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Design, synthesis, and analysis of antagonists of GPR55: Piperidine-substituted 1,3,4-oxadiazol-2-ones

Maria Elena Meza-Aviña [a,†], Mary A. Lingerfelt [a,†], Linda M. Console-Bram [b], Thomas F. Gamage [b], Haleli Sharir [b], Kristen E. Gettys [a], Dow P. Hurst [a], Evangelia Kotsikorou [c], Derek M. Shore [a], Marc G. Caron [d], Narasinga Rao [a], Larry S. Barak [d], Mary E. Abood [b,*], Patricia H. Reggio [a,*], Mitchell P. Croatt [a,*]

[a] Department of Chemistry and Biochemistry, Natural Products and Drug Discovery Center, University of North Carolina at Greensboro, Greensboro, NC 27402, United States
[b] Center for Substance Abuse Research, Temple University, Philadelphia, PA 19140, United States
[c] Department of Chemistry, University of Texas—Pan American, Edinburg, TX 78539, United States
[d] Duke University Medical Center, Durham, NC 27709, United States

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ABSTRACT

A series of 1,3,4-oxadiazol-2-ones was synthesized and tested for activity as antagonists at GPR55 in cellular beta-arrestin redistribution assays. The synthesis was designed to be modular in nature so that a sufficient number of analogues could be rapidly accessed to explore initial structure–activity relationships. The design of analogues was guided by the docking of potential compounds into a model of the inactive form of GPR55. The results of the assays were used to learn more about the binding pocket of GPR55. With this oxadiazolone scaffold, it was determined that modification of the aryl group adjacent to the oxadiazolone ring was often detrimental and that the distal cyclopropane was beneficial for activity. These results will guide further exploration of this receptor.

GPR55, a recently deorphanized, rhodopsin-like (class A) G protein-coupled receptor (GPCR), is a receptor for L-α-lysophosphatidylinositol (LPI, Fig. 1) which serves as the endogenous agonist (GenBank entry NM 005683).[1] Initial studies noted that a variety of CB1 and CB2 ligands bind to GPR55[2,3] and more recent studies have focused on physiological roles for GPR55 in inflammatory pain,[2] neuropathic pain,[2] bone development,[3] and the potential for activation of GPR55 being pro-carcinogenic.[4–8] Despite the important potential biological functions of GPR55, the research is limited by the lack of both potent and selective agonists and antagonists.[9,10]

Based on a high-throughput, high-content screen of approximately 300,000 compounds from the Molecular Libraries Probe Production Centers Network initiative,[11] a few molecular scaffolds were identified that had relatively good selectivity and potency as antagonists at GPR55. These structures were then docked into the inactive state model of GPR55[12] to visualise the key features of the antagonists. Of the compounds that exhibited selective and moderate activity as antagonists at GPR55, three different structural families were identified as illustrated by ML191, ML192, and ML193 (Fig. 1).

The docking of the structures in Figure 1 into the inactive state model of GPR55 indicated a few important interactions as we previously reported.[12] Briefly, the primary interaction was hydrogen bonding between the lysine at position 2.60(80)[13] and the oxadiazolone carbonyl in ML191, the amide carbonyl in ML192, or an oxygen of the sulfonamide in ML193. The hypothesised interactions with K2.60(80) positioned the bottom aryl rings of all three structures, as represented in Figure 1, to maintain the toggle switch interaction between M3.36(105) and F6.48(239). The remaining interactions of the ligands presented in Figure 1 and GPR55 are primarily aromatic stacking with various residues. Specifically for ML191, the toluene ring attached to the cyclopropane stacks with F169 and the phenyl group attached to the oxadiazolone stacks with F6.55(246) and F3.33(102; Fig. 2). In addition to these interactions, moderate beneficial van der Waals interactions were identified between the oxadiazolone and both M7.39(274) and Y3.32(101). Since the interactions between ML191 and GPR55 centred on the three aromatic rings of ML191,

* Corresponding authors.
E-mail addresses: mabood@temple.edu (M.E. Abood), phreggio@uncg.edu (P.H. Reggio), mpcroatt@uncg.edu (M.P. Croatt).
† These authors contributed equally to this work.

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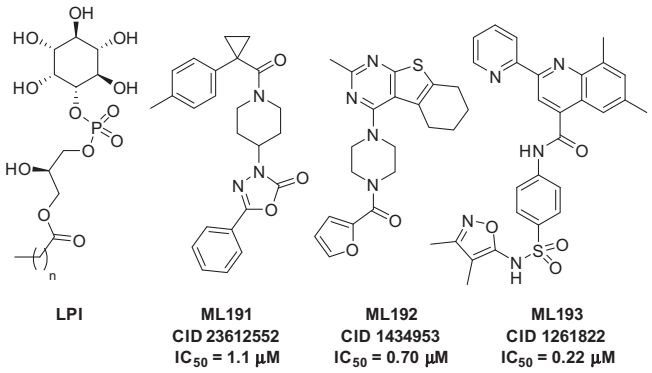


Figure 1. LPI and lead antagonists of GPR55.[12]

compounds were desired that modified the electronics and sterics of these areas. Hence, the ML191 synthetic studies reported herein were undertaken to explore the SAR of this oxadiazolone class of compounds. ML191 was also chosen as the lead antagonist since there are very few structurally related compounds that could be purchased and screened compared to the available compounds for ML192 and ML193.

