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Design, synthesis and SAR analysis of novel potent and selective small molecule antagonists of NPBWR1 (GPR7)

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Abstract

Novel small molecule antagonists of NPBWR1 (GPR7) are herein reported. A high-throughput screening (HTS) of the Molecular Libraries-Small Molecule Repository library identified 5-chloro-4-(4-methoxyphenoxy)-2-(*p*-tolyl)pyridazin-3(2*H*)-one as a NPBWR1 hit antagonist with micromolar activity. Design, synthesis and structure–activity relationships study of the HTS-derived hit led to the identification of 5-chloro-2-(3,5-dimethylphenyl)-4-(4-methoxyphenoxy)pyridazin-3(2*H*)-one lead molecule with submicromolar antagonist activity at the target receptor and high selectivity against a panel of therapeutically relevant off-target proteins. This lead molecule may provide a pharmacological tool to clarify the molecular basis of the in vivo physiological function and therapeutic utility of NPBWR1 in diverse disease areas including inflammatory pain and eating disorders.

Keywords

NPBWR1 (GPR7); Selective small molecule antagonists; Feeding behavior and energy homeostasis; Inflammatory pain

In 1995 O'Dowd et al. reported the existence of two structurally related orphan G-protein coupled receptors termed GPR7 and GPR8 which were identified by the cloning of opioid-somato-statin-like receptor genes from human genomic DNA.¹ GPR7 and GPR8 share a

70% nucleotide and a 64% amino acid identity with each other. Although orthologs of GPR7 and GPR8 have been isolated by PCR from many species, in rodents GPR8 has not been found.² Neuropeptide W (NPW) and neuropeptide B (NPB) were successively identified as the endogenous ligands for these receptors.^{3,4} NPB mRNA is widely distributed throughout the mouse brain and is present in the hippocampus, hypothalamic nucleus, Edinger-Westphal nucleus (EW), locus coeruleus, inferior olive and lateral parabrachial nucleus.^{3,5} NPW expression is more confined to EW, ventral tegmental area (VTA), periaqueductal gray and dorsal raphe nucleus.⁶

Following the receptor deorphanizing, GPR7 and GPR8 were reclassified by IUPHAR as NPBWR1 (GPR7) and NPBWR2 (GPR8).⁷ NPW and NPB activate both receptors with varying degree of affinity. NPBWR1 has a slightly higher affinity for NPB as compared with the two NPW isoforms NPW23 and NPW30, whereas NPBWR2 shows a potency rank order of NPW23 > NPW30 > NPB.^{3,4,8} Both NPBWR1 and NPBWR2 couple to the Gi-class of GPCRs and decrease intracellular cAMP.³

In situ hybridization in rodents and tissue binding studies showed that NPBWR1 mRNA is localized in the suprachiasmatic nucleus, hippocampus, arcuate nucleus, bed nucleus of the stria terminalis, extended amygdala, VTA.^{2,3}

Based on the distribution of peptides (NPW and NPB) and receptors (NPBWR1, NPBWR2) it was hypothesized that this neuropeptide system plays an important role in modulating feeding behavior and energy homeostasis,^{3,4} although its effects are incompletely understood and some discrepancies are found in the literature. Indeed, intracerebroventricular (i.c.v.) administration of NPW to fasting rats or free-feeding rats before dark phase suppressed food intake and increased energy expenditure as a result of augmented heat production, oxygen consumption and body temperature.⁹ However, two groups demonstrated an orexigenic effect in rats followed by acute administration of NPW.^{4,10} I.c.v. injection of NPB into rats induced a biphasic feeding effect at low doses with early and mild orexigenic action followed by marked anorexia, whereas higher doses caused anorexia at all time points.³ Although further studies are needed to resolve the molecular mechanisms of its anorectic properties, NPBWR1 has been recently hypothesized to play an important role in the regulation of feeding and energy metabolism by stimulating lipolysis and inhibiting leptin secretion.^{11,12} Remarkably, NPBWR1 knockout mice developed adult-onset obesity and had elevated plasma leptin concentration.¹³ Additionally, rats injected with NPW showed decreased plasma insulin and leptin concentration.¹⁴ Compatible with these studies, NPBWR1 has been demonstrated to be expressed in rat adipocytes, while NPW and NPB are detectable in preadipocytes and macrophages.¹¹

