Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

N-Benzylbenzamides: A Novel Merged Scaffold for Orally Available Dual Soluble Epoxide Hydrolase/Peroxisome Proliferator-Activated Receptor γ Modulators

René Blöcher,[†] Christina Lamers,[†] Sandra K. Wittmann,[†] Daniel Merk,[†] Markus Hartmann,[†] Lilia Weizel,[†] Olaf Diehl,[†] Astrid Brüggerhoff,[†] Marcel Boß,[‡] Astrid Kaiser,[†] Tim Schader,[†] Tamara Göbel,[†] Manuel Grundmann,[§] Carlo Angioni,[∥] Jan Heering,[⊥] Gerd Geisslinger,[∥] Mario Wurglics,[†] Evi Kostenis,[§] Bernhard Brüne,[‡] Dieter Steinhilber,[†] Manfred Schubert-Zsilavecz,[†] Astrid S. Kahnt,[†] and Ewgenij Proschak*[,†]

[†]Institute of Pharmaceutical Chemistry, Goethe-University Frankfurt, Max-von-Laue-Strasse 9, D-60438 Frankfurt am Main, Germany

[‡]Institute of Biochemistry I, Goethe-University Frankfurt, Theodor-Stern-Kai 7, D-60590 Frankfurt am Main, Germany

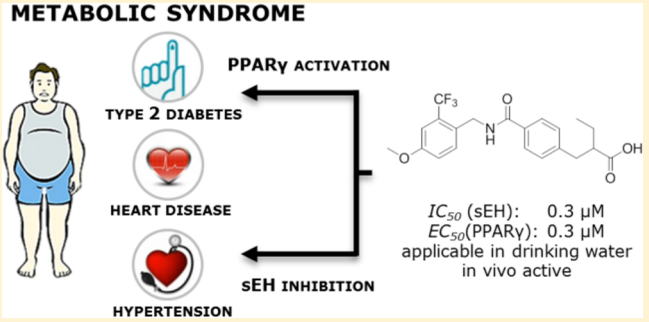
[§]Institute of Pharmaceutical Biology, Rheinische Friedrich-Wilhelms-Universität Bonn, Nussallee 6, D-53115 Bonn, Germany

[∥]Institute of Clinical Pharmacology, Goethe-University Frankfurt, Theodor-Stern-Kai 7, D-60590 Frankfurt am Main, Germany

[⊥]Project Group Translational Medicine and Pharmacology TMP, Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Theodor-Stern-Kai 7, D-60590 Frankfurt am Main, Germany

Supporting Information

ABSTRACT: Metabolic syndrome (MetS) is a multifactorial disease cluster that consists of dyslipidemia, cardiovascular disease, type 2 diabetes mellitus, and obesity. MetS patients are strongly exposed to polypharmacy; however, the number of pharmacological compounds required for MetS treatment can be reduced by the application of multitarget compounds. This study describes the design of dual-target ligands that target soluble epoxide hydrolase (sEH) and the peroxisome proliferator-activated receptor type γ (PPARγ). Simultaneous modulation of sEH and PPARγ can improve diabetic conditions and hypertension at once. *N*-Benzylbenzamide derivatives were determined to fit a merged sEH/PPARγ pharmacophore, and structure−activity relationship studies were performed on both targets, resulting in a submicromolar (sEH IC_{50} = 0.3 μM/PPARγ EC_{50} = 0.3 μM) modulator **14c**. In vitro and in vivo evaluations revealed good ADME properties qualifying **14c** as a pharmacological tool compound for long-term animal models of MetS.



INTRODUCTION

Metabolic syndrome (MetS) names a group of risk factors such as central obesity, atherogenic dyslipidemia, insulin resistance, and endothelial dysfunction that lead to arteriosclerotic cardiovascular diseases (ASCVD) such as coronary heart disease, stroke, peripheral vascular disease, and type 2 diabetes (T2D).[1] In addition, patients affected by T2D develop long-term microvascular complications. Two-thirds of T2D patients suffer from neuropathic pain,[2] and one-third of them develop diabetic nephropathy.[3] MetS has a very complex pathophysiology that is only partially understood. Epidemiological evidence shows that the rising prevalence of MetS in Western societies is due to Western lifestyle factors such as misbalanced, high caloric food intake, sedentary lifestyle, and stress.[4] To date, the first-line treatment of MetS that simultaneously addresses all risk factors is a change in lifestyle, i.e., weight reduction, increased physical activity, and an antiatherogenic diet.[5] Nevertheless, previously developed individual disorders such as endothelial dysfunction and T2D cannot be completely reversed by this approach and symptoms will worsen with advancing age. Therefore, patients who accumulate various risk factors over time also accumulate quite a number of medications to separately treat each disorder. Treatment of the MetS risk factors and follow-up diseases often requires multiple drugs leading to the phenomenon of polypharmacy. Here, the pharmacokinetic and pharmacologic situation in patients reaches an unfavorable complexity, and unpredictable drug−drug interactions can occur. In addition, medical compliance is at risk. While therapy costs rise, the probability for medication errors increases.[4] In this situation, it is advisible to focus drug research on compounds capable of treating more than one aspect of MetS. The advantages and

Received: April 2, 2015
Published: November 23, 2015

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81

drawbacks of multitarget compounds have been exhaustively discussed; however, multitarget ligands addressing more than one risk factor at once may find a reasonable application in this study.[6] Herein, we present a multitarget approach that involves the simultaneous modulation of soluble epoxide hydrolase (sEH) and peroxisome proliferator-activated receptor γ (PPARγ) for the treatment of the MetS.

PPARγ, a member of the PPAR nuclear receptor family, plays a key role in adipogenesis, regulation of lipid metabolism and glucose homeostasis, as well as in anti-inflammatory processes, and is therefore targeted in the treatment of T2D.[7] The physiological and pathophysiological role of PPARγ has been the focus of research for several decades, which has been exhaustively reviewed.[8] Pharmacological activation of the receptor by thiazolidinediones (TZDs) such as rosiglitazone and pioglitazone induces beneficial effects on insulin action and blood-glucose levels. However, the clinical use of TZDs is limited because of excessive weight gain, fluid retention, and increased risk of osteoporosis in treated patients. Treatment with rosiglitazone led to an increase in cardiovascular complications as indicated by meta-analysis of clinical trials.[9] Troglitazone was withdrawn from the market due to hepatotoxicity, and pioglitazone seems to trigger bladder cancer.[9] Another drawback is the poor effect of TZDs on the occurrence of macrovascular events, although the equilibration of blood glucose levels reduces microvascular complications.[10] In this context, it is important to mention that some of the adverse events that were observed with TZD, such as cancer development and hepatotoxicity, seem to be a characteristic of single compounds rather than a class-specific phenomenon.[11]

The second target chosen in this study is the soluble epoxide hydrolase (sEH), which is abundantly expressed in adipose tissue and whose expression and activity increases with obesity.[12] sEH is an enzyme of the arachidonic acid cascade, promoting the hydrolysis of cytochrome P450 derived epoxyeicosatrienoic acids (EETs) to their less bioactive corresponding diols, the dihydroxyepoxyeicosatrienoic acids (DHETs).[13] Through sEH inhibition, EET levels are increased. Endothelial cell-derived EETs activate calcium-activated potassium channels on smooth muscle cells, leading to hyperpolarization and vascular relaxation.[14] Numerous studies show EET-derived effects on various MetS associated disorders such as cardiovascular disease (CVD), dyslipidemia, neuropathy, and nephropathy.[15] Recent studies have shown improved angiogenesis by endothelial progenitor cells derived from patients with acute myocardial infarction through sEH inhibition and subsequent activation of PPARγ by accumulating EETs, indicating a significant degree of crosstalk between PPARγ and sEH.[16,17] In this context, it is remarkable that PPARα and PPARγ agonists induce sEH expression.[12] Furthermore, the impaired functionality of pancreatic islet β-cells is one of the underlying mechanisms that cause T2D. In this context it was shown that sEH inhibition can prevent hyperglycemia and augment islet glucose stimulated insulin secretion in diabetic mice, and sEH- knockout mice displayed attenuated islet cell apoptosis in streptozotocin (STZ)-induced diabetes.[18]

Because there is an unmet medical need for safer PPARγ modulating drugs that possess additional cardio and kidney protective properties, the combination of PPARγ agonism with sEH inhibition in one compound might be beneficial for the treatment of T2D. The main side effect of known PPARγ activators is water retention, which results in weight gain and edema. Fortunately, sEH inhibition and EETs are natriuretic and positively influence water and electrolyte homeostasis.[19] Imig et al. previously showed in spontaneously hypertensive obese (SHROB) rats that combination therapy using an sEH inhibitor (t-AUCB) and a PPARγ agonist (rosiglitazone) lowered blood pressure and reduced systemic glucose, triglycerides and free fatty acids. The study also demonstrated the renoprotective effects of the regiment by showing that it attenuated renal injury. Remarkably, an additional positive synergistic effect of the combination compared to the single sEH/PPARγ therapies was also reported.[20] These experiments motivated us to investigate the potential of dual sEH/PPARγ therapeutics. Recently, we presented the in vitro proof of principle for sEH/PPARγ dual modulation.[21] However, to achieve in vivo application capability, these compounds needed improvement. In this study, the optimization process and its evaluation were explored.

DESIGN OF A MERGED SEH/PPARγ PHARMACOPHORE

The identification of a common pharmacophore is a challenging task in the design process of dual modulators. GlaxoSmithKline published in 2011 a PPARγ agonist (*R*)-1-((3,5-difluoropyridin-2-yl)methyl)-2-methyl-*N*-(1-phenylpropyl)-1*H*-benzo[*d*]imidazole-5-carboxamide (GSK1997132B) without the commonly used acidic headgroup, for blood−brain barrier penetration reasons (Figure 1).[22] The binding mode of the cocrystallized

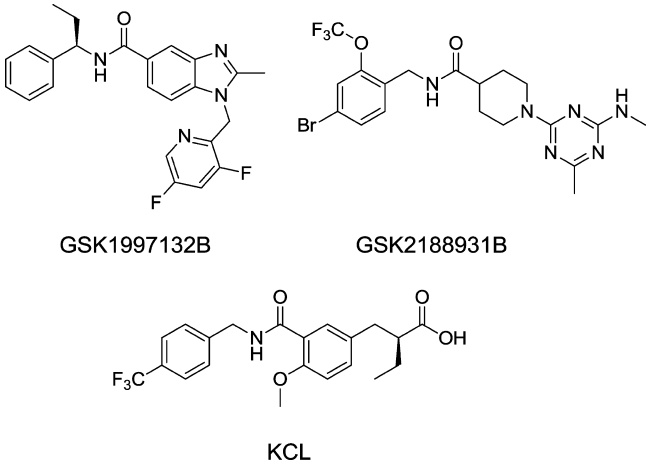
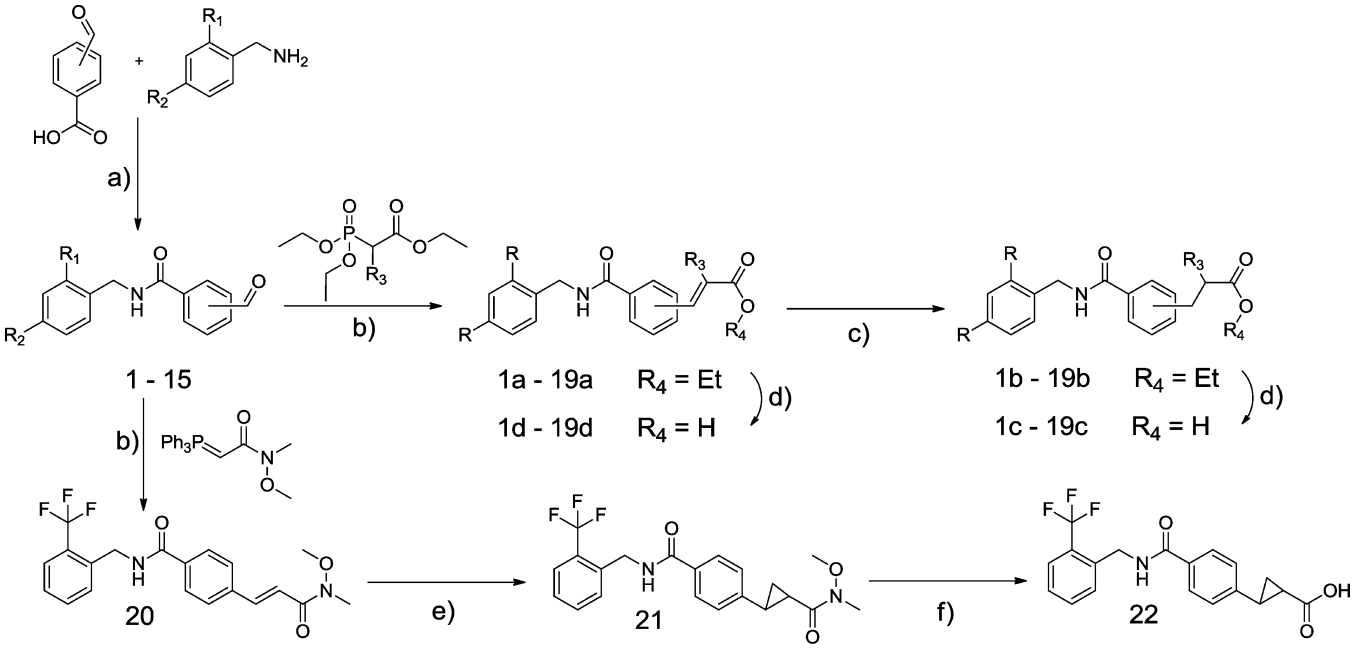


Figure 1. Landmark structures for design of novel dual ligand.

ligand indicates that a benzylamide moiety is able to replace the acidic headgroup while retaining full agonist properties of the ligand. Almost all reported sEH inhibitors are epoxide mimetics, containing a urea or an amide structure as pharmacophore. In this situation the benzylamide structure would represent a merged pharmacophore for sEH and PPARγ, which is the best starting point in dual ligand design. Several benzylamides were reported as sEH inhibitors, the most advanced compound of this study is (*N*-({4-bromo-2-[(trifluoromethyl)oxy]phenyl}-methyl)-1-[4-methyl-6-(methylamino)-1,3,5-triazin-2-yl]-4-piperidinecarboxamide) (GSK2188931B, Figure 1).[23] On the basis of the reported SAR, we adapted the ortho trifluoromethylbenzyl substitution important for inhibitory activity on sEH and metabolic stability of the compounds. Finally, several studies describe *N*-benzylbenzamides as PPARα, PPARγ, PPARδ, or pan-agonists, represented by KCL (Figure 1).[24,25] These compounds exhibit the classical PPAR binding mode, with the acidic headgroup responsible for the interaction with helix 12 and receptor activation. Nevertheless, this information motivated us

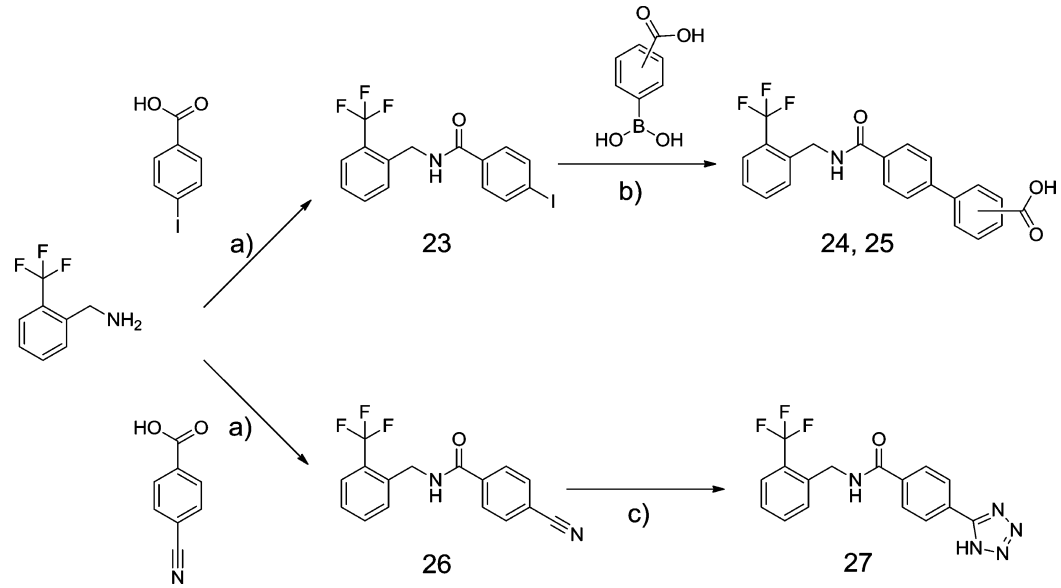
DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81

Scheme 1[a]



[a](a) IBCF, TEA, dry DCM, 12 h; (b) NaH, THF, 0 °C, 2 h; (c) H_2, Pd/C, EtOH, 12 h; (d) MeOH/H_2O/THF, KOH, MW, 100 °C, 30 min; (e) $Me_3SO^+I^-$, NaH, DMSO, 6 h; (f) KOH, EtOH/H_2O, 16 h; (g) diethyl benzylphosphonate, NaH, THF, 0 °C, 2 h.

Scheme 2[a]



[a](a) EDC, DMAP, dry DCM, 12 h; (b) $Pd(AcO)_2$, K_2CO_3, acetone/H_2O, 65 °C, 1 h; (c) NaN_3, NH_3Cl, DMF, 12 h.

toward molecular design, using the *N*-benzylbenzamide moiety as a merged pharmacophore.

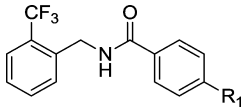
SYNTHESIS

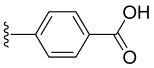
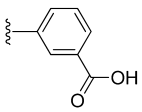
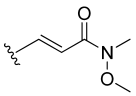
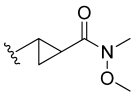
Synthetic routes to all investigated *N*-benzylbenzamide derivatives are shown in Schemes 1 and 2. α-Substituted *N*-benzylbenzamide propionic acids (**1c**–**19c**) were prepared in four steps. Some of the *N*-benzylbenzamide ethyl cinnamates (**1a**–**19a**) were also hydrolyzed to their corresponding *N*-benzylbenzamide cinnamic acids (**1d**–**19d**) in order to extend the structure–activity relationship data.

The synthesis of *N*-benzylbenzamide propionic acids (**1c**–**19c**) (Scheme 1) started with the activation of either 4-formylbenzoic acid or 3-formylbenzoic acid with isobutyl chloroformate (IBCF) in DCM under dry basic conditions, followed by the addition of various 2- or 2,4-substituted benzylamines to produce **1**–**15**.[26] Compounds **1**–**15** were subsequently turned into their corresponding *N*-benzylbenzamide ethyl cinnamate derivatives (**1a**–**15a**) by a Wittig reaction, using triethyl 2-phosphonobutyrate.[27] Using the same reaction type, four different α-substituents (hydrogen, methyl, propyl, and phenyl) were introduced to the *N*-benzylbenzamide cinnamate scaffold, while the benzylamine fragment was kept constant at 2-trifluoromethyl substitution (**16a**–**19a**).

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61–81

Table 1. In Vitro Activity Values of Dual sEH/PPAR Modulators: Diversity Screening[a]



compd.	R_1	w.s. [μM]	IC_{50} sEH [μM]	EC_{50} PPARα [μM] (E_{max} - %)	EC_{50} PPARδ [μM] (E_{max} - %)	EC_{50} PPARγ [μM] (E_{max} - %)
	GW7647	n.t.	n.t.	0.2 ± 0.05	n.t.	n.t.
	pioglitazone	n.t.	n.t.	n.t.	n.t.	0.2 ± 0.05 μM
	L-165041	n.t.	n.t.	n.t.	0.039 ± 0.008	n.t
	AUDA	n.t.	107 ± 12.8	n.t.	n.t.	n.t.
24		5-3.75	0.17 ± 0.006	ia.	ia.	@ 10 μM (30%)
25		n.t.	1 ± 0.1	ia.	ia.	@ 10 μM (28%)
27		500-375	5 ± 0.7	ia.	ia.	ia.
20		n.t.	0.07 ± 0.02	ia.	ia.	ia.
21		n.t.	0.08 ± 0.004	ia.	ia.	@10 μM (30%)

[a]ia = inactive. n.t. = not tested. E_{max} = maximum activation in percent of control. w.s. = water solubility. compd = compound.

All α,β-unsaturated carbonyl compounds (**1a−19a**) were reduced with palladium on carbon catalyst in dry EtOH under hydrogen atmosphere to maintain the *N*-benzylbenzamide ethyl propanoates (**1b−19b**).[27] The deprotection of either the *N*-benzylbenzamide cinnamate (**1a−19a**) or the *N*-benzylbenzamide propionate (**1b−19b**) to their corresponding acids **1d−19d** and **1c−19c** was carried out by a microwave reaction under basic conditions with a solvent mixture of MeOH/H_2O/THF in the ratio 1/2/1.[28]

The α,β-cyclopropane derivative **22** was synthesized in three steps (Scheme 1).[29] Starting with a Wittig reaction of **1** and *N*-methoxy-*N*-methyl(triphenylphosphoranylidene)acetamide, intermediate **20** was obtained. **21** was synthesized by a Corey−Chaykovsky reaction. A basic deprotection was peformed in an EtOH/H_2O solvent mixture.[30]

The biphenyl ortho- and meta-benzoic acid derivatives **24** and **25** were synthesized in a two-step route shown in Scheme 2. In the first step 4-iodobenzoic acid was activated by EDC under DMAP catalysis and combined with 2-trifluoromethylbenzylamine to compound **24** which was subsequently coupled with 4-carboxy as well as 3-carboxybenzeneboronic acid under Suzuki conditions[31] to yield the desired biphenyl acid derivatives **24** and **25**.

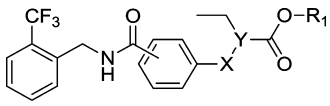
Tetrazole **27** was also produced in a two-step synthesis (Scheme 2). The nitrile intermediate **26** was prepared under the same conditions as compound **23**. For the tetrazole synthesis NaN_3 and NH_4Cl in DMF were used.[32]

RESULTS AND DISCUSSION

On the basis of the previously described hypothesis a series of diverse compounds with an acidic headgroup (**24**, **25**) or a bioisostere (**20**, **21**, **27**) were synthesized. After the reintroduction of the acidic headgroup and extension of the aromatic core a new set of two isomeric compounds was prepared (**24**, **25**). The sEH inhibition dropped almost 1 order of magnitude from

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81

Table 2. In Vitro Activity Values of Dual sEH/PPAR Modulators: Variation of the Substitution Pattern of the Central Phenyl Moiety[a]



a) X-Y:CH=C; R_1:CH_2CH_3

b) X-Y: CH_2-CH; R_1= CH_2CH_3

c) X-Y: CH_2-CH; R_1=H

d) X-Y: CH=C; R_1=H

compd	subst	ws [μM]	IC_{50} sEH [μM]	EC_{50} PPARα [μM] (E_{max}, %)	EC_{50} PPARδ [μM] (E_{max}, %)	EC_{50} PPARγ [μM] (E_{max}, %)
1a	para	nt	0.063 ± 0.003	ia	ia	at 10 μM (20%)
1b	para	5−2.5	0.044 ± 0.005	ia	ia	1.8 ± 0.2 (86%)
1c	para	100−75	1.6 ± 0.2	ia	ia	4.8 ± 2.1 (127%)
1d	para	nt	0.12 ± 0.01	ia	ia	2.2 ± 0.3 (117%)
15a	meta	nt	0.04 ± 0.006	nt	nt	nt
15b	meta	nt	0.027 ± 0.002	ia	ia	at 10 μM (40%)
15c	meta	nt	0.9 ± 0.08	at 10 μM (34%)	ia	6.4 ± 1.3 (60%)
15d	meta	nt	0.4 ± 0.1	nt	nt	nt

[a]ia = inactive. nt = not tested. E_{max} = maximum activation in percent of control. ws = water solubility. compd = compound.

