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Discovery of Potent Inhibitors of Human and Mouse Fatty Acid Amide Hydrolases

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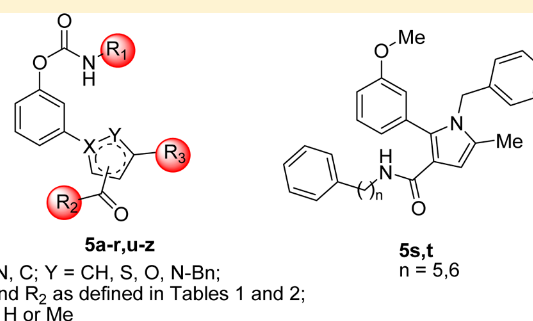
Supporting Information

ABSTRACT: Fatty acid amide hydrolase (FAAH, EC 3.5.1.99) is the main enzyme catabolizing endocannabinoid fatty acid amides. FAAH inactivation promotes beneficial effects upon pain and anxiety without the side effects accompanying agonists of type-1 cannabinoid receptors. Aiming at discovering new selective FAAH inhibitors, we developed a series of compounds (**5a−u**) characterized by a functionalized heteroaromatic scaffold. Particularly, **5c** and **5d** were identified as extremely potent, noncompetitive, and reversible FAAH inhibitors endowed with a remarkable selectivity profile and lacking interaction with the hERG channels. In vivo antinociceptive activity was demonstrated for **5c**, **5d**, and **5n** at a dose much lower than that able to induce either striatal and limbic stereotypies or anxiolytic activity, thus outlining their potential to turn into optimum preclinical candidates. Aiming at improving pharmacokinetic properties and metabolic stability of **5d**, we developed a subset of nanomolar dialyzable FAAH inhibitors (**5v−z**), functionalized by specific polyethereal lateral chains and fluorinated aromatic rings.



INTRODUCTION

Endogenous cannabinoids (endocannabinoids, ECs) are a class of signaling lipids such as *N*-arachidonoylethanolamine (anandamide, AEA, **1**, Chart 1), oleamide, and 2-arachidonoylglycerol (2-AG), which activate type-1 and type-2 cannabinoid receptors (CB_1R and CB_2R) to modulate a range of responses and processes including pain, inflammation, appetite, motility, sleep, thermoregulation, and cognitive and emotional states.[1,2] The actions of these signaling lipids are rapidly terminated by cellular reuptake through a purported AEA membrane transporter (AMT),[3,4] trafficking through intracellular transporters,[5,6] and subsequent hydrolysis operated by a number of enzymes. Among the latter, the fatty acid amide hydrolase (FAAH, EC 3.5.1.99) was originally identified as the enzyme responsible for rat oleamide hydrolysis,[7] while in humans it preferably hydrolyses **1** and is also the main regulator of the endogenous tone of **1** in vivo.[8] In addition to FAAH, *N*-acylethanolamine acid amidase (NAAA)[9] and monoacylglycerol lipase (MAGL)[10] were later identified as additional ECs-metabolizing enzymes. A current therapeutically attractive approach aims at eliciting the desirable effects of CBRs activation while avoiding the negative effects of global CB_1R stimulation. Accordingly, inactivation of FAAH would be expected to elevate the endogenous concentrations of all its substrates and consequently prolong and potentiate their beneficial (therapeutic) effects on pain and anxiety without eliciting the classical CB_1R agonists side effects (hypomotility, hypothermia, and catalepsy).[11] In humans, FAAH was mainly detected in pancreas, brain, kidney, skeletal muscle, placenta, and less abundantly in liver.[12] The importance of FAAH was demonstrated by the generation of *faah* knockout mice that showed elevated resting brain concentration of **1** and manifested: (i) analgesic phenotype in both the carrageenan model of inflammatory pain and in the formalin model of spontaneous pain,[13] (ii) reduction in inflammatory responses,[14] and (iii) improvements in slow wave sleep and memory acquisition.[15,16] Of the two isoforms of the enzyme, FAAH1 is a

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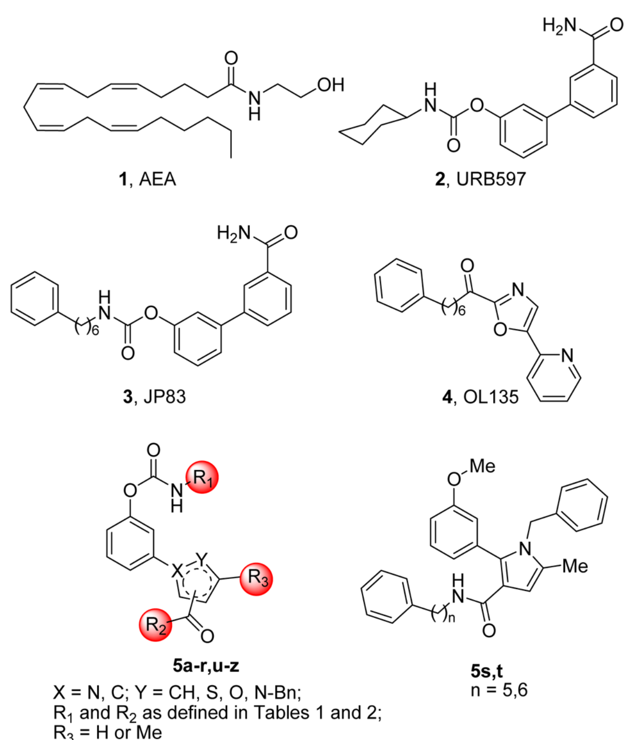
Chart 1. Substrate and Representative Reversible and Irreversible FAAH Inhibitors and Title Compounds



membrane-bound enzyme belonging to the amidase family. The analysis of its crystal structure[17] revealed a core composed of a characteristic Ser-Ser-Lys catalytic triad that is buried deep within the enzyme and can be accessed by two narrow channels. The right side hydrophobic tunnel, termed acyl binding channel (ACB), leads to the hydrophilic catalytic triad from the membrane-bound surface and bifurcates into two subchannels. The left side channel, known as cytoplasmic access channel (CA) or cytosolic port (CP), is exposed to the solvent, emerges at about 80° angle from the ACB, and represents the back door by which the hydrolysis product leaves the enzyme.[17]

The FAAH inhibitors known so far, depending on their mechanism of action, can be classified as irreversible and reversible inhibitors. While the early irreversible inhibitors (e.g., methoxyarachidonoyl fluorophosphonate, MAFP, IC_{50} = 1−3 nM) were substrate-inspired ligands,[18] a more recently characterized class of inhibitors was the carbamate-based compounds, represented by URB597 (**2**, Chart 1, IC_{50} = 4.6 nM).[19] The early structure−activity relationship (SAR) studies on the URB series of analogues demonstrated the presence of an activated carbonyl as a key feature for FAAH inhibition. Kinetic studies evidenced a noncompetitive and nondialyzable (thus considered "irreversible") mechanism of inhibition, suggesting a covalent binding with FAAH that was later confirmed by mass spectrometry analysis.[20,21] Biochemical evidence suggest that **2** and related analogues covalently bind the FAAH active site by adopting an orientation in which the *O*-biaryl substituents reside in the CA of the enzyme, where they would be susceptible to enzyme-catalyzed protonation to enhance their function as leaving groups.[21] On the basis of this background, the *N*-6-phenylhexylcarbamate analogue JP83 (**3**, Chart 1, IC_{50} = 14 nM) was later synthesized to confirm the theoretical binding mode.[22] In the context of FAAH reversible inhibitors, the major breakthrough was represented by the development of a series of α-ketoheterocycles.[23−25] These ligands were characterized by the presence of a properly activated ketone functionality, suitable for the active site Ser241 nucleophilic attack. Among them, a potent and selective FAAH reversible inhibitor (OL135, **4**, Chart 1, K_i = 4.7 nM)[26] was identified as a valuable tool for the investigation of FAAH pharmacology.[27]

With the aim of discovering new scaffolds for selective FAAH inhibition, we recently designed, on the model of the known FAAH inhibitors, a series of ligands where specific heterocyclic systems were introduced to modulate potency and lipophilicity. We herein present a series of new potent and selective FAAH inhibitors (**5a−z**, Chart 1, and Tables 1 and 2), characterized by a phenylheterocyclic structure.[28] Starting from a small set of analogues (**5a−d**), comprising the structure of our 1-phenylpyrrole-based lead compound **5d**, the rational SAR studies were performed by varying and combining: (i) the length and steric hindrance of the distal chain at the carbamoyl system (R_1 of compounds **5a−r,u** of Chart 1 and Table 1: *n*-butyl, cyclohexyl, undecynyl, phenylhexyl), (ii) the amide/ester replacement (R_2 of compounds **5e−i**, Table 1), (iii) the nature of the heteroaryl moiety (2-furan, **5j,l,n**, 2-thiophene, **5k,m,o**, and 2-benzylpyrrole, **5s−u**, Table 1) and the position of the amide functionality on the heteroaryl system (**5p−r**, Table 1), and (iv) the introduction of a hydrophilic lateral chain at the carbamoyl system ((mono/di/trifluoro)phenoxyethoxyethyl substituted analogues, R_2 of compounds **5v−z**, Table 2). Synthesis, molecular modeling, and in vitro and in vivo biological properties (efficacy and toxicity profiles) of the new class of FAAH inhibitors are discussed in the present article.

Chemistry. The chemistry for the synthesis of compounds **5a−z** is described in Schemes 1−5. The isocyanates were prepared as reported in Scheme 1. Alcohols **6a,b** were submitted to a Mitsunobu reaction in the presence of phtalimide, triphenylphosphine, and diisopropyl azodicarboxylate (DIAD), and the resulting products were treated with hydrazine monohydrate in refluxing ethanol to afford amines **7a,b**. Reaction of **7a** with phosgene furnished the 6-phenyl-1-hexylisocyanate **8**. 11-Isocyanatoundec-1-yne **11** was obtained from alcohol **9**. The latter compound was converted into the corresponding azide (via the mesylate). Subsequent reduction of the azido-derivative to amine with iron(III) chloride and sodium iodide followed by treatment with phosgene led to **11**. Phenoxyethoxyethyl isocyanates **15a−e** were obtained starting from diethylene glycol **12** that was monobrominated in the presence of carbon tetrabromide and triphenylphosphine to generate **13**. This bromide was reacted with the suitable phenols in the presence of potassium iodide and potassium hydroxide to provide alcohols **14a−e**. Mitsunobu reaction followed by treatment with hydrazine monohydrate and then phosgene led to the desired isocyanates **15a−e**.

The synthesis of compounds **5a−i** and **5v−z** is reported in Scheme 2. 2,5-Dimethoxytetrahydrofuran-3-carboxaldehyde **16**, after treatment with iodine and ammonium hydroxide, led to its corresponding 3-cyanoderivative **17**.[29] Subsequent Clauson−Kaas reaction with 3-aminophenol generated the 1-phenyl-3-cyanopyrrole derivative **18**, which was hydrolyzed to the corresponding amide **19** in the presence of hydrogen peroxide. Reaction of **19** with the suitable alkylisocyanates afforded the title compounds **5a−d**, whereas reaction with the differently functionalized phenoxyethoxyethyl isocyanates afforded the final products **5v−z**. Clauson−Kaas reaction of 3-aminophenol

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Table 1. Inhibition Activity towards Mouse Brain FAAH (K_i and IC_{50}, nM) for Compounds 5a−u and towards Human Recombinant FAAH (IC_{50}, nM) for Selected Compounds

Compd	Chemical Structure	R_1	R_2	X	*m*FAAH K_i (nM)[a]	*m*FAAH IC_{50} (nM)[a]	*hr*FAAH IC_{50} (nM)[a]
5a		$(\,)_3$Me	NH_2	-	60	240	NT[b]
5b			NH_2	-	32	120	NT[b]
5c		$(\,)_9$	NH_2	-	**0.18**	0.67	170
5d		$(\,)_6$	NH_2	-	**0.16**	0.60	NT[b]
5e	O, O, N, H, R_1, N, O, R_2	$(\,)_3$Me	O $(\,)_9$	-	16[c] 9[d] 7[e]	>70	NT[b]
5f		$(\,)_3$Me	O $(\,)_6$	-	18[c] 7[d] 7[e]	>200	NT[b]
5g			O $(\,)_9$	-	53[c] 46[d] 37[e]	>10000	NT[b]
5h			O $(\,)_6$		69[c] 63[d] 57[e]	>50000	NT[b]
5i		$(\,)_6$	O $(\,)_9$	-	11[c] 6[d] 5[e]	>200	NT[b]
5j	HN, R_1, O, O, X, Me, O, R_2	$(\,)_3$Me	NH_2	O	140	530	NT[b]
5k		$(\,)_3$Me	NH_2	S	30[c] 21[d] 21[e]	>200	NT[b]
5l			NH_2	O	9[c] 10[d] 9[e]	>70	NT[b]
5m			NH_2	S	8[c] 9[d] 8[e]	>200	NT[b]
5n		$(\,)_6$	NH_2	O	**0.49**	1.7	**102**
5o		$(\,)_6$	NH_2	S	**0.50**	1.9	NT[b]
5p	O, O, N, H, R_1, O, Me, R_2, O	$(\,)_3$Me	NH_2	-	17[c] 12[d] 15[e]	>200	NT[b]
5q			NH_2	-	19[c] 19[d] 13[e]	>200	NT[b]
5r		$(\,)_6$	NH_2	-	**4.8**	18	NT[b]
5s	O, R_1, N, Me, R_2, O	Me	H N $(\,)_5$	-	84[c] 91[d] 69[e]	>50000	NT[b]
5t		Me	H N $(\,)_6$	-	33[c] 29[d] 20[e]	>200	NT[b]
5u		O, H N	H N $(\,)_6$	-	39[c] 27[d] 15[e]	>200	NT[b]
2[20]		-	-	-	1.2	4.6	
3[22]		-	-	-	-	14	36[f]
4[26]		-	-	-	4.7	-	628[f]

[a]Each value is the mean of at least three experiments (all SD are within 10%); [b]NT not tested; [c]FAAH inhibition as residual FAAH activity % after preincubation with 10 μM of the tested compound (**2** inhibition % was 16% at 10 μM); [d]FAAH inhibition as residual FAAH activity % after preincubation with 25 μM of the tested compound (**2** inhibition % was 16% at 10 μM); [e]FAAH inhibition as residual FAAH activity % after preincubation with 50 μM of the tested compound (**2** inhibition % was 16% at 10 μM).; [f]done at Caliper (see: www.CaliperLS.com).

