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STRUCTURE-ACTIVITY RELATIONSHIPS OF AGONISTS FOR THE ORPHAN G PROTEIN-COUPLED RECEPTOR GPR27

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Abstract. GPR27 belongs, with GPR85 and GPR173, to a small subfamily of three receptors called "Super-Conserved Receptors Expressed in the Brain" (SREB). It has been postulated to participate in key physiological processes such as neuronal plasticity, energy metabolism, and pancreatic ß-cell insulin secretion and regulation. Recently, we reported the first selective GPR27 agonist, 2,4-dichloro-*N*-(4-(*N*-phenylsulfamoyl) phenyl)benzamide (I, pEC_{50} 6.34, E_{max} 100%). Here, we describe the synthesis and structureactivity relationships of a series of new derivatives and analogs of I. All products were evaluated for their ability to activate GPR27 in an arrestin recruitment assay. As a result, agonists were identified with a broad range of efficacies including partial and full agonists, showing higher efficacies than the lead compound I. The most potent agonist was 4-chloro-2,5-difluoro-*N*-(4-(*N*-phenylsulfamoyl)phenyl)benzamide (7y, pEC_{50} 6.85, E_{max} 37%), and the agonists with higher efficacies were 4-chloro-2-methyl-*N*-(4-(*N*-phenylsulfamoyl)phenyl)benzamide (7p, pEC_{50} 6.04, E_{max} 123%), and 2-bromo-4-chloro-*N*-(4-(*N*-phenylsulfamoyl) phenyl)benzamide (7r, pEC_{50} 5.99, E_{max} 123%). Docking studies predicted the putative binding site and interactions of agonist 7p with GPR27. Selected potent agonists were found to be soluble and devoid of cellular toxicity within the range of their pharmacological activity. Therefore, they represent important new tools to further characterize the (patho)physiological roles of GPR27.

Keywords: Agonist, GPR27, GPR85, GPR173, Orphan GPCR, Super-conserved receptors expressed in the brain, SREB, Atypical GPCR, Sulfonamide.

1. Introduction

G protein-coupled receptors (GPCRs) comprise the largest and most diverse group of cell-membrane receptors (>800) encoded by the human genome. GPCRs mediate a variety of cellular signaling pathways responding to chemical and physical stimuli such as classical neurotransmitters, peptides, hormones, lipids, sugars, proteins, and light. Due to their versatile and widespread functions in many physiological processes, they constitute a major source for the development of therapeutic drugs, and represent the focus of extensive research efforts by academia and pharma industry. Indeed, approximately 34% of all modern drugs approved by the Food and Drug Administration act by binding to GPCRs [1] and account for a global sales volume of over 180 billion US dollars annually. In addition to almost 300 described receptors, about 100 of these are poorly characterized orphan receptors whose natural ligand and physiological functions are still unknown or unconfirmed [2,3]. Hitherto, the discovery of a GPCR function has often led to new paradigms and concepts in health and disease. Thus, orphan receptors are expected to play unsuspected roles in diverse cellular and organ functions [4].

Super-conserved receptors expressed in the brain (SREB) are a family of three orphan G protein-coupled receptors: GPR27 (SREB1), GPR85 (SREB2), and GPR173 (SREB3) [5,6]. They are mainly expressed in the central nervous system (CNS) [7,8], and have a high degree of sequence conservation in vertebrates [5]. Among the SREBs, GPR27 has attracted much attention due to its putative role in pancreatic ß-cell insulin transcription [9,10] and glucose-stimulated insulin secretion [9]. However, its

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precise role in insulin activity remains elusive as recent whole-body GPR27 knockout mouse studies revealed that loss of GPR27 has only minor effects on glucose tolerance, although its deletion reduces insulin mRNA levels [10]. Recently, Nath et al. reported the potential role of GPR27 in lipid metabolism, insulin signaling, and glucose homeostasis [11]. In addition, the high expression levels of GPR27 in the brain, especially in the hippocampus, suggests a significant role in neural plasticity [7]. The whole body zebrafish gpr27 knockout revealed elevated glucose levels. Notably, gpr27 deletion elevated medium-chain acylcarnitines, lipid species known to be involved in insulin resistance in humans. Given its potential as a novel drug target, further investigation is warranted for understanding the (patho) physiological and pharmacological functions of GPR27. However, the absence of knowledge of the endogenous ligand and the paucity of surrogate ligands complicate the examination of its functional role.

Our group recently reported GPR27 agonists I and II (Fig. 1), discovered upon a screening campaign with an assay developed inhouse based on the recruitment of **ß-**arrestin-2 by a chimeric form of GPR27 termed GPR27V2 [12]. This construct bears the vasopressin V2 receptor C-terminal tail to increase the sensitivity of the assay, which is a standard strategy for the detection of GPCR ligands [13]. The agonists we identified were able to specifically recruit **ß-**arrestin-2 to the unmodified receptor, and were selective compared to the other SREBs, GPR85 and GPR173. Interestingly, compounds containing pyrazole and coumarine scaffolds were reported as nonselective inverse agonists for GPR27 [14]. However, these substances were inactive as inverse agonists in our test system [12].

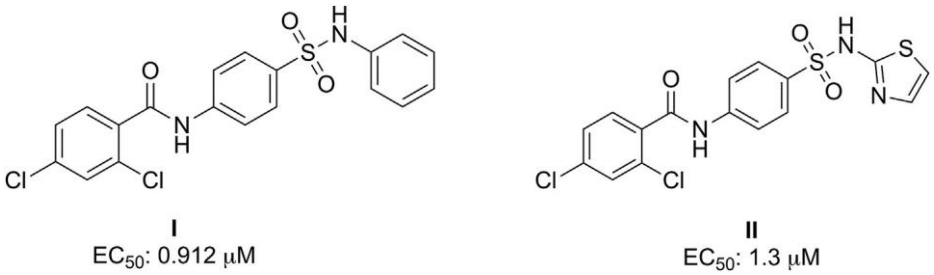

Fig. 1. Structure and activity of GPR27 agonists. The EC_{50} of **I** and **II** at GPR27V2 are given.

In the present study, we aimed to investigate the structure-activity relationships (SARs) of I and define a pharmacophore for GPR27 agonism. We synthesized a variety of derivatives and analogs of **I** and evaluated them for their ability to recruit ß-arrestin 2 by activation of the GPR27V2 receptor in a firefly luciferase complementation assay.

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2. Results and discussion

2.1. COMPOUNDS DESIGN

To explore the SARs of the lead molecule 2,4-dichloro-*N*-(4-(*N*-phenylsulfamoyl)phenyl)benzamide (I), the following systematic structural modifications were performed (Fig. 2): Exploration of different substitutions on ring C; (B) the role of the sulfonamide NH probing the importance of hydrogen bond interaction between NH and the receptor; (C) replacement of the amide with a sulfonamide between ring A and B; (D) introduction of a reversed amide group between ring A and B; (E) replacement of the sulfonamide with an amide between ring B and C; (F) insertion of a methylene unit between ring A and the amide group; (G) substitution of ring A in various positions, and (H) replacement of the monocyclic ring A with bicyclic ring systems.

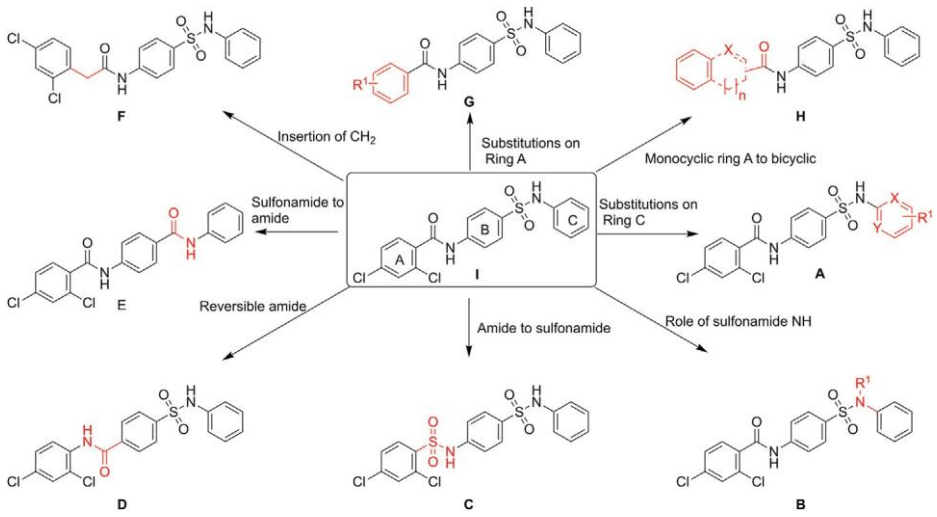

Fig. 2. Systematic structural modifications of lead molecule **I**.

2.2. CHEMISTRY

At first, the key sulfonamide intermediates **4a-m** (Scheme 1) were synthesized according to published procedures [15e18]. Briefly, condensation of commercially available acetamidobenzenesulfonyl chlorides (**1a-b**) with a variety of amines **2a-m** in the presence of triethylamine in dichloromethane (DCM) yielded the corresponding sulfamoylphenylacetamides **3a-m** in excellent yields. Subsequently, these acetamides were deprotected to obtain the amines **4a-m** by treating with concentrated hydrochloric acid under reflux conditions.

Target compounds **7a-l** were synthesized by coupling reaction of sulfonamide derivatives **4a-m** with 2,4-dichlorobenzoyl chloride (**6a**), which was previously prepared from the corresponding carboxylic acid **5a** by reaction with thionyl chloride under reflux (Scheme 1). The intermediate 4-amino-*N*-

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phenylbenzenesulfonamide (**4a**) reacted with 2,4dichlorobenzenesulfonyl chloride (**5b**) in the presence of triethylamine to produce compound **7m** (Scheme 1). The reversed amide derivative **7n** was synthesized by reaction of 2,4-dichloroaniline (**6c**) with 4-(**N**-phenylsulfamoyl)benzoic acid (**4n**) in the presence of (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-**b**]pyridinium 3-oxide hexafluorophosphate (HATU), and diisopropylethylamine (DIPEA) in DMF in a good yield (Scheme 1).

Compound **7o** was prepared from 2,5-dichloroacetic acid (**5d**) via its carboxylic acid chloride (**6d**) and subsequently coupled with **4a** (Scheme 2).

Compounds **7p-z** and **7aa** (Scheme 2) were prepared from various aryl carboxylic acids (**5e-p**). First, the appropriate carboxylic acids were converted to the corresponding acid chlorides **6e-p** and subsequently treated with **4a** in the presence of $NaHCO_3$ to produce 7**p-z** and **7aa** in excellent yields.

In a further series of compounds, the monocyclic ring was replaced by a bicyclic ring system. The appropriate bicyclic carboxylic acids **5q-r** were converted to their acid chlorides **6q-r** in the presence of $SOCl_2$ and instantly reacted with **4a** to produce compounds **7 ab-ac** (Scheme 2).

To probe the role of the sulfonamide functionality in the lead compound, a corresponding amide derivative **7ad** was synthesized by coupling of **5a** with 4-amino-N-phenylbenzamide (**6s**) in the presence of HATU and DIPEA at room temperature [19].

