LIGAND-BASED DRUG DESIGN APPROACHES FOR THE IDENTIFICATION OF NOVEL GPR55 MODULATORS

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INTRODUCTION

GPR55 is an orphan Class A G-protein coupled receptor that recognizes a sub-set of cannabinoid CB1 and CB2 ligands, suggesting that GPR55 could belong to the endocannabinoid system. Lysophosphatidylinositol (LPI) has been proposed to be endogenous ligand for GPR55. This receptor is involved in diverse physiological and pathological processes such as inflammatory and neuropathic pain, metabolic disorder, bone and neuronal development, and cancer. Few potent GPR55 ligands have been identified to date. High throughput screening of a large library of compounds from the Molecular Libraries Probe Production Centers Network (MLPCN) allowed the identification of different GPR55 chemical scaffolds, including ML192, a GPR55 antagonist. However, their potency and selectivity needs to be optimized in order to develop appropriate pharmacological tools or novel drugs to continue with the challenging goal of the validation of this receptor

AIMS

In this work, we aim to identify novel potent GPR55 antagonists based on the thienopyrimidine scaffold (hit compound ML192, Fig 1) developing a pharmacophore model that will be used as input for scaffold hopping approaches.

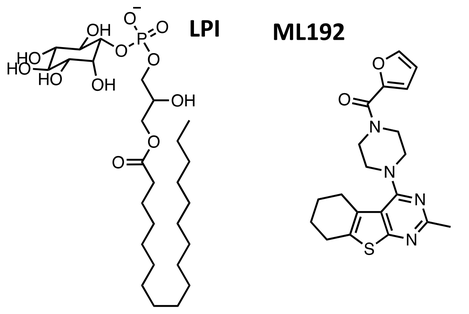


Fig. 1. Structure of the putative endogenous GPR55 ligand **LPI**, and the GPR55 hit antagonists **ML192** identified by a HTS.[1]

BACKGROUND

DOCKING AND SAR STUDIES

Previous exploration of the structure-activity relationship (SAR) of the thienopyrimidine scaffold as GPR55 antagonist was focused on modifications at positions R^1, R^2 and R^3. These modifications were proposed according to the interactions elucidated using docking studies on our GPR55 inactive state model (Figure 2). These compounds were synthesized and evaluated using a β-arrestin recruitment assay in CHO cells overexpressing human GPR55 (Table 1).

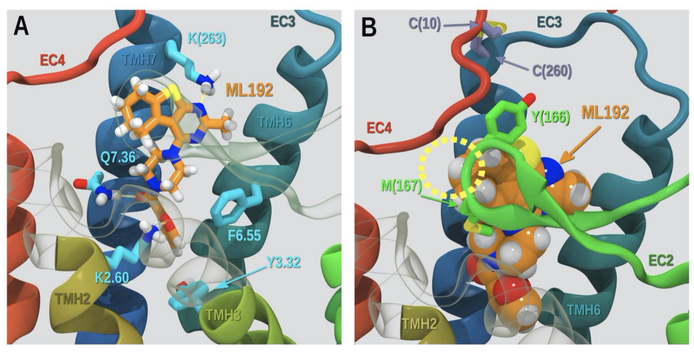
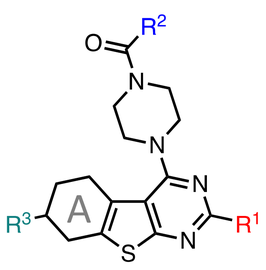


Fig. 2. Docking studies of thienopyrimidine in the GPR55 R state. **(A)** *h*GPR55 R/**ML192** complex; residues interacting with the ligand are shown in blue tubes. **(B)** *h*GPR55 R/**ML192** complex in which the ligand is shown is Van der Waals; green tubes highlight residues that establish hydrophobic interactions; purple tubes represent the EC4-EC3 disulfide bridge.



5 membered aromatic heterocycles are prefered. Bulkier or aliphatic substituents lead to a dramatic loss of activity

Subtituents such as methyl or H are prefered. Bulkier substituents such as morphonylethyl lead to loss of activity.