0.17 to 1 μM, by switching the acidic headgroup from para to meta position. The para position of the acidic headgroup seems to fit more properly in the lipophilic tunnel-shaped sEH binding pocket.[33] PPARγ activation of the para and meta derivatives at a concentration of 10 μM was around 30% (compared to 1 μM pioglitazone), indicating that acidic functionality or at least an H-bond acceptor is still necessary for full PPARγ activation. In **27** the core fragment was reduced to one aromatic ring, and the carboxylic acid was replaced by a tetrazole bioisostere. These changes caused a loss of PPARγ activation, and sEH inhibition in the micromolar range (IC_{50} = 5 μM) was achieved (Table 1). To improve the PPARγ activation without exhaustive expansion of molecular weight, the introduction of the α-substituted propionic acid analogous to KCL (Figure 1) has been employed. As mentioned in the synthesis paragraph, four types of carbonyl derivatives were produced for each substitution pattern. In the first quartet (**1a−d**), a potent modulator, with full agonistic PPARγ properties and single digit micromolar potency on both sEH and PPARγ, was found (**1c**) (Table 2). In this structural class, sEH inhibition improved by 1 order of magnitude from acid to ester derivative, which can be explained by the mainly lipophilic sEH binding site. Except **1a**, all derivatives of this series showed similar activity on PPARγ.

A set of central meta substituted isomers (**15a−d**) showed no improvement of activities compared with the para congeners (Table 2). Also, compound **15c** showed loss of PPARγ selectivity over other PPAR subtypes. Potency on both targets in a low micromolar range, small molecular weight, PPARγ subtype selectivity, and reasonable water solubility under assay conditions qualified compound **1c** as a good starting point for pharmacological profiling.

Compound **1c** did not impair cell viability of HepG2 cells up to a concentration of 30 μM, indicated by the WST-1 assay.[34] In Sprague-Dawley rat liver microsomes the in vitro metabolic stability of compound **1c** has been determined, and after 1 h ~92% of **1c** remained intact (see Supporting Information, Figure S6a). PPARγ activation by **1c** was evaluated in different cellular systems by measuring the effect on adipocyte differentiation. The capability of **1c** to trigger adipocyte differentiation in murine 3T3-L1 fibroblasts and human primary preadipocytes was determined and compared to rosiglitazone (PPARγ agonist) and *N*-cyclohexyl-*N*′-iodophenylurea (CIU, sEH inhibitor).[35] In 3T3-L1 fibroblasts, a dose-dependent effect (1−10 μM) of **1c** on adipocyte differentiation could be demonstrated (Figure 2a). Differentiated adipocytes were visualized using Oil Red O staining. At a 10 μM concentration of **1c** a lower amount of adipocytes accumulated lipids compared to a 2 μM concentration of rosiglitazone. Surprisingly CIU was also able to start adipocyte differentiation with no direct PPARγ activation. A hypothesis to this phenomenon is the subsequent PPARγ activation through an EET−PPARγ pathway.[36] In human adipocytes, a similar effect of **1c** was observed (see Supporting Information, Figure S2). By Oil Red O staining, a dose dependent (1−10 μM) effect to the adipocyte differentiation was determined, which was also lower compared to 2 μM rosiglitazone. In contrast to murine 3T3-L1 fibroblasts, CIU was not able to start differentiation in human adipocytes, which could be explained by the decreased inhibitory activity of CIU at human sEH.[37] In addition, the expression of four PPARγ target genes (GLUT4, glucose transporter type 4; adiponectin; FABP4, fatty acid binding protein 4; LPL, lipoprotein lipase) in the differentiated murine and human adipocytes was determined by qPCR analysis as a measure of target activation.[38] In murine 3T3-L1 fibroblasts (Figure 2b) **1c** dose-dependently activated expression of all target genes analyzed. At a concentration of 10 μM **1c** showed a slightly lower expression of all four PPARγ target genes compared to the rosiglitazone (2 μM) control. In human adipocytes the effect of **1c** on the PPARγ target expression was more diverse (see Supporting Information, Figure S3). Here, the upregulation of the GLUT4 expression at a **1c** concentration of 10 μM was comparable to the rosiglitazone (2 μM) control. In contrast, adiponectin, FABP4, and LPL showed only minor effects in the upregulation caused by **1c** stimulation. The diverse effects of **1c** on the expression of the PPARγ target genes will need more detailed research. It is known that certain PPARγ agonists can selectively transactivate a number of PPARγ target genes while

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81

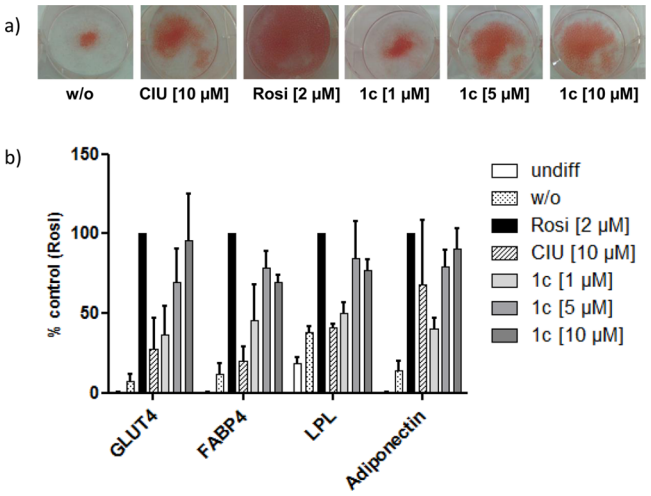


Figure 2. 3T3-L1 mouse fibroblasts were differentiated in the presence of the different compounds. Subsequently, (a) cells were stained with Oil Red O or (b) PPARγ target gene expression (GLUT4, adiponectin, FABP4, LPL) was determined by quantitative PCR analysis. Shown are mean values ± SEM (b) or one representative experiment (a) out of three independent experiments.

sparing others. The physiological consequence of this is not completely understood yet and is the subject of intensive research.

On the basis of this favorable in vitro profile, two in vivo PK/PD studies were carried out in mice. To achieve a prodrug effect, compound **1b**, the ethyl ester derivative of **1c**, was characterized in vivo. After a single oral application of 30 mg/kg bodyweight (bw) to nine (RijOrl, SWISS/CD-1) mice (gavage) **1b** was not detected in the plasma of the animal at all time points, indicating rapid ester hydrolysis. The corresponding acid (**1c**) appeared in plasma with C_{max} = 787 ng/mL (~2 μM) after 0.5 h (t_{max}), $AUC_{0\rightarrow\infty}$ = 4026 ng·h/ml, Cl/*f* = 7.5 L h^{-1} kg^{-1}, and V_z/f = 54.3 l/kg (see Supporting Information, Figure S4). Recently it was shown that PPARγ activation in the CNS is involved in the increased weight gain associated with marketed PPARγ activators by controlling food intake and energy expenditure.[39] Therefore, to establish the blood–brain barrier diffusion capacity of **1c**, its concentration was determined in the brains of mice. Here, the concentration of **1c** did not exceed 30 ng/g brain tissue (see Supporting Information, Figure S5). This led to the assumption that **1c** only poorly penetrates the blood–brain barrier. Unfortunately, a C_{max} = 787 ng/mL of **1c** after 30 mg/kg dosing of **1b** was lower than the in vitro activity values of **1c**. Thus, the second PK/PD study in mice with per oral application of 30 mg/kg bw of **1c** (the acidic derivative of **1b**) to nine (RijOrl, SWISS/CD-1) mice (gavage) was performed. **1c** reached a maximum concentration in the mouse plasma of 7200 ng/mL (~20 μM) after 0.5 h (t_{max}), which is 1 order of magnitude higher than the C_{max} of **1c** after the oral administration of **1b** and almost 1 order of magnitude higher than the in vitro EC_{50} values on both targets (Figure 4a). All pharmacokinetic profiles were also improved ($AUC_{0\rightarrow\infty}$ = 15847 ng·h/mL, Cl/*f* = 1.9 L h^{-1} kg^{-1}, V_z/f = 8 L/kg).

The EET to DHET ratio in plasma gives direct information about the effectivity of sEH inhibition.[13] At 8 h after application of **1c** to the mice the plasma EET/DHET ratio increased by at least 2-fold (Figure 3b). For determination of PPARγ activation in vivo, the expression of the PPARγ target gene CD36 in liver tissue of the treated mice was quantified by qPCR analysis. The expression increased by at least 2-fold compared to non-treated mice (Figure 3a). In vitro and in vivo characterization of **1c** was suboptimal, with capacity to improve in potency and bioavailability. Therefore, the following SAR study was conducted.

We explored the SAR of α-substituted benzylbenzamide propionic acid derivatives having in mind the application in an animal model of metabolic syndrome. Thus, two main optimization criteria have been identified. The first aim was the improvement of water solubility to fit a long-term drinking water application. The second aim was to achieve sufficient potency in a concentration range below the steady state concentration in plasma. The substitution at the α-position of the carboxyl function plays a key role in PPARγ activation, assuming the classical PPAR binding mode.[40] On the basis of that knowledge, the first variations of the compound were modifications of the α-ethyl group. Neither the reduction to methyl or complete removal of the α-substituent nor the extension to propyl or phenyl substitution showed any major effects on PPARγ activation (Table 3). We interpret these SARs as a possible indication of the alternative binding mode to PPARγ accommodated by this compound series. An α,β-cyclopropyl derivative **22** showed no enhanced potency on any target. The synthesis path of **22** yielded certain nonacidic prestages (**21**, **22**; Table 1) which were evaluated on the two investigated targets. As expected, they showed good inhibitory potency toward sEH in a double-digit nanomolar range. Surprisingly, compound **21** with a similar scaffold as **22**, however lacking an acidic moiety, showed a slightly higher activation of PPARγ. This again led to the assumption of a minor role of the acidic headgroup and the possible appearance of an alternative binding mode. Subsequently, variations on the ortho position of the benzyl ring were investigated (**2b–6c**) (Table 4). Notably,

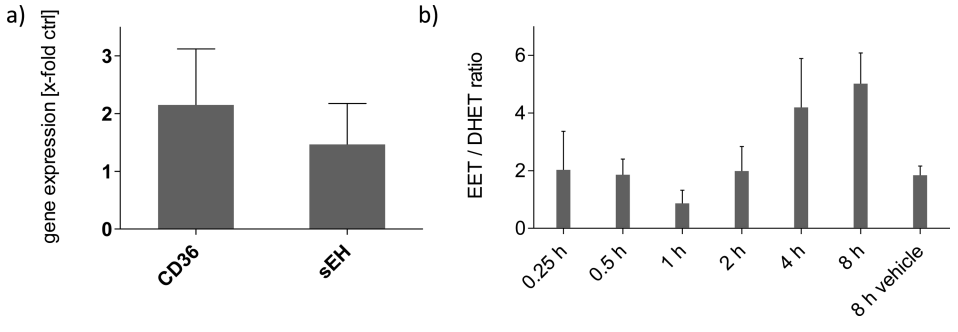


Figure 3. (a) Expression of the PPARγ target genes CD36 and sEH in mouse liver after single application of compound **1c** (30 mg/kg bw; 8 h; three animals). (b) EET/DHET ratio in mouse plasma after a single po application of compound **1c** (30 mg/kg bw; three animals per two time points).

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61–81

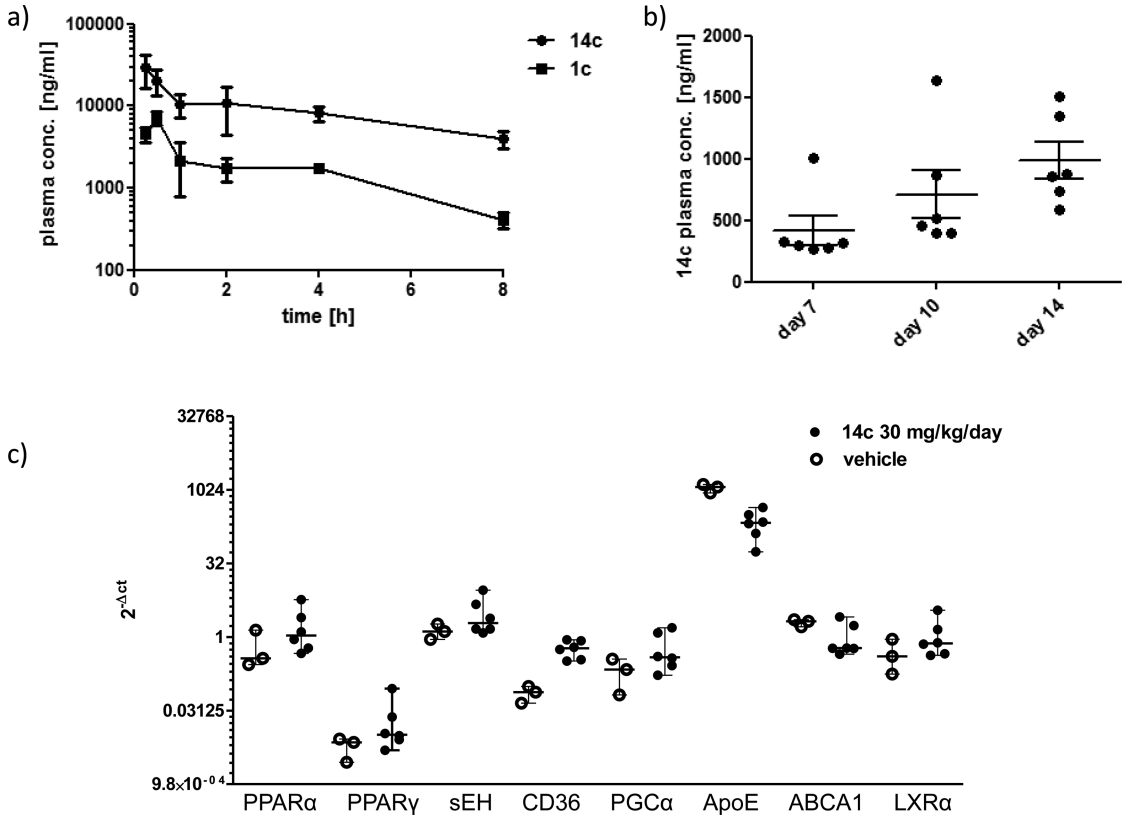
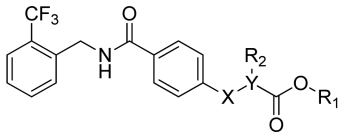


Figure 4. (a) Plasma concentration of compounds **1c** and **14c**, in mice (30 mg/kg po; 3 animals per compound at every second time point). (b) Plasma concentration of compound **14c**, in mice (30 mg/kg in drinking water; 6 animals). (c) Expression of the PPARγ target genes in mouse liver after 14 days of application with compound **14c** (30 mg kg^{-1} day^{-1} in drinking water; 6 animals; 3 control animals).

Table 3. In Vitro Activity Values of Dual sEH/PPAR Modulators: Variation of the α-Substituent[a]



b) R_1: CH_2CH_3

c) R_1:H

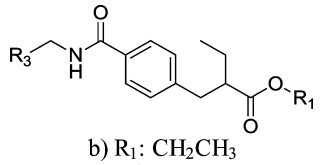
compd.	R_2	X-Y	w.s. [μM]	IC_{50} sEH [μM]	EC_{50} PPARα [μM] (E_{max}-%)	EC_{50} PPARδ [μM] (E_{max}-%)	EC_{50} PPARγ [μM] (E_{max}-%)
16b	H	CH_2-CH	5-3.75	0.11 ± 0.003	ia.	ia.	@ 10 μM (38 %)
16c	H	CH_2-CH	500-375	9 ± 1.7	ia.	ia.	@ 10 μM (38 %)
17b	CH_3	CH_2-CH	10-7.5	0.25 ± 0.04	ia.	ia.	8 ± 1.5 μM (110 %)
17c	CH_3	CH_2-CH	500-375	8 ± 1.6	ia.	ia.	3 ± 0.5 μM (90 %)
1b	CH_2CH_3	CH_2-CH	5-2.5	0.044 ± 0.005	ia.	ia.	1.8 ± 0.2 (86%)
1c	CH_2CH_3	CH_2-CH	100-75	1.6 ± 0.2	ia.	ia.	4.8 ± 2.1 (127%)
18b	$CH_2CH_2CH_3$	CH_2-CH	5-3.75	0.17 ± 0.04	ia.	ia.	0.9 ± 0.2 (132 %)
18c	$CH_2CH_2CH_3$	CH_2-CH	500-375	5 ± 1.3	ia.	ia.	1.5 ± 0.4 (180 %)
19b	Phenyl	CH_2-CH	5-3.75	0.12 ± 0.013	ia.	ia.	2 ± 0.4 (53 %)
19c	Phenyl	CH_2-CH	100-75	2.5 ± 0.5	ia.	ia.	3 ± 0.9 μM (68 %)
22	H	cyclopropyl	n.t.	5.5 ± 0.2	ia.	ia.	@ 10 μM (24 %)

[a]ia = inactive. nt = not tested. E_{max} = maximum activation in percent of control. ws = water solubility. compd = compound.

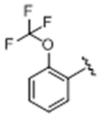
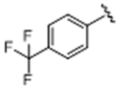
a substituent in this position is additionally important for the metabolic protection of the amide and the reactive benzyl methylene. The -CF_3 group was substituted by -H, -CH_3, -Cl, -Br, and -OCF_3, which all led to a loss of potency. Only the -OCF_3 ester derivative (**6b**) showed a marginal sEH inhibition improvement. With the absence of an ortho substitution sEH inhibition almost vanished and PPAR activity dropped for nearly 1 order of magnitude. This highlights the importance of the ortho CF_3 substitution. The next group of derivatives (**7b**−**11c**) (Table 4) were prepared to investigate the influence of the para substitution of the benzyl moiety. With the introduction of sterically demanding groups (-CF_3, -OCF_3, and -O-phenyl) in

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81

Table 4. In Vitro Activity Values of Dual sEH/PPAR Modulators: Variation of the Terminal Benzyl Substitution[a]

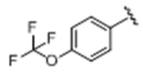
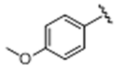
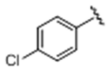
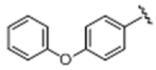
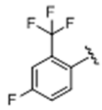
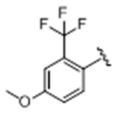


b) R_1: CH_2CH_3
c) R_1:H

compd.	R_3	w.s. [μM]	IC_{50} sEH [μM]	EC_{50} PPARα [μM] (E_{max} - %)	EC_{50} PPARδ [μM] (E_{max} - %)	EC_{50} PPARγ [μM] (E_{max} - %)
1b		5-2.5	0.044 ± 0.005	ia.	ia.	1.8 ± 0.2 (86%)
1c		100-75	1.6 ± 0.2	ia.	ia.	4.8 ± 2.1 (127%)
2b		n.t.	8.5 ± 2.9	ia.	ia.	16 ± 1.7 (94%)
2c		n.t.	@ 10 μM (4%)	ia.	ia.	13.5 ± 2.0 (123%)
3b		n.t.	0.9 ± 0.1	@ 10 μM (15 %)	ia.	@ 10 μM (15%)
3c		n.t.	@ 10 μM (25%)	ia.	ia.	4 ± 0.5 (106%)
4b		n.t.	3.8 ± 0.2	ia.	ia.	@ 10 μM (14%)
4c		n.t.	@ 10 μM (20%)	ia.	ia.	@ 10 μM (40%)
5b		25-20	4 ± 0.7	ia.	ia.	ia.
5c		500-375	@ 10 μM (34%)	ia.	ia.	@ 10 μM (40%)
6b		100-75	0.03 ± 0.008	ia.	ia.	3.5 ± 0.6 (88%)
6c		500-375	@ 10 μM (23%)	ia.	ia.	8 ± 1.3 (110%)
7b		n.t.	2.2 ± 0.2	ia.	ia.	11 ± 1.9 (74%)
7c		n.t.	@ 10 μM (28%)	ia.	ia.	@ 10 μM (23%)
8b		n.t.	0.57 ± 0.007	@ 10μM (22%)	ia.	4.2 ± 1.5 (76%)
8c		n.t.	7.2 ± 0.7	7 ± 0.8 (89%)	ia.	6.3 ± 2.7 (192%)

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81

Table 4. continued

compd.	R_3	w.s. [μM]	IC_{50} sEH [μM]	EC_{50} PPARα [μM] (E_{max} - %)	EC_{50} PPARδ [μM] (E_{max} - %)	EC_{50} PPARγ [μM] (E_{max} - %)
9b		n.t.	0.9 ± 0.42	3 ± 0.1 (58%)	ia.	3 ± 0.5 (68%)
9c		n.t.	14 ± 2	2 ± 0.3 (89%)	ia.	2 ± 0.3 (125%)
10b		10-7.5	0.62 ± 0.02	ia.	ia.	@ 10 μM (40%)
10c		500-375	@ 10 μM (34%)	ia.	ia.	7 ± 2 (110%)
11b		10-7.5	1.7 ± 0.1	ia.	ia.	@ 10 μM (40%)
11c		500-375	1.5 ± 0.2	ia.	ia.	4 ± 1 (171%)
12b		n.t.	n.t.	4 ± 0.7 (110%)	ia.	1.4 ± 0.3 (141%)
12c		n.t.	12 ± 1	0.9 ± 0.3 (106%)	@ 10 μM (20%)	0.3 ± 0.08 (181%)
13b		n.t.	0.12 ± 0.07	ia.	ia.	2.8 ± 0.9 (118%)
13c		n.t.	1.2 ± 0.2	ia.	ia.	0.6 ± 0.2 (158%)
14b		n.t.	0.03 ± 0.001	@ 10 μM (22%)	ia.	2 ± 0.3 (136%)
14c		500-375	0.33 ± 0.05	@ 10 μM (29%)	ia.	0.3 ± 0.09 (160%)

[a]ia = inactive. n.t. = not tested. E_{max} = maximum activation in percent of control. w.s. = water solubility. compd = compound.

the para position of the benzyl ring the PPARγ subtype selectivity got lost and no major improvements on PPARγ were accomplished. The activation of the PPARα subtype by introduction of larger moieties in the para benzyl ring position on similar scaffolds can also be found in literature but mostly without effects on PPARγ activation.[24] Nevertheless, the para-O-phenyl derivative (**12c**) showed, as the only compound in this study, full activation of both PPARα and PPARγ subtypes. **12c** also reached the highest PPARγ potency, with an EC_{50} of 0.3 μM and a peak activation of 181% compared to 1 μM pioglitazone. The sEH inhibition decreased 1 order of magnitude for all para substituted derivatives lacking an ortho substituent. **12c** represents a good PPARα/γ dual agonist; however, it lacks appropriate sEH inhibitory potency. The use of smaller substituents at the benzyl para position (-F, -O-CH_3, -Cl) did not improve the potency on either one of the targets but kept PPARγ subtype selectivity. In the next step ortho, para combined substitution pattern of the benzyl moiety was created (**13b**–**14c**) (Table 4). The impact of the benzyl-ring ortho-CF_3 substitution has already been explored. As para substitution partner in this combination -F and -OCH_3 were chosen, referring to their subtype selective activation on PPARγ in the previous data. For the -OCH_3 substituent an increase in water solubility was also assumed. The ortho-CF_3, para-F substitution pattern improved subtype-selective PPARγ activation but had no enhancing effect on sEH inhibition. With compound **14c** (ortho-CF_3, meta-O-CH_3) potency on both targets got improved by almost 1 order of magnitude (sEH IC_{50} = 0.3 μM, PPARγ EC_{50} = 0.3 μM/160%). The full PPARγ activation by the ester **14b** could be an indication for ester hydrolysis in the cellular system. Therefore, we measured the hydrolysis rate of **14b** and detected almost 50% conversion toward **14c** in the supernatant of COS7 cells (Figure S1). Furthermore, we measured the direct interaction of **14b** and **14c** with PPARγ LBD using differential scanning fluorimetry.[41] While **14c** was able to stabilize PPARγ LBD comparable to the full agonist rosiglitazone, **14b** did not exhibit any stabilization effect (Figure S9).