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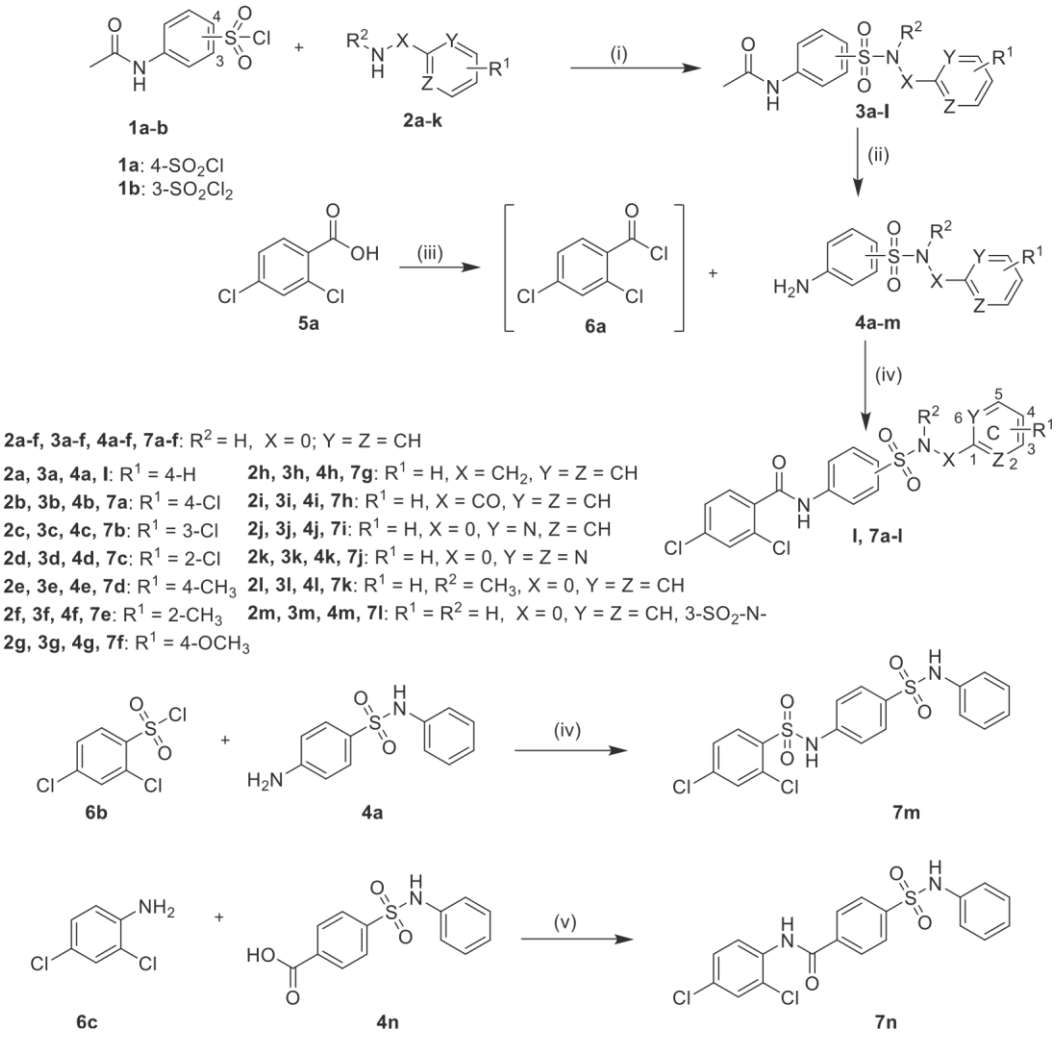

Scheme 1. Synthesis of sulfonamide-derived products **7a-n**. Reagents and conditions: (i) DCM, Et_3N, RT, 2 h, 62e78%; (ii) concd. HCl, 100 C, 2 h, 69e90%; (iii), DCM, $SOCl_2$, 50 C, 1 h; (iv) acetone, $NaHCO_3$, RT, 12 h, 79e92%; (v) HATU, DIPEA, DMF, RT, 12 h, 96%.

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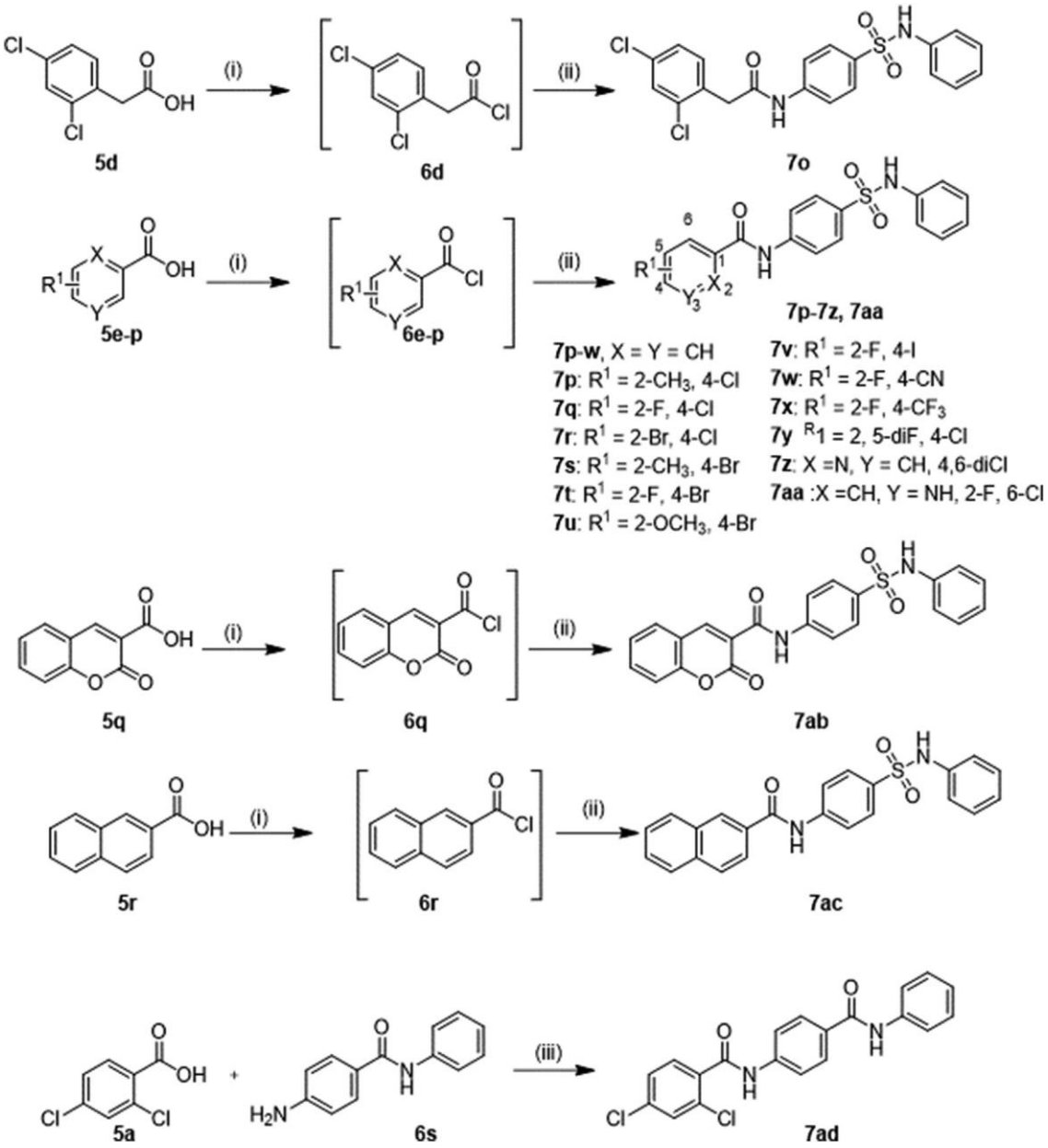

Scheme 2. Synthesis of compounds **7o**-**7z**, **7aa-ad**. Reagents and conditions: (i) DCM, $SOCl_2$, 50 C, 1 h; (ii) **4a**, acetone, $NaHCO_3$, RT, 12 h; 71e89% (iii) HATU, DIPEA, DMF, RT, 12 h, 85%.

^1H, and ^{13}C NMR, spectra confirmed the structures of all synthesized final products. The purity of the compounds was determined by high-performance liquid chromatography (HPLC) coupled to UV and electrospray ionization mass spectrometry (ESIMS) confirming a purity of at least 95%.

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2.3. PHARMACOLOGICAL EVALUATION

The agonistic activity at GPR27 of all final compounds **7a-z**, **7aa-ad** (Table 1) was investigated according to our previously reported procedure [12]. In our previous study, we could not detect a coupling of GPR27 with G proteins and assumed that the receptor displays an atypical pharmacology. Accordingly, we resorted for the present study to a validated firefly luciferase complementation assay to detect ß-arrestin 2 recruitment to the activated receptor in HEK293 cells stably transfected with GPR27V2 [12]. Each compound was tested at several concentrations ranging from 33 nM to 33 µM. pEC_{50} values were calculated for each compound concentration-response curve that reached saturation. When possible, the efficacy of the compounds was measured by comparing their maximal effects to the maximal signal induced by the lead compound **I** (pEC_{50} 6.34, E_{max} 100%).

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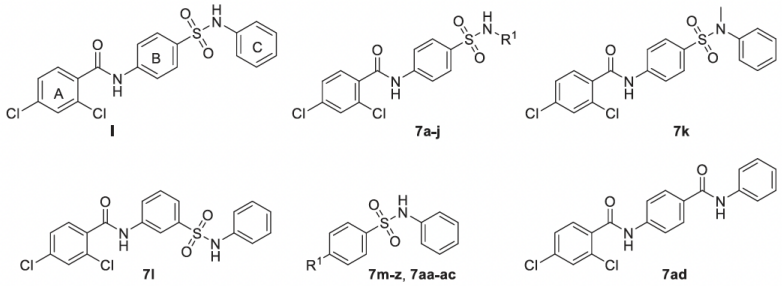
Table 1
Agonistic activities of **7a**-**z**, **7aa**-**ad** at the GPR27 determined in a firefly luciferase complementation assay.



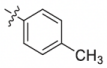
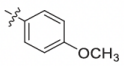
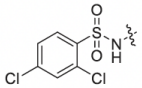
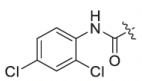
Compounds No.	Substituent	Human GPR27	
		pEC_{50}[a]	E_{max} (%)[b]
I	see above for structure	**6.34** ± 0.27 [12]	100
7a		< **4**	n.d.
7b		**6.24** ± 0.15	40
7c		**5.90** ± 0.17	53
7d		< **4**	n.d.
7e		**5.91** ± 0.28	32
7f		< **4**	n.d.
7g		< **4**	n.d.
7h		< **4**	n.d.
7i		**5.52** ± 0.20	40
7j		< **4**	n.d.
7k	See above for structure	**5.59** ± 0.14	50
7l	See above for structure	< **4**	n.d.
7m		< **4**	n.d.
7n		< **4**	n.d.

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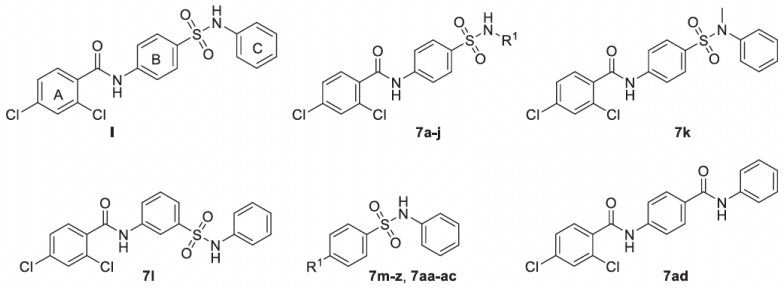
Table 1 (*continued*)



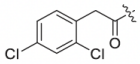
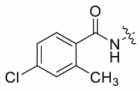
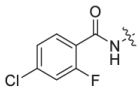
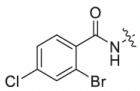
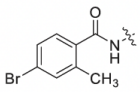
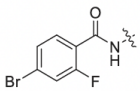
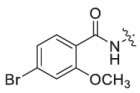
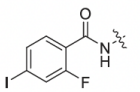
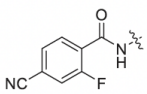
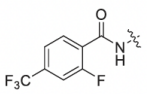
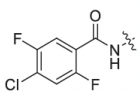
Compounds No.	Substituent	Human GPR27	
		pEC_{50}[a]	E_{max} (%)[b]
7o		< **4**	n.d.
7p		**6.04** ± 0.17	123
7q		**5.40** ± 0.30	57
7r		**5.99** ± 0.12	123
7s		**6.07** ± 0.07	103
7t		**6.32** ± 0.12	67
7u		**5.99** ± 0.26	n.d.
7v		**5.29** ± 0.55	36
7w		**6.64** ± 0.17	29
7x		**6.44** ± 0.13	50
7y		**6.85** ± 0.27	37

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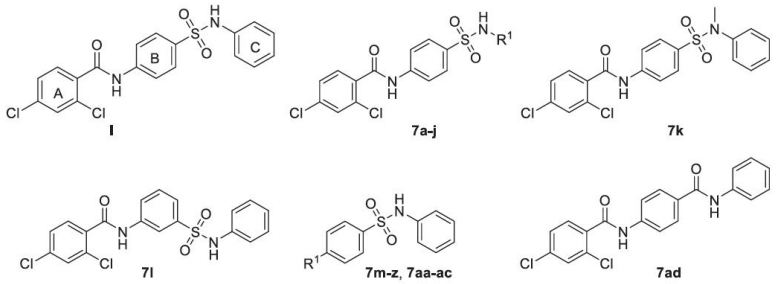
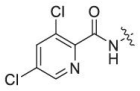
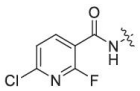
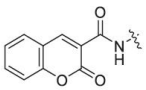
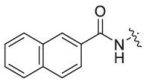
Table 1 (*continued*)



Compounds No.	Substituent	Human GPR27	
		pEC_{50}[a]	E_{max} (%)[b]
7z		**5.24** ± 0.12	70
7aa		**5.64** ± 0.12	78
7 ab		**5.11** ± 0.18	53
7ac		**5.07** ± 0.06	115
7ad	See above for structure	< **5**	n.d.