Intrathecal administration of either NPW23 or NPB produced analgesic effect in inflammatory pain models in rats (plantar injection of formalin or carrageenan) but had no effects in thermal and mechanical nociceptive tests.¹⁵ Although the precise mechanisms responsible for the analgesic effect elicited by NPW23 or NPB in the rat formalin and carrageenan tests are not known, these data suggested that both spinally-applied NPW23 and NPB suppressed the input of nociceptive information to the spinal dorsal horn and indicated a new potential therapeutic approach to treating inflammatory pain.¹⁵ Interestingly, samples taken from patients with inflammatory/immunomediated neuropathies showed a higher expression of NPBWR1 restricted to myelin-forming Schwann cells where the receptor is constitutively present at low levels. Consistently, animal models of acute inflammatory and trauma-induced neuropathic pain exhibited an increased myelin-associated expression of NPBWR1, suggesting a therapeutic role of this receptor in analgesia and as a marker of inflammatory neuropathies.¹⁶ I.c.v. injection of NPB in male rats elevated prolactin and corticosterone, and decreased growth hormone (GH) levels in circulation, suggesting that

this peptide may play a physiological role in the neuroendocrine response to stress via the activation of the hypothalamus-pituitary-adrenal axis.¹⁷ Additionally, i.c.v. injection of NPB has been recently shown to induce slow wave sleep (SWS) in wild-type mice in a dose-dependent manner when administered during the dark period. Interestingly, the NPB did not impact SWS in NPBWR1 knockout mice thus suggesting that NPBWR1 may have a role in modulating the sleep/waking state.¹⁸

Remarkably, consistent with the dual analgesic and anti-epileptic activity of several anticonvulsant neuropeptides, i.c.v. administration of NPW in the brain reduced seizure activity in mice, suggesting that NPBWR1 may be a novel target for epilepsy.¹⁹

Despite the accumulating evidence proving the implication of NPBWR1 in the regulation of feeding behavior, neuroendocrine function and pain, aspects of the receptor biological role remain unclear, partly due to the lack of in vivo pharmacological tools. Recently, a series of novel small molecule antagonists of NPBWR1 have been disclosed; however, these molecules were found to be P-glycoprotein substrates and suffered from poor metabolic stability having less than 10% parent remaining at 30 min in mouse liver microsomes.²⁰

Herein we report on the discovery, synthesis and structure–activity relationships (SAR) of novel NPBWR1 antagonists which may allow experiments aimed to elucidate the biological profile and the therapeutic application of the target receptor. The 5-chloro-4-(4-methoxyphenoxy)-2-(*p*-tolyl)pyridazin-3(2*H*)-one **1a** (Fig. 1) was identified by in house high-throughput screening (HTS) of the Molecular Libraries-Small Molecule Repository (MLSMR) library as a NPBWR1 antagonist hit with an IC₅₀ value of 2.2 μM at the target receptor.²¹ The structural integrity of the hit was confirmed by its re-synthesis via condensation of *p*-tolyl hydrazine **2a** with mucochloric acid **3**. The 4,5-dichloro pyridazin-3(2*H*)-one **4a** thus obtained underwent nucleophilic addition at position 4 with phenol **5a** using anhydrous 1,4-dioxane (Scheme 1).²²

Our SAR studies on **1a** involved the preparation and testing of analogs **1b–1ae** varying the *p*-tolyl region (A) while maintaining the 4-methoxyphenoxy group (C) and the pyridazin-3(2*H*)-one central core (B) as constant moieties (Scheme 1, Table 1).²³ The target compounds were obtained analogously to **1a** starting from commercially available or readily synthesized aryl hydrazines **2b–2ae**.²⁴

