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# Discovery of Benzamidine- and 1-Aminoisoquinoline-Based Human MAS-Related G-Protein-Coupled Receptor X1 (MRGPRX1) Agonists

Eva Prchalová,[†,‡] Niyada Hin,[†] Ajit G. Thomas,[†] Vijayabhaskar Veeravalli,[†,‡] Justin Ng,[†] Jesse Alt,[†] Rana Rais,[†,‡] Camilo Rojas,[†,§] Zhe Li,[∥] Hiroe Hihara,[⊥] Mika Aoki,[⊥] Kyoko Yoshizawa,[⊥] Tomoki Nishioka,[⊥] Shuichi Suzuki,[⊥] Theresa Kopajtic,[#] Sheena Chatrath,[▽] Qin Liu,[▽] Xinzhong Dong,[∥] Barbara S. Slusher,[†,‡,∥] and Takashi Tsukamoto*[,†,‡]

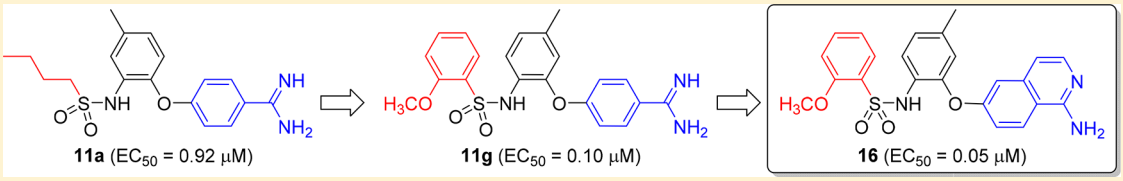
[†]Johns Hopkins Drug Discovery, [‡]Department of Neurology, [§]Department of Molecular and Comparative Pathobiology, and [∥]Solomon H. Snyder Department of Neuroscience, Johns Hopkins University, Baltimore, Maryland 21205, United States

[⊥]Tsukuba Research Laboratories, Eisai Co., Ltd., Tsukuba, Ibaraki 300-2635, Japan

[#]Biobehavioral Imaging and Molecular Neuropsychopharmacology Unit, National Institute on Drug Abuse-Intramural Research Program, National Institutes of Health, Baltimore, Maryland 21224, United States

[▽]Department of Anesthesiology and Center for the Study of Itch, Washington University School of Medicine, St. Louis, Missouri 63110, United States

Supporting Information




**ABSTRACT:** Mas-related G-protein-coupled receptor X1 (MRGPRX1) is a human sensory neuron-specific receptor and has been actively investigated as a therapeutic target for the treatment of pain. By use of two HTS screening hit compounds, 4-(4-(benzyloxy)-3-methoxybenzylamino)benzimidamide (**5a**) and 4-(2-(butylsulfonamido)-4-methylphenoxy)benzimidamide (**11a**), as molecular templates, a series of human MRGPRX1 agonists were synthesized and evaluated for their agonist activity using HEK293 cells stably transfected with human MrgprX1. Conversion of the benzamidine moiety into a 1-aminoisoquinoline moiety carried out in the later stage of structural optimization led to the discovery of a highly potent MRGPRX1 agonist, *N*-(2-(1-aminoisoquinolin-6-yloxy)-4-methylphenyl)-2-methoxybenzenesulfonamide (**16**), not only devoid of positively charged amidinium group but also with superior selectivity over opioid receptors. In mice, compound **16** displayed favorable distribution to the spinal cord, the presumed site of action for the MRGPRX1-mediated analgesic effects.

## INTRODUCTION

A subset of Mas-related G-protein-coupled receptors (Mrgprs) is expressed specifically in the small diameter DRG sensory neurons and is implicated in the modulation of nociception.[1] Among these, human homologue MRGPRX1 has gained increased interest as a promising therapeutic target partly owing to the analgesic effects of BAM8-22 (Figure 1),[2] a proteolytic product derived from proenkephalin A with full agonist activity at human MRGPRX1 and its closest rodent orthologues, mouse MRGPRC11 and rat MRGPRC.[3] For example, intrathecal (spinal cord) injection of BAM8-22 was found to attenuate both mechanical and thermal hyperalgesia in mice[4] and rats.[5] We have also found that JHU-58 (Figure 1), an Arg-Phe-$NH_2$ peptidomimetic with full agonist activity at mouse MRGPRC11 and rat MRGPRC,[6] exhibits analgesic effects in rodent models of neuropathic pain.[5] JHU-58, however, displayed negligible agonist activity at human MRGPRX1, hindering its ability to serve as a molecular template for further structural optimization efforts aimed at clinical translation. Human MRGPRX1 agonists **1** and **2** (Figure 1) reported by GSK[7] and ACADIA,[8] respectively, may have a potential to be explored as new leads though their high molecular weight (>500) likely contributes to unfavorable CNS drug-like molecular properties and solubility. Indeed, compound **1** could not be tested in vitro at concentrations above 2 $\mu$M due to its limited solubility.[6]

This prompted us to conduct high throughput screening of Eisai's compound library in HEK293 cells stably transfected with human MRGPRX1 by using FLIPR calcium 4 assay kit (Molecular Devices) to measure intracellular calcium responses.[9] We identified two submicromolar hits **5a** and **11a** (Figure 2) as full agonists with $EC_{50}$ values of 0.51 $\mu$M and 0.92 $\mu$M, respectively. The common structural feature of the two lead compounds is obviously the presence of benzamidine moiety, which is known as a bioisostere of a guanidyl group of

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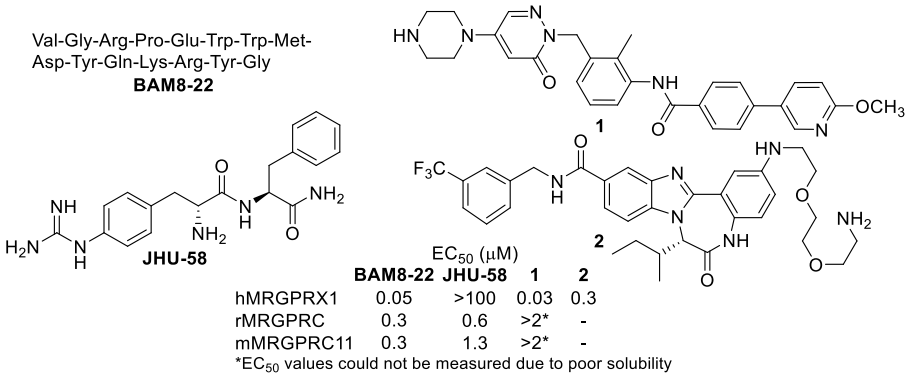



**Figure 1.** Representative Mrgpr agonists.

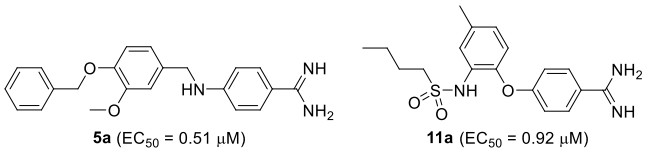



**Figure 2.** New lead human MRGPRX1 agonists **5a** and **11a** identified by HTS.

arginine. Thus, it is conceivable that the benzamidine portion of these lead compounds shares a binding site with the Arg residue of BAM8-22. While compound **1** is more potent than **5a** and **11a**, what differentiates these HTS hits from the existing human MRGPRX1 agonists **1** and **2** is their higher ligand efficiency[10] (LE = 0.32−0.33) values owing to their lower molecular weight (MW = 361) as compared to those of **1** (LE = 0.27) and **2** (LE = 0.19).

Here, we describe the design, synthesis, and structure−activity relationships (SARs) of a new series of human MRGPRX1 agonists using the new leads **5a** and **11a** as molecular templates. Our optimization efforts aimed at not only improving agonist potency but also replacing the benzamidine group with other functional groups with reduced basicity in an attempt to improve CNS permeability. These efforts led to the discovery of a highly potent and CNS-penetrant agonist potentially valuable in exploring the therapeutic utility of human MRGPRX1 activation in neuropathic pain.

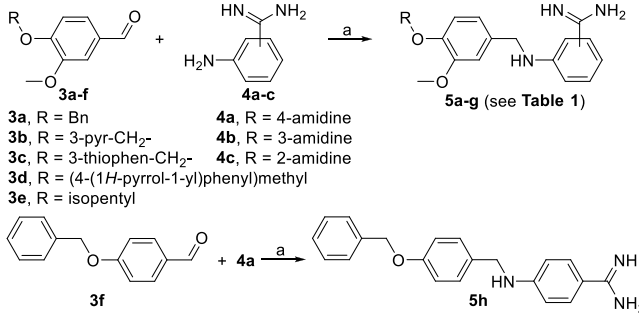
## CHEMISTRY

Compounds **5a−h** were synthesized by reductive amination of substituted benzaldehydes **3a−f** with aminobenzamidines **4a−c** mediated by $NaCNBH_3$ (Scheme 1).

Compounds **11a−i** were synthesized in four steps starting with nucleophilic aromatic substitution of 1-fluoro-2-nitrobenzene derivatives **6a−c** with hydroxybenzonitriles **7a−c** in the presence of potassium carbonate as a base (Scheme 2).[11] The nitro group of resulting 2-nitrodiphenyl ethers **8a−e** underwent catalytic hydrogenation, yielding the corresponding amines **9a−e**. Reaction of **9a−e** with sulfonyl chlorides afforded sulfonamides **10a−i**. Conversion of the nitrile group of **10a−i** into amidine group via Pinner reaction followed by treatment with ammonia[12] gave the desired compounds **11a−i**.

As shown in Scheme 3, we also synthesized analogs of compounds **5a** and **11a**, in which the highly basic amidine moiety is replaced by less basic bioisosteres. Compound **13** containing an 1-aminoisoquinoline was synthesized by reductive amination of benzaldehyde **3a** with isoquinoline-

**Scheme 1. Synthesis of Compounds 5a−h**[a]




[a]Reagents and conditions: (a) $NaCNBH_3$, DIEA, AcOH, EtOH, rt.