[a] pEC_{50} values were measured in a β-arrestin 2 recruitment—based firefly luciferase complementation assay. Data points represent the mean ± S.E.M. of three replicates. The activity of all compounds has been verified in at least 2 independent experiments.
[b] Emax is expressed as the percentage of the maximal activity of the lead compound **I**. n.d. = not determined.

3. Structure activity relationships

In the present study, 30 new sulfonamide derivatives and analogs were synthesized and tested as agonists for GPR27.

To gain insights into the SARs, we initially focused on substitutions of ring C. The following substituents were introduced: 4-, 3-, or 2-Cl (**7a-c**), 4- or 2-CH_3 (**7d**, **7e**), and 4-OCH_3 (**7f**). Activity was abolished with 4-Cl (**7a**), 4-CH_3 (**7d**) and 4-OCH_3 (**7f**) substituents, while 3-Cl (**7b**, pEC_{50} 6.24) maintained, and 2-Cl (**7c**, pEC_{50} 5.90) and 2-CH_3 (**7e**, pEC_{50} 5.91 reduced the agonistic activity compared to the lead compound **I**.

Next, we inserted a methylene or carbonyl group between the sulfonamide and ring C. However, the resulting analogs **7g** and **7h** were inactive, suggesting that ring C should be directly connected to the sulfonamide functionality. The replacement of ring C in lead compound **I** with pyridine (**7i**, pEC_{50} 5.2) showed moderate activity, while pyrimidine (**7j**) lost the agonistic activity. These results indicated that the hydrophobic ring C was favorable for interaction with the receptor.

Methylation of the sulfonamide NH hydrogen in lead structure **I** (pEC_{50} 6.34) yielding the corresponding N-methylated compound **7k** (pEC_{50} 5.59) slightly reduced the agonistic activity. When

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the position of the sulfonamide group was changed from position 4 to 3 on ring B, a complete loss of agonistic activity was observed with the resulting analog **7l**, indicating that the position of the sulfonamide group on ring B of the lead compound was important for receptor activation.

In the next effort, the amide group between ring A and ring B in lead compound **I** was replaced with a sulfonamide (**7m**), a reversed amide (**7n**), or an acetyl group (**7o**). However, none of the tested compounds showed agonistic activity at GPR27. These results indicated that the presence of an amide group is crucial for the agonistic activity.

In the next set of experiments, we investigated the substituent effect on ring A. At first, we replaced the chlorine at position 2 of lead compound **I** with 2-CH_3, 2-F, or 2-Br. The 2-methyl- (**7p**, pEC_{50} 6.04) and 2-bromo- (**7r**, pEC_{50} 5.99) substituted derivatives showed equipotent agonistic activities to lead compound I, while the 2-fluoro-substituted derivative (**7q**, pEC_{50} 5.40) reduced the potency. To obtain deeper insight into the SARs of sulfonamide derivatives, we expanded the study by investigating different substituents on ring A of lead structure **I**. The rank order of potency for substituents in various positions of ring A is as follows: 2,5-diF, 4-Cl (**7y**, pEC_{50} 6.85) ~ 2-F, 4-CN (**7w**, pEC_{50} 6.64) ~ 2-F, 4-CF_3 (**7x**, pEC_{50} 6. 44) ~ 2-F, 4-Br (**7t**, pEC_{50} 6.32) > 2-CH_3, 4-Br (**7s**, pEC_{50} 6.07) ~ 2-OMe, 4-Br (**7u**, pEC_{50} pEC_{50} 5.99) > 2-F, 4-I (**7v**, pEC_{50} 5.29). Compounds **7t**, **7w**, **7x**, and **7y** were about equipotent to the lead compound. Compounds **7u** and **7v** slightly reduced agonistic activity.

Introduction of a heteroatom, like nitrogen, in ring A yielded compounds **7z** (pEC_{50} 5.24) and **7aa** (pEC_{50} 5.64), both showing reduced agonistic activity. Next, ring A in the lead compound was replaced with bicyclic structures such chromen-2-one (**7 ab**, pEC_{50} 5.11), and naphthalene (**7ac**, pEC_{50} 5.07). However, the resulting analogs **7 ab**, and **7ac** displayed reduced potency. We subsequently examined the role of the sulfonamide linker by replacing it with an amide group. However, the resulting analog **7ad** (pEC_{50}<5) showed reduced agonistic activity, indicating that the presence of a sulfonamide group is favorable for activating the receptor GPR27.

The structure-activity relationships of sulfonamide derivatives at GPR27 are summarized in Fig. 3. In general, the results indicated steep SARs. Substitutions like 2-F, 4-CN (**7w**, pEC_{50} 6.64), 2-F, 4-CF_3 (**7x**, pEC_{50} 6.44), and 2,5-diF, 4-Cl (**7y**, pEC_{50} 6.85) on ring A resulted in compounds that were equipotent to the lead structure. The amide group between ring A and B and the sulfonamide functionality are crucial for agonistic activity. Changing the substitution pattern of the sulfonamide on ring B from the p-to the m-position led to a loss of activity (compare **I** and **7l**). Exchange of the sulfonamide by an amide function reduced agonistic activity (compare **I** and **7ad**). The direct connection of ring C to the sulfonamide is important (compare **I** and **7g**, **7h**). Substitution on ring C reduced the agonistic activity. Replacement of ring C with heteroaryl rings diminished potency (compare **I** and **7i**, **7j**).

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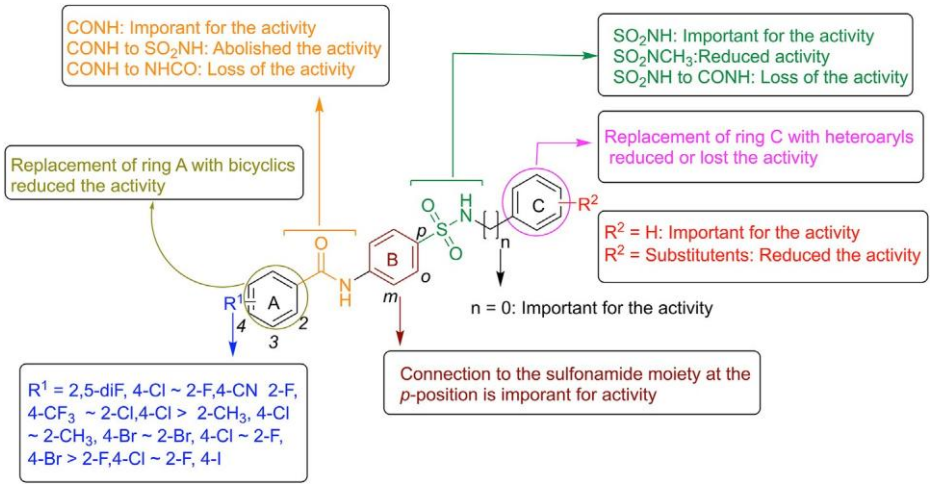

Fig. 3. Structure-activity relationships of sulfonamide-derived GPR27 agonists.

Efficacy. In the ß-arrestin 2 recruitment assay, compounds **7p** (E_{max}: 123%), **7r** (E_{max}: 123%), and **7ac** (E_{max}: 115%) showed somewhat higher efficacy compared to the lead compound **I** (E_{max}: 100%). Compound **7s** (E_{max}: 103%) was similarly efficacious as **I**. Other active agonists such as **7t** (E_{max}: 67%), (**7v**, E_{max}: 36%), **7w** (E_{max}: 29%), **7x** (E_{max}: 50%), **7y** (E_{max}: 37%), **7z** (E_{max}: 70%), **7aa** (E_{max}: 78%), and **7ad** (E_{max}: 53%) showed markedly lower efficacies ranging from 29 to 78%.

Concentration-response-curves for the most interesting compounds **7p**, **7s**, **7x**, **7w**, **7y** as well as for the lead compound **I** are displayed in Fig. 4.

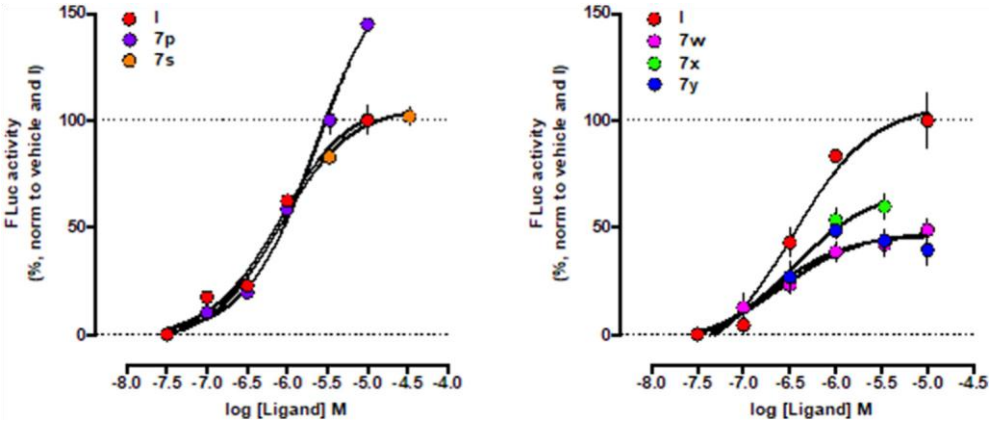

Fig. 4. Concentration-response curve for the most potent compounds to detect ß-arrestin 2 recruitment to the activated receptor in HEK cells stably transfected with GPR27: **7p** (pEC_{50} 6.04 ± 0.17, E_{max} 123%), **7s** (pEC_{50} 6.07 ± 0.07, E_{max} 103%), **7w** (pEC_{50} 6.64 ± 0.17, E_{max} 29%), **7x** (pEC_{50} 6.44 ± 0.13, E_{max} 50%), **7y** (pEC_{50} 6.85 ± 0.27, E_{max} 37%). Data are mean ± SEM, n ¼ 3, representative of at least two independent experiments.

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To exclude potential artifacts due to limited solubility of some of the compounds at higher concentration, we performed additional experiments (see SI, Fig. S1). Our data clearly showed that at concentrations up to 10 µM and in many cases up to >30 µM, no significant effects of the compounds solubility on the readout was visible. Some compounds showed sign of precipitation at higher concentration and should be used with caution at concentrations above 10 µM.

4. Cytotoxicity studies

To further assess the properties of the compounds we synthesized, we evaluated their cellular toxicity with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Fig. S2 displays the data which we obtained for the selected compounds. In general, the compounds were well tolerated in our cell cultures. None of the compounds was cytotoxic at concentrations up to 33.3 µM. Interestingly, some compounds such as **I**, **7p**, **7s** or **7x** tended to slightly increase the signal at high concentrations (Fig. S2). This effect was statistically significant with the compound **7x** at the concentration of 33.3 µM.

