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Original article

Fibrate-derived *N*-(methylsulfonyl)amides with antagonistic properties on PPARα

Alessandra Ammazzalorso, Alessandra D'Angelo, Antonella Giancristofaro, Barbara De Filippis, Mauro Di Matteo, Marialuigia Fantacuzzi, Letizia Giampietro, Pasquale Linciano, Cristina Maccallini, Rosa Amoroso*

Dipartimento di Farmacia, Università "G. d'Annunzio", via dei Vestini, Chieti 66100, Italy

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ABSTRACT

The identification of novel PPAR ligands represents an attractive research to fully understand the complex biological pathways regulated by these receptors. Selective PPAR modulators, inverse agonists and antagonists of three PPAR isoforms could help to clarify biological effects on lipid and glucose homeostasis. Here we describe the identification of a group of *N*-(methylsulfonyl)amides, derived from PPARα agonist carboxylic acids. Transactivation and FRET assay confirmed an antagonist behaviour on PPARα for some of these compounds, with submicromolar IC_{50}. A preliminary analysis on selectivity α/γ revealed different profiles of inhibition or activation.

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear receptor superfamily [1]. The three PPAR isoforms, PPARα, γ, and β/δ, vary in their tissue distributions, selectivity and responsiveness to ligands. PPARα plays a role in the clearance of circulating or cellular lipids via the regulation of gene expression involved in lipid metabolism in liver and skeletal muscle [2]. PPARγ promotes adipocyte differentiation to enhance blood glucose uptake [3], whereas PPARβ/δ is involved in lipid oxidation and cell proliferation [4]. PPAR subtypes share a similar structure: an N-terminal ligand-independent transactivation domain (AF1), followed by a highly conserved DNA binding domain (DBD) and a C-terminal ligand binding domain (LBD) containing a ligand-dependent transactivation function (AF2). PPAR ligands induce binding to PPAR responsive elements (PPREs) in the DNA after dimerization with another nuclear receptor, the retinoid-X receptor (RXR) [5].

A number of potent and selective PPAR agonists have been disclosed [6], but to date it remains the need for additional small regulators of PPARs, in order to further decipher the biological pathways regulated by these receptors. Selective PPAR modulators (SPPARMs) [7], inverse agonists [8], and antagonists [9] of the three PPAR isoforms have attracted much interest as investigative tools, to fully understand the biology and pharmacology of PPARs. To date only few selective PPARα antagonists have been described: GW6471 [10], and triazolone-based compounds from Lilly [11], able to revert the activity of full agonists. The strategy of blocking PPARα activation has found several applications in elucidating the biological pathways controlled by PPARα [12]. In addition, a new study by Laurenti et al. suggested an interesting role of PPARα antagonists in glioblastoma models in reducing lipid droplets, related to tumour malignancy grade [13].

In the search for novel compounds acting on PPARα, we recently reported the synthesis and biological evaluation of a family of *N*-(phenylsulfonyl)amides containing a benzothiazole scaffold, found to be PPARα inhibitors [14]; compound **1** was found the best compound, showing a dose-dependent inhibition profile on PPARα activation promoted by GW7647, with a micromolar potency (IC_{50} = 6.5 μM); it was able to decrease CPT1A gene expression (Fig. 1).

By comparing results obtained from our studies and Lilly's sulfonimidic triazolones from literature, we suppose the crucial role of *N*-phenylsulfonyl moiety in determining an antagonistic behaviour on PPARα, suggesting the steric hindrance of sulfonimide could be the key feature determining activation or block of the receptor. We thought to replace the phenyl ring with a less hindered substituent, such a methyl, to verify that hypothesis.

* Corresponding author. Tel.: +39 0871 3554686; fax: +39 0871 3554911.
E-mail address: ramoroso@unich.it (R. Amoroso).

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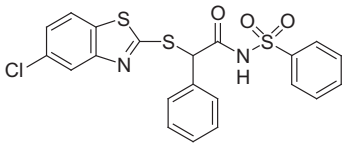

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Fig. 1. Benzothiazole-based *N*-(phenylsulfonyl)amide, PPARα antagonist.

In this work we present the synthesis of new *N*-(methylsulfonyl) amides, derived from PPARα agonist carboxylic acids. The bioisosteric replacement of the carboxylic group with a sulfonimidic one is a useful strategy in drug discovery. The sulfonimidic moiety shows a very similar profile to carboxylic group in terms of acidity and H-bond properties. It might be interesting to evaluate the influence of this bioisosteric modification in PPARα ligands, where the carboxylic head has been described as a structural key involved in a H-bond network within the ligand binding domain [15].

2. Results and discussion

In this paper we describe the synthesis and biological evaluation of a first group of benzothiazole-based compounds (**2a**–**g**), and a second group derived from classical fibrates clofibrate, fenofibrate, bezafibrate and gemfibrozil (**3**–**6**). We selected clofibric acid (**7**) and GW7647 (**8**) as reference compounds (Fig. 2).