1,6-diamine **12** mediated by $NaCNBH_3$. Sulfonamide-based agonists **16** and **19** were obtained from **6b**, which was first coupled with **14** or **17** in the presence of potassium carbonate to give **15** or **18**, respectively. Catalytic hydrogenation of the nitro group of **15** and **18** followed by reaction with 2-methoxybenzene-1-sulfonyl chloride in pyridine provided the desired compounds **16** and **19**.

## RESULTS AND DISCUSSION

In vitro experiments measuring agonist activity at MRGPRX1 were performed in a FLIPR assay using HEK293 cells stably transfected with human MrgprX1. The results are summarized in Table 1. All of the compounds acted as full MRGPRX1 agonists ($E_{max}$ > 95% of BAM8-22) except for compounds **5c** and **19**, which showed negligible agonist activity at concentrations up to 100 $\mu$M. It appears that the position of the amidine group has significant impact on the agonist activity. Meta- (compound **5b**) and ortho-substitution (compound **5c**) resulted in 6-fold and >100-fold decreases in potency, respectively, as compared to **5a**. This suggests the critical role played by the para-amidine group of compound **5a** in strong interaction with MRGPRX1. Replacement of the terminal benzyl group with a 3-pyridinylmethyl group led to 10-fold loss of potency (compound **5d**). In contrast, substitution with 3-thiophenylmethyl group (compound **5e**) and [4-(1*H*-pyrrol-1-yl)phenyl]methyl (compound **5f**) resulted in similar potency to **5a**. Meanwhile, both compound **5g** containing an aliphatic group instead of the benzyl group and compound **5h** devoid of the methoxy group from the linker region exhibited decreased agonist potency.

Given the preference for para-amidine substitution observed in analogs of **5a**, SAR studies on lead compound **11a** focused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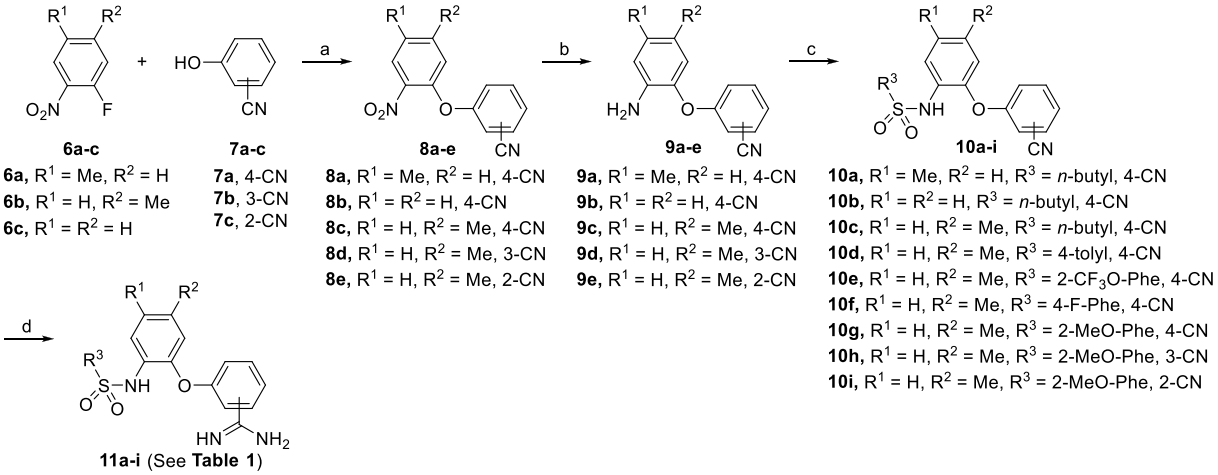
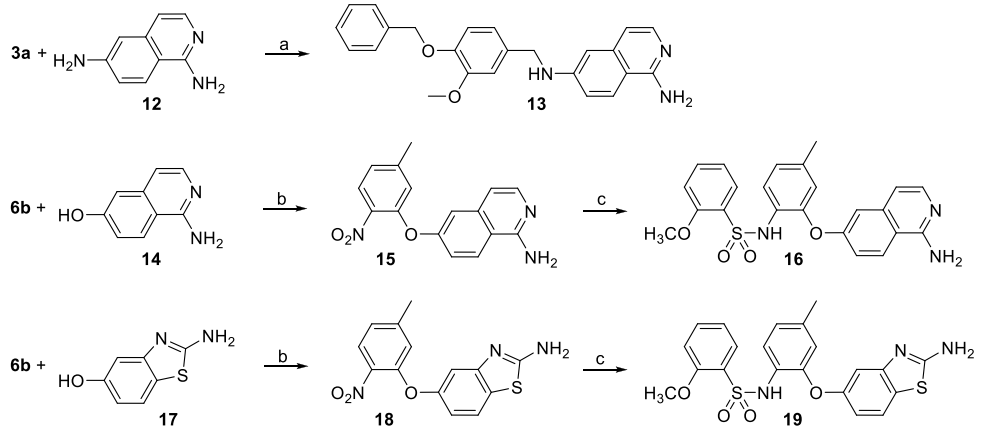
**Scheme 2. Synthesis of Compounds 11a–i**[a]




[a]Reagents and conditions: (a) $K_2CO_3$, DMF, 50 °C; (b) $H_2$, 10% Pd/C, MeOH, 20–30 psi, 30 min; (c) $R_2$-$SO_2Cl$, pyridine, 50 °C, 1.5 h; (d) (i) HCl (gas), MeOH, 0 °C to rt, (ii) 7 N $NH_3$ in MeOH, 65 °C, 1 h.

**Scheme 3. Synthesis of Compounds 13, 16, and 19**[a]




[a]Reagents and conditions: (a) $NaCNBH_3$, DIEA, AcOH, EtOH, rt; (b) ArOH, $K_2CO_3$, DMF, 50 °C; (c) (i) $H_2$, Pd/C, MeOH, 20–30 psi, 30 min; (ii) 2-methoxybenzene-1-sulfonyl chloride, pyridine, 50 °C, 1.5 h.

on its non-benzamidine portion. Structural changes in the internal linker and/or the terminal alkylsulfonamide group had various impact in MRGPRX1 agonist potency as seen in compounds **11a–g**. The most potent agonists within this series, compounds **11e** and **11g**, exhibited $EC_{50}$ values of 150 and 100 nM, respectively. Using compound **11g** as a template, we moved the amidine group to the meta (compound **11h**) and the ortho (compound **11i**) positions. Both compounds exhibited decreased agonist potency compared to **11g**, confirming the critical role played by the para-amidine group in MRGPRX1 agonist potency.

Compounds **13**, **16**, and **19** possess a potential bioisostere of the benzamidine group, an effective strategy often used in inhibitors of serine proteases involved in the coagulation cascade.[13−16] Compound **13** contains a 1-aminoisoquinoline instead of the benzamidine group of compound **5a**. This modification resulted in decline of agonist potency from 0.51 $\mu$M (compound **3a**) to 3.7 $\mu$M (compound **13**). In contrast, similar modification to compound **11g** resulted in the most potent agonist **16** with an $EC_{50}$ value of 0.05 $\mu$M. Compound **19** containing a 2-aminobenzothiazole, however, showed no agonist activity at concentrations up to 100 $\mu$M. The discovery of agonist **16** is significant not only because of its high agonist potency but also because of its substantially lower p$K_a$ value for the 1-amino group of the aminoisoquinoline (p$K_a$ ∼ 7.5) as compared to that of the benzamidine moiety (p$K_a$ ∼ 12.5) of compound **11g**.[13−16]

Given the complete loss of agonist activity for ortho-amidine analog **5c**, structurally close compounds **5a** and **5c** served as ideal positive and negative controls in target engagement studies. Activation of MRGPRX1 is known to inhibit high-voltage-activated (HVA) $Ca^{2+}$ currents, which reduces neurotransmitter release and attenuates spinal nociceptive transmission.[17] To this end, we assessed BAM8-22, potent agonist **5a**, and compound **5c** devoid of MRGPRX1 agonist activity for their inhibitory effect on HVA $Ca^{2+}$ currents in cultured human MRGPRX1-expressing DRG neurons obtained from humanized *MrgprX1* mice.[17] As shown in Figure 3, both BAM8-22 and compound **5a** inhibited HVA $Ca^{2+}$ currents while compound **5c** showed no inhibitory effects, indicating the MRGPRX1-dependent mechanism involved in inhibition of HVA $Ca^{2+}$ currents.

Given the close relation between opioid and MrgC receptors in regard to ligand pharmacology[2,3] and function,[18,19] we


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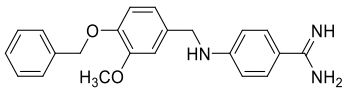
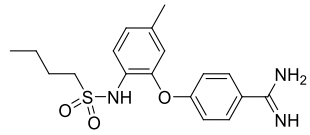
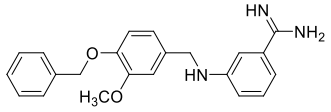
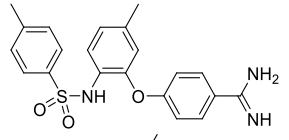
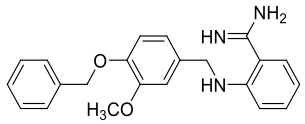
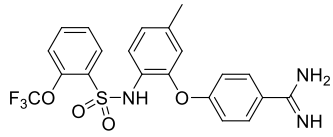
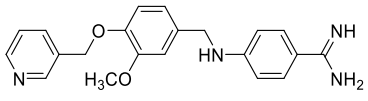
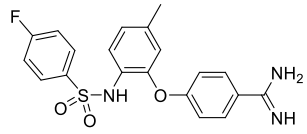
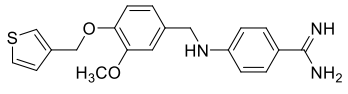
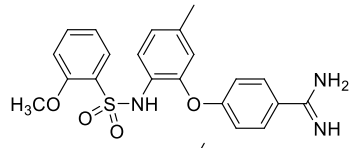
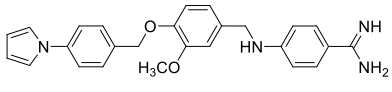
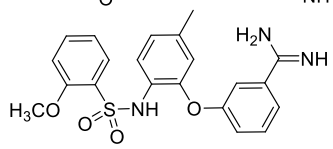
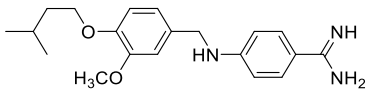
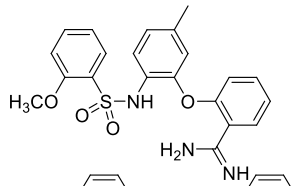
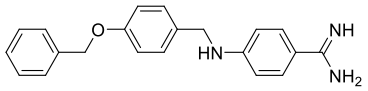
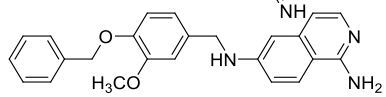
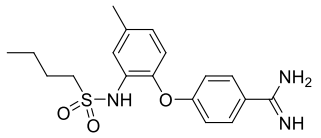
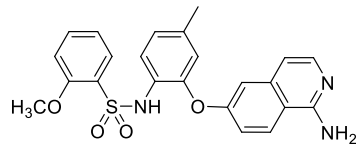
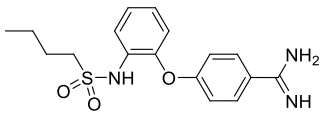
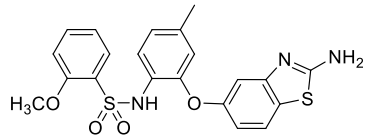
**Table 1. In Vitro MRGPRX1 Agonist Potency of Compounds 5a–h, 11a–i, 13, 16, and 19**