Submicromolar potency on both targets and improved solubility of **14c** motivated us to perform a second pharmacological

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61–81

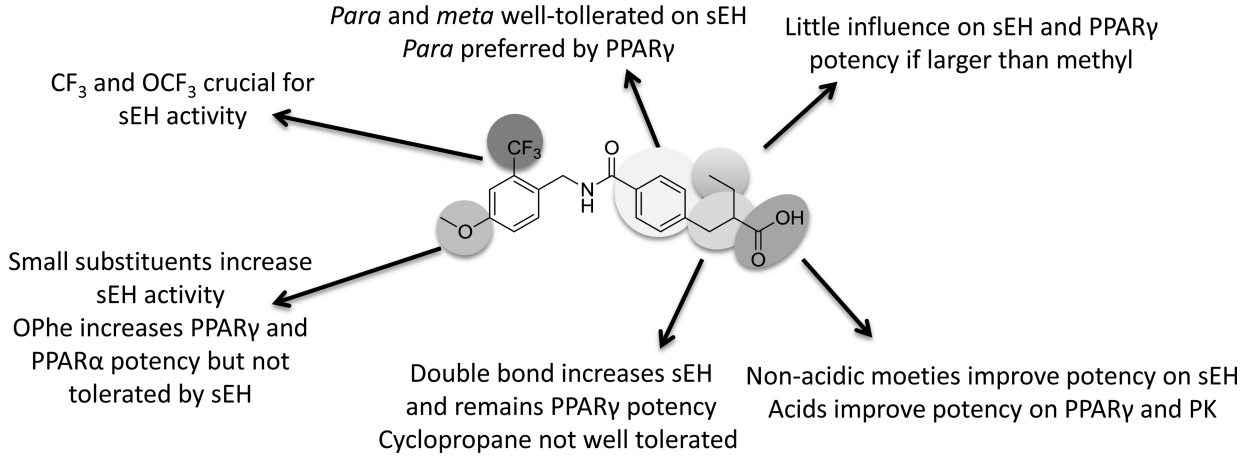


Figure 5. Summary of structure−activity relationship studies of *N*-benzylbenzamides.

profiling. Driven by structural similarities, the impact of compounds **1c** and **14c** on free fatty acid receptor 1 (FFA1, formerly GPR40) activation was determined. FFA1 or GPR40 is a receptor relevant for pancreatic β-cell insulin secretion. The partial agonistic effect, shown in Supporting Information (Figures S7 and S8), is assumed to be secondary in the pathogenic interference. Compound **14c** did not impair cell viability of HepG2 cells up to a concentration of 30 μM, indicated by the WST-1 assay. After a 1 h incubation of **14c** with Spargue-Dawley rat liver microsomes ~96% of the compound remained intact (Supporting Information, Figure S6b). A PK study in mice with oral application of 30 mg/kg bw of **14c** to nine (RijOrl, SWISS/CD-1) mice (gavage) was performed. **14c** exhibited a superior pharmacokinetic profile compared to **1c** (C_{max} = 29145 ng/mL, $AUC_{0\rightarrow\infty}$ = 94 412 ng·h/mL, Cl/f = 0.3 L h^{-1} kg^{-1}, V_z/f = 2 L/kg).

In a 2-week in vivo pharmacokinetic study in six mice, with drinking water application of **14c** (30 mg/kg bw), a final plasma concentration of 986 ± 363 ng/mL (~3 ± 1.1 μM) was achieved (Figure 4b). Quantitative PCR analysis of the mouse livers after 2 weeks of treatment showed an upregulation of PPARα and PPARγ as well as the PPARγ target genes sEH, CD36, PGC-1α, and LXRα. Interestingly, the LXRα regulated target genes apolipoprotein E (ApoE) and the cholesterol transporter ABCA1 were not upregulated (Figure 4c). sEH levels were also slightly increased as expected for a PPARγ agonist. As the plasma concentration of **14c** was 1 order of magnitude higher than the in vitro sEH IC_{50} and PPARγ EC_{50} values and in vivo efficacy could be demonstrated, the compound **14c** qualifies as a pharmacological tool for diabetic animal models.

CONCLUSION

This study was able to create a series of well characterized sEH/PPARγ dual modulators. Along a hit (**1c**) to lead (**14c**) compound development, potency and PK/PD parameters were improved. Information about drug−target interaction properties for sEH and PPARγ has also been generated. A clear trend of improved sEH inhibition by nonacidic derivatives can be recognized. This phenomenon fits the common knowledge about the character of the sEH binding pocket. The preferred placement of an acidic headgroup at the para position of a lipophilic linear shaped molecule (**24**) compared to a side-facing acidic headgroup (**25**) is consistent with previous studies.[42] The importance of an ortho-CF_3 group at the benzyl moiety, for sEH inhibition and metabolic stability, at this particular type of scaffold has also been explored by Thalji et al.[23] In the case of PPARγ activity values, no clear preference of carboxylic acid derivatives can be recognized due to the hydrolytic activity of COS7 cells. Comparing this work with the research done on *N*-benzylbenzamides as PPAR agonists, a difference in the activity profiles can be recognized. However, the introduction of sterically demanding moieties in the para position of the benzyl ring caused a shift in the activity profile analogous to KCL. It was shown that the space for structural variations to fulfill the desired aims of this project is rather tight. Figure 5 summarizes the SAR of *N*-benzylbenzamides as dual sEH/PPARγ modulators.

Compound **14c** is a pharmacological tool with interesting features for the investigation as experimental agent to treat the metabolic syndrome. There are high expectations on the sEH/PPARγ combinational therapy of diabetes mellitus type 2. The advantages of multitarget therapeutics have been broadly discussed by Peters.[43] Imig et al. have already shown that the combined application of rosiglitazone (PPARγ agonist) and t-AUCB (sEH inhibitor) produces a positive synergistic effect on kidney injury in spontaneous hypertensive obese (SHROB) rats.[20] In this context, compound **14c** could be valuable for preclinical investigation of the simultaneous modulation of the sEH/PPARγ axis in MetS.

One of the shortcomings of certain single PPARγ agonists, especially TZDs, is the frequently observed sodium and water retention. This effect can be dangerous to patients with congestive heart failure.[44] sEH and EETs are natriuretic and auxiliary to maintain fluid and electrolyte homeostasis. Although safe approved diuretics are available, dual sEH/PPARγ modulators could solve this problem without the neccesity of additional therapeutic agents. Inferentially, the combination of a PPARγ agonist with a sEH inhibitor might overcome existing side effects by keeping the beneficial features as well as extending them with new ones.

EXPERIMENTAL SECTION

Chemistry. General. All educts, reagents, and solvents were purchases from the companies Alfa-Aesar GmbH & Co KG (Karlsruhe, Germany), Sigma-Aldrich Chemie GmbH (Hannover, Germany), Apollo Scientific Ltd. (Manchester, England), JRD Fluorochemicals, Ltd.. (Surrey, England) and used without further purification. The companies guaranteed purity above 97%. TLC was performed by silica coated aluminum foil (particle size 60 μm) purchased from Merck KGaA (Darmstadt, Germany). For purification of synthesized compounds an Intelli Flash 310 chromatograph by the firm Varian Medical Systems Deutschland GmbH (Darmstadt, Germany) was used. Two kinds of packed columns have been used: SF25-80g and SF25-60g, both loaded with silica gel (particle size 50 μm) and also purchased

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81

from firm Varian Medical Systems Deutschland GmbH (Darmstadt, Germany). ^{1}H (250/400 MHz) and ^{13}C (64 MHz) were measured on DPX250 and AV400 nuclear magnetic resonance spectrometer from Bruker (Karlsruhe, Germany). All spectra were analyzed with the TopSpin software from Bruker (Karlsruhe, Germany). Tetramethylsilane was used as internal standard. DMSO-d_6 and methanol-d_4 were used as solvents. HPLC and mass analyses were performed by a LCMS 2020 from Shimadzu (Duisburg, Germany), under the use of a MultoHigh 100 RP 18, 3 μm, 100 mm × 2 mm column from CS Chromatography-Service GmbH (Langerwehe, Germany). Eluation was maintained by an acetonitrile/water gradient from 20% to 75%. The electron spray ionization produced positive (+) as well as negative (−) spectra, and the UV chromatogram measured two wavelengths (λ = 254 and 280 nm). High-resolution mass spectrometry was performed by a Thermo Scientific MALDI LTQ ORBITRAP XL instrument. All final compounds had a purity of ≥95% as determined by HPLC.

General Procedure for the Preparation of the Compounds 1−17, Using the Example of 4-Formyl-*N*-(2-(trifluoromethyl)-benzyl)benzamide (1). 1 g (6.7 mmol) of 4-formylbenzoic acid, 0.9 mL (6.7 mmol) of triethylamine, and 1 mL (7.3 mmol) of isobutyl chloroformate were dissolved in 30 mL of chloroform at 0 °C under an argon atmosphere. After 1 h 0.9 mL (6.7 mmol) of 2-(trifluoromethyl)benzylamine was added. The solution was allowed to warm to room temperature and stirred for 12 h. The reaction mixture was washed three times with each 20 mL of 2 M HCl solution, 20 mL of 1 M NaOH solution and one time with 20 mL of brine. The organic layer was dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude product was recrystallized from an ethyl acetate/hexane (EE/Hex) mixture. A white solid remained. Yield: 1.43 g (70%). ^{1}H NMR (DMSO-d_6, δ): 10.17 (s, 1H, Ph_1-CHO), 9.38 (t, J = 5.9 Hz, 1H, Ph_1-OCNH), 9.08−8.19 (m, 4H, CHO-Ph_1), 7.83−7.52 (m, 4H, OCNH-CH_2-Ph_2), 4.75 (d, J = 5.6 Hz, 2H, Ph_1-OCNH-CH_2). MS-ESI: m/z 342 [M + Cl^-].

General Procedure for the Preparation of the Compounds 1a−19a, Using the Example of Ethyl (*E*)-4-[*N*-((2-(Trifluoromethyl)-benzyl)benzamide]-α-ethylcinnamate (1a). To a solution of 156 mg (6.5 mmol) of NaH in 5 mL of dry THF under an argon atmosphere at 0 °C was added slowly 1.2 mL (4.9 mmol) of triethyl 2-phosphonobutyrate. After 30 min a solution of 1 g (3.3 mmol) of 4-formyl-*N*-(2-(trifluoromethyl)benzyl)benzamide (**1**) in 10 mL of dry THF was added to the reaction mixture and stirred for 2 h. To quench the reaction, an amount of 25 mL of water was used. The resulting mixture was diluted with 10 mL of EE. The organic layer was washed three times with brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. After recrystallization from EE/Hex a white solid remained. Yield: 0.92 g (70%). ^{1}H NMR (DMSO-d_6, δ): 9.25 (t, J = 5.8 Hz, 1H, Ph_2-OCNH), 8.09−7.38 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 7.67 (s, 1H, OCNH-Ph_1-CH), 4.74 (d, J = 5.4 Hz, 2H, Ph_1-OCNH-CH_2), 4.30 (q, J = 7.1 Hz, 2H, C-COO-CH_2), 2.40 (q, J = 6.9 Hz, 2H, CH-C-CH_2), 1.36 (t, J = 7.06 Hz, 3H, COO-CH_2-CH_3), 1.18 (t, J = 7.38 Hz, 3 H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 169.4, 167.3, 136.9, 136.5, 136.3, 135.8, 135.7, 131.5, 129.4, 128.8, 128.4, 127.3, 126.3, 125.1, 125, 124.9, 124.1, 60.5, 40.2, 23.7, 13.3, 10. HRMS: measured m/z [M + H]$^+$ 405.1550 (theoretical, 405.1551).

General Procedure for the Preparation of the Compounds 1b−19b, Using the Example of Ethyl 2-Ethyl 3-[4-(*N*-((2-trifluoromethyl)benzyl)benzamide)]propanoate (1b). 250 mg (0.617 mmol) of **1a** and 9.8 mg (0.1 mmol) of palladium on carbon were suspended in dry ethanol and stirred under hydrogen atmosphere for 12 h. Reaction mixture was filtered over Celite and the solvent removed under reduced pressure. Without further purification clear resinous oil occurred. Yield: 0.2 g (90%). ^{1}H NMR (DMSO-d_6, δ): 9.10 (t, J = 5.8 Hz, 1H, Ph_2-OCNH), 7.91−7.34 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.71 (d, J = 5.5 Hz, 2H, Ph_1-OCNH-CH_2), 4.11−4.01 (m, 2H, CH-COO-CH_2), 2.97−2.83 (m, 2H, Ph_1-CH_2), 2.73−2.64 (m, 1H, Ph_1-CH_2-CH), 1.65−1.56 (m, 2H, Ph_1-CH_2-CH-CH_2), 1.14 (t, J = 6.6 Hz, 3H, COO-CH_2-CH_3), 0.93 (t, J = 7.4, 3H, CH-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 175.5, 168.8, 143.8, 137, 132.6, 132.04, 131.9, 128.2, 127.1, 127, 125.7, 125.6, 125.5, 125.4, 124.9, 60, 49, 39.8, 37.7, 25.2, 13.2, 10.5. HRMS: measured m/z [M + H]$^+$ 408.1781 (theoretical, 408.1781).

2-Ethyl-3-[4-(*N*-((2-trifluoromethyl)benzyl)benzamide)]-propionic Acid (1c). Yield: 0.06 g (60%). ^{1}H NMR (DMSO-d_6, δ): 12.2 (s, COOH), 9.10 (t, J = 5.9 Hz, 1H, Ph_2-OCNH), 7.93−7.36 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.72 (d, 2H, J = 5.6 Hz, Ph_1-OCNH-CH_2), 2.98−2.78 (m, 2H, CH_2-CH-CH_2), 2.59−2.50 (m, 1H, Ph_1-CH_2-CH), 1.64−1.53 (m, Ph_1-CH_2), 0.94 (t, J = 7.4 Hz, CH-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 177.6, 168.9, 144.1, 137.1, 132.1, 131.9, 128.8, 128.1, 127.0, 127.4, 125.7, 125.6, 125.5, 125.4, 122.8, 49.0, 39.8, 37.6, 24.9, 10.5. HRMS: m/z 380.1469 (theoretical, 380.1468).

General Procedure for the Preparation of the Compounds 1d−19d and 1c−19c, Using the Example of (*E*)-4-[*N*-((2-Trifluoromethyl)benzyl)benzamide]-α-ethylcinnamic Acid (1d). 100 mg (0.2 mmol) of ethyl (*E*)-4-[*N*-((2-trifluoromethyl)-benzyl)benzamide]-α-ethylcinnamate (**1a**) and 69 mg KOH (1.2 mmol) were dissolved in 2 mL of a mixture THF/H_2O/MeOH in the ratio 1:2:1 and stirred in a microwave for 30 min at 70 °C and 35 W. The organic layer was removed under reduced pressure. The aqueous layer was diluted with 1 mL of H_2O, acidified with 12 M HCl solution, and stored at 4 °C. The pure product precipitated and no further purification was needed. Yield: 0.06 g (60%). ^{1}H NMR (DMSO-d_6, δ): 12.71 (s, 1H, COOH), 9.24 (t, J = 5.9 Hz, 1H, Ph_2-OCNH), 8.06−7.47 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 4.74 (d, J = 5.2 Hz, 2H, Ph_1-OCNH-CH_2), 2.51 (q, J = 8 Hz, 2H, CH-C-CH_2), 1.17 (t, J = 7.5 Hz, 3H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 169.8, 168.4, 139.3, 137, 136.7, 133.5, 132.1, 128.9, 128.3, 127.9, 127.7, 127.3, 127.1, 127, 126.9, 125.7, 125.6, 40.0, 20.4, 12.8. HRMS: measured m/z [M + H]$^+$ 378.1312 (theoretical, 378.1313).

4-Formyl-*N*-(benzyl)benzamide (2). Yield: 0.99 g (68%). ^{1}H NMR (DMSO-d_6, δ): 10.1 (s, 1H, Ph_1-CHO), 9.27 (t, J = 5.9 Hz, 1H, Ph_1-OCNH), 8.11−7.99 (m, 4H, CHO-Ph_1), 7.37−7.23 (m, 4H, OCNH-CH_2-Ph_2), 4.52 (d, J = 5.8 Hz, 2H, Ph_1-OCNH-CH_2). MS-ESI: m/z 240 [M + H^+].

Ethyl (*E*)-4-[*N*-Benzylbenzamide]-α-ethylcinnamate (2a). Yield: 0.92 g (65%). ^{1}H NMR (DMSO-d_6, δ): 9.12 (t, J = 6.1 Hz, 1H, Ph_2-OCNH), 7.98−7.21 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 7.33 (s, 1H, OCNH-Ph_1-CH), 4.50 (d, J = 5.7 Hz, 2H, Ph_1-OCNH-CH_2), 4.23 (q, J = 7 Hz, 2H, C-COO-CH_2), 2.41 (q, J = 7 Hz, 2H, CH-C-CH_2), 1.30 (t, J = 7.8 Hz, 3H, COO-CH_2-CH_3), 1.12 (t, J = 7.3 Hz, 3 H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 167.5, 165.7, 137.9, 137.5, 137.1, 136.1, 135.9, 131.5, 129, 128.3, 127.5, 127.4, 126.7, 125.6, 125.5, 124.4, 61.7, 39.8, 24.9, 14.2, 10.5. HRMS: measured m/z [M + H]$^+$ 338.1752 (theoretical, 338.1750).

Ethyl 2-Ethyl-3-[4-(*N*-benzylbenzamide)]propanoate (2b). Yield: 0.21 g (84%). ^{1}H NMR (methanol-d_4, δ): 7.69−7.11 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.46 (s, 2H, Ph_1-OCNH-CH_2), 3.98−3.88 (m, 2H, CH-COO-CH_2), 2.86−2.72 (m, 2H, Ph_1-CH_2), 2.58−2.48 (m, 1H, Ph_1-CH_2-CH), 1.62−1.47 (m, 2H, Ph_1-CH_2-CH-CH_2), 1.02 (t, J = 6.3 Hz, 3H, COO-CH_2-CH_3), 0.83 (t, J = 8, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 175.5, 168.6, 143.6, 138.8, 132.4, 128.8, 128.1, 127.1, 127, 126.8, 125.6, 125.5, 125.4, 125.3, 60, 49.1, 43, 37.7, 25, 13.3, 10.7. HRMS: measured m/z [M + H]$^+$ 340.191 (theoretical, 340.1907).

2-Ethyl-3-[4-(*N*-benzylbenzamide)]propionic Acid (2c). Yield: 0.05 g (55%). ^{1}H NMR (methanol-d_4, δ): 7.83−6.92 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.46 (s, 2H, Ph_1-OCNH-CH_2), 2.89−2.67 (m, 2H, Ph_1-CH_2), 2.51−2.42 (m, 1H, Ph_1-CH_2-CH), 1.62−1.39 (m, 2H, Ph_1-CH_2-CH-CH_2), 0.85 (t, J = 7.6, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 178.2, 168.7, 144.1, 138.9, 132.1, 128.8, 128.8, 128.1, 128.1, 127.1, 127.1, 127.0, 126.7, 49.5, 43.1, 37.8, 25.2, 10.7. HRMS: measured m/z [M + H]$^+$ 312.1601 (theoretical, 312.1594).

(*E*)-4-[*N*-Benzylbenzamide]-α-ethylcinnamic Acid (2d). Yield: 0.06 g (65%). ^{1}H NMR (DMSO-d_6, δ): 12.61 (s, 1H, COOH), 9.11 (t, J = 5.9 Hz, 1H, Ph_2-OCNH), 7.99−7.22 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 7.33 (s, 1H, Ph_1-CH), 4.50 (d, J = 6.1 Hz, 2H, Ph_1-OCNH-CH_2), 2.47 (q, J = 7.7 Hz, 2H, CH-C-CH_2), 1.11 (t, J = 7.2 Hz, 3H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 168.9, 165.7, 139.7, 138, 136.4, 136.3, 128.9, 128.3, 127.5, 127.2, 126.7, 125.2, 125.1,

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81

124.0, 123.5, 123.0, 42.7, 20.3, 13.3. HRMS: measured m/z $[M + H]^+$ 310.1438 (theoretical, 310.1438).

4-Formyl-*N*-(2-(methyl)benzyl)benzamide (3). Yield: 1 g (65%). ^{1}H NMR (DMSO-d_6, δ): 10.10 (s, 1H, Ph_1-CHO), 9.14 (t, J = 5.4 Hz, 1H, Ph_1-OCNH), 8.13−7.99 (m, 4H, CHO-Ph_1), 7.31−7.14 (m, 4H, OCNH-CH_2-Ph_2), 4.49 (d, J = 5.6 Hz, 2H, Ph_1-OCNH-CH_2), 2.34 (s, 3H, Ph_2-CH_3). MS-ESI: m/z 254 $[M + H^+]$.

Ethyl (*E*)-4-[*N*-((2-Methyl)benzyl)benzamide]-α-ethylcinnamate (3a). Yield: 0.9 g (66%). ^{1}H NMR (DMSO-d_6, δ): 9.10 (t, J = 6 Hz, 1H, Ph_2-OCNH), 7.70−7.21 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 7.20 (s, 1H, OCNH-Ph_1-CH), 4.30 (d, J = 5.7 Hz, 2H, Ph_1-OCNH-CH_2), 4.18 (q, J = 6.9 Hz, 2H, C-COO-CH_2), 2.39 (q, J = 6.8 Hz, 2H, CH-C-CH_2), 2.41 (s, 3H, Ph_2-CH_3), 1.25 (t, J = 7.7 Hz, 3H, COO-CH_2-CH_3), 1.10 (t, J = 7.2 Hz, 3 H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 167.4, 165.3, 136.8, 136.3, 136.1, 136, 135.9, 132.5, 129, 128.4, 128.5, 127.4, 126.5, 125.8, 125.3, 124.1, 60.7, 40.1, 23.9, 18.7, 13.2, 10.1. HRMS: measured m/z $[M + H]^+$ 352.1907 (theoretical, 352.1907).