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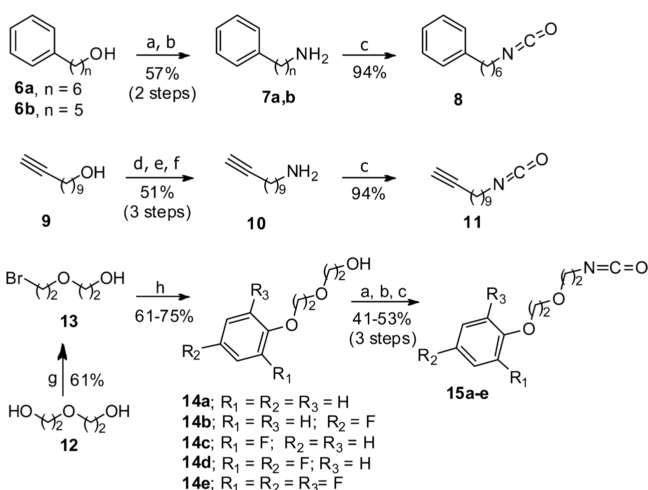
Table 2. Determination of the Inhibition Activity towards Mouse Brain FAAH (as K_i and IC_{50}, nM) for Polyethereal Compounds 5v−z and Calculated Solubility Properties

Compd	Chemical Structure	R_1	K_i (nM)[a]	IC_{50} (nM)[a]	LogP[b]	LogS in water at pH = 7[b]
5d			**0.16**	0.60	4.75	-5.69
5v			23	87	**2.42**	**-4.42**
5w			8	31	**2.71**	**-4.72**
5x			18	69	4.70	-5.65
5y			7	28	4.74	-5.80
5z			11	43	4.68	-5.86

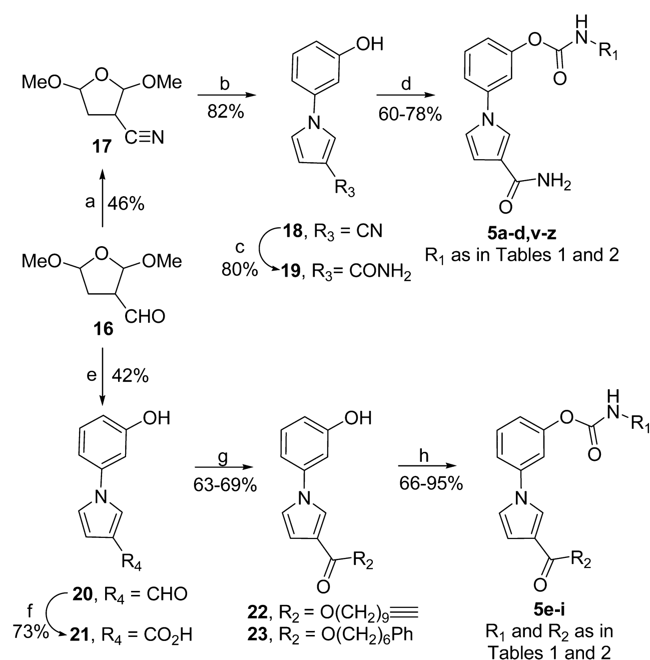
[a]Each value is the mean of at least three independent experiments (all SD are within 10%). [b]ACD/Labs V12.0, Toronto, Canada.

Scheme 1. Synthesis of Isocyanates 8, 11, and 15a−e[a]



[a]Reagents and conditions: (a) phthalimide, DIAD, PPh_3, THF, from 0 °C to rt, 10 h; (b) $N_2H_4 \cdot H_2O$, EtOH, reflux, 4 h; (c) from **7a**: $COCl_2$ (20% in toluene), $NaHCO_3$, H_2O, DCM, 0 °C, 15 min; (d) MsCl, TEA, THF, rt, 15 min; (e) NaN_3, DMF, 85 °C, 4 h; (f) $FeCl_3$, NaI, MeCN, reflux, 1 h; (g) CBr_4, PPh_3, MeCN, rt, 12 h; (h) appropriate phenol, KI, KOH, MeCN, 60 °C, 11 h.

Scheme 2. Synthesis of Compounds 5a−i and 5v−z[a]

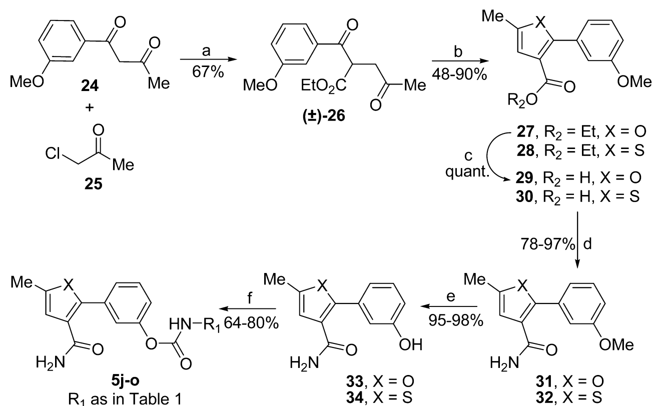


[a]Reagents and conditions: (a) I_2, NH_4OH, THF, rt, 1 h; (b) 3-aminophenol, dioxane, 5 N HCl, reflux, 10 min; (c) 30% H_2O_2, 6 N NaOH, EtOH, reflux 12 h; (d) appropriate isocyanate, TEA, dry THF, rt, 16 h; (e) 3-aminophenol, AcOH, H_2O, 100 °C, 15 min; (f) $AgNO_3$, MeOH, 2 N NaOH, reflux, 1 h; (g) appropriate alcohol, PPh_3, DIAD, dry THF, 0 °C to rt, 48 h; (h) appropriate isocyanate, TEA, dry THF, rt, 16 h.

with 2,5-dimethoxytetrahydrofuran-3-carboxaldehyde **16** afforded the corresponding 1-phenylpyrrole derivative **20**. The aldehyde functionality was then oxidized using silver nitrate. The resulting acid **21** was treated with undecyn-1-yl alcohol and 6-phenyl-1-hexyl alcohol, in the presence of triphenylphosphine and DIAD, to afford esters **22** and **23**, respectively. Reaction of the latter compounds with the appropriate isocyanate yielded the corresponding carbamates **5e−i**.

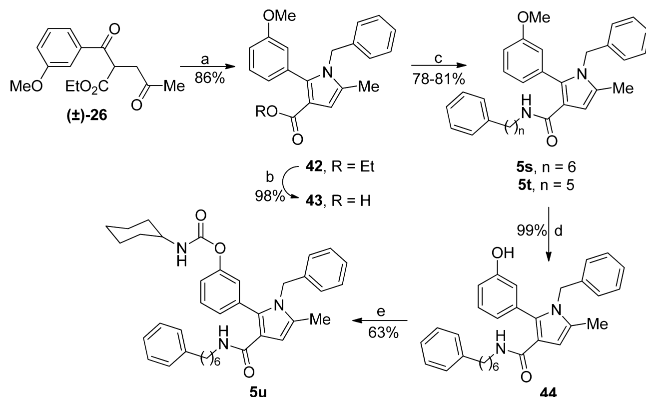
Alkylation of the 1,3-diketone **24** (Scheme 3) with chloroacetone **25** in the presence of sodium hydride afforded the key intermediate **26**. The microwave (MW)-assisted Paal−Knorr reaction of the latter substances in the presence of concentrated hydrochloric acid resulted in the formation of phenylfuran **27**. Analogously, MW-assisted Paal−Knorr reaction of **26** in the presence of Lawesson's reagent afforded thiophene-derivative **28**.[30] The hydrolysis of the ester groups led to the corresponding acids **29** and **30**. Subsequent treatment with thionyl chloride and ammonium hydroxide afforded the corresponding amides **31** and **32**. Methoxy groups deprotection with boron tribromide followed by reaction of the corresponding phenols with the appropriate isocyanate afforded carbamates **5j−o**.

Scheme 3. Synthesis of Compounds 5j−o[a]



[a]Reagents and conditions: (a) NaH, dry THF, 0 °C, 30 min then rt, 48 h; (b) X = O, concd HCl, EtOH, MW, 10 min; X = S, Lawesson's reagent, toluene, MW, 10 min; (c) NaOH, EtOH, H_2O, 7 h; (d) $SOCl_2$, reflux, 30 min then concd NH_4OH, THF, rt, 7 h; (e) BBr_3, dry DCM, −78 °C to rt, 8 h; (f) appropriate isocyanate, TEA, dry THF, rt, 16 h.

Scheme 5. Synthesis of Final Compounds 5s−u[a]



[a]Reagents and conditions: (a) benzylamine, AcOH, 130 °C, 150 W, 12 min; (b) NaOH, EtOH/THF 1:1 reflux, 16 h; (c) amine **7a** or **7b**, TEA, HOBt, EDCI, dry DCM, from 0 °C to rt, 16 h; (d) BBr_3, dry DCM, −78 °C to rt, 12 h; (e) **8**, TEA, dry THF, rt, 16 h.

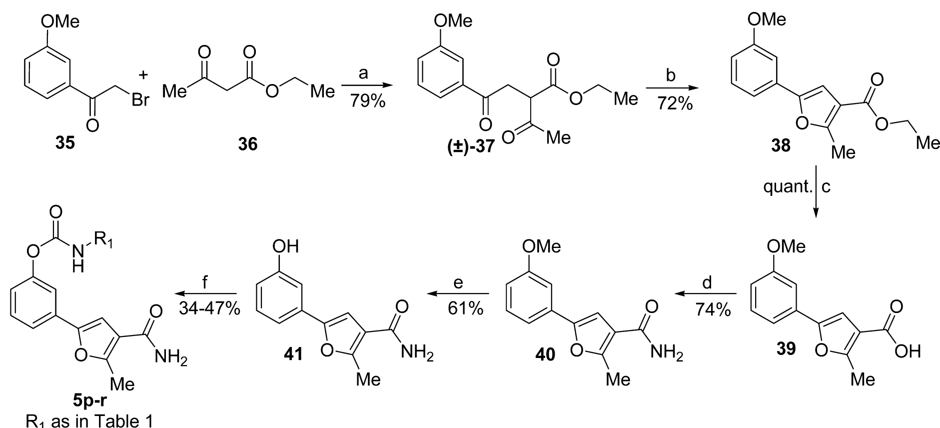
The 1,4-diketo intermediate **37** (Scheme 4) was obtained by alkylation of ethylacetoacetate **36** with the bromide **35**. Treatment of **37** with concentrated hydrochloric acid under MW irradiation afforded the phenylfuran **38**. After saponification of the ester functionality of **38**, the resulting acid **39** was converted into the amide **40** by reaction with thionyl chloride and ammonium hydroxide. The methoxy group was cleaved by treatment with boron tribromide to afford the phenol-derivative **41**. Reaction of **41** with the appropriate isocyanate gave compounds **5p−r**.

Compounds **5s−u** were prepared following Scheme 5. MW-assisted Paal−Knorr cyclization reaction of **26** in the presence of benzylamine and acetic acid led to the benzylpyrrole derivative **42**.[30] After hydrolysis, compounds **5s** and **5t** were prepared by coupling the acid **43** with the suitable amines in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDCI) and *N*-hydroxybenzotriazole (HOBt). Removal of the methoxy group of compound **5s** afforded phenol **44**, which was treated with cyclohexylisocyanate to generate compound **5u**.

RESULTS AND DISCUSSION

Biological Assays. The potency of inhibition of FAAH for the newly developed compounds (**5a−u**, Table 1, and **5v−z**, Table 2) was assessed through inhibition studies performed on the mouse brain enzyme; the relative IC_{50} and K_i values are given in Tables 1 and 2. The inhibitory activity of the most interesting inhibitors of the series (**5c** and **5n**) was confirmed at Caliper with the human recombinant enzyme (Table 1). Molecular modeling studies supported the SAR (Figure 1) and helped in the rationalization of the observed potencies with mouse and human enzymes. For compounds **5d**, **5o**, **5w**, and **5y** (Table 3 and Figure 2) dialysis studies were performed in order to evaluate the reversibility of FAAH inhibition. A subset of compounds (**5c**, **5d**, **5n**, and **5o**) were further examined for their selectivity toward CB_1, CB_2, and vanilloid ($TRPV_1$) receptors (Table 3). The latter are an additional target of **1**.[6] Moreover, the lack of interference of **5c**, **5d**, **5n**, and **5o** with the biosynthesis of AEA and 2-AG and with the hydrolysis of 2-AG was assessed as well as the absence of interaction with a panel of more than 50 CNS monoamine receptors and transporters (Table 1 of the Supporting Information (SI)).

Scheme 4. Synthesis of Compounds 5p[a]



[a]Reagents and conditions: (a) NaH, dry THF, 0 °C, 30 min then rt, 8 h; (b) HCl, EtOH, MW, 10 min; (c) NaOH, EtOH, H_2O, 7 h; (d) $SOCl_2$, reflux, 30 min then NH_4OH, THF, rt, 7 h; (e) BBr_3, dry DCM, −78 °C to rt, 8 h; (f) appropriate isocyanate, TEA, dry THF, rt, 16 h.

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Figure 1. Three-dimensional model of dimeric-FAAH enzyme (turquoise ribbon for A and B chains) in complex with **5c** (A) and **5y** (B) (purple and orange ball and sticks, respectively). Ligand–protein interaction plot of **5c** (C) and **5y** (D) (pink line, H-bond backbone; pink–dot line, H-bond side chain; green spheres are hydrophobic residues, light-blue are polar residues, violet are positively charged residues, red are negatively charged residues, and gray ones are Gly residues).

Table 3. Further Characterization of Mouse FAAH Inhibition, Selectivity, and hERG Activity for Selected Compounds

compd	type of inhibition[a]	CB_1, CB_2, TRPV1	NAPE-PLD, DAGL	MAGL	hERG IC_{50} (μM)[b]
5c	NT[c]	no aff.[d]	no interf.[e]	no interf.[e]	>10
5d	rev. non comp.[f]	no aff.[d]	no interf.[e]	no interf.[e]	>10
5n	NT[c]	no aff.[d]	no interf.[e]	no interf.[e]	>10
5o	rev. non comp.[f]	no aff.[d]	no interf.[e]	no interf.[e]	>10

[a]Determined by microdialysis studies; [b]done at Cerep; [c]NT not tested. [d]no aff. = no affinity; [e]no interf. = no interference; [f]rev. non comp. = reversible non competitive.

Figure 2. (Ir)reversible inhibition of FAAH by reference compounds (**2**, **3**, and **4**) and selected analogues (**5d**, **5o**, **5w**, and **5y**) (A) without dialysis, (B) with dialysis. (Substrate = 10 μM [^{3}H]AEA, control (CT) = 578 ± 16 pmol/min per mg of protein).

Finally, hERG potassium channel activation was assessed for **5c**, **5d**, and **5n** (Table 3).

Moreover, the Irwin test (Table 4) was performed for a preliminary in vivo characterization of orally administered

Table 4. In Vivo Preliminary Behavioral Studies and Analgesic Activity Evaluation after Oral Administration in Mice

		Irwin test limbic and striatal stereotypies		elevated plus maze		Randall−Selitto	
compd	mg/kg	60 min	240 min	mg/kg	anxiolytic effect	mg/kg	analgesia
5c	10	+	absent	3	absent	10	absent
	30	+	absent	10	absent	30	+
	100	+	absent	30	absent	100	+
5d	10	+	+	3	absent	10	absent
	30	+	+	10	absent	30	+
	100	+	+	30	absent	100	+
5n	10	+	absent	3	absent	10	absent
	30	+	absent	10	absent	30	+
	100	+	absent	30	absent	100	+

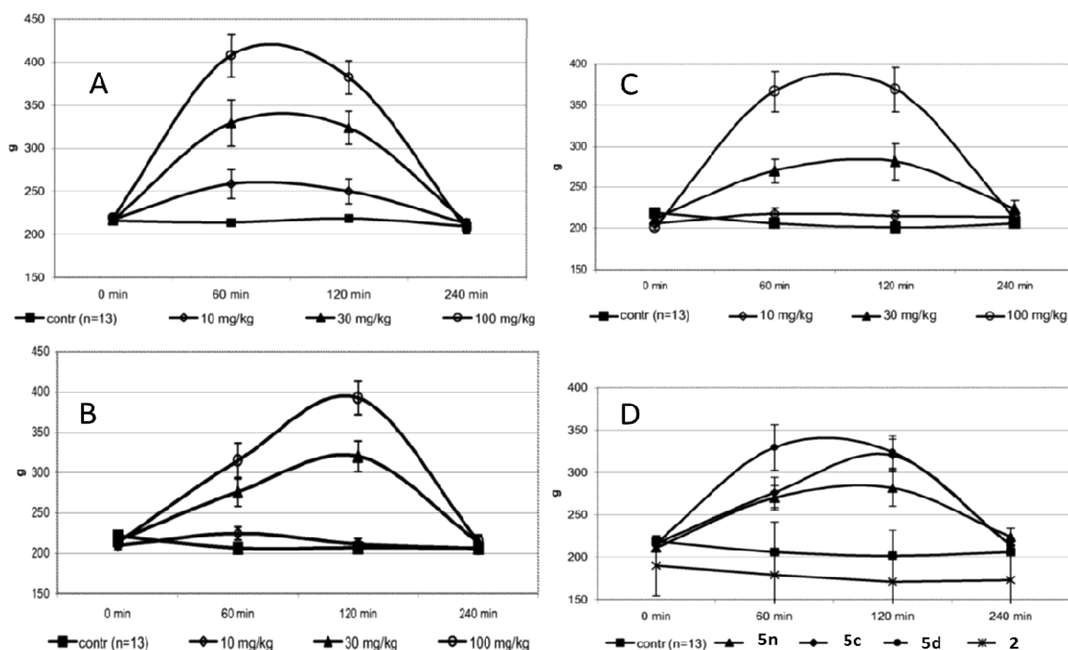

Figure 3. Analgesic effect of the selective FAAH inhibitors. Dose−response curves of the analgesic effect of the selective FAAH inhibitors **5d**, **5c**, and **5n** at 10, 30, and 100 mg/kg (A−C, respectively). Area under curve 0−240 min. Kruskal−Wallis one way analysis of variance on ranks. (A) H = 33.431; DF = 3; P = 0.001. Dunn's test: $P < 0.05$, 30, and 100 mg/kg vs control. (B) H = 35.063; DF = 3; P = 0.001. Dunn's test: $P < 0.05$, 30, and 100 mg/kg vs control. (C) H = 36.183; DF = 3; P = 0.001. Dunn's test: $P < 0.05$, 30, and 100 mg/kg vs control. Analgesic effect of **5c**, **5d**, and **5n** at a dose of 30 mg/kg compared with compound **2** at a dose of 50 mg/kg (D).

compounds **5c**, **5d**, and **5n**, and the Randall−Selitto test (Table 4 and Figure 3) was used for the evaluation of their antalgic activity.