| Cmpd | Structure | $EC_{50}$ (μM)[a] | Cmpd | Structure | $EC_{50}$ (μM)[a] |
|---|---|---|---|---|---|
| **5a** |  | 0.51 ± 0.49 | **11c** |  | 2.2 ± 1.0 |
| **5b** |  | 3.3 ± 1.8 | **11d** |  | 0.16 ± 0.08 |
| **5c** |  | >100 | **11e** |  | 0.15 ±0.04 |
| **5d** |  | 5.2 ± 0.9 | **11f** |  | 2.1 ± 0.4 |
| **5e** |  | 0.29 ±0.06 | **11g** |  | 0.10 ±0.09 |
| **5f** |  | 0.68 ± 0.20 | **11h** |  | 3.6 ± 1.5 |
| **5g** |  | 1.9 ± 0.9 | **11i** |  | 23 ± 7 |
| **5h** |  | 5.2 ± 1.1 | **13** |  | 3.7 ± 0.6 |
| **11a** |  | 0.92 ± 0.47 | **16** |  | 0.05 ± 0.02 |
| **11b** |  | 0.30 ± 0.12 | **19** |  | >100 |

[a]Values are the mean ± SD of at least four experiments.

assessed the selectivity of the two most potent MRGPRX1 agonists **11g** and **16** over δ-, μ-, and κ-opioid receptors by radioligand binding assays. As shown in Table 2, benzamidine-based agonist **11g** exhibited substantial affinity to δ- and μ-opioid receptors. Compound **16**, however, exhibited 50-fold or greater selectivity over all three receptors, unmasking additional advantage brought by the 1-aminoisoquinoline moiety. Compound **16** was also found to be inactive against mouse MRGPRC11 even at concentration of 40 μM (see the Supporting Information).

Given its reduced basicity, potent MRGPRX1 agonist activity, and good selectivity over opioid receptors, we selected compound **16** for in vivo pharmacokinetics studies in mice. In addition to plasma levels, we measured drug levels in spinal cord, which is the site of action for MRGPRX1 agonists to attenuate spinal nociceptive transmission. As shown in Figure 4, substantial levels of **16** were detected in plasma and spinal



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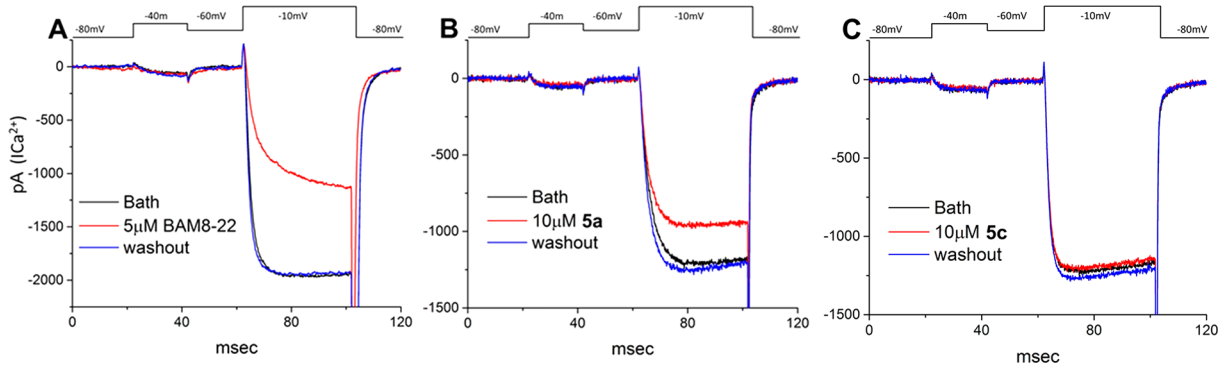


**Figure 3.** Traces of HVA $Ca^{2+}$ currents evoked by depolarization before (black) and after (red) bath application of 5 $\mu$M BAM8-22 (A), 10 $\mu$M **5a** (B), and 10 $\mu$M **5c** (C). Currents were recorded by whole-cell patch clamp recording of DRG neurons from *MrgprX1* mice. HVA $Ca^{2+}$ currents are inward currents (negative nA) evoked by −10 mV depolarization.

**Table 2. Binding Affinities ($K_i$) of 11g and 16 to Opioid Receptors**

| | $K_i$ (nM) | | |
|---|---|---|---|
| compd | $\delta$ ([$^3$H]DADLE) | $\mu$ ([$^3$H]DAMGO) | $\kappa$ ([$^3$H]U-69,593) |
| **11g** | 220 | 72.7 | 1430 |
| **16** | 2470 | 3730 | 12100 |

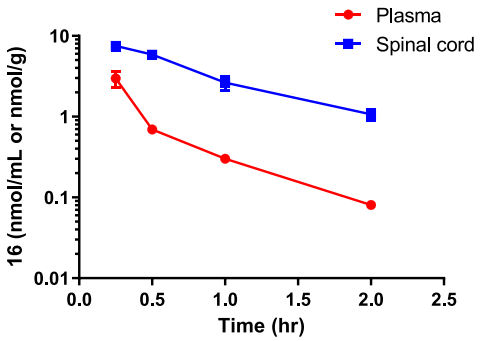


**Figure 4.** Pharmacokinetics of **16** in mice (10 mg/kg, iv).

cord (AUC: 0.90 ± 0.09 nmol·h/mL plasma vs 5.66 ± 0.33 nmol·h/g spinal cord) following intravenous administration of **16** at 10 mg/kg dose in male CD1 mice ($n$ = 3 for each time point), resulting in a spinal cord to plasma ratio of 6.3. While this is encouraging, **16** had a half-life of approximately 0.33 h. Subsequent studies revealed that **16** is extensively metabolized ($t_{1/2}$ < 15 min) in mouse liver microsomes (results not shown), which likely contributed to the short half-life observed in mice after intravenous administration.

## CONCLUSIONS

Chronic pain remains one of the most prevalent yet undertreated health problems. Furthermore, given the ongoing opioid crisis, there is a critical unmet medical need for new chronic pain therapies devoid of abuse potential and adverse effects. Restricted expression of human MRGPRX1 in primary nociceptive neurons could provide a substantial advantage to its agonists over opioid-based analgesics in treating pain. By use of two amidine-based hit compounds **5a** and **11a** as molecular templates, systematic lead optimization was carried out in an attempt to improve potency and drug-like molecular properties. In particular, successful conversion of the benzamidine moiety of **11g** into a 1-aminoisoquinoline moiety without loss of agonist potency led to a highly potent MRGPRX1 agonist **16** not only devoid of positively charged amidinium group but also with superior selectivity over opioid receptors. Compound **16** displayed favorable distribution to the spinal cord, presumably due to the reduced p$K_a$ value for the 1-amino group of the aminoisoquinoline as compared to that of the benzamidine moiety. Although compound **16** showed a high degree of clearance, the preferential distribution to the spinal cord over the circulatory system is particularly appealing since it will minimize the itch side effect caused by peripheral MRGPRX1 activation. Given its high selectivity over opioid receptors, compound **16** appears to be a promising agent that merits further investigation. Compound **16**, however, failed to act as an agonist at mouse MrgprC11, limiting its utility in conventional mouse models of pain. It should be noted, however, that humanized *MrgprX1* mice have been developed to overcome the limited cross-species reactivity of human MRGPRX1 agonists on the murine receptors.[17] These mice lack a cluster containing most MrgprA and MrgprC genes including MrgprC11 and are well suited for studying the pharmacology of human MRGPRX1 modulation. Indeed, intrathecal injection of BAM8-22 was found to inhibit heat hypersensitivity and spontaneous pain in *MrgprX1* mice after peripheral nerve injury,[17] demonstrating the utility of this strain in evaluating human MRGPRX1 agonists. Studies are underway to evaluate our human MRGPRX1 agonists in the humanized mice and improve the metabolic stability in liver microsomes to achieve sustained exposure. Collectively, these efforts may lead to the development of viable treatment options for chronic pain.