Interestingly, the unsubstituted phenyl analog **1b** was ~sixfold less potent than the hit. When the 4-methyl group of the hit was exchanged by fluorine (**1c**), chlorine (**1g**) or bromine (**1f**) the potency varied inversely to the electronegativity of the halogen. Indeed, the bromide **1f** was threefold more potent than the hit, whereas the chloride **1g** and fluoride **1c** were slightly more and less potent than the hit respectively. The 4-ethylcarboxylate analog **1h** was slightly more potent than the hit. Moderately polar substituents such as trifluoromethyl (**1d**) and isopropoxy (**1e**) groups in the *para* position were also tolerable with a ~threefold increase in potency. A 3- to 4-fold increase in potency over the hit was observed when in the same position were inserted bulkier and/or longer substituents such as ethyl (**1i**) *sec*-butyl (**1l**), *tert*-butyl (**1n**). A greater increase of activity (eightfold) was observed for the isopropyl derivative **1j**, whereas the *n*-pentyl analog **1m** was slightly less potent than **1a**. Interestingly, the presence of a π - or π -like system was tolerated but did not significantly improve the potency as observed for the phenyl **1o** and cyclopropyl **1k** analogs. Adding a methyl group in the *ortho* position led to a slight increment of potency compared to the hit as observed for the 2,4-, 2,5- and 2,3-dimethyl substituted derivatives **1p–1r**. Remarkably, 3,4-dimethyl substituted analog **1s** was ~fourfold more potent than **1a** and slightly more potent than the 2,4-dimethyl analog **1p**. 3-Monosubstituted analogs bearing a small group such as methyl (**1t**), chlorine (**1u**) or ethyl (**1x**) were 2- to 4-fold more potent than their 4-substituted

counterparts. In the same trend, the 3,5-dimethyl derivative **1z** was twofold more potent than its 3,4-substituted analog **1s**. However, similar activity was found for 3- and 4-bromo substituted analogs (**1v**, **1f**) as well as for 3- and 4-isopropyl derivatives (**1y**, **1j**). The 3-methoxyphenyl derivative **1w** was slightly less potent than the 3-ethylphenyl analog **1x** indicating a minor negative impact of the electron donating properties of the methoxy group. The 3-methyl-4-isopropylphenyl analog **1aa** was almost equipotent to the 4-isopropylphenyl analog **1j**. Surprisingly, tetrahydronaphthalen-1-yl **1ab** was equipotent to tetrahydronaphthalen-2-yl analog **1ac** while naphthalen-2-yl **1ae** was twofold more potent than the naphthalen-1-yl analog **1ad**.

We selected the aromatic coil A of representative examples (**1a**, **1j**, **1z**) to investigate the SAR at the 4-methoxyphenoxy head C. The analogs **6a–6n** were prepared similarly to the hit (Scheme 2). The biological results are reported in Table 2.²³ First, the *para* substituent of C was varied while keeping the *p*-tolyl coil A (**6a–6h**). Removing the methoxy group from **1a** led to ninefold loss in potency (**6a**). Interestingly, the hydroxyphenoxy analog **6c** was slightly more potent than **1a**, while the potency remained unchanged by installing a trifluoromethoxy (**6d**) or hydroxymethyl (**6g**) group. The ethoxy (**6b**) as well as trifluoromethyl (**6e**) and ethyl (**6f**) substituted compounds were ~2- to 3-fold more potent than the hit, thus suggesting that a hydrogen bond interaction in this position is not fundamental for the potency. The 3,4-dimethoxyphenoxy analog **6h** was ninefold less potent than the hit. The results from the molecules containing the 4-isopropylphenyl coil (**6i–6l**) did not parallel those from the *p*-tolyl series as observed for the 4-hydroxyphenoxy **6i** and 4-trifluoromethoxyphenoxy **6j** derivatives which were less potent than **1j** by ~18- and 45-fold, respectively. Moreover, the 4-hydroxymethylphenoxy analog **6k** was only ~2-fold less potent than **1j** and ~3-fold more potent than **1a**. In the presence of 4-aminoacetyl substituent (**6l**) the potency decreased by eightfold as compared to **1j**.

Within the 3,5-dimethyl series (**6m** and **6n**), the 4-ethoxyphenoxy **6m** was almost equipotent to the 4-methoxyphenoxy derivative **1z** while the 4-hydroxymethylphenoxy analog **6n** was less than twofold less potent than **1z**. Interestingly, since some polar groups were tolerated, the *para* position of C may be exploited for installing solubility-enhancing features and improving physicochemical properties of the hit class.