In Vitro FAAH Inhibition, Structure−Activity Relationship (SAR), and Molecular Modeling Studies. With the aim of discovering new scaffolds for selective FAAH inhibition, we designed a series of compounds (**5a**−**z**, Chart 1, and Tables 1 and 2) characterized by a differently decorated phenylheterocyclic structure. Starting from a biaryl structure (on the model of **2** and **3**), and on our 1-phenylpyrrole-based lead compound **5d**, SAR studies were performed by varying and combining: (i) the length and hindrance of the chain attached to the carbamoyl system (n-butyl, cyclohexyl, undecynyl, and phenylhexyl, Table 1), (ii) the amide/ester replacement (**5e**−**i**, Table 1), (iii) the nature of the heteroaryl moiety (2-furan, **5j**,**l**,**n**, 2-thiophene, **5k**,**m**,**o**, and 2-benzylpyrrole, **5u**−**z**, Table 1), and the position of the amide functionality on the heteroaryl system (**5p**−**r**, Table 1), and (iv) the nature of the lateral chain attached to the carbamoyl system (phenoxyethoxyethyl, **5v** and mono/di/trifluorophenoxyethoxyethyl, **5w**−**z**, Table 2). Also, docking studies were performed in order to establish the binding mode of the new inhibitors (Figure 1).

(i). Evaluation of the Effect of Varying Length and Hindrance of the Chain Attached to the Carbamoyl System. Among the 1-phenylpyrrole-based compounds, all the 1-phenylpyrrole-3-carboxamido analogues (**5a**−**d**) were extremely potent FAAH inhibitors. In particular, compounds **5c** and **5d**, bearing the highly hydrophobic, long, and flexible chains (undecinyl and phenylhexyl, respectively) were subnanomolar FAAH inhibitors (**5c**, K_i = 0.18 nM, and **5d**, K_i = 0.16 nM, Table 1). Introduction of the more hindering cyclohexyl moiety lowered the activity by 3 orders of magnitude (**5b**, K_i = 32 nM). To further investigate this effect for 1-phenylpyrrole analogues, the n-butyl analogue **5a** was synthesized and tested, and it was found to be able to inhibit the enzyme with just half of the potency of the parent analogue **5b**. This finding confirms that length and hydrophobicity of the chain at the carbamoyl moiety (rather than the steric hindrance) is of pivotal importance in determining a high affinity for the FAAH active site.

To investigate the binding mode of these new inhibitors, a docking procedure was applied by using a 3D model of mouse dimeric-FAAH1 enzyme. Indeed, in line with literature data, after cocrystallization of MAPF with the enzyme,[17] and as hypothesized for the natural substrate **1**, the hydrophobic interaction engaged by the carbamoyl chain along the FAAH ACB channel is of crucial importance to potently modulate the inhibition potency of these analogues. In general, the molecules bind a pocket that spans from the CP, with the *N*-heteroaryl group, to the ACB channel, with the alkyl (or cycloalkyl) chain. This binding mode suggests the importance of the amide group making H-bonds with Leu380 and Gln273. Furthermore, the pyrrole and alkyl chain could favorably interact with Leu192; this residue is replaced by a Phe in human FAAH1 isoform and could explain the lower inhibition potency observed when **5c** was tested on the human enzyme (**5c**, $K_{ihrFAAH}$ = 170 nM). In Figure 1A,C, the results of the docking studies performed on the picomolar analogue **5c** are reported. H-Bonds between the carbamate and protein backbone stabilize the protein−ligand interaction in the catalytic pocket. In particular, the carbamate carbonyl O is engaged in an H-bond with Gly239 and Ile238 backbone (in the anionic hole), while the carbamate NH establishes an H-bond with the Met191 carbonyl. A series of hydrophobic contacts are possible along the gorge (green spheres surrounding the alkyl chain in Figure 1C), which may further contribute to stabilize the proposed binding mode. Moreover, the presence of an aromatic group at the end of the O-alkyl chain, as in **5d**, could reinforce the binding by means of $\pi-\pi$ stabilization with Phe381.

(ii). Amide/Ester Replacement. Given that FAAH catalytic residues are accessible by two narrow channels, though slightly different in terms of size and hydrophilicity, we reasoned that double-chained analogues could shape these channels, thus giving information about the room available in the proximity of the amide moiety. Starting from the potent inhibitors **5a−d**, we synthesized and tested a small set of analogues replacing the amide moiety by the chemically accessible ester functionality. Although much less efficient than the parent compounds, the ester analogues (**5e−i**) disclosed a number of interesting SAR. The more potent inhibitors were obtained when the phenylhexyl or undecynyl ester chains were combined with a *n*-butyl substituent on the carbamoyl moiety. Compound **5e** was identified as the most active of the series, being equipotent to compound **2**, when tested under the same experimental conditions; instead, the phenylhexyl analogue **5f** was found to be slightly less potent. Increasing the bulk of the carbamoyl substituent (cyclohexyl in place of *n*-butyl, **5g,h**) was detrimental for activity, as already observed for the amide counterparts. Interestingly, compound **5i**, which combines the presence of the two long chained substituents, appeared to accommodate quite well both alkyl chains, being slightly less potent than **2** in inhibiting the enzyme.

(iii). Evaluation of the Effect of Varying the Nature of the Heteroaryl Moiety and Different Positioning of the Heteroaryl Amide Functionality. The effect of introducing different five-membered heteroaryls, in place of the pyrrol-1-yl system of compounds **5a−d**, was widely explored by means of the design of three subsets of analogues: the 2-methyl-4,5-difunctionalyzed furans and the thiophene analogues (**5j,l,n** and **5k,m,o**, respectively) and the 2-methyl-3,5-difunctionalyzed furan-based compounds (**5p−r**). All the synthesized compounds confirmed the previously observed SAR, and the FAAH inhibition potency followed the trend *n*-butyl ≤ cyclohexyl < phenylhexyl. The three phenylhexyl analogues were indeed the most potent of the series (**5n**, K_i = 0.49 nM; **5o**, K_i = 0.50 nM; **5r**, K_i = 4.80 nM). The activity of **5n** was also measured on human FAAH (**5n**, $K_{ihrFAAH}$ = 102 nM). Analysis of the FAAH inhibition data revealed that the 2-methyl-4,5-difunctionalyzed furan and thiophene were slightly preferred over the 2-methyl-3,5-difunctionalyzed furan moiety. The insertion of an extra volume by means of an *N*-benzylpyrrole moiety reduced the inhibitor potency (**5u**). The same detrimental effect was observed when replacing the urethane system with a methoxy group (**5s** and **5t**).

(iv). Effect of the Insertion of Arylpolyethereal Chains Attached to the Carbamoyl System. As a further step aimed at improving the hydrophilic and pharmacokinetic properties of the parent compounds, while retaining their in vitro pharmacology and selectivity profile, we designed and synthesized a subset of analogues (**5v−z**), characterized by the 1-phenylpyrrole scaffold functionalized by specific polyethereal lateral chains. The latter analogues, as for **5d** and **5o**, were also fully characterized as potent, selective, and dialyzable analogues (Table 3 and Figure 2). Furthermore, although the pharmacological properties of AEA and other fatty acid amides underscore the potential therapeutic interest of FAAH inhibition, the presence of a lipid-like chain may confer to AEA-like compounds a number of unfavorable biopharmaceutical properties, including low water solubility, high plasma protein binding, and high accumulation in fat tissues. Moreover, the marked similarity among the natural substrates of the ECs-metabolizing enzymes renders selectivity a crucial issue for the development of a novel drug candidate. Keeping this in mind, and aiming at improving the hydrophilic and pharmacokinetic properties of our lead compound **5d** while retaining its in vitro pharmacology and selectivity profiles, we developed a subset of analogues (**5v−z**, Table 2) where the 1-phenylpyrrole core structure was maintained and the *n*-hexyl spacer of the moiety attached to the carbamoyl functionality of **5d** was replaced by a polyethereal chain of equal length. The distal phenyl system of **5d** was also replaced by polyfluorinated moieties, placing the F atoms at *ortho* and *para* positions of the phenyl system, which are known to be the more suitable positions for metabolic inactivation. Altogether, these modifications influenced a series of parameters (e.g., Log*P* and Log*S*, Table 2) and allowed the identification of a subset of potent FAAH inhibitors potentially endowed with better pharmacokinetic properties and improved metabolic stability. In particular, the *p*-F (**5w**) and the *o,p*-di-F (**5y**) substituted analogues were identified as the most potent inhibitors of the series (**5w**, K_i = 8 nM; **5y**, K_i = 7 nM). Interestingly, the calculated water solubility (ACD/Labs V12.0,Toronto, Canada) for **5w** was also the highest of the subseries of analogues and was incremented by about one logarithmic unit with respect to that predicted for **5d** (Log*S* **5w** = −4.72 vs Log*S* **5d** = −5.69, Table 2, ACD/Labs). Notably, **5w** maintained FAAH inhibition potency in the nanomolar range, being only 1 order of magnitude less potent than **5d** (Table 2).

Docking studies, in line with the observed FAAH inhibition potency, confirmed that the polyethereal-chained analogues share their binding mode with **5c**. Accordingly, as reported in parts B and D of Figure 1, the binding mode of compound **5y** highlights the H-bonds established by both the amide (with Leu380 and Gln273) and the carbamate moieties (with Ile238, Met191, and Gly239), which are identical to those already discussed for **5c**.

On the basis of these data, compounds **5w** and **5y** were further characterized as potent, noncompetitive, and dialyzable analogues (Figure 2).

Dialysis Studies. As shown in Figure 2, the newly developed analogues **5d**, **5o**, **5w**, and **5y**, as well as reference irreversible (**2** and **3**) and reversible (**4**) FAAH inhibitors, were incubated with mouse FAAH. The results obtained show that the nondialyzed enzyme was inhibited by each of the compounds tested in this study (Figure 2A). After dialysis, the reversible inhibitors were washed out, and the enzyme recovered its activity (Figure 2B) unless the compounds remained firmly bound to the protein. Comparing the bars relative to **4** (reversible inhibitor), **2**, and **3** (irreversible inhibitors) with those of the analogues **5d**, **5o**, **5w**, and **5y**, it clearly emerged the reversibility of binding of all the new compounds tested.

Selectivity Profile. A wide pharmacological in vitro investigation of compounds **5c**, **5d**, and **5n** (Table 3) on a variety of receptors (CB_1, CB_2, and TRPV1), metabolic enzymes (*N*-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD), diacylglycerol lipase (DAGL), and MAGL, and AMT transporter, as well as on a panel of more than 50 proteins of the CNS such as GPCRs and monoamine transporters (see Table 1, SI), documented the high selectivity profile of the new compounds **5c** and **5d**. Notably, the tested compounds did not show any interference either with AEA and 2-AG biosynthesis by NAPE-PLD and DAGL, respectively, or with 2-AG hydrolysis by MAGL. We could also assess that **5c**, **5d**, and **5n** fail to interact with hERG channels, thus excluding interference at the cardiac level, which is a valuable property for centrally active molecules.

Analgesic in Vivo Activity and Preliminary Safety Studies. *Preliminary Safety Studies.* Prior to the in vivo evaluation of the antalgic properties of the selected compounds **5c**, **5d**, and **5n**, preliminary safety studies were performed for excluding central effects that could have behavioral implications and that might interfere with the analgesic evaluation or negatively affect the therapeutic index. The Irwin test originally described in 1968[31] is currently employed for assessing the general effects of a drug or drug candidate and to estimate the minimum lethal dose of a test substance, the dose range for CNS responses, and the primary effects on behavior and physiological functions. Data from the Irwin test were used to assess the risks associated with the use of each substance (safety pharmacology) and also to select doses for subsequent tests of efficacy. Basically, rats or mice are administered the test substance and then they are observed for the next several hours. The animals are assessed for behaviors related to neurotoxicity (such as convulsions and tremor) or related to CNS stimulation (such as excitation, Straub tail, jumping, hypersensitivity to external stimuli, stereotypies, and aggressive behavior) and depression (such as sedation, rolling gait, loss of balance, loss of traction, motor incoordination, hyposensitivity to external stimuli, decreased muscle tone, akinesia, catalepsy, and hypothermia). Effects on autonomic functions (respiration, pupil diameter, body temperature, salivation, and defecation) are also noted, along with the eventual lethality of the tested compounds. Animals are initially administered a large dose of the tested drug, and the subsequent doses are selected following the responses to the initial exposure. Accordingly, preliminary tests demonstrated, as outlined in Table 4, that after oral administration to mice of 100, 30, and 10 mg/kg of **5c**, **5d**, and **5n**, limbic, and striatal stereotypies were very weak after 1 h and were absent after 4 h with the exception of **5d**. The anxiolytic effect and motor disturbances were absent at any time of observation. The same dose range was used in the induced pain model.

Randall-Selitto. Induction of Mechanical Hyperalgesia in Rats. Mechanical nociceptive thresholds were measured in the rat paw pressure test by applying increasing pressure to the left and right hind paws using a Randall–Selitto analgesimeter (Ugo Basile, Varese, Italy).[32] The parameter used to quantify the nociceptive threshold was defined as the pressure (grams) at which the rat withdrew its paw.

When **5c**, **5d**, and **5n** (10, 30, and 100 mg/kg) were tested in a dose–response experiment, all three compounds did exert analgesic activity at 30 and 100 mg/kg dose regimen, the latter dosage conferring a higher effect (Figure 3A–C). Notably, oral treatment with a 30 mg/kg dose of **5c**, **5d**, and **5n** demonstrated a significant analgesic activity, whereas the reference compound **2** at a dose of 50 mg/kg did not show any activity (Figure 3D).

CONCLUSIONS

In summary, we developed a new class of potent and selective FAAH reversible inhibitors. Besides the high selectivity, assessed for selected compounds on a large panel of receptors, including endocannabinoid receptors, enzymes, and transporters, the potent FAAH inhibitors **5c** and **5d** fail to interact also with the human ERG channel. The most potent inhibitors were further analyzed by in vivo studies to evaluate their antalgic properties. Results indicated that oral administration of 30 mg/kg dose of **5c**, **5d**, and **5n** determined a significant analgesic effect, and the profile was much better than that obtained with the reference compound **2**, which, at a dose of 50 mg/kg, did not show any activity. No behavioral effects were detected for the tested compounds (in vivo Irwin tests), and the absence of adverse effects at the analgesic dose highlighted a wide therapeutic window for the compounds **5c,d** and **5n**. Starting from the lead **5c**, we rationally synthesized a subset of analogues which allowed to select potent and reversible FAAH inhibitors potentially endowed with better solubility properties and improved metabolic stability. Analogue **5w** was the best performing compound of this subset. Taken together, the data herein presented evidence a trajectory for the discovery of a new class of potent, reversible, and safe FAAH inhibitors potentially useful as valuable pharmacological tools to be added to the armamentarium of agents potentially useful for the treatment of (neuropathic) pain.