## EXPERIMENTAL SECTION

**General.** All solvents were reagent grade or HPLC grade. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Syntheses of compounds **3**,[20] **4c**,[21] and **14**[22] were previously reported. Compounds **3c**, **3d**, and **3e** were obtained by Mitsunobu reactions of 4-hydroxy-3-methoxybenzaldehyde with the corresponding alcohols (ROH) by following the method previously reported for similar reactions.[23] Melting points were obtained on a Mel-Temp apparatus and are uncorrected. $^1$H NMR spectra were recorded at 400 or 500 MHz. $^{13}$C NMR spectra were recorded at 101 or 125 MHz. The HPLC solvent system consisted of deionized water and acetonitrile, both containing 0.1% formic acid. Preparative HPLC purification was performed on an Agilent 1200 series HPLC system equipped with an Agilent G1315D DAD detector using a Phenomenex Luna 5 $\mu$m C18 column (21.2 mm × 250 mm, 5 $\mu$m). Analytical HPLC was performed on an Agilent 1200 series HPLC system equipped with an Agilent G1315D DAD detector (detection at 220 nm) and an Agilent 6120 quadrupole


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MS detector. Unless otherwise specified, the analytical HPLC conditions involve the following: (A) for nonpolar compounds 20% acetonitrile/80% water for 0.25 min followed by gradient to 85% acetonitrile/15% water over 1.5 min and continuation of 85% acetonitrile/15% water for 2.25 min with a Luna C18 column (2.1 mm × 50 mm, 3.5 μm) at a flow rate of 1.25 mL/min; (B) for polar compounds 5% acetonitrile/95% water for 0.25 min followed by gradient to 40% acetonitrile/60% water over 1.5 min and continuation of 85% acetonitrile/15% water for 2.25 min with a Luna C18 column (2.1 mm × 50 mm, 3.5 μm) at a flow rate of 1.25 mL/min. Unless otherwise noted, all final compounds biologically tested were confirmed to be of ≥95% purity by the HPLC methods described above.

**4-(4-(Benzyloxy)-3-methoxybenzylamino)benzimidamide (5a).** 4-(Benzyloxy)-3-methoxybenzaldehyde **3a** (300 mg, 1.24 mmol), 4-aminobenzimidamide dihydrochloride **4a** (184 mg, 1.36 mmol), DIEA (2.48 mmol, 0.43 mL), and a trace of bromocresol green were dissolved in ethanol (5 mL) at rt, and the mixture was stirred for 15 min. Acetic acid was added dropwise until the blue solution turned slightly green and the resulting mixture was stirred at rt for 15 min. Sodium cyanoborohydride (311 mg, 4.95 mmol) was added portionwise at rt, and the reaction was stirred overnight. Volatiles were removed under reduced pressure, and the residue was dissolved in EtOAc, washed with 2 N NaOH, water and brine. The organic layer was dried over magnesium sulfate, filtered and solvents were evaporated to give a white solid which was purified using preparative HPLC (method 20−70%: flow rate of 25 mL/min; gradient of 20% acetonitrile/80% $H_2O$ for 5.0 min followed by an increase to 70% acetonitrile/30% $H_2O$ over 40 min and continuation of 70% acetonitrile/30% $H_2O$ until 50 min) to give 94 mg (21% yield) of the title compound as white solid; mp 181 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.75 (s, 3H), 4.28 (d, $J$ = 6.1 Hz, 2H), 5.04 (s, 2H), 6.69 (d, $J$ = 9.1 Hz, 2H), 6.82 (dd, $J$ = 1.8, 8.2 Hz, 2H), 6.96 (d, $J$ = 8.1 Hz, 1H), 7.00 (d, $J$ = 1.8, 1H), 7.32 (m, 1H), 7.36−7.43 (m, 5H), 7.61 (d, $J$ = 9.1 Hz, 2H), 8.74 (bs, 3H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 45.6, 55.5, 69.9, 111.5, 113.5, 113.7, 119.2, 127.7, 128.4, 129.2, 131.9, 137.2, 146.7, 149.1, 153.0, 164.9. LCMS: (A) retention time 1.53 min, $m/z$ = 362 $[M + H]^+$.

**3-(4-(Benzyloxy)-3-methoxybenzylamino)benzimidamide (5b).** Compound **5b** was prepared as described for the preparation of **5a** except 3-aminobenzimidamide **4b** was used in place of 4-aminobenzimidamide **4a** and the compound was obtained by recrystallization in EtOAc/hexanes. Light blue solid (34% yield); mp 153 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.75 (s, 3H), 4.22 (d, $J$ = 6.1 Hz, 2H), 5.04 (s, 2H), 6.49 (m, 1H), 6.78−6.88 (m, 3H), 6.94−7.00 (m, 3H), 7.17 (m, 1H), 7.32−7.41 (m, 4H), 8.24 (bs, 3H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 46.1, 55.5, 69.9, 110.5, 111.6, 113.5, 114.5, 116.1, 119.3, 127.8, 128.4, 129.1, 132.5, 137.3, 146.7, 148.9, 149.1, 165.7. LCMS: (A) retention time 1.55 min, $m/z$ = 362 $[M + H]^+$.

**2-(4-(Benzyloxy)-3-methoxybenzylamino)benzimidamide (5c).** Compound **5c** was prepared as described for the preparation of **5a** except 2-aminobenzimidamide dihydrochloride **4c** was used in place of 4-aminobenzimidamide dihydrochloride **4a** and the residue was purified using a Biotage Isolera One flash purification system with a silica gel cartridge (EtOAc/hexanes, 2% $Et_3N$), followed by a second purification by preparative HPLC (method 20−70%: flow rate of 25 mL/min; gradient of 20% acetonitrile/80% $H_2O$ for 5.0 min followed by an increase to 70% acetonitrile/30% $H_2O$ over 40 min and continuation of 70% acetonitrile/30% $H_2O$ until 50 min). Off-white solid foam (4% yield); mp 172 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.76 (s, 3H), 4.28 (bs, 2H), 5.04 (s, 2H), 6.59 (dd, $J$ = 8.8, 17.2 Hz, 2H), 6.89 (dd, $J$ = 8.3, 19.7 Hz, 2H), 7.05 (d, $J$ = 1.5, 1H), 7.23 (m, 2H), 7.31 (m, 1H), 7.36−7.43 (m, 4H), 8.44 (s, 1H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 46.2, 55.5, 69.9, 111.4, 111.7, 113.4, 115.2, 115.5, 119.0, 127.8, 128.4, 129.0, 132.4, 137.3, 145.8, 146.6, 149.1, 166.4, 167.9. LCMS: (B) retention time 2.50 min, $m/z$ = 362 $[M + H]^+$.

**4-(3-Methoxy-4-(pyridin-3-ylmethoxy)benzylamino)-benzimidamide (5d).** Compound **5d** was prepared as described for the preparation of **5a** except 3-methoxy-4-(pyridin-3-ylmethoxy)-benzaldehyde **3b** was used in place of 4-(benzyloxy)-3-methoxybenzaldehyde **3a** and the compound was purified using preparative HPLC (method 20−70%: flow rate of 25 mL/min; gradient of 20% acetonitrile/80% $H_2O$ for 5.0 min followed by an increase to 70% acetonitrile/30% $H_2O$ over 40 min and continuation of 70% acetonitrile/30% $H_2O$ until 50 min). White fluffy solid (35% yield); mp 87 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.75 (s, 3H), 4.29 (d, $J$ = 6.1 Hz, 2H), 5.09 (s, 2H), 6.69 (d, $J$ = 9.1 Hz, 2H), 6.83 (dd, $J$ = 2.0, 7.8 Hz, 1H), 7.00 (dd, $J$ = 2.5, 4.3 Hz, 2H), 7.32 (d, $J$ = 6.3 Hz, 1H), 7.41 (dd, $J$ = 4.8, 7.8 Hz, 1H), 7.58 (d, $J$ = 8.8 Hz, 2H), 7.83 (d, $J$ = 8.1 Hz, 1H), 8.32 (bs, 2H), 8.53 (dd, $J$ = 1.5, 4.8 Hz, 1H), 8.63 (d, $J$ = 2.0 Hz, 1H), 8.74 (bs, 1H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 45.5, 55.6, 67.8, 111.6, 113.0, 113.9, 119.2, 123.6, 129.6, 132.2, 132.8, 135.7, 146.5, 149.1, 149.2, 153.3, 164.3. LCMS: (C) retention time 1.63 min, $m/z$ = 363 $[M + H]^+$.

**4-(3-Methoxy-4-(thiophen-3-ylmethoxy)benzylamino)-benzimidamide (5e).** Compound **5e** was prepared as described for the preparation of **5a** except 3-methoxy-4-(thiophen-3-ylmethoxy)-benzaldehyde **3c** was used in place of 4-(benzyloxy)-3-methoxybenzaldehyde **3a** and the compound was purified using preparative HPLC (method 20−70%: flow rate of 25 mL/min; gradient of 20% acetonitrile/80% $H_2O$ for 5.0 min followed by an increase to 70% acetonitrile/30% $H_2O$ over 40 min and continuation of 70% acetonitrile/30% $H_2O$ until 50 min). White fluffy solid (28% yield); mp 112 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.74 (s, 3H), 4.29 (d, $J$ = 5.6 Hz, 2H), 5.02 (s, 2H), 6.69 (d, $J$ = 8.8 Hz, 2H), 6.82 (dd, $J$ = 2.3, 5.6 Hz, 1H), 6.98 (m, 2H), 7.14 (dd, $J$ = 2.0, 3.8 Hz, 1H), 7.31 (t, $J$ = 5.8 Hz, 1H), 7.53 (m, 2H), 7.58 (d, $J$ = 8.8 Hz, 2H), 8.41 (bs, 2H), 8.72 (bs, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 45.8, 55.7, 65.7, 111.8, 113.0, 113.8, 119.4, 124.3, 126.8, 128.0, 129.9, 132.0, 138.3, 146.9, 149.4, 153.6, 164.5. LCMS: (A) retention time 1.40 min, $m/z$ = 368 $[M + H]^+$.

