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Sulfonyl Fluoride Inhibitors of Fatty Acid Amide Hydrolase

Shakiru O. Alapafuja[†], **Spyros P. Nikas**[*,†], **Indu Bharatan**[†], **Vidyanand G. Shukla**[†], **Mahmoud L. Nasr**[†], **Anna L. Bowman**[†], **Nikolai Zvonok**[†], **Jing Li**[†], **Xiaomeng Shi**[#], **John R. Engen**[#], and **Alexandros Makriyannis**[*,†]

[†]Center for Drug Discovery and Departments of Chemistry and Chemical Biology and Pharmaceutical Sciences, Northeastern University, Boston, MA, 02115, USA

[#]Department of Chemistry and Chemical Biology and Barnett Institute of Chemical and Biological Analysis, Northeastern University, Boston, MA, 02115, USA

Abstract

Sulfonyl fluorides are known to inhibit esterases. Early work from our laboratory has identified hexadecyl sulfonylfluoride (AM374) as a potent in vitro and in vivo inhibitor of fatty acid amide hydrolase (FAAH). We now report on later generation sulfonyl fluoride analogs that exhibit potent and selective inhibition of FAAH. Using recombinant rat and human FAAH we show that 5-(4-hydroxyphenyl)pentanesulfonyl fluoride (AM3506) has similar inhibitory activity for both the rat and the human enzyme, while rapid dilution assays and mass spectrometry analysis suggest that the compound is a covalent modifier for FAAH and inhibits its action in an irreversible manner. Our SAR results are highlighted by molecular docking of key analogs.

Introduction

Fatty acid amide hydrolase (FAAH) is an intracellular membrane-bound enzyme that degrades and inactivates members of the fatty acid amide (FAA) family of endogenous signaling lipids, including anandamide (**1**, Figure 1) and oleamide (**2**).[1,2] Anandamide[3] binds and activates the CB1 and CB2 cannabinoid receptors,[4] the molecular targets of plant-derived (−)-Δ^9-terahydrocannabinol ((−)-Δ^9-THC), while oleamide induces physiological sleep[5] and modulates serotonergic systems[6] and GABAergic transmission.[7] Fatty acid amide hydrolase is currently the only characterized mammalian enzyme that is in the amidase signature (AS) family bearing the unusual Ser-Ser-Lys catalytic triad, as confirmed by the crystal structure of the enzyme after reaction with methyl arachidonoyl fluorophosphonate (MAFP).[1,2]

The pharmacological effects of FAAH inhibition have been demonstrated in FAAH knockout mice[8] as well as by chemical inhibition.[9,10] Increased central and peripheral neuronal levels of anandamide and other FAAs produce physiological effects including analgesia,[10,11] apoptosis in various cancer cells,[12-14] modulation of memory processes,[15,16] neuroprotection,[9,17-19] epilepsy,[20] feeding,[21] and prevention of neurotoxicity of the human amyloid-β peptide in Alzheimer's disease.[22] In addition, anti-depressant, anxiolytic, anti-inflammatory, anti-hypertensive, gastrointestinal and sleep-inducing effects have been

[*]To whom correspondence should be addressed. Alexandros Makriyannis (Ph.D) Center for Drug Discovery and Departments of Chemistry and Chemical Biology and Pharmaceutical Sciences, Northeastern University, Boston, MA, 02115, USA. Tel.: +1-617-373-4200; fax: +1-617-373-7493; a.makriyannis@neu.edu. Spyros P. Nikas (Ph.D) Center for Drug Discovery, Northeastern University, Boston, MA, 02115, USA. Tel.: +1-617-373-7620; fax: +1-617-373-7493; s.nikas@neu.edu. .

Supporting information available Elemental analysis results for compounds **11a-11f**, **20a-20d**, **21a-21d**, **26** and **27**. This material is available free of charge via the Internet at http://pubs.acs.org.

observed.[10,23-26] These pharmacological effects are devoid of unwanted central "cannabinoid effects" such as hypomotility, hypothermia, catalepsy, and weight gain which accompany directly acting exogenous cannabinoid agonists such as (−)-Δ^9-THC.[27] Thus, there is significant therapeutic potential for FAAH inhibitors as analgesic, neuroprotective, anti-inflammatory and anti-anxiety drugs, and as agents for the treatment of metabolic and sleep disorders.

