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Brief Article

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Design and Synthesis of Dual Modulators of Soluble Epoxide Hydrolase and Peroxisome Proliferator-Activated Receptors

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Supporting Information

ABSTRACT: Metabolic syndrome is a complex condition which often requires the use of multiple medications as a treatment. The resulting problems of polypharmacy are increase in side effects, drug−drug interactions, and its high economic cost. Development of multitarget compounds is a promising strategy to avoid the complications arising from administration of multiple drugs. Modulators of peroxisome proliferator-activated receptors (PPARs) are established agents in the treatment of dyslipidaemia, hyperglycaemia, and insulin resistance. Inhibitors of soluble epoxide hydrolase (sEH) are under evaluation for their use in cardiovascular diseases. In the present study, a series of dual sEH/PPAR modulators containing a pyrrole acidic headgroup and a urea pharmacophore were designed, synthesized, and evaluated in vitro using recombinant enzyme and cell-based assays. Compounds with different activity profiles were obtained which could be used in the treatment of metabolic syndrome.

INTRODUCTION

The metabolic syndrome (MetS)[1] is a clustering of factors mainly consisting of the so-called "deadly quartet" of hyperglycaemia, hypertriglyceridemia, hypertension, and obesity. MetS results in an increased risk for atherosclerosis and diabetes. Because of its complex nature, the current therapy strategies of MetS require multiple treatments regulating lipid and glucose homeostasis as well as blood pressure and coagulation. Up-to-date treatment for MetS follows a lifestyle change of the patient to increase the physical activity, initiation of drug therapy with statins to reduce LDL, blood pressure reducing agents, oral antidiabetics, and compounds to handle obesity. Nevertheless, the combination of several drugs for individual risk factors can decrease the efficacy and enhance the toxicity of each drug. Thus, there is a strong unmet medical need in reliable and efficient drugs targeting multiple symptoms of MetS over the long term, thereby minimizing problems with polypharmacy.[2]

PPARs contribute to the regulation of glucose, lipid, and cholesterol metabolism, therefore they seem to be a valuable target to treat MetS.[3] With the hypolipidemic fibrates and the antidiabetic thiazolidindiones (TZD), two drug classes had entered the market. PPARγ agonists like troglitazone, rosiglitazone, and most recently pioglitazone were suspended by some authorities due to severe adverse events. However, a very promising finding had been made when Choi et al.[4] showed that the antidiabetic effect of the TZDs is partially mediated by the inhibition of the cyclin-dependent kinase 5 mediated phosphorylation of PPARγ. With this discovery, a promising new development regarding PPARγ as a drug target arises.

The fibrate-derived PPARα agonists (e.g., clofibrate, bezafibrate) are used in the treatment of dyslipidaemia associated with atherosclerosis and dyslipidaemia primarily linked to type 2 diabetes mellitus. The remaining subtype PPARδ stimulates fatty acid oxidation in heart and skeletal muscle and plays a role in cell differentiation and atherosclerosis. Reviewing recent patent literature implied that the development of PPARδ agonists is a promising target toward MetS associated pathologies.[5]

Soluble epoxide hydrolase (sEH) metabolizes epoxyeicosatrienoic acids (EETs), previously produced by epoxygenases, to the corresponding dihydroxyeicosatrienoic acids (DHETs). Inhibition or deletion of soluble epoxide hydrolase prevents hyperglycaemia, promotes insulin secretion, reduces islet apoptosis, and decreases adipogenesis.[6,7] On the basis of this knowledge, sEH has attired the attention as possible target to treat MetS[8] in conjunction with selective PPAR activation. This current study presents dual modulators that target sEH and PPAR, an interesting therapeutic approach to treat MetS related pathologies. PPARs and sEH are related through the modulatory action of sEH substrates and metabolites on PPAR.

Latest investigations demonstrated that EETs[9] are PPARγ and PPARα ligands. Competition as well as direct binding assays revealed that 14,15-EET[10] binds to the ligand-binding domain of PPARα.