**4-(4-(4-(1*H*-Pyrrol-1-yl)benzyloxy)-3-methoxy-benzylamino)benzimidamide (5f).** Compound **5f** was prepared as described for the preparation of **5a** except 4-(4-(1*H*-pyrrol-1-yl)benzyloxy)-3-methoxybenzaldehyde **3d** was used in place of 4-(benzyloxy)-3-methoxybenzaldehyde **3a** and the compound was purified using preparative HPLC (method 20−70%: flow rate of 25 mL/min; gradient of 20% acetonitrile/80% $H_2O$ for 5.0 min followed by an increase to 70% acetonitrile/30% $H_2O$ over 40 min and continuation of 70% acetonitrile/30% $H_2O$ until 50 min). White solid (45%); mp 225 °C (dec). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.76 (s, 3H), 4.28 (d, $J$ = 5.8 Hz, 2H), 5.06 (s, 2H), 6.26 (t, $J$ = 2.3 Hz, 2H), 6.67 (d, $J$ = 8.8 Hz, 2H), 6.83 (dd, $J$ = 1.5, 7.8 Hz, 1H), 6.98 (m, 2H), 7.25 (t, $J$ = 6.1 Hz, 1H), 7.37 (t, $J$ = 2.3 Hz, 2H), 7.48 (m, 2H), 7.57−7.60 (m, 4H), 8.43 (s, 1H), 8.52 (bs, 2H), 10.22 (bs, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 45.6, 55.6, 69.4, 110.5, 111.5, 113.6, 119.0, 119.2, 129.2, 131.9, 134.1, 139.5, 146.7, 149.2, 153.1, 164.8, 167.6. LCMS: (A) retention time 1.73 min, $m/z$ = 427 $[M + H]^+$.

**4-(4-(Isopentyloxy)-3-methoxybenzylamino)-benzimidamide (5g).** Compound **5g** was prepared as described for the preparation of **5a** except 4-(isopentyloxy)-3-methoxy-benzaldehyde **3e** was used in place of 4-(benzyloxy)-3-methoxybenzaldehyde **3a** and the compound was purified using preparative HPLC (method 20−70%: flow rate of 25 mL/min; gradient of 20% acetonitrile/80% $H_2O$ for 5.0 min followed by an increase to 70% acetonitrile/30% $H_2O$ over 40 min and continuation of 70% acetonitrile/30% $H_2O$ until 50 min). White hygroscopic solid (37% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.90 (d, $J$ = 6.6 Hz, 6H), 1.55 (qt, $J$ = 6.8, 13.4 Hz, 2H), 1.76 (m, 1H), 3.73 (s, 3H), 3.90 (t, $J$ = 6.8 Hz, 2H), 4.27 (d, $J$ = 5.8 Hz, 2H), 6.68 (d, $J$ = 8.8 Hz, 2H), 6.82 (m, 1H), 6.89 (d, $J$ = 8.3 Hz, 1H), 6.96 (d, $J$ = 1.8 Hz, 1H), 7.28 (t, $J$ = 6.1 Hz, 1H), 7.57 (d, $J$ = 9.1 Hz, 2H), 8.45 (s, 1H), 8.71 (bs, 1H), 9.33 (bs, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 22.5, 24.5, 37.6, 46.0, 55.5, 66.7, 111.5, 113.0, 113.5, 119.3, 129.3, 131.4, 147.2, 149.0, 153.1, 164.6, 168.0. LCMS: (A) retention time 1.57 min, $m/z$ = 342 $[M + H]^+$.

**4-(4-(Benzyloxy)benzylamino)benzimidamide (5h).** Compound **5h** was prepared as described for the preparation of **5a** except 4-(benzyloxy)benzaldehyde **3f** was used in place of 4-(benzyloxy)-3-


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methoxybenzaldehyde **3a** and the compound was purified using preparative HPLC (method 20−70%: flow rate of 25 mL/min; gradient of 20% acetonitrile/80% $H_2O$ for 5.0 min followed by an increase to 70% acetonitrile/30% $H_2O$ over 40 min and continuation of 70% acetonitrile/30% $H_2O$ until 50 min). White solid (57% yield), mp 222 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 4.28 (d, $J$ = 5.8 Hz, 2H), 5.07 (s, 2H), 6.85 (d, $J$ = 8.8 Hz, 2H), 6.96 (d, $J$ = 8.6 Hz, 2H), 7.25 (m, 3H), 7.32 (m, 1H), 7.36 (m, 2H), 7.42 (m, 2H), 7.56 (d, $J$ = 8.8 Hz, 2H), 8.47 (s, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 45.6, 69.6, 114.1, 115.2, 128.1, 128.3, 128.9, 128.9, 129.7, 131.6, 137.6, 153.4, 157.8, 165.3, 168.0. LCMS: (A) retention time 1.76 min, $m/z$ = 332 $[M + H]^+$.

**4-(4-Methyl-2-nitrophenoxy)benzonitrile (8a).** A mixture of 1-fluoro-4-methyl-2-nitrobenzene **6a** (7.5 g, 48.4 mmol), 4-hydroxybenzonitrile **7a** (5.76 g, 48.4 mmol), and potassium carbonate (20.0 g, 145.0 mmol) in DMF (100 mL) was heated at 50 °C overnight. After cooling, solvent was removed under reduced pressure, 10% $KHSO_4$ solution in water and EtOAc were added, and the layers were separated. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated. The residue was triturated in a EtOAc−hexanes mixture to give 11 g of product as a yellow solid (89% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.43 (s, 3H), 7.12 (d, $J$ = 8.8 Hz, 2H), 7.33 (d, $J$ = 8.3 Hz, 1H), 7.63 (dd, $J$ = 0.8, 8.3 Hz, 1H), 7.85 (d, $J$ = 9.1 Hz, 2H), 8.00 (d, $J$ = 1.0 Hz, 1H).

**4-(2-Nitrophenoxy)benzonitrile (8b).** Compound **8b** was prepared as described for the preparation of **8a** except 1-fluoro-2-nitrobenzene **6b** was used in place of 1-fluoro-4-methyl-2-nitrobenzene **6a**. Yellow solid (85% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.16 (d, $J$ = 8.8 Hz, 2H), 7.40 (d, $J$ = 8.3 Hz, 1H), 7.51 (m, 1H), 7.79 (m, 1H), 7.87 (d, $J$ = 9.1 Hz, 2H), 8.15 (m, 1H).

**4-(5-Methyl-2-nitrophenoxy)benzonitrile (8c).** Compound **8c** was prepared as described for the preparation of **8a** except 2-fluoro-4-methyl-1-nitrobenzene **6c** was used in place of 1-fluoro-4-methyl-2-nitrobenzene **6a**. Tan solid (76% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.45 (s, 3H), 6.99−7.04 (m, 3H), 7.18 (dd, $J$ = 1.8, 8.3 Hz, 1H), 7.64 (m, 2H), 7.98 (d, $J$ = 8.6 Hz, 1H).

**3-(5-Methyl-2-nitrophenoxy)benzonitrile (8d).** Compound **8d** was prepared as described for the preparation of **8a** except 2-fluoro-4-methyl-1-nitrobenzene **6c** was used in place of 1-fluoro-4-methyl-2-nitrobenzene **6a** and 3-hydroxybenzonitrile **7b** in place of 4-hydroxybenzonitrile **7a**. Light yellow solid (80% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.44 (s, 3H), 6.93 (s, 1H), 7.15 (m, 1H), 7.20 (m, 1H), 7.24 (m, 1H), 7.43 (m, 1H), 7.48 (m, 1H), 7.97 (d, $J$ = 8.6 Hz, 1H).

**2-(5-Methyl-2-nitrophenoxy)benzonitrile (8e).** Compound **8e** was prepared as described for the preparation of **8a** except 2-fluoro-4-methyl-1-nitrobenzene **6c** was used in place of 1-fluoro-4-methyl-2-nitrobenzene **6a** and 2-hydroxybenzonitrile **7c** in place of 4-hydroxybenzonitrile **7a**. Light tan solid (65% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.47 (s, 3H), 6.77 (d, $J$ = 8.3 Hz, 1H), 7.09 (d, $J$ = 8.3 Hz, 1H), 7.17 (td, $J$ = 1.0, 7.8 Hz, 1H). 7.45 (m, 2H), 7.88 (dd, $J$ = 1.8, 7.8 Hz, 1H), 7.88 (d, $J$ = 2.3 Hz, 1H).

**4-(2-Amino-4-methylphenoxy)benzonitrile (9a).** To a solution of **8a** in methanol was added a spatula tip of 5% Pd/C, and the mixture was hydrogenated for 40 min at 30 psi. The reaction was filtered through Celite, and the filtrate was concentrated in vacuo. Beige solid (90% yield). $^1$H NMR (400 MHz, CDCl3) $\delta$ 2.19 (s, 3H), 4.94 (s, 2H), 6.38 (d, $J$ = 7.8 Hz, 1H), 6.64 (s, 1H), 6.76 (d, $J$ = 7.8 Hz, 1H), 6.95 (d, $J$ = 8.1 Hz, 2H), 7.76 (d, $J$ = 8.1 Hz, 2H).

**4-(2-Aminophenoxy)benzonitrile (9b).** The title compound was prepared as described for the preparation of **9a** except compound **8b** was used in place of **8a**. Light tan solid (quantitative yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 5.04 (s, 2H), 6.58 (m, 1H), 6.84 (m, 2H), 6.98 (m, 3H), 7.79 (m, 2H).

**4-(2-Amino-5-methylphenoxy)benzonitrile (9c).** The title compound was prepared as described for the preparation of **9a** except compound **8c** was used in place of **8a**. Yellow solid (quantitative yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.15 (s, 3H), 4.80 (s, 2H), 6.72 (m, 2H), 6.81 (m, 1H), 6.96 (m, 2H), 7.77 (m, 2H).

**3-(2-Amino-5-methylphenoxy)benzonitrile (9d).** The title compound was prepared as described for the preparation of **9a** except compound **8d** was used in place of **8a**. Beige solid (75% yield);. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.25 (s, 3H), 3.62 (s, 2H), 6.72 (d, $J$ = 2.0 Hz, 1H), 6.76 (d, $J$ = 8.1 Hz, 1H), 6.87 (m, 1H), 7.18 (m, 1H), 7.20 (m, 1H), 7.32 (m, 1H), 7.39 (m, 1H).

**2-(2-Amino-5-methylphenoxy)benzonitrile (9e).** The title compound was prepared as described for the preparation of **9a** except compound **8e** was used in place of **8a**. Light tan solid (87% yield). $^1$H NMR (400 MHz DMSO-$d_6$) $\delta$ 2.20 (s, 3H), 5.01 (s, 2H), 6.38 (m, 1H), 6.65 (m, 2H), 6.77 (d, $J$ = 8.1 Hz, 1H), 7.13 (td, $J$ = 1.0, 7.6 Hz, 1H), 7.56 (m, 1H), 7.80 (dd, $J$ = 1.5, 7.3 Hz, 1H).