Over the last thirteen years an increasing number of irreversible and reversible FAAH inhibitors were disclosed.[10,28] Irreversible inhibitors include sulfonyl fluorides[29] (e.g., **3** and **4**) as well as aryl carbamates and ureas[24,30-32] (e.g., **7**). Reversible inhibitors include a number of synthetic agents bearing electrophilic carbonyl groups such as trifluoromethyl ketones (e.g., **5**), α-keto-esters and amides, aldehydes, α-halo-ketones, and the α-keto-heterocyclic type of inhibitors (e.g. **6**).[33-36] Additionally, ester derivatives of azetidinone, (thio)hydantoin analogs as well as boronic acids have been reported to inhibit FAAH.[28,37,38]

Work from our and other laboratories had provided evidence that the catalytic serine in FAAH is a more reactive nucleophile compared to the serine residues in other esterases. This has served as a basis for the development of more selective FAAH inhibitors. In the course of our program,[9,17-19,25,26,29,39-46] aimed at developing potent and selective inhibitors for the endocannabinoid deactivating enzymes, we have examined the abilities of a series of second generation sulfonyl fluorides (Table 1) to inhibit FAAH. Structural features of the irreversible inhibitors hexadecyl sulfonylfluoride **3** (AM374),[29] an early generation FAAH inhibitor developed in our laboratory, and phenylmethane sulfonyl fluoride **4** (PMSF), a generic esterase inhibitor, were incorporated into a phenylalkyl template (analogs **11a**-**11f**, Table 1). Furthermore, a hydrophilic hydroxyl group was added to the phenyl ring (analogs **21a**-**21d**) and the benzylic methylene group was replaced by the polar oxygen atom (analog **26**). Extension of our structure activity relationship (SAR) study to include synthetic intermediates (analogs **20a**-**20d**), shows that addition of the bulky benzyloxy group on the phenyl ring successfully modifies the phenylalkyl template resulting in potent FAAH inhibitors. All analogs synthesized were tested for their inhibitory activity on fatty acid amide hydrolase. In addition, initial testing for selectivity was carried out by also comparing FAAH activities of the most potent compounds against three endocannabinoid targets, namely, CB1 and CB2 receptors as well as the other major endocannabinoid inactivating enzyme monoacylglycerol lipase (MGL).

One of the most successful analogs identified in this study, 5-(4-hydroxyphenyl)pentane sulfonyl fluoride **21d** (**AM3506**),[25,41] has served as a valuable pharmacological tool to explore the cardiovascular, gastrointestinal and amygdala-mediated fear extinction effects related to FAAH inhibition.[25,26,40] Additionally, as reported earlier,[25] **21d** exhibited low "off target" effects when tested against a large number of serine hydrolases using activity-based proteomic methods.

Chemistry

Synthesis of phenylalkyl sulfonyl fluorides **11a**-**11f** was accomplished by a reaction sequence shown in Scheme 1. Commercially available phenylalkyl alcohols **8b**-**8f** were converted to the respective iodides **9b**-**9f** in very good yields (72-85%) using the triphenylphosphine, iodine, imidazole method.[47] Low temperature lithium-iodine exchange[48] between the primary alkyl iodides **9a**-**9f** and *t*-BuLi afforded the corresponding primary alkyllithium reagents that were treated[49] *in situ* with sulfuryl chloride to produce phenylalkyl sulfonyl chlorides **10a**-**10f** in moderate yields (19-23%). Treatment of these intermediates with NH_4F in refluxing acetone gave phenylalkyl sulfonyl fluorides **11a**-**11f** in excellent yields (91-93%).