Ethyl 2-Ethyl-3-[4-(*N*-((2-methyl)benzyl)benzamide)]-propanoate (3b). Yield: 0.19 g (75%). ^{1}H NMR (DMSO-d_6, δ): 8.85 (t, J = 5.7 Hz, 1H, Ph_2-OCNH), 7.85−7.15 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.46 (d, J = 6.2, 2H, Ph_1-OCNH-CH_2), 4.16−3.96 (m, 2H, CH-COO-CH_2), 2.92−2.77 (m, 2H, Ph_1-CH_2), 2.68−2.59 (m, 1H, Ph_1-CH_2-CH), 2.34 (s, 3H, Ph_2-CH_3), 1.61−1.5 (m, 2H, Ph_1-CH_2-CH-CH_2), 1.09 (t, J = 7.5 Hz, 3H, COO-CH_2-CH_3), 0.88 (t, J = 8.1, 3H, CH-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 167.8, 165.4, 137.8, 136.2, 136, 135.8, 129.0, 128.2, 128.1, 127.4, 125.9, 125.8, 125.3, 124.2, 124, 60.3, 40.1, 38.3, 23.9, 18.9, 13.2, 12.1. HRMS: measured m/z $[M + H]^+$ 354.2065 (theoretical, 354.2064).

2-Ethyl-3-[4-(*N*-((2-methyl)benzyl)benzamide)]propionic Acid (3c). Yield: 0.02 g (20%). ^{1}H NMR (methanol-d_4, δ): 8.01−7.03 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.47 (s, 2H, Ph_1-OCNH-CH_2), 2.91−2.69 (m, 2H, Ph_1-CH_2), 2.55−2.45 (m, 1H, Ph_1-CH_2-CH), 2.26 (s, 3H, Ph_2-CH_3), 1.63−1.42 (m, 2H, Ph_1-CH_2-CH-CH_2), 0.86 (t, J = 7.4, 3H, CH-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 176.1, 167.3, 166.1, 145.1, 143.2, 137.3, 135.6, 132.3, 129.8, 129.3, 129, 128.7, 127.3, 126.6, 125.6, 48.1, 37.2, 24.7, 18.5, 11.4. HRMS: measured m/z $[M + H]^+$ 326.1752 (theoretical, 326.1751).

(*E*)-4-[*N*-((2-Methyl)benzyl)benzamide]-α-ethylcinnamic Acid (3d). Yield: 0.02 g (19%). ^{1}H NMR (DMSO-d_6, δ): 12.62 (s, 1H, COOH), 8.97 (t, J = 5.8 Hz, 1H, Ph_2-OCNH), 7.98−7.13 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 7.17 (s, 1H, Ph_1-CH), 4.48 (d, J = 5.7 Hz, 2H, Ph_1-OCNH-CH_2), 2.48 (q, J = 8.5 Hz, 2H, CH-C-CH_2), 2.34 (s, 3H, Ph_2-CH_3), 1.12 (t, J = 7.1 Hz, 3H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 169.8, 168.1, 139.1, 137.0, 136.6, 136, 135.9, 133.9, 129.9, 128.9, 128.6, 127.9, 127.4, 127.2, 126.9, 126.9, 41.4, 20.3, 18.7, 12.8. HRMS: measured m/z $[M + H]^+$ 324.1595 (theoretical, 324.1594).

4-Formyl-*N*-(2-(chloro)benzyl)benzamide (4). Yield: 1.16 g (70%). ^{1}H NMR (DMSO-d_6, δ): 10.14 (s, 1H, Ph_1-CHO), 9.28 (t, J = 6 Hz, 1H, Ph_1-OCNH), 8.13−8.02 (m, 4H, CHO-Ph_1), 7.50−7.28 (m, 4H, OCNH-CH_2-Ph_2), 4.58 (d, J = 5.8 Hz, 2H, Ph_1-OCNH-CH_2). MS-ESI: m/z 274 $[M + H^+]$.

Ethyl (*E*)-4-[*N*-((2-Chloro)benzyl)benzamide]-α-ethylcinnamate (4a). Yield: 0.87 g (64%). ^{1}H NMR (DMSO-d_6, δ): 9.15 (t, J = 5.8 Hz, 1H, Ph_2-OCNH), 8.02−7.27 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 7.40 (s, 1H, OCNH-Ph_1-CH), 4.57 (d, J = 5.7 Hz, 2H, Ph_1-OCNH-CH_2), 4.24 (q, J = 7.2 Hz, 2H, C-COO-CH_2), 2.38 (q, J = 6.9 Hz, 2H, CH-C-CH_2), 1.30 (t, J = 7.5 Hz, 3H, COO-CH_2-CH_3), 1.11 (t, J = 6.7 Hz, 3 H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 167.3, 165.1, 138.1, 137.4, 137.2, 135.8, 135.7, 132.2, 129.3, 128.1, 127.4, 127.3, 126.5, 125.6, 125.2, 123.9, 60.5, 39.6, 24.7, 14.1, 10. HRMS: measured m/z $[M + H]^+$ 372.1363 (theoretical, 372.1361).

Ethyl 2-Ethyl-3-[4-(*N*-((2-chloro)benzyl)benzamide)]-propanoate (4b). Yield: 0.2 g (90%). ^{1}H NMR (methanol-d_4, δ): 7.83−7.26 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.61 (s, 2H, Ph_1-OCNH-CH_2), 4.11−4.00 (m, 2H, CH-COO-CH_2), 3.00−2.84 (m, 2H, Ph_1-CH_2), 2.71−2.62 (m, 1H, Ph_1-CH_2-CH), 1.74−1.59 (m, 2H, Ph_1-CH_2-CH-CH_2), 1.15 (t, J = 6.8 Hz, 3H, COO-CH_2-CH_3), 0.96 (t, J = 8.7, 3H, CH-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 167.8, 166.2, 137.4, 136.4, 135.9, 135.8, 129.2, 128.5, 128.3, 127.6, 126.9, 126.8, 125.4, 124.5, 124, 58.3, 40, 38.6, 23.9, 18.5, 13.1, 11.7 . HRMS: measured m/z $[M + H]^+$ 375.1423 (theoretical, 375.1422).

2-Ethyl-3-[4-(*N*-((2-chloro)benzyl)benzamide)]propionic Acid (4c). Yield: 0.05 g (50%). ^{1}H NMR (methanol-d_4, δ): 7.68−7.1 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.46 (s, 2H, Ph_1-OCNH-CH_2), 2.89−2.69 (m, 2H, Ph_1-CH_2), 2.54−2.44 (m, 1H, Ph_1-CH_2-CH), 1.63−1.41 (m, 2H, Ph_1-CH_2-CH-CH_2), 0.86 (t, J = 7.6, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 177.6, 168.7, 143.8, 138.9, 132.2, 128.8, 128.8, 128.1, 128.1, 127.1, 127.0, 126.9, 126.8, 126.5, 49, 43.1, 37.5, 25.0, 10.6. HRMS: measured m/z $[M + H]^+$ 346.1206 (theoretical, 346.1205).

(*E*)-4-[*N*-((2-Chloro)benzyl)benzamide]-α-ethylcinnamic Acid (4d). Yield: 0.06 g (62%). ^{1}H NMR (DMSO-d_6, δ): 12.69 (s, 1H, COOH), 9.17 (t, J = 6 Hz, 1H, Ph_2-OCNH), 8.05−7.35 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 4.62 (d, J = 5.8 Hz, 2H, Ph_1-OCNH-CH_2), 2.50 (q, J = 6.4 Hz, 2H, CH-C-CH_2), 1.17 (t, J = 7.2 Hz, 3H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 169.8, 168.3, 139.2, 137, 136.6, 135.7, 133.7, 132.9, 129.1, 128.9, 128.6, 128.4, 127.9, 127.3, 126.9, 126.4, 41.2, 20.3, 12.7. HRMS: measured m/z $[M + H]^+$ 344.1052 (theoretical, 344.1048).

4-Formyl-*N*-(2-(bromo)benzyl)benzamide (5). Yield: 1.33 g (69%). ^{1}H NMR (DMSO-d_6, δ): 10.11 (s, 1H, Ph_1-CHO), 9.29 (t, J = 5.6 Hz, 1H, Ph_1-OCNH), 8.14−8.01 (m, 4H, CHO-Ph_1), 7.67−7.02 (m, 4H, OCNH-CH_2-Ph_2), 4.55 (d, J = 5.8 Hz, 2H, Ph_1-OCNH-CH_2). MS-ESI: m/z 319 $[M + H^+]$.

Ethyl (*E*)-4-[*N*-((2-Bromo)benzyl)benzamide]-α-ethylcinnamate (5a). Yield: 0.85 g (65%). ^{1}H NMR (DMSO-d_6, δ): 9.19 (t, J = 5.8 Hz, 1H, Ph_2-OCNH), 8.06−7.23 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 7.20 (s, 1H, OCNH-Ph_1-CH), 4.58 (d, J = 5.6 Hz, 2H, Ph_1-OCNH-CH_2), 4.29 (q, J = 7.3 Hz, 2H, C-COO-CH_2), 2.37 (q, J = 7 Hz, 2H, CH-C-CH_2), 1.35 (t, J = 6.8 Hz, 3H, COO-CH_2-CH_3), 1.17 (t, J = 5.4 Hz, 3 H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 168.3, 166.1, 137.8, 137.4, 137.1, 135.7, 135.5, 132.1, 129.5, 128.2, 127.5, 127.2, 126.3, 125.8, 125.3, 122.9, 61.5, 40.6, 25.7, 14.4, 10.5. HRMS: measured m/z $[M + H]^+$ 416.0855 (theoretical, 416.0856).

Ethyl 2-Ethyl-3-[4-(*N*-((2-bromo)benzyl)benzamide)]-propanoate (5b). Yield: 0.18 g (70%). ^{1}H NMR (methanol-d_4, δ): 7.81−7.25 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.59 (s, 2H, Ph_1-OCNH-CH_2), 4.09−4.00 (m, 2H, CH-COO-CH_2), 2.90−2.85 (m, 2H, Ph_1-CH_2), 2.69−2.61 (m, 1H, Ph_1-CH_2-CH), 1.72−1.58 (m, 2H, Ph_1-CH_2-CH-CH_2), 1.14 (t, J = 7.9 Hz, 3H, COO-CH_2-CH_3), 0.95 (t, J = 8.1, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 176.0, 167.5, 144.9, 130.2, 129.5, 128.5, 128.4, 128.2, 127.2, 127.0, 126, 125.7, 125.6, 125.5, 61.4, 50.4, 44.4, 39.1, 26.6, 14.5, 11.9. HRMS: measured m/z $[M + H]^+$ 418.1013 (theoretical, 418.1012).

2-Ethyl-3-[4-(*N*-((2-bromo)benzyl)benzamide)]propionic Acid (5c). Yield: 0.05 g (51%). ^{1}H NMR (DMSO-d_6, δ): 12.12 (s, 1H, COOH), 8.96 (t, J = 6.1 Hz, 1H, Ph_2-OCNH), 7.81−7.19 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.46 (d, J = 6.1, 2H, Ph_1-OCNH-CH_2), 2.90−2.71 (m, 2H, Ph_1-CH_2), 2.60−2.50 (m, 1H, Ph_1-CH_2-CH), 1.60−1.41 (m, 2H, Ph_1-CH_2-CH-CH_2), 0.87 (t, J = 7.6, 3H, CH-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 176.4, 166.5, 143.7, 140.2, 132.7, 129.2, 128.7, 128.1, 128.1, 127.7, 127.6, 126.9, 126.8, 126.5, 48.5, 40.5, 39.7, 25.1, 11.9. HRMS: measured m/z $[M + H]^+$ 390.07 (theoretical, 390.0699).

(*E*)-4-[*N*-((2-Bromo)benzyl)benzamide]-α-ethylcinnamic Acid (5d). Yield: 0.06 g (61%). ^{1}H NMR (DMSO-d_6, δ): 12.68 (s, 1H, COOH), 9.18 (t, J = 5.7 Hz, 1H, Ph_2-OCNH), 8.08−7.26 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 4.58 (d, J = 5.4 Hz, 2H, Ph_1-OCNH-CH_2), 2.50 (q, J = 8.2 Hz, 2H, CH-C-CH_2), 1.18 (t, J = 7 Hz, 3H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 169.8, 168.3, 139.2, 137.2, 137, 136.6, 133.7, 132.4, 128.9, 128.6, 127.9, 127.4, 127.3, 126.9, 125.6, 122.7, 43.7, 20.4, 12.8. HRMS: measured m/z $[M + H]^+$ 388.0544 (theoretical, 388.0543).

4-Formyl-*N*-(2-(trifluoromethoxy)benzyl)benzamide (6). Yield: 1.18 g (70%). ^{1}H NMR (DMSO-d_6, δ): 10.11 (s, 1H, Ph_1-CHO), 9.27 (t, J = 5.7 Hz, 1H, Ph_1-OCNH), 8.11−8.02 (m, 4H, CHO-Ph_1), 7.51−7.37 (m, 4H, OCNH-CH_2-Ph_2), 4.59 (d, J = 5.7 Hz, 2H, Ph_1-OCNH-CH_2). MS-ESI: m/z 324 $[M + H^+]$.

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81

Ethyl (*E*)-4-[*N*-((2-Trifluoromethoxy)benzyl)benzamide]-α-ethylcinnamate (6a). Yield: 0.86 g (66%). ^{1}H NMR (DMSO-d_6, δ): 9.10 (t, J = 5.8 Hz, 1H, Ph_2-OCNH), 7.96−7.33 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 7.40 (s, 1H, OCNH-Ph_1-CH), 4.69 (d, J = 5.5 Hz, 2H, Ph_1-OCNH-CH_2), 4.30 (q, J = 7.2 Hz, 2H, C-COO-CH_2), 2.57 (q, J = 7.5 Hz, 2H, CH-C-CH_2), 1.37 (t, J = 7 Hz, 3H, COO-CH_2-CH_3), 1.18 (t, J = 7.6 Hz, 3 H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 173.3, 168.5, 167.1, 138.8, 138.4, 137.1, 136.7, 135.4, 132.2, 129.2, 128.4, 127.4, 127.3, 125.3, 125.1, 124.1, 123.1, 60.5, 42.6, 24.7, 14.7, 10.1. HRMS: measured m/z [M + H]$^+$ 421.1501 (theoretical, 421.1503).

Ethyl 2-Ethyl-3-[4-(*N*-((2-trifluoromethoxy)benzyl)-benzamide)]propanoate (6b). Yield: 0.2 g (90%). ^{1}H NMR (methanol-d_4, δ): 7.69−7.17 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.55 (s, 2H, Ph_1-OCNH-CH_2), 3.97−3.89 (m, 2H, CH-COO-CH_2), 2.86−2.73 (m, 2H, Ph_1-CH_2), 2.58−2.51 (m, 1H, Ph_1-CH_2-CH), 1.61−1.47 (m, 2H, Ph_1-CH_2-CH-CH_2), 1.02 (t, J = 7 Hz, 3H, COO-CH_2-CH_3), 0.84 (t, J = 7.6, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 177.5, 175.0, 166.5, 145.1, 133.5, 132.6, 130.4, 130.3, 129.9, 128.6, 128.4, 127, 125.8, 125.6, 61.4, 50.5, 44.4, 39.1, 26.5, 14.7, 12.0. HRMS: measured m/z [M + H]$^+$ 423.1667 (theoretical, 423.1665).

2-Ethyl-3-[4-(*N*-((2-trifluoromethoxy)benzyl)benzamide)]-propionic Acid (6c). Yield: 0.07 g (66%). ^{1}H NMR (DMSO-d_6, δ): 12.18 (s, 1H, COOH), 9.03 (t, J = 6 Hz, 1H, Ph_2-OCNH), 7.88−7.26 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.53 (d, J = 6, 2H, Ph_1-OCNH-CH_2), 2.96−2.76 (m, 2H, Ph_1-CH_2), 2.63−2.59 (m, 1H, Ph_1-CH_2-CH), 1.64−1.50 (m, 2H, Ph_1-CH_2-CH-CH_2), 0.90 (t, J = 7.6, 3H, CH-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 177.5, 176.4, 166.5, 143.7, 140.2, 133.1, 132.7, 129.2, 129.0, 128.7, 127.7, 127.6, 127.1, 126.8, 126.5, 48.5, 40.3, 39.1, 26.5, 11.9. HRMS: measured m/z [M + H]$^+$ 396.1417 (theoretical, 396.1417).

(*E*)-4-[*N*-((2-Trifluoromethoxy)benzyl)benzamide]-α-ethylcinnamic Acid (6d). Yield: 0.06 g (65%). ^{1}H NMR (DMSO-d_6, δ): 12.71 (s, 1H, COOH), 9.15 (t, J = 6.7 Hz, 1H, Ph_2-OCNH), 8.03−7.4 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 4.62 (d, J = 5.9 Hz, 2H, Ph_1-OCNH-CH_2), 2.51 (q, J = 6.9 Hz, 2H, CH-C-CH_2), 1.16 (t, J = 7.9 Hz, 3H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 171.3, 169.2, 166.5, 166.4, 149.7, 146.6, 138.8, 136.9, 134.1, 133.1, 132.2, 129.7, 129.5, 129.1, 128.2, 128, 128, 37.7, 20.8, 14.1. HRMS: measured m/z [M + H]$^+$ 394.1261 (theoretical, 394.1261).

4-Formyl-*N*-(4-fluorobenzyl)benzamide (7). Yield: 0.97 g (70%). ^{1}H NMR (DMSO-d_6, δ): 10.15 (s, 1H, Ph_1-CHO), 9.33 (t, J = 5.7 Hz, 1H, Ph_1-OCNH), 8.16−8.04 (m, 4H, CHO-Ph_1), 7.47−7.18 (m, 4H, OCNH-CH_2-Ph_2), 4.54 (d, J = 5.9 Hz, 2H, Ph_1-OCNH-CH_2). MS-ESI: m/z 258 [M + H$^+$].

Ethyl (*E*)-4-[*N*-((4-fluoro)benzyl)benzamide]-α-ethylcinnamate (7a). Yield: 0.93 g (67%). ^{1}H NMR (DMSO-d_6, δ): 9.18 (t, J = 6 Hz, 1H, Ph_2-OCNH), 8.02−7.18 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 7.66 (s, 1H, OCNH-Ph_1-CH), 4.53 (d, J = 6.1 Hz, 2H, Ph_1-OCNH-CH_2), 4.28 (q, J = 7 Hz, 2H, C-COO-CH_2), 2.54 (q, J = 7.9 Hz, 2H, CH-C-CH_2), 1.34 (t, J = 7.11 Hz, 3H, COO-CH_2-CH_3), 1.18 (t, J = 7.6 Hz, 3 H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 167.5, 167.2, 161.8, 139.7, 138.3, 138.1, 133.4, 131.2, 128.2, 128.1, 127.2, 127, 126.1, 125.1, 124.5, 118.9, 61.5, 42.6, 16.7, 14.6, 14.1. HRMS: measured m/z [M + H]$^+$ 356.1657 (theoretical, 356.1657).

Ethyl 2-Ethyl 3-[4-(*N*-((4-fluoro)benzyl)benzamide)]-propanoate (7b). Yield: 0.23 g (91%). ^{1}H NMR (DMSO-d_6, δ): 8.91 (t, J = 5.8 Hz, 1H, OCNH), 7.75−7.03 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.37 (d, J = 6, 2H, Ph_1-OCNH-CH_2), 3.98−3.86 (m, 2H, CH-COO-CH_2), 2.84−2.69 (m, 2H, Ph_1-CH_2), 2.60−2.48 (m, 1H, Ph_1-CH_2-CH), 1.53−1.41 (m, 2H, Ph_1-CH_2-CH-CH_2), 1.01 (t, J = 7.4 Hz, 3H, COO-CH_2-CH_3), 0.79 (t, J = 7.4, 3H, CH-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 177.6, 165.6, 143.4, 138.6, 133.4, 133.2, 132.1, 127.5, 127.1, 126.7, 126.6, 126.5, 125.4, 125.2, 56.0, 48.2, 45.0, 37.6, 24.0, 13.1, 11.7. HRMS: measured m/z [M + H]$^+$ 358.1814 (theoretical, 358.1813).

2-Ethyl-3-[4-(*N*-((4-fluoro)benzyl)benzamide)]propionic Acid (7c). Yield: 0.05 g (50%). ^{1}H NMR (methanol-d_4, δ): 7.68−6.91 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.43 (s, 2H, Ph_1-OCNH-CH_2), 2.89−2.69 (m, 2H, Ph_1-CH_2), 2.54−2.44 (m, 1H, Ph_1-CH_2-CH), 1.62−1.41 (m, 2H, Ph_1-CH_2-CH-CH_2), 0.86 (t, J = 7.4, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 177.6, 168.7, 163.7, 160.5, 143.9, 135.0, 135.0, 132.1, 129.1, 129.0, 129.0, 127.0, 114.9, 114.6, 49.1, 42.4, 37.6, 25.0, 10.6. HRMS: measured m/z [M + H]$^+$ 330.1503 (theoretical, 330.15).

(*E*)-4-[*N*-((4-Fluoro)benzyl)benzamide]-α-ethylcinnamic Acid (7d). Yield: 0.07 g (70%). ^{1}H NMR (DMSO-d_6, δ): 12.63 (s, 1H, COOH), 9.12 (t, J = 6.3 Hz, 1H, Ph_2-OCNH), 7.97−7.12 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 4.48 (d, J = 5.6 Hz, 2H, Ph_1-OCNH-CH_2), 2.44 (q, J = 8.9 Hz, 2H, CH-C-CH_2), 1.11 (t, J = 6.9 Hz, 3H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 169.8, 168.1, 139.1, 137.0, 136.6, 134.9, 133.8, 129.1, 129.0, 128.9, 127.9, 127.2, 126.8, 125.6, 114.9, 114.6, 42.4, 20.3, 12.7. HRMS: measured m/z [M + H]$^+$ 328.1345 (theoretical, 328.1344).

4-Formyl-*N*-(4-(trifluoromethyl)benzyl)benzamide (8). Yield: 1.3 g (70%). ^{1}H NMR (DMSO-d_6, δ): 10.10 (s, 1H, Ph_1-CHO), 9.37 (t, J = 5.9 Hz, 1H, Ph_1-OCNH), 8.12−8 (m, 4H, CHO-Ph_1), 7.74−7.54 (m, 4H, OCNH-CH_2-Ph_2), 4.60 (d, J = 5.8 Hz, 2H, Ph_1-OCNH-CH_2). MS-ESI: m/z 308 [M + H$^+$].

Ethyl (*E*)-4-[*N*-((4-Trifluoromethyl)benzyl)benzamide]-α-ethylcinnamate (8a). Yield: 0.92 g (65%). ^{1}H NMR (DMSO-d_6, δ): 9.23 (t, J = 6.5 Hz, 1H, Ph_2-OCNH), 7.99−7.53 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 7.62 (s, 1H, OCNH-Ph_1-CH), 4.59 (d, J = 6.3 Hz, 2H, Ph_1-OCNH-CH_2), 4.24 (q, J = 7.4 Hz, 2H, C-COO-CH_2), 2.50 (q, J = 7.9 Hz, 2H, CH-C-CH_2), 1.30 (t, J = 7.4 Hz, 3H, COO-CH_2-CH_3), 1.12 (t, J = 7.6 Hz, 3 H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 167.4, 167, 164.8, 139.3, 137.1, 136.7, 131.3, 132.4, 130.2, 128.6, 128.4, 127.4, 127.1, 126.1, 124.9, 124.3, 117.9, 61.3, 41.6, 17.7, 14.1, 14.0. HRMS: measured m/z [M + H]$^+$ 406.1626 (theoretical, 406.1625).