5. Molecular modelling

Given the availability of the homology model for GPR27, GPR85 and GPR173, in the proposed active conformation, we aimed to predict the binding mode of our compounds, as well as suggest a mechanism for the apparent selectivity of our lead compound **I**. In this sense, we calculated the potential binding affinity (using MM-GBSA) of **I**, as well as the most potent full agonist (**7p**) and partial agonist (**7y**), against the homology model structures of the three most relevant GPCR structures from the same family ((https:// gpcrdb.org, accessed on August 2021). Compounds were docked within the hydrophobic main cavity of each receptor, using flexible docking, and had their binding energy calculated after minimization. Fig. 5 for the docking poses and Table S1 displaying the calculated binding energies. The selectivity of the lead compound towards GPR27 can be potentially explained by its increased predicted binding affinity (63.24 kcal/mol), when compared to the other receptors (56.93 for GPR85 and -50.98 for GPR173, Table S1). We also hypothesize that this preference should also be observed, to a lesser extent, with **7p** and **7y**.

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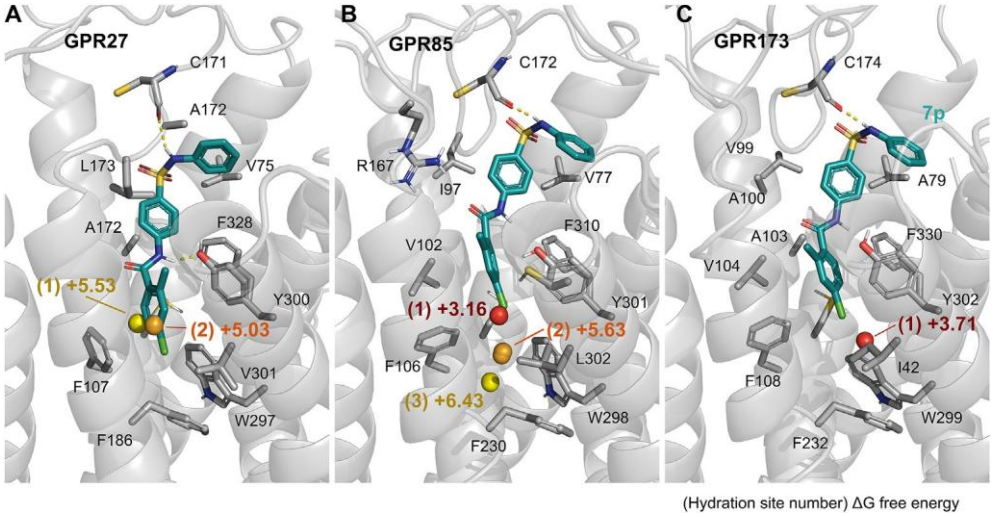

Fig. 5. Conserved hydration sites in the hydrophobic pocket of each SREB (GPR27, GPR85 and GPR173) can explain the differences in the compound's energy binding. All calculations were performed based on apostructure receptor, around the ligand coordinates generated by docking, and the agonist **7p** potential binding modes are superimposed as reference. Conserved water molecules are presented as colored spheres (see Table S2 for individual energy values), and have the average of their WaterMap based free energy values (ΔG, kcal/ mol) shown. The highlighted spheres represent regions where ligand occupancy could contribute to binding affinity by energy gain.

Since the chemical changes between these compounds lie in ring A, which is buried in the pocket, we suggest that the differences in binding affinity do not originate from enthalpic interactions, but rather arising from solvent displacement and entropy energy gain. Therefore, we proceeded with WaterMap calculations, which provides an estimate of potential hydration sites and their energies, along with short MD simulations. We analyzed the hydration sites in proximity to where the ring A would be positioned (Fig. 5 and Table S2), where high energy sites represent regions where ligand occupancy could contribute to binding affinity by energy gain.

As observed in Fig. 5, GPR27 high energy hydration sites are fully occupied by ring A, while the most relevant sites of GPR85 and GPR173 are unoccupied. This can explain the higher calculated binding affinity of the compounds for GPR27. These regions could be relevant for later ligand development with longer substituents in ring A. However, the accurate prediction of potential modifications is limited by the quality of the available homology models and the short simulation times.

6. Conclusion

In conclusion, a series of 30 new sulfonamide derivatives and analogs was synthesized to analyze their SARs at the poorly investigated orphan receptor GPR27. Starting from compound **I** as a lead structure, our goal was to define the pharmacophore, study its SARs and improve its potency as GPR27 agonist. We investigated the activity of all the compounds with a firefly luciferase complementation

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assay and gathered information on the solubility and toxicity of the most interesting compounds. The most active compounds showed good potencies (pEC_{50} >6) as agonists of GPR27 with E_{max} values ranging from 30 to 120% compared to the reference compound **I**. In addition, the estimated solubility and cellular toxicity was not problematic at pharmacologically relevant concentrations. Thus, these new derivatives will be crucial tool compounds for elucidating the physiologic roles and therapeutic potential of GPR27.

7. Experimental section

7.1. CHEMISTRY

7.1.1. GENERAL METHODS

All commercially available reagents were used as purchased (Acros, Alfa Aesar, Sigma-Aldrich, ABCR or TCI). Solvents were used without additional purification or drying except for dichloromethane, which was distilled over calcium hydride. The reactions were monitored by thin-layer chromatography (TLC) using aluminum sheets with silica gel 60 F254 (Merck). Column chromatography was performed with 0.060e0.200 mm silica gel with pore diameter of ca. 6 nm. All synthesized compounds were finally dried in vacuum at 8e12 Pa (0.08e0.12 mbar) using a sliding vane rotary vacuum pump (Vacuubrand GmbH). ^{1}H and ^{13}C NMR data were collected on a Bruker Avance 500 MHz NMR spectrometer at 500 and 126 MHz, respectively. If indicated, NMR data were collected on a Bruker Ascend 600 MHz NMR spectrometer at 600 MHz (^{1}H) and 151 MHz (^{13}C). DMSO-d_6 was employed as a solvent at 303 K, unless otherwise noted. Chemical shifts are reported in parts per million (ppm) relative to the deuterated solvent, that is, DMSO, ∂ 1 H: 2.49 ppm; ^{13}C: 39.7 ppm. Coupling constants J are given in hertz, and spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), sext. (sextet), m (multiplet), and br (broad). Melting points were determined on a Büchi 530 melting point apparatus and are uncorrected. The purities of isolated products were determined by ESI-mass spectra obtained on a liquid chromatography-mass spectrometry (LC-MS) instrument (Applied Biosystems API 2000 LCMS/MS, HPLC Agilent 1100) using the following procedure: the compounds were dissolved at a concentration of 1.0 mg/mL in acetonitrile containing 2 mM ammonium acetate. Then,10 µL of the sample was injected into an HPLC column (Macherey-Nagel Nucleodur 3 µ C18, 50 x 2.00 mm^2). Elution was performed with a gradient of water/acetonitrile (containing 2 mM ammonium acetate) from 90:10 to 0:100 for 20 min at a flow rate of 300 µL/min, starting the gradient after 10 min. UV absorption was detected from 200 to 950 nm using a diode array detector. Purity of all compounds was determined at 254 nm. The purity of the compounds was generally 95%.

7.2. GENERAL PROCEDURE FOR THE SYNTHESIS OF 4A-M

3-/4-Acetamidobenzenesulfonyl chloride (**1**, 1 equiv.) was dissolved in dichloromethane (80 mL) at 0 C. An appropriate aniline (1.05 equiv.) and triethylamine (2 equiv.) were added to the solution dropwise. Then, the mixture was stirred for 2 h at room temperature. TLC monitored reaction progress.

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After reaction completed, 2 N HCl and Brine solution were added to the mixture. The residue was obtained by washing the mixture with water and dichloromethane and the solid recovered by filtration. The compound was added to 12 N HCl, heated to reflux and stirred for 2 h. Cooled to room temperature, the mixture was washed by NaOH, and extracted with dichloromethane (3 x 30 mL) and methanol (3 x 30 mL). Combined organic layers were dried over magnesium sulfate and concentrated under vacuum. The crude products were purified by a silica-gel column chromatography. **4a** [15], **4b** [20], **4c** [21], **4d** [21], **4e** [17], **4f** [22], **4g** [17], **4h** [15], **4i** [23], **4j** [21], **4k** [24], **4l** [25], **4m** [26].

7.3. GENERAL PROCEDURE FOR THE SYNTHESIS OF I, 7A-L

To the solution of 2,4-dichlorobenzoic acid (**5a**, 0.300 g, 15.7 mmol) in dichloromethane (30 mL), 3 drops of dimethylformamide and 1 mL of thionylchloride were added dropwise one by one. After reaction completed, monitored by TLC, the mixture was concentrated under vacuum and dichloromethane (4 x 10 mL) was added to remove the excess thionyl chloride during evaporation. The resulting 2,4-dichlorobenzoyl chloride (**6a**) was subsequently treated with appropriate sulfonamide derivatives **4a-m** (17 mmol) in the presence of $NaHCO_3$ (50 mmol) in acetone at room temperature overnight. After reaction completed, acetone was evaporated and the residue was poured onto ice-water. The mixture was then extracted with ethyl acetate (3 x 30 mL). The collected organic layer was washed by brine solutions, dried over magnesium sulfate, and concentrated under vacuum. The product was purified by column chromatography by using 40e50% ethyl acetate in petroleum ether.

7.3.1. 2,4-DICHLORO-N-(4-(N-PHENYLSULFAMOYL)PHENYL)BENZAMIDE (I)

Compound **I** was synthesized between the reaction of 2,4-dichlorobenzoyl chloride (**6a**) obtained from 2,4-dichlorobenzoic acid (**5a**, 0.300 g, 15.7 mmol) and 4-amino-N-phenyl-benzenesulfonamide (**4a**, 0.428 g, 17 mmol). Colorless solid (86% yield). ^{1}H NMR (500 MHz, DMSO-d_6) ∂ 10.86 (s, 1H), 10.17 (s, 1H), 7.81 (d, J ¼ 8.8 Hz, 2H), 7.77e7.68 (m, 3H), 7.63 (d, J ¼ 8.3 Hz, 1H), 7.55 (dd, J ¼ 8.3, 2.0 Hz, 1H), 7.27e7.16 (m, 2H), 7.09 (dt, J ¼ 8.7, 1.7 Hz, 2H), 7.06e6.95 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) ∂ 164.60, 142.54, 137.92, 135.31, 134.34, 131.34, 130.50, 129.37, 129.24, 128.10, 127.63, 124.10, 120.13, 119.50. LC-MS: positive [m/z] ¼ 421.0 [M+H]$^+$. Purity by HPLC-UV (254 nm) ESI-MS: 98.0%.

7.3.2. 2,4-DICHLORO-N-(4-(N-(4-CHLOROPHENYL)SULFAMOYL)PHENYL) BENZAMIDE (7A)

Compound **7a** was synthesized between the reaction of 2,4-dichlorobenzoyl chloride (**6a**) obtained from 2,4-dichlorobenzoic acid (**5a**, 0.300 g, 15.7 mmol) and 4-amino-N-(4-chlorophenyl) benzenesulfonamide (**4b**, 0.480 g, 17 mmol). Colorless solid (79% yield), mp 178e180 C. ^{1}H NMR (600 MHz, DMSO-d_6) ∂ 11.11 (s, 1H, NH), 8.04e7.99 (m, 3H), 7.79 (d, J ¼ 1.9 Hz, 1H), 7.69 (d, J ¼ 8.2 Hz, 1H), 7.58 (dd, J ¼ 8.2,1.9 Hz,1H), 7.54 (s,1H), 7.54 (d, J ¼ 8.3 Hz,1H), 7.43 (q, J ¼ 8.9 Hz, 3H), 7.32 (dd, J ¼ 8.3, 1.9 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) ∂ 164.94, 144.33, 135.58, 135.23, 134.95, 134.07, 133.24, 132.14, 131.91, 131.42, 130.64, 130.32, 130.31, 129.59, 129.51, 127.77, 127.52, 119.47. LC-MS: positive [m/z] ¼ 455.0, 457.0 [M+H]$^+$. Purity by HPLC-UV (254 nm) ESI-MS: 96.0%.