Preparation of the benzyloxy and hydroxyl substituted phenylalkyl sulfonyl fluorides **20a**-**20d** and **21a**-**21d** is summarized in Scheme 2. Commercially available phenoxyalkyl bromides **12a** and **12b** were reacted with triphenylphosphine in refluxing benzene[47] to give the respective (phenoxyalkyl)triphenylphosphonium bromides **13a** and **13b** in very good yields (84-85%). These were treated with potassium *bis*(trimethylsilyl)amide and the generated phosphoranes were coupled with 4- or 3- or 2-anisaldehyde in an olefination reaction to furnish intermediate alkenes **14a**-**14d** in 91-93% yields. This Wittig reaction afforded isomeric mixtures of alkenes favoring the *cis* isomer (*cis*:*trans* = 92-94:8-6 by ^{1}H NMR).

Catalytic hydrogenation of **14a**-**14d** led to the corresponding phenolic methyl ethers **15a**-**15d** in 94-96% yields. Exposure of these compounds to boron tribromide in methylene chloride[47,50] cleaved the two ether groups and introduced the bromo group at the terminal carbon atom of the alkyl moiety, producing bromides **16a**-**16d** in very good yields (90-93%). Protection of the phenolic hydroxyl in **16a**-**16d** led to benzyl ethers **17a**-**17d** (77-81%) that upon reaction with sodium sulfite in refluxing EtOH/H_2O for 24 hours afforded sodium sulfonates **18a**-**18d**. Subsequent treatment with thionyl chloride in the presence of catalytic amounts of DMF gave sulfonyl chlorides **19a**-**19d** in 37-40% overall yields for the combined steps. During the course of this work we found that use of microwave irradiation remarkably decreases the time required for the conversion of bromides to sodium sulfonates and enhances the yields of the respective sulfonyl chlorides. Optimal conditions involve microwave heating (160°C) of the bromides and sodium sulfite in a mixture of THF/EtOH/H_2O (1:2:2) for 15 min. For example, treatment of **17d** with sodium sulfite under our optimized conditions and exposure of the respective sulfonate **18d** to thionyl chloride in the presence of catalytic amounts of DMF, gave sulfonyl chloride **19d** in 50% yield. Details of this work were published separately.[39]

Intermediate sulfonyl chlorides **19a**-**19d** were treated with NH_4F to produce the respective sulfonyl fluorides **20a**-**20d** in 90-93% yields. However, debenzylation of the phenolic hydroxyl group in **20a**-**20d** using palladium on active carbon was unsuccessful. The problem was solved by using a mixture of $HS(CH_2)_2SH$ and $BF_3{\cdot}Et_2O$ for cleavage of the benzyl group,[51] to give sulfonyl fluorides **21a**-**21d** in 66-70% yields.

The arylalkyl ether analog **26** and ester **27** were synthesized as shown in Scheme 3. In a similar fashion, etherification of commercially available 4-benzyloxyphenol (**22**) with 1,4-dibromobutane gave bromide **23** in 50% yield. Microwave heating of **23** with sodium sulfite and exposure of the resulting sodium sulfate **24** to $SOCl_2$/DMF led to **25** in 51% overall yield. Subsequent treatment with NH_4F gave the required sulfonyl fluoride **26** (93% yield). Sulfonic ester **27** was produced by reacting sulfonyl chloride **19a** with methanol.

Results and discussion

Structure-activity relationships

Inhibition data (Table 1) for FAAH and MGL were determined using fluorescent assay protocols as described in the experimental. The principle of these protocols is based on monitoring the fluorescence produced by enzyme catalyzed hydrolysis of a fluorogenic substrate to give a highly fluorescent compound. A fluorescent assay protocol to monitor FAAH activity has already been described.[52] This methodology uses human FAAH expressed in Chinese Hamster Ovary (CHO) cell lines and arachidonyl-7-amino, 4-methyl-coumarin amide (AAMCA) as the fluorogenic substrate. As detailed under Experimental, our modified protocol involves transmembrane domain-deleted rat FAAH (ΔTM rFAAH) expressed in *E. coli*[53] and AAMCA as the substrate. MGL activity was monitored with a

simple, sensitive, and amenable to high-throughput screening, fluorometric assay recently developed in our laboratory.[43,45,46] This methodology involves recombinant hexa-histidine-tagged human MGL (hMGL) overexpressed in *E. coli* and the novel fluorogenic substrate arachidonoyl, 7-hydroxy-6-methoxy-4-methyl-coumarin ester (AHMMCE).[43,45,46] Each compound's inhibition is presented in Table 1 as an IC_{50} value from a full eight point curve performed in triplicate.