The *N*-(methylsulfonyl)amides **2a**–**g** were easily obtained by the direct coupling of corresponding carboxylic acids with methanesulfonamide, in the presence of 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) and 4-dimethylamino pyridine (DMAP) (Scheme 1). The synthesis of starting acids has been reported elsewhere [16]. Commercial fibrates (clofibric acid, bezafibrate and gemfibrozil) were reacted as described, whereas fenofibrate was previously hydrolyzed by aqueous NaOH 2 N, in THF at 60 °C.

We performed the biological evaluation of derivatives **2a**–**g** and **3**–**6** by a standard transactivation assay [17], combined with a competitive time-resolved fluorescence resonance energy transfer (TR-FRET) assay [18]. In the transactivation assay, GAL4-PPAR chimeric receptors were expressed in eukaryotic cells HEK293; we utilized firefly luciferase reporter gene technology to provide optimal assay sensitivity and dynamic range when quantifying nuclear receptor activity. The assays were performed using agonist and antagonist formats. In the antagonist mode the experimental procedure is the same except that cells are incubated with an agonist before the addition of tested compounds. New compounds were tested at 150 μM, and reference compounds **7** and **8** at 150 μM and 1 μM, respectively. The results, expressed as fold activation, are shown in Table 1. Among benzothiazole-based compounds, all derivatives show an antagonistic profile, except for **2a**, that activates PPARα (EC_{50} = 1.59 μM). Some of these compounds exhibit a good inhibition of the receptor, with submicromolar IC_{50} values, as for **2c**, **2e**, and **2g** (IC_{50} = 0.6, 0.8 and 0.9 μM, respectively). Methanesulfonimidic derivatives of fibrates **3**–**6** gave a different profile in PPARα activation: fenofibrate analogue **4** does not produce a notable activation or repression of basal activity, clofibrate and gemfibrozil derivatives **3** and **6** are agonists (EC_{50} = 10.7 and 7.1 μM, respectively), whereas bezafibrate analogue shows an antagonistic profile, also if with a poor potency (IC_{50} = 68.5 μM).

The dose-dependent antagonistic profile of **2e** and **5** is illustrated in Fig. 3: both decrease the activation promoted by **8** (2 μM) at concentrations 1–150 μM, without showing PPARα activation when used alone. Similar trends were found for all compounds showing an antagonistic behaviour.

The different effect produced by tested compounds on PPARα does not follow a clear trend of structure–activity relationship. The nature of substituent R_3, among benzothiazole derivatives **2a**–**g**, could be a structural feature involved in the activation or block of the receptor, with only methyl derivative **2a** acting as agonist. It is also evident that steric hindrance on sulfonimidic moiety does not represent the key factor modulating the receptor activity: both

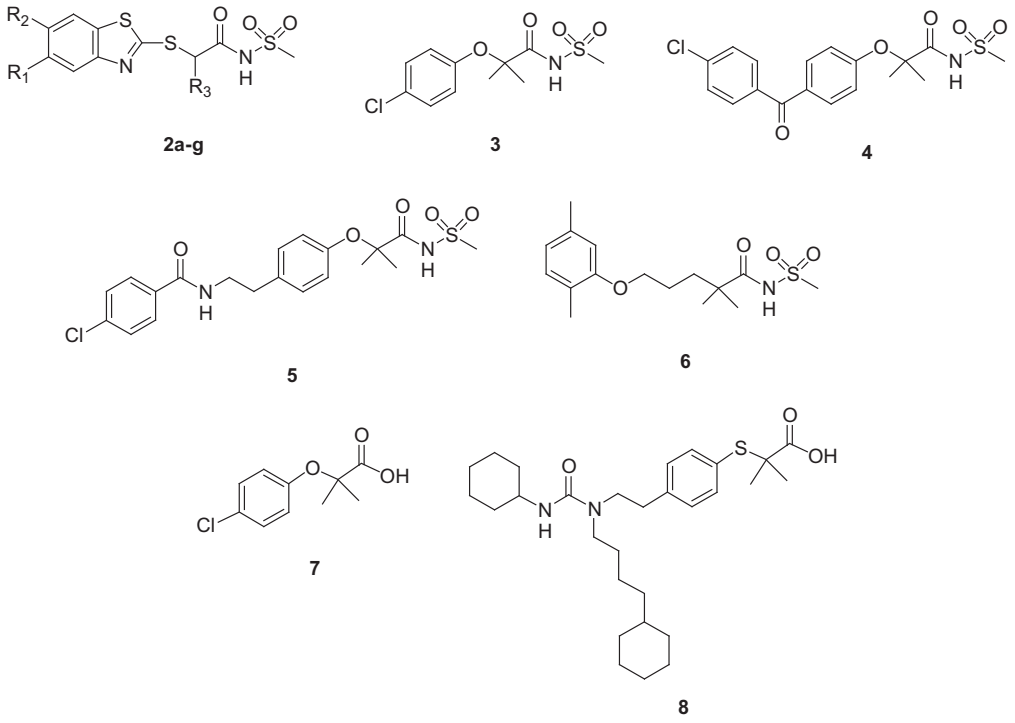
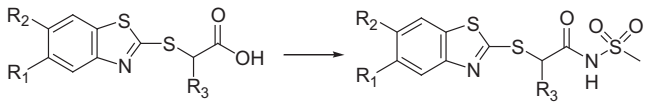


Fig. 2. *N*-(Methylsulfonyl)amides benzothiazole-based or derived from classical fibrates and reference compounds.