Next, we modified the pyridazin-3(2*H*)-one region B while maintaining constant the *p*-tolyl or 4-isopropyl phenyl in A and the 4-methoxy or 4-ethoxy phenoxy in C. The synthesis and biological results of compounds **9a–9u** are reported in Schemes 3-6 and Table 3.²³ The 5-bromopyridazin-3(2*H*)-ones **9a–9c** were synthesized analogously to the hit (Scheme 3). Mucobromic acid **7** was condensed with aryl hydrazine **2a**, **2j** or **2t** under acidic conditions to yield 4,5-dibromo pyridazin-3(2*H*)-ones **8a–8c** which underwent nucleophilic addition at position 4 with the phenoxide of **5a** or **5c**. Suzuki coupling of boronic acids **10a–10c** with **9a** led to derivatives **9d–9f** (Scheme 3). Stille coupling of bromide **9b** with tetramethyltin **11** or tributyl(vinyl)tin **12** furnished **9g** and **9h** (Scheme 3). Reduction of the olefin **9h** using palladium/carbon (Pd/C) under hydrogen atmosphere yielded the ethyl derivative **9i**. Oxidative cleavage of **9h** using sodium periodate and osmium tetroxide led to the formation of the aldehyde **9j** which was reduced to the alcohol **9k** using sodium borohydride. Conversion of **9j** into the corresponding oxime followed by dehydration using mesylate chloride furnished the nitrile **9l**. **9j** was oxidized with sodium hypochlorite to the carboxylic acid **9m** which was esterified to **9n** (Scheme 3).

1j was dechlorinated using Pd/C and hydrogen to give **9o** (Scheme 4)^{22b} **1j** reacted with sodium azide to furnish the corresponding azide derivative which was reduced with Pd/C and hydrogen to the corresponding primary amine **9p**. Reaction of **9p** with acetyl chloride furnished **9q** (Scheme 4).

Condensation of 3-methylphenyl hydrazine **2t** with dichloromaleic anhydride **13** under acidic conditions followed by O-methylation using methyl sulfate afforded **15**. Nucleophilic substitution of **5a** on **15** led to the formation of the final product **9r** (Scheme 5).²⁵

The synthesis of **9s** involved the condensation of 4-isopropylphenyl hydrazine **2j** with diethyl ketomalonate **16** followed by acetylation using acetyl chloride/zinc chloride and cyclization using lithium bis(trimethylsilyl)amide at low temperature to furnish the intermediate **17**. Subsequent bromination at position 4 of **17** using bromine followed by chlorination at position 5 using phosphoryl chloride and selective substitution of bromine with **5a** under mild basic conditions provided the title compound (Scheme 6). Hydrolysis of **9s** gave the carboxylic derivative **9t** which was methylated to **9u**. The *p*-tolyl bromide **9a** was ~threefold more potent than **1a**, while the 4-isopropylphenyl **9b** was ~2.5-fold less potent than its chloride analog **1j** and equipotent to the 4-ethoxyphenoxy analog **9c**. Derivatization of **9a** by installing an aromatic ring such as phenyl (**9d**) or furanyl (**9e**) led to drastic loss in potency. Installing a cyclopropyl (**9f**) led to ~eightfold loss in potency compared to **9a**. Within the 4-isopropylphenyl series, the methyl **9g**, vinyl **9h** and ethyl **9j** were ~28-, 9-, and 40-fold less potent than **1j**. Interestingly, hydrogen bond acceptors such as carbaldehyde (**9j**), nitrile (**9l**) and methyl ester (**9n**) were ~23- and ~15- and 10-fold less potent than **1j**, respectively. The primary alcohol **9k** was ~21-fold less potent than **1j** while the carboxylic acid **9m** was inactive. Total loss of potency was found for the unsubstituted **9o**, the amine **9p** and acetamide **9q** analogs. When a methoxy group was introduced in position 6 (**9r**) the potency decreased by ~3.5-fold compared to **1t**. Electron-withdrawing groups in the same position were also detrimental for the activity with the ethyl and methyl carboxylate analogs (**9s**, **9u**) being 13-fold less potent than **1j**. Total loss of activity was observed for the carboxylic acid **9t**.

In order to assess the potential of drug developability, the selectivity profile of **1z** was investigated against a Ricerca panel of 35 therapeutically relevant off-target proteins including GPCRs, enzymes and ion channels. Remarkably, the compound was found to be highly selective against the pool of tested targets (stimulation/inhibition <50% at 10 μ M). A moderate response of 53% inhibition at 10 μ M concentration was only found for CYP450 3A4.²¹ The molecule was non cytotoxic in a viability assay on HT29 cells at the final tested concentration of 20 μ M and was chemically stable in a phosphate buffered saline (PBS) at pH 7.4 with half-life higher than 48 h, thus indicating the lack of reactive sites.²¹ However, **1z** displays poor water solubility (2 μ M in PBS at pH 7.4, room temperature).²¹ Overall, **1z** is as suitable lead molecule for optimization studies addressed at enhancing its in vitro potency and physicochemical properties.