Ethyl 2-Ethyl 3-[4-(*N*-((4-trifluoromethyl)benzyl)benzamide)]-propanoate (8b). Yield: 0.23 g (87%). ^{1}H NMR (methanol-d_4, δ): 7.70−7.17 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.54 (s, 2H, Ph_1-OCNH-CH_2), 3.99−3.88 (m, 2H, CH-COO-CH_2), 2.87−2.72 (m, 2H, Ph_1-CH_2), 2.59−2.49 (m, 1H, Ph_1-CH_2-CH), 1.62−1.46 (m, 2H, Ph_1-CH_2-CH-CH_2), 1.03 (t, J = 7.5 Hz, 3H, COO-CH_2-CH_3), 0.83 (t, J = 7.2, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 175.6, 166.3, 143.5, 132.7, 132.3, 131.5, 130.4, 128.8, 127.5, 127.1, 125.1, 125.1, 125.0, 125, 124.9, 60.0, 49.0, 42.7, 37.7, 25.1, 13.2, 10.7. HRMS: measured m/z [M + H]$^+$ 408.178 (theoretical, 408.1781).

2-Ethyl-3-[4-(*N*-((4-trifluoromethyl)benzyl)benzamide)]-propionic Acid (8c). Yield: 0.05 g (53%). ^{1}H NMR (methanol-d_4, δ): 7.7−7.1 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.61 (s, 2H, Ph_1-OCNH-CH_2), 3.03−2.82 (m, 2H, Ph_1-CH_2), 2.66−2.56 (m, 1H, Ph_1-CH_2-CH), 1.76−1.53 (m, 2H, Ph_1-CH_2-CH-CH_2), 0.99 (t, J = 8.3, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 177.5, 168.8, 144.0, 132.5, 132.2, 132.1, 131.1, 129.1, 129.0, 127.5, 127.0, 125.0, 125.0, 124.9, 124.6, 49.0, 47.4, 37.5, 25.0, 10.6. HRMS: measured m/z [M + H]$^+$ 380.1467 (theoretical, 380.1468).

(*E*)-4-[*N*-((4-Trifluoromethyl)benzyl)benzamide]-α-ethylcinnamic Acid (8d). Yield: 0.05 g (50%). ^{1}H NMR (methanol-d_4, δ): 8.08−7.29 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 4.70 (s, 2H, Ph_1-OCNH-CH_2), 2.57 (q, J = 7.5 Hz, 2H, CH-C-CH_2), 1.22 (t, J = 7.2 Hz, 3H, C-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 167.2, 166.5, 144.2, 138.4, 138.1, 137.0, 136.5, 133.8, 133.6, 132.2, 129.6, 129.4, 128.6, 128.5, 127.1, 127.0, 126.8, 29.5, 19.4, 12.6. HRMS: measured m/z [M + H]$^+$ 377.1245 (theoretical, 377.1246).

4-Formyl-*N*-(4-(trifluoromethoxy)benzyl)benzamide (9). Yield: 1.37 g (70%). ^{1}H NMR (DMSO-d_6, δ): 10.15 (s, 1H, Ph_1-CHO), 9.36 (t, J = 6 Hz, 1H, Ph_1-OCNH), 8.18−8.04 (m, 4H, CHO-Ph_1), 7.55−7.36 (m, 4H, OCNH-CH_2-Ph_2), 4.58 (d, J = 6 Hz, 2H, Ph_1-OCNH-CH_2). MS-ESI: m/z 324 [M + H$^+$].

Ethyl (*E*)-4-[*N*-((4-Trifluoromethoxy)benzyl)benzamide]-α-ethylcinnamate (9a). Yield: 0.83 g (65%). ^{1}H NMR (DMSO-d_6, δ): 9.14 (t, J = 5.9 Hz, 1H, Ph_2-OCNH), 7.99−7.27 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 7.42 (s, 1H, OCNH-Ph_1-CH), 4.5 (d, J = 5.4 Hz, 2H, Ph_1-OCNH-CH_2), 4.21 (q, J = 7.2 Hz, 2H, C-COO-CH_2), 2.40 (q, J = 7.8 Hz, 2H, CH-C-CH_2), 1.27 (t, J = 7.3 Hz, 3H, COO-CH_2-CH_3), 1.09 (t, J = 6.6 Hz, 3 H, C-CH_2-CH_3). ^{13}C NMR

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81

(DMSO-d_6, δ): 173.1, 169, 168.3, 155.8, 140.3, 136.7, 135.1, 131.4, 131.2, 128.7, 128.3, 127.6, 127.1, 125.1, 123.9, 122.3, 116.9, 59.3, 40.6, 16.7, 14.5, 14.1. HRMS: measured m/z $[M + H]^+$ 422.1574 (theoretical, 422.1574).

Ethyl 2-Ethyl 3-[4-(*N*-((4-trifluoromethoxy)benzyl)-benzamide)]propanoate (9b). Yield: 0.24 g (94%). ^{1}H NMR (methanol-d_4, δ): 7.69−7.12 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.48 (s, 2H, Ph_1-OCNH-CH_2), 3.98−3.88 (m, 2H, CH-COO-CH_2), 2.87−2.71 (m, 2H, Ph_1-CH_2), 2.58−2.48 (m, 1H, Ph_1-CH_2-CH), 1.64−1.42 (m, 2H, Ph_1-CH_2-CH-CH_2), 1.02 (t, J = 5.7 Hz, 3H, COO-CH_2-CH_3), 0.84 (t, J = 7.4, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 177.8, 175.6, 168.6, 143.8, 138.2, 132.1, 130.2, 130.3, 128.8, 128.7, 127, 125.3, 125.2, 125.1, 120.7, 60.1, 49.2, 42.5, 37.7, 25.2, 13.1, 10.6. HRMS: measured m/z $[M + H]^+$ 424.1726 (theoretical, 424.1730).

2-Ethyl-3-[4-(*N*-((4-trifluoromethoxy)benzyl)benzamide)]-propionic Acid (9c). Yield: 0.06 g (60%). ^{1}H NMR (methanol-d_4, δ): 7.82−7.24 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.61 (s, 2H, Ph_1-OCNH-CH_2), 3.03−2.82 (m, 2H, Ph_1-CH_2), 2.66−2.56 (m, 1H, Ph_1-CH_2-CH), 1.76−1.53 (m, 2H, Ph_1-CH_2-CH-CH_2), 0.99 (t, J = 8.3, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 178.0, 175.6, 168.7, 148.1, 144.1, 138.3, 132.0, 128.8, 128.7, 128.5, 127, 125.3, 125.2, 125.1, 120.7, 49.3, 47.0, 37.6, 25.0, 10.6. HRMS: measured m/z $[M + H]^+$ 396.1416 (theoretical, 396.1417).

(*E*)-4-[*N*-((4-Trifluoromethoxy)benzyl)benzamide]-α-ethyl-cinnamic Acid (9d). Yield: 0.07 g (69%). ^{1}H NMR (methanol-d_4, δ): 7.96−7.24 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 4.64 (s, 2H, Ph_1-OCNH-CH_2), 2.56 (q, J = 7.4 Hz, 2H, CH-C-CH_2), 1.19 (t, J = 9.6 Hz, 3H, C-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 169.7, 168.2, 165.5, 145.2, 139.2, 138.2, 137.0, 136.6, 134.1, 133.7, 132.2, 129.7, 129.5, 129.1, 128.2, 128.0, 120.8, 28.5, 20.4, 12.7. HRMS: measured m/z $[M + H]^+$ 394.1258 (theoretical, 394.1261).

4-Formyl-*N*-(4-(methoxy)benzyl)benzamide (10). Yield: 1.3 g (70%). ^{1}H NMR (DMSO-d_6, δ): 10.14 (s, 1H, Ph_1-CHO), 9.24 (t, J = 5.5 Hz, 1H, Ph_1-OCNH), 8.15−8.03 (m, 4H, CHO-Ph_1), 7.34−6.93 (m, 4H, OCNH-CH_2-Ph_2), 4.49 (d, J = 5.9 Hz, 2H, Ph_1-OCNH-CH_2). MS-ESI: m/z 270 $[M + H^+]$.

Ethyl (*E*)-4-[*N*-((4-Methoxy)benzyl)benzamide]-α-ethylcinna-mate (10a). Yield: 0.91 g (67%). ^{1}H NMR (methanol-d_4, δ): 7.80−6.60 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 7.50 (s, 1H, OCNH-Ph_1-CH), 4.41 (s, 2H, Ph_1-OCNH-CH_2), 4.17 (q, J = 7 Hz, 2H, C-COO-CH_2), 3.67 (s, Ph_2-O-CH_3), 2.44 (q, J = 7.5 Hz, 2H, CH-C-CH_2), 1.24 (t, J = 7.6 Hz, 3H, COO-CH_2-CH_3), 1.05 (t, J = 5 Hz, 3 H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 167, 166.3, 140.4, 138.3, 135.7, 135.1, 131.7, 130.3, 128.7, 128.5, 127.3, 127.1, 124.2, 123.1, 122.1, 115.9, 58.3, 55.1, 40.1, 16.5, 14.6, 14.1. HRMS: measured m/z $[M + H]^+$ 367.1783 (theoretical, 367.1784).

Ethyl 2-Ethyl 3-[4-(*N*-((4-methoxy)benzyl)benzamide)]-propanoate (10b). Yield: 0.2 g (90%). ^{1}H NMR (methanol-d_4, δ): 7.78−6.88 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.45 (s, 2H, Ph_1-OCNH-CH_2), 4.10−3.98 (m, 2H, CH-COO-CH_2), 3.78 (s, 3H, Ph_2-O-CH_3), 2.96−2.83 (m, 2H, Ph_1-CH_2), 2.68−2.61 (m, 1H, Ph_1-CH_2-CH), 1.73−1.56 (m, 2H, Ph_1-CH_2-CH-CH_2), 1.14 (t, J = 7.1 Hz, 3H, COO-CH_2-CH_3), 0.95 (t, J = 7.5, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 177.1, 160.5, 144.9, 133.8, 132.2, 130.1, 129.9, 128.4, 127.2, 127.0, 125.3, 125.2, 125.1, 114.9, 61.4, 55.8, 44.0, 39.1, 37.7, 26.6, 14.6, 11.9. HRMS: measured m/z $[M + H]^+$ 370.2017 (theoretical, 370.2013).

2-Ethyl-3-[4-(*N*-((4-methoxy)benzyl)benzamide)]propionic Acid (10c). Yield: 0.06 g (62%). ^{1}H NMR (DMSO-d_6, δ): 12.19 (s, 1H, COOH), 8.95 (t, J = 6.7 Hz, 1H, Ph_2-OCNH), 7.86−6.91 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.45 (d, J = 5.8, 2H, Ph_1-OCNH-CH_2), 3.78 (s, 3H, Ph_2-O-CH_3), 2.96−2.77 (m, 2H, Ph_1-CH_2), 2.63−2.60 (m, 1H, Ph_1-CH_2-CH), 1.64−1.49 (m, 2H, Ph_1-CH_2-CH-CH_2), 0.93 (t, J = 7.6, 3H, CH-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 176.4, 166.4, 158.6, 143.6, 132.8, 132.2, 129.1, 129, 127.6, 127.0, 125.3, 125.2, 125.1, 114.1, 55.5, 42.4, 40.8, 37.5, 25.1, 11.9. HRMS: measured m/z $[M + H]^+$ 342.17 (theoretical, 342.17).

(*E*)-4-[*N*-((4-Methoxy)benzyl)benzamide]-α-ethylcinnamic Acid (10d). Yield: 0.06 g (64%). ^{1}H NMR (DMSO-d_6, δ) 12.51 (s, 1H, COOH), 8.93 (t, J = 6.2 Hz, 1H, Ph_2-OCNH), 7.87−6.78 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 4.33 (d, J = 5.7 Hz, 2H, Ph_1-OCNH-CH_2), 3.64 (s, 3H, Ph_2-O-CH_3), 2.36 (q, J = 7.9 Hz, 2H, CH-C-CH_2), 1.01 (t, J = 7.1 Hz, 3H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 169.8, 168.0, 159.0, 139.0, 137.0, 136.5, 133.9, 130.8, 128.9, 128.7, 128.5, 128.5, 127.9, 127.2, 126.8, 113.5, 54.3, 46.8, 20.3, 12.7. HRMS: measured m/z $[M + H]^+$ 340.1544 (theoretical, 340.1543).

4-Formyl-*N*-(4-chlorobenzyl)benzamide (11). Yield: 1.14 g (69%). ^{1}H NMR (DMSO-d_6, δ): 10.10 (s, 1H, Ph_1-CHO), 9.30 (t, J = 6.4 Hz, 1H, Ph_1-OCNH), 8.10−8.01 (m, 4H, CHO-Ph_1), 7.43−7.35 (m, 4H, OCNH-CH_2-Ph_2), 4.50 (d, J = 6 Hz, 2H, Ph_1-OCNH-CH_2). MS-ESI: m/z 274 $[M + H^+]$.

Ethyl (*E*)-4-[*N*-((4-Chloro)benzyl)benzamide]-α-ethylcinna-mate (11a). Yield: 0.93 g (69%). ^{1}H NMR (methanol-d^4, δ): 7.93−7.34 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 7.36 (s, 1H, OCNH-Ph_1-CH), 4.58 (s, 2H, Ph_1-OCNH-CH_2), 4.30 (q, J = 7.5 Hz, 2H, C-COO-CH_2), 2.56 (q, J = 7.4 Hz, 2H, CH-C-CH_2), 1.37 (t, J = 7.5 Hz, 3H, COO-CH_2-CH_3), 1.18 (t, J = 7.2 Hz, 3 H, C-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 166.6, 165.3, 154.4, 137.4, 136.1, 135.5, 131.6, 130.2, 129.1, 128.6, 126.9, 126.8, 125.3, 123.1, 122.8, 115.9, 59.3, 40.2, 16.1, 14.6, 14.1. HRMS: measured m/z $[M + H]^+$ 371.1296 (theoretical, 371.1298).

Ethyl 2-Ethyl 3-[4-(*N*-((4-chloro)benzyl)benzamide)]-propanoate (11b). Yield: 0.2 g (91%). ^{1}H NMR (methanol-d_4, δ): 7.68−7.12 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.46 (s, 2H, Ph_1-OCNH-CH_2), 3.98−3.87 (m, 2H, CH-COO-CH_2), 2.85−2.73 (m, 2H, Ph_1-CH_2), 2.57−2.50 (m, 1H, Ph_1-CH_2-CH), 1.62−1.45 (m, 2H, Ph_1-CH_2-CH-CH_2), 1.02 (t, J = 7.1 Hz, 3H, COO-CH_2-CH_3), 0.83 (t, J = 7.4, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 177.0, 164.1, 145.0, 140.2, 130.2, 129.5, 128.5, 128.5, 128.4, 127.0, 125.5, 125.3, 125.1, 114.4, 61.4, 50.5, 44.5, 39.0, 26.6, 14.5, 12.1. HRMS: measured m/z $[M + H]^+$ 374.1518 (theoretical, 374.1518).

2-Ethyl-3-[4-(*N*-((4-chloro)benzyl)benzamide)]propionic Acid (11c). Yield: 0.05 g (50%). ^{1}H NMR (DMSO-d_6, δ): 12.13 (s, 1H, COOH), 8.97 (t, J = 6 Hz, 1H, Ph_2-OCNH), 7.83−7.27 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.53 (d, J = 6.4, 2H, Ph_1-OCNH-CH_2), 2.90−2.71 (m, 2H, Ph_1-CH_2), 2.57−2.52 (m, 1H, Ph_1-CH_2-CH), 1.58−1.43 (m, 2H, Ph_1-CH_2-CH-CH_2), 0.88 (t, J = 7.1, 3H, CH-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 176.4, 166.7, 146.6, 143.9, 132.4, 132.4, 129.6, 129.2, 129.1, 128.0, 127.7, 125.2, 125.1, 121.1, 48.5, 39.4, 37.5, 25.1, 11.9. HRMS: measured m/z $[M + H]^+$ 346.1206 (theoretical, 346.1205).

(*E*)-4-[*N*-((4-Chloro)benzyl)benzamide]-α-ethylcinnamic Acid (11d). Yield: 0.06 g (66%). ^{1}H NMR (DMSO-d_6, δ) 12.54 (s, 1H, COOH), 9.03 (t, J = 5.8 Hz, 1H, Ph_2-OCNH), 7.87−7.23 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 4.39 (d, J = 6.1 Hz, 2H, Ph_1-OCNH-CH_2), 2.37 (q, J = 7.4 Hz, 2H, CH-C-CH_2), 1.01 (t, J = 7.6 Hz, 3H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 168.7, 166.1, 137.1, 137.0, 135.6, 133.9, 133.6, 129.4, 129.2, 128.9, 128, 127.6, 126.4, 125.4, 114.8, 114.6, 37.4, 21.3, 12.7. HRMS: measured m/z $[M + H]^+$ 344.1045 (theoretical, 344.1048).

4-Formyl-*N*-4-(phenoxybenzyl)benzamide (12). Yield: 1.39 g (69%). ^{1}H NMR (DMSO-d_6, δ): 10.10 (s, 1H, Ph_1-CHO), 9.26 (t, J = 5.9 Hz, 1H, Ph_1-OCNH), 8.17−7.99 (m, 4H, CHO-Ph_1), 7.42−6.96 (m, 9H, OCNH-CH_2-Ph_2 + Ph_2-O-Ph_3), 4.50 (d, J = 6 Hz, 2H, Ph_1-OCNH-CH_2). MS-ESI: m/z 332 $[M + H^+]$.

Ethyl (*E*)-4-[*N*-((4-Phenoxy)benzyl)benzamide]-α-ethylcinna-mate (12a). Yield: 0.85 g (69%). ^{1}H NMR (DMSO-d_6, δ): 9.11 (t, J = 5.9 Hz, 1H, Ph_1-OCNH), 7.97−6.98 (m, 12H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_2-O-Ph_3), 7.40 (s, 1H, OCNH-Ph_1-CH), 4.49 (d, J = 6.5, 2H, Ph_1-OCNH-CH_2), 4.24 (q, J = 7.2 Hz, 2H, C-COO-CH_2), 2.45 (q, J = 7.3 Hz, 2H, CH-C-CH_2), 1.30 (t, J = 7.7 Hz, 3H, COO-CH_2-CH_3), 1.12 (t, J = 7.8 Hz, 3 H,C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 166.1, 164.2, 156.6, 155.4, 137.4, 136.1, 135.5, 131.6, 130.2, 129.1, 128.7, 128.6, 128.3, 126.9, 126.8, 125.3, 123.1, 122.8, 121.5, 118.1, 118.7, 115.9, 59.3, 40.2, 16.2, 14.5, 14. HRMS: measured m/z $[M + H]^+$ 430.2017 (theoretical, 430.2013).

Ethyl 2-Ethyl 3-[4-(*N*-((4-phenoxy)benzyl)benzamide)]-propanoate (12b). Yield: 0.2 g (90%). ^{1}H NMR (methanol-d_4, δ): 7.68−7.12 (m, 13H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.46

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81

(s, 2H, Ph_1-OCNH-CH_2), 3.98−3.87 (m, 2H, CH-COO-CH_2), 2.85−2.73 (m, 2H, Ph_1-CH_2), 2.57−2.50 (m, 1H, Ph_1-CH_2-CH), 1.62−1.45 (m, 2H, Ph_1-CH_2-CH-CH_2), 1.02 (t, J = 7.1 Hz, 3H, COO-CH_2-CH_3), 0.83 (t, J = 7.4, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 175.7, 168.5, 157.4, 156.5, 143.7, 136.1, 134.0, 132.4, 130.2, 129.5, 128.8, 128.7, 127.0, 126.9, 126.8, 125.3, 123.1, 122, 121.5, 118.5, 118.3, 115.9, 60.1, 42.2, 25.2, 13.2, 10.5. HRMS: measured m/z [M + H]$^+$ 370.2017 (theoretical, 370.2013).

2-Ethyl-3-[4-(*N*-((4-phenoxy)benzyl)benzamide)]propionic Acid (12c). Yield: 0.03 g (30%). ^{1}H NMR (methanol-d_4, δ): 7.69−6.82 (m, 13H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.44 (s, 2H, Ph_1-OCNH-CH_2), 2.89−2.69 (m, 2H, Ph_1-CH_2), 2.54−2.44 (m, 1H, Ph_1-CH_2-CH), 1.62−1.41 (m, 2H, Ph_1-CH_2-CH-CH_2), 0.85 (t, J = 7.6, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 177.5, 168.6, 157.4, 156.4, 133.9, 132.4, 132.2, 129.4, 128.8, 128.7, 127.0, 126.9, 126.8, 125.3, 123.1, 122.9, 121.5, 118.5, 118.3, 118, 47.6, 42.2, 37.5, 25.0, 10.6. HRMS: measured m/z [M + H]$^+$ 404.1858 (theoretical, 404.1856).

(*E*)-4-[*N*-((4-Phenoxy)benzyl)benzamide]-α-ethylcinnamic Acid (12d). Yield: 0.02 g (16%). ^{1}H NMR (methanol-d_4, δ) 7.96−6.95 (m, 14H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH + Ph_2-O-Ph_3), 4.60 (s, 2H, Ph_1-OCNH-CH_2), 2.56 (q, J = 7.1 Hz, 2H, CH-C-CH_2), 1.20 (t, J = 7.5 Hz, 3H, C-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 169.8, 168.1, 157.4, 155.4, 139.1, 137, 136.5, 133.9, 133.8, 133.8, 129.5, 128.9, 128.8, 127.9, 127.2, 126.9, 122.9, 122.8, 121.5, 118.5, 118.3, 115.9, 42.8, 20.3, 12.8. HRMS: measured m/z [M + H]$^+$ 402.1696 (theoretical, 402.1699).

4-Formyl-*N*-(4-fluoro-2-(trifluoromethyl)benzyl)benzamide (13). Yield: 1.37 g (67%). ^{1}H NMR (DMSO-d_6, δ): 10.11 (s, 1H, Ph_1-CHO), 9.34 (t, J = 5.5 Hz, 1H, Ph_1-OCNH), 8.17−8.01 (m, 4H, CHO-Ph_1), 7.68−7.51 (m, 3H, OCNH-CH_2-Ph_2), 4.66 (d, J = 5.5 Hz, 2H, Ph_1-OCNH-CH_2). MS-ESI: m/z 326 [M + H$^+$].

Ethyl (*E*)-4-[*N*-((4-Fluoro(2-trifluoromethyl))benzyl)-benzamide]-α-ethylcinnamate (13a). Yield: 0.85 g (70%). ^{1}H NMR (DMSO-d_6, δ): 9.19 (t, J = 5.3 Hz, 1H, Ph_2-OCNH), 8.00−7.53 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 4.65 (d, J = 4.6 Hz, 2H, Ph_1-OCNH-CH_2), 4.24 (q, J = 7 Hz, 2H, C-COO-CH_2), 2.48 (q, J = 8.5 Hz, 2H, CH-C-CH_2), 1.30 (t, J = 7.4 Hz, 3H, COO-CH_2-CH_3), 1.13 (t, J = 7.1 Hz, 3 H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 175.4, 165.3, 141.9, 136.3, 135.3, 135.1, 132.6, 132.5, 129.4, 128.8, 128.2, 126.1, 126, 125.3, 125.1, 124.8, 124.1, 60.4, 40.1, 24.6, 14.1, 11. HRMS: measured m/z [M + H]$^+$ 424.1530 (theoretical, 424.1530).