Furthermore, latest publications discovered that DHETs have a high potency to activate PPARα,[10] suggesting that DHETs may have additional vascular actions as a result of their effect on PPARα. Figure 1 summarizes the expected mode of action of dual acting compounds which should lead to increased EETs levels due to sEH inhibition and substitute the beneficial activation of PPAR by DHETs. Fang et al. discovered an activation of PPARα by substituted urea-derived sEHIs after cellular metabolism.[11]

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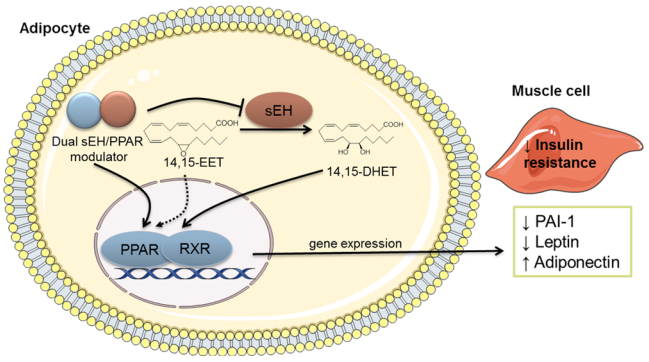


Figure 1. Mode of action of dual sEH/PPAR modulators. The sEH inhibitor leads to a decreased DHETs level, which might lead to decreased PPAR activation. This effect is compensated by the sEH/PPAR dual modulation.

In the present work, we describe novel compounds that are able to inhibit sEH and activate PPAR regardless their metabolism. Further development of these compounds could lead to agents with beneficial action on hyperglycaemia, hypertriglyceridemia, and hypertension, which can serve as a starting point for the development of polypharmacological compounds as a treatment of MetS.[12]

CHEMISTRY

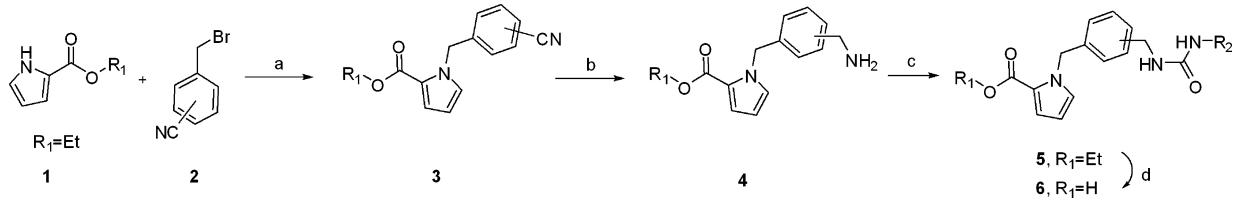
We prepared the compounds **5a−i**, **6a−i** that are connected through a methylbenzyl linker between the pyrrole ring and the urea pharmacophore. An adamantyl group has been used in many sEH inhibitors[13] with high in vitro efficacy (e.g., AUDA), and Ph-*p*OCF_3 substituted moiety showed improved pharmacokinetic properties.[14] On the basis of this knowledge, we developed derivatives carrying pyrrole structures linked to urea containing compounds (sEH pharmacophore) that end in a carboxylic group needed for activation of all PPAR subtypes. We followed the previous strategy[15] to synthetize potent PPAR agonists: acidic headgroup−aromatic core−linker−hydrophobic tail.[16] In the present study, we explored the effect of the substitution pattern (*o*-, *m*-, *p*-) on the activity at the different targets. We synthesized the compounds **6a−i**, with an acidic group and **5a−i** as ethyl carboxylate esters, as seen in Scheme 1. Starting from ethyl 1*H*-pyrrole-2-carboxylate (**1**) that reacted with the *o*-, *m*-, or *p*-(bromomethyl) benzonitrile (**2**) via phase transfer catalysis using TBAI in a mixture of DCM/aqueous NaOH (1/1), we obtained the following *N*-alkylated products (**3a−c**) that underwent reduction with Raney Ni, the corresponding amines (**4a−c**) remaining which reacted with three different isocyanates: cyclohexylisocyanate, adamantylisocyanate, and *p*-OCF_3-phenylisocyanate, yielding the corresponding urea derivatives (**5a−i**). To obtain the compounds (**5a−i**) as carboxylic acid, they were solved in a mixture of THF/MeOH/water (1:1:2) and treated with KOH under microwave radiation to yield (**6a−i**).