In summary, we have reported the design, synthesis and SAR analysis of novel small molecule NPBWR1 antagonists based on 5-chloro-4-(4-alkoxyphenoxy)-2-(aryl)pyridazin-3(2*H*)-one chemotype. Systematic SAR studies of the MLSMR hit **1a** led to the identification of a lead molecule **1z** (CYM50557) with submicromolar antagonist activity at NPBWR1 and high selectivity against a panel of off-targets. **1z** may provide a novel valuable pharmacological tool to explore the effects of NPBWR1 signaling cascade and probe the molecular basis of the in vivo physiological function and therapeutic utility of the target receptor. Details of further research efforts will be communicated in due course.

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23. The biological inhibition assay employed a chimeric cell line that forces the receptor to use Gq α 3; therefore the assay readout was calcium release. HEK cells stably co-transfected with the human NPBWR1 and Gq α 3 (hGPR7 HEK293T/Gq α 3 cell line) were used for this study. Cells were plated at 10,000 cells/well of a 384 well plate in 25 μ L media and incubated overnight. Next, 25 μ L of Fluo8 NW (ABD Bioquest) was added to all wells and the assay plate incubated for 50 min at 37 $^{\circ}$ C, 5% CO $_2$ and 95% relative humidity. Test compounds were added and the assay plate was incubated for 15 min at room temperature. The assay was started by performing a basal read of fluorescence (495 nm excitation and 515 nm emission) for 15 s on the FLIPR Flexstation II 384 (Molecular Devices). Next, 5.5 μ L of GPR7 agonist (20 nM final concentration = EC $_{80}$) in FLIPR Buffer (HBSS/20 mM Hepes/0.1% BSA) or FLIPR Buffer alone were dispensed to the appropriate wells. Then a real time fluorescence measurement was immediately performed for the remaining 45 s of the assay. Tested compounds were assayed in triplicate in an 8-point 1:3 dilution series starting at a nominal concentration of 20 μ M. For each test compound, percent inhibition was plotted against the log of the compound concentration. A three parameter equation describing a sigmoidal dose-response curve was then fitted using GraphPad Prism (GraphPad Software Inc) normalized from 0 to 100 for each assay. In cases where the highest concentration tested (i.e. 20 μ M) did not result in greater than 50% activation, the IC $_{50}$ was determined manually as greater than 20 μ M.
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25. The regiochemistry of **9r** was verified by 2D ROSE experiment recorded using spectrometer Bruker DRX-600. NOE effects were observed between the protons of the methoxy group on the 4-methoxyphenoxy moiety C (CH $_3$ -reg.C) and the aromatic protons in *ortho* position to this group. The protons of the methoxy group (CH $_3$ - reg.B) at position 6 of the pyridazin-3(2*H*)-one core B gave rise to a single cross-peak with the aromatic proton (Ha) on carbon 2 of the 3-methylphenyl group. A cross-peak was also observed between Ha and the vicinal methyl group. No NOE effects were observed between CH $_3$ - reg.B and the protons of the 4-methoxyphenoxy system.

Figure 1.
In vitro potency of the NPBWR1 antagonist hit.

Scheme 1. Synthesis of **1a-1ae**.

Reagents and conditions: (i) (a) **2a-2ae** (1 equiv.), **3** (1 equiv.), EtOH, rt, 3 h; (b) AcOH, reflux, 3 h; (ii) NaH (1equiv.), **5a** (1 equiv.), **4a-4ae** (1 equiv.), 1,4-dioxane, 0°C to rt, overnight.

Scheme 2. Synthesis of **6a-6n**.Reagents and conditions: (i) NaH (1 equiv.), **5b-5o** (1 equiv.), **4a** or **4j** or **4z** (1 equiv.), 1,4-dioxane, 0°C to rt, overnight.

Scheme 3.
Synthesis of **9a-9n**.