***N*-(2-(4-Cyanophenoxy)-5-methylphenyl)butane-1-sulfonamide (10a).** To a solution of compound **9a** (298 mg, 1.33 mmol) in pyridine (10 mL) was added butane-1-sulfonyl chloride (208 mg, 1.61 mmol) at rt. The reaction was stirred at rt for 10 min, then heated at 50 °C for 1.5 h. Pyridine was removed in vacuo, and the crude material was partitioned between water and EtOAc. The organic layer was washed with brine, dried over sodium sulfate, and concentrated in vacuo. The residue was purified using a Biotage Isolera One flash purification system with a silica gel cartridge (EtOAc/hexanes) to give 256 mg (56% yield) of title compound as a light brown solid. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.87 (dd, $J$ = 7.8, 6.9 Hz, 3H), 1.38 (q, $J$ = 7.4 Hz, 2H), 1.74 (td, $J$ = 7.6, 7.0, 3.0 Hz, 2H), 2.38 (s, 3H), 3.16−2.92 (m, 2H), 6.56 (s, 1H), 6.88 (d, $J$ = 8.3 Hz, 1H), 6.95 (dd, $J$ = 8.3, 2.0 Hz, 1H), 7.03 (d, $J$ = 8.6 Hz, 2H), 7.50 (d, $J$ = 2.0 Hz, 1H), 7.64 (d, $J$ = 8.7 Hz, 2H).

***N*-(2-(4-Cyanophenoxy)phenyl)butane-1-sulfonamide (10b).** The title compound was prepared as described for the preparation of **10a** except compound **9b** was used in place of **9a** and the crude material was purified using DCM as mobile phase. Brown oil (46% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.76 (t, $J$ = 7.3 Hz, 3H), 1.25 (m, 2H), 1.59 (m, 2H), 2.98 (t, $J$ = 7.8 Hz, 2H), 7.03 (dd, $J$ = 1.5, 7.8 Hz, 1H), 7.09 (d, $J$ = 8.8 Hz, 2H), 7.15 (m, 1H), 7.21 (m, 1H), 7.49 (dd, $J$ = 1.8, 8.1 Hz, 1H), 7.83 (d, $J$ = 8.8 Hz, 2H), 8.42 (s, 1H).

***N*-(2-(4-Cyanophenoxy)-4-methylphenyl)butane-1-sulfonamide (10c).** The title compound was prepared as described for the preparation of **10a** except compound **9c** was used in place of **9a**. Dark orange oil (57% yield). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.87 (td, $J$ = 7.4, 1.1 Hz, 3H), 1.37 (q, $J$ = 7.5 Hz, 2H), 1.79−1.69 (m, 2H), 2.30 (s, 3H), 3.08−3.02 (m, 2H), 6.50 (s, 1H), 6.78 (d, $J$ = 1.8 Hz, 1H), 7.08−7.00 (m, 3H), 7.56 (d, $J$ = 8.3 Hz, 1H), 7.66 (d, $J$ = 8.8 Hz, 1H).

***N*-(2-(4-Cyanophenoxy)-4-methylphenyl)-4-methylbenzenesulfonamide (10d).** The title compound was prepared as described for the preparation of **10a** except compound **9c** was used in place of **9a** and 4-tolylsulfonyl chloride in place of butane-1-sulfonyl chloride. Beige solid (98% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.26 (s, 3H), 2.39 (s, 3H), 6.61 (m, 3H), 6.75 (s, 1H), 7.00 (dd, $J$ = 1.5, 8.3 Hz, 1H), 7.10 (d, $J$ = 7.8 Hz, 2H), 7.45 (m, 2H), 7.52 (m, 2H), 7.63 (d, $J$ = 8.1 Hz, 1H).

***N*-(2-(4-Cyanophenoxy)-4-methylphenyl)-2-(trifluoromethoxy)benzenesulfonamide (10e).** The title compound was prepared as described for the preparation of **10a** except compound **9c** was used in place of **9a** and 2-(trifluoromethoxy)-benzene-1-sulfonyl chloride in place of butane-1-sulfonyl chloride. Off white solid (82% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.23 (s, 3H), 6.65 (d, $J$ = 1.8 Hz, 1H), 6.78 (m, 2H), 6.94 (m, 1H), 7.09 (s, 1H), 7.11 (m, 1H), 7.31 (m, 1H), 7.53 (m, 4H), 7.93 (dd, $J$ = 1.8, 7.8 Hz, 1H).

***N*-(2-(4-Cyanophenoxy)-4-methylphenyl)-4-fluorobenzenesulfonamide (10f).** The title compound was prepared as described for the preparation of **10a** except compound **9c** was used in place of **9a** and 4-fluorobenzene-1-sulfonyl chloride in place of butane-1-sulfonyl chloride. White solid (85% yield). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 2.26 (s, 3H), 6.62 (s, 1H), 6.71−6.64 (m, 3H), 6.99−7.04 (m, 3H), 7.53 (dd, J = 7.8, 1.7 Hz, 2H), 7.60 (d, J = 8.4 Hz, 1H), 7.72−7.64 (m, 2H).



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***N*-(2-(4-Cyanophenoxy)-4-methylphenyl)-2-methoxybenzenesulfonamide (10g).** The title compound was prepared as described for the preparation of **10a** except compound **9c** was used in place of **9a** and 2-methoxybenzene-1-sulfonyl chloride in place of butane-1-sulfonyl chloride. Light tan solid (quantitative yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.18 (s, 3H), 3.65 (s, 3H), 6.73 (m, 3H), 6.96 (m, 3H), 7.25 (d, $J$ = 8.1 Hz, 1H), 7.47 (m, 1H), 7.57 (m, 1H), 7.71 (m, 2H), 9.31 (s, 1H).

***N*-(2-(3-Cyanophenoxy)-4-methylphenyl)-2-methoxybenzenesulfonamide (10h).** The title compound was prepared as described for the preparation of **10a** except compound **9d** was used in place of **9a** and 2-methoxybenzene-1-sulfonyl chloride in place of butane-1-sulfonyl chloride. Tan solid (83% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.17 (s, 3H), 2.28 (s, 3H), 3.61 (s, 3H), 6.68 (m, 2H), 6.75 (m, 2H), 6.94 (m, 1H), 6.98 (m, 1H), 7.27 (d, $J$ = 8.1 Hz, 1H), 7.43 (dd, $J$ = 17.8, 16.9 Hz, 2H), 7.51 (m, 1H), 9.15 (s, 1H).

***N*-(2-(2-Cyanophenoxy)-4-methylphenyl)-2-methoxybenzenesulfonamide (10i).** The title compound was prepared as described for the preparation of **10a** except compound **9e** was used in place of **9a** and 2-methoxybenzene-1-sulfonyl chloride in place of butane-1-sulfonyl chloride. Tan solid (quantitative yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.23 (s, 3H), 3.73 (s, 3H), 6.37 (d, $J$ = 8.6 Hz, 1H), 6.83 (d, $J$ = 8.3 Hz, 1H), 6.97 (m, 3H), 7.17 (td, $J$ = 0.8, 7.6 Hz, 1H), 7.22 (d, $J$ = 2.0 Hz, 1H), 7.48 (m, 2H), 7.66 (dd, $J$ = 1.8, 7.8 Hz, 1H), 7.78 (dd, $J$ = 1.8, 7.8 Hz, 1H), 9.55 (s, 1H).

**4-(2-(Butylsulfonamido)-4-methylphenoxy)benzimidamide (11a).** A solution of **10a** (230 mg, 0.668 mmol) in mixture of anhydrous methanol (10 mL) and anhydrous dioxane (1 mL) was subjected to a stream of bubbling HCl (gas) at 0 °C for 30 min. The flask was capped with a septum and the mixture was stirred at rt overnight. Methanol was removed in vacuo and the residue was dried under high vacuum. The obtained material was redissolved in a 7 N solution of ammonia in methanol (20 mL), and the mixture was heated at 65 °C for 1 h before cooling to rt and stirring overnight. The solvent was removed in vacuo. The resulting residue was purified using reverse phase preparative HPLC (20% MeCN/80% water followed by an increase to 70% MeCN over 40 min and an increase to 100% MeCN over 10 min; flow rate 15 mL/min) to give title compound (15% yield) as a white solid; mp 82 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.77 (t, $J$ = 7.3 Hz, 3H), 1.24 (h, $J$ = 7.4 Hz, 2H), 1.54−1.36 (m, 2H), 2.23 (s, 3H), 2.73−2.65 (m, 2H), 6.54 (dd, $J$ = 8.2, 2.2 Hz, 1H), 6.78 (d, $J$ = 7.9 Hz, 1H), 6.92−6.83 (m, 2H), 7.24 (d, $J$ = 2.1 Hz, 1H), 7.77−7.68 (m, 2H), 9.18 (s, 4H). LCMS: (A) retention time 1.40 min, $m/z$ 362 [M + H]$^+$. $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 14.0, 21.1, 21.3, 25.8, 52.2, 117.4, 121.3, 123.3, 126.9, 127.1, 128.5, 129.3, 130.4, 135.4, 162.3, 165.4. LCMS: (A) retention time 1.40 min, $m/z$ 362 [M + H]$^+$.

**4-(2-(Butylsulfonamido)phenoxy)benzimidamide (11b).** The title compound was prepared as described for the preparation of **11a** except compound **10b** was used in place of **10a**. White solid (25% yield); mp 50 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.75 (t, $J$ = 7.3 Hz, 3H), 1.26 (m, 2H), 1.58 (m, 2H), 2.97 (t, $J$ = 7.8 Hz, 2H), 7.02 (dd, $J$ = 1.5, 7.8 Hz, 1H), 7.08 (d, $J$ = 8.8 Hz, 2H), 7.15 (m, 1H), 7.20 (m, 1H), 7.48 (dd, $J$ = 1.8, 8.1 Hz, 1H), 7.82 (d, $J$ = 8.8 Hz, 2H), 8.41 (s, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 13.5, 20.9, 24.9, 117.1, 119.4, 122.9, 124.6, 126.0, 128.8, 130.4, 132.8, 146.3, 165.5. LCMS: (A) retention time 1.45 min, $m/z$ 348 [M + H]$^+$.