RESULTS AND DISCUSSION

Known PPARα, γ, and δ selective agonists (GW7647,[17] pioglitazone,[18] L-165041[19]) and the sEH inhibitor AUDA[13] were evaluated and used as references. To obtain dual modulators of sEH/PPAR, it was necessary to link both pharmacophores via an aromatic spacer and maintain the lipophilic core which is characteristic of PPAR agonists. Adamantyl and cyclohexyl ureas have been shown to exhibit high sEH inhibitory properties. In terms of sEH inhibition, the ethyl ester derivatives with cyclohexyl moieties (**5a−c**) are better tolerated than adamantyl (**5d−f**) or Ph-*p*OCF_3 (**5g−i**), reaching IC_{50} values in the range from 23 to 39 nM. In general, the introduction of a Ph-*p*OCF_3 substituent decreased the inhibitory activity, yielding inactive (**5h**, **6g**) or slightly active compounds (**5g**, **5i**, **6h**, **6i**) (Table 1). The comparison of carboxylic acids with the corresponding esters in terms of sEH inhibition shows that cyclohexyl substituted esters (**5a−c**) and adamantyl (**5d−f**) are more potent than their carboxylic acids counterparts (**6a−c**, **6d−f**). Regarding the substitution pattern, the inhibitory potency of cyclohexyl carrying esters remains unaffected (**5a−c**, 23 nM < IC_{50} < 39 nM). This tendency holds true for adamantyl substituted esters (**5d−f**, 43 nM < IC_{50} < 87 nM). This behavior stands in contrast with the inhibitory potency of the acids, possibly due to an alternative binding mode which already has been described for sEH.[19] Regarding cyclohexyl acid derivatives *o*- (**6a**, IC_{50} = 747 nM) and *p*- (**6c**, IC_{50} = 252 nM), substituted compounds were more potent than the *m*-substituted moiety (**6b**, IC_{50} = 1923 nM). In the case of adamantyl, the *p*- (**6f**, IC_{50} = 73 nM) was more potent than *o*-adamantyl (**6d**, IC_{50} = 178 nM) and *m*- (**6e**, IC_{50} = 304 nM) derivatives, possibly due to sterical hindrance.

The PPAR activation potency of the compounds showed a wide diversity from modulatory effects at 10 μM to full agonistic properties with certain EC_{50} values (Table 2). Regarding the PPAR activities, only two ethyl ester derivatives showed a partial activation of PPARγ at 10 μM (**5b**, 21%; **5c**, 16%). As expected, the majority of carboxylic acids (**6a−i**) were able to activate PPARs. We observed that adamantyl derivatives (**6d−f**) were able to activate PPARγ selectively, probably due to the larger left distal ligand binding pocket compared to the other receptor subtypes.[16] The selectivity was impaired when testing Ph-*p*OCF_3 derivatives that activated both PPARα and PPARγ concerning *o*-substitution (**6g**, 38% (PPARα) and 128%, EC_{50} = 2 μM (PPARγ)). While *m*- and *p*-substituted compounds (**6h**, **6i**) yield a PPARα full agonism, only partial agonism was observed for the *o*-substituted Ph-*p*OCF_3

Scheme 1. Synthesis of sEH/PPAR Dual Modulators 5a−i, 6a−i[a]



[a]Reagents and conditions: (a) tetrabutylammonium iodide, NaOH 50% aqueous, DCM, 0 °C to rt, 12 h; (b) Raney Ni, H_2, MeOH; (c) R-NCO, DIPEA, DCM, rt, 12 h; (d) THF/MeOH/H_2O (1:2:2), KOH, MW, 90 °C, 15 min.

