European Journal of Medicinal Chemistry 107 (2016) 119–132

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Potent and selective *N*-(4-sulfamoylphenyl)thiourea-based GPR55 agonists

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ARTICLE INFO

Article history:
Received 22 April 2015
Received in revised form
15 October 2015
Accepted 28 October 2015
Available online 2 November 2015

Keywords:
G protein-coupled receptor 55
Molecular modeling
Structure–activity relationships

ABSTRACT

To date, many known G protein-coupled receptor 55 (GPR55) ligands are those identified among the cannabinoids. In order to further study the function of GPR55, new potent and selective ligands are needed. In this study, we utilized the screening results from PubChem bioassay AID 1961 which reports the results of Image-based HTS for Selective Agonists of GPR55. Three compounds, CID1792579, CID1252842 and CID1011163, were further evaluated and used as a starting point to create a series of nanomolar potency GPR55 agonists with *N*-(4-sulfamoylphenyl)thiourea scaffold. The GPR55 activity of the compounds were screened by using a commercial β-arrestin PathHunter assay and the potential compounds were further evaluated by using a recombinant HEK cell line exhibiting GPR55-mediated effects on calcium signalling. The designed compounds were not active when tested against various endocannabinoid targets (CB1R, CB2R, FAAH, MGL, ABHD6 and ABHD12), indicating compounds' selectivity for the GPR55. Finally, structure–activity relationships of these compounds were explored.

1. Introduction

G protein-coupled receptor 55 (GPR55) has emerged as an interesting novel target for a subset of cannabinoid ligands [1]. However, there are several features of GPR55 which do not support its classification as a cannabinoid receptor. Firstly, the strongest candidate for an endogenous GPR55 ligand is the non-cannabinoid lipid-mediator L-α-lysophosphatidylinositol (LPI), and especially its arachidonic acid derivate [2,3]. Secondly, results concerning the activation of GPR55 by cannabinoids have been controversial, with little consensus between groups [1]. Thirdly, it has even been suggested that GPR55 could act as an "anti-cannabinoid" receptor because GPR55 and cannabinoid receptor type 1 (CB1R) exhibit opposite roles in some systems, for example SR141716A is an inverse agonist/antagonist of the CB1R and an agonist at GPR55 [4,5]. Functional selectivity may also add to the complexity, where GPR55's pharmacology and downstream signalling vary in ligand- and system-dependent manners [1,6].

Despite the open questions related to GPR55 pharmacology, the receptor has been shown to have a role in an increasing array of physiological and pathological processes, including inflammation

Abbreviations: ABHD6/12, α/β-hydrolase domain containing 6/12; 2-AG, 2-arachidonoylglycerol; AEA, *N*-arachidonoylethanolamine; ARA-LPI, 1-arachidonoyl-2-hydroxy-*sn*-glycero-3-phosphoinositol; CB1/2R, cannabinoid receptor 1/2; CoMFA, comparative molecular field analysis; EA, ethyl acetate; EC, extra cellular; GlyT1, glycine transporter subtype 1; GPR55, G protein-coupled receptor 55; LPI, lysophosphatidylinositol; MGL, monoacylglycerol lipase; TMH, transmembrane helix.

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http://dx.doi.org/10.1016/j.ejmech.2015.10.050
0223-5234/

and pain [7–9], synaptic transmission [10], bone development [11], cancer [12–15], and gastrointestinal functions [16]. The GPR55/LPI system may also be associated with obesity in humans [17] whereas functional polymorphism in the GPR55 gene has been linked with anorexia nervosa [18].

To date, only a small set of selective GPR55 ligands have been reported in the literature because many ligands have been identified among the cannabinoids. In addition, even LPI has been shown to interact with certain plasma membrane ion channels and possibly additional GPCRs [19]. More selective and potent synthetic GPR55 ligands are thus needed as research tools to further study the pharmacology and function of this receptor.

Some new scaffolds for GPR55 agonists have been found by a high-content, high-throughput β-arrestin screen of 290,000 compounds (PubChem AID 1961) [20,21]. The study highlighted three new scaffolds represented by the compounds: ML184 (PubChem Compound ID: CID2440433), ML185 (CID1374043) and ML186 (CID15945391). The EC_{50} values for the compounds at GPR55 were reported to be 263 nM, 658 nM and 305 nM, respectively. The compounds were also demonstrated to be selective over CB1R, CB2R and GPR35. Three agonists found in the above mentioned screen, CID1792197, CID1172084 (analog of ML185) and ML184, have been used to identify the GPR55 agonist binding site by computational modeling [22]. Structurally similar compounds to ML184 have also been found in a diversity screening study conducted by GlaxoSmithKline [23]. However, the compounds have both GPR55 agonist and glycine transporter subtype 1 (GlyT1) inhibitor activity. The most potent GPR55 agonists of the study were GSK494581A (IC_{50} = 20 nM for GlyT1 and EC_{50} = 316 nM for GPR55) and GSK575594A (IC_{50} = 10 μM for GlyT1 and EC_{50} = 158 nM for GPR55) [23]. The structures of the above mentioned GPR55 agonists are shown in Fig. 1.