R3 accommodates small substituents but enhanced potency was found for the dimethyl group favoring hydrophobic interactions

PHARMACOLOGICAL EVALUATION

Compound	R1	R2	R3	IC50 (mM)
ML192	Me	Furan-2-yl	H	0.70 ± 0.22#
CID3193014	Me	Furan-2-yl	Me	0.43 ± 0.11#
CID655864	Cyclopropyl	Furan-2-yl	H	0.86 ± 0.37#
CID3193022	Me	Cyclopropyl	Me	10.6 ± 2.9#
CID1434957	Me	Cyclohexyl	H	9.24 ± 3.76
CID3193015	Me	Phenyl	Me	21.5 ± 4.6#
CID1434959	Me	M-tolyloxymethyl	H	>32#
CID3197465	Me	1,2-dimethyl-1H-benzo[*d*]imidazol-6-yl	Me	>32#
CID4877555	Me	Tetrahydrofuran-2-yl	H	>32#
CID46864267	Me	Pyridin-4-yl	Me	>32#
(PM1.4) 15	H	Furan-2-yl	H	0.68 (0.1-3.5)
(CID1434956) 16	Me	Thiophen-2-yl	H	3.7 (1.8-4.9)
(LJ114) 17	Me	1-methyl-1H-pyrrol-2-yl	H	13.1 (6.3-14.9)
(KC52) 18	Me	Furan-2-yl	diMe	0.28 (0.1-0.9)
(KC59) 19	Morpholinylmethyl	Furan-2-yl	H	2.3 (0.1-10.2)
(SS172) 26*	Me	Furan-2-yl	Me	8.0 (0.7-8.8)
(SS171) 27*	Me	Furan-2-yl	H	3.0 (0.1-9.5)
(LFA258) 34	2-hydroxyethyl	Furan-2-yl	diMe	11.7 (3.5-39.1)
(LFA287B) 35	Hydroxymethyl	Furan-2-yl	diMe	7.16 (4.5-11.3)
(LFA273) 36	Morpholinylethyl	Furan-2-yl	diMe	>32#

Table 1. *Ring A = benzene. # Data previously reported (IC_{50} ± SEM)[1]. Data represent the mean of at least 3 experiments, and 95% confidence intervals (CI) for the IC_{50} values are given in parentheses.

DRUG DESIGN

PHARMACOPHORE MODEL

The PHASE module integrated in the Maestro software (Schrödinger, LLC, NY 2019) was used to develop of a pharmacophore model (Figure 3) based on the previous SAR data (Table 1).