Reagents and conditions: (i) (a) EtOH, rt, 3 h; (b) AcOH, reflux, 3h; (ii) NaH, **5a** or **5c** (1 equiv.), 1,4-dioxane, 0°C to rt, overnight; (iii) **9a** (1 equiv.), **10a-10b** (3 equiv.), Pd(PPh₃)₄, Na₂CO₃ (2 equiv.), 1,4-dioxane/H₂O, 80°C, 6h; (iv) **9b** (1 equiv.), **11** (2 equiv.), PdCl₂[P(*o*-tolyl)]₂ (0.1 equiv.), DMA, 90°C, 6h; (v) **9b** (1 equiv.), **12** (1 equiv.), PdCl₂(PPh₃)₂ (0.1 equiv.), toluene, 110°C, 6h; (vi) **9h** (1 equiv.), H₂, Pd/C 10%, EtOH, rt, 8h; (vii) **9h** (1 equiv.), OsO₄ (0.1 equiv.), NaIO₄ (2.5 equiv.), THF/H₂O (2/1), rt, overnight; (viii) **9j** (1 equiv.), NaBH₄ (0.33 equiv.), EtOH, rt, 1h.; (ix) (a) **9j** (1 equiv.), NH₂OH·HCl (1 equiv.), EtOH, 70°C, 2h.; (b) MeSO₂Cl, DCE, 80°C, 5h; (x) **9j** (1 equiv.), NaOCl₂ (1.5 equiv.), NaH₂PO₄ (3 equiv.), H₂O₂ (1.2 equiv.), tBuOH/DMF, 0°C to rt, 3h; (xi) **9m** (1 equiv.), EDCI (1.5 equiv.), HOBt (1.5 equiv.), MeOH (2 equiv.), CH₂Cl₂, rt, 4h.

Scheme 4. Synthesis of **9o**–**9q**.

Reagents and conditions: (i) **1j** (1 equiv.), H_2 , Pd/C 10%, EtOH, rt, 2h; (ii) (a) **1j** (1 equiv.), NaN_3 (5 equiv.), DMF, 1h; (b) H_2 , Pd/C 10%, EtOH, rt, 4h; (iii) **9p** (1 equiv.), AcCl (1.3 equiv.), DIPEA (1.3 equiv.), CH_2Cl_2 , rt, overnight.

Scheme 5. Synthesis of **9r**.

Reagents and conditions: (i) **2t** (1 equiv.), **13** (1 equiv.), 20%, HCl, 100°C, 3 h; (ii) **14** (1 equiv.), Me₂SO₄ (1.3 equiv.), K₂CO₃ (3 equiv.), acetone, reflux, 3 h; (iii) **15** (1 equiv.), **5a** (1 equiv.), K₂CO₃ (1 equiv.), THF, reflux, overnight.

Scheme 6. Synthesis of **9s**–**9u**.

Reagents and conditions: (i) **2j** (1 equiv.), **16** (1.1 equiv.), MgSO_4 (1.1 equiv.), CHCl_3 , 0°C to rt, overnight; (ii) AcCl (21 equiv.), ZnCl_2 (7.3 equiv.), rt, 48h; (iii) LHMDS (1.1 equiv.), THF, -78°C to -40°C , 90 min; (iv) **17** (1 equiv.), Br_2 (1.1 equiv.), EtOH, 0°C , 7 min; (v) POCl_3 , 85°C , 12h; (vi) **5a** (1 equiv.), K_2CO_3 (1 equiv.), THF, reflux, overnight; (vii) **9s** (1 equiv.), 1N NaOH (5 equiv.), EtOH, rt, 30 min; (viii) **9t** (1 equiv.), K_2CO_3 (5 equiv.), MeI (5 equiv.), DMF, rt, overnight.