EXPERIMENTAL PROCEDURES

Materials. Chemicals were of the purest analytical grade. AEA, resinferatoxin (RTX), CP55.940, 1-stearoyl-2-arachidonoyl-*sn*-glycerol and 2-oleoylglycerol (2-OG) were from Sigma-Aldrich; *N*-arachidonoylphosphatidylethanolamine (NArPE), URB597, OL-135, and JP83 were from Alexis Corporation. [^{3}H]NArPE (200 Ci/mmol), [^{3}H]2-OG (20 Ci/mmol), [^{3}H-ethanolamine]AEA (60 Ci/mmol), [^{3}H-arachidonyl]AEA (200 Ci/mmol), and [^{14}C]DAG (55 mCi/mmol) were from ARC. [^{3}H]CP55.940 (163 Ci/mmol) and [^{3}H]RTX (43 Ci/mmol) were from Perkin-Elmer Life Sciences.

Chemistry. Reagents were purchased from Aldrich and were used as received. Reaction progress was monitored by TLC using Merck Silica Gel 60 F_{254} (0.040–0.063 mm) with detection by UV. Merck Silica Gel 60 (0.040–0.063 mm) was used for column chromatography. Melting points were determined in Pyrex capillary tubes using an Electrothermal 8103 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian 300 MHz instrument

using the residual signal of the deuterated solvent as internal standard. Splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), and broad (br); the value of chemical shifts (δ) are given in ppm and coupling constants (*J*) in hertz (Hz). ES-MS, APCI-MS spectra were performed by an Agilent 1100 series LC/MSD spectrometer and by LCQDeca-Thermofinnigan spectrometer. Elemental analyses were performed in a Perkin-Elmer 240C elemental analyzer, and the results were within ±0.4% of the theoretical values, unless otherwise noted.

Yields refer to purified products and are not optimized. All moisture-sensitive reactions were performed under argon atmosphere using oven-dried glassware and anhydrous solvents. All the organic layers were dried using anhydrous sodium sulfate.

6-Phenyl-1-hexylamine (7a). **6a** (320 μL, 1.68 mmol), triphenylphosphine (441 mg, 1.68 mmol), and phthalimide (247 mg, 1.68 mmol) were vigorously stirred in 2.4 mL of dry THF while cooling to 0 °C. DIAD (320 μL, 1.67 mmol) in 2.4 mL of dry THF was added dropwise to the cooled mixture. After stirring at room temperature for 12 h, the solvent was removed under reduced pressure. Column chromatography (25% ethyl acetate in *n*-hexane) afforded the pure 2-(6-phenylhexyl)isoindole-1,3-dione (71% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (m, 4H), 1.64 (m, 4H), 2.58 (t, 2H, *J* = 2.6 Hz), 3.66 (t, 2H, *J* = 7.2 Hz), 7.15 (m, 3H), 7.23 (m, 2H), 7.68 (m, 2H), 7.82 (m, 2H). ESI-MS *m*/*z* $[M + H]^+$ 308 (100). To a solution of 2-(6-phenylhexyl)isoindole-1,3-dione (364 mg, 1.19 mmol) in 18.0 mL of ethanol, hydrazine monohydrate (180 μL, 3.71 mmol) was added. The solution was stirred under reflux for 5 h and then cooled to room temperature, and the white precipitate removed by filtration. The solvent was removed from the filtrate under reduced pressure. The residue was dissolved in 1 N sodium hydroxide (50 mL) and extracted with chloroform (3 × 40 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. Column chromatography (10% MeOH in chloroform with 2% of TEA) afforded the pure compound **7a** as a yellow oil (80% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (m, 4H), 1.45 (m, 2H), 1.62 (m, 2H), 2.35 (s, 2H), 2.60 (t, 2H, *J* = 2.6 Hz), 3.68 (t, 2H, *J* = 7.0 Hz), 7.16 (m, 3H), 7.26 (m, 2H). ESI-MS *m*/*z* $[M + H]^+$ 178 (100).

6-Phenyl-1-pentylamine (7b). Title compound was prepared as described for **7a** starting from **6b**. Column chromatography (10% MeOH in chloroform with 2% of TEA) afforded the pure compound **7b** as a yellow oil (80% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (m, 4H), 1.47 (m, 2H), 2.33 (s, 2H), 2.60 (t, 2H, *J* = 2.5 Hz), 3.68 (t, 2H, *J* = 7.2 Hz), 7.16 (m, 3H), 7.28 (m, 2H).

(6-Isocyanatohexyl)benzene (8). Compound **7a** (165 mg, 0.93 mmol) was dissolved in 9.3 mL of DCM. The solution was cooled to 0 °C, and a saturated aqueous solution of sodium bicarbonate (9.3 mL) was added. The biphasic mixture was stirred for 10 min at 0 °C, the layers were allowed to separate, and a solution of phosgene (20% in toluene, 980 μL, 1.86 mmol) was added directly to the organic layer via syringe. After 15 min, the aqueous layer was extracted with DCM (3 × 10 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated. The compound was used in the following step without further purification (94% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (m, 4H), 1.51 (m, 4H), 2.51 (t, 2H, *J* = 7.7 Hz), 3.14 (t, 2H, *J* = 6.6 Hz), 7.07 (m, 3H), 7.17 (m, 2H).

Undec-10-ynyl-1-amine (10). To a solution of **9** (1.14 mL, 5.9 mmol) in dry THF (40.0 mL), TEA (910 μL, 6.50 mmol) and methanesulfonyl chloride (1.37 mL, 17.7 mmol) were added dropwise. After stirring for 15 min, the suspension was filtered through Celite and the filtrate was washed with brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated. Undec-10-ynyl methanesulfonate was used in the following step without further purification. ^{1}H NMR (300 MHz, acetone-d_6) δ 1.20−1.44 (m, 14H), 1.83 (t, 1H, *J* = 2.6 Hz), 2.03−2.09 (m, 2H), 2.88 (s, 3H), 4.10 (t, 2H, *J* = 6.4 Hz).

To a solution of the mesylate (1.45 g, 5.9 mmol) in 40.0 mL of dry DMF, sodium azide (1.91 g, 29.0 mmol) was added. The mixture was stirred at 85 °C for 4 h. After cooling to room temperature, water and *n*-hexane were added. The organic layer was washed with a saturated aqueous solution of sodium bicarbonate and with brine and then dried over anhydrous sodium sulfate, filtered, and concentrated. Column chromatography (10% ethyl acetate in *n*-hexane) afforded the pure 11-azidoundec-1-yne (72% yield). ^{1}H NMR (300 MHz, acetone-d_6) δ 1.24−1.55 (m, 14H), 1.86 (t, 1H, *J* = 2.6 Hz), 2.08−2.13 (m, 2H), 3.18 (t, 2H, *J* = 7.04 Hz).

To a solution of the azide (826 mg, 4.27 mmol) in 44.0 mL of MeCN, iron(III) chloride (1.04 g, 6.41 mmol) and sodium iodide (5.77 g, 38.46 mmol) were added. The reaction mixture was refluxed for 1 h. After cooling, chloroform was added, and the resulting solution was washed with a saturated aqueous solution of sodium thiosulfate and then with a saturated aqueous solution of sodium bicarbonate. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The crude was purified by means of flash chromatography (10% MeOH in chloroform with 0.2% of TEA). Title compound **10** was obtained as an amorphous pale-yellow solid (72% yield). ^{1}H NMR (300 MHz, acetone-d_6) δ 1.30−1.64 (m, 14H), 1.94 (t, 1H, *J* = 2.6 Hz), 2.15−2.20 (m, 2H), 2.50 (t, 2H, *J* = 7.6 Hz), 4.72 (brs, 2H). ESI-MS *m*/*z* $[M + H]^+$ 194 (100).

11-Isocyanatoundec-1-yne (11). Title compound was prepared as previously described for compound **8** starting from amine **10** and phosgene. The compound was used in the following step without further purification (94% yield). ^{1}H NMR (300 MHz, acetone-d_6) δ 1.25−1.63 (m, 14H), 1.89−1.91 (m, 1H), 2.13−2.18 (m, 2H), 3.26 (t, 2H, *J* = 6.7 Hz).

2-(2-Bromoethoxy)ethanol (13). To a solution of **12** (1.79 mL, 18.85 mmol) in 30.0 mL of MeCN, carbon tetrabromide (6.88 g, 20.74 mmol), and triphenylphosphine (4.95 g, 18.85 mmol) were sequentially added, and the resulting mixture was stirred at room temperature for 11 h. Then 1 N sodium hydroxide (25 mL) was added and MeCN was removed by rotary evaporation. The aqueous residue was extracted with ethyl acetate (3 × 30 mL), dried over anhydrous sodium sulfate, filtered, and concentrated. The crude residue was purified by column chromatography on silica gel (33% *n*-hexane in ethyl acetate) to afford the product as yellow oil (61% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.63 (s, 1H), 3.42 (t, *J* = 6.0 Hz, 2H), 3.54−3.57 (m, 2H), 3.67−3.70 (m, 2H), 3.75 (t, *J* = 5.7 Hz, 2H).

2-(2-Phenoxyethoxy)ethanol (14a). To a solution of phenol (52 μL, 0.59 mmol) in 4.0 mL of MeCN, potassium hydroxyde (68 mg, 1.18 mmol) was added and the resultant mixture was heated to 60 °C. After 2 h, potassium iodide (99 mg, 0.59 mmol) and a solution of bromide **13** (200 mg, 1.18 mmol) in 3.0 mL of MeCN were added and the reaction mixture was stirred for additional 10 h at 60 °C. Then the mixture was diluted with water, extracted with ethyl acetate, and the organic layers dried over anhydrous sodium sulfate, filtered, and concentrated. Purification by silica gel column chromatography (40% ethyl acetate in *n*-hexane) afforded the title compound **14a** (72% yield) as a pale-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.75 (brs, 1H), 3.37−3.67 (m, 2H), 3.73−3.76 (m, 2H), 3.83−3.86 (m, 2H), 3.11−3.14 (m, 2H), 6.91−6.96 (m, 3H), 7.25−7.31 (m, 2H).

2-(2-(4-Fluorophenoxy)ethoxy)ethanol (14b). Title compound was prepared according to the procedure previously described for **14a** starting from 4-fluorophenol and **13**. Compound was obtained as pale-yellow oil (68% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.87 (brs, 1H), 3.59−3.62 (m, 2H), 3.69−3.73 (m, 2H), 3.76−3.80 (m, 2H), 4.02−4.05 (m, 2H), 6.79−6.84 (m, 2H), 6.89−6.93 (m, 2H).

2-(2-(2-Fluorophenoxy)ethoxy)ethanol (14c). Title compound was prepared according to the procedure previously described for **14a** starting from 2-fluorophenol and **13**. Compound was obtained as pale-yellow oil (61% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.54 (brs, 1H), 3.63−3.67 (m, 2H), 3.71−3.75 (m, 2H), 3.84−3.87 (m, 2H), 4.16−4.20 (m, 2H), 6.87−7.09 (m, 4H).

2-(2-(2,4-Difluorophenoxy)ethoxy)ethanol (14d). Title compound was prepared according to the procedure previously described for **14a** starting from 2,4-difluorophenol and **13**. Compound was obtained as pale-yellow oil (75% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.80 (brs, 1H), 3.59−3.63 (m, 2H), 3.68−3.72 (m, 2H), 3.78−3.82 (m, 2H), 4.10−4.13 (m, 2H), 6.69−6.95 (m, 3H).

2-(2-(2,4,6-Trifluorophenoxy)ethoxy)ethanol (14e). Title compound was prepared according to the procedure previously

described for **14a** starting from 2,4,6-trifluorophenol and **13**. Compound was obtained as pale-yellow oil (75% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.61 (brs, 1H), 3.56−3.60 (m, 2H), 3.65−3.67 (m, 2H), 3.73−3.76 (m, 2H), 4.16−4.19 (m, 2H), 6.58−6.66 (m, 2H).

2-(2-Phenoxyethoxy)ethanisocyanate (15a). 2-(2-Phenoxyethoxy)ethanamine was prepared as described for compound **7a**. Title compound **15a** was prepared according to the procedure previously described for **8** starting from 2-(2-phenoxyethoxy)-ethanamine and phosgene. Compound **15a** was obtained as pale-yellow oil and used for the subsequent step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.42−3.54 (m, 2H), 3.69−3.73 (m, 2H), 3.87−3.90 (m, 2H), 4.14−4.18 (m, 2H), 6.93−6.99 (m, 3H), 7.27−7.32 (m, 2H).

4-Fluoro-1-(2-(2-isocyanatoethoxy)ethoxy)benzene (15b). Title compound was prepared according to the procedure previously described for **15a** starting from 2-(2-(4-fluorophenoxy)ethoxy)-ethanamine and phosgene, and it was obtained as pale-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.45−3.48 (m, 2H), 3.70−3.74 (m, 2H), 3.88−3.90 (m, 2H), 4.12−4.16 (m, 2H), 6.92−6.94 (m, 2H), 6.99−7.03 (m, 2H).

2-Fluoro-1-(2-(2-isocyanatoethoxy)ethoxy)benzene (15c). Title compound was prepared according to the procedure previously described for **15a** starting from 2-(2-(2-fluorophenoxy)ethoxy)-ethanamine and phosgene, and pale-yellow oil was obtained. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.41−3.45 (m, 2H), 3.70−3.74 (m, 2H), 3.88−3.91 (m, 2H), 4.20−4.23 (m, 2H), 6.90−7.08 (m, 4H).

2,4-Difluoro-1-(2-(2-isocyanatoethoxy)ethoxy)benzene (15d). Title compound was prepared according to the procedure previously described for **15a** starting from 2-(2-(2,4-difluorophenoxy)-ethoxy)ethanamine and phosgene, and pale-yellow oil was obtained. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.40−3.44 (m, 2H), 3.68−3.72 (m, 2H), 3.85−3.88 (m, 2H), 4.16−4.19 (m, 2H), 6.73−6.99 (m, 3H).

2,4,6-Trifluoro-1-(2-(2-isocyanatoethoxy)ethoxy)benzene (15e). Title compound was prepared according to the procedure previously described for **15a** starting from 2-(2-(2,4,6-trifluorophenoxy)ethoxy)ethanamine and phosgene and pale-yellow oil was obtained. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.39−3.43 (m, 2H), 3.66−3.70 (m, 2H), 3.81−3.84 (m, 2H), 4.22−4.25 (m, 2H), 6.64−6.70 (m, 2H).

2,5-Dimethoxytetrahydrofuran-3-carbonitrile (17). Compound **16** (5.0 g, 31.2 mmol) was dissolved in 40.0 mL of dry THF. Then 10% aqueous ammonium hydroxide (40.0 mL) and iodine (10.3 g, 40.6 mmol) were sequentially added. The reaction mixture was stirred at 25 °C for 1.5 h. Then a 10% aqueous solution of sodium thiosulfate was added to quench the iodine excess. The layers were separated, and the aqueous phase was extracted with DCM. The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated. The crude was purified by means of flash chromatography (33% DCM in petroleum ether). Title compound was obtained as a yellow oil (46% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.05−2.43 (m, 2H), 3.15 (m, 1H), 3.31 (s, 3H), 3.36 (s, 3H), 5.02−5.19 (m, 2H).

1-(3-Hydroxyphenyl)-1*H*-pyrrole-3-carbonitrile (18). To a solution of 3-aminophenol (632 mg, 5.78 mmol) in 10.0 mL of 1,4-dioxane, compound **17** (1.0 g, 6.36 mmol) dissolved in 5.0 mL of 1,4-dioxane was added. The reaction mixture was refluxed for 5 h, and then 8.0 mL of 5 N hydrochloric acid were added dropwise. The reaction was refluxed for additional 10 min. After cooling to room temperature, the reaction mixture was partitioned between water and DCM. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated. Flash chromatography on silica gel (50% *n*-hexane in ethyl acetate) afforded compound **18** as an amorphous white solid (82% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.57−6.59 (m, 1H), 6.70 (brs, 1H), 6.83−6.92 (m, 3H), 7.01−7.03 (m, 1H), 7.26−7.34 (m, 1H), 7.49−7.51 (m, 1H). ESI-MS m/z $[M + Na]^+$ 207 (100).