Ethyl 2-Ethyl 3-[4-(*N*-(4-fluoro(2-trifluoromethyl)benzyl)-benzamide)]propanoate (13b). Yield: 0.2 g (90%). ^{1}H NMR (methanol-d_4, δ): 7.83−7.18 (m, 7H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.63 (s, 2H, Ph_1-OCNH-CH_2), 4.01−3.86 (m, 2H, CH-COO-CH_2), 2.88−2.72 (m, 2H, Ph_1-CH_2), 2.59−2.49 (m, 1H, Ph_1-CH_2-CH), 1.65−1.43 (m, 2H, Ph_1-CH_2-CH-CH_2), 1.03 (t, J = 7.04 Hz, 3H, COO-CH_2-CH_3), 0.84 (t, J = 7.7, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 175.6, 168.8, 163.0, 159.7, 143.8, 133.1, 131.9, 131.0, 130.9, 128.9, 127.1, 118.9, 118.6, 113.3, 112.9, 60.0, 49.1, 39.3, 37.4, 25.2, 13.0, 10.6. HRMS: measured m/z [M + H]$^+$ 426.1686 (theoretical, 426.1687).

2-Ethyl 3-[4-(*N*-(4-Fluoro(2-trifluoromethyl)benzyl)-benzamide)]propionic Acid (13c). Yield: 0.05 g (50%). ^{1}H NMR (methanol-d_4, δ): 7.84−7.18 (m, 7H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.64 (s, 2H, Ph_1-OCNH-CH_2), 2.91−2.71 (m, 2H, Ph_1-CH_2), 2.55−2.45 (m, 1H, Ph_1-CH_2-CH), 1.64−1.42 (m, 2H, Ph_1-CH_2-CH-CH_2), 0.86 (t, J = 7.6, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 177.5, 168.9, 159.7, 144.8, 143.1, 133.1, 131.8, 131.0, 131.0, 128.8, 127.1, 118.8, 118.6, 112.9, 112.9, 60.0, 46.7, 37.5, 25.0, 10.6. HRMS: measured m/z [M + H]$^+$ 398.137 (theoretical, 398.1374).

(*E*)-4-[*N*-((4-Fluoro(2-trifluoromethyl))benzyl)benzamide]-α-ethylcinnamic Acid (13d). Yield: 0.06 g (60%). ^{1}H NMR (methanol-d_4, δ) 8.11−7.36 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 4.79 (s, 2H, Ph_1-OCNH-CH_2), 2.57 (q, J = 7.43 Hz, 2H, CH-C-CH_2), 1.20 (t, J = 7.4 Hz, 3H, C-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 169.7, 168.4, 141.9, 139.4, 136.9, 136.7, 133.5, 131.2, 131.0, 128.9, 128.2, 127.3, 126, 125.3, 125.1, 124.8, 124.1, 39.4, 20.4, 12.8. HRMS: measured m/z [M + H]$^+$ 396.1215 (theoretical, 396.1217).

4-Formyl-*N*-(4-methoxy-2-(trifluoromethyl)benzyl)-benzamide (14). Yield: 1.41 g (70%). ^{1}H NMR (DMSO-d_6, δ): 10.09 (s, 1H, Ph_1-CHO), 9.23 (t, J = 5.9 Hz, 1H, Ph_1-OCNH), 8.1−8 (m, 4H, CHO-Ph_1), 7.48 (d, J = 9.5 Hz, 1H, OCNH-CH_2-Ph_2-3H), 7.26−7.2 (m, 2H, OCNH-CH_2-Ph_2^-2,5H), 4.60 (d, J = 5.7 Hz, 2H, Ph_1-OCNH-CH_2), 3.81 (s, 1H, CH_2-Ph_2-4-OCH_3). MS-ESI: m/z 338 [M + H$^+$].

Ethyl (*E*)-4-[*N*-((4-Methoxy(2-trifluoromethyl))benzyl)-benzamide]-α-ethylcinnamate (14a). Yield: 0.9 g (70%). ^{1}H NMR (DMSO-d_6, δ): 9.10 (t, J = 4.7 Hz, 1H, Ph_2-OCNH), 8.02−7.24 (m, 7H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 7.40 (s, 1H, OCNH-Ph_1-CH), 4.61 (d, J = 5.1 Hz, 2H, Ph_1-OCNH-CH_2), 4.25 (q, J = 7.1 Hz, 2H, C-COO-CH_2), 2.45 (q, J = 7.7 Hz, 2H, CH-C-CH_2), 1.30 (t, J = 8.5 Hz, 3H, COO-CH_2-CH_3), 1.12 (t, J = 7.2 Hz, 3 H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 168.4, 166.3, 158.9, 136.4, 136.3, 135.9, 135.6, 132.5, 129.4, 128.8, 128.2, 126.3, 126.1, 125.3, 125, 124.8, 124.1, 60.1, 55.3, 40.1, 23.6, 13.0, 11.0. HRMS: measured m/z [M + H]$^+$ 436.1728 (theoretical, 436.1730).

Ethyl 2-Ethyl 3-[4-(*N*-(4-methoxy(2-trifluoromethyl)benzyl)-benzamide)]propanoate (14b). Yield: 0.2 g (90%).^{1}H NMR (methanol-d_4, δ): 7.71−7.02 (m, 7H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.59 (s, 2H, Ph_1-OCNH-CH_2), 4.01−3.86 (m, 2H, CH-COO-CH_2), 3.74 (s, 3H, Ph_2-O-CH_3), 2.87−2.72 (m, 2H, Ph_1-CH_2), 2.59−2.49 (m, 1H, Ph_1-CH_2-CH), 1.65−1.43 (m, 2H, Ph_1-CH_2-CH-CH_2), 1.03 (t, J = 7 Hz, 3H, COO-CH_2-CH_3), 0.84 (t, J = 7.3, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 175.7, 168.8, 158.8, 151.2, 143.9, 132.1, 130.3, 128.8, 128.7, 128.6, 128.3, 127.2, 116.7, 111.8, 111.7, 67.9, 59.9, 55.1, 39.5, 37.9, 25.1, 13.2, 10.8. HRMS: measured m/z [M + H]$^+$ 438.1882 (theoretical, 438.1887).

2-Ethyl-3-[4-(*N*-(4-methoxy(2-trifluoromethyl)benzyl)-benzamide)]propionic Acid (14c). Yield: 0.7 g (79%). ^{1}H NMR (DMSO-d_6, δ): 12.15 (s, CH_2-CH-COOH), 8.97 (t, J = 5.6 Hz, 1H, Ph_2-OCNH), 7.87−7.20 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.58 (d, 2H, J = 5.5, Ph_1-OCNH-CH_2), 3.81 (s, 3H, Ph_2-O-CH_3), 2.94−2.72 (m, 2H, CH_2-CH-CH_2), 2.90−2.72 (m, 1H, Ph_1-CH_2-CH), 1.57−1.46 (m, Ph_1-CH_2), 0.90 (t, J = 7.5 Hz, CH-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 176.8, 166.8, 158.5, 144.3, 132.4, 130.8, 129.6, 129.3, 128, 127.8, 127.4, 126.5, 122.8, 118.2, 112.1, 56.3, 50.5, 48.4, 37.5, 25.1, 11.8. HRMS: m/z 410.1572 (theoretical, 410.1573).

(*E*)-4-[*N*-((4-Methoxy(2-trifluoromethyl))benzyl)benzamide]-α-ethylcinnamic Acid (14d). Yield: 0.05 g (55%). ^{1}H NMR (methanol-d_4, δ) 7.97−6.65 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 4.75 (s, 2H, Ph_1-OCNH-CH_2), 3.87 (s, 3H, Ph_2-CH_3), 2.57 (q, J = 7.6 Hz, 2H, CH-C-CH_2), 1.20 (t, J = 7.4 Hz, 3H, C-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 169.7, 168.3, 158.9, 139.3, 137.1, 136.6, 133.6, 130.5, 128.9, 128.1, 127.9, 127.3, 126.9, 126.1, 116.7, 112, 111.8, 54.7, 20.3, 12.6. HRMS: measured m/z [M + H]$^+$ 408.1415 (theoretical, 408.1417).

3-Formyl-*N*-(2-(trifluoromethyl)benzyl)benzamide (15). Yield: 1.38 g (70%). ^{1}H NMR (DMSO-d_6, δ): 10.11 (s, 1H, Ph_1-CHO), 9.36 (t, J = 5.9 Hz, 1H, Ph_1-OCNH), 8.27−8.10 (m, 4H, CHO-Ph_1), 7.79−7.46 (m, 4H, OCNH-CH_2-Ph_2), 4.71 (d, J = 5.8 Hz, 2H, Ph_1-OCNH-CH_2). MS-ESI: m/z 308 [M + H$^+$].

Ethyl (*E*)-3-[*N*-((2-Trifluoromethyl)benzyl)benzamide]-α-ethylcinnamate (15a). Yield: 0.91 g (70%). ^{1}H NMR (methanol-d_4, δ): 7.82−7.32 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 7.48 (s, 1H, OCNH-Ph_1-CH), 4.70 (s, 2H, Ph_1-OCNH-CH_2), 4.18 (q, J = 7.22 Hz, 2H, COO-CH_2), 2.45 (q, J = 7.6 Hz, 2H, CH-C-CH_2), 1.25 (t, J = 7.1 Hz, 3H, COO-CH_2-CH_3), 1.07 (t, J = 7.6 Hz, 3 H, C-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 169.9, 169.4, 138.5, 137.5, 137.4, 135.8, 133.5, 133.2, 130, 129.8, 128.5, 128.3, 127.1, 127, 125, 124.8, 124.1, 62.1, 41.3, 21.7, 14.6, 14.1. HRMS: measured m/z [M + H]$^+$ 405.1552 (theoretical, 405.1553).

Ethyl 2-Ethyl 3-[3-(*N*-((2-trifluoromethyl)benzyl)benzamide)]-propanoate (15b). Yield: 0.14 g (55%). ^{1}H NMR (methanol-d_4, δ): 7.65−7.27 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.69 (s, 2H, Ph_1-OCNH-CH_2), 3.93 (q, J = 7.2, 2H, CH-COO-CH_2), 2.87−2.74

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81

(m, 2H, Ph_1-CH_2), 2.59−2.51 (m, 1H, Ph_1-CH_2-CH), 1.64−1.46 (m, 2H, Ph_1-CH_2-CH-CH_2), 1.01 (t, J = 7.1 Hz, 3H, COO-CH_2-CH_3), 0.84 (t, J = 7.4 Hz, 3H, CH-CH_2-CH_3). ^{13}C NMR (methanol-d_4, δ): 177.1, 168.8, 141.6, 140.5, 135.4, 133.5, 133.5, 129.7, 129.1, 128.5, 127.1, 126.5, 125.5, 125.4, 124.9, 61.5, 50.7, 39.8, 39.2, 26.5, 14.6, 12.1. HRMS: measured m/z $[M + H]^+$ 408.1781 (theoretical, 408.1781).

2-Ethyl-3-[3-(*N*-((2-trifluoromethyl)benzyl)benzamide)]-propionic Acid (15c). Yield: 0.06 g (60%). ^{1}H NMR (DMSO-d_6, δ): 12.15 (s, COOH), 9.13 (t, J = 5.5 Hz, 1H, Ph_2-OCNH), 7.85−7.43 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.72 (d, 2H, J = 5.1 Hz, Ph_1-OCNH-CH_2), 2.99−2.78 (m, 2H, CH_2-CH-CH_2), 2.66−2.60 (m, 1H, Ph_1-CH_2-CH), 1.66−1.50 (m, Ph_1-CH_2), 0.95 (t, J = 7.3 Hz, CH-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 176.5, 167.0, 140.5, 138.2, 134.4, 133.1, 132.4, 128.7, 128.7, 128.2, 127.7, 126.2, 126.1, 125.6, 122.8, 48.7, 39.4, 37.6, 25.1, 11.9. HRMS: measured m/z $[M + H]^+$ 380.1473 (theoretical, 380.1468).

(*E*)-3-[*N*-((2-Trifluoromethyl)benzyl)benzamide]-α-ethylcinnamic Acid (15d). Yield: 0.05 g (50%). ^{1}H NMR (DMSO-d_6): 12.66 (s, 1H, COOH), 9.22 (t, J = 5.8, 1H, OCNH), 8.02−7.47 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + OCNH-Ph_1-CH), 4.73 (d, J = 6, 2H, Ph_1-OCNH-CH_2), 2.54−2.43 (m, 2H, CH-C-CH_2), 1.17 (t, J = 7.2 Hz, 3 H, C-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 170.0, 165.0, 137.5, 137.0, 136.0, 135.6, 133.4, 133.0, 130.0, 129.4, 128.3, 128.3, 127.2, 127.0, 124.9, 124.8, 124.1, 38.2, 20.1, 14.2. HRMS: measured m/z $[M + H]^+$ 377.3571 (theoretical, 377.3572).

Ethyl (*E*)-4-[*N*-((2-Trifluoromethyl)benzyl)benzamide]-cinnamate (16a). Yield: 0.78 g (67%). ^{1}H NMR (DMSO-d_6, δ): 9.19 (t, J = 6 Hz, 1H, Ph_2-OCNH), 8.00−7.47 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 6.77 (d, J = 16.2 Hz, 1H, Ph_1-CH-CH), 4.69 (d, J = 5.7 Hz, 2H, Ph_1-OCNH-CH_2), 4.33 (q, J = 7.5 Hz, 2H, CH-COO-CH_2), 1.28 (t, J = 7.4 Hz, 3H, COO-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 170.9, 168.4, 137.5, 136.5, 136.4, 135.8, 132.5, 131.2, 130, 129.6, 128.4, 128.1, 127.5, 127.3, 125.1, 124.8, 124.2, 62.3, 41.1, 14.1. HRMS: measured m/z $[M + H]^+$ 378.1312 (theoretical, 378.1312).

Ethyl 3-[4-(*N*-((2-Trifluoromethyl)benzyl)benzamide)]-propanoate (16b). Yield: 0.24 g (95%). ^{1}H NMR (DMSO-d_6, δ): 9.10 (t, J = 5.8 Hz, 1H, OCNH), 7.94−7.35 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.71 (d, J = 6.2 Hz, 2H, Ph_1-OCNH-CH_2), 4.10 (q, J = 7 Hz, 2H, CH_2-COO-CH_2), 2.97 (t, J = 7.6 Hz, 2H, Ph_1-CH_2), 2.71 (t, J = 7.1 Hz, 2H, Ph_1-CH_2-CH_2), 1.21 (t, J = 7 Hz, 3H, COO-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 177.1, 168.8, 141.6, 140.5, 135.4, 133.5, 133.5, 129.7, 129.1, 128.5, 127.1, 126.5, 125.5, 125.4, 124.9, 61.5, 50.7, 39.8, 39.2. HRMS: measured m/z $[M + H]^+$ 380.1468 (theoretical, 380.1468).

3-[4-(*N*-((2-Trifluoromethyl)benzyl)benzamide)]propionic Acid (16c). Yield: 0.05 g (50%). ^{1}H NMR (DMSO-d_6, δ): 12.21 (s, 1H, COOH), 9.04 (t, J = 6.8 Hz, 1H, OCNH), 7.86−7.34 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.65 (d, J = 5.6 Hz, 2H, Ph_1-OCNH-CH_2), 2.88 (t, J = 8, 2H, Ph_1-CH_2), 2.57 (t, J = 8.6 Hz, 2H, Ph_1-CH_2-CH_2). ^{13}C NMR (DMSO-d_6, δ): 174.0, 166.7, 145.0, 138.3, 135.4, 133.1, 132.2, 129.7, 129.1, 128.8, 127.8, 126.3, 125.5, 125.4, 123.4, 50.7, 39.8, 35.5. HRMS: measured m/z $[M + H]^+$ 352.1156 (theoretical, 352.1155).

(*E*)-4-[*N*-((2-Trifluoromethyl)benzyl)benzamide]cinnamic Acid (16d). Yield: 0.07 g (72%). ^{1}H NMR (DMSO-d_6, δ): 12.4 (s, 1H, COOH), 9.1 (t, J = 5.5 Hz, 1H, Ph_2-OCNH), 7.91−7.38 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 6.58 (d, J = 16 Hz, 1H, H), 4.60 (d, J = 5.7 Hz, 2H, Ph_1-OCNH-CH_2). ^{13}C NMR (DMSO-d_6, δ): 169.8, 168.3, 139.3, 137.0, 136.7, 133.5, 132.1, 128.9, 128.3, 127.9, 127.3, 127.1, 127.0, 126.9, 126.7, 125.7, 125.6, 40.0. HRMS: measured m/z $[M + H]^+$ 350.1 (theoretical, 350.1).

Ethyl (*E*)-4-[*N*-((2-Trifluoromethyl)benzyl)benzamide]-α-methylcinnamate (17a). Yield: 0.83 g (65%). ^{1}H NMR (DMSO-d_6, δ): 9.23 (t, J = 6 Hz, 1H, Ph_2-OCNH), 8.06−7.51 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 4.73 (d, J = 5.6 Hz, 2H, Ph_1-OCNH-CH_2), 4.28 (q, J = 7.2 Hz, 2H, C-COO-CH_2), 3.37 (d, J = 2.1, 3H, CH-C-CH_3), 1.34 (t, J = 7 Hz, 3H, COO-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 168, 165, 139.5, 136.4, 136.3, 135.8, 129.5, 129.1, 128.9, 127.4, 127.1, 126.5, 126.3, 125.9, 125.1, 124.4, 124.2, 59.9, 42.1, 13.7, 11.9. HRMS: measured m/z $[M + H]^+$ 392.1469 (theoretical, 392.1468).

Ethyl 2-Methyl 3-[4-(*N*-((2-trifluoromethyl)benzyl)benzamide)]-propanoate (17b). Yield: 0.2 g (93%). ^{1}H NMR (DMSO-d_6, δ): 9.04 (t, J = 6 Hz, 1H, OCNH), 7.86−7.27 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.65 (d, J = 5.5 Hz, 2H, Ph_1-OCNH-CH_2), 4.01 (q, J = 6.8 Hz, 2H, CH-COO-CH_2), 3.00−2.88 (m, 1H, Ph_1-CH_2-CH), 2.82−2.7 (m, 2H, Ph_1-CH_2), 1.14−1.05 (m, 6H, COO-CH_2-CH_3 + CH_2-CH-CH_3). ^{13}C NMR (DMSO-d_6, δ): 177.4, 166.8, 143.5, 140.5, 133.1, 132.5, 131.5, 129.7, 129.3, 128.6, 127.8, 126.5, 125.5, 125.4, 124.9, 60.2, 50.7, 39.8, 39.2, 17.2, 14.6. HRMS: measured m/z $[M + H]^+$ 394.1625 (theoretical, 394.1525).

2-Methyl 3-[4-(*N*-((2-Trifluoromethyl)benzyl)benzamide)]-propionic Acid (17c). Yield: 0.05 g (50%). ^{1}H NMR (DMSO-d_6, δ): 12.17 (s, 1H, COOH), 9.04 (t, J = 6.2 Hz, 1H, OCNH), 7.86−7.30 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.65 (d, J = 6.6 Hz, 2H, Ph_1-OCNH-CH_2), 3.00−2.90 (m, 1H, Ph_1-CH_2-CH), 2.73−2.64 (m, 2H, Ph_1-CH_2), 1.05 (d, J = 6.4, 3H, CH_2-CH-CH_3). ^{13}C NMR (DMSO-d_6, δ): 177.1, 166.7, 143.9, 140.5, 133.2, 132.4, 131.5, 129.4, 129.3, 128.6, 127.7, 126.5, 125.4, 125.4, 124.8, 50.7, 40.8, 39.1, 17.2. HRMS: measured m/z $[M + H]^+$ 366.1314 (theoretical, 366.1312).

(*E*)-4-[*N*-((2-Trifluoromethyl)benzyl)benzamide]-α-methylcinnamic Acid (17d). Yield: 0.07 g (71%). ^{1}H NMR (DMSO-d_6, δ): 12.62 (s, 1H, COOH), 9.23 (t, J = 5.8 Hz, 1H, Ph_2-OCNH), 8.06−7.51 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 4.74 (d, J = 5.6 Hz, 2H, Ph_1-OCNH-CH_2), 2.12 (d, J = 1.4, 3H, CH-C-CH_3). ^{13}C NMR (DMSO-d_6, δ): 169.1, 166, 138.6, 137.5, 136.7, 134.9, 133.5, 132.7, 130.2, 130.0, 130.0, 129.6, 128.2, 127.5, 127.4, 127.3, 126.8, 41.1, 12.3. HRMS: measured m/z $[M + H]^+$ 364.1158 (theoretical, 364.1155).

Ethyl (*E*)-4-[*N*-((2-Trifluoromethyl)benzyl)benzamide]-α-propylcinnamate (18a). Yield: 0.81 g (65%). ^{1}H NMR (DMSO-d_6, δ): 9.18 (t, J = 6 Hz, 1H, Ph_2-OCNH), 8.00−7.30 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 4.67 (d, J = 5.3 Hz, 2H, Ph_1-OCNH-CH_2), 4.23 (q, J = 7.4 Hz, 2H, C-COO-CH_2), 2.49−2.30 (m, 2H, CH-C-CH_2), 1.56−1.47 (m, 2H, C-CH_2-CH_2), 1.34 (t, J = 7 Hz, 3H, COO-CH_2-CH_3), 0.90 (t, J = 7 Hz, 3H, CH_2-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 167.0, 165.5, 138.5, 136.3, 136.2, 135.7, 130.5, 129.1, 128.7, 127.1, 127.0, 126.5, 126.2, 126.1, 125.7, 125.0, 124.6, 60.0, 42.1, 29.0, 20.1, 14.2, 13.7. HRMS: measured m/z $[M + H]^+$ 420.1780 (theoretical, 420.1781).

Ethyl 2-Propyl 3-[4-(*N*-((2-trifluoromethyl)benzyl)benzamide)]-propanoate (18b). Yield: 0.17 g (66%). ^{1}H NMR (DMSO-d_6, δ): 9.06 (t, J = 5.75 Hz, 1H, OCNH), 7.85−7.28 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.65 (d, J = 4.25 Hz, 2H, Ph_1-OCNH-CH_2), 3.99 (q, J = 7.3 Hz, 2H, CH-COO-CH_2), 3.07−2.90 (m, 2H, Ph_1-CH_2), 2.82−2.76 (m, 1H, Ph_1-CH_2-CH), 1.61−1.39 (m, 2H, Ph_1-CH_2-CH-CH_2), 1.07 (t, J = 7.2 Hz, 3H, COO-CH_2-CH_3), 1.35−1.16 (m, 2H, Ph_1-CH_2-CH-CH_2-CH_2), 0.85 (t, J = 7.13 Hz, 3H, CH-CH_2-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 175.0, 166.7, 143.5, 138.1, 132.9, 132.4, 131.5, 129.7, 129.2, 128.6, 127.7, 126.2, 125.5, 125.4, 124.9, 42.7, 38.1, 34.6, 34.4, 20.3, 14.5, 14.2. HRMS: measured m/z $[M + H]^+$ 422.1936 (theoretical, 422.1937).

2-Propyl-3-[4-(*N*-((2-trifluoromethyl)benzyl)benzamide)]-propionic Acid (18c). Yield: 0.02 g (20%). ^{1}H NMR (DMSO-d_6, δ): 12.47 (s, 1H, CCOH), 9.04 (t, J = 4.7 Hz, 1H, OCNH), 7.85−7.29 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.65 (d, J = 5.5 Hz, 2H, Ph_1-OCNH-CH_2), 2.91−2.71 (m, 2H, Ph_1-CH_2), 2.65−2.45 (m, 1H, Ph_1-CH_2-CH), 1.59−1.20 (m, 4H, Ph_1-CH_2-CH-CH_2 + CH_2-CH-CH_2-CH_2), 1.35−0.85 (t, J = 7.1 Hz, 3H, CH-CH_2-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 176.6, 167.1, 144.0, 138.4, 133.2, 132.4, 131.5, 129.4, 129.2, 128.1, 127.7, 126.2, 125.9, 125.4, 124.9, 38.0, 34.6, 34.1, 20.3, 14.5. HRMS: measured m/z $[M + H]^+$ 394.1623 (theoretical, 394.1625).