2a-g

Scheme 1. Reagents and conditions: methanesulfonamide, EDC, DMAP, dry CH_2Cl_2, 0 °C–r.t., 24 h.

methane- and benzenesulfonimidic derivatives can act as antagonists, or, in same cases, as agonists.

We decided to further analyze the binding of our compounds to PPARα by means of a TR-FRET assay, a robust and well established method based on the fluorescence resonance energy transfer. The competitive ligand binding to PPARα results in a loss of FRET signal, observed at proper wavelengths. In a first experiment we performed FRET assay testing our compounds at 100 μM, with **7** and **8** (1 μM) as positive controls. Also compound **1** was included as a positive control: we demonstrated elsewhere its ability to produce an antagonistic effect on PPARα activation at micromolar concentration [14]. Results are expressed as the ratio of fluorescence intensity at 520 nm (fluorescein emission excitated by terbium emission) and 495 nm (terbium emission). Relative FRET values were normalized for **7**–100%. All tested compounds show a significant decrease in FRET ratio, with affinity profiles similar or better than positive controls. Among benzothiazole derivatives, higher binding affinities were found for **2b** and **2e** (137.4 and 116.7%, respectively); all fibrate derivatives, except for **5**, showed positive binding affinities compared to clofibric acid (128.5, 121.2 and 118.0% for **4**, **3** and **6**, respectively) (Fig. 4).

Starting from these data, we selected compounds showing a high FRET ratio in order to derive the IC_{50} in the FRET-based competitive ligand binding assay. Selected compounds (**2b**, **2c**, **2e** and **2g**) were tested at five concentrations, in three independent experiments. Results are summarized in Table 2, where combined data from transactivation and FRET are reported. The FRET assay fully confirmed the transactivation results, as it is evident from the nice agreement among the calculated IC_{50} values. Only a slight difference, in terms of IC_{50} value, was observed for compound **2b**, that appears the more potent derivative in the FRET assay, but not in the transactivation experiment (IC_{50} = 0.48 and 8.87 μM, respectively). The comparison of these data allowed us to point out the good binding affinities to PPARα of the newly synthesized *N*-(methylsulfonyl)amides, with a quite good potency profile.

Table 1
In vitro PPARα transactivation of synthesized compounds.

Compd	R_1	R_2	R_3	FA[a]	IC_{50} (μM)[b]	EC_{50} (μM)[c]
2a	H	OEt	Me	1.84 ± 0.07	–	1.59 ± 0.04
2b	H	OEt	*n*-Pr	0.36 ± 0.07	8.87 ± 0.08	–
2c	Cl	H	*n*-Pr	0.28 ± 0.06	0.6 ± 0.01	–
2d	Cl	H	*i*-Pr	0.47 ± 0.12	46.5 ± 1.31	–
2e	Cl	H	*n*-But	0.08 ± 0.02	0.8 ± 0.01	–
2f	Cl	H	*n*-Hex	0.04 ± 0.01	5.1 ± 0.51	–
2g	Cl	H	Ph	0.07 ± 0.03	0.9 ± 0.02	–
3				1.70 ± 0.10	–	10.7 ± 0.61
4				1.10 ± 0.10	–	–
5				0.44 ± 0.04	68.5 ± 3.21	–
6				1.55 ± 0.05	–	7.1 ± 0.32
7				1.6 ± 0.22	–	55.0 ± 3.9
8				2.8 ± 0.23	–	0.2 ± 0.02

[a] FA: Fold activation. Compounds were tested in at least three separate experiments at 150 μM. Only **8** was tested at 1 μM. The results are expressed ± SEM.
[b] Compounds were tested in at least three separate experiments at five concentrations, from 1 to 150 μM, in the presence of **8** (2 μM). The results are expressed ± SEM.
[c] Compounds were tested in at least three separate experiments at five concentrations, from 1 to 150 μM. The results are expressed ± SEM.

A preliminary overview on subtype selectivity was also addressed by a transactivation experiment on PPARγ isoform. Each compound was tested on PPARγ at the concentration corresponding to IC_{50} (or EC_{50}) on alpha isoform. The comparison of FA values outlined different behaviours for tested compounds (Fig. 5).