Only a few selective GPR55 receptor antagonists have been recently discovered. These include CID16020046 by Kargl and coworkers [24], compounds ML193, ML192 and ML191 by Heynen-Genel et al. [25,26] identified in collaboration with the Molecular Libraries Probe Production Centers Network initiative, and coumarin derivatives by Rempel et al. [27] In addition, some magnolol derivatives have been recently reported to behave as GPR55 antagonist, though retaining activity at additional receptors [28].

The aim of our study was to design and synthesize a series of selective and potent GPR55 agonists which do not interact with various endocannabinoid targets. At first, we utilized the screening results from PubChem bioassay AID 1961 and selected *N*-(4-sulfamoylphenyl)thiourea based structures [CID1792579 (**1**) and CID1252842 (**2**) and CID1011163 (**3**)] as a starting point for ligand development. Consequently, we screened a small set of commercial analogues having similarity with the ligands **1**–**3** for their GPR55 activity by using commercial β-arrestin PathHunter assay. The active compounds were further evaluated by using a recombinant HEK239-GPR55 cell line exhibiting GPR55-mediated effects on calcium signalling [29]. Based on the results, a total of 16 compounds were designed, synthesized and evaluated for their ability to activate the GPR55 receptor. The compounds were not active when tested against various endocannabinoid targets (CB1R, CB2R, FAAH, MGL, ABHD6 and ABHD12), indicating selectivity for the GPR55 receptor.

Overall, we report here the pharmacological evaluation of 27 GPR55 ligands, of which several turned out to be low nanomolar, high potency agonists. Structure–activity relationships (SAR) of the compounds were explored to define important features for receptor activation.

2. Results and discussion

2.1. Search and evaluation of the hit structures

A starting point for the ligand design was found from PubChem bioassay AID 1961 which reports the results of Image-based HTS for selective agonists of GPR55 [20,21]. Two compounds, CID1792579 (**1**) and CID1252842 (**2**) (Fig. 2), were selected among the active compounds based on structural similarity, potency and synthetic feasibility. The assay (AID 1961) also included one inactive compound, CID1011163 (**3**) (Fig. 2), an *N*-unsubstituted sulfonamide analogue of **1** and **2**. All three compounds were first screened by using commercial β-arrestin PathHunter assay and then further

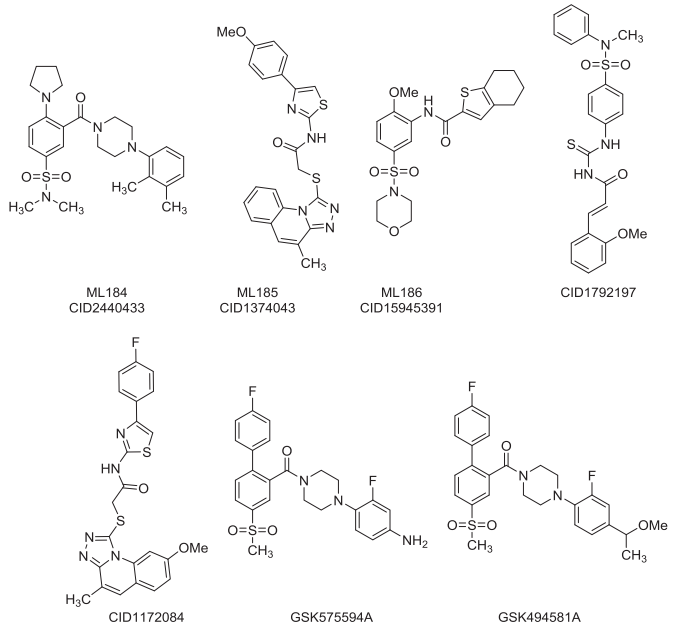
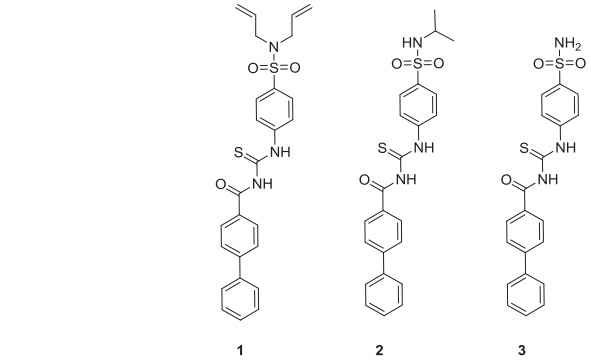


Fig. 1. Some GPR55 agonists mentioned in literature.



	1 CID1792579	2 CID1252842	3 CID1011163
EC_{50} reported in PubChem AID 1961:	500 nM	500 nM	inactive
PathHunter beta-arrestin screen for GPR55:	119[a] (105, 134)	155[a] (148, 162)	103[a] (83, 122)
Ca^{2+} signaling assay for GPR55, EC_{50}:	11.9 nM[b] (8.4-17.0 nM)	122.3 nM[b] (97.4-153.5 nM)	76.2 nM[b] (40.1-144.8 nM)

Fig. 2. The GPR55 activity data for structures **1**–**3** obtained from PubChem bioassay (AID 1961) [20,21]. (a) GPR55 agonism at 10 μM ligand concentration. Results are reported as a percentage of the luminescence given by the reference compound LPI at 1 μM concentration (mean with range of two experiments performed in duplicate). (b) Measured EC_{50} values for effects on calcium signalling (mean with 95% confidence intervals, derived from concentration-response curves, with each point representing pooled data from 24 individual cells).