(*E*)-4-[*N*-((2-Trifluoromethyl)benzyl)benzamide]-α-propylcinnamic Acid (18d). Yield: 0.02 g (18%). ^{1}H NMR (DMSO-d_6, δ): 12.67 (s, 1H, COOH), 9.17 (t, J = 5.9 Hz, 1H, Ph_2-OCNH), 8.05−7.41 (m, 9H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH), 4.69 (d, J = 5.2 Hz, 2H, Ph_1-OCNH-CH_2), 2.55−2.33 (m, 2H, CH-C-CH_2), 1.60−1.42 (m, 2H, C-CH_2-CH_2), 0.91 (t, J = 7.7 Hz, 3H,

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81

CH_2-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 168.9, 166.0, 138.5, 137.5, 137.4, 136.8, 135.1, 133.8, 133.5, 132.7, 129.3, 129.0, 128.5, 128.2, 127.6, 127.3, 125.9, 42.1, 29, 18.4, 14.0. HRMS: measured m/z $[M + H]^+$ 392.1471 (theoretical, 392.1468).

Ethyl (*E*)-4-[*N*-((2-Trifluoromethyl)benzyl)benzamide]-α-phenylcinnamate (19a). Yield: 0.62 g (60%). ^{1}H NMR (DMSO-d_6, δ): 9.06 (t, J = 6 Hz, 1H, Ph_2-OCNH), 7.98−7.15 (m, 14H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH + C-Ph_4), 4.62 (d, J = 6 Hz, 2H, Ph_1-OCNH-CH_2), 4.23 (q, J = 6.6 Hz, 2H, C-COO-CH_2), 1.24 (t, J = 7.4 Hz, 3H, COO-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 166.5, 165.3, 136.5, 136.3, 136.2, 135.7, 134.2, 130.5, 129.1, 128.8, 128.6, 128.5, 128.4, 127.7, 127.1, 126.6, 126.5, 126.6, 126.2, 126.1, 125.7, 125, 124.6, 60.0, 42.1, 13.7. HRMS: measured m/z $[M + H]^+$ 454.1622 (theoretical, 454.1625).

Ethyl 2-Phenyl 3-[4-(*N*-((2-trifluoromethyl)benzyl)benzamide)]-propanoate (19b). Yield: 0.1 g (40%). ^{1}H NMR (DMSO-d_6, δ): 9.02 (t, J = 6 Hz, 1H, OCNH), 7.81−7.24 (m, 13H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + CH_2-CH-Ph_4), 4.63 (d, J = 5.5 Hz, 2H, Ph_1-OCNH-CH_2), 4.08−3.92 (m, 2H, CH-COO-CH_2), 3.4−3.31 (m, 2H, Ph_1-CH_2), 3.11−3.03 (m, 1H, Ph_1-CH_2-CH), 1.07 (t, J = 7.1 Hz, 3H, COO-CH_2-CH_3). ^{13}C NMR (DMSO-d_6, δ): 173.0, 166.8, 143.1, 139, 138.2, 138.1, 133.1, 132.5, 129.4, 129.0, 128.6, 128.3, 127.7, 127.7, 127.7, 126.6, 126.5, 126.6, 126.2, 126.1, 125.7, 125, 124.6, 60.9, 19.0, 14.5. HRMS: measured m/z $[M + H]^+$ 456.1785 (theoretical, 456.1781).

2-Phenyl-3-[4-(*N*-((2-trifluoromethyl)benzyl)benzamide)]-propionic Acid (19c). Yield: 0.2 g (16%). ^{1}H NMR (DMSO-d_6, δ): 12.47 (s, 1H, COOH), 9.08 (t, J = 6.9 Hz, 1H, OCNH), 7.87−7.27 (m, 13H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + CH_2-CH-Ph_4), 4.70 (d, J = 5.4 Hz, 2H, Ph_1-OCNH-CH_2), 3.45−3.05 (m, 2H, Ph_1-CH_2), 4.00 (t, J = 7.8, 1H, Ph_1-CH_2-CH). ^{13}C NMR (DMSO-d_6, δ): 174.7, 166.9, 143.7, 139.4, 138.2, 138.1, 133.2, 132.2, 129.3, 128.8, 128.6, 128.3, 127.7, 127.7, 127.7, 126.6, 126.5, 126.6, 126.2, 126.1, 125.7, 125, 124.6, 52.7. HRMS: measured m/z $[M + H]^+$ 428.1465 (theoretical, 428.1468).

(*E*)-4-[*N*-((2-Trifluoromethyl)benzyl)benzamide]-α-phenylcinnamic Acid (19d). Yield: 0.02 g (17%). ^{1}H NMR (DMSO-d_6, δ): 8.94 (t, J = 5.8 Hz, 1H, Ph_2-OCNH), 7.79−6.91 (m, 14H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-CH + C-Ph_4), 4.54 (d, J = 5.5 Hz, 2H, Ph_1-OCNH-CH_2). ^{13}C NMR (DMSO-d_6, δ): 166.2, 155.5, 129.6, 129.2, 129, 128.9, 128.3, 128.2, 128.1, 128.0, 128.0, 128.0, 127.5, 127.2, 126.9, 126.2, 125.8, 125.8, 126.1, 126.0, 125.3, 125.0, 124.6, 41.0. HRMS: measured m/z $[M + H]^+$ 426.1309 (theoretical, 426.1312).

(*E*)-*N*-Methoxy-*N*-methyl 3-[4-(*N*-((2-trifluoromethyl)benzyl)-benzamide)]but-2-enamide (20). To a solution of 1 g (3.3 mmol) of 4-formyl-*N*-(2-(trifluoromethyl)benzyl)benzamide (**1**) in 20 mL of chloroform under argon atmosphere was added 1.3 g (3.6 mmol) *N*-methoxy-*N*-methyl(triphenylphosphoranylidene)acetamide. After 16 h the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography with solvent mixture of EE/Hex in the ratio 1:1. A white solid remained as pure product. Yield: 0.6 g (47%). ^{1}H NMR (DMSO-d_6, δ): 9.20 (t, J = 5.8 Hz, 1H, Ph_1-OCNH), 8.00−7.20 (m, 10H, OCNH-Ph_1 + OCNH-CH_2-Ph_2 + Ph_1-CH + Ph_1-CH-CH), 4.70 (d, J = 5.4 Hz, 2H, Ph_1-OCNH-CH_2), 3.78 (s, 3H, OCN-O-CH_3), 3.25 (s, 3H, OCN-CH_3). MS-ESI: m/z 393 $[M + H^+]$.

***N*-Methoxy-*N*-methyl 3-[4-(*N*-((2-trifluoromethyl)benzyl)-benzamide)]cyclopropanecarboxamide (21).** To a solution of 561 mg (2.6 mmol) of trimethylsulfonium iodide in 3.15 mL of dry DMSO under argon atmosphere was added 97 mg (2.55 mmol) NaH in small portions. After the reaction mixture was stirred for 1 h, a solution of 500 mg (1.3 mmol) of (*E*)-*N*-methoxy-*N*-methyl 3-[4-(*N*-((2-trifluoromethyl)benzyl)benzamide)]but-2-enamide (**20**) in 1.05 mL of dry DMSO was injected. The reaction was quenched with 10 mL if saturated NH_4Cl solution after 6 h. The product was extracted three times with 5 mL of DCM. The collected organic layers were washed once with 4 mL of brine and dried over $MgSO_4$. The solvent was removed under reduced pressure. The pure product was recrystallized from a EE/Hex mixture and occurred as white solid. Yield: 0.62 g (60%). ^{1}H NMR (DMSO-d_6, δ): 9.06 (t, J = 5.4 Hz, 1H, OCNH), 7.88−7.31 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.67 (d, J = 5.4 Hz, 2H, Ph_1-OCNH-CH_2), 3.66 (s, 3H, OCN-O-CH_3), 3.16 (s, 3H, OCN-CH_3), 2.57−2.36 (m, 2H, Ph_1-CH + Ph_1-CH-CH), 1.54−1.40 (m, 2H, Ph_1-CH-CH_2). ^{13}C NMR (DMSO-d_6, δ): 167.4, 143.8, 135.7, 130.8, 130.6, 126.9, 126.4, 126.2, 126.1, 125.7, 124.6, 124.3, 123.0, 122.3, 122.0, 38.5, 37.4, 32, 27.8, 24, 20.6. HRMS: measured m/z $[M + H]^+$ 407.1578 (theoretical, 407.1577).

2-[4-(*N*-((2-Trifluoromethyl)benzyl)benzamide)]-cyclopropanecarboxylic Acid (22). To a solution of 100 mg (0.25 mmol) of *N*-methoxy-*N*-methyl 3-[4-(*N*-((2-trifluoromethyl)-benzyl)benzamide)]cyclopropanecarboxamide (**21**) in 3 mL of EtOH was added 3 mL of KOH solution (10%). The reaction mixture was refluxed for 24 h. EtOH was removed from the reaction solution under reduced pressure, and the remaining aqueous solution was washed three times with DEE. The aqueous solution pH was adjusted to 1 with 12 M HCl solution. The pure white product precipitated and was collected by filtration. Yield: 0.05 g (55%). ^{1}H NMR (DMSO-d_6, δ): 12.27 (s, 1H, COOH), 8.99 (t, J = 5.4 Hz, 1H, OCNH), 7.80−7.20 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.59 (d, J = 5.5 Hz, 2H, Ph_1-OCNH-CH_2), 2.30−2.40 (m, 1H, Ph_1-CH), 1.86−1.80 (m, 1H, Ph_1-CH-CH), 1.44−1.31 (m, 2H, Ph_1-CH-CH_2). ^{13}C NMR (DMSO-d_6, δ): 173.7, 166.2, 144.2, 137.7, 137.1, 132.6, 132.2, 131.8, 128.1, 128, 127.4, 127.3, 125.9, 125.9, 125.8, 39.7, 37.4, 25.1, 24.5. HRMS: measured m/z $[M + H]^+$ 364.1158 (theoretical, 364.1155).

General Procedure for the Preparation of the Compounds 23 and 26, Using the Example of 4-Iodo-*N*-(2-(trifluoromethyl)-benzyl)benzamide (23). 1 g (6.7 mmol) of 4-iodobenzoic acid, 1.5 g (8 mmol) of EDC, and 0.16 g (1.3 mmol) of DMAP were mixed under argon atmosphere in 25 mL of dry DCM and stirred as a suspension for 1 h at 0 °C. Then 0.9 g (7.3 mmol) of 2-trifluoromethylbenzylamine was added in one portion. The mixture was allowed to warm to room temperature and was further stirred for 24 h. The organic solution was washed twice with 20 mL of 2 M HCl solution and one time with 20 mL of brine. The organic solvent was dried over $MgSO_4$ and then removed under reduced pressure. The crude product was recrystallized from a EE/Hex mixture, and a white solid remained. Yield: 0.89 g (64%). ^{1}H NMR (DMSO-d_6, δ): 9.38 (t, J = 6.3 Hz, 1H, Ph_1-OCNH), 8.11−8.00 (m, 4H, CHO-Ph_1), 7.79−7.48 (m, 4H, OCNH-CH_2-Ph_2), 4.70 (d, J = 5.7 Hz, 2H, Ph_1-OCNH-CH_2). MS-ESI: m/z 405 $[M + H^+]$.

General Procedure for the Preparation of the Compounds 24 and 25, Using the Example of 4-[*N*-(2-(Trifluoromethyl)benzyl))-benzamide]-(1,1′-biphenyl) 4-Acid (24). 250 mg (1.5 mmol) of 4-carboxybenzenboronic acid, 555 mg (1.4 mmol) of 4-iodo-[*N*-(2-(trifluoromethyl)benzyl))benzamide] (**23**), 9.2 mg (0.04 mmol) of palladium(II) acetate, and 568 mg (4.1 mmol) of K_2CO_3 were dissolved in a mixture of acetone/H_2O in the ratio 1:1. The reaction was stirred for 1 h at 65 °C. The mixture was then filtered through Celite, and acetone was evaporated under reduced pressure. After acidifying the aqueous layer with 12 M HCl solution, the product precipitated. A white solid remained, and no further purification was needed. Yield: 0.36 g (66%). ^{1}H NMR (DMSO-d_6, δ): 13.03 (s, 1H, COOH), 9.21 (t, J = 6.1 Hz, 1H, OCNH), 8.09−7.48 (m, 12H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-Ph_7), 4.71 (d, J = 5.5 Hz, 2H, Ph_1-OCNH-CH_2). ^{13}C NMR (DMSO-d_6, δ): 167.0, 166.0, 162.4, 143.2, 142.1, 141.8, 137.9, 134.7, 133.5, 132.7, 130.2, 130.0, 128.9, 128.2, 127.4, 127.3, 127.1, 127.1, 127.0, 125.1, 125.0, 48.1. HRMS: measured m/z $[M + H]^+$ 400.1156 (theoretical, 400.1155).

4-[*N*-(2-(Trifluoromethyl)benzyl))benzamide]-(1,1′-biphenyl)-3-carboxylic Acid (25). Yield: 0.37 g (67%). ^{1}H NMR (DMSO-d_6, δ): 13.08 (s, 1H, COOH), 9.14 (t, J = 5.7 Hz, 1H, OCNH), 8.20−7.39 (m, 12H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2 + Ph_1-Ph_7), 4.63 (d, J = 5.5 Hz, 2H, Ph_1-OCNH-CH_2). ^{13}C NMR (DMSO-d_6, δ): 168.1, 166.0, 144.0, 143.1, 142.1, 140.8, 136.8, 134.8, 133.4, 132.9, 130.0, 130.0, 128.8, 128.1, 127.7, 127.5, 127.1, 126.1, 126.0, 125.1, 124.0, 49.1. HRMS: measured m/z $[M + H]^+$ 400.1155 (theoretical, 400.1155).

4-Cyano-*N*-(2-(trifluoromethyl)benzyl)benzamide (26). Yield: 0.92 g (65%). ^{1}H NMR (DMSO-d_6, δ): 9.38 (t, J = 6.3 Hz, 1H, Ph_1-OCNH), 8.11−8.00 (m, 4H, CHO-Ph_1), 7.79−7.48 (m, 4H, OCNH-CH_2-Ph_2), 4.70 (d, J = 5.7 Hz, 2H, Ph_1-OCNH-CH_2). MS-ESI: m/z 305 $[M + H^+]$.

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81

[*N*-(2-(Trifluoromethyl)benzyl)benzamide]-4-(1*H*-tetrazole) (27). 100 mg (0.3 mmol) of 4-cyano-*N*-(2-(trifluoromethyl)benzyl))-benzamide (**26**), 43 mg (0.7 mmol) of NaN_3, and 23 mg (0.4 mmol) of NH_4Cl were dissolved in 2 mL of dry DMF under argon atmosphere and stirred for 12 h at 150 °C. After the reaction mixture reached room temperature 1 mL of H_2O was added. To the aqueous layer 12 M HCl solution was added and the product precipitated. Through filtration the slightly yellow solid, which did not need further purification, was collected. Yield: 0.11 g (92%). ^{1}H NMR (DMSO-d_6, δ): 9.35 (t, J = 5.6 Hz, 1H, OCNH), 8.25−7.54 (m, 8H, OCNH-Ph_1 + Ph_1-OCNH-CH_2-Ph_2), 4.76 (d, J = 5.6 Hz, 2H, Ph_1-OCNH-CH_2). ^{13}C NMR (DMSO-d_6, δ): 166.6, 160.3, 137.9, 136.9, 135.5, 132.7, 132.3, 129.2, 128.6, 128.2, 127.7, 127.2, 126.7, 125.5, 125.1, 125.0, 48.2. HRMS: measured m/z $[M + H]^+$ 348.1067 (theoretical, 348.1067).

sEH Activity Assay. The IC_{50} values of the compounds were determined by a fluorescence-based assay system of 96-well format. As substrate, nonfluorescent PHOME (3-phenylcyano-(6-methoxy-2-naphthalenyl)methyl ester 2-oxiraneacetic acid, Cayman Chemicals) was used, which can be hydrolyzed by the sEH to the fluorescent 6-methoxynaphthaldehyde.[45] The formation of the product was measured (λ_{em} = 330 nm, λ_{ex} = 465 nm) by a Tecan Infinite F200 Pro plate reader. Therefore, recombinant human sEH (2 μg/well) in Bis-Tris buffer, pH 7, with 0.1 mg/mL BSA containing a final concentration of 0.01% Triton-X 100 was used. An amount of 100 μL of protein was incubated with different concentrations of compounds (DMSO with final concentration of 1%) for 30 min at room temperature. After that an amount of 10 μL of substrate was added (final concentration 50 μM). The hydrolyzed substrate was measured for 30 min (one point every minute). A blank control (no protein and no compound) as well as a positive control (no compound) was executed. All measurements were performed in triplicate.

PPAR Activity Assay. The assay was performed according to the procedure published before.[46] COS-7 cells were grown in DMEM high glucose, supplemented with 10% fetal calf serum (FCS), 1% sodium pyruvate (SP), and 1% penicillin/streptomycin (PS) at 37 °C and 5% CO_2. Used plasmids for PPAR transactivation assay are shown in Supporting Information. The day before transfection, COS-7 cells were seeded in 96-well plates with a density of 30 000 cells per well. Transient transfection was carried out using Lipofectamine LTX reagent (Invitrogen, Carlsbad, CA, USA) 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 PPAR subtype. At 5 h after transfection, medium was changed to DMEM without phenol red and 10% FCS, supplemented with 1% SP, 1% PS, and 1% L-glutamine, now additionally containing 0.1% DMSO and the respective test compound or 0.1% DMSO alone as untreated control. Each concentration was tested in triplicate wells, and each experiment was repeated independently at least three times. Following overnight incubation with the test compounds, cells were assayed for luciferase activity using Dual-Glo luciferase assay system (Promega) according to the manufacturer's protocol. Luminescence was measured using a microplate reader (Infinite M200, Tecan Group Ltd., Crailsheim, Germany). Each concentration of the compounds was tested in triplicate wells. 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 (pioglitazone for PPARγ, GW7647 for PPARα,[47] and L165041 for PPARδ,[48] each at 1 μM). Data are expressed as mean ± SE; $n \geq 3$.

WST-Cytotoxicity Assay. The WST-1 assay (Roche Diagnostic GmbH, Mannheim, Germany) was used to determine the cell viability after treatment with the compounds. For this purpose, Hela and HepG2 cells were seeded each in 96-well plates at a density of 1×10^4 per well in DMEM with phenol red and in prescence of 10% FCS. After 24 h the medium was changed. Fresh DMEM with 10% FCS was added, and the cells were treated with the compounds for 48 h. Cell viability was assessed according to the manufacturer's protocol using a microplate reader (Infinite M200, Tecan Group Ltd., Crailsheim, Germany). All experiments were performed at least in triplicate.

Water Solubility Approximation. PBS at pH 7.4 with 0.01% polysorbate 20 (Tween) was combined with 1% of a DMSO solution of the inquired compound in a 96-well transparent flat bottom microtiter plate. Precipitation of the compound was measured at 650 nm using a microplate reader (Infinite M200, Tecan Group Ltd., Crailsheim, Germany).

In Vitro Drug Metabolism in Rat Liver Microsomes. A solution of the test compound (1 mM) was prepared in 100% DMSO. 432 μL of phosphate buffer (0.1 M, pH 7.4) and 50 μL of NADPH-regenerating system (30 mM glucose 6-phosphate, 4 U/mL glucose 6-phosphate dehydrogenase, 10 mM NADP, 30 mM $MgCl_2$) and 5 μL of the corresponding test compound were preincubated at 37 °C. The final concentration of the investigated compound is 10 μM. After 5 min the reaction was started by the addition of 13 μL of microsome mix from the liver of Sprague-Dawley rats (Gibco, Darmstadt, Germany; 20 mg of protein/mL in 0.1 M phosphate buffer). The incubation was performed in a shaking water bath at 37 °C. The reaction was stopped by the addition of 500 μL of ice-cold methanol at 0, 15, 30, and 60 min. The samples were centrifuged at 10 000g for 5 min at 4 °C. The supernatants were analyzed and quantified by HPLC. Control samples were always performed to check the stability of the compounds in the reaction mixture. First control was without NADPH, which is needed for the enzymatic activity of the microsomes. Second control was with inactivated microsomes (microsomes that were incubated for 20 min at 90 °C). Third control was without test compounds (to determine the baseline). As positive control, a solution of 7-ethoxycoumarin (1 mM) was used. The final concentration of the control compound, under assay conditions, was again 10 μM. The amounts of the test compounds were quantified by an external calibration curve.

Differentiation of Murine 3T3-L1 Cells. 3T3-L1 cells were subcultured in DMEM containing 10% newborn calf serum in a humidified atmosphere at 37 °C, 5% CO_2. Cells were differentiated into adipocytes for 14 days according to the method of Zebisch et al.[49] Briefly, cells were seeded in 6-well plates (2.5×10^6/well). Differentiation was started at day 3 by addition of 1 μg/mL insulin, 0.25 μM dexamethasone, and 0.5 mM isobutylmethylxanthine in DMEM supplemented with 10% fetal calf serum. At day 5 medium was replaced by medium containing only insulin for 2 more days. After this, cells were kept for lipid droplet accumulation in basal medium without additions until day 15. Rosiglitazone (2 μM) and *N*-cyclohexyl-*N*′-(iodophenyl)urea (CIU) (10 μM) were used as PPARγ and sEH positive controls, respectively. Differentiation of 3T3-L1 cells was confirmed by Oil Red O staining. Cells were washed with PBS and subsequently fixed for 60 min with a formaldehyde solution (4% in PBS). After this, cells were rinsed with 60% isopropanol and incubated with Oil Red O solution (0.3%) for 120 min.

Quantitative Polymerase Chain Reaction (qPCR). 3T3-L1 cells or homogenized mice tissues were lysed using TRIzol reagent (Ambion, Life Technologies, Carlsbad, CA, USA), and mRNA was isolated following the manufacturer's protocol. DNA contaminations were digested using DNase (DNase I, RNase-free kit; Thermo Scientific, Waltham, MA, USA) and mRNA concentrations were measured with a NANODROP2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Subsequently, reverse transcription was performed using the high capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA, USA). PCR was performed using specific primers for ABCA1, adiponectin, ApoE, CD36, FABP-4, GLUT-4, LPL, LXRα, PGC-1α, PPARa, PPARg, sEH (shown in Supporting Information) with a StepOnePlus real-time PCR system (Applied Biosystems, Foster City, CA, USA). Non-POU domain-containing octamer binding protein (NoNo) and β-actin were used as reference genes for 3T3-L1 and mouse tissue, respectively. All samples were measured in triplicate and were analyzed using the ΔCT method.

In Vivo Studies. All mice PK studies were performed by Pharmacelsus GmbH (Saarbrücken, Germany), a commercial research organization, and were approved by and conducted in accordance with the regulations of the local animal welfare authorities (Landesamt für Gesundheit and Verbraucherschutz, Abteilung Lebensmittel- and

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81

Veterinärwesen, Saarbrücken, Germany). For a detailed description see the Supporting Information. The sEH PD data were generated through determination of epoxyeicosatrienoic acids (EETs) and their metabolites dihydroxyepoxyeicosatrienoic acids (DHETs) by LC/MS−MS.[50] Experimental details are described in Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01239.