1-(3-Hydroxyphenyl)-1*H*-pyrrole-3-carboxamide (19). Compound **18** (460 mg, 2.50 mmol) was dissolved in 2.0 mL of 6 N sodium hydroxide and 2.0 mL of 30% aqueous H_2O_2. The reaction mixture was refluxed for 12 h, and then the solvents were removed in vacuo. The crude was purified by flash chromatography (100% ethyl acetate to 5% MeOH in ethyl acetate). Pure compound **19** was obtained as a white solid (80% yield). ^{1}H NMR (300 MHz, acetone-d_6) δ 6.47 (brs, 1H), 6.66 (brs, 1H), 6.76−6.84 (m, 2H), 6.99−7.04(m, 2H), 7.20−7.31 (m, 2H), 7.85 (s, 1H), 9.40 (brs, 1H). ESI-MS m/z $[M - H]^-$ 201 (100).

(3-(3-Carbamoyl-1*H*-pyrrol-1-yl)phenoxycarbonyl)-butylamine (5a). To a solution of **19** (40 mg, 0.20 mmol) in 4.0 mL of dry THF, *n*-butylisocyanate (90 μL, 0.79 mmol) and TEA (110 μL, 0.79 mmol) were added. The reaction mixture was stirred at room temperature for 16 h, and then the solvent was removed and the residue was purified by silica gel column chromatography (2% MeOH in chloroform) to afford title compound **5a** as a white amorphous solid (63% yield). ^{1}H NMR (300 MHz, acetone-d_6) δ 0.94 (t, 3H, J = 7.2 Hz), 1.34−1.47 (m, 2H), 1.52−1.62 (m, 2H), 3.22 (q, 2H, J = 6.6 Hz), 6.35 (brs, 1H), 6.74 (s, 1H), 6.91 (brs, 2H), 7.08 (d, 1H, J = 7.8 Hz), 7.28−7.50 (m, 4H), 7.85 (s, 1H). ^{13}C NMR (75 MHz, acetone-d_6) δ 13.4, 19.9, 32.0, 40.9, 110.6, 114.0, 116.6, 119.7, 120.2, 121.8, 122.7, 130.5, 140.9, 152.9, 154.3, 165.6. Anal. ($C_{16}H_{19}N_3O_3$): C, H, N.

(3-(3-Carbamoyl-1*H*-pyrrol-1-yl)phenoxycarbonyl)-cyclohexylamine (5b). Title compound was prepared according to the procedure described for **5a** starting from **19**, the commercially available cyclohexylisocyanate, and TEA. Column chromatography (2% MeOH in chloroform) provided **5b** as a white solid (62% yield); mp 175−176 °C (MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 1.19−1.39 (m, 5H), 1.65 (d, 1H, J = 12.6 Hz), 1.78 (d, 2H, J = 11.7 Hz), 1.95 (d, 2H, J = 10.8 Hz), 3.39−3.48 (m, 1H), 6.72 (s, 1H), 7.07 (d, 1H, J = 7.8 Hz), 7.21−7.38 (m, 3H), 7.47 (t, 1H, J = 7.8 Hz), 7.80 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 25.0 (2), 25.4, 32.8 (2), 50.5, 110.2, 114.2, 116.9, 119.8, 120.5, 121.0, 122.3, 130.4, 140.8, 152.5, 154.7, 168.5. Anal. ($C_{18}H_{21}N_3O_3$): C, H, N.

(3-(3-Carbamoyl-1*H*-pyrrol-1-yl)phenoxycarbonyl)-1-undec-10-ynylamine (5c). Title compound was prepared according to the procedure described for **5a** starting from **19**, **11**, and TEA. Column chromatography (2% MeOH in chloroform) provided compound **5c** as a white solid (78% yield); mp 129−130 °C (MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 1.35−1.56 (m, 14H), 2.13 (s, 1H), 2.16 (s, 2H), 3.18 (t, 2H, J = 6.9 Hz), 6.72 (s, 1H), 7.08 (d, 1H, J = 7.2 Hz), 7.22−7.39 (m, 3H), 7.48 (t, 1H, J = 8.1 Hz), 7.80 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 17.8, 26.7, 28.5, 28.6, 29.0, 29.2, 29.37, 29.5, 40.9, 68.2, 83.9, 110.2, 114.1, 117.0, 119.8, 120.5, 121.0, 122.3, 130.4, 140.9, 152.6, 155.5, 168.5. Anal. ($C_{23}H_{29}N_3O_3$): C, H, N.

(3-(3-Carbamoyl-1*H*-pyrrol-1-yl)phenoxycarbonyl)-(6-phenylhexyl)amine (5d). Title compound was prepared according to the procedure described for **5a** starting from **19**, **8**, and TEA. Column chromatography (2% MeOH in chloroform) provided **5d** as a white solid (60% yield); mp 139−140 °C (MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 1.38−1.40 (m, 4H), 1.53−1.66 (m, 4H), 2.60 (t, 2H, J = 15.0 Hz), 3.17 (t, 2H, J = 13.8 Hz), 6.72 (s, 1H), 7.05−7.37 (m, 9H), 7.46 (t, 1H, J = 8.4 Hz), 7.79 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 26.5, 28.8, 29.5, 31.5, 35.6, 40.9, 110.2, 114.1, 117.0, 119.8, 120.5, 121.0, 122.3, 125.5, 128.1 (2), 128.2 (2), 130.4, 140.8, 142.7, 152.5, 155.5, 168.5. ESI-MS m/z $[M + H]^+$ 406, $[M + Na]^+$ 428 (100), $[M + K]^+$ 444, $[2M + H]^+$ 811, $[2M + Na]^+$ 833. Anal. ($C_{24}H_{27}N_3O_3$): C, H, N.

1-(3-Hydroxyphenyl)-1*H*-pyrrole-3-carbaldehyde (20). To a solution of 3-aminophenol (588 mg, 5.39 mmol) in acetic acid (10.0 mL) and water (2.0 mL), dimethoxytetrahydrofuran carboxaldehyde **16** (950 mg, 5.93 mmol) in acetic acid (1.0 mL) was added dropwise. The solution was heated at 100 °C for 15 min. After removal of the solvent, the dark-brown reaction mixture was diluted with ethyl acetate and neutralized with an aqueous saturated solution of sodium carbonate. Then, it was extracted with ethyl acetate (3 × 100 mL), dried over anhydrous sodium sulfate, filtered, and concentrated. Column chromatography (50% ethyl acetate in *n*-hexane) afforded the pure product as a yellow solid (42% yield). ^{1}H NMR (300 MHz, acetone-d_6) δ 6.70 (s, 1H), 6.87 (d, 1H, J = 7.8 Hz), 7.08−7.12 (m, 2H), 7.35−7.37 (m, 2H), 8.00 (s, 1H), 8.84 (s, 1H), 9.84 (s, 1H). ESI-MS m/z $[M - H]^-$ 186 (100).

1-(3-Hydroxyphenyl)-1*H*-pyrrole-3-carboxylic Acid (21). To a solution of **20** (255 mg, 1.36 mmol) in 3.0 mL of MeOH and 3.0 mL

of 6 N sodium hydroxide, $AgNO_3$ (370 mg, 2.18 mmol) was added. The reaction mixture was then refluxed for 1 h. After cooling, the solvent was removed. The residue was diluted with ethyl acetate. The aqueous layer was then acidified to pH = 1 using concentrated hydrochloric acid and subsequently extracted with ethyl acetate (3 × 20 mL). The organic layer was then dried over anhydrous sodium sulfate, filtered, and concentrated to afford the pure product as a white solid (73% yield). ^{1}H NMR (300 MHz, acetone-d_6) δ 6.67 (dd, 1H, *J* = 1.8, 3.0 Hz), 6.84 (ddd, 1H, *J* = 7.8, 2.1, 0.9 Hz), 7.04−7.11 (m, 2H), 7.25 (dd, 1H, *J* = 2.1, 3.0 Hz), 7.34 (t, 1H, *J* = 8.4 Hz), 7.78 (dd, 1H, *J* = 1.8, 2.4 Hz,), 8.78 (s, 1H), 10.49 (brs, 1H). ESI-MS *m*/*z* $[M - H]^-$ 202 (100).

Undec-10-ynyl-1-(3-hydroxyphenyl)-1*H*-pyrrole-3-carboxylate (22). To a stirred solution of acid **21** (100 mg, 0.49 mmol) and **9** (90 μL, 0.49 mmol) in 2.0 mL of dry THF at 0 °C, triphenylphosphine (129 mg, 0.49 mmol), and DIAD (90 μL, 0.49 mmol) were added. The reaction mixture was stirred at room temperature for 48 h. The solvent was then removed under reduced pressure. Saturated aqueous sodium carbonate was added to the residue, and it was extracted with ethyl acetate (3 × 25 mL), dried over anhydrous sodium sulfate, filtered, and concentrated. Purification by means of flash chromatography (10% ethyl acetate in *n*-hexane) afforded the pure product **22** as a white solid (69% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32−1.55 (m, 12H), 1.70−1.75 (m, 2H), 1.93 (t, 1H, *J* = 2.7 Hz), 2.17 (dd, 2H, *J* = 2.7, 7.2 Hz), 4.25 (t, 2H, *J* = 6.9 Hz), 5.52 (brs, 1H), 6.74 (q, 1H , *J* = 1.8 Hz), 6.79 (dd, 1H *J* = 2.1, 8.1 Hz), 6.92 (t, 1H, *J* = 2.1 Hz), 6.95−7.00 (m, 2H), 7.30 (t, 1H, *J* = 8.1 Hz), 7.66 (dd, 1H, *J* = 1.5, 2.1 Hz).

6-Phenylhexyl-1-(3-hydroxyphenyl)-1*H*-pyrrole-3-carboxylate (23). The title compound was prepared according to the procedure previously described for **22** starting from acid **21**, **6a**, triphenylphosphine, and DIAD. Column chromatography (10% ethyl acetate in *n*-hexane) afforded the pure product as a white solid (63% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38−1.48 (m, 4H), 1.49−1.76 (m, 4H), 2.62 (t, 2H, *J* = 6.9 Hz), 4.25 (t, 2H, *J* = 6.9 Hz), 6.73−6.99 (m, 6H), 7.16−7.30 (m, 6H), 7.67−7.68 (m, 1H).

(3-((3-(Undec-10-ynyloxy)carbonyl)-1*H*-pyrrol-1-yl)-phenoxycarbonyl)butylamine (5e). Title compound was prepared according to the procedure previously described for **5a** starting from **22**, *n*-butylisocyanate, and TEA. Column chromatography (40% *n*-hexane in chloroform) afforded **5e** as a white solid (94% yield); mp 52−53 °C (ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, 3H, *J* = 7.2 Hz), 1.30−1.74 (m, 18H), 1.93 (s, 1H), 2.16 (dd, 2H, *J* = 4.5, 6.3 Hz), 3.26 (q, 2H, *J* = 6.3 Hz), 4.22 (t, 2H, *J* = 6.3 Hz), 5.22 (brs, 1H), 6.73 (s, 1H), 6.99 (s, 1H), 7.07 (d, 1H, *J* = 8.1 Hz), 7.19−7.26 (m, 2H), 7.39 (t, 1H, *J* = 8.1 Hz), 7.65 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 18.6, 20.1, 26.2, 28.7, 28.9, 29.1, 29.2, 29.5, 29.6, 32.1, 41.3, 64.3, 68.3, 85.0, 111.9, 114.8, 117.7, 118.6, 120.2, 120.7, 124.5, 130.5, 140.8, 152.2, 154.3, 165.0. ESI-MS *m*/*z* $[M + H]^+$ 453, $[M + Na]^+$ 475 (100), $[M + K]^+$ 491. Anal. ($C_{27}H_{36}N_2O_4$): C, H, N.

(3-(3-((6-Phenylhexyloxy)carbonyl)-1*H*-pyrrol-1-yl)-phenoxycarbonyl)butylamine (5f). Title compound was prepared according to the procedure previously described for **5a** starting from **23**, *n*-butylisocyanate, and TEA. Column chromatography (40% *n*-hexane in chloroform) afforded **5f** as a low melting white solid (95% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, 3H, *J* = 7.2 Hz), 1.34−1.81 (m, 11H), 2.62 (t, 3H, *J* = 7.2 Hz), 3.27 (q, 2H, *J* = 6.6 Hz), 4.24 (t, 2H, *J* = 6.6 Hz), 5.21 (t, 1H, *J* = 5.4 Hz), 6.75 (s, 1H), 6.99−7.43 (m, 10H), 7.67 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 20.1, 26.2, 29.0, 29.2, 31.6, 32.1, 36.1, 41.3, 64.3, 112.0, 114.9, 117.7, 118.6, 120.2, 120.8, 124.5, 125.8, 128.5 (2), 128.6 (2), 130.6, 140.8, 142.9, 152.2, 154.3, 165.0. ESI-MS *m*/*z* $[M + H]^+$ 461, $[M + Na]^+$ 485 (100), $[M + K]^+$ 501. Anal. ($C_{28}H_{34}N_2O_4$): C, H, N.

(3-(3-((Undec-10-ynyloxy)carbonyl)-1*H*-pyrrol-1-yl)-phenoxycarbonyl)cyclohexylamine (5g). Title compound was prepared according to the procedure previously described for **5a** starting from **22**, cyclohexylisocyanate, and TEA. Column chromatography (40% *n*-hexane in chloroform) afforded **5g** as a white amorphous solid (83% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16−1.54 (m, 17H), 1.60−1.72 (m, 5H), 1.93 (t, *J* = 2.7 Hz, 1H), 2.01 (d, 2H, *J* = 9.6 Hz), 2.16 (td, 2H, *J* = 2.4, 6.9 Hz), 3.54−3.57 (m, 1H), 4.23 (t, 2H, *J* = 6.6 Hz), 5.06 (d, 1H, *J* = 7.5 Hz), 6.73 (q, 1H, *J* = 1.8 Hz), 6.99 (t, 1H, *J* = 2.7 Hz), 7.08 (d, 1H, *J* = 8.7 Hz), 7.20−7.22 (m, 2H), 7.40 (t, 1H, *J* = 8.4 Hz), 7.66 (t, 1H, *J* = 1.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.6, 25.0 (2), 25.6, 26.2, 28.7, 28.9, 29.1, 29.2, 29.5, 29.6, 33.4 (2), 50.5, 64.3, 68.3, 85.0, 111.9, 114.9, 117.7, 118.6, 120.2, 120.7, 124.5, 130.5, 140.8, 152.2, 153.4, 164.9. ESI-MS *m*/*z* $[M + H]^+$ 479, $[M + Na]^+$ 501 (100), $[M + K]^+$ 517. Anal. ($C_{29}H_{38}N_2O_4$): C, H, N

(3-(3-((6-Phenylhexyloxy)carbonyl)-1*H*-pyrrol-1-yl)-phenoxycarbonyl)cyclohexylamine (5h). Title compound was prepared according to the procedure previously described for **5a** starting from **23**, cyclohexylisocyanate, and TEA. Column chromatography (40% *n*-hexane in chloroform) afforded **5h** as a white solid (66% yield); mp 112−113 °C (ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22−1.71 (m, 9H), 1.60−1.77 (m, 7H), 2.00−2.04 (m, 2H), 2.62 (t, 2H, *J* = 7.2 Hz), 3.55−3.58 (m, 1H), 4.24 (t, 2H, *J* = 6.6 Hz), 5.05 (d, 1H, *J* = 7.5 Hz), 6.74 (q, 1H, *J* = 1.5 Hz), 7.01 (t, 1H, *J* = 2.7 Hz), 7.10−7.27 (m, 8H), 7.40 (t, 1H, *J* = 8.1 Hz), 7.67 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.0 (2), 25.7, 26.2, 29.1, 29.2, 31.6, 33.4 (2), 36.1, 50.5, 64.3, 112.0, 114.9, 117.7, 118.6, 120.2, 120.8, 124.5, 125.9, 128.5 (2), 128.6 (2), 130.5, 140.8, 142.9, 152.3, 153.4, 165.0. ESI-MS *m*/*z* $[M + H]^+$ 489, $[M + Na]^+$ 511 (100), $[M + K]^+$ 527. Anal. ($C_{30}H_{36}N_2O_4$): C, H, N.