Compounds **1** and **2e** were found mainly inhibitors of PPARα, without a notable influence on gamma isoform; **2f** and **2g** showed inhibition of both PPAR subtypes, whereas four derivatives (**2b**, **2c**, **2d** and **5**) showed mixed properties, producing inhibition of PPARα and activation of PPARγ. At last, a group of compounds (**2a**, **3** and **6**) gave a different, interesting profile, producing strong activation of PPARγ with a moderate PPARα activity, so promising a dual agonistic activity.

These results look of some interest, with benzothiazole derivatives **2a**–**g** showing a correlation between chemical structure and selectivity. We observed a decreasing ability to activate PPARα by increasing the steric hindrance of substituent R_3, in alpha to carbonylic group. The presence of a *n*-propylic group or more hindered alkyls, or a phenyl ring, produces antagonism on PPARα. The nature of this substituent, indeed, has a different impact on PPARγ: if R_3 is less hindered, the compound is able to activate the receptor, whereas *n*-hexyl or phenyl derivatives are antagonists.

Reported data confirm the possibility to obtain PPAR ligands lacking in carboxylic head, so disrupting the classical H-bond network present in carboxylic agonists. The different steric hindrance on sulfonimidic moiety is probably important, but it does not represent the key factor modulating the receptor activity. Whereas benzenesulfonimidic derivatives were found mainly antagonists, the methylsulfonyl analogues here presented show activation or inhibition on PPARα.

3. Conclusions

A group of novel *N*-(methylsulfonyl)amides were designed and synthesized as bioisosteric derivatives of fibrates. The combined biological approach, including transactivation and FRET assay, revealed an interesting profile for some of these compounds. A preliminary screening on PPARγ isoform revealed different behaviour; we identified PPARα antagonists, with a dose-dependent inhibition profile and submicromolar IC_{50}, but also potential dual α/γ antagonists and agonists.

4. Experimental section

4.1. Chemistry

Melting points were determined on a Büchi B-540 apparatus and are uncorrected. Infrared spectra were recorded on a FT-IR 1600 Perkin–Elmer spectrometer. NMR spectra were run at 300 MHz on a Varian instrument; chemical shifts (δ) are reported in ppm. Microanalyses were carried out with an Eurovector Euro EA 3000 model analyser and the analytical results were within 0.4% of the theoretical values. Commercial reagents were used as received from Aldrich or Fluka.

4.2. General procedure for the synthesis of N-(methylsulfonyl) amides

To a cooled mixture (0–5 °C) of proper acids (1.0 mmol) in dry dichloromethane (15 mL), 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC, 1.0 mmol, 177.0 μL) and 4-dimethylaminopyridine (DMAP, 1.0 mmol, 122.2 mg) were added, under stirring in a nitrogen atmosphere. After 15 min, methanesulfonamide (1.1 mmol) was added, and the mixture was allowed to warm to room temperature. After stirring overnight, the

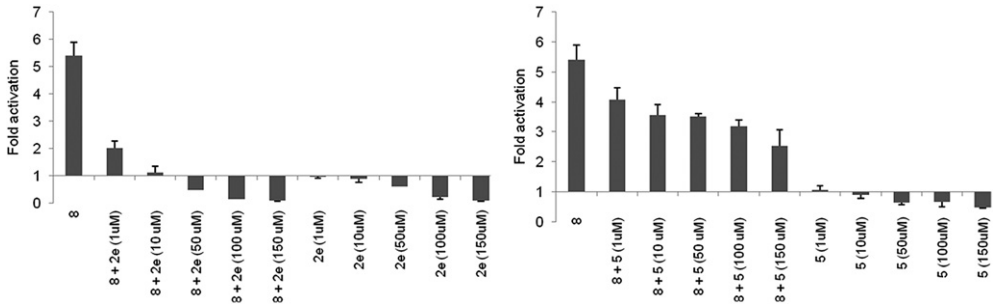


Fig. 3. Dose-dependent antagonistic profile of **2e** and **5**.

reaction was diluted with dichloromethane (15 mL), washed with 2 N HCl (3 × 30 mL), dried on Na_2SO_4. After evaporation of solvent under reduced pressure, crude products were purified on silica gel (eluent dichloromethane/methanol 9:1).

4.2.1. 2-[(6-Ethoxy-1,3-benzothiazol-2-yl)thio]-N-(methylsulfonyl) propanamide (**2a**)

Yellowish needles, 62% yield, m.p. 136–138 °C. IR (KBr) 3210, 1726, 1685, 1558 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.44 (t, 3H, J = 6.9 Hz, CH_3CH$_2$OAr), 1.61 (d, 3H, J = 7.5 Hz, CH_3CH), 3.26 (s, 3H, CH_3SO$_2$), 4.07 (q, 2H, J = 6.9 Hz, ArOCH_2CH$_3$), 4.32 (q, 1H, J = 7.5 Hz, CHCH$_3$), 7.06 (dd, 1H, J = 9.0 Hz, J = 2.4 Hz, CH Ar), 7.22 (d, 1H, J = 2.4 Hz, CH Ar), 7.81 (d, 1H, J = 9.0 Hz, CH Ar), 12.49 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 15.0 (CH$_3$CH$_2$OAr), 15.3 (CH$_3$CH), 41.3 (CH$_3$SO$_2$), 45.1 (CHCH$_3$), 64.4 (ArOCH$_2$CH$_3$), 105.0, 116.5 and 122.2 (CH Ar), 136.4, 145.9, 157.5 and 163.4 (C Ar), 170.6 (C=O). Anal. ($C_{13}H_{16}N_2O_4S_3$) C, H, N.