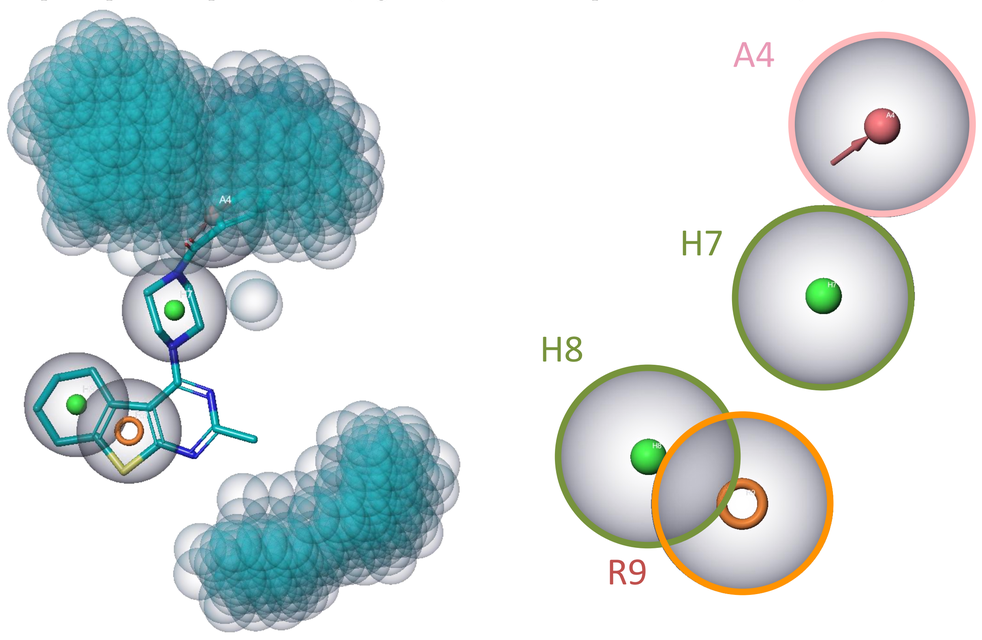


Fig. 3. **(A)** Visual representation of the thienopyrimidine pharmacophore model. The model contained "excluded volumes" (Green-blue spheres) simulating the atoms of the binding site surrounding the ligand and, thus, preventing compounds to be placed in these space points during screening. **(B)** Pharmacophore features are color-coded (pink, hydrogen bond acceptor; orange, aromatic ring; green, hydrophobic region).

SCAFFOLD HOPPING

Scaffold Hopping was used to design novel derivatives of the hit compound ML192 based on previous known structural features (Table 1). Isosteric matching core hopping (Maestro software, Schrödinger, LLC, NY 2019) using our developed pharmacophore model was used to explore fragment replacement at specified positions. The piperazine (core A, **Fig. 4**) and the thienopyrimidine moieties (core B, **Fig. 4**) were independently studied. Workflow schemes for each core, as well as filtering criteria are detailed in figure 4. Selected final molecules will be synthesized; their structure is not displayed due to potential patentability concerns.

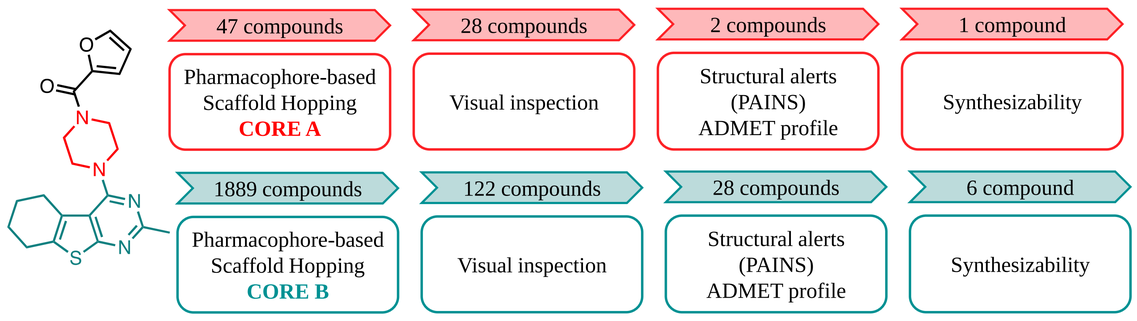


Fig. 4. Workflow scheme of the scaffold hopping process. Filtering criteria used to analyze the molecules generated through the isosteric matching core-hopping approach are detailed on each box.

CONCLUSIONS

Different computer aided drug design tecniques have been combined to identify novel GPR55 modulators based on the hit antagonist ML192. Derivatization of this compound was approached using isosteric matching core hopping tecniques based on a pharmacophore model developed upon previous SAR studies. Seven different chemotypes have been selected for the initial development following different criteria including structural diversity, ADMET profile and synthetic accessibility. These molecules will be soon synthesized; their structure is not shown due to potential patentability requirements.

References

1. Heynen-Genel, S. et al. (2010) Screening for Selective Ligands for GPR55: Antagonists; NIH; 2. Lingerfelt, M. et al. (2017) *Biochem.* 56, 473-486; 3. Meza-Avina, E. et al. (2016) *Bioorg. Med. Chem. Lett.*, 26, 1827-1830; 4. Kotsikorou, E. et al. . (2013) *Biochem.* 52, 9456-9469

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