The present study involves sulfonyl fluoride derivatives where the sulfonyl fluoride group is connected to a substituted or non-substituted phenyl ring through a linear alkyl linker. Substituents of the phenyl ring comprise the hydrophilic hydroxyl and the hydrophobic and bulky benzyloxy groups. Examination of the IC_{50} values of the analogs carrying a non-substituted phenyl ring (**11a**-**11f**, Table 1) reveals that a five to seven carbon long linker is optimal for FAAH inhibition.

It can be postulated that, within the FAAH's catalytic channel, this linking methylene chain places the phenyl ring in a position that mimics the π-unsaturations of the endogenous substrates anandamide ($\Delta^{8,9}$ double bond) and oleamide ($\Delta^{9,10}$ double bond). It should be noted that similar trends were observed earlier with α-keto-heterocyclic type of inhibitors where the phenyl-binding region appears to constitute a special site at the intersection of the FAAH's membrane access channel and the chain-binding pocket.[34,54,55] Thus, the phenylpentyl (**11c**), phenylhexyl (**11d**) and phenylheptyl (**11e**) analogs block FAAH activity with IC_{50}'s of 37-50 nM. Of these three compounds, the analog with the lowest IC_{50} value (**11e**) was selected as the main template for further studies aimed at exploring the effect of phenyl ring substitution on FAAH inactivation. As can be seen from the inhibition data depicted in Table 1, presence of a hydroxyl or benzyloxy group at the phenyl ring of **11e** can results in significant enhancement of the compound's ability to inhibit FAAH. More specifically, addition of *ortho*-, *meta*- or *para*-hydroxy groups (**21c**, **21b** and **21a**) leads to 15- to 31-fold increases in the compounds' potencies. However, the *ortho*-hydroxy analog (**21c**) exhibits the lowest selectivity for FAAH (47-fold) over MGL. Thus, in terms of inhibitory activity and selectivity for FAAH, the order for the hydroxyl substituted phenylheptyl analogs is as follows: **21a** > **21b** > **21c**. Interestingly, the successful addition of a *para*-hydroxy group on the phenylheptyl template (compound **11e**) seems to work equally well for the phenylpentyl prototype (compound **11c**). Thus, the less lipophilic analog **21d**, with the pentyl linker, exhibits a similar inhibitory activity and selectivity profile as **21a**.

Extension of our SAR study shows that presence of the bulky benzyloxy group at the *ortho*-, *meta*- or *para*-position of the phenylheptyl template (**20c**, **20b** and **20a**) enhances FAAH inhibitory activity by 9-10 fold. This suggests additional favorable binding contacts within FAAH's active site. Again, the *ortho*-substituted analog (**20c**) shows the lowest selectivity for FAAH (27-fold) over MGL. The order of inhibitory activity/selectivity for the benzyloxy substituted phenylheptyl analogs is as follows: **20b** > **20a** > **20c**. A comparison of the IC_{50} values of **20a** and **20d** indicates that modification of the length of the linker from seven carbon atoms to five carbon atoms retains inhibitory activity for FAAH and slightly improves selectivity over MGL. Furthermore, as seen in analog **26**, replacement of the benzylic methylene group with the polar oxygen atom does not affect the compound's potency and selectivity for FAAH. Conversely, replacement of the sulfonyl fluoride group with a sulfonyl ester moiety (compound **27**) leads to a significant decrease (73-fold) in the compound's inhibitory activity.

Our SAR suggests that within the FAAH's catalytic channel and around the phenyl ring binding region, there is suitable space and flexibility to accommodate the hydrophilic hydroxyl and the bulky benzyloxy groups. Presumably, the benzyloxy groups, that represent conformationally defined π-unsaturated systems, are capable of mimicking the length,

conformational properties, and the π-characteristics of the terminal double bonds (e.g. $\Delta^{11,12}$ and $\Delta^{14,15}$ in anandamide) in the endogenous lipids that are recognized by FAAH.