4.2.2. 2-[(6-Ethoxy-1,3-benzothiazol-2-yl)thio]-N-(methylsulfonyl) pentanamide (**2b**)

White needles, 57% yield, m.p. 136–138 °C. IR (KBr) 3163, 1715, 1692, 1335 and 1136 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.95 (t, 3H, J = 7.2 Hz, CH_3CH$_2$CH$_2$), 1.44 (t, 3H, J = 6.9 Hz, CH_3CH$_2$OAr), 1.42–1.63 (m, 2H, CH$_3$CH_2CH$_2$), 1.78–2.17 (m, 2H, CH$_3$CH$_2$CH_2), 3.26 (s, 3H, CH_3SO$_2$), 4.07 (q, 2H, J = 6.9 Hz, ArOCH_2CH$_3$), 4.13 (t, 1H, J = 7.2 Hz, CHCH$_2$), 7.06 (dd, 1H, J = 9.0 Hz, J = 2.4 Hz, CH Ar), 7.22 (d, 1H, J = 2.4 Hz, CH Ar), 7.82 (d, 1H, J = 9.0 Hz, CH Ar), 12.29 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 13.8 (CH$_3$CH$_2$CH$_2$), 14.9 (CH$_3$CH$_2$OAr), 20.5 (CH$_2$CH$_2$CH$_3$), 31.3 (CH$_2$CH$_2$CH$_3$), 41.3 (CH$_3$SO$_2$), 50.4 (CHCH$_2$), 64.4 (ArOCH$_2$CH$_3$), 105.0, 116.5 and 122.3 (CH Ar), 136.4, 146.0, 157.5 and 163.2 (C Ar), 170.4 (C=O). Anal. ($C_{15}H_{20}N_2O_4S_3$) C, H, N.

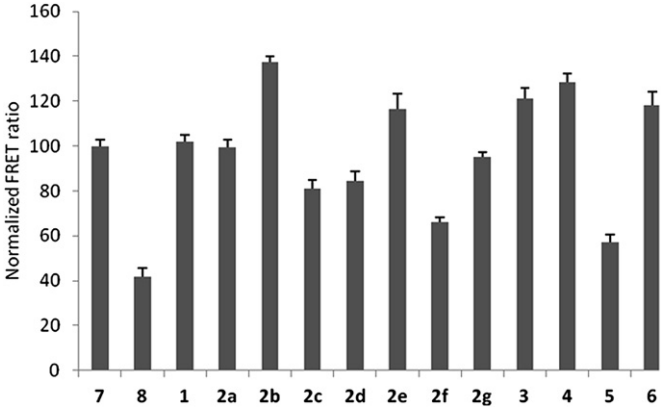


Fig. 4. Normalized FRET ratios (520/495 nm) obtained for tested compounds.

4.2.3. 2-[(5-Chloro-1,3-benzothiazol-2-yl)thio]-N-(methylsulfonyl) pentanamide (**2c**)

White needles, 53% yield, m.p. 150–151 °C. IR (KBr) 1716, 1549, 1345 and 1171 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.97 (t, 3H, J = 7.2 Hz, CH_3CH$_2$), 1.43–1.62 (m, 2H, CH_2CH$_3$), 1.80–2.17 (m, 2H, CH_2CH), 3.27 (s, 3H, CH_3SO$_2$), 4.22 (t, 1H, J = 7.5 Hz, CH), 7.36 (dd, 1H, J = 8.7 Hz, J = 2.1 Hz, CH Ar), 7.69 (d, 1H, J = 8.7 Hz, CH Ar), 7.93 (d, 1H, J = 2.1 Hz, CH Ar), 11.69 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 13.8 (CH$_3$CH$_2$), 20.5 (CH$_2$CH$_3$), 31.2 (CH$_2$CH), 41.4 (CH$_3$SO$_2$), 50.2 (CH), 121.6, 122.1 and 126.3 (CH Ar), 133.3, 133.5, 152.4 and 169.1 (C Ar), 169.9 (C=O). Anal. ($C_{13}H_{15}ClN_2O_3S_3$) C, H, N.