**4-(2-(Butylsulfonamido)-5-methylphenoxy)benzimidamide (11c).** The title compound was prepared as described for the preparation of **11a** except compound **10c** was used in place of **10a**. White solid (57% yield); mp 178 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.77 (t, $J$ = 7.6 Hz, 3H), 1.25 (m, 2H), 1.58 (m, 2H), 2.24 (s, 3H), 2.10 (m, 2H), 6.85 (d, $J$ = 1.3 Hz, 1H), 7.01 (dd, $J$ = 1.3, 8.5 Hz, 1H), 7.08 (d, $J$ = 8.8 Hz, 2H), 7.34 (d, $J$ = 8.2 Hz, 1H), 8.44 (s, 1H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 13.5, 20.4, 20.8, 25.3, 51.6, 117.4, 121.0, 122.7, 126.0, 128.7, 129.9, 165.1, 167.3. LCMS: (A) retention time 1.60 min, $m/z$ 362 [M + H]$^+$.

**4-(5-Methyl-2-(4-methylphenylsulfonamido)phenoxy)-benzimidamide (11d).** The title compound was prepared as described for the preparation of **11a** except compound **10d** was used in place of **10a**. White powder (22% yield); mp 140 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.19 (s, 3H), 2.29 (s, 3H), 6.75 (m, 3H), 6.96 (dd, $J$ = 2.0, 8.3 Hz, 1H), 7.20 (dd, $J$ = 6.3, 8.6 Hz, 3H), 7.51 (d, $J$ = 8.1 Hz, 2H), 7.73 (d, $J$ = 8.8 Hz, 2H), 9.17 (bs, 2H), 9.71 (bs, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 20.3, 20.9, 116.9, 121.1, 121.9, 125.5, 126.0, 126.6, 129.3, 129.7, 138.3, 142.4, 147.2, 161.4, 165.0, 167.7. LCMS: (A) retention time 1.44 min, $m/z$ 396 [M + H]$^+$.

**4-(5-Methyl-2-(2-(trifluoromethoxy)phenylsulfonamido)-phenoxy)benzimidamide (11e).** The title compound was prepared as described for the preparation of **11a** except compound **10e** was used in place of **10a**. White fluffy solid (80% yield); mp 110 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.17 (s, 3H), 6.71 (s, 1H), 6.81 (dd, $J$ = 1.5, 8.6 Hz, 2H), 6.86 (d, $J$ = 8.6 Hz, 1H), 7.15 (d, $J$ = 8.1 Hz, 1H), 7.33 (m, 2H), 7.54 (t, $J$ = 7.8 Hz, 1H), 7.72 (m, 3H), 8.28 (s, 1H), 9.07 (bs, 2H), 9.61 (bs, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 20.3, 116.7, 118.6 120.7 121.1, 121.2, 121.4, 125.9, 126.8, 129.8, 130.1, 133.4, 145.2, 161.8, 164.9, 166.4. LCMS: (A) retention time 1.60 min, $m/z$ 466 [M + H]$^+$.

**4-(2-(4-Fluorophenylsulfonamido)-5-methylphenoxy)-benzimidamide (11f).** The title compound was prepared as described for the preparation of **11a** except compound **10f** was used in place of **10a**. White fluffy solid (43% yield); mp 185 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.10 (s, 3H), 6.61 (d, $J$ = 2.3 Hz, 1H), 6.65 (m, 1H), 6.80 (m, 2H), 7.10 (m, 3H), 7.56 (dd, $J$ = 5.7, 8.8 Hz, 2H), 7.71 (m, 2H), 8.91 (bs, 3H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) 20.4, 115.8, 116.1, 116.8, 121.3, 122.1, 125.9, 126.1, 128.3, 129.4, 129.5, 129.8, 135.4, 137.8, 137.8, 147.3, 161.4, 162.7, 165.2, 165.2. LCMS: (A) retention time 1.35 min, $m/z$ 400 [M + H]$^+$.

**4-(2-(2-Methoxyphenylsulfonamido)-5-methylphenoxy)-benzimidamide (11g).** The title compound was prepared as described for the preparation of **11a** except compound **10g** was used in place of **10a**. White needles (58% yield); mp 149 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.17 (s, 3H), 3.67 (s, 3H), 6.72 (d, $J$ = 1.3 Hz, 1H), 6.79 (d, $J$ = 8.8 Hz, 2H), 6.94−7.01 (m, 3H), 7.23 (d, $J$ = 8.3 Hz, 1H), 7.47 (m, 1H), 7.58 (dd, $J$ = 1.5, 7.8 Hz, 1H), 7.74 (d, $J$ = 8.8 Hz, 2H), 9.23 (bs, 2H), 9.78 (bs, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 20.3, 55.8, 112.7, 117.2, 119.8, 120.8 122.9, 125.8, 129.4, 130.0, 134.7, 147.5, 156.3, 161.5, 164.8. LCMS: (A) retention time 1.34 min, $m/z$ 412 [M + H]$^+$.

**3-(2-(2-Methoxyphenylsulfonamido)-5-methylphenoxy)-benzimidamide (11h).** The title compound was prepared as described for the preparation of **11a** except compound **10h** was used in place of **10a**. Brown solid (14% yield); mp 117 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.16 (s, 3H), 3.66 (s, 3H), 6.65 (d, $J$ = 1.3 Hz, 1H), 6.88 (m, 2H), 6.97 (m, 2H), 7.15 (s, 1H), 7.18 (d, $J$ = 8.3 Hz, 1H), 7.48 (m, 3H), 7.62 (m, 1H), 8.40 (bs, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 20.4, 55.8, 112.6, 117.1, 119.7, 120.2, 122.1, 125.3, 129.5, 130.4, 130.5, 134.5, 156.3, 157.0, 165.4. LCMS: (A) retention time 1.62 min, $m/z$ 412 [M + H]$^+$.

**2-(2-(2-Methoxyphenylsulfonamido)-5-methylphenoxy)-benzimidamide (11i).** The title compound was prepared as described for the preparation of **11a** except compound **10i** was used in place of **10a**. Light tan solid (21% yield); mp 232 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.04 (s, 3H), 3.56 (s, 3H), 6.36 (d, $J$ = 7.1 Hz, 1H), 6.81 (m, 1H), 6.92 (m, 4H), 7.16 (m, 1H), 7.29 (m, 1H), 7.47 (m, 1H), 7.54 (d, $J$ = 7.1 Hz, 1H), 7.58 (d, $J$ = 6.8 Hz, 1H), 8.49 (bs, 2H), 10.98 (bs, 1H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 21.2, 55.6, 99.5, 112.3, 116.4, 116.6, 119.0, 120.0 122.0, 122.7, 129.7, 129.8, 131.5, 133.6, 134.1, 143.7, 156.6, 157.5, 163.7. LCMS: (A) retention time 1.37 min, $m/z$ 412 [M + H]$^+$.

***N*$^6$-(4-(Benzyloxy)-3-methoxybenzyl)isoquinoline-1,6-diamine (13).** The title compound was prepared as described for the preparation of **5a** except isoquinoline-1,6-diamine **12** was used in place of 4-aminobenzimidamide **4a**. Crude material was purified using a Biotage Isolera One flash purification system with a silica gel cartridge ($CHCl_3$/MeOH, 0.5% $Et_3N$). Tan powder (10% yield); mp 155 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.76 (s, 3H), 4.26 (d, $J$ = 5.6 Hz, 2H), 5.04 (s, 2H), 6.39 (s, 2H), 6.50 (d, $J$ = 2.0 Hz, 1H), 6.55


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(d, $J$ = 5.8 Hz, 1H), 6.71 (t, $J$ = 5.8 Hz, 1H), 6.87 (m, 2H), 6.96 (d, $J$ = 8.1 Hz, 1H), 7.04 (d, $J$ = 1.5 Hz, 1H), 7.31 (m, 1H), 7.36−7.43 (m, 4H), 7.52 (d, $J$ = 5.8 Hz, 1H), 7.84 (d, $J$ = 9.1 Hz, 1H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 46.0, 55.5, 69.9, 102.0, 109.3, 109.4, 111.6, 113.5, 116.1, 119.3, 125.0, 127.8, 128.4, 132.3, 137.3, 139.1, 140.9, 146.7, 149.1, 149.7, 156.7. LCMS: (A) retention time 1.65 min, $m/z$ 386 $[M + H]^+$.

**6-(5-Methyl-2-nitrophenoxy)isoquinolin-1-amine (15).** The title compound was prepared as described for the preparation of **8a** except 1-fluoro-2-nitrobenzene **6c** was used in place of 1-fluoro-4-methyl-2-nitrobenzene **6a** and 1-aminoisoquinolin-6-ol **14** in place of 4-hydroxybenzonitrile **4a**. Crude material was purified using a Biotage Isolera One flash purification system with a silica gel cartridge (DCM/EtOAc, 0.5% $Et_3N$). Yellow powder (92% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.37 (s, 3H), 6.81 (m, 3H), 7.12 (d, $J$ = 2.5 Hz, 1H), 7.15 (s, 1H), 7.21 (dd, $J$ = 2.8, 9.1 Hz, 1H), 7.26 (m, 1H), 7.74 (d, $J$ = 5.8 Hz, 1H), 8.04 (d, $J$ = 8.3 Hz, 1H), 8.24 (d, $J$ = 9.1 Hz, 1H).