Figures showing human adipocyte differentiations and qPCR, PK study of compound **1b** after po application in mice, brain concentration of compound **1b**, conversion of **14b** to **14c** in COS7 cells, differential scanning fluorimetry with PPARγ LBD and **14b** and **14c**, metabolic stability of compound **14c** in rat liver microsomes, effect of compound **1c** and **14c** on GPR40 (FFA1) by IMP measurement; methods for plasmids used in PPAR transactivation assay, human adipocyte differentiation and analysis, procedures of in vivo PK studies, EET/DHET analysis by LC/MS−MS (PDF)

Molecular formula strings (CSV)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: proschak@pharmchem.uni-frankfurt.de. Phone: +49 69 798 29301.

Author Contributions

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.

■ ACKNOWLEDGMENTS

This work was supported by the Else-Kröner-Fresenius Foundation graduate college for Translational Research Innovation - Pharma (TRIP) and Deutsche Forschungsgemeinschaft (DFG; Sachbeihilfe PR1405/1-2; SFB 1039 Teilprojekte A02, A07 and Z01). R.B., O.D., and M.B. express thanks to the graduate college for Translational Research Innovation - Pharma (TRIP) for the Ph.D. fellowship. The table of contents graphic is Courtesy of University of California Television.

■ ABBREVIATIONS USED

ABCA1, ATP binding cassette transporter 1; ADME, absorption, distribution, metabolism, and excretion; AMI, acute myocardial infarction; aP2, human adipocyte fatty acid binding protein; ASCVD, arteriosclerotic cardiovascular disease; ATP, adenosine triphosphate; $AUC_{0\rightarrow\infty}$, area under the concentration−time curve extrapolated to infinity; Bis-Tris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol; -Br, bromine substituent; BSA, bis(trimethylsilyl)-acetamide; bw, body weight; CD36, fatty acid translocase; $-CH_3$, methyl substituent; CIU, *N*-cyclohexyl-*N*′-iodophenylurea; -Cl, chlorine substituent; Cl/*f*, total body clearance normalized to bioavailability; C_{max}, maximal concentration; CNS, central nervous system; compd, compound; COS7, CV-1 (simian) in Origin and carrying the SV40 genetic material; CVD, cardiovascular disease; DCM, dichloromethane; DEE, diethyl ether; DHET, dihydroxyepoxyeicosatrienoic acid; DIPEA, diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMEM, Dulbecco's modified Eagle medium; DMF, dimethylformamide; DMSO-d_6, deuterated dimethyl sulfoxide; DNA, deoxyribonucleic acid; EC_{50}, half maximal effective concentration; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; EE, ethyl acetate; EET, epoxyeicosatrienoic acid; E_{max}, maximum activation in percent; EPC, endothelial progenitor cell; ESI, electrospray ionization; EtOH, ethanol; -F, fluorine substituent; FABP4, fatty acid binding protein 4; FATP, fatty acid transporter protein; FCS, fetal calf serum; FFA, free fatty acid; FFA1/GPR40, free fatty acid receptor 1; GLUT-4, glucose transporter type 4; GSIS, glucose stimulated insulin secretion; -H, hydrogen substituent; H_2O, water; HCl, hydrochloric acid; HDL, high density lipoprotein; HDL-C, high density lipoprotein cholesterol; HepG2, hepatocyte carcinoma; Hex, hexane; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectrometry; ia, inactive; IBCF, isobutyl chloroformate; IC_{50}, half maximal inhibitory concentration; K_2CO_3, potassium carbonate; KOH, potassium hydroxide; LBD, ligand binding domain; LC−MS, liquid chromatography−mass spectrometry; LC−MS/MS, liquid chromatography−mass spectrometry/mass spectrometry; LDL-C, low density lipoprotein cholesterol; LPL, lipoprotein lipase; M, molar; *m*/*z*, mass to charge ratio; MALDI, matrix-assisted laser desorption/ionization; $Me_3SO^+I^-$, trimethylsulfoxonium iodide; MeOH, methanol; methanol-d_4, deuterated methanol; MetS, metabolic syndrome; $MgCl_2$, magnesium chloride; $MgSO_4$, magnesium sulfate; mRNA, messenger ribonucleic acid; MW, microwave; nt, not tested; NADPH, nicotinamide adenine dinucleotide phosphate; NaH, sodium hydride; NaN_3, sodium azide; NaOH, sodium hydroxide; NH_4Cl, ammonium chloride; NMR, nuclear magnetic resonance spectrometry; $-OCF_3$, trifluoromethoxy substituent; $-O-CH_3$, methoxy substituent; -O-phenyl, oxophenyl substituent; po, per oral; P/S, penicillin/streptomycin; PBS, phosphate buffer system; $Pd(AcO)_2$, palladium(II) acetate; PEPCK, phosphoenolpyruvate carboxykinase; PHOME, (3-phenylcyano-(6-methoxy-2-naphthalenyl)methyl ester 2-oxiraneacetic acid; PK/PD, pharmacokinetic/pharmacodynamics; PPAR, peroxisome proliferator-activated receptor; qPCR, real-time polymerase chain reaction; Oil Red O, 1-[2,5-dimethyl-4-(2,5-dimethylphenylazo)phenylazo]-2-naphthol; RCT, reverse cholesterol transport; RP, reversed phase; RXR, retionid X receptor; SAR, structure−activity relationship; sEH, soluble epoxide hydrolase; sEH-KO, sEH knockout; SHROB, spontaneous hypertensive obese; SP, sodium pyruvate; STZ, streptozocin; T2D, type 2 diabetes; t-AUCB, *trans*-4-[4-(3-adamantan-1-y1ureido)cyclohexyloxy]benzoic acid; TG, triglyceride; THF, tetrahydrofuran; TLC, thin-layer chromatography; t_{max}, time to reach the maximum concentration; TNFα, tumor necrosis factor α; TZD, thiazolidinedione; UV, ultraviolet; V_z/f, volume of distribution normalized to bioavailability; ws, water solubility; WAT, white adipose tissue; WST-1, water soluble tetrazolium/(4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzol-disulfonate)

■ REFERENCES

(1) Eckel, R. H.; Alberti, K.; Grundy, S. M.; Zimmet, P. Z. The Metabolic Syndrome. *Lancet* **2010**, *375*, 181−183.

(2) Javed, S.; Petropoulos, I. N.; Alam, U.; Malik, R. A. Treatment of Painful Diabetic Neuropathy. *Ther. Adv. Chronic Dis.* **2015**, *6*, 15−28.

(3) Chan, G. C. W.; Tang, S. C. W. Diabetic Nephropathy: Landmark Clinical Trials and Tribulations. *Nephrol., Dial., Transplant.* **2015**, 1−10.

(4) Grundy, S. M. Drug Therapy of the Metabolic Syndrome: Minimizing the Emerging Crisis in Polypharmacy. *Nat. Rev. Drug Discovery* **2006**, *5*, 295−309.

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81

(5) Grundy, S. M.; Cleeman, J. I.; Daniels, S. R.; Donato, K. A.; Eckel, R. H.; Franklin, B. A.; Gordon, D. J.; Krauss, R. M.; Savage, P. J.; Smith, S. C.; Spertus, J. A.; Costa, F. Diagnosis and Management of the Metabolic Syndrome. *Circulation* **2005**, *112*, 2735−2752.
(6) Morphy, R.; Rankovic, Z. Designed Multiple Ligands. An Emerging Drug Discovery Paradigm. *J. Med. Chem.* **2005**, *48*, 6523−6543.
(7) Tontonoz, P.; Spiegelman, B. M. Fat and beyond: The Diverse Biology of PPAR Gamma. *Annu. Rev. Biochem.* **2008**, *77*, 289−312.
(8) Staels, B.; Fruchart, J.-C. Therapeutic Roles of Peroxisome Proliferator-activated Receptor Agonists. *Diabetes* **2005**, *54*, 2460.
(9) Ahmadian, M.; Suh, J. M.; Hah, N.; Liddle, C.; Atkins, A. R.; Downes, M.; Evans, R. M. PPARγ Signaling and Metabolism: The Good, the Bad and the Future. *Nat. Med.* **2013**, *99*, 557−566.
(10) Rohatgi, A.; McGuire, D. K. Effects of the Thiazolidinedione Medications on Micro- and Macrovascular Complications in Patients with Diabetes - Update 2008. *Cardiovasc. Drugs Ther.* **2008**, *22*, 233−240.
(11) Cariou, B.; Charbonnel, B.; Staels, B. Thiazolidinediones and PPARγ Agonists: Time for a Reassessment. *Trends Endocrinol. Metab.* **2012**, *23*, 205−215.
(12) Taeye, B. M. De; Morisseau, C.; Coyle, J.; Covington, J. W.; Yang, J.; Murphy, S. B.; Friedman, D. B.; Hammock, B. B.; Vaughan, E. Expression and Regulation of Soluble Epoxide Hydrolase in Adipose Tissue. *Obesity* **2010**, *18*, 489−498.
(13) Imig, J. D.; Hammock, B. D. Soluble Epoxide Hydrolase as a Therapeutic Target for Cardiovascular Diseases. *Nat. Rev. Drug Discovery* **2009**, *8*, 794−805.
(14) Huang, A.; Sun, D.; Jacobson, A.; Carroll, M. a.; Falck, J. R.; Kaley, G. Epoxyeicosatrienoic Acids Are Released to Mediate Shear Stress-Dependent Hyperpolarization of Arteriolar Smooth Muscle. *Circ. Res.* **2005**, *96*, 376−383.
(15) Fleming, I. The Pharmacology of the Cytochrome P450 Epoxygenase/soluble Epoxide Hydrolase Axis in the Vasculature and Cardiovascular Disease. *Pharmacol. Rev.* **2014**, *66*, 1106−1140.
(16) Liu, Y.; Zhang, Y.; Schmelzer, K.; Lee, T.-S.; Fang, X.; Zhu, Y.; Spector, A. A.; Gill, S.; Morisseau, C.; Hammock, B. D.; Shyy, J. Y.-J. The Antiinflammatory Effect of Laminar Flow: The Role of PPARgamma, Epoxyeicosatrienoic Acids, and Soluble Epoxide Hydrolase. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 16747−16752.
(17) Xu, D. Y.; Davis, B. B.; Wang, Z. H.; Zhao, S. P.; Wasti, B.; Liu, Z. L.; Li, N.; Morisseau, C.; Chiamvimonvat, N.; Hammock, B. D. A Potent Soluble Epoxide Hydrolase Inhibitor, T-AUCB, Acts through PPARγ to Modulate the Function of Endothelial Progenitor Cells from Patients with Acute Myocardial Infarction. *Int. J. Cardiol.* **2013**, *167*, 1298−1304.
(18) Luo, P.; Chang, H.-H.; Zhou, Y.; Zhang, S.; Hwang, S. H.; Morisseau, C.; Wang, C.-Y.; Inscho, E. W.; Hammock, B. D.; Wang, M.-H. Inhibition or Deletion of Soluble Epoxide Hydrolase Prevents Hyperglycemia, Promotes Insulin Secretion, and Reduces Islet Apoptosis. *J. Pharmacol. Exp. Ther.* **2010**, *334*, 430−438.
(19) Capdevila, J. H.; Falck, J. R.; Imig, J. D. Roles of the Cytochrome P450 Arachidonic Acid Monooxygenases in the Control of Systemic Blood Pressure and Experimental Hypertension. *Kidney Int.* **2007**, *72*, 683−689.
(20) Imig, J. D.; Walsh, K. a; Hye Khan, M. A.; Nagasawa, T.; Cherian-Shaw, M.; Shaw, S. M.; Hammock, B. D. Soluble Epoxide Hydrolase Inhibition and Peroxisome Proliferator Activated Receptor γ Agonist Improve Vascular Function and Decrease Renal Injury in Hypertensive Obese Rats. *Exp. Biol. Med.* **2012**, *237*, 1402−1412.
(21) Buscató, E.; Blöcher, R.; Lamers, C.; Klingler, F.-M.; Hahn, S.; Steinhilber, D.; Schubert-Zsilavecz, M.; Proschak, E. Design and Synthesis of Dual Modulators of Soluble Epoxide Hydrolase and Peroxisome Proliferator-Activated Receptors. *J. Med. Chem.* **2012**, *55*, 10771−10775.
(22) Sime, M.; Allan, A. C.; Chapman, P.; Fieldhouse, C.; Giblin, G. M. P.; Healy, M. P.; Lambert, M. H.; Leesnitzer, L. M.; Lewis, A.; Merrihew, R. V.; Rutter, R. a.; Sasse, R.; Shearer, B. G.; Wilson, T. M.; Xu, R. X.; Virley, D. J. Discovery of GSK1997132B a Novel Centrally Penetrant Benzimidazole PPARγ Partial Agonist. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5568−5572.
(23) Thalji, R. K.; McAtee, J. J.; Belyanskaya, S.; Brandt, M.; Brown, G. D.; Costell, M. H.; Ding, Y.; Dodson, J. W.; Eisennagel, S. H.; Fries, R. E.; Gross, J. W.; Harpel, M. R.; Holt, D. a.; Israel, D. I.; Jolivette, L. J.; Krosky, D.; Li, H.; Lu, Q.; Mandichak, T.; Roethke, T.; Schnackenberg, C. G.; Schwartz, B.; Shewchuk, L. M.; Xie, W.; Behm, D. J.; Douglas, S. a.; Shaw, A. L.; Marino, J. P. Discovery of 1-(1,3,5-Triazin-2-Yl)piperidine-4-Carboxamides as Inhibitors of Soluble Epoxide Hydrolase. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3584−3588.
(24) Nomura, M.; Tanase, T.; Ide, T.; Tsunoda, M.; Suzuki, M.; Uchiki, H.; Murakami, K.; Miyachi, H. Design, Synthesis, and Evaluation of Substituted Phenylpropanoic Acid Derivatives as Human Peroxisome Proliferator Activated Receptor Activators. Discovery of Potent and Human Peroxisome Proliferator Activated Receptor Alpha Subtype-Selective Activators. *J. Med. Chem.* **2003**, *46*, 3581−3599.
(25) Ohashi, M.; Nakagome, I.; Kasuga, J. I.; Nobusada, H.; Matsuno, K.; Makishima, M.; Hirono, S.; Hashimoto, Y.; Miyachi, H. Design, Synthesis and in Vitro Evaluation of a Series of Alpha-Substituted Phenylpropanoic Acid PPARgamma Agonists to Further Investigate the Stereochemistry-Activity Relationship. *Bioorg. Med. Chem.* **2012**, *20*, 6375−6383.
(26) Schmitt, M. L.; Hauser, A. T.; Carlino, L.; Pippel, M.; Schulz-Fincke, J.; Metzger, E.; Willmann, D.; Yiu, T.; Barton, M.; Schüle, R.; Sippl, W.; Jung, M. Nonpeptidic Propargylamines as Inhibitors of Lysine Specific Demethylase 1 (LSD1) with Cellular Activity. *J. Med. Chem.* **2013**, *56*, 7334−7342.
(27) Hieke, M.; Ness, J.; Steri, R.; Greiner, C.; Werz, O.; Schubert-Zsilavecz, M.; Weggen, S.; Zettl, H. SAR Studies of Acidic Dual Gamma-secretase/PPARgamma Modulators. *Bioorg. Med. Chem.* **2011**, *19*, 5372−5382.
(28) 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.
(29) Giannetti, A. M.; Zheng, X.; Skelton, N. J.; Wang, W.; Bravo, B. J.; Bair, K. W.; Baumeister, T.; Cheng, E.; Crocker, L.; Feng, Y.; Gunzner-Toste, J.; Ho, Y.-C.; Hua, R.; Liederer, B. M.; Liu, Y.; Ma, X.; O'Brien, T.; Oeh, J.; Sampath, D.; Shen, Y.; Wang, C.; Wang, L.; Wu, H.; Xiao, Y.; Yuen, P.; Zak, M.; Zhao, G.; Zhao, Q.; Dragovich, P. S. Fragment-Based Identification of Amides Derived from Trans-2-(Pyridin-3-Yl)cyclopropanecarboxylic Acid as Potent Inhibitors of Human Nicotinamide Phosphoribosyltransferase (NAMPT). *J. Med. Chem.* **2014**, *57*, 770−792.
(30) Reichelt, A.; Gaul, C.; Frey, R. R.; Kennedy, A.; Martin, S. F. Design, Synthesis, and Evaluation of Matrix Metalloprotease Inhibitors Bearing Cyclopropane-Derived Peptidomimetics as P1′ and P2′ Replacements. *J. Org. Chem.* **2002**, *67*, 4062−4075.
(31) Morini, G.; Comini, M.; Rivara, M.; Rivara, S.; Bordi, F.; Plazzi, P. V.; Flammini, L.; Saccani, F.; Bertoni, S.; Ballabeni, V.; Barocelli, E.; Mor, M. Synthesis and Structure-Activity Relationships for Biphenyl H3 Receptor Antagonists with Moderate Anti-Cholinesterase Activity. *Bioorg. Med. Chem.* **2008**, *16*, 9911−9924.
(32) Zhao, H. The Synthesis and Structures of Deuterium-Labeled 5-Substituted 1 H -Tetrazoles. *J. Labelled Compd. Radiopharm.* **2008**, *51*, 293−296.
(33) Xing, L.; McDonald, J. J.; Kolodziej, S. a; Kurumbail, R. G.; Williams, J. M.; Warren, C. J.; O'Neal, J. M.; Skepner, J. E.; Roberds, S. L. Discovery of Potent Inhibitors of Soluble Epoxide Hydrolase by Combinatorial Library Design and Structure-Based Virtual Screening. *J. Med. Chem.* **2011**, *54*, 1211−1222.
(34) Ishiyama, M.; Tominaga, H.; Shiga, M.; Sasamoto, K.; Ohkura, Y.; Ueno, K. A Combined Assay of Cell Viability and in Vitro Cytotoxicity with a Highly Water-Soluble Tetrazolium Salt, Neutral Red and Crystal Violet. *Biol. Pharm. Bull.* **1996**, *19*, 1518−1520.
(35) Morisseau, C.; Goodrow, M. H.; Newman, J. W.; Wheelock, C. E.; Dowdy, D. L.; Hammock, B. D. Structural Refinement of Inhibitors

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81

of Urea-Based Soluble Epoxide Hydrolases. *Biochem. Pharmacol.* **2002**, *63*, 1599−1608.
(36) Shen, L.; Peng, H.; Zhao, S.; Xu, D. A Potent Soluble Epoxide Hydrolase Inhibitor, T-AUCB, Modulates Cholesterol Balance and Oxidized Low Density Lipoprotein Metabolism in Adipocytes in Vitro. *Biol. Chem.* **2014**, *395*, 443−451.
(37) Gomez, G. A.; Morisseau, C.; Hammock, B. D.; Christianson, D. W. Structure of Human Epoxide Hydrolase Reveals Mechanistic Inferences on Bifunctional Catalysis in Epoxide and Phosphate Ester Hydrolysis. *Biochemistry* **2004**, *43*, 4716−4723.
(38) Guan, Y.; Breyer, M. D. Peroxisome Proliferator-Activated Receptors (PPARs): Novel Therapeutic Targets in Renal Disease. *Kidney Int.* **2001**, *60*, 14−30.
(39) Lu, M.; Sarruf, D. A.; Talukdar, S.; Sharma, S.; Li, P.; Bandyopadhyay, G.; Nalbandian, S.; Fan, W.; Gayen, J. R.; Mahata, S. K.; Webster, N. J.; Schwartz, M. W.; Olefsky, J. M. Brain PPARγ Promotes Obesity and Is Required for the Insulin− Sensitizing Effect of Thiazolidinediones. *Nat. Med.* **2011**, *17*, 618−622.
(40) Lamers, C.; Schubert-Zsilavecz, M.; Merk, D. Therapeutic Modulators of Peroxisome Proliferator-Activated Receptors (PPAR): A Patent Review (2008-Present). *Expert Opin. Ther. Pat.* **2012**, *22*, 803−841.
(41) Niesen, F. H.; Berglund, H.; Vedadi, M. The use of differential scanning fluorimetry to detect ligand interactions that promote protein stability. *Nat. Protoc.* **2007**, *2*, 2212−21.
(42) Huang, S. X.; Li, H. Y.; Liu, J. Y.; Morisseau, C.; Hammock, B. D.; Long, Y. Q. Incorporation of Piperazino Functionality into 1,3-Disubstituted Urea as the Tertiary Pharmacophore Affording Potent Inhibitors of Soluble Epoxide Hydrolase with Improved Pharmacokinetic Properties. *J. Med. Chem.* **2010**, *53*, 8376−8386.
(43) Peters, J. U. Polypharmacology − Foe or Friend? *J. Med. Chem.* **2013**, *56*, 8955−8971.
(44) Yang, T.; Soodvilai, S. Renal and Vascular Mechanisms of Thiazolidinedione-Induced Fluid Retention. *PPAR Res.* **2008**, *2008*, 943614.
(45) Wolf, N. M.; Morisseau, C.; Jones, P. D.; Hock, B.; Hammock, B. D. Development of a High-Throughput Screen for Soluble Epoxide Hydrolase Inhibition. *Anal. Biochem.* **2006**, *355*, 71−80.
(46) Rau, O.; Wurglics, M.; Paulke, A.; Zitzkowski, J.; Meindl, N.; Bock, A.; Dingermann, T.; Abdel-Tawab, M.; Schubert-Zsilavecz, M. Carnosic Acid and Carnosol, Phenolic Diterpene Compounds of the Labiate Herbs Rosemary and Sage, Are Activators of the Human Peroxisome Proliferator-Activated Receptor Gamma. *Planta Med.* **2006**, *72*, 881−887.
(47) Brown, P. J.; Stuart, L. W.; Hurley, K. P.; Lewis, M. C.; Winegar, D. A.; Wilson, J. G.; Wilkison, W. O.; Ittoop, O. R.; Willson, T. M. Identification of a Subtype Selective Human PPARalpha Agonist through Parallel-Array Synthesis. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1225−1227.
(48) Berger, J.; Leibowitz, M. D.; Doebber, T. W.; Elbrecht, A.; Zhang, B.; Zhou, G.; Biswas, C.; Cullinan, C. A.; Hayes, N. S.; Li, Y.; Tanen, M.; Ventre, J.; Wu, M. S.; Berger, G. D.; Mosley, R.; Marquis, R.; Santini, C.; Sahoo, S. P.; Tolman, R. L.; Smith, R. G.; Moller, D. E. Novel Peroxisome Proliferator-Activated Receptor (PPAR) Gamma and PPARdelta Ligands Produce Distinct Biological Effects. *J. Biol. Chem.* **1999**, *274*, 6718−6725.
(49) Zebisch, K.; Voigt, V.; Wabitsch, M.; Brandsch, M. Protocol for Effective Differentiation of 3T3-L1 Cells to Adipocytes. *Anal. Biochem.* **2012**, *425*, 88−90.
(50) Sisignano, M.; Park, C.-K.; Angioni, C.; Zhang, D. D.; von Hehn, C.; Cobos, E. J.; Ghasemlou, N.; Xu, Z.-Z.; Kumaran, V.; Lu, R.; Grant, A.; Fischer, M. J. M.; Schmidtko, A.; Reeh, P.; Ji, R.-R.; Woolf, C. J.; Geisslinger, G.; Scholich, K.; Brenneis, C. 5,6-EET Is Released upon Neuronal Activity and Induces Mechanical Pain Hypersensitivity via TRPA1 on Central Afferent Terminals. *J. Neurosci.* **2012**, *32*, 6364−6372.

DOI: 10.1021/acs.jmedchem.5b01239
J. Med. Chem. 2016, 59, 61−81