4.2.4. 2-[(5-Chloro-1,3-benzothiazol-2-yl)thio]-3-methyl-N-(methylsulfonyl)butanamide (**2d**)

White solid, 67% yield, m.p. 129–130 °C. IR (KBr) 3191, 1686, 1461, 1348, 1136 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.15 (dd, 6H, J = 6.9 Hz, CH_3CHCH_3), 2.50 (m, 1H, CH$_3$CHCH$_3$), 3.27 (s, 3H, CH_3SO$_2$), 3.98 (t, 1H, J = 7.2 Hz, CHS), 7.37 (dd, 1H, J = 8.7 Hz, J = 2.1 Hz, CH Ar), 7.70 (d, 1H, J = 8.7 Hz, CH Ar), 7.92 (d, 1H, J = 2.1 Hz, CH Ar), 11.56 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 20.0 and 21.0 (CH$_3$CHCH$_3$), 28.6 (CH$_3$CHCH$_3$), 41.4 (CH$_3$SO$_2$), 58.2 (CHS), 121.6, 122.1 and 126.3 (CH Ar), 133.3, 133.5, 152.5 and 168.7 (C Ar), 169.8 (C=O). Anal. ($C_{13}H_{15}ClN_2O_3S_3$) C, H, N.

4.2.5. 2-[(5-Chloro-1,3-benzothiazol-2-yl)thio]-N-(methylsulfonyl) hexanamide (**2e**)

White solid, 63% yield, m.p. 120–122 °C. IR (KBr) 3194, 1694, 1460, 1345, 1134 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.91 (t, 3H, J = 7.2 Hz, CH_3CH$_2$), 1.24–1.59 (m, 4H, CH_2), 1.78–2.25 (m, 2H, CH_2), 3.27 (s, 3H, CH_3SO$_2$), 4.21 (t, 1H, J = 7.2 Hz, CHS), 7.37 (dd, 1H, J = 8.7 Hz, J = 2.1 Hz, CH Ar), 7.69 (d, 1H, J = 8.7 Hz, CH Ar), 7.93 (d, 1H, J = 2.1 Hz, CH Ar), 11.69 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 14.0 (CH$_3$CH$_2$), 22.4, 29.0 and 29.3 (CH$_2$), 41.4 (CH$_3$SO$_2$), 50.4 (CH), 121.6, 122.1 and 126.3 (CH Ar), 133.3, 133.5, 152.4 and 169.1 (C Ar), 169.9 (C=O). Anal. ($C_{14}H_{17}ClN_2O_3S_3$) C, H, N.

4.2.6. 2-[(5-Chloro-1,3-benzothiazol-2-yl)thio]-N-(methylsulfonyl) octanamide (**2f**)

White solid, 70% yield, m.p. 102–104 °C. IR (KBr) 3213, 1706, 1430, 1346, 1146 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (t, 3H, J = 7.2 Hz, CH_3CH$_2$),

Table 2
Combined data obtained for selected compounds by transactivation and FRET assay.

Compd	Transactivation assay	FRET assay	
	IC_{50} (μM)	Normalized FRET ratio % (520/495 nm)	IC_{50} (μM)
2b	8.87 ± 0.08	137.4	0.48 ± 0.01
2c	0.6 ± 0.01	80.9	0.89 ± 0.03
2e	0.8 ± 0.01	116.7	0.60 ± 0.01
2g	0.9 ± 0.02	95.3	0.95 ± 0.07

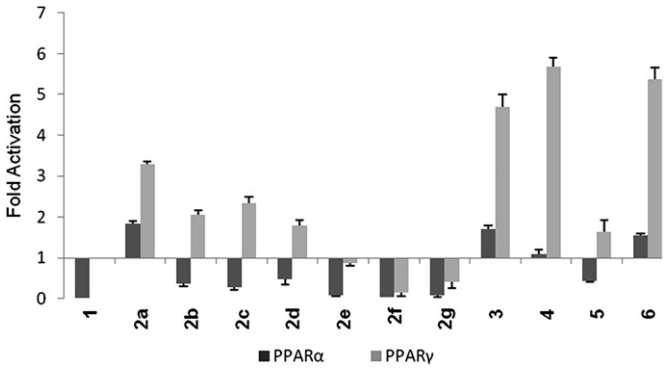


Fig. 5. Activation or inhibition profile on PPARα and PPARγ isoforms.

1.24–2.13 (m, 10H, C*H*$_2$), 3.27 (s, 3H, C*H*$_3$SO$_2$), 4.21 (t, 1H, *J* = 7.2 Hz, C*H*S), 7.37 (dd, 1H, *J* = 8.7 Hz, *J* = 2.1 Hz, C*H* Ar), 7.69 (d, 1H, *J* = 8.7 Hz, C*H* Ar), 7.93 (d, 1H, *J* = 2.1 Hz, C*H* Ar), 11.70 (bs, 1H, N*H*); ^{13}C NMR (CDCl$_3$) *δ* 14.2 (*C*H$_3$CH$_2$), 22.7, 27.2, 29.0, 29.3 and 31.6 (*C*H$_2$), 41.4 (*C*H$_3$SO$_2$), 50.5 (*C*H), 121.6, 122.1 and 126.3 (*C*H Ar), 133.3, 133.5, 152.4 and 169.1 (*C* Ar), 169.9 (*C*=O). Anal. ($C_{16}H_{21}ClN_2O_3S_3$) C, H, N.