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Table 1. Inhibition and Activation Values of dual sEH/PPAR Modulators 5a−i, 6a−i[a]

name	R_1	R_2	subst	IC_{50} (nM) sEH	% activation @10 μM PPARα	% activation @10 μM PPARγ	% activation @10 μM PPARδ
GW7647				nt	100	nt	nt
pioglitazone				nt	nt	100	nt
L-165041				nt	nt	nt	100
AUDA				107 ± 12.8	nt	nt	nt
5a	Et	cyclohexyl	*o*-	39 ± 1.4	ia	ia	ia
5b	Et	cyclohexyl	*m*-	27 ± 8.0	ia	21 ± 4.1	ia
5c	Et	cyclohexyl	*p*-	23 ± 1.0	ia	16 ± 4.6	ia
5d	Et	adamantyl	*o*-	43 ± 10.0	ia	ia	ia
5e	Et	adamantyl	*m*-	46 ± 4.2	ia	ia	ia
5f	Et	adamantyl	*p*-	87 ± 8.4	ia	ia	ia
5g	Et	Ph-*p*OCF_3	*o*-	2009 ± 1165.6	ia	ia	ia
5h	Et	Ph-*p*OCF_3	*m*-	ia	ia	ia	ia
5i	Et	Ph-*p*OCF_3	*p*-	611 ± 248.4	ia	ia	ia
6a	H	cyclohexyl	*o*-	747 ± 194.9	ia	ia	ia
6b	H	cyclohexyl	*m*-	1923 ± 724.7	ia	ia	ia
6c	H	cyclohexyl	*p*-	252 ± 17.1	ia	ia	ia
6d	H	adamantyl	*o*-	178 ± 55.4	ia	45 ± 14.7	ia
6e	H	adamantyl	*m*-	304 ± 78.3	ia	16 ± 5.7	ia
6f	H	adamantyl	*p*-	73 ± 4.5	ia	18 ± 7.8	ia
6g	H	Ph-*p*OCF_3	*o*-	ia	38 ± 1.9	105 ± 14.4	ia
6h	H	Ph-*p*OCF_3	*m*-	943 ± 658.3	58 ± 5.7	43 ± 12.5	25 ± 4.5
6i	H	Ph-*p*OCF_3	*p*-	258 ± 48.0	67 ± 13.4	84 ± 24.1	ia

[a]nt = not tested, ia = inactive.

Table 2. EC_{50} Values of Dual sEH/PPAR Modulators[a]

name	IC_{50} (nM) sEH	EC_{50} (max activation) PPARα	EC_{50} (max activation) PPARγ	EC_{50} (max activation) PPARδ
GW7647	nt	0.2 ± 0.05 μM	nt	nt
pioglitazone	nt	nt	0.2 ± 0.05 μM	nt
L-165041	nt	nt	nt	0.039 ± 0.008 μM
AUDA	107 ± 12.8	nt	nt	nt
6d	178 ± 55.4	ia	6 ± 0.8 μM (60 ± 1.3%)	ia
6g	ia	7 ± 1.5 μM (55 ± 9.5%)	2 ± 0.4 μM (128 ± 14.4%)	ia
6h	943 ± 658.3	6 ± 0.5 μM (97 ± 38.9%)	nd	nd
6i	258 ± 48.0	5 ± 0.6 μM (90 ± 11.4%)	nd	ia

[a]nt = not tested; ia = inactive; nd = not determinable.

derivative (**6g**), which can be probably explained by the sterical hindrance. A pan-agonist was obtained (**6h**) that activated all classes of PPAR (58% on PPARα, 43% on PPARγ. and 25% on PPARδ). When considering *p*-substituted compounds, another dual PPARα/γ agonist was found (**6i**, 67% on PPARα and 84% on PPARγ at 10 μM). Surprisingly, cyclohexyl moieties (**6a−c**) did not lead to sustainable PPAR activation, indicating that this building block is not suitable for incorporation into dual acting sEH/PPAR modulators.

