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# Discovery of non-peptidergic MrgX1 and MrgX2 receptor agonists and exploration of an initial SAR using solid-phase synthesis

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ABSTRACT

A class of small molecules displaying comparable activities with peptide ligands BAM22 and corticostatin-14 at both the human and rhesus monkey MrgX1 and MrgX2 receptors, respectively, was discovered. A comparative study to compare solid-phase and solution-phase chemistries for the efficient synthesis of the active class, tetracyclic benzimidazoles, was undertaken. The solid-phase chemistry was found to be superior both for the synthesis of analogs and for the synthesis of gram quantities.



Mas-related G-protein coupled receptors (Mrg receptors), also known as sensory-neuron specific receptors (SNSRs), are a large family of over 50 subtypes of orphan receptors expressed almost exclusively in small diameter sensory neurons.[1,2] The physiological functions of these receptors are unknown but their expression pattern suggests a role in nociception. MrgX1 and MrgX2 are human receptor subtypes for which peptide ligands have been identified,[2–5] and for which more direct evidence for a role in nociception exists.[6] However, despite their obvious potential as drug targets, little progress has been made exploiting MrgX1 or MrgX2 receptors therapeutically. This is partly because rodent orthologs of these receptors do not exist necessitating the use of higher species such as rhesus monkey to perform in vivo studies,[7,8] and partly because the known peptide ligands (e.g., BAM22)[2] and (e.g., cortistatin-14)[3] for MrgX1 and MrgX2 receptors, respectively, activate other receptors complicating target validation. Recently small molecule antagonists for the MrgX1 receptor were reported[9] but further development of MrgX receptor selective non-peptidergic agonists and antagonists will be necessary to fully elucidate the complex pharmacology of the Mrg family.

Herein we report the discovery and initial structure–activity relationship (SAR) of potent and selective MrgX1 and MrgX2 receptor agonists. A high-throughput screen of 250,000 small molecules was performed using recombinant human MrgX1 receptors expressed in the mammalian cell-based functional assay receptor selection and amplification technology (R-SAT).[7,10] The high-throughput screen resulted in the identification of a number of agonist hits from different structure classes. Subsequent pharmacological profiling revealed several compounds from one chemical class that also had agonist activity at human MrgX2 receptors. Two of the compounds that showed selectivity between MrgX1 and MrgX2 receptors were selected as starting points for exploring the SAR. Compounds **1** and **2** showed full efficacy at the MrgX1 receptor ($pEC_{50}$ 5.6) and the MrgX2 receptor ($pEC_{50}$ 6.5), respectively (see Fig. 1).

A solid-phase chemistry protocol was used to resynthesize **1** and **2** and to further synthesize a focused compound library (Scheme 1).[11] The focused library was tested for activity at the MrgX1 and MrgX2 receptors using R-SAT, a representative set of compounds is shown in Table 1 (compounds **1**, **2**, and **12–19**). The first step in the synthesis of tetracyclic benzimidazoles (Scheme 1) requires attachment of benzhydrylamine (MBHA) resin to the selected amino acid. Coupling of the *N*-protected amino acid to MBHA resin using *N,N′*-diisopropylcarbodiimide (DIC) and *N*-

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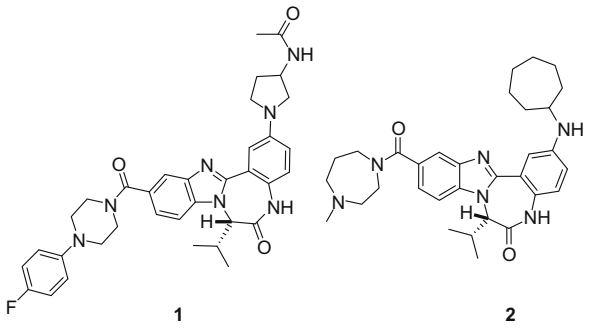
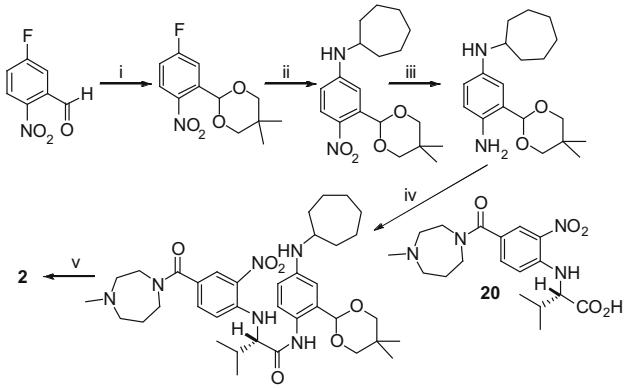


**Figure 1.** Identified MrgX1 and MrgX2 receptor agonists **1** and **2**.

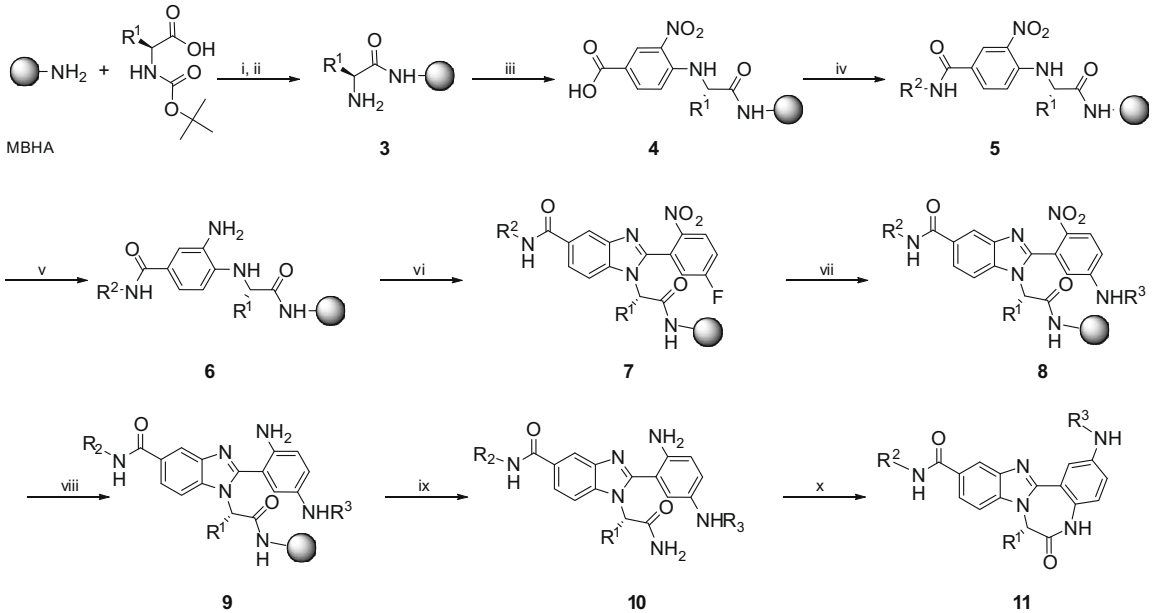


**Scheme 2.** Reagents and conditions: (i) 2,2-dimethylpropane-1,3-diol, TsOH (cat) in toluene, reflux 3 h, 99% yield; (ii) cycloheptanamine, in EtOH, $^{i}Pr_2EtN$ (1.5 equiv), MW 180 °C, 20 min, 69% yield; (iii) $Na_2S_2O_4$ (5 equiv), $Na_2CO_3$ (5 equiv), EtOH/$H_2O$, 75% yield; (iv) carboxylic acid **20**, HOBt (1.5 equiv), EDCI·HCl (1.5 equiv), $Et_3N$ (2 equiv), DMAP (0.1 equiv). $CH_3CN$, MW 120 °C, 5 min, 28% yield; (v) Fe, 50 °C, 45 min, EtOH/$H_2O$/HCl (4:3:1), 50 °C, 90 min, 12% yield.

hydroxybenzotriazole (HOBt) in DMF, followed by deprotection of the *N*-Boc group provided the resin bound compound **3**. The resin was then heated in a solution of 4-fluoro-3-nitrobenzoic acid and diisopropylethylamine (DIEA) in *N*-methylpyrrolidinone (NMP) affording compound **4**. Coupling of **4** with an amine using DIC and HOBt in DMF resulted in compound **5** with two diversity points. Reduction of the nitro group to the amine **6** was achieved using a 2-M solution of $SnCl_2$ in *N*-methylpyrrolidinone, then reaction with 5-fluoro-2-nitrobenzaldehyde afforded the desired trisubstituted benzimidazole **7**. Displacement of the fluoro substituent with appropriate amines followed by reduction of the nitro group resulted in compound **9**. This was then cleaved from the resin utilizing a mixture with trifluoromethanesulfonic acid (TFMSA) to form the corresponding non-cyclic benzimidazole **10**. Unfortunately the ring closure did not go to completion during the cleavage, although this might have been expected from previous reports.[11] Ring closure was finally obtained by heating in the microwave (MW) for 5 min in concd HCl. All the compounds were purified by preparative MS–HPLC and verified by NMR and LC/MS. The overall yield of this solid-phase synthesis protocol was between 5% and 25% over 10 steps.

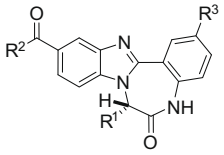
Meanwhile to meet the expected demand for the synthesis of larger quantities and to speed up the resynthesis, a considerable effort was put into developing a solution-phase protocol (Schemes 2 and 3). While the synthetic route in Scheme 3 basically follows the solid-phase protocol, the strategy depicted in Scheme 2 differs from the solid-phase protocol in having the formation of the benzimidazole as the last step. Although both solution-phase methods gave the target molecules, neither of the methods was superior to the solid-phase protocol. The major obstacles were the time consuming and yield reducing purifications between reaction steps, which demanded extensive chromatography to yield pure compounds. Maybe not unexpectedly, this shows that a well validated solid-phase protocol is a powerful method to use. Hence, the solid-phase protocol was further used in the resynthesis and in the synthesis of gram quantities of material (Scheme 1).

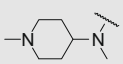
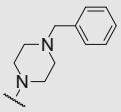
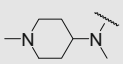
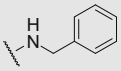
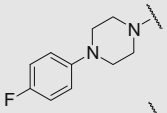
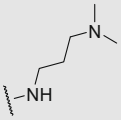
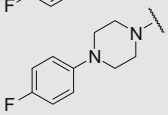
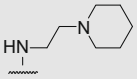
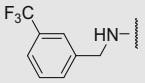
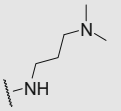
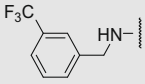
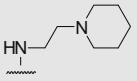
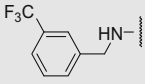
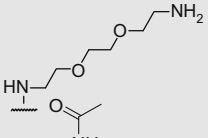
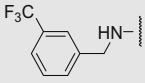
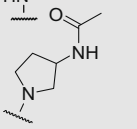
The library initially screened included about 500 compounds of this class with R1 based on the L-amino acids glycine, alanine, valine, isoleucine, phenylalanine, methionine, glutamine, and the non-endogenous amino acid 2-amino-2-phenylacetic acid. In the screen, 30 confirmed hits were found (6% hit rate) and all of the



**Scheme 1.** Reagents and conditions: (i) HOBt, DIC, DMF, rt, 12 h; (ii) 55% TFA, DCM, rt, 40 min; (iii) 4-fluoro-3-nitrobenzoic acid, DIEA, NMP, 70 °C, 24 h; (iv) $R^2NH_2$, DIC, HOBt, DMF, rt, 24 h; (v) $SnCl_2$, NMP, rt, 24 h; (vi) 5-fluoro-2-nitrobenzoic acid, NMP, AcOH, 75 °C, 72 h; (vii) $R^3NH_2$, NMP, rt, 24 h; (viii) $SnCl_2$, NMP, rt, 24 h; (ix) mixture of 84% TFA, 8% thioanisole 8% TFMSA, rt, 2 h; (x) concd HCl, MW 100 °C, 5 min, 5–25% overall yield.

**Table 1**
In vitro activation of human and rhesus monkey MrgX1 and MrgX2 receptors using R-SAT



| Compound | $R_1$ | $R_2$ | $R_3$ | MrgX1 (human) | | MrgX2 (human) | | MrgX1 (monkey) | | MrgX2 (monkey) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $pEC_{50}$ | Eff (%) | $pEC_{50}$ | Eff (%) | $pEC_{50}$ | Eff (%) | $pEC_{50}$ | Eff (%) |
| BAM22[a] | | | | 6.1 ± 0.6 | 100 ± 14 | — | NA[b] | 4.5 ± 0.6 | 50 ± 25 | — | NA |
| Cortistatin-14 | | | | — | NA | 6.3 ± 0.6 | 100 ± 28 | — | NA | 6.1 ± 0.4 | 86 ± 25 |
| **1** | | | | 5.6 ± 0.3 | 98 ± 16 | — | NA | 5.6 ± 0.7 | 89 ± 3 | — | NA |
| **2** | | | | — | NA | 6.5 ± 0.5 | 110 ± 36 | — | NA | 6.4 ± 0.2 | 50 ± 23 |
| **12** |  |  |  | — | NA | 6.4 ± 0.6 | 216 ± 82 | — | NA | 6.7 ± 0.4 | 133 ± 66 |
| **13** |  |  |  | — | NA | 6.8 ± 0.4 | 87 ± 26 | | | | |
| **14** |  |  |  | 6.2 ± 1.1 | 108 ± 41 | | 60 ± 19[c] | 5.8 ± 0.6 | 102 ± 1 | — | NA |
| **15** |  |  |  | 5.9 ± 0.4 | 94 ± 27 | — | NA | 6.0 ± 0.2 | 57 ± 10 | — | NA |
| **16** |  |  |  | 7.2 ± 0.5 | 147 ± 20 | 6.2 ± 0.4 | 103 ± 5 | 6.1 ± 0.0 | 157 ± 0 | 5.4 ± 0.2 | 85 ± 32 |
| **17** |  |  |  | 6.7 ± 0.5 | 133 ± 35 | 5.7 ± 0.7 | 28 ± 19 | 6.1 ± 0.3 | 127 ± 30 | — | NA |
| **18** |  |  |  | 6.5 ± 0.3 | 135 ± 32 | — | NA | 6.1 ± 0.2 | 126 ± 21 | — | NA |
| **19** |  |  |  | 6.5 ± 0.6 | 129 ± 27 | 5.9 ± 0.4 | 41 ± 13 | 6.4 ± 0.3 | 124 ± 30 | 5.7 ± 0.2 | 27 ± 4 |

[a] BAM22: bovine adrenal medulla peptide 22 and Cortistatin-14 (Ref. 7) were used as reference ligands and set to 100% Eff at the MrgX1 receptor and the MrgX2 receptor, respectively.
[b] NA: no activity at $pEC_{50} > 5.0$. $pEC_{50}$ and efficacy values are the average of at least two independent experiments ± SD, where each experiment is carried out with duplicate determinations for each data point.
[c] Max efficacy at concentrations <10 μM.

hits had R1 originating from either glycine, or preferably valine or isoleucine. The glycine analog of compound **12** was 50 times less potent at the MrgX2 receptor.[12] Therefore, R1 was limited by using the amino acids valine and isoleucine in the new analogs synthesized (Table 1). In this limited set of compounds, two selective compounds **18** and **12** displayed increased activity at the MrgX1 and MrgX2 receptors, respectively, compared to compounds **1** and **2** (Table 1 and Fig. 2). Both compounds had similar potency as the peptide reference compounds BAM22 and cortistatin-14. The initial SAR indicated that R1 should be an alkyl group such as isopropyl or isobutyl. In general, molecules where R3 equaled aryl (**12** and **13**) or R2 equaled non-aryl (**2**, **12**, and **13**) provided compounds that were either inactive or showed weak agonist activity at the MrgX1 receptor. This trend was also seen when the original hits were analyzed. In addition, although the library contained several molecules where

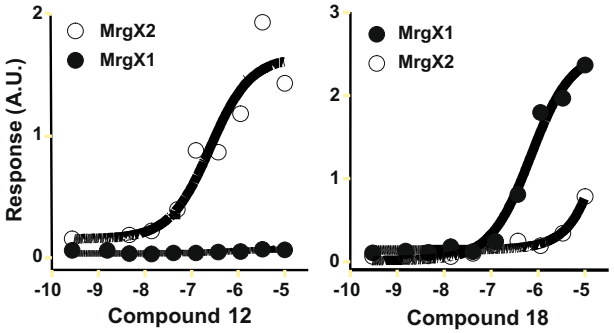
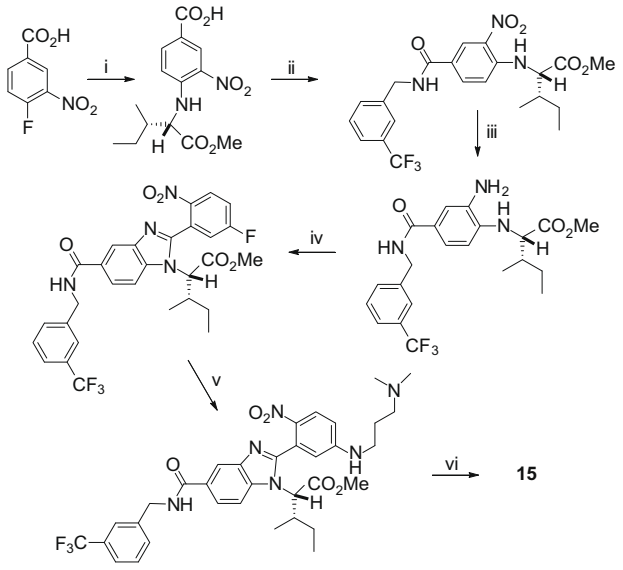



**Figure 2.** Receptor selectivity of MrgX receptor agonists. Each compound was tested in concentration response experiments in R-SAT at human MrgX1 and MrgX2 receptors.




**Scheme 3.** Reagents and conditions: (i) isoleucine methylester, MeOH, reflux 72 h; (ii) DIC, HOBt, 3-trifluoromethyl-benzylamine, DMF, rt, 48 h, 80% yield over two steps; (iii) EtOH, 5% Pd/C, $H_2$, 30% yield; (iv) 5-fluoro-2-nitrobenzaldehyde, NMP/AcOH 1:1, MW 70 °C, 10 min, 32% yield; (v) $N^1$,$N^1$-dimethylpropane-1,3-diamine, NMP, MW 100 °C, 10 min 34% yield; (vi) $SnCl_2$, NMP, rt, after 24 h, HCl, MW 100 °C, 10 min, 22% yield.

both R2 and R3 equaled aryl substituents, including permutations of, for example, **12**, **13**, and **14**, none of these compounds were identified as actives at the MrgX1 receptor in the initial screen. MrgX1 receptor agonists were preferred when R2 equals phenylpiperazine (**14** or **15**). While most meta-$CF_3$-benzyl amine containing compounds activated both receptors (**16**, **17**, and **19**), compound **18** was a potent and selective MrgX1 receptor agonist.

The agonist activity of compound **12** was also confirmed in $Ca^{2+}$ mobilization assay.[12] In addition compound **12** was also found to be stable in human liver microsomes ($Cl_{int}$ 5 μL/min mg). These compounds also activated the homologous monkey receptor subtypes with similar potencies and selectivities as the human subtypes (Table 1).

Thus, despite the fact that rodent homologues of MrgX1 and MrgX2 receptors do not exist, these compounds, or analogs of them, could be used preclinically to probe the physiological functions of MrgX1 and MrgX2 receptors.

In conclusion, a new class of potent and selective non-peptidergic MrgX1 and MrgX2 receptor agonists has been identified which display activities comparable with the reference peptides BAM22 and corticostatin-14.

## Acknowledgment

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12. Result not shown.