Table 1

NPBWR1 antagonist activity of **1a–1ae**

Compd.	Ar	IC ₅₀ (μM) ^a
1a	4-Methylphenyl	2.20
1b	Phenyl	13.00
1c	4-Fluorophenyl	3.60
1d	4-Trifluoromethylphenyl	0.75
1e	4-Isopropoxyphenyl	0.77
1f	4-Bromophenyl	0.69
1g	4-Chlorophenyl	1.77
1h	4-Ethylcarboxylatephenyl	1.20
1i	4-Ethylphenyl	0.58
1j	4-Isopropylphenyl	0.27
1k	4-Cyclopropylphenyl	1.10
1l	4-(<i>sec</i> -Butyl)phenyl	0.65
1m	4-(<i>n</i> -Pentyl)phenyl	2.57
1n	4-(<i>tert</i> -Butyl)phenyl	0.68
1o	4-Phenylphenyl	1.71
1p	2,4-Dimethylphenyl	0.98
1q	2,5-Dimethylphenyl	1.40
1r	2,3-Dimethylphenyl	1.30
1s	3,4-Dimethylphenyl	0.59
1t	3-Methylphenyl	0.57
1u	3-Chlorophenyl	0.69
1v	3-Bromophenyl	0.71
1w	3-Methoxyphenyl	0.41
1x	3-Ethylphenyl	0.23
1y	3-Isopropylphenyl	0.28
1z	3,5-Dimethylphenyl	0.27
1aa	3-Methyl-4-isopropylphenyl	0.30
1ab	5,6,7,8-Tetrahydronaphthalen-1-yl	0.65
1ac	5,6,7,8-Tetrahydronaphthalen-2-yl	0.65
1ad	Naphthalen-1-yl	0.36
1ae	Naphthalen-2-yl	0.16

^aData are reported as mean of *n* = 3 determinations.

Table 2NPBWR1 antagonist activity of **6a–6n**

Compd.	Ar	Ar ¹	IC ₅₀ (μM) ^a
6a	4-Methylphenyl	Phenyl	20.00
6b	4-Methylphenyl	4-Ethoxyphenyl	0.99
6c	4-Methylphenyl	4-Hydroxyphenyl	1.30
6d	4-Methylphenyl	4-Trifluoromethoxyphenyl	2.20
6e	4-Methylphenyl	4-Trifluoromethyl	0.88
6f	4-Methylphenyl	4-Ethylphenyl	0.68
6g	4-Methylphenyl	4-Hydroxymethylphenyl	2.30
6h	4-Methylphenyl	3,4-Dimethoxyphenyl	20.00
6i	4-Isopropylphenyl	4-Hydroxyphenyl	4.80
6j	4-Isopropylphenyl	4-Trifluoromethoxyphenyl	12.20
6k	4-Isopropylphenyl	4-Hydroxymethylphenyl	0.65
6l	4-Isopropylphenyl	4-Aminoacetylphenyl	2.10
6m	3,5-Dimethylphenyl	4-Ethoxyphenyl	0.21
6n	3,5-Dimethylphenyl	4-Hydroxymethylphenyl	0.44

^aData are reported as mean of *n* = 3 determinations.

Table 3

NPBWR1 antagonist activity of **9a–9u**

Compd.	Ar	R	R ¹	R ²	IC ₅₀ (μM) ^a
9a	4-Methylphenyl	Methoxy	Bromine	H	0.78
9b	4-Isopropylphenyl	Methoxy	Bromine	H	0.72
9c	4-Isopropylphenyl	ethoxy	Bromine	H	0.72
9d	4-Methylphenyl	Methoxy	Phenyl	H	>20
9e	4-Methylphenyl	Methoxy	Furan-2-yl	H	14.28
9f	4-Methylphenyl	Methoxy	Cyclopropyl	H	6.20
9g	4-Isopropylphenyl	Methoxy	Methyl	H	7.50
9h	4-Isopropylphenyl	Methoxy	Vinyl	H	2.40
9i	4-Isopropylphenyl	Methoxy	Ethyl	H	10.70
9j	4-Isopropylphenyl	Methoxy	Carbaldehyde	H	6.20
9k	4-Isopropylphenyl	Methoxy	Hydroxymethyl	H	5.60
9l	4-Isopropylphenyl	Methoxy	Nitrile	H	4.20
9m	4-Isopropylphenyl	Methoxy	Carboxylic acid	H	>20
9n	4-Isopropylphenyl	Methoxy	Methyl carboxylate	H	2.7
9o	4-Isopropylphenyl	Methoxy	H	H	>20
9p	4-Isopropylphenyl	Methoxy	Amine	H	>20
9q	4-Isopropylphenyl	Methoxy	Acetamide	H	>20
9r	3-Methylphenyl	Methoxy	Chlorine	Methoxy	2.10
9s	4-Isopropylphenyl	Methoxy	Chlorine	Ethyl Carboxylate	3.60
9t	4-Isopropylphenyl	Methoxy	Chlorine	Carboxylate	>20
9u	4-Isopropylphenyl	Methoxy	Chlorine	Methyl carboxylate	3.60

^aData are reported as mean of *n* = 3 determinations.