Overall, this systematic SAR study suggests that: 1) for a phenylalkyl template, a five to seven carbon long linker is optimal for FAAH inhibition; 2) addition of a hydroxyl or benzyloxyl group in the phenyl ring enhances the inhibitory activity by 9- to 31-fold; and 3) the most successful compounds in terms of inhibitory activity and selectivity for FAAH over MGL are the hydroxyl substituted analogs **21a**, **21d** and the benzyloxy substituted analogs **20b**, **20d** and **26**.

Subsequently, the most successful sulfonyl fluorides, **21a**, **21d**, **20b**, **20d** and **26** were tested for their CB1 and CB2 receptor binding affinities[50,56] to determine selectivity over the other two endocannabinoid proteins and the results are depicted in Table 2. We observe that the tested compounds had moderate to low affinities for binding to rat CB1 and mouse CB2 with the benzyloxy substituted analogs (**20b**, **20d**, **26**) exhibiting slightly higher CB receptor binding affinities when compared to the hydroxyl substituted ones (**21a** and **21d**). These receptor binding affinity data reported here were carried out using a radioligand displacement assay indicating that our FAAH inhibitors interact with CB1 and CB2 at their respective orthosteric sites. We have no information on whether these ligand-receptor interactions are reversible or of a covalent nature. It should be pointed out that unlike their sulfonyl chloride counterparts, sulfonyl fluorides exhibit relatively low to moderate electrophilic properties and are, thus, capable of exhibiting remarkable selectivities for their targets. This is clearly demonstrated in our recently published results for **21d**.[25] Activity based protein profiling experiments in mouse brain and liver demonstrate that this compound has selectivity for FAAH over a large number of serine hydrolases.

Of the two most successful compounds (**21a** and **21d**) identified here, we chose the less lipophilic **21d** to probe the interaction of FAAH with sulfonyl fluorides in both *in vitro* and *in vivo* models. Experiments with the recombinant human enzyme, studies on the mode of inhibition, and molecular modeling work are following, while assessment of target selectivity and *in vivo* studies with **21d** were published recently.[25,26,40]

Inhibition of recombinant human FAAH

Rat FAAH exhibits 84% sequence identity with the human enzyme.[57] This divergent nature of rat and human FAAH could possibly result in species-based differences in inhibitory potency. For this reason, the key inhibitor **21d** was examined against the human enzyme and the results are shown in Table 3. We observe that the tested compound exhibits similar inhibitory activity for both rat and the human FAAH.

Enzyme inhibition studies

We utilized the rapid dilution assay[58] to assess whether **21d** inhibits FAAH through a reversible or irreversible mechanism. Under the conditions of the rapid dilution assay, incubation of the enzyme with a reversible inhibitor leads to an equilibrated system. Rapid dilution of this system perturbs the equilibrium between the inhibitor and the enzyme and results in virtually complete recovery of enzymatic activity. In contrast, incubation of the enzyme with an irreversible inhibitor results in the formation of an irreversible enzyme-inhibitor complex with very little or no enzymatic activity being recovered after dilution of the assay mixture. As detailed in the experimental, two purified FAAH enzyme aliquots were pre incubated for one hour with equal volumes of DMSO without inhibitor (control) or excess (40-fold higher than its IC_{50} value) of **21d**. As shown in Figure 2, after rapid dilution, a negligible recovery of FAAH activity (8%) was observed for **21d** after 80 min and did not

essentially change after 24 hours incubation (data not shown), suggesting that **21d** inhibits FAAH through an irreversible mechanism.

To further explore the inhibition mode of the sulfonyl fluorides reported here, we next carried out liquid chromatography-time of flight mass spectrometry (LC-QTOF) experiments to directly test whether **21d** covalently modified ΔTM rFAAH. It should be noted that mass spectrometric characterization of the FAAH-inhibitor complex with sulfonyl fluorides is reported here for first