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7.3.3. 2,4-DICHLORO-N-(4-(N-(3-CHLOROPHENYL)SULFAMOYL)PHENYL) BENZAMIDE (7B)

Compound **7b** was synthesized between the reaction of 2,4-dichlorobenzoyl chloride (**6a**) obtained from 2,4-dichlorobenzoic acid (**5a**, 0.300 g, 15.7 mmol) and 4-amino-*N*-(3-chlorophenyl) benzenesulfonamide (**4c**, 0.480 g, 17 mmol). Colorless solid (81% yield), mp 162e164 C. ^{1}H NMR (500 MHz, DMSO-d_6) ∂ 10.91 (d, *J* ¼ 11.9 Hz, 1H), 7.82 (t, *J* ¼ 15.6 Hz, 2H), 7.79e7.72 (m, 3H), 7.63 (d, *J* ¼ 8.2 Hz, 1H), 7.55 (dd, *J* ¼ 8.3, 2.0 Hz, 1H), 7.25 (d, *J* ¼ 8.0 Hz, 1H), 7.11 (s, 1H), 7.07e7.04 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) ∂ 164.49, 142.84, 139.61, 135.31, 133.91, 133.53, 131.38, 131.09, 130.55, 129.42, 128.19, 127.68, 123.78, 119.67, 119.13, 118.07. LC-MS: positive [*m*/*z*] ¼ 455.0, 457.0 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 95.5%.

7.3.4. 2,4-DICHLORO-N-(4-(N-(2-CHLOROPHENYL)SULFAMOYL)PHENYL) BENZAMIDE (7C)

Compound 7c was synthesized between the reaction of 2,4-dichlorobenzoyl chloride (**6a**) obtained from 2,4-dichlorobenzoic acid (**5a**, 0.300 g, 15.7 mmol) and 4-amino-*N*-(2-chlorophenyl) benzenesulfonamide (**4d**, 0.480 g, 17 mmol). Colorless solid (86% yield), mp 171e173 C. ^{1}H NMR (500 MHz, DMSO-d_6) ∂ 10.90 (s,1H), 7.84 (d, *J* ¼ 8.8 Hz, 2H), 7.81e7.67 (m, 3H), 7.63 (d, *J* ¼ 8.2 Hz, 1H), 7.55 (dd, *J* ¼ 8.3, 2.0 Hz,1H), 7.25 (d, *J* ¼ 8.0 Hz,1H), 7.11 (t, *J* ¼ 2.0 Hz, 1H), 7.11e6.91 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) ∂ 164.69, 142.84, 139.61, 135.31, 133.91, 133.53, 131.38, 131.09, 130.55, 129.42, 128.19, 127.68, 119.67, 119.13. LC-MS: positive [*m*/*z*] ¼ 455.0, 457.0 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 96.0%.

7.3.5. 2,4-DICHLORO-N-(4-(N-(P-TOLYL)SULFAMOYL)PHENYL)BENZAMIDE (7D)

Compound **7d** was synthesized between the reaction of 2,4-dichlorobenzoyl chloride (**6a**) obtained from 2,4-dichlorobenzoic acid (**5a**, 0.300 g, 15.7 mmol) and 4-amino-*N*-(p-tolyl)benzenesulfonamide (**4e**, 0.445 g, 17 mmol). Colorless solid (78% yield), mp 290e292 C. ^{1}H NMR (500 MHz, DMSO-d_6) ∂ 10.42 (s, 1H), 10.06 (s, 1H), 7.71 (d, *J* ¼ 1.9 Hz, 1H), 7.61 (d, *J* ¼ 8.3 Hz, 2H), 7.56 (d, *J* ¼ 8.2 Hz,1H), 7.55e7.46 (m, 3H), 7.33 (d, *J* ¼ 8.0 Hz, 2H), 7.13e6.95 (m, 2H), 2.32 (s, 3H).^{13}C NMR (126 MHz, DMSO-d_6) ∂ 163.92, 143.32, 136.78, 135.83, 135.32, 134.96, 133.71, 131.35, 130.44, 129.79, 129.31, 127.58, 126.89, 121.30, 120.56. LC-MS: positive [*m*/*z*] ¼ 435.0, 437.0 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 98.0%.

7.3.6. 2,4-DICHLORO-N-(4-(N-(O-TOLYL)SULFAMOYL)PHENYL)BENZAMIDE (7E)

Compound **7e** was synthesized between the reaction of 2,4-dichlorobenzoyl chloride (**6a**) obtained from 2,4-dichlorobenzoic acid (**5a**, 0.300 g, 15.7 mmol) and 4-amino-*N*-(o-tolyl)benzenesulfonamide (**4f**, 0.445 g, 17 mmol). Light yellow solid (79% yield), mp 212e214 C. ^{1}H NMR (400 MHz, DMSO-d_6) ∂ 10.93 (s, 1H), 9.50 (s, 1H), 7.84 (d, *J* ¼ 8.7 Hz, 2H), 7.79 (d, *J* ¼ 1.9 Hz, 1H), 7.67 (t, *J* ¼ 8.2 Hz, 3H), 7.58 (dd, *J* ¼ 8.3, 2.0 Hz, 1H), 7.21e7.12 (m, 1H), 7.13e7.04 (m, 2H), 6.99 (dt, *J* ¼ 5.4, 3.0 Hz,1H), 2.04 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) ∂ 164.98, 142.76, 135.81, 135.68, 135.64, 135.35, 134.51, 131.69, 131.21, 130.90, 129.75, 128.32, 128.01, 126.82, 126.73, 119.77, 18.15. LC-MS: positive [*m*/*z*] ¼ 435.0, 437.0 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 99.0%.

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7.3.7. 2,4-DICHLORO-N-(4-(N-(4-METHOXYPHENYL)SULFAMOYL)PHENYL) BENZAMIDE (7F)

Compound **7f** was synthesized between the reaction of 2,4-dichlorobenzoyl chloride (**6a**) obtained from 2,4-dichlorobenzoic acid (**5a**, 0.300 g, 15.7 mmol) and 4-amino-*N*-(4-methoxyphenyl) benzenesulfonamide (**4g**, 0.473 g, 17 mmol). Colorless solid (72% yield), mp 286e288 C. ∂ 10.40 (s, 1H), 10.11 (s, 1H), 7.82 (d, *J* ¼ 8.8 Hz, 2H), 7.71e7.62 (m, 2H), 7.51 (d, *J* ¼ 8.1 Hz, 1H), 7.30 (t, *J* ¼ 18.2 Hz,1H), 7.30e7.20 (m, 3H), 7.15e7.06 (m, 2H), 7.02e6.85 (m, 1H), 3.86 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) ∂ 163.51, 157.41, 143.01, 137.92, 133.90, 130.24, 129.21, 125.21, 124.02, 124.12, 123.62, 120.20, 118.58, 114.47, 56.64. LC-MS: positive [*m*/*z*] ¼ 451.0, 453.1 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 96.0%.

7.3.8. N-(4-(N-BENZYLSULFAMOYL)PHENYL)-2,4-DICHLOROBENZAMIDE (7G)

Compound **7g** was synthesized between the reaction of 2,4-dichlorobenzoyl chloride (**6a**) obtained from 2,4-dichlorobenzoic acid (**5a**, 0.300 g, 15.7 mmol) and 4-amino-*N*-benzylbenzenesulfonamide (**4h**, 0.445 g, 17 mmol). Colorless solid (83% yield), mp 218e220 C. ^{1}H NMR (500 MHz, DMSO-d_6) ∂ 10.89 (s, 1H), 8.03 (s, 1H), 7.86 (d, *J* ¼ 8.8 Hz, 2H), 7.83e7.72 (m, 3H), 7.66 (d, *J* ¼ 8.2 Hz, 1H), 7.57 (dd, *J* ¼ 8.2, 2.0 Hz, 1H), 7.35e7.10 (m, 5H), 3.96 (s, 2H). ^{13}CNMR (126 MHz, DMSO-d_6) ∂ 164.63, 137.86, 135.59, 135.43, 135.34, 131.39, 130.55, 129.43, 128.38, 127.89, 127.73, 127.70, 127.28, 119.59, 46.27. LC-MS: positive [*m*/*z*] ¼ 435.0, 437.0 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 99.0%.

7.3.9. N-(4-(N-BENZOYLSULFAMOYL)PHENYL)-2,4-DICHLOROBENZAMIDE (7H)

Compound **7h** was synthesized between the reaction of 2,4-dichlorobenzoyl chloride (**6a**) obtained from 2,4-dichlorobenzoic acid (**5a**, 0.300 g, 15.7 mmol) and *N*-((4-aminophenyl)sulfonyl) benzamide (**4i**, 0.470 g,17 mmol). Light brown solid (89% yield), mp 321e323 C.^{1}H NMR (600 MHz, DMSO-d_6) ∂ 10.75 (s,1H), 7.94e7.80 (m, 4H), 7.74 (t, *J* ¼ 5.2 Hz, 3H), 7.63 (d, *J* ¼ 8.2 Hz, 1H), 7.55 (dd, *J* ¼ 8.2, 1.9 Hz, 1H), 7.42 (t, *J* ¼ 7.3 Hz, 1H), 7.34 (t, *J* ¼ 7.6 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) ∂ 168.65, 164.45, 140.95, 139.70, 137.40, 135.68, 135.19, 131.42, 130.91, 130.53, 129.39, 128.51, 128.34, 127.86, 127.67, 118.72. LC-MS: positive [*m*/*z*] ¼ 449.0, 451.0 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 99.5%.

7.3.10. 2,4-DICHLORO-N-(4-(N-(PYRIDIN-2-YL)SULFAMOYL)PHENYL) BENZAMIDE (7I)

Compound **7i** was synthesized between the reaction of 2,4-dichlorobenzoyl chloride (**6a**) obtained from 2,4-dichlorobenzoic acid (**5a**, 0.300 g, 15.7 mmol) and 4-amino-*N*-(pyridin-2-yl)benzenesulfonamide (**4j**, 0.430 g, 17 mmol). Colorless solid (87% yield), mp 232e234 C. ^{1}H NMR (500 MHz, DMSO-d_6) ∂ 10.84 (s, 1H), 8.15e7.91 (m, 1H), 7.86 (d, *J* ¼ 8.8 Hz, 2H), 7.79 (dd, *J* ¼ 17.1, 10.5 Hz, 2H), 7.75 (d, *J* ¼ 2.0 Hz, 1H), 7.70 (dd, *J* ¼ 8.9, 7.2 Hz, 1H), 7.62 (t, *J* ¼ 10.2 Hz, 1H), 7.59e7.50 (m, 1H), 7.13 (d, *J* ¼ 8.6 Hz, 1H), 6.87 (t, *J* ¼ 6.2 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) ∂ 164.59, 153.16, 144.32, 144.15, 142.09, 140.33, 135.42, 135.31, 131.38, 130.53, 129.41, 128.01, 127.67, 119.38, 115.81, 114.64, 113.77. LC-MS: positive [*m*/*z*] ¼ 422.0, 424.0 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 97.0%.

