforded the amine analog **1j** (X = H, H).

The synthesis of the reverse ether linked analog **1k** is illustrated in Scheme 3. First, the purchased phenol **6** was chlorinated with sulfuryl chloride on the sterically least hindered *ortho*-phenolic site. Then, potassium carbonate promoted alkylation of the phenol with bromoacetonitrile provided the ether **7**. Finally, base catalyzed condensation of the commercially available *ortho*-dianiline **8a** with the nitrile **7** yielded the ether linked analog **1k**. The differentially substituted benzimidazole analogs **1s**–**1v** were prepared in a completely analogous manner from the commercially available *ortho*-dianilines **8b**–**8e**.

The linker derivatives **1l**–**1n** with all carbon connections were prepared as depicted in Scheme 4. First, Horner–Wadsworth–Emmons stabilized ylide coupling of the aldehyde **4** with the commercially available phosphorus reagents provided the olefin esters (R = H or R = Me). Hydrolysis of the ester (R = H) with sodium hydroxide then afforded the α,β-unsaturated acid **9b**. Alternatively, metal catalyzed hydrogenation of the alkenes, followed by hydrolysis of the esters yielded the saturated acids **9a** and **9c**. Condensation of these acids **9a**–**9c** with the *ortho*-dianiline **8a** and finally, acid catalyzed cyclization gave the carbon linked analogs **1l**–**1n**.

As shown in Table 1, the amide linkage **1i** was not tolerated by the receptor, inducing no activation at concentrations up to 32,000 nM. Possibly, the carbonyl group cannot be accommodated in the receptor binding pocket, but more likely, the *trans* configuration of the amide bond displays the two appendages in the



Scheme 5. Reagents and conditions: (a) **10a–10d**, LDA, THF, 0 °C to −78 °C; **3f**, −78 °C to rt, 13–39%.

wrong orientation for receptor activation (vide infra). This implies that one of the two possible *gauche* rotamers is the active conformer. In addition, the amine linker analog **1j** is not a BRS-3 agonist. The positive charge at physiological pH is likely not accepted by the protein. In contrast, the reverse ether analog **1k** (EC_{50} = 510 nM, %Max = 71%) is an equipotent BRS-3 agonist as the thioether **1h**. Likewise, the carbon linked derivatives **1l** (EC_{50} = 210 nM, %Max = 82%) and **1n** (EC_{50} = 350 nM, %Max = 83%) exhibit similar potency to the thioether **1h**. Furthermore, analog **1n** shows that carbon substitution of the linker is permissible as well. In contrast, similar to the amide derivative **1i**, the *trans* olefin **1m** is inactive, further supporting the hypothesis that an *anti* rotamer population is not the preferred conformation for efficacy.

With two potential linker replacements with perceived greater metabolic stability identified, focus shifted to exploration of the benzimidazole pharmacophore. Benzimidazole replacements were synthesized as shown in Scheme 5. The commercially available benzoxazoles **10a** (X = O, Y = N) and **10c** (X = N, Y = O) and benzthiazoles **10b** (X = S, Y = N) and **10d** (X = N, Y = S) were alkylated with the chloride **3f** to give the carbon linked derivatives **1o**–**1r**.

As shown in Table 1, both benzimidazole nitrogens are absolutely required for agonist activity as replacement of either one of them with oxygen or sulfur, as in analogs **1o**-**1r**, destroys all agonist activity. It is possible that a positive charge on the benzimidazole is necessary for receptor activation, but with only a moderate basicity (calculated pK_a = ~5.9), it is likely that the benzimidazole is not charged. Furthermore, analog **1j** (calculated pK_a = ~7.4) was not an agonist despite the ability to delocalize its charge to either ring nitrogen. Therefore, it seems more likely that a hydrogen bond donor is required for agonist activity and the benzimidazole NH fulfills this requirement.