Regarding dual modulation of sEH/PPAR, we obtained two compounds that partially activated PPARγ (**6e**, **f**) and inhibited sEH with moderate potency (IC_{50} values of 304 and 73 nM, respectively). Compound **6h** inhibited sEH (IC_{50} = 943 nM) and activated PPARα,γ,δ (EC_{50} = 6 μM on PPARα, 43% at 10 μM on PPARγ, 25% at 10 μM on PPARδ). Compound **6i** inhibited sEH (IC_{50} = 258 nM) and activated PPARα,γ (EC_{50} = 5 μM on PPARα, 84% at 10 μM on PPARγ), resulting in an interesting compound to be evaluated in further experiments.

CONCLUSION

This work describes the synthesis of dual sEH/PPAR modulators as potential agents for the treatment of metabolic syndrome. Following a combinatorial approach, an acidic headgroup, known as a pharmacophore important for PPAR dual agonistic activity, was combined with different hydrophobic urea derivatives in order to introduce an epoxide mimetic.

The resulting compounds displayed high inhibition on sEH and different patterns of PPAR agonistic activity.

This study demonstrates that the pharmacophores of PPAR agonists and sEH inhibitors can be easily combined, resulting in a simplified blueprint of a dual sEH/PPAR modulator. Further in vivo pharmacological evaluation studies are needed in order to evaluate which pattern of PPAR activation shows the most promising profile for treatment of metabolic syndrome.

EXPERIMENTAL SECTION

General. All reagents and solvents were purchased from the suppliers Alfa-Aesar GmbH & Co. KG (Karlsruhe, Germany) and Sigma-Aldrich Chemistry GmbH (Hannover, Germany) and used without further purification. Retention factors were determined by thin layer chromatography with silica coated aluminum foil (particle size 60 μm) obtained from Merck KGaA (Darmstadt, Germany). Flash chromatography was performed on packed silica columns (particle size 50 μm) from Varian Medical Systems GmbH (Darmstadt, Germany). ^{1}H (250/400 MHz) and ^{13}C (64 MHz) spectra were measured on AV 250 and AMX 400 nuclear magnetic resonance spectrometers from

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Bruker. Mass spectra were measured using electrospray ionization (+) with a VG Plattform II spectrometer from Fisons. High resolution mass spectra were measured by a MALDI LTQ Orbitrap XL spectrometer from Thermo Scientific. All compounds were characterized by NMR and MS. All final compounds had a purity of ≥95% as determined by HPLC (LC2020, Shimadzu, Duisburg, Germany), except for **6f** (92%). Data are expressed as mean values with SE. All IC_{50} and EC_{50} values are means with SE of the IC_{50} or EC_{50} values obtained from measurements at five different concentrations of the compounds in 3−5 independent experiments. IC_{50} and EC_{50} values were determined using a sigmoidal dose response (variable slope) equation from GraphPad Prism (GraphPad Software, LaJolla, CA) software.

General Procedure for the Preparation of Compounds 3a−c (*o*-, *m*-, *p*-). A solution of ethyl 1*H*-pyrrole-2-carboxylate (**1**) (7.2 mmol) in DCM (50 mL) was cooled to 0 °C. Tetrabutylammonium iodide (0.22 mmol) and 50 mL of sodium hydroxide solution (50%) were added. The mixture was stirred for 30 min at 0 °C before (bromomethyl)benzonitrile (**2**) (7.9 mmol) was added. The mixture was allowed to warm to room temperature and stirred vigorously overnight. The reaction was quenched by adding concentrated hydrochloric acid until a pH of 1 was reached. The aqueous layer was extracted three times with 20 mL DCM. The collected organic layers were washed twice with 10 mL of brine, dried over $MgSO_4$, and concentrated under reduced pressure. After purification by flash chromatography (Hex:EE 0−15%), white crystals remained.