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7.3.11. 2,4-DICHLORO-N-(4-(N-(PYRIMIDIN-2-YL)SULFAMOYL)PHENYL) BENZAMIDE (7J)

Compound **7j** was synthesized between the reaction of 2,4-dichlorobenzoyl chloride (**6a**) obtained from 2,4-dichlorobenzoic acid (**5a**, 0.300 g, 15.7 mmol) and 4-amino-*N*-(pyrimidin-2-yl) benzenesulfonamide (**4k**, 0.432 g, 17 mmol). Colorless solid (84% yield), mp 203e205 C. ^{1}H NMR (500 MHz, DMSO-d_6) ∂ 10.89 (s, 1H), 8.48 (dd, *J* ¼ 12.1, 4.9 Hz, 2H), 7.97 (d, *J* ¼ 8.8 Hz, 2H), 7.85 (d, *J* ¼ 8.7 Hz, 2H), 7.75 (d, *J* ¼ 1.9 Hz,1H), 7.62 (dd, *J* ¼ 15.4, 8.5 Hz,1H), 7.55 (dd, *J* ¼ 8.3, 2.0 Hz, 1H), 7.01 (dt, *J* ¼ 16.2, 4.8 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) ∂ 164.57, 158.48, 157.09, 153.21, 142.61, 135.31, 135.17, 131.39, 130.55, 129.96, 129.42, 129.06, 127.68, 119.18, 115.83, 112.24. LC-MS: positive [*m*/*z*] ¼ 423.0, 425.0 [M+H]$^{+}$. Purity by HPLC-UV (254 nm) ESI-MS: 97.0%.

7.3.12. 2,4-DICHLORO-N-(4-(N-METHYL-N-PHENYLSULFAMOYL)PHENYL) BENZAMIDE (7K)

Compound **7k** was synthesized between the reaction of 2,4-dichlorobenzoyl chloride (**6a**) obtained from 2,4-dichlorobenzoic acid (**5a**, 0.300 g, 15.7 mmol) and 4-amino-*N*-methyl-N-phenylbenzenesulfonamide (**4l**, 0.470 g, 17 mmol). Light yellow solid (85% yield), mp 169e171 C. ^{1}H NMR (500 MHz, DMSO-d_6) ∂ 10.94 (s,1H), 7.84 (t, *J* ¼ 12.0 Hz, 2H), 7.77 (t, *J* ¼ 4.6 Hz, 1H), 7.71e7.62 (m, 1H), 7.56 (dd, *J* ¼ 8.2, 2.1 Hz, 1H), 7.53e7.45 (m, 2H), 7.41e7.31 (m, 2H), 7.31e7.24 (m, 1H), 7.12 (ddd, *J* ¼ 12.5, 6.9, 4.5 Hz, 2H), 3.13 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) ∂ 164.71, 142.95, 141.38, 135.39, 135.31, 131.36, 130.88, 130.56, 129.44, 129.05, 128.87, 127.70, 127.30, 126.31, 119.41, 119.18, 38.00. LC-MS: positive [*m*/*z*] ¼ 435.0, 437.0 [M+H]$^{+}$. Purity by HPLC-UV (254 nm) ESI-MS: 99.0%.

7.3.13. 2,4-DICHLORO-N-(3-(N-PHENYLSULFAMOYL)PHENYL)BENZAMIDE (7L)

Compound **7l** was synthesized between the reaction of 2,4-dichlorobenzoyl chloride (**6a**) obtained from 2,4-dichlorobenzoic acid (**5a**, 0.300 g, 15.7 mmol) and 3-amino-*N*-phenylbenzenesulfonamide (**4m**, 0.430 g, 17 mmol). Light yellow solid (79% yield), mp 192e194 C. ^{1}H NMR (600 MHz, DMSO-d_6) ∂ 10.82 (s,1H),10.35 (s,1H), 8.31 (s,1H), 7.77 (dd, *J* ¼ 8.1, 5.0 Hz, 2H), 7.64 (d, *J* ¼ 8.2 Hz, 1H), 7.55 (dd, *J* ¼ 8.2, 1.7 Hz, 1H), 7.53e7.41 (m, 2H), 7.22 (t, *J* ¼ 7.8 Hz, 2H), 7.10 (d, *J* ¼ 7.9 Hz, 2H), 7.02 (t, *J* ¼ 7.4 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) ∂ 164.50, 140.45, 139.46, 137.75, 135.35, 135.30, 131.39, 130.55, 129.97, 129.41, 129.31, 127.66, 124.23, 123.59, 122.11, 120.20, 117.57. LC-MS: positive [*m*/*z*] ¼ 421.0, 423.0 [M+H]$^{+}$. Purity by HPLC-UV (254 nm) ESI-MS: 96.0%.

7.4. SYNTHESIS OF 2,4-DICHLORO-N-(4-(N-PHENYLSULFAMOYL)PHENYL) BENZENESULFONAMIDE (7M)

To the solution of 2,4-dichlorobenzenesulfonyl chloride (**6b**, 0.200 g, 0.81 mmol) in acetone (10 mL) at room temperature, $NaHCO_3$ (0.205 g, 2.4 mmol) was added and the mixture was stirred for 10 min. A solution of 4-amino-*N*-phenylbenzenesulfonamide (**4a**, 0.204 g, 0.81 mmol) in acetone (5 mL) was added and the reaction was continued overnight. After reaction completed, acetone was evaporated and the residue was poured onto ice-water. The mixture was then extracted with ethyl acetate (3 x 30 mL). The collected organic layer was washed with brine solutions, dried over $MgSO_4$, and concentrated under vacuum. The product was purified by column chromatography by using 50% ethyl acetate in petroleum ether. Light brown solid (90% yield), mp 183e185 C. ^{1}H NMR (600 MHz, DMSO-d_6)

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∂ 11.28 (s,1H),10.09 (s,1H), 8.05 (d, *J* ¼ 8.6 Hz, 1H), 7.82 (d, *J* ¼ 2.1 Hz, 1H), 7.72e7.42 (m, 3H), 7.25e7.04 (m, 4H), 7.04e6.77 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) ∂ 141.11, 139.26, 137.73, 135.21, 134.22, 133.17, 132.11, 131.72, 129.23, 128.54, 128.26, 124.31, 120.44, 118.38. LC-MS: positive [*m*/*z*] ¼ 457.0, 459.0 [M+H]$^+$. Purity by HPLC-UV (254 nm) ESI-MS: 99.0%.

7.5. SYNTHESIS OF N-(2,4-DICHLOROPHENYL)-4-(N-PHENYLSULFAMOYL) BENZAMIDE (7N)

To the solution of 2,4-dichloroaniline (**6c**, 0.200 g,1.25 mmol), 4-(N-phenylsulfamoyl)benzoic acid (**4n**, 0.346 g, 1.25 mmol), and HATU (0.522 g, 1.37 mmol) in DMF (10 mL), DIPEA (0.509 m, 3.12 mmol) was added. The mixture was allowed to stir overnight. After reaction completed, the mixture was then extracted with ethyl acetate (3 x 30 mL). The collected organic layer was dried over magnesium sulfate and concentrated under vacuum. The product was purified by column chromatography by using 50% ethyl acetate in petroleum ether. Colorless solid (90% yield), mp 172e174 C. ^{1}H NMR (500 MHz, DMSO-d_6) ∂ 10.40 (s, 1H), 10.30 (s, 1H), 8.16e7.95 (m, 2H), 7.98e7.79 (m, 2H), 7.71 (d, *J* ¼ 2.4 Hz, 1H), 7.58 (d, *J* ¼ 8.6 Hz, 1H), 7.46 (dd, *J* ¼ 8.6, 2.4 Hz, 1H), 7.28e7.19 (m, 2H), 7.10 (dd, *J* ¼ 8.8, 1.7 Hz, 2H), 7.09e6.95 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) ∂ 164.58, 142.50, 137.73, 137.56, 134.06, 131.28, 130.74, 129.71, 129.40, 129.26, 128.83, 127.86, 127.06, 124.51, 120.46. LC-MS: positive [*m*/*z*] ¼ 421.0, 423.0 [M+H]$^+$. Purity by HPLC-UV (254 nm) ESI-MS: 96.0%.

7.6. SYNTHESIS OF COMPOUNDS 7O-7Z, 7AA-AC

Compounds **7o**-**7z**, **7aa-ac** were synthesized using the same procedure for the synthesis of **I**, **7a-l**.

7.6.1. 2-(2,4-DICHLOROPHENYL)-N-(4-(N-PHENYLSULFAMOYL)PHENYL) ACETAMIDE (7O)

Compound **7o** was synthesized between the reaction of 2-(2,4-dichlorophenyl)acetyl chloride (**6d**) obtained from 2-(2,4-dichlorophenyl)acetic acid (**5d**, 0.321 g, 15.7 mmol) and 4-amino-*N*-phenylbenzenesulfonamide (**4a**, 0.430 g, 17 mmol). Light yellow solid (74% yield), mp 229e231 C. ^{1}H NMR (500 MHz, DMSO-d_6) ∂ 10.55 (s, 1H), 7.67 (s, 4H), 7.58 (d, J ¼ 2.1 Hz, 1H), 7.47e7.32 (m, 2H), 7.29e7.12 (m, 2H), 7.13e7.00 (m, 2H), 6.94 (dd, *J* ¼ 10.5, 4.2 Hz, 1H), 3.84 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) ∂ 168.45, 142.57, 139.07, 134.83, 134.37, 133.79, 132.90, 132.48, 129.06, 128.59, 128.02, 127.35, 123.39, 119.97, 118.84, 39.20. LC-MS: positive [*m*/*z*] ¼ 435.0, 437.0 [M+H]$^+$. Purity by HPLC-UV (254 nm) ESI-MS: 96.0%.

7.6.2. 4-CHLORO-2-METHYL-N-(4-(N-PHENYLSULFAMOYL)PHENYL)BENZAMIDE (7P)

Compound **7p** was synthesized between the reaction of 4-chloro-2-methylbenzoyl chloride (**6e**) obtained from 4-chloro-2-methylbenzoic acid (**5e**, 0.270 g, 15.7 mmol) and 4-amino-*N*-phenylbenzenesulfonamide (**4a**, 0.430 g, 17 mmol). Light yellow solid (81% yield), mp 251e253 C. ^{1}H NMR (500 MHz, DMSO-d_6) ∂ 10.65 (s, 1H), 10.18 (s, 1H), 7.83 (d, *J* ¼ 8.7 Hz, 2H), 7.72 (d, *J* ¼ 8.8 Hz, 2H), 7.48 (d, *J* ¼ 8.2 Hz,1H), 7.40 (s,1H), 7.35 (dd, *J* ¼ 8.2,1.9 Hz, 1H), 7.21 (t, *J* ¼ 7.9 Hz, 2H), 7.12e7.05 (m, 2H), 7.00 (t, *J* ¼ 7.4 Hz, 1H), 2.35 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) ∂ 167.44, 143.02, 138.39,

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138.21, 138.02,135.41,134.61,134.04,130.42,129.40,129.30,128.05,125.79, 124.10, 120.10, 119.58, 18.30. LC-MS: positive [m/z] ¼ 401.0, 403.0 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 99.0%.

7.6.3. 4-CHLORO-2-FLUORO-N-(4-(N-PHENYLSULFAMOYL)PHENYL)BENZAMIDE (7Q)

Compound **7q** was synthesized between the reaction of 4-chloro-2-fluorobenzoyl chloride (**6f**) obtained from 4-chloro-2-fluorobenzoic acid (**5f**, 0.274 g, 15.7 mmol) and 4-amino-*N*-phenylbenzenesulfonamide (**4a**, 0.430 g, 17 mmol). Colorless solid (79% yield), mp 181e183 C. ^{1}H NMR (500 MHz, DMSO-d_6) ∂ 10.67 (s, 1H, NH), 7.73 (d, J ¼ 8.8 Hz, 2H), 7.71e7.64 (m, 3H), 7.60 (dd, J ¼ 10.0, 1.9 Hz, 1H), 7.41 (dd, J ¼ 8.4, 1.9 Hz, 1H), 7.05 (dt, J ¼ 8.7, 7.9 Hz, 2H), 6.94 (d, J ¼ 7.5 Hz, 2H), 6.77 (t, J ¼ 7.2 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) ∂ 162.28, 159.11 (d, J ¼ 253.1 Hz), 141.22, 136.41 (d, J ¼ 10.5 Hz), 131.44 (d, J ¼ 3.5 Hz), 128.89 (d, J ¼ 35.7 Hz), 128.75e128.50 (m), 127.71 (s), 125.85 (s), 125.08 (d, J ¼ 3.2 Hz), 123.73 (d, J ¼ 14.9 Hz), 123.20 (s), 120.95 (s), 120.41 (s), 119.97 (s), 119.65 (s), 119.41 (s), 116.98 (d, J ¼ 25.8 Hz). LC-MS: positive [m/z] ¼ 405.0, 407.0 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 97.0%.