In contrast to benzimidazole replacement, substitution of the methoxy group with small lipophilic moieties was permissible. Both the chloro and trifluoromethyl derivatives **1s** (EC_{50} = 570 nM, %Max = 59%) and **1t** (EC_{50} = 240 nM, %Max = 69%) were equipotent BRS-3 agonists as the methoxy analog **1k**. Larger substituents, like the phenoxy group in derivative **1v**, have no measured activation and if able to bind likely disturb the receptor conformation, preventing its recruitment of G proteins for signal transduction. Furthermore, substitution of the 6-position of the benzimidazole ring, as in analog **1u**, also abolished agonist activity (compare **1s** with **1u**). Representative BRS-3 analogs were profiled versus NMB-R (BB_1)[25] and GRP-R (BB_2),[26] but none of them exhibited any agonist efficacy as measured by intracellular calcium mobilization in a FLIPR-based assay. Although a homology receptor model of BRS-3, based on the principles of comparative protein modelling, using the X-ray crystal structure of the seven transmembrane receptor bovine rhodopsin, was developed, its predictions did not prove fruitful for further target design. Nor did it provide any deeper understanding of the structure/activity relationships of these analogs.

A representative BRS-3 agonist **1k** was dosed i.v. in fasted rats to ascertain its pharmacokinetic properties. It exhibited a moderate volume of distribution (V_{SS} = 1200 mL/kg) with a short terminal half-life ($t_{1/2}$ = 30 min) and a high clearance (C_l = 61 mL/min/kg) that approached hepatic blood flow. Although these inhibitors conform to Lipinski's Rule of 5 for good bioavailability, analog **1k** was not dosed orally, because of its poor i.v. exposure.

In summary, a directed screening approach identified a weak partial BRS-3 agonist, the proton pump inhibitor omeprazole **1a**. Replacements of the pyridine fragment with various groups led to the identification of a 1,3-benzodioxole submicromolar BRS-3 agonist **1h**. Further modifications to the linker region, as in **1k** and **1l**, helped improve metabolic stability, eliminating the omeprazole rearrangement liability and removing the proton pump inhibition liability. Finally, modifications to the benzimidazole revealed the importance of the NH for hydrogen bond donation and showed that the chloride and trifluoromethane analogs **1s** and **1t** were also submicromolar BRS-3 agonists. These BRS-3 modulators may prove useful in further defining the physiological roles of BRS-3.

References and notes

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25. Activation of BB_1 in recombinant BacMam Baculovirus transduced HEK 293 cells grown in EMEM media supplemented with 10% fetal calf serum, 2 nM L-glutamine, and 1% non-essential amino acids at 37 °C, 5% CO_2, and 95% humidity for 24 h. Growth media is removed via aspiration and replaced with HBSS media containing 2.5 mM probenicid and 0.1% bovine serum albumin (w/v) and FLIPR dye. The assay is initiated by the measurement of a background reading, followed by the addition of the test compound. Activation induced by the test compound is recorded for 2 min. The data points are fitted to a curve using non-linear regression analysis. The EC_{50} values are the mean of at least two assays. D-Phe-Gln-Trp-Ala-Val-β-Ala-His-Phe-Nle-NH_2 had an EC_{50} = 0.55 nM in this assay.
26. Activation of BB_2 in recombinant BacMam Baculovirus transduced HEK 293 cells grown in EMEM media supplemented with 10% fetal calf serum, 2 nM L-glutamine, and 1% non-essential amino acids at 37 °C, 5% CO_2, and 95% humidity for 24 h. Growth media is removed via aspiration and replaced with HBSS media containing 2.5 mM probenicid and 0.1% bovine serum albumin (w/v) and FLIPR dye. The assay is initiated by the measurement of a background reading, followed by the addition of the test compound. Activation induced by the test compound is recorded for 2 min. The data points are fitted to a curve using non-linear regression analysis. The EC_{50} values are the mean of at least two assays. D-Phe-Gln-Trp-Ala-Val-β-Ala-His-Phe-Nle-NH_2 had an EC_{50} = 0.060 nM in this assay.