General Procedure for the Preparation of the Compounds 4a−c. A mixture of ethyl (cyanobenzyl)-1*H*-pyrrole-2-carboxylate (**3a−c**) (5.9 mmol, 1 equiv) and Raney nickel (5.9 mmol) in 100 mL of dry ammoniacal methanol was stirred overnight at room temperature at H_2 atmosphere under a pressure of 6 bar. The catalyst was filtered off through Celite, and the solvent was removed under reduced pressure to give a yellow oil.

General Procedure for the Preparation of the Compounds 5a−i. A solution of ethyl 1-(aminomethyl)benzyl)-1*H*-pyrrole-2-carboxylate (**4a−c**) (0.97 mmol) and *N*,*N*-diisopropylethylamine (2.9 mmol) in 10 mL of dry DCM was stirred at room temperature under argon. An isocyanate (0.97 mmol) was added, and the mixture was stirred overnight. The solvent was evaporated under reduced pressure. The crude product was purified by hot filtration in hexane and recrystallized from ethanol.

General Procedure to Obtain Carboxylic Acid Compounds 6a−i. The ethyl ester (**5a−i**) was treated with potassium hydroxide (0.652 mmol) in a solvent mixture of THF/MeOH/H_2O (1:2:2) and accomplished under microwave irradiation at 90 °C (35 W) during 15 min. The solvent was removed under reduced pressure and the residue solved in water. For precipitation, 1 M hydrochloric acid was added. After filtration, the white solid was lyophilized. 1-(2-((3-Cyclohexylureido)methyl)benzyl)-1*H*-pyrrole-2-carboxylic acid (**6a**) was obtained from ethyl 1-(((ureido)methyl)benzyl)-1*H*-pyrrole-2-carboxylate **5a** (46 mg, 0.12 mmol) and yielded 25 mg (50% yield). ^{1}H NMR (CH_3OH-d_4): δ 7.19 (d, J = 7.2 Hz, 1H), 7.08−7.04 (m, 2H), 6.9 (m, 1H), 6.77 (m, 1H), 6.39 (d, J = 7.2 Hz, 1H), 6.09 (m, 1H), 5.54 (s, 2H), 4.29 (s, 2H), 3.39 (m 1H), 1.79 (m, 2H), 1.64 (m, 2H), 1.51 (m, 1H) and 1.25 (m, 5H) ppm. ^{13}C NMR (CH_3OH-d_4): δ 160.0, 162.6, 137.7, 135.0, 130.1, 127.3, 126.9, 125.5, 119.9, 117.3, 110.1, 55.9, 52.6, 42.5, 34.3, 31.4, 26.2, 25.3, and 24.8 ppm. HPLC (98% purity). HRMS: measured m/z [M + H$^+$] 356.1967 (theoretical, 356.1969).

Activity Assays. sEH activity assay. For the recombinant affinity purified sEH, we used a fluorescent-based assay[19] that uses PHOME (3-phenyl-cyano(6-methoxy-2-naphthalenyl)methyl ester-2-oxirane-acetic acid) in a 96-well format assay to determine IC_{50} values. Recombinant sEH (2 μg/well) was incubated with inhibitors for 10 min at room temperature in 25 mM Bis-Tris/HCl and 0.1 mg/mL BSA buffer (110 μL, pH 7.0) before substrate (PHOME) was added ($[S]_{final}$ = 50 μM). Activity was evaluated by measuring the appearance of the fluorescent product 6-methoxynaphthaldehyde (λ_{em} = 330 nm, λ_{ex} = 465 nm).

PPAR Transactivation Assay. COS7 cells were grown in DMEM supplemented with 10% FCS, sodium pyruvate, and penicillin/streptomycin at 37 °C and 5% CO_2. The day before transfection, cells were seeded in 96-well plates at a density of 30000 cells per well. Transient transfection was carried out by Lipofectamine LTX reagent (Invitrogen) according to the manufacturer's protocol with pFR-Luc (Stratagene), pRL-SV40 (Promega), and the Gal4-fusion receptor plasmids (pFA-CMV-hPPAR-LBD) of the respective subtype. Five h after transfection, the medium was changed to DMEM without phenol red and 10% FCS, containing 0.1% DMSO and the respective concentrations of the test compounds.