(3-(3-((Undec-10-ynyloxy)carbonyl)-1*H*-pyrrol-1-yl)-phenoxycarbonyl)-(6-phenylhexyl)amine (5i). Title compound was prepared according to the procedure previously described for **5a** starting from **22**, **8**, and TEA. Column chromatography (40% *n*-hexane in chloroform) afforded **5i** as a colorless viscous oil (85% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32−1.75 (m, 22H), 1.94 (t, 1H, *J* = 2.4 Hz), 2.18 (td, 2H, *J* = 2.4, 6.9 Hz), 2.62 (t, 2H, *J* = 7.5 Hz), 3.26 (q, 2H, *J* = 6.6 Hz), 4.24 (t, 2H, *J* = 6.6 Hz), 5.16 (brs, 1H), 6.75 (s, 1H), 7.00 (s, 1H), 7.08 (d, 1H, *J* = 8.7 Hz), 7.16−7.30 (m, 7H), 7.41 (t, 1H, *J* = 8.7 Hz), 7.67 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.6, 26.3, 26.8, 28.7, 29.0, 29.1 (2), 29.3, 29.5, 29.6, 29.9, 31.6, 36.1, 41.5, 64.4, 68.3, 85.0, 111.9, 114.9, 117.8, 118.7, 120.2, 120.8, 124.5, 125.9, 128.5 (2), 128.6 (2), 130.6, 140.9, 142.8, 152.2, 154.3, 165.0. ESI-MS *m*/*z* $[M + H]^+$ 557, $[M + Na]^+$ 579 (100), $[M + K]^+$ 595. Anal. ($C_{35}H_{44}N_2O_4$): C, H, N.

(±)-Ethyl-2-acetyl-4-(3-methoxyphenyl)-4-oxobutanoate ((±)-26). To a suspension of sodium hydride (36 mg, 1.49 mmol) in 8.0 mL of dry THF at 0 °C, **24** (262 μL, 1.35 mmol) was added dropwise. After 30 min, **25** (119 μL, 1.49 mmol) was added dropwise at 0 °C and the reaction mixture was stirred at room temperature for 48 h. The reaction was quenched with 1 N hydrochloric acid (3.0 mL), THF was evaporated, and the residue was extracted with ethyl acetate (3 × 25 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated. Column chromatography (25% ethyl acetate in *n*-hexane) afforded the pure product (±)-**26** (67% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.14 (t, 3H, *J* = 6.9 Hz), 2.21 (s, 3H), 3.06 (m, 2H), 3.83 (s, 3H), 4.10 (q, 2H, *J* = 6.9 Hz), 4.85 (t, 1H, *J* = 7.0 Hz), 7.09−7.13 (m, 1H), 7.33−7.39 (m, 1H), 7.51 (s, 1H), 7.59 (d, 1H, *J* = 7.8 Hz).

Ethyl-2-(3-methoxyphenyl)-5-methylfuran-3-carboxylate (27). Compound (±)-**26** (735 mg, 2.60 mmol) was dissolved in 8.0 mL of ethanol. Concentrated hydrochloric acid (0.73 mL) was added, and the reaction mixture was submitted to MW irradiation (150 W) (CEM Discovery Microwave System) and refluxed (maximum internal temperature 100 °C) for 10 min. The reaction mixture was cooled, diluted with ethyl acetate, neutralized with saturated aqueous sodium bicarbonate, and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated. Column chromatography (3% ethyl acetate in *n*-hexane) afforded the pure product as colorless viscous oil (90% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (t, 3H, *J* = 6.9 Hz), 2.34 (d, 3H, *J* = 0.9 Hz), 3.84 (s, 3H), 4.28 (q, 2H, *J* = 7.2 Hz), 6.44 (d, 1H, *J* = 1.2 Hz), 6.92 (ddd, 1H, *J* = 0.6, 2.1, 8.1 Hz), 7.32 (t, 1H, *J* = 7.8 Hz), 7.56−7.59 (m, 1H), 7.62−7.64 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.5, 14.5, 55.5, 60.6, 109.2, 113.5, 115.0, 115.2, 120.7,

129.2, 131.4, 151.21, 155.8, 159.5, 163.9. ESI-MS m/z [M − OEt]$^+$ 215, [M + Na]$^+$ 283 (100).

Ethyl-2-(3-methoxyphenyl)-5-methylthiophene-3-carboxylate (28). Compound (±)-**26** (500 mg, 1.8 mmol) was dissolved in 5.0 mL of toluene. Lawesson's reagent (2.00 g) was added, and the reaction mixture was submitted to MW irradiation (150W) (CEM Discovery Microwave System) and refluxed (maximum internal temperature 100 °C) for 10 min. After cooling, the mixture was filtered through Celite and the solvent was removed under reduced pressure. Flash column chromatography (1% ethyl acetate in n-hexane) afforded the pure product as colorless oil (48% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (t, 3H, J = 7.2 Hz), 2.47 (d, 3H, J = 0.9 Hz), 3.82 (s, 3H), 4.17 (q, 2H, J = 6.9 Hz), 6.90 (ddd, 1H, J = 0.9, 2.7, 8.4 Hz), 7.00−7.05 (m, 2H), 7.15 (d, 1H, J = 0.9 Hz), 7.28 (t, 1H, J = 7.8 Hz). ESI-MS m/z [M − OEt]$^+$ 231, [M + Na]$^+$ 299 (100).

2-(3-Methoxyphenyl)-5-methylfuran-3-carboxylic Acid (29). To a solution of **27** (42 mg, 0.16 mmol) in ethanol (3.0 mL) and water (1.0 mL), sodium hydroxide (161 mg, 4.03 mmol) was added and the reaction mixture was stirred at room temperature for 7 h, then ethanol was removed and the residue was acidified with 6 N HCl to pH = 1. The mixture was extracted with ethyl acetate (3 × 20 mL). The organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated. The title compound was used for the following step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.37 (s, 3H), 3.86 (s, 3H), 6.48 (s, 1H), 6.95 (dd, 1H, J = 7.8, 3.9 Hz,), 7.34 (t, 1H, J = 7.9 Hz), 7.54−7.59 (m, 2H). ESI-MS m/z [M − H]$^-$ 231 (100).

2-(3-Methoxyphenyl)-5-methylthiophene-3-carboxylic Acid (30). Title compound was prepared according to the procedure described for **29** starting from **28** and sodium hydroxide. Compound was used in the following step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.46 (d, 3H, J = 0.6 Hz), 3.81 (s, 3H), 6.92 (dd, 1H, J = 2.4, 8.4 Hz), 7.03−7.07 (m, 2H), 7.19 (d, 1H, J = 0.9 Hz), 7.28 (t, 1H, J = 8.1 Hz).

2-(3-Methoxyphenyl)-5-methylfuran-3-carboxamide (31). Acid **29** (68 mg, 0.29 mmol) was added portionwise to thionyl chloride (300 μL) at room temperature. Then the reaction mixture was refluxed for 30 min and then evaporated to dryness. The residue was dissolved in THF (3.0 mL), and concentrated ammonium hydroxide (1.0 mL) was added cautiously at room temperature and the solution stirred for 1 h. Then the reaction mixture was extracted with ethyl acetate. The organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated. Column chromatography on silica gel (40% ethyl acetate in n-hexane) afforded the pure product as a white solid (97% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.34 (d, 3H, J = 0.6 Hz), 3.83 (s, 3H), 5.79 (brs, 1H), 5.90 (brs, 1H), 6.33 (d, 1H, J = 0.9 Hz), 6.92 (dt, 1H, J = 3.0, 7.5 Hz), 7.26−7.38 (m, 3H).

2-(3-Methoxyphenyl)-5-methylthiophene-3-carboxamide (32). Title compound was prepared according to the procedure described for **31** starting from **30**, thionyl chloride, and ammonium hydroxide concentrated solution. Flash column chromatography (40% ethyl acetate in n-hexane) afforded the pure product as a white solid (78% yield). ^{1}H NMR (300 MHz, acetone-d_6) δ 2.46 (s, 3H), 3.82 (s, 3H), 6.50 (brs, 2H), 6.93 (dd, 1H, J = 1.8, 8.4 Hz), 6.99 (d, 1H, J = 1.2 Hz), 7.06−7.11 (m, 2H), 7.32 (t, 1H, J = 8.1 Hz).

2-(3-Hydroxyphenyl)-5-methylfuran-3-carboxamide (33). To a suspension of **31** (188.0 mg, 0.81 mmol) in dry DCM (6.0 mL), boron tribromide (1 M solution in DCM, 2.4 mL, 2.4 mmol) was added at −78 °C. The reaction mixture was then allowed to warm to room temperature and stirred for 8 h. A saturated aqueous solution of sodium carbonate was added to quench the reaction. The residue was extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated to afford the pure product as a brownish solid (98% yield). ^{1}H NMR (300 MHz, acetone-d_6) δ 2.33 (d, 3H, J = 0.9 Hz), 6.42 (d, 1H, J = 0.9 Hz), 6.56 (brs, 1H), 6.81 (ddd, 1H, J = 0.9, 2.7, 4.8 Hz), 6.94 (brs, 1H), 7.22 (t, 1H, J = 7.8 Hz), 7.45 (dt, 1H, J = 7.8, 1.5 Hz), 7.53 (t, 1H, J = 1.8 Hz), 8.48 (s, 1H).

2-(3-Hydroxyphenyl)-5-methylthiophene-3-carboxamide (34). Title compound was prepared according to the procedure described for **33** starting from **32** and boron tribromide. Pure product was obtained as white solid (95% yield). ^{1}H NMR (300 MHz, acetone-d_6) δ 2.45 (d, 3H, J = 0.9 Hz), 6.49 (brs, 2H), 6.84 (ddd, 1H, J = 0.9, 2.4, 8.1 Hz), 6.96−7.00 (m, 3H), 7.22 (t, 1H, J = 7.5 Hz), 8.55 (s, 1H).

(3-(3-Carbamoyl-5-methylfuran-2-yl)phenoxycarbonyl)-butylamine (5j). Title compound was prepared according to the procedure described for **5a** starting from **33**, n-butylisocyanate, and TEA. Column chromatography (1% MeOH in chloroform) afforded the pure product as a white solid (77% yield); mp 170−171 °C (MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 0.96 (t, 3H, J = 7.5 Hz), 1.36−1.43 (m, 2H), 1.50−1.57 (m, 2H), 2.35 (s, 3H), 3.15−3.21 (m, 2H), 6.38 (d, 1H, J = 0.9 Hz), 7.07 (ddd, 1H, J = 0.9, 2.4, 4.8 Hz), 7.38 (t, 1H, J = 7.8 Hz), 7.63 (t, 1H, J = 1.8 Hz), 7.70 (dt, 1H, J = 1.5, 5.4 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ 12.0, 12.9, 19.8, 31.7, 40.5, 107.9, 118.2, 120.2, 121.6, 123.7, 129.0, 131.6, 151.4, 151.5, 151.9, 155.9, 168.0. Anal. ($C_{17}H_{20}N_2O_4$): C, H, N.

(3-(3-Carbamoyl-5-methylthiophen-2-yl)phenoxycarbonyl)-butylamine (5k). The title compound was prepared according to the procedure previously described for **5a** starting from **34**, n-butylisocyanate, and TEA. Column chromatography (1% MeOH in chloroform) afforded **5k** as a white solid (64% yield); mp 162−163 °C (MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.87 (t, 3H, J = 7.5 Hz), 1.26−1.35 (m, 2H), 1.38−1.46 (m, 2H), 2.42 (d, 3H, J = 0.6 Hz), 3.03 (q, 2H, J = 6.9 Hz), 6.93 (d, 1H, J = 1.2 Hz), 7.05 (ddd, 1H, J = 1.2, 2.1, 7.8 Hz), 7.14 (t, 1H, J = 1.8 Hz), 7.24−7.38 (m, 3H), 7.57 (brs, 1H), 7.76 (t, 1H, J = 5.7 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.3, 15.4, 20.1, 32.0, 41.0, 121.8, 121.9, 125.4, 127.9, 129.9, 135.0, 135.1, 139.2, 139.8, 151.6, 154.8, 167.0. ESI-MS m/z [M + Na]$^+$ 355 (100), [2M + Na]$^+$ 687. Anal. ($C_{17}H_{20}N_2O_3S$): C, H, N.

(3-(3-Carbamoyl-5-methylfuran-2-yl)phenoxycarbonyl)-cyclohexylamine (5l). The title compound was prepared according to the procedure previously described for **5a** starting from **33**, cyclohexylisocyanate and TEA. Column chromatography (1% MeOH in chloroform) afforded **5l** as a white solid (79% yield); mp 202−203 °C (MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.13−1.28 (m, 5H), 1.52−1.59 (m, 1H), 1.67−1.70 (m, 2H), 1.79−1.83 (m, 2H), 2.31 (s, 3H), 3.28−3.30 (m, 1H), 6.48 (s, 1H), 7.04 (dd, 1H, J = 0.9, 7.5 Hz), 7.27 (brs, 1H), 7.37 (t, 1H, J = 8.1 Hz), 7.63−7.74 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.8, 25.2 (2), 25.8, 33.2 (2), 50.4, 109.3, 119.3, 120.6, 122.3, 123.7, 129.7, 131.8, 150.7, 151.5, 151.6, 154.0, 165.7. ESI-MS m/z [M + Na]$^+$ 365 (100), [2M + Na]$^+$ 707. Anal. ($C_{19}H_{22}N_2O_4$): C, H, N.

(3-(3-Carbamoyl-5-methylthiophen-2-yl)phenoxycarbonyl)-cyclohexylamine (5m). The title compound was prepared according to the procedure previously described for **5a** starting from **34**, cyclohexylisocyanate and TEA. Column chromatography (1% MeOH in chloroform) afforded **5m** as a white solid (72% yield); mp 183−184 °C (MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 1.22−1.39 (m, 5H), 1.64 (brd, 1H, J = 12.6 Hz), 1.78 (brd, 2H, J = 12.0 Hz), 1.94 (brd, 2H, J = 10.5 Hz), 2.47 (s, 3H), 3.38−3.48 (m, 1H), 6.97 (m, 1H), 7.09 (d, 1H, J = 8.1 Hz), 7.21 (s, 1H), 7.30−7.41 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD) δ 13.7, 25.0 (2), 25.4, 32.8 (2), 50.5, 121.5, 122.1, 125.6, 126.8, 129.3, 132.9, 134.7, 139.7, 141.4, 151.5, 155.0, 167.1. ESI-MS m/z [M + Na]$^+$ 381 (100), [2M + Na]$^+$ 739. Anal. ($C_{19}H_{22}N_2O_3S$): C, H, N.

(3-(3-Carbamoyl-5-methylfuran-2-yl)phenoxycarbonyl)-(6-phenylhexyl)amine (5n). Title compound was prepared according to the procedure previously described for **5a** starting from **33**, **8**, and TEA. Column chromatography (1% MeOH in chloroform) afforded the pure product as a white solid (71% yield); mp 156−157 °C (MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (brs, 4H), 1.55−1.66 (m, 4H), 2.33 (s, 3H), 2.61 (t, 2H, J = 7.5 Hz), 3.24 (q, 2H, J = 6.6 Hz), 5.10 (brs, 1H), 5.61 (brs, 1H), 5.82 (brs, 1H), 6.32 (s, 1H), 7.11−7.19 (m, 4H), 7.25−7.30 (m, 2H), 7.39 (d, 1H, J = 7.8 Hz), 7.58 (s, 1H), 7.64 (d, 1H, J = 7.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.6, 26.8, 29.1, 29.9, 31.6, 36.1, 41.5, 108.5, 118.1, 121.2, 122.3, 124.7, 125.9, 128.5 (2), 128.6 (2), 129.8, 131.3, 142.8, 151.3, 151.5, 152.1, 154.6, 165.9. Anal. ($C_{25}H_{28}N_2O_4$): C, H, N.