*4.2.7. 2-[(5-Chloro-1,3-benzothiazol-2-yl)thio]-N-(methylsulfonyl)-2-phenylacetamide (**2g**)*

White solid, 58% yield, m.p. 176–178 °C. IR (KBr) 1697, 1548, 1351, 1177, 1128 cm^{-1}; ^{1}H NMR (CDCl$_3$) *δ* 3.29 (s, 3H, C*H*$_3$SO$_2$), 5.48 (s, 1H, C*H*S), 7.34–7.49 (m, 6H, C*H* Ar), 7.69 (d, 1H, *J* = 8.4 Hz, C*H* Ar), 7.94 (d, 1H, *J* = 1.8 Hz, C*H* Ar), 11.21 (bs, 1H, N*H*); ^{13}C NMR (CDCl$_3$) *δ* 41.5 (*C*H$_3$SO$_2$), 54.4 (*C*HS), 121.6, 122.2, 126.2, 129.1, 129.4 and 129.6 (*C*H Ar), 132.2, 133.4, 133.5, 152.6 and 168.2 (*C* Ar), 168.3 (*C*=O). Anal. ($C_{16}H_{13}ClN_2O_3S_3$) C, H, N.

*4.2.8. 2-(4-Chlorophenoxy)-2-methyl-N-(methylsulfonyl) propanamide (**3**)*

White solid, 65% yield, m.p. 153–155 °C. IR (KBr) 3221, 1703, 1334, 1165 cm^{-1}; ^{1}H NMR (CDCl$_3$) *δ* 1.51 (s, 6H, C(C*H*$_3$)$_2$), 3.34 (s, 3H, C*H*$_3$SO$_2$), 6.87 (d, 2H, *J* = 8.7 Hz, C*H* Ar), 7.28 (d, 2H, *J* = 8.7 Hz, C*H* Ar), 9.00 (bs, 1H, N*H*); ^{13}C NMR (CDCl$_3$) *δ* 24.5 (C(*C*H$_3$)$_2$), 41.6 (*C*H$_3$SO$_2$), 82.4 (*C*(CH$_3$)$_2$), 123.5 and 129.8 (*C*H Ar), 130.1 and 151.6 (*C* Ar), 173.7 (*C*ONH). Anal. ($C_{11}H_{14}ClNO_4S$) C, H, N.

*4.2.9. 2-[4-(4-Chlorobenzoyl)phenoxy]-2-methyl-N-(methylsulfonyl)propanamide (**4**)*

White solid, 73% yield, m.p. 159–161 °C. IR (KBr) 3077, 1708, 1642, 1288, 1121 cm^{-1}; ^{1}H NMR (CDCl$_3$) *δ* 1.62 (s, 6H, C(C*H*$_3$)$_2$), 3.33 (s, 3H, C*H*$_3$SO$_2$), 6.99 (d, 2H, *J* = 8.4 Hz, C*H* Ar), 7.47 (d, 2H, *J* = 8.4 Hz, C*H* Ar), 7.72 (d, 2H, *J* = 8.4 Hz, C*H* Ar), 7.78 (d, 2H, *J* = 8.4 Hz, C*H* Ar), 8.88 (bs, 1H, N*H*CO); ^{13}C NMR (CDCl$_3$) *δ* 24.7 (C(*C*H$_3$)$_2$), 41.6 (*C*H$_3$SO$_2$), 82.2 (*C*(CH$_3$)$_2$), 120.4, 128.9, 131.4 and 132.2 (*C*H Ar), 133.0, 136.0, 139.1 and 157.3 (*C* Ar), 173.5 (*C*ONH), 194.3 (*C*OPh). Anal. ($C_{18}H_{18}ClNO_5S$) C, H, N.

*4.2.10. 4-Chloro-N-[2-(4-{1,1-dimethyl-2-[(methylsulfonyl)amino]-2 oxoethoxy}phenyl)ethyl]benzamide (**5**)*

White solid, 77% yield, m.p. 184–185 °C. IR (KBr) 3400, 1710, 1629, 1334, 1126 cm^{-1}; ^{1}H NMR (DMSO) *δ* 1.43 (s, 6H, C(C*H*$_3$)$_2$), 2.76 (t, 2H, *J* = 7.2 Hz, C*H*$_2$Ph), 3.23 (s, 3H, C*H*$_3$SO$_2$), 3.41–345 (m, 2H, C*H*$_2$NH), 6.78 (d, 2H, *J* = 8.4 Hz, C*H* Ar), 7.14 (d, 2H, *J* = 8.4 Hz, C*H* Ar), 7.51 (d, 2H, *J* = 8.4 Hz, C*H* Ar), 7.80 (d, 2H, *J* = 8.4 Hz, C*H* Ar), 8.62 (t, 1H, N*H*CO), 11.93 (bs, 1H, N*H*SO$_2$); ^{13}C NMR (DMSO) *δ* 24.7 (C(*C*H$_3$)$_2$), 34.8 (*C*H$_2$Ph), 41.5 (*C*H$_2$NH), 41.6 (*C*H$_3$SO$_2$), 80.7 (*C*(CH$_3$)$_2$), 120.2, 129.0, 129.7 and 130.1 (*C*H Ar), 133.9, 134.2, 136.5 and 153.4 (*C* Ar), 165.7 (*C*ONH), 174.9 (*C*ONHSO$_2$). Anal. ($C_{20}H_{23}ClN_2O_5S$) C, H, N.