Following overnight incubation with the test compounds, cells were assayed for reporter gene activity using Dual-Glo luciferase assay system (Promega, Mannheim, Germany) according to the manufacturer's protocol. Luminescence was measured with a GENios Pro luminometer (Tecan Deutschland GmbH, Crailsheim, Germany). Each concentration of the compounds was tested in triplicate wells, and each experiment was repeated independently at least three times. Normalization for transfection efficacy and cell growth was done by division of the firefly luciferase data by renilla luciferase data, resulting in relative light units. Activation factors were obtained by dividing by DMSO control. EC_{50} and standard deviation values were calculated by mean values of at least three determinations by SigmaPlot 2001 (Systat Software GmbH, Erkrath, Germany) using a four-parameter logistic regression. All compounds were evaluated by comparison of the achieved maximum effect to that of the reference compound (GW 7647 for PPARα, pioglitazone for PPARγ, and L165,041 for PPARδ each with 1 μM).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Assay protocols, purity determination method, and NMR and mass analysis data of compounds **5a−i** and **6b-i**. This material is available free of charge via the Internet at http://pubs.acs.org..

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Author Contributions

†These authors contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AUDA, 12-(3-adamantan-1-yl-ureido)dodecanoic acid; DHETs, dihydroxyeicosatrienoic acids; EETs, epoxyeicosatrienoic acids; FCS, fetal calf serum; LDL, low-densitiy lipoprotein; MetS, metabolic syndrome; PPAR, peroxisome proliferator-activated receptor; sEH, soluble epoxide hydrolase; sEHI, soluble epoxide hydrolase inhibitor; TBAI, tetrabutyl ammonium iodide; TZD, thiazolidinedione

REFERENCES

(1) Eckel, R. H.; Grundy, S. M.; Zimmet, P. Z. The metabolic syndrome. *Lancet 365*, 1415−1428.

(2) Page , J. Nonalcoholic fatty liver disease: the hepatic metabolic syndrome. *J. Am. Acad. Nurse Pract.* **2012**, *24*, 345−351.

(3) Grundy, S. M. Drug therapy of the metabolic syndrome: minimizing the emerging crisis in polypharmacy. *Nature Rev. Drug Discovery* **2006**, *5*, 295−309.

(4) Pirat, C.; Farce, A.; Lebègue, N.; Renault, N.; Furman, C.; Millet, R.; Yous, S.; Speca, S.; Berthelot, P.; Desreumaux, P.; Chavatte, P. Targeting peroxisome proliferator-activated receptors (PPARs): development of modulators. *J. Med. Chem.* **2012**, *55*, 4027−4061.

(5) Choi, J. H.; Banks, A. S.; Estall, J. L.; Kajimura, S.; Boström, P.; Laznik, D.; Ruas, J. L.; Chalmers, M. J.; Kamenecka, T. M.; Blüher, M.; Griffin, P. R.; Spiegelman, B. M. Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPARgamma by Cdk5. *Nature* **2010**, *466*, 451−456.

(6) Lamers, C.; Schubert-Zsilavecz, M.; Merk, D. Therapeutic modulators of peroxisome proliferator-activated receptors (PPAR): a patent review (2008−present). *Exp. Opin. Ther. Pat.* **2012**, *22*, 803−841.

(7) Vanella, L.; Kim, D. H.; Sodhi, K.; Barbagallo, I.; Burgess, A. P.; Falck, J. R.; Schwartzman, M. L.; Abraham, N. G. Crosstalk between EET and HO-1 downregulates Bach1 and adipogenic marker expression in mesenchymal stem cell derived adipocytes. *Prostaglandins Other Lipid Mediators* **2011**, *96*, 54−62.