(3-(3-Carbamoyl-5-methylthiophen-2-yl)phenoxycarbonyl)-(6-phenylhexyl)amine (5o). Title compound was prepared

according to the procedure previously described for **5a** starting **34**, **8**, and TEA. Column chromatography (1% MeOH in chloroform) afforded the pure product as a white solid (80% yield); mp 152−153 °C (MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36−1.40 (m, 4H), 1.54−1.69 (m, 4H), 2.45 (d, 3H, J = 1.2 Hz), 2.61 (t, 2H, J = 7.5 Hz), 3.24 (q, 2H, J = 6.9 Hz), 5.13 (t, 1H, J = 5.4 Hz), 5.53 (brs, 2H), 7.12−7.19 (m, 5H), 7.25−7.32 (m, 4H), 7.39 (t, 1H, J = 7.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.3, 26.8, 29.1, 29.9, 31.6, 36.1, 41.5, 122.3, 123.1, 125.9, 126.6, 127.9, 128.5 (2), 128.6 (2), 129.9, 132.6, 134.3, 139.5, 141.7, 142.8, 151.4, 154.5, 166.0. Anal. ($C_{25}H_{28}N_2O_3S$): C, H, N.

(±)-Ethyl-2-acetyl-4-(3-methoxyphenyl)-4-oxobutanoate (±)-(37). Title compound was prepared according to the procedure described for (±)-**26** starting from **35**, **36**, and sodium hydride. Column chromatography (33% diethyl ether in petroleum ether) afforded the pure product as colorless viscous oil (79% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (t, 3H, J = 7.2 Hz), 2.45 (s, 3H), 3.51 (dd, 1H, J = 18.6, 5.7 Hz), 3.71 (dd, 1H, J = 18.6, 8.3 Hz), 3.85 (s, 3H), 4.23 (m, 3H), 7.12 (m, 1H), 7.37 (m, 1H), 7.47 (m, 1H), 7.58 (m, 1H). ESI-MS m/z [M + H]$^+$ 279, [M + Na]$^+$ 301 (100).

Ethyl-5-(3-methoxyphenyl)-2-methylfuran-3-carboxylate (38). Title compound was prepared according to the procedure described for **27** starting from (±)-**37** and concentrated hydrochloric acid. Column chromatography (50% diethyl ether in petroleum ether) afforded the pure product as colorless viscous oil (72% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (t, 3H, J = 7.2 Hz), 2.62 (s, 3H), 3.81 (s, 3H), 4.29 (q, 2H, J = 7.2 Hz), 6.80 (m, 1H), 6.86 (s, 1H), 7.15−7.28 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.6, 55.4, 60.4, 106.0, 109.1, 113.6, 115.6, 116.4, 129.9, 131.5, 151.74, 158.8, 160.1, 164.1.

5-(3-Methoxyphenyl)-2-methylfuran-3-carboxylic Acid (39). Title compound was prepared according to the procedure described for **29** starting from compound **38** and sodium hydroxide. Compound was carried on without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.69 (s, 3H), 3.86 (s, 3H), 6.84 (m, 1H), 6.92 (s, 1H), 7.18−7.33 (m, 3H), 10.83 (brs, 1H).

5-(3-Methoxyphenyl)-2-methylfuran-3-carboxamide (40). Title compound was prepared according to the procedure described for **31** starting from acid **39**, thionyl chloride, and ammonium hydroxide concentrated solution. Column chromatography (30% n-hexane in ethyl acetate) afforded the pure product as a white solid (74% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.67 (s, 3H), 3.85 (s, 3H), 5.68 (brs, 2H), 6.67 (s, 1H), 6.83 (m, 1H), 7.16 (m, 1H), 7.21 (m, 1H), 7.26−7.32 (m, 1H). ESI-MS m/z [M + Na]$^+$ 254 (100), [M + K]$^+$ 270, [2M + Na]$^+$ 485.

5-(3-Hydroxyphenyl)-2-methylfuran-3-carboxamide (41). Title compound was prepared according to the procedure previously described for **33** starting from **40** and boron tribromide. Pure product was obtained as a pale-yellow solid (61% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 2.58 (s, 3H), 6.70 (m, 1H), 6.92 (s, 1H), 7.09 (m, 2H), 7.17 (m, 1H). ESI-MS m/z [M + Na]$^+$ 240 (100).

(3-(4-Carbamoyl-5-methylfuran-2-yl)phenoxycarbonyl)-butylamine (5p). The title compound was prepared according to the procedure previously described for **5a** starting from **41**, n-butylisocyanate, and TEA. Column chromatography (1% MeOH in chloroform) afforded the pure product as a white solid (47% yield); mp 162−163 °C (MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 0.97 (t, 3H, J = 7.4 Hz), 1.37−1.58 (m, 4H), 2.61 (s, 3H), 3.19 (t, 2H, J = 7.1), 7.01 (m, 2H), 7.38 (m, 2H), 7.49 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 12.5, 12.9, 19.8, 31.7, 40.6, 104.9, 116.7, 117.3, 120.1, 120.8, 129.7, 131.5, 150.9, 152.0, 155.9, 157.2, 167.3. ESI-MS m/z [M + H]$^+$ 317, [M + Na]$^+$ 339, [2M + Na]$^+$ 655 (100). Anal. ($C_{17}H_{20}N_2O_4$): C, H, N.

(3-(4-Carbamoyl-5-methylfuran-2-yl)phenoxycarbonyl)-cyclohexylamine (5q). Title compound was prepared according to the procedure previously described for **5a** starting from **41**, cyclohexylisocyanate, and TEA. Column chromatography (10% MeOH in chloroform) afforded the pure product as a white solid (34% yield); mp 182−183 °C (MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 1.19−1.39 (m, 5H), 1.65 (m, 1H), 1.79 (m, 2H), 1.96 (m, 2H), 2.61 (s, 3H), 3.38 (m, 1H), 7.02 (m, 2H), 7.39 (m, 2H), 7.50 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 12.5, 24.9, 25.4, 32.8, 50.5, 104.9, 116.7, 117.3, 120.1, 120.8, 129.6, 131.5, 150.9, 152.0, 155.0, 157.2, 167.3. ESI-MS m/z [M + Na]$^+$ 365(100), [2M + Na]$^+$ 707. Anal. ($C_{19}H_{22}N_2O_4$): C, H, N.

(3-(4-Carbamoyl-5-methylfuran-2-yl)phenoxycarbonyl)-(6-phenylhexyl)amine (5r). Title compound was prepared according to the procedure previously described for **5a** starting from **41**, **8**, and TEA. Column chromatography (10% MeOH in chloroform) afforded the pure product as a white solid (40% yield); mp 139−140 °C (MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 1.37 (m, 4H), 1.52−1.63 (m, 4H), 2.58 (m, 5H), 3.16 (t, 2H, J = 6.9 Hz), 6.99 (m, 2H), 7.12 (m, 3H), 7.22 (m, 2H), 7.35 (m, 2H), 7.46 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 12.6, 26.5, 28.8, 29.5, 31.5, 35.7, 40.9, 104.9, 116.7, 117.3, 120.1, 120.8, 125.5, 128.1, 128.2, 129.7, 131.6, 142.7, 150.9, 152.0, 155.8, 157.2, 167.3. ESI-MS m/z [M + Na]$^+$ 443 (100), [M + K]$^+$ 459, [2M + Na]$^+$ 863. Anal. ($C_{25}H_{28}N_2O_4$): C, H, N.

Ethyl-1-benzyl-2-(3-methoxyphenyl)-5-methyl-1*H*-pyrrole-3-carboxylate (42). (±)-**26** (141 mg, 0.507 mmol) was dissolved in acetic acid (500 μL) in a 25 mL round bottomed flask. Benzylamine (277 μL, 2.54 mmol) was added, and the flask was inserted into the cavity of a CEM Discovery microwave system apparatus and refluxed (maximum internal temperature 130 °C), employing a microwave power of 150W for 12 min. Then the reaction mixture was cooled and diluted with ethyl acetate. It was then neutralized with saturated sodium bicarbonate aqueous solution and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated. Column chromatography (20% ethyl acetate in n-hexane) afforded the pure product as pale-yellow oil (86% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (t, 3H, J = 7.2 Hz), 2.12 (s, 3H), 3.60 (s, 3H), 4.11 (q, 2H, J = 7.2 Hz), 4.92 (s, 2H), 6.50 (s, 1H), 6.73 (s, 1H), 6.84−6.87 (m, 4H), 7.19−7.27 (m, 4H).

1-Benzyl-2-(3-methoxyphenyl)-5-methyl-1*H*-pyrrole-3-carboxylic Acid (43). Compound **42** (123 mg, 0.475 mmol) was dissolved in 4.0 mL of ethanol/THF in the ratio 1:1. Then, sodium hydroxide (4 pellets) was added and the reaction mixture was refluxed. After 16 h, the solvent was removed under reduced pressure. The residue was partitioned between ethyl acetate and water. The aqueous layer was then acidified to pH = 2 with 4 N hydrochloric acid and extracted with ethyl acetate (3 × 25 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated. The product was then recrystallized from DCM and hexane and obtained as a white amorphous solid (98% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.11 (s, 3H), 3.60 (s, 3H), 4.90 (s, 2H), 6.52 (s, 1H), 6.73 (s, 1H), 6.83−6.86 (m, 4H), 7.17−7.28 (m, 4H).

1-Benzyl-2-(3-methoxyphenyl)-5-methyl-*N*-(5-phenylpentyl)-1*H*-pyrrole-3-carboxamide (5s). The title compound was prepared according to the procedure described for **5s** starting from **43** and **7b**. Column chromatography (30% ethyl acetate in n-hexane) afforded the pure product as a white amorphous solid (78% yield.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01−1.11 (m, 2H), 1.21−1.31 (m, 2H), 1.43−1.54 (m, 2H), 2.14 (s, 3H), 2.51 (t, 2H, J = 7.8 Hz), 3.13−3.20 (m, 2H), 3.59 (s, 3H), 4.86 (s, 2H), 5.24−5.26 (m, 1H), 6.53 (s, 1H), 6.71−6.73 (m, 1H), 6.79−6.93 (m, 4H), 7.10−7.29 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.5, 26.6, 29.4, 31.3, 36.0, 39.2, 47.8, 55.3, 108.6, 115.7, 116.0, 117.6, 123.5, 125.8, 125.9, 127.4, 128.5, 128.6, 128.9, 129.4, 130.11, 133.0, 133.5, 138.3, 142.8, 159.9, 165.1. ESI-MS m/z [M + H]$^+$ 467, [M + Na]$^+$ 489, [M + K]$^+$ 505, [2M + Na]$^+$ 955 (100). Anal. ($C_{31}H_{34}N_2O_2$): C, H, N.

1-Benzyl-2-(3-methoxyphenyl)-5-methyl-*N*-(6-phenylhexyl)-1*H*-pyrrole-3-carboxamide (5t). To a solution of acid **43** (45 mg, 0.184 mmol) in 4.0 mL of dry DCM at 0 °C were added TEA (52 μL, 0.368 mmol), EDCI (53 mg, 0.276 mmol), and HOBt (40 mg, 0.258 mmol) and the mixture was stirred at 0 °C for 10 min. Then, **7a** (39 mg, 0.220 mmol) in 1.0 mL of dry DCM was added. The stirring was continued at 0 °C for 30 min, and then the reaction mixture was allowed to warm to room temperature within 16 h. The solvent was subsequently removed in vacuo. Column chromatography (30% ethyl acetate in n-hexane) afforded the pure product as a white amorphous

solid (81% yield.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98−1.11 (m, 2H), 1.15−1.25 (m, 4H), 1.44−1.57 (m, 2H), 2.13 (s, 3H), 2.55 (t, 2H, J = 7.5 Hz), 3.12−3.21 (m, 2H), 3.57 (s, 3H), 4.85 (s, 2H), 5.21−5.23 (m, 1H), 6.53 (s, 1H), 6.70−6.73 (m, 1H), 6.79−6.89 (m, 4H), 7.14−7.29 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.5, 26.8, 28.9, 29.4, 31.5, 36.0, 38.2, 48.0, 54.3, 106.6, 115.7, 116.0, 117.6, 123.3, 125.8, 125.9, 127.3, 128.7, 128.8, 128.9, 129.4, 130.2, 133.0, 133.5, 138.3, 142.8, 159.7, 164.5. ESI-MS m/z [M + H]$^+$ 481 (100), [M + Na]$^+$ 503, [2M + H]$^+$ 962, [2M + Na]$^+$ 983. Anal. ($C_{32}H_{36}N_2O_2$): C, H, N.

1-Benzyl-2-(3-hydroxyphenyl)-5-methyl-*N*-(6-phenylhexyl)-1*H*-pyrrole-3-carboxamide (44). The title compound was prepared according to the procedure previously described for **33** starting from **5s** and boron tribromide (99% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88−1.07 (m, 2H), 1.10−1.19 (m, 4H), 1.40−1.55 (m, 2H), 2.02 (s, 3H), 2.55 (t, 2H, J = 7.5 Hz), 3.11 (m, 2H), 4.82 (s, 2H), 5.41 (m, 1H), 6.53 (s, 1H), 6.65 (m, 1H), 6.70−6.91 (m, 4H), 7.15−7.29 (m, 9H).

(3-(3-(6-Phenylhexylcarbamoyl)-1-benzyl-5-methyl-1*H*-pyrrol-2-yl)phenoxycarbonyl)cyclohexylamine (5u). The title compound was prepared according to the procedure previously described for **5a** starting from **44**, cyclohexylisocyanate, and TEA. Column chromatography (30% ethyl acetate in *n*-hexane) afforded the pure product as colorless oil (63% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01−1.39 (m, 12H), 1.43−1.79 (m, 6H), 1.90−2.02 (m, 2H), 2.16 (s, 3H), 2.55 (t, 2H, J = 7.8 Hz), 3.18 (m, 2H), 3.51 (m, 1H), 4.88 (m, 3H), 5.24 (m, 1H), 6.50 (s, 1H), 6.80 (m, 2H), 6.98−7.30 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.5, 25.0, 25.6, 26.8, 29.2, 29.5, 29.9, 31.6, 33.4, 36.1, 39.3, 47.9, 50.4, 101.0, 105.0, 108.6, 117.8, 122.3, 124.6, 125.8, 125.9, 127.3, 127.9, 128.5, 128.6, 128.9, 129.8, 132.3, 133.3, 138.1, 143.0, 151.4, 153.4, 159.4, 165.0, 180.7, 183.3, 184.8. Anal. ($C_{38}H_{45}N_3O_3$): C, H, N.

(3-(3-Carbamoyl-1*H*-pyrrol-1-yl)phenoxycarbonyl)-(2-(2-phenoxyethoxy)ethyl)amine (5v). Title compound was prepared according to the procedure described for **5a** starting from **19**, **15a**, and TEA. Column chromatography (100% chloroform to 2% MeOH in chloroform) afforded **5v** as a white amorphous solid (65% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.46−3.52 (m, 2H), 3.69−3.73 (m, 2H), 3.85−3.88 (m, 2H), 4.10−4.16 (m, 2H), 5.85−6.00 (m, 3H), 6.55 (m, 1H), 6.92−7.03 (m, 4H), 7.06 (m, 1H), 7.15−7.19 (m, 2H), 7.25−7.39 (m, 3H), 7.61 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 41.3, 67.4, 69.8, 70.1, 110.0, 114.7, 114.8 (2), 117.6, 120.1, 120.7, 121.1, 121.3, 122.7, 129.8 (2), 130.5, 140.8, 152.1, 154.5, 158.8, 166.8. ESI-MS m/z [M + Na]$^+$ 432 (100). Anal. ($C_{22}H_{23}N_3O_5$): C, H, N.