*4.2.11. 5-(2,5-Dimethylphenoxy)-2,2-dimethyl-N-(methylsulfonyl) pentanamide (**6**)*

White solid, 66% yield, m.p. 97–98 °C. IR (KBr) 3456, 1714, 1331, 1158 cm^{-1}; ^{1}H NMR (CDCl$_3$) *δ* 1.26 (s, 6H, C(C*H*$_3$)$_2$), 1.74–1.76 (m, 4H C*H*$_2$C*H*$_2$), 2.17 (s, 3H, C*H*$_3$Ph), 2.29 (s, 3H, C*H*$_3$Ph), 3.26 (s, 3H, C*H*$_3$SO$_2$), 3.93 (t, 2H, C*H*$_2$O), 6.60 (s, 1H, C*H* Ar), 6.66 (d, 1H, *J* = 7.5 Hz, C*H* Ar), 6.99 (d, 1H, *J* = 7.5 Hz, C*H* Ar), 8.25 (bs, 1H, CON*H*); ^{13}C NMR (CDCl$_3$) *δ* 16.0 and 21.6 (*C*H$_3$Ph), 25.1 (C(*C*H$_3$)$_2$), 37.2 (OCH$_2$CH$_2$), 41.6 (*C*H$_3$SO$_2$), 43.6 (OCH$_2$CH$_2$CH$_2$), 67.5 (OCH$_2$), 82.4 (*C*(CH$_3$)$_2$), 112.2 and 121.1 (*C*H Ar), 123.7 (*C* Ar), 130.6 (*C*H Ar), 136.8 and 156.9 (*C* Ar), 176.8 (*C*ONH). Anal. ($C_{16}H_{25}NO_4S$) C, H, N.

4.3. In vitro PPAR transactivation assay

HEK293A cells were maintained in growth medium composed of DMEM (Sigma) supplemented with 10% FBS (Gibco), 1% penicillin/streptomycin (Sigma), 1% MEM non-essential amino acid (Sigma) and 1% sodium pyruvate MEM 100 mM (Sigma). The PPARα or PPARγ ligand-binding activity of the test compounds was determined using transient transfection assay. The HEK293A cells were plated in white 96-well plates and cultured until 70–80% confluency for 16 h. Before transfection, the culture medium was replaced by fresh serum-free medium. The cells were transiently transfected with 50 ng of reporter plasmid, 20 ng of renilla, 40 ng of pGEM, and 30 ng of each receptor expression plasmid per well by the calcium phosphate coprecipitation method. Test compounds were added after 6 h. After 16–18 h treatment, the cellular luciferase activity was determined using commercial firefly luciferase assay according to the supplier's instructions (Promega). The results were normalized to the renilla activity to correct the transfection efficiencies.

4.4. Time-resolved fluorescence resonance energy transfer (TR-FRET) analysis

TR-FRET assay was performed by using LanthaScreen™ TR-FRET PPARα competitive binding assay kit (Invitrogen, PV4892). This ligand-binding assay is based on the competitive displacement of a labelled pan-agonist, as described by the manufacturer (Invitrogen). Briefly, for the PPARα assay a terbium-labelled, anti-glutathione S-transferase (GST) antibody is used to indirectly label the PPARα LBD. When a fluorescent ligand (tracer; the PPAR pan-agonist) binds to the receptor, energy transfer from the antibody to the tracer occurs, and a high TR-FRET ratio is observed. Competitive ligand binding to the PPARα LBD is detected by a test compound's ability to displace the tracer from PPARα, resulting in a loss of FRET signal between the antibody and the tracer. Compounds, except for **8** (1 μM), were screened at 100 μM and the assay performed following the manufacturer's instructions. After a 2 h incubation, fluorescence was measured using a fluorescence counter (Spectramax Gemini-XS, Molecular Devices) at emission wavelengths of 495 or 520 nm, with excitation at 340 nm. TR-FRET signal was measured. The data were calculated as TR-FRET ratio by dividing the emission signal of the acceptor (fluorescein, 520 nm) by the emission signal of the donor (terbium, 495 nm). Binding activity is reported as Inhibitory Concentration (IC_{50}) calculated in a concentration range of 1–100 μM. This parameter represents test ligand concentration at which 50% of the labelled pan-agonist is displaced by the test ligand.

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