7.6.4. 2-BROMO-4-CHLORO-N-(4-(N-PHENYLSULFAMOYL)PHENYL)BENZAMIDE (7R)

Compound **7r** was synthesized between the reaction of 2-bromo-4-chlorobenzoyl chloride (**6g**) obtained from 2-bromo-4-chlorobenzoic acid (**5g**, 0.368 g, 15.7 mmol) and 4-amino-*N*-phenylbenzenesulfonamide (**4a**, 0.430 g, 17 mmol). Colorless solid (74% yield), mp 277e279 C. ^{1}H NMR (500 MHz, DMSO-d_6) ∂ 10.75 (s, 1H), 7.86 (s, 1H), 7.71 (dd, J ¼ 18.3, 8.5 Hz, 4H), 7.58 (s, 2H), 7.09 (t, J ¼ 7.5 Hz, 2H), 6.96 (d, J ¼ 7.7 Hz, 2H), 6.80 (d, J ¼ 7.0 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) ∂ 165.40, 141.41, 137.69, 135.12, 132.17, 130.35, 128.80, 128.02, 127.77, 120.30, 120.08, 119.25. LC-MS: positive [m/z] ¼ 465.0, 467.0 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 96.0%.

7.6.5. 4-BROMO-2-METHYL-N-(4-(N-PHENYLSULFAMOYL)PHENYL)BENZAMIDE (7S)

Compound **7s** was synthesized between the reaction of 4-bromo-2-methylbenzoyl chloride (**6h**) obtained from 4-bromo-2-methylbenzoic acid (**5h**, 0.337 g, 15.7 mmol) and 4-amino-*N*-phenylbenzenesulfonamide (**4a**, 0.430 g, 17 mmol). Light yellow solid (72% yield), mp 216e218 C. ^{1}H NMR (500 MHz, DMSO-d_6) ∂ 10.69 (s, 1H), 7.83 (d, J ¼ 8.7 Hz, 2H), 7.72 (d, J ¼ 8.7 Hz, 2H), 7.64 (s, 1H), 7.61e7.54 (m, 1H), 7.26 (d, J ¼ 8.2 Hz,1H), 7.22 (t, J ¼ 7.6 Hz, 2H), 7.09 (d, J ¼ 8.5 Hz, 2H), 7.01 (t, J ¼ 7.4 Hz, 1H), 2.30 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) ∂ 173.07, 166.41, 142.94, 138.55, 137.53, 135.04, 133.97, 132.97, 132.80, 129.79, 129.29, 128.01, 124.02, 119.97, 119.63, 118.49, 18.58. LC-MS: positive [m/z] ¼ 445.0, 447.0 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 96.0%.

7.6.6. 4-BROMO-2-FLUORO-N-(4-(N-PHENYLSULFAMOYL)PHENYL)BENZAMIDE (7T)

Compound **7t** was synthesized between the reaction of 4-bromo-2-fluorobenzoyl chloride (**6i**) obtained from 4-bromo-2-fluorobenzoic acid (**5i**, 0.343 g, 15.7 mmol) and 4-amino-*N*-phenylbenzenesulfonamide (**4a**, 0.430 g,17 mmol). Colorless solid (83% yield), mp 200e202 C. ^{1}H NMR (400 MHz, DMSO) ∂ 10.81 (s, 1H), 10.21 (s, 1H), 7.83 (d, J ¼ 8.9 Hz, 2H), 7.76 (dd, J ¼ 7.1, 5.2 Hz, 3H), 7.63 (d, J ¼ 7.5 Hz, 1H), 7.57 (dd, J ¼ 8.3, 1.7 Hz, 1H), 7.37e7.18 (m, 2H), 7.17e7.07 (m, 2H), 7.02 (t, J ¼ 7.4 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) ∂ 162.90, 159.30 (d, J ¼ 254.4 Hz), 142.87, 138.22, 134.58,

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131.92 (d, J ¼ 3.2 Hz), 129.60, 128.35 (d, J ¼ 3.5 Hz), 128.36, 128.33, 125.13 (d, J ¼ 9.7 Hz), 124.49, 124.18 (d, J ¼ 14.9 Hz), 120.52, 120.25, 119.98. LC-MS: positive [m/z] ¼ 449.0, 451.1 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 96.0%.

7.6.7. 4-BROMO-2-METHOXY-N-(4-(N-PHENYLSULFAMOYL)PHENYL)BENZAMIDE (7U)

Compound **7u** was synthesized between the reaction of 4-bromo-2-methoxybenzoyl chloride (**6j**) obtained from 4-bromo-2-methoxybenzoic acid (**5j**, 0.362 g, 15.7 mmol) and 4-amino-N-phenylbenzenesulfonamide (**4a**, 0.430 g, 17 mmol). Light yellow solid (79% yield), mp 212e214 C. ^{1}H NMR (500 MHz, DMSO-d_6) ∂ 10.41 (s, 1H), 10.14 (s, 1H), 7.81 (d, J ¼ 8.8 Hz, 2H), 7.76e7.63 (m, 2H), 7.50 (d, J ¼ 8.1 Hz, 1H), 7.35 (t, J ¼ 18.2 Hz, 1H), 7.30e7.16 (m, 3H), 7.15e7.06 (m, 2H), 7.06e6.88 (m, 1H), 3.87 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) ∂ 164.55, 157.40, 142.78, 137.97, 133.91, 131.26, 129.28, 125.24, 124.25, 124.16, 123.63, 120.21, 119.58, 115.47, 56.64. LC-MS: positive [m/z] ¼ 461.0, 463.0 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 99.0%.

7.6.8. 2-FLUORO-4-IODO-N-(4-(N-PHENYLSULFAMOYL)PHENYL)BENZAMIDE (7V)

Compound **7v** was synthesized between the reaction of 2-fluoro-4-iodobenzoyl chloride (**6k**) obtained from 2-fluoro-4-iodobenzoic acid (**5k**, 0.417 g, 15.7 mmol) and 4-amino-N-phenylbenzenesulfonamide (**4a**, 0.430 g, 17 mmol). Colorless solid (86% yield), mp 224e226 C. ^{1}H NMR (400 MHz, DMSO-d_6) ∂ 10.78 (s,1H, NH), 10.21 (s, 1H), 7.84 (dd, J ¼ 13.9, 5.1 Hz, 3H), 7.78e7.63 (m, 3H), 7.44 (t, J ¼ 7.8 Hz,1H), 7.23 (t, J ¼ 7.9 Hz, 2H), 7.14e7.06 (m, 2H), 7.02 (t, J ¼ 7.4 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) ∂ 163.12, 158.89 (d, J ¼ 254.9 Hz), 142.89, 138.22, 134.55, 134.15 (d, J ¼ 3.3 Hz), 131.78 (d, J ¼ 2.9 Hz), 129.60, 128.43, 125.51 (d, J ¼ 24.1 Hz), 124.43 (d, J ¼ 11.0 Hz), 120.53, 119.96, 98.63, 98.55. LC-MS: positive [m/z] ¼ 497.0, 499.0 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 97.0%.

7.6.9. 4-CYANO-2-FLUORO-N-(4-(N-PHENYLSULFAMOYL)PHENYL)BENZAMIDE (7W)

Compound **7v** was synthesized between the reaction of 2-fluoro-4-iodobenzoyl chloride (**6l**) obtained from 2-fluoro-4-iodobenzoic acid (**5l**, 0.417 g, 15.7 mmol) and 4-amino-N-phenylbenzenesulfonamide (**4a**, 0.430 g, 17 mmol). Colorless solid (81% yield), mp 319e331 C.^{1}H NMR (400 MHz, DMSO-d_6) ∂ 10.81 (s,1H), 10.20 (s, 1H), 7.83 (d, J ¼ 8.9 Hz, 2H), 7.79e7.67 (m, 3H), 7.69e7.60 (m, 1H), 7.57 (dd, J ¼ 8.3, 1.7 Hz, 1H), 7.36e7.16 (m, 2H), 7.18e7.06 (m, 2H), 7.07e6.84 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) ∂ 162.89, 159.33 (d, J ¼ 267.7 Hz), 142.87, 138.23, 134.59, 131.92 (d, J ¼ 3.4 Hz), 129.60, 128.40 (d, J ¼ 7.7 Hz), 128.36, 125.05 (d, J ¼ 12.9 Hz), 124.19 (d, J ¼ 15.1 Hz), 120.52, 120.25, 119.98. LC-MS: positive [m/z] ¼ 396 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 96.0%.

7.6.10. 2-FLUORO-N-(4-(N-PHENYLSULFAMOYL)PHENYL)-4-(TRIFLUOROMETHYL)BENZAMIDE (7X)

Compound **7x** was synthesized between the reaction of 2-fluoro-4-(trifluoromethyl)benzoyl chloride (**6m**) obtained from 2-fluoro-4-(trifluoromethyl)benzoic acid (**5m**, 0.326 g, 15.7 mmol) and 4-amino-N-phenylbenzenesulfonamide (**4a**, 0.430 g,17 mmol). Colorless solid (77% yield), mp 291e293 C. ^{1}H NMR (400 MHz, DMSO-d_6) ∂ 10.97 (s, 1H), 10.22 (s, 1H), 7.99e7.87 (m, 2H), 7.84 (d, J ¼ 8.7 Hz, 2H), 7.82e7.66 (m, 3H), 7.24 (t, J ¼ 7.7 Hz, 2H), 7.16e7.07 (m, 2H), 7.04 (d, J ¼ 7.5 Hz, 1H). ^{13}C NMR

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(101 MHz, DMSO-d_6) ∂ 162.57, 160.33, 157.83, 142.71, 138.22, 134.79, 131.66, 129.61, 128.50, 124.49, 122.08, 120.54, 120.01, 114.52, 114.26. LC-MS: positive [m/z] ¼ 439.0, 441.0 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 96.0%.

7.6.11. 4-CHLORO-2,5-DIFLUORO-N-(4-(N-PHENYLSULFAMOYL)PHENYL)BENZAMIDE (7Y)

Compound **7y** was synthesized between the reaction of 4-chloro-2,5-difluorobenzoyl chloride (**6n**) obtained from 4-chloro-2,5-difluorobenzoic acid (**5n**, 0.302 g, 15.7 mmol) and 4-amino-*N*-phenylbenzenesulfonamide (**4a**, 0.430 g, 17 mmol). Colorless solid (82% yield), mp 205e207 C. ^{1}H NMR (400 MHz, DMSO-d_6) ∂ 10.88 (s, 1H), 10.22 (s,1H), 7.84 (dq, J ¼ 8.6, 6.0 Hz, 4H), 7.76 (d, J ¼ 8.9 Hz, 2H), 7.24 (dd, J ¼ 11.2, 4.6 Hz, 2H), 7.15e7.07 (m, 2H), 7.03 (t, J ¼ 7.4 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) ∂ 161.62, 155.78 (dd, J ¼ 116.9, 2.6 Hz), 153.33 (dd, J ¼ 111.6, 2.9 Hz), 142.69, 138.21, 134.75, 129.60, 128.47, 124.99 (dd, J ¼ 17.5, 6.4 Hz), 124.50, 123.40 (dd, J ¼ 19.9, 11.2 Hz), 120.55, 120.04, 119.43, 119.15, 118.00, 117.90 (dd, J ¼ 25.0, 3.8 Hz). LC-MS: positive [m/z] ¼ 423.0, 425.0 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 98.0%.