(8) Kim, D. H.; Vanella, L.; Inoue, K.; Burgess, A.; Gotlinger, K.; Manthati, V. L.; Koduru, S. R.; Zeldin, D. C.; Falck, J. R.; Schwartzman, M. L.; Abraham, N. G. Epoxyeicosatrienoic acid agonist regulates human mesenchymal stem cell-derived adipocytes through activation of HO-1-pAKT signaling and a decrease in PPARγ. *Stem Cells Dev.* **2010**, *19*, 1863−1873.

(9) Iyer, A.; Kauter, K.; Alam, M. A.; Hwang, S. H.; Morisseau, C.; Hammock, B. D.; Brown, L. Pharmacological inhibition of soluble epoxide hydrolase ameliorates diet-induced metabolic syndrome in rats. *Exp. Diabetes Res.* **2012**, *2012*, 758614.

(10) Ng, V. Y.; Huang, Y.; Reddy, L. M.; Falck, J. R.; Lin, E. T.; Kroetz, D. L. Cytochrome P450 eicosanoids are activators of peroxisome proliferator-activated receptor alpha. *Drug Metab. Dispos.* **2007**, *35*, 1126−1134.

(11) Cowart, L. A.; Wei, S.; Hsu, M.-H.; Johnson, E. F.; Krishna, M. U.; Falck, J. R.; Capdevila, J. H. The CYP4A isoforms hydroxylate epoxyeicosatrienoic acids to form high affinity peroxisome proliferator-activated receptor ligands. *J. Biol. Chem.* **2002**, *277*, 35105−35112.

(12) Fang, X.; Hu, S.; Xu, B.; Snyder, G. D.; Harmon, S.; Yao, J.; Liu, Y.; Sangras, B.; Falck, J. R.; Weintraub, N. L.; Spector, A. A. 14,15-Dihydroxyeicosatrienoic acid activates peroxisome proliferator-activated receptor-alpha. *Am. J. Physiol., Heart Circul. Physiol.* **2006**, *290*, H55−H63.

(13) Fang, X.; Hu, S.; Watanabe, T.; Weintraub, N. L.; Snyder, G. D.; Yao, J.; Liu, Y.; Shyy, J. Y.-J.; Hammock, B. D.; Spector, A. A. Activation of peroxisome proliferator-activated receptor alpha by substituted urea-derived soluble epoxide hydrolase inhibitors. *J. Pharmacol. Exp. Ther.* **2005**, *314*, 260−270.

(14) Schulman, I. G. Nuclear receptors as drug targets for metabolic disease. *Adv. Drug Delivery Rev.* **2010**, *62*, 1307−1315.

(15) Morisseau, C.; Goodrow, M. H.; Dowdy, D.; Zheng, J.; Greene, J. F.; Sanborn, J. R.; Hammock, B. D. Potent urea and carbamate inhibitors of soluble epoxide hydrolases. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 8849−8854.

(16) Tsai, H.-J.; Hwang, S. H.; Morisseau, C.; Yang, J.; Jones, P. D.; Kasagami, T.; Kim, I.-H.; Hammock, B. D. Pharmacokinetic screening of soluble epoxide hydrolase inhibitors in dogs. *Eur. J. Pharm. Sci.* **2010**, *40*, 222−238.

(17) Proschak, E.; Sander, K.; Zettl, H.; Tanrikulu, Y.; Rau, O.; Schneider, P.; Schubert-Zsilavecz, M.; Stark, H.; Schneider, G. From molecular shape to potent bioactive agents II: fragment-based de novo design. *ChemMedChem* **2009**, *4*, 45−48.

(18) Pirard, B. Peroxisome Proliferator-Activated Receptors target family landscape: a chemometrical approach to ligand selectivity based on protein binding site analysis. *J. Comput.-Aided Mol. Des.* **2003**, *17*, 785−796.

(19) Gomez, G. A.; Morisseau, C.; Hammock, B. D.; Christianson, D. W. Human soluble epoxide hydrolase: structural basis of inhibition by 4-(3-cyclohexylureido)-carboxylic acids. *Protein Sci.* **2006**, *15*, 58−64.