***N*-(2-(1-Aminoisoquinolin-6-yloxy)-4-methylphenyl)-2-methoxybenzenesulfonamide (16).** To a solution of compound **15** (219 mg, 0.825 mmol) in pyridine (10 mL) was added 2-methoxybenzenesulfonyl chloride (341 mg, 1.65 mmol) at rt. The reaction was stirred at rt for 10 min, then heated at 65 °C overnight. Pyridine was removed in vacuo, and the crude material was partitioned between water and EtOAc. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified using a Biotage Isolera One flash purification system with a silica gel cartridge ($CHCl_3$/MeOH) to give **16** as yellow solid (98% yield); mp 238 °C (dec). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.26 (s, 3H), 3.61 (s, 3H), 6.83 (d, $J$ = 2.5 Hz, 1H), 6.85 (d, $J$ = 8.6 Hz, 1H), 6.91 (m, 2H), 7.00 (m, 2H), 7.15 (dd, $J$ = 2.5, 9.1 Hz, 1H), 7.24 (d, $J$ = 2.0 Hz, 1H), 7.36 (m, 1H), 7.58 (d, $J$ = 7.1 Hz, 1H), 7.61 (dd, $J$ = 1.8, 7.8 Hz, 1H), 8.43 (d, $J$ = 9.3 Hz, 1H), 8.88 (bs, 2H), 9.42 (s, 1H), 12.93 (s, 1H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 20.6, 55.8, 110.9, 111.3, 112.5, 119.5, 119.8, 121.1, 126.2, 127.2, 127.3, 127.8, 129.1, 129.3, 134.7, 135.1, 139.2, 144.5, 153.5, 156.2, 158.4, 162.2. LCMS: (A) retention time 1.65 min, $m/z$ 436 $[M + H]^+$.

**5-(5-Methyl-2-nitrophenoxy)benzo[*d*]thiazol-2-amine (18).** The title compound was prepared as described for the preparation of **8a** except 1-fluoro-2-nitrobenzene **6c** was used in place of 1-fluoro-4-methyl-2-nitrobenzene **6a** and 2-aminobenzo[$d$]thiazol-5-ol **17** in place of 4-hydroxybenzonitrile **4a**. Crude material was purified using a Biotage Isolera One flash purification system with a silica gel cartridge (DCM/EtOAc, 0.5% $Et_3N$). Yellow powder (84% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.30 (s, 3H), 6.85 (s, 1H), 6.95 (dd, $J$ = 2.8, 8.8 Hz, 1H), 7.09 (d, $J$ = 8.8 Hz, 1H), 7.34 (d, $J$ = 8.6 Hz, 1H), 7.49 (m, 3H), 7.94 (d, $J$ = 8.3 Hz, 1H).

***N*-(2-(2-Aminobenzo[d]thiazol-5-yloxy)-4-methylphenyl)-2-methoxybenzenesulfonamide (19).** To a solution of compound **18** (228 mg, 0.840 mmol) in pyridine (10 mL) was added 2-methoxybenzenesulfonyl chloride (347 mg, 1.68 mmol) at rt. The reaction was stirred at rt for 10 min, then heated at 65 °C overnight. Pyridine was removed in vacuo and the crude material was partitioned between water and EtOAc. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified using a Biotage Isolera One flash purification system with a silica gel cartridge ($CHCl_3$/MeOH) to give **19** as an orange solid (97% yield), mp 198 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.20 (s, 3H), 3.67 (s, 3H), 6.58 (d, $J$ = 8.3 Hz, 1H), 6.65 (dd, $J$ = 2.5, 8.6 Hz, 1H), 6.85 (dd, $J$ = 2.0, 8.3 Hz, 1H), 6.96−7.01 (m, 3H), 7.20 (d, $J$ = 2.0 Hz, 1H), 7.28 (d, $J$ = 8.8 Hz, 1H), 7.47 (m, 1H), 7.64 (dd, $J$ = 1.8, 7.8 Hz, 1H), 8.43 (bs, 2H), 9.07 (s, 1H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 20.4, 55.9, 111.6, 112.7, 116.8, 117.3, 118.1, 119.7, 125.7, 126.9, 127.2, 127.5, 129.4, 132.6, 134.7, 147.6, 150.8, 151.8, 156.5, 158.1, 167.2. LCMS: (A) retention time 1.78 min, $m/z$ 442 $[M + H]^+$.

**In Vitro MrgV1 Receptor Assay.** HEK293 cells stably transfected with human *MrgprX1* were plated in 96 well plates at 25 000 cell/well and incubated 2 days before imaging. On the day of imaging, cells were incubated in 100 $\mu$L of HBSS with 2 $\mu$M Fluo 4AM and 1% Trypan Red for 50 min at 37 °C. The cells were then equilibrated for 10 min at room temperature before imaging. Test compounds were dissolved in HBSS and diluted in a serial dilution. Test compounds, BAM 8-22 (positive control), or HBSS (negative control) were added (50 $\mu$L into 100 $\mu$L), and cells were imaged on the FLIPR for 2 min. Data were exported as maximum−minimum fluorescent signal.

**DRG Sensory Neuronal Cultures.** DRGs from 3- to 4-week-old *MrgprX1* mice were collected in cold DH10 medium (DMEM/F-12 with 10% fetal bovine serum and 1% penicillin/streptomycin, Gibco) and treated with enzyme solution (5 mg/mL dispase and 1 mg/mL collagenase type I in HBSS without $Ca^{2+}$ and $Mg^{2+}$, Gibco) at 37 °C. After trituration and centrifugation, cells were resuspended in DH10 with nerve growth factor (50 ng/mL, Upstate Biotechnology, Lake Placid, NY) and glial cell line-derived neurotrophic factor (25 ng/mL, R&D Systems), plated on glass coverslips coated with poly-D-lysine (100 $\mu$g/mL, Biomedical Technologies) and laminin (10 $\mu$g/mL, Invitrogen), cultured at 37 °C, and used after 20−40 h.[24]

**Whole-Cell Recordings of Cultured DRG Neurons.** Whole cell currents of cultured DRG neurons from *MrgprX1* mice with MrgprA3-GFP marker were recorded with an Axon 700B amplifier and pCLAMP 9.2 software (Molecular Devices, Sunnyvale, CA, USA). Extracellular solution contained (in mM) 130 *N*-methyl-D-glucamine chloride (NMDG-Cl), 5 $BaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 glucose, with pH of 7.4 adjusted with 1 M NMDG. Osmolality was adjusted to 310 mOsm/kg with sucrose. Electrodes were pulled (model pp-830; Narishige, East Meadow, NY, USA) from borosilicate glass (World Precision Instruments, Sarasota, FL, USA) with resistance of 2−4 MΩ. Pipette solution contained (in mM) 140 tetraethylammonium chloride, 10 EGTA, 1 $MgCl_2$, 10 HEPES, 0.5 GTP, and 3 ATP, with pH of 7.4 and osmolality of approximately 300 mOsm/kg. The voltage protocol was modified from a previously published method.[3] Briefly, cells were held at −80 mV and evoked to −40 mV for 20 ms (ms) to activate low-voltage $Ca^{2+}$ channels and then held to −60 mV for 20 ms and evoked to −10 mV for 40 ms to activate HVA $Ca^{2+}$ channels. Leak currents were subtracted with P/4 protocol. Liquid junction potentials and whole cell capacitances were offset, and series resistances were compensated by 70%. All experiments were performed at room temperature (21−23 °C).

**Opioid Receptor Binding Affinity.** As previously reported in detail,[25] the binding affinities of compounds **11g** and **16** to $\delta$-, $\mu$-, and $\kappa$-opioid receptor sites were measured using [$^3$H]DADLE, [$^3$H]-DAMGO, or [$^3$H]U69,593 as ligands, respectively.

**In Vivo Pharmacokinetic Studies in Mice.** All pharmacokinetic studies in mice were conducted according to protocols approved by the Animal Care and Use Committee at Johns Hopkins University. Male CD 1 mice between 25 and 30 g were obtained from Harlan, Inc., and maintained on a 12 h light−dark cycle with ad libitum access to food and water. Compound **16** was administered to mice ($n$ = 3) as a single intravenous dose of 10 mg/kg. The mice were sacrificed at predetermined time points after drug administration. For collection of plasma and spinal cord tissue, animals were euthanized with $CO_2$, and blood samples were collected in heparinized microtubes by cardiac puncture. Spinal cords were dissected and immediately flash frozen (−80 °C). Plasma was obtained by centrifugation of blood at 3000$g$ for 15 min and stored at −80 °C until LC/MS analysis.

Prior to extraction, frozen samples were thawed on ice. The calibration curves were developed using plasma and spinal cord from naive animals as a matrix. Plasma samples (50 $\mu$L) were processed using a protein precipitation method by addition of 300 $\mu$L of acetonitrile as with internal standard (losartan [5 $\mu$M]), followed by vortexing for 30 s and then centrifugation at 10 000 rcf for 10 min. For spinal cord tissue, homogenized samples were vortexed and centrifuged as above. A 10 $\mu$L aliquot of supernatant was diluted with 40 $\mu$L of water containing 0.5 $\mu$M losartan as internal standard. Extracts were centrifuged at 10 000 rcf at 4 °C for 10 min. Supernatants were transferred to 250 $\mu$L polypropylene autosampler vials sealed with a Teflon cap. A volume of 3 $\mu$L was injected onto the ultraperformance liquid chromatography (UPLC) instrument for quantitative analysis by LC/MS/MS. Calibration curves over the


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range of 0.01−10 nmol/mL in plasma and spinal cord were constructed from the peak area ratio of the analyte to the internal standard using linear regression with a weighting factor of 1/(nominal concentration). Correlation coefficient of greater than 0.99 was obtained in all analytical runs for all analytes.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.9b01003.

$^{1}$H and $^{13}$C spectra of the target compounds and agonist activity of JHU-58 and compound **16** at MRGPRC11 (PDF)

Molecular formula strings and some data (CSV)

## ■ AUTHOR INFORMATION


### Corresponding Author

*Phone: (410) 614-0982. Fax: (410) 614-0659. E-mail: ttsukamoto@jhmi.edu.

### ORCID

Rana Rais: 0000-0003-4059-2453

Barbara S. Slusher: 0000-0001-9814-4157

Takashi Tsukamoto: 0000-0002-0216-7520


### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

MRGPRX1, Mas-related G-protein-coupled receptor member X1; DRG, dorsal root ganglion; HTS, high throughput screening; HVA, high-voltage activated

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