7.6.12. 3,5-DICHLORO-N-(4-(N-PHENYLSULFAMOYL)PHENYL)PICOLINAMIDE (7Z)

Compound **7z** was synthesized between the reaction of 4,6-dichloronicotinoyl chloride (**6o**) obtained from 4,6-dichloronicotinic acid (**5o**, 0.301 g, 15.7 mmol) and 4-amino-*N*-phenylbenzenesulfonamide (**4a**, 0.430 g, 17 mmol). Colorless solid (89% yield), mp 225e227 C. ^{1}H NMR (400 MHz, DMSO-d_6) ∂ 11.07 (s, 2H), 8.75 (dd, J ¼ 17.0, 2.0 Hz, 2H), 8.46 (dd, J ¼ 15.7, 2.0 Hz, 2H), 7.88 (d, J ¼ 8.8 Hz, 4H), 7.77 (d, J ¼ 8.8 Hz, 4H), 7.38e7.16 (m, 4H), 7.10 (d, J ¼ 7.6 Hz, 4H), 7.02 (t, J ¼ 7.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) ∂ 163.26, 149.28, 146.53, 142.53, 138.70, 138.37, 134.97, 133.30, 129.70, 129.60, 128.48, 124.41, 120.56, 120.05. LC-MS: positive [m/z] ¼ 422.0, 424.0 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 98.0%.

7.6.13. 6-CHLORO-2-FLUORO-N-(4-(N-PHENYLSULFAMOYL)PHENYL)NICOTINAMIDE (7AA)

Compound **7aa** was synthesized between the reaction of 6-chloro-2-fluoronicotinoyl chloride (**6p**) obtained from 6-chloro-2-fluoronicotinic acid (**5p**, 0.301 g, 15.7 mmol) and 4-amino-*N*-phenylbenzenesulfonamide (**4a**, 0.274 g, 17 mmol). Light yellow solid (81% yield), mp 199e201 C. ^{1}H NMR (400 MHz, DMSO-d_6)) ∂ 10.94 (s, 1H), 10.22 (s, 1H), 8.39e8.23 (m,1H), 7.82 (d, J ¼ 8.8 Hz, 2H), 7.76 (d, J ¼ 8.9 Hz, 2H), 7.69 (d, J ¼ 7.9 Hz,1H), 7.22 (d, J ¼ 7.7 Hz, 2H), 7.10 (d, J ¼ 7.7 Hz, 2H), 7.03 (t, J ¼ 7.3 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) ∂ 161.55, 158.30 (d, J ¼ 246.3 Hz), 157.08, 149.40 (d, J ¼ 14.1 Hz), 144.86 (d, J ¼ 3.0 Hz), 142.61, 138.19, 134.83, 129.61, 128.51, 124.52, 123.00 (d, J ¼ 5.0 Hz), 122.97, 120.57, 120.04, 118.39 (d, J ¼ 28.0 Hz). LC-MS: positive [m/z] ¼ 406.0, 408.0 $[M+H]^+$. Purity by HPLC-UV (254 nm) ESI-MS: 96.0%.

7.6.14. 2-OXO-N-(4-(N-PHENYLSULFAMOYL)PHENYL)-2H-CHROMENE-3CARBOXAMIDE (7 AB)

Compound **7 ab** was synthesized between the reaction of 2-oxo-2H-chromene-3-carbonyl chloride (**6q**) obtained from 2-oxo-2H-chromene-3-carboxylic acid (**5q**, 0.298 g, 15.7 mmol) and 4-amino-*N*-phenylbenzenesulfonamide (**4a**, 0.274 g, 17 mmol). Colorless solid (71% yield), mp 271e273 C. ^{1}H NMR (400 MHz, DMSO-d_6) ∂ 10.89 (s, 1H), 10.23 (s, 1H), 8.88 (s, 1H), 7.95 (s, 1H), 7.91e7.83 (m, 2H),

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7.82e7.71 (m, 3H), 7.54 (d, J ¼ 8.4 Hz, 1H), 7.46 (dt, J ¼ 15.7, 4.1 Hz, 1H), 7.24 (dd, J ¼ 11.2, 4.6 Hz, 2H), 7.10 (dd, J ¼ 8.7, 4.5 Hz, 2H), 7.06e6.97 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) ∂ 161.06, 160.56, 154.40, 148.09, 142.17, 138.18, 134.94, 134.83, 130.82, 129.61, 128.56, 125.81, 124.56, 120.64, 120.36, 120.22, 118.82, 116.75. LC-MS: positive [m/z] ¼ 447.7 [M+H]$^+$. Purity by HPLC-UV (254 nm) ESI-MS: 96.0%.

7.6.15. N-(4-(N-PHENYLSULFAMOYL)PHENYL)-2-NAPHTHAMIDE (7AC)

Compound **7 ab** was synthesized between the reaction of 2-naphthoyl chloride (**6r**) obtained from 2 2-naphthoic acid (**5r**, 0.272 g, 15.7 mmol) and 4-amino-N-phenylbenzenesulfonamide (**4a**, 0.274 g, 17 mmol). Colorless solid (80% yield), mp 224e226 C. ^{1}H NMR (400 MHz, DMSO-d_6) ∂ 10.73 (s,1H), 8.58 (s,1H), 8.16e8.04 (m, 2H), 8.04e7.97 (m, 2H), 7.99e7.90 (m, 2H), 7.75 (s, 2H), 7.69e7.59 (m, 2H), 7.20 (t, J ¼ 7.8 Hz, 2H), 7.07 (d, J ¼ 7.7 Hz, 2H), 6.95 (t, J ¼ 7.0 Hz, 1H). 13C NMR (101 MHz, DMSO-d_6) ∂ 166.18, 134.86, 132.48, 129.46, 128.72, 128.58, 128.16, 127.41, 124.87, 120.59, 120.30. LC-MS: positive [m/z] ¼ 403.3 [M+H]$^+$. Purity by HPLC-UV (254 nm) ESI-MS: 96.0%.

7.6.16. 2,4-DICHLORO-N-(4-(PHENYLCARBAMOYL)PHENYL)BENZAMIDE (7AD, K960)

Compound **7ad** was synthesized between the reaction of 2,4-dichlorobenzoic acid (**5a**, 0.300 g, 15.7 mmol) and 4-amino-N-phenylbenzamide (**6s**, 0.332 g, 17 mmol) in the presence of HATU (0.647 g,17 mmol), and DIPEA (0.677 mL, 40 mmol) in DMF (10 mL). Colorless solid (89% yield), mp 231e233 C. ^{1}H NMR (600 MHz, DMSO) ∂ 10.80 (s, 1H), 10.13 (s, 1H), 7.99 (t, J ¼ 7.3 Hz, 2H), 7.83 (d, J ¼ 8.7 Hz, 2H), 7.77 (dd, J ¼ 6.9, 4.9 Hz, 3H), 7.67 (d, J ¼ 8.2 Hz, 1H), 7.56 (dd, J ¼ 7.6, 5.6 Hz, 1H), 7.45e7.20 (m, 2H), 7.09 (t, J ¼ 7.4 Hz, 1H). ^{13}C NMR (151 MHz, DMSO) ∂ 164.93, 164.47, 141.75, 139.37, 135.62, 135.22, 131.40, 130.55, 130.15, 129.41, 128.82, 128.72, 127.67, 123.70, 120.53, 119.00. LC-MS: positive [m/z] ¼ 385.0, 387.0 [M+H]$^+$. Purity by HPLC-UV (254 nm) ESI-MS: 98.0%.

8. Measurement of ß-arrestin 2 recruitment by firefly luciferase complementation

The procedure that was used was described previously [12]. Briefly, we used HEK293 cells stably expressing ß-arrestin 2 fused with the N-terminal part of the firefly luciferase (FLuc) and GPR27V2 fused with the C-terminal part of the luciferase (HEK293.FnLArrb2.GPR27V2LFc) to measure the compounds activity. The cells were cultured at 37 C with 5% CO_2 atmosphere with DMEM (Lonza, Verviers, Belgium) supplemented with 10% fetal bovine serum, 1% $_L$-glutamine (Lonza, Verviers, Belgium), 1% penicillin and streptomycin (Lonza, Verviers, Belgium), puromycin (1 μ) g/mL, A&G Scientific) and hygromycin (200 μg/mL, Invivogen, San Diego, California, USA). Fresh culture medium was added the day before the experiment. To obtain concentration-response curves, cells were first detached with trypsin, washed and resuspended in HBSS (120 mM NaCl, 5.4 mM KCl, 0.8 mM MgSO4,10 mM HEPES, pH 7.4). 1 μl of test compounds (at the appropriate concentration) in DMSO was disposed in white 96 wells plates (655083, Greiner Bio-One, Kremsmünster, Oberosterreich, Ger-€ many) and 100 μl of the cell suspension was added at a final density of 35,000 cells/well. The plate was incubated for 10 min at room temperature and, after addition of D-luciferin (500 μM, Sanbio,

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Uden, The Netherlands), luminescence was read immediately on a Centro XS^3 LB960 plate reader (Berthold Technologies, Bad Wildbad, Deutschland).

9. Solubility

1 μl of each tested compounds (in appropriate concentration or vehicle control) in DMSO was distributed into 96-well clear plate (ThermoFisher Scientific, Waltham, Massachusetts, USA) with 100 μl of HBSS medium and incubated for 10 min at 37 C and 5% of CO_2 in a humidified chamber. After incubation time, the absorbance (expressed as optical density, OD) of the solutions was directly measured with a SpectraMax plus (Molecular Devices, San Jose, California, USA) plate reader at 500 nm.

10. Protein preparation, induced-fit docking and MM/GBSA

The system preparation and docking calculations were performed using the Schrodinger Drug Discovery suite for molecular€ modeling (version 2020.4). The GPR27, GPR85 and GPR173 homology models were obtained from the GPCRdb (https://gpcrdb. org, accessed on August 2021). The structures were prepared with the Protein preparation wizard (Schrodinger 2020.4, [€ 27]) to fix protonation states of amino acids residues, adding hydrogens and also fixing missing side-chain atoms.

Docking studies with the prepared ligands were performed using Glide [28] (Glide V7.7), with the flexible modality of Inducedfit docking with extra precision (XP), followed by a side-chain minimization step using Prime [29,30]. Ligands were docked within a grid around 12 Å from the centroid of the hydrophobic pocket, determined by the residues Phe107 and Tyr300 (numbering of GPR27) generating 10 poses per ligand. The highest score poses, with the sulfonamide moiety pointing towards the solvent was further refined. The refinement of protein-ligand complexes was then performed using Prime with standard options. In this step, all side chains within 5 Å of each docked ligand pose were minimized. This step was also employed to calculate the potential binding energy for each conformation using MM/GBSA [31].

11. WaterMap calculations

WaterMap calculations [32] were performed using the same initial apostructure protein system treated for docking. The system was solvated in TIP3P water box extending at least 10 Å beyond the proposed ligand-binding pocket and amino acids being this cutoff were restrained. A single 5 ns molecular dynamics simulation was performed, and the waters molecules trajectories were then clustered into distinct hydration sites. Enthalpy values of the hydration sites were obtained by averaging the non-bonded interaction for each water molecule in the cluster. Entropy and enthalpy

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values for each hydration site were calculated using inhomogeneous solvation theory. Hydration sites around the base of the hydrophobic pocket (Phe106 and Tyr300) were chosen to be further analyzed.

12. Cytotoxicity

MTT cell proliferation assay was used to evaluate the cytotoxicity of the tested compounds in the HEK293.FnLArrb2.GPR27V2LFc cell line. Cells were seeded into 96-well clear plate (ThermoFisher Scientific, Waltham, Massachusetts, USA) at a final density of 30,000 cells/100mL/well with DMEM medium (without phenol red) and cultured for 24 h at 37 C with 5% of CO_2. Afterwards,1 μl of test compounds solution in DMSO (at appropriate concentration) was added and incubated for 10 min at 37 C. Then, 10 μl of a solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 11465007001, Sigma-Aldrich, Saint-Louis, Missouri, USA) was added to each well. The plate was incubated for 4 h at 37 C in a humidified incubator. After incubation time, 25 μl of supernatant was carefully removed from the wells and the formazan crystals were dissolved in 50 μL DMSO for 10 min in 37 C. Finally, the absorbance (optical density, OD) of the resultant solution was measured at 540 nm with a SpectraMax plus plate reader (Molecular Devices, San Jose, California, USA).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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