(3-(3-Carbamoyl-1*H*-pyrrol-1-yl)phenoxycarbonyl)-(2-(2-(4-fluorophenoxy)ethoxy)ethyl)amine (5w). Title compound was prepared according to the procedure described for **5a** starting from **19**, **15b**, and TEA. Column chromatography (100% chloroform to 2% MeOH in chloroform) afforded **5w** as a white amorphous solid (73% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.44−3.53 (m, 2H), 3.69−3.72 (m, 2H), 3.84−3.87 (m, 2H), 4.10−4.13 (m, 2H), 5.65−5.77 (m, 3H), 6.55 (m, 1H), 6.89−7.08 (m, 6H), 7.18−7.26 (m, 2H), 7.37−7.43 (m, 1H), 7.63 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 41.3, 68.2, 69.9, 70.1, 109.9, 114.8, 115.8, 115.9 (2), 116.0, 116.3, 117.7, 120.1, 120.9, 122.8, 130.6, 140.8, 152.1, 154.4, 154.9, 159.2, 166.6. ESI-MS m/z [M + Na]$^+$ 447 (100). Anal. ($C_{22}H_{22}FN_3O_5$): C, H, N.

(3-(3-Carbamoyl-1*H*-pyrrol-1-yl)phenoxycarbonyl)-(2-(2-(2-fluorophenoxy)ethoxy)ethyl)amine (5x). Title compound was prepared according to the procedure described for **5a** starting from **19**, **15c**, and TEA. Column chromatography (100% chloroform to 2% MeOH in chloroform) afforded **5x** as a white amorphous solid (78% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.48−3.52 (m, 2H), 3.69−3.73 (m, 2H), 3.86−3.90 (m, 2H), 4.20−4.23 (m, 2H), 5.79−5.99 (m, 3H), 6.56 (m, 1H), 6.85−7.19 (m, 8H), 7.36 (m, 1H), 7.63 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 41.3, 69.2, 69.7, 70.1, 109.9, 114.8, 115.8, 116.5, 116.7, 120.1, 120.9, 122.0 (2), 122.8, 124.6 (2), 130.5, 140.8, 146.9, 152.2, 154.5, 166.6. ESI-MS m/z [M + Na]$^+$ 447 (100). Anal. ($C_{22}H_{22}FN_3O_5$): C, H, N.

(3-(3-Carbamoyl-1*H*-pyrrol-1-yl)phenoxycarbonyl)-(2-(2-(2,4-difluorophenoxy)ethoxy)ethyl)amine (5y). Title compound was prepared according to the procedure described for **5a** starting from **19**, **15d**, and TEA. Column chromatography (100% chloroform to 2% MeOH in chloroform) afforded **5y** as a white amorphous solid (75% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.46−3.52 (m, 2H), 3.68−3.72 (m, 2H), 3.84−3.87 (m, 2H), 4.16−4.20 (m, 2H), 5.72−5.90 (m, 3H), 6.56 (m, 1H), 6.74−7.07 (m, 5H), 7.17 (m, 2H), 7.35−7.41 (m, 1H), 7.63 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 41.3, 69.7, 70.1, 70.2, 105.2, 110.0, 110.9, 114.7, 116.7, 117.6, 120.1, 121.0, 122.8, 130.5, 140.7, 143.5, 151.3, 152.2, 154.5, 155.4, 158.6, 166.9. ESI-MS m/z [M + H]$^+$ 446, [M + Na]$^+$ 468 (100), [M + K]$^+$ 484. Anal. ($C_{22}H_{21}F_2N_3O_5$): C, H, N.

(3-(3-Carbamoyl-1*H*-pyrrol-1-yl)phenoxycarbonyl)-(2-(2-(2,4,6-trifluorophenoxy)ethoxy)ethyl)amine (5z). Title compound was prepared according to the procedure described for **5a** starting from **19**, **15e**, and TEA. Column chromatography (100% chloroform to 2% MeOH in chloroform) afforded **5z** as a white amorphous solid (78% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.45−3.53 (m, 2H), 3.65−3.69 (m, 2H), 3.79−3.82 (m, 2H), 4.23−4.26 (m, 2H), 5.67−5.71 (m, 1H), 5.90 (brs, 2H), 6.56 (m, 1H), 6.63−6.73 (m, 2H), 6.98−7.08 (m, 2H), 7.17−7.21 (m, 2H), 7.38 (m, 1H), 7.63 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 41.3, 70.1, 70.3, 74.1, 101.1 (2), 110.0, 114.7, 117.6, 120.1, 120.8, 121.0, 122.8, 130.6, 140.8, 152.2 (2), 154.5, 155.8, 158.0, 158.9, 166.9, ESI-MS m/z [M + H]$^+$ 464, [M + Na]$^+$ 486 (100). Anal. ($C_{22}H_{20}F_3N_3O_5$): C, H, N.

Experimental Section for Modeling and Biochemical Assays. *Sequence Alignment, Model Building, and Optimization.* The mouse FAAH1 sequence was taken in fasta format from UniProtKB/Swiss-Prot [www.uniprot.org/uniprot] (entry: O08914). A protein−protein BLAST search (applying BLOSUM62 similarity matrix) [www.ncbi.nlm.nih.gov/blast/Blast.cgi] on the Protein Data Bank (PDB) [www.pdb.org] was conducted using the target sequence to identify and align structural homologues. Crystal structure of FAAH1 from *Rattus norvegicus* (PDB code: 1MT5, resolution 2.80 Å),[17] complexed with the irreversible inhibitor MAPF and sharing 91% and 95% of sequence identity and similarity, respectively, was chosen as template.

Before 3D model building step, the template structure was optimized using *Protein Preparation Wizard tool* implemented in Maestro (version 9.1, Schrödinger, LLC, New York, NY, 2010). Also, a missing atom building step was performed using the *Prime-Refinement tool* (Side Chain Prediction):[33] beginning with the first residue to be predicted, the side-chain rotamer library was used to find rotamers with the lowest energy while keeping all other side chains fixed. Then, the 3D model of mouse FAAH1 was built by *Prime*. The inhibitor MAPF was incorporated in the model generation process to preserve catalytic site conformation. Side chain rotamers of conserved residues were retained and all side chains were optimized. Amino acid side chains not derived from the template were minimized as well. The refined model was subjected to structural check program (PROCHECK[34,35]) to gauge its quality. To optimize the CA channel, a *m*FAAH1/transition intermediate (TI) of **2** complex was built. The TI of **2** was made based on crystallized ligand MAPF, being the phosphonate group an analogue of the tetrahedral intermediate. The inhibitor was orientated as described according to biochemical evidence that carbamates covalently modify the active site of FAAH, by an irreversible (or slowly reversible) mechanism involving carbamylation of the nucleophile Ser241[22] and on theoretical QM/MM calculations by Lodola et al.[36]

A molecular dynamic was carried out at 300 K with time step of 1.5 fs: the bounded-ligand and a set of side chain around 8 Å from it were allowed to move. The system was equilibrated for a period of 10 ps, and then a production run of 1 ns was carried out. One hundred sampled structures were cauterized and minimized. The lowest energy one was used for homology model validation. All the calculations were carried out by using MacroModel, version 9.8 (Schrödinger, LLC: New York, 2010). The bounded-**2** atoms were removed, and the hydrogen atoms of the catalytic site residues were reassigned, restoring the native state of the catalytic triad (Ser241, Ser217, and Lys142).

The 3D structures of all the molecules used in this study were built using Maestro (version 9.1, Schrödinger, LLC, New York, NY, 2010) and minimized by MacroModel (version 9.8, Schrödinger, LLC, New

York, NY, 2010) using the OPLS-2005 as force field. All molecules were then pretreated by LigPrep application (version 2.4, Schrödinger, LLC, New York, NY, 2010), implemented in Maestro, generating the most probable ionization state and tautomers at physiological pH and any possible enantiomer. Compound **2** and our inhibitors were docked using Glide sp protocol (version 5.5, Schrödinger, LLC, New York, NY, 2009), and bounded-**2** was used as reference ligand.

FAAH Assay. The activity of FAAH (E.C.3.5.1.4) was assayed by measuring the release of [^{3}H]ethanolamine from 10 μM [^{3}H-ethanolamine]AEA.[37] Mouse brain homogenates (40 μg per test) were preincubated for 20 min with each compounds, and then they were incubated with [^{3}H-ethanolamine]AEA for 15 min at 37 °C, in 500 μL of 50 mM Tris−HCl buffer (pH 9.0). The reaction was stopped by the addition of 800 μL ice-cold methanol/chloroform (2:1, v/v) with vortexing. The mixture was centrifuged at 3000*g* for 5 min, the upper aqueous layer was put in a vial containing liquid scintillation cocktail (Ultima Gold XR, Perkin-Elmer Life Sciences), and radioactivity was quantified in a β-counter. FAAH activity was expressed as pmol [^{3}H]ethanolamine released/min per mg of protein.

Kinetic Analysis of FAAH. The IC_{50} (half-maximal inhibitory concentration) values of the compounds toward FAAH activity were calculated through nonlinear regression analysis of dose−response curves, performed with the Prism4 program (GraphPAD Software for Science, San Diego, CA). The type of inhibition and the apparent inhibition constant (K_i) values of the compounds were ascertained using different concentrations of substrate ([^{3}H-ethanolamine]AEA, 0−20 μM range) and inhibitor (0−5 μM range). The kinetic data were fitted to Michaelis−Menten curves, which were subjected to nonlinear regression analysis through the Prism4 program in order to calculate apparent Michaelis−Menten constant (K_m), maximal velocity (V_{max}), and K_i values. The same Michaelis−Menten curves were also analyzed by Lineweaver−Burk diagrams (double reciprocal plots) in order to further visualize the type of inhibition.[37]

Dialysis of FAAH. Dialysis was performed on mouse brain homogenates (40 μg per test), preincubated for 20 min with selected compounds, and then dialyzed for 18 h at 4 °C against an excess (2 L) of 20 mM Tris-HCl buffer (pH 7.0).[37] Then, FAAH activity was assayed as described above.

Assays of Other ECS Elements. In all assays, homogenates were preincubated for 20 min with each compounds, then activity was measured as detailed below. The synthesis of AEA through the activity of NAPE-PLD was assayed with mouse brain homogenates (200 μg per test), using 100 μM [^{3}H]NArPE as substrate.[37] The uptake of 500 nM [^{3}H-arachidonyl]AEA was measured in intact human keratinocytes (HaCaT cells) (5×10^5 per test), and the synthesis of 2-AG by DAGL was assayed with mouse brain homogenates (100 μg protein per test) using 100 μM [^{14}C]DAG as substrate.[37] The hydrolysis of 2-AG by MAGL was measured with mouse brain supernatants (100 μg protein per test) using 10 μM [^{3}H]2-OG as substrate.[37]

Receptor Binding Assays. Membrane fractions were isolated from mouse brain (as a source of CB_1R) or mouse spleen (as a source of CB_2R), and were used in rapid filtration assays (100 μg per test) with the synthetic cannabinoid [^{3}H]CP55.940 (500 pM). Membrane fractions prepared from mouse brain were used in rapid filtration assays (100 μg per test) also to test the effect of the compounds on the binding of [^{3}H]RTX (500 pM) to TRPV1. In all experiments, unspecific binding was determined in the presence of an excess (1 μM) of "cold" agonist (CP55.940 or RTX, respectively).[37]

Experimental Details for the in Vivo Tests. *Irwin Test.* General behavioral observations were recorded by camera in CD-1 mice (Charles River, Calco, Italy), 5−6 week old, (4 animals/group) 60 and 240 min after oral 10, 30, and 100 mg/kg **5c**, **5d**, and **5n** administration by Irwin test[28]. Limbic stereotypies as sniffing and grooming while striatal as licking and biting were considered. The compounds were suspended in a solution containing 0.1% Tween 80 in 0.5 CMC (medium viscosity) (Sigma-Aldrich, Milan), which was used as vehicle, and administered in a volume of 10 mL/kg in mice.

Randall−Selitto. Mechanical nociceptive thresholds were measured in the rat paw pressure test[32] by applying increasing pressure to the left and right hind paws using a Randall−Selitto analgesimeter (Ugo Basile, Varese, Italy). The parameter used to quantify the nociceptive threshold was defined as the pressure (grams) at which the rat withdrew its paw. The day before testing, rats received three training sessions. Pressure was gradually applied to the right hind paw, and paw withdrawal thresholds (PWTs) were assessed as the pressure (grams) required for eliciting paw withdrawal. A cutoff point of 250 g was used to prevent any tissue damage to the paw.

Elevated Plus Maze. The apparatus was of gray Plexiglas and consisted of two open and two closed arms linked by a common central platform. The maze was elevated 40 cm above floor level and dimly lit. Animals were individually placed on the central platform of the maze facing an open arm. A standard 5 min test was employed. The amount of time spent by each animal in either open or closed arm was recorded by an Any-maze video tracking system (Ugo Basile), as well as the number of entries and the total distance walked by each animal into either arm.

hERG (Automated Patch-Clamp). CHO-K1 cells were originally obtained from American Tissue Culture Collection (ATCC). hERG cDNA (GenBank sequence NM_000238) was subcloned into pSI vector (Promega) at Cerep. The CHO-K1 cells were cotransfected with this construct and pPUR (containing puromycin selective marker, BD Bioscience). After selection in puromycin for 10 days, single colonies were selected and verified with hERG potassium currents. The stably transfected cells were used in this study. The stably transfected cells were cultured in F-12 Kaighn's Nutrient Mixture medium (Invitrogen) + 10% FBS at 37 °C for 1−3 days. Cells were kept at 30 °C for 12−24 h before patch clamp experiment in order to increase the hERG current amplitude. Subsequently, the cells were harvested by trypsination, and kept in serum-free medium for up to 6 h at room temperature before recording. The cells were washed and resuspended in extracellular solution before being applied to the patch clamp site. After whole cell configuration was achieved, the cells were held at −80 mV. A 50 ms pulse to −40 mV was delivered to measure the leaking current, which was subtracted from the tail current on line. Then the cell was depolarized at +20 mV for 2 s, followed by a 1 s pulse to −40 mV to reveal hERG tail current. This paradigm was delivered once every 5 s to monitor the current amplitude. The extracellular solution (control) was applied first, and the cell was stabilized in extracellular solution for 5 min. Then the test compound was applied from low concentrations to high concentrations cumulatively. The cell was incubated with each test concentration for 5 min. During the incubation, the cell was repetitively stimulated using the voltage protocol described above, and the tail current amplitude was continuously monitored. The degree of inhibition (%) was obtained by measuring the tail current amplitude before and after drug incubation.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Biological assays to define the selectivity profile of compounds **5c** and **5d** on a panel of different targets. Elemental analysis results for final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ECs, endocannabinoids; AEA, anandamide; CBR, cannabinoid receptor; 2-AG, 2-arachidonoylglycerol; AMT, AEA membrane transporter; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; ACB, acyl binding channel; CA, cytoplasmic access; CP, cytosolic port; CNS, central nervous system; PNS, peripheral nervous systems; MAFP, methoxyarachidonoyl fluorophosphonate; MW, microwave; SAR, structure−activity relationship; THF, tetrahydrofuran; TEA, triethylamine; DMF, dimethylformamide; DCM, dichloromethane; DMSO, dimethylsulfoxide; DIAD, diisopropyl azodicarboxylate; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, *N*-hydroxybenzotriazole; SAR, structure−activity relationship; TRPV, vanilloid receptor; hERG, human ether-a-go-go related gene; CT, control; NAPE-PLD, *N*-acyl phosphatidylethanolamine phospholipase D; DAGL, diacylglycerol lipase; PWTs, paw withdrawal thresholds; AA, arachidonic acid; RP-HPLC, reverse phase high pressure liquid chromatography; RTX, resinferatoxin; 2-OG, 2-oleoylglycerol; PDB, Protein Data Bank; TI, transition intermediate

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