Our synthetic approach to GPR55 antagonists was designed so that many different structures could be accessed to rapidly explore initial SAR, along with validating or modifying our current model (Fig. 2).[11] The synthesis begins with the coupling of a carboxylic acid to 4-piperidone by first forming the acid chloride (Scheme 1). The different acids chosen, based on the initial hit, modify the electronics and sterics of this section of the molecule. Relative to ML191, compound **2a** reduces the steric impact, **2b** increases the electron-density in the aromatic ring, and compounds **2c** and **2d** decrease the electron-density. Compounds **2e** and **2f** were selected to examine the influence of steric bulk at the position of the cyclopropane ring. The largest change in overall structure relates to the 1-naphthoic acid derivative (**2f**). Although the naphthalene ring is structurally different, this analogue can position the distal aromatic ring in a similar position as the phenyl rings of the other

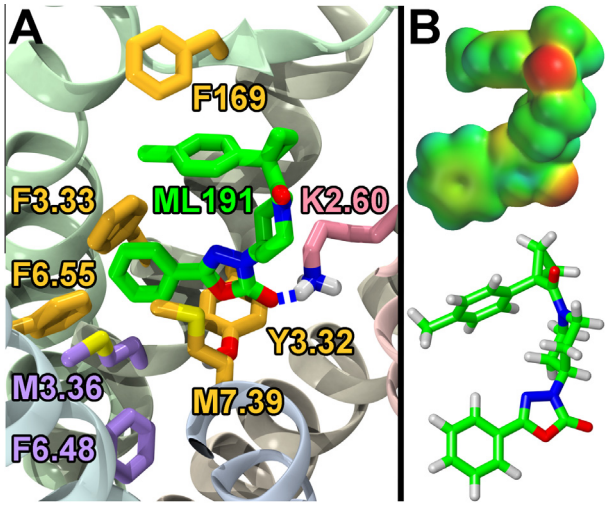
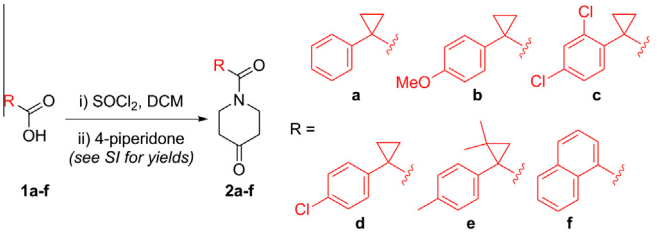


Figure 2. (A) Docking and key interactions between ML191 and GPR55. ML191 (green) has a key H-bond interaction with K2.60 (pink). ML191 also has π-stacking or other van der Waals interactions with F169, F3.33, F6.55, M7.39, and Y3.32 (all mustard). The interactions with M7.39 and F6.55 appear to hinder the rotation of M3.36 and F6.48 (both purple) which are considered the toggle switch for GPR55. (B) Electrostatic potential map of ML191. This figure is adapted from previously published work, see Ref. 12.



Scheme 1. Synthesis of acylated piperidones.

analogues since the bond angle for the Cα will be similar to that of the cyclopropane analogues, however, this structure is much flatter.

With a handful of acylated piperidones prepared, the final two steps first involved a reductive coupling of aryl hydrazides (**3t**–**z**) with the previously synthesized piperidones (**2a**–**f**) to yield hydrazides **4** (Scheme 2).[14] These compounds were then cyclocarbonylated using triphosgene to yield oxadiazolones **5**.[15] The reductive coupling reactions proceeded smoothly but the products of that step were often unstable to silica gel chromatography. Therefore, the unpurified products were treated with triphosgene without further purification. This modification of the synthesis typically improved the yields of the final compounds (see Supplementary data for individual yields).

Similar to the cyclopropane starting materials (**1a**–**f**), the hydrazides (**3t**–**z**) were selected to probe the electronic and steric opportunities in the binding site. Based on the current model (Fig. 2), the aromatic ring adjacent to the oxadiazolone is involved in an interaction with M3.36(105) and F6.48(239). Additionally, the oxadiazolone contributes as the key interaction between the basic carbonyl oxygen with the ammonium of K2.60(80). Thus, electron rich aromatic rings adjacent to the oxadiazolone should make the carbonyl oxygen more basic and strengthen this interaction.

A targeted exploration of the SAR of all six acids (**1a**–**f**) with hydrazide **3t** and all seven hydrazides (**3t**–**z**) with acid **1a** (Fig. 3 and Table 1) was performed instead of synthesizing and exploring the biological activity of all 42 permutations of the six acids and seven hydrazides. Acid **1a** and hydrazide **3t** were chosen as the constants since these were the most simplified pieces consisting of an unsubstituted phenyl ring. Unfortunately, there were solubility issues with some of the compounds (e.g., **5bt** and **5bv**), so additional combinations were required to elucidate the effect of the different areas of the scaffold.

Compounds were initially screened via an image-based cell assay to identify antagonist activity. The rationale for using the β-arrestin recruitment assay was to provide a fair comparison of IC_{50} values since our initial report employed this assay.[11,12] Briefly, U2OS cells overexpressing GPR55 and βarr2-GFP were exposed to LPI (6 μM; EC_{80}) resulting in the recruitment of β-arrestin. Antagonist activity was evaluated by ligand-mediated inhibition of LPI-induced receptor activation. This strategy quickly identified the compounds that had IC_{50} values higher than 15 μM which were excluded from further analysis (Fig. 3).

Concentration response curves were generated for compounds that were active at concentrations below 15 μM employing both the image-based β-arrestin recruitment assay and the DiscoveRx PathHunter® chemiluminescent β-arrestin complementation assay. In the DiscoveRx PathHunter® system, CHO-K1 cells stably expressing GPR55 (fused with a β-galactosidase enzyme fragment), and β-arrestin (fused to an N-terminal deletion mutant of β-galactosidase) were used to quantitate the inhibition of LPI-induced β-arrestin activity. (Fig. 4, Table 1). Hence, antagonist activity was evaluated through the use of two differential means of β-arrestin quantitation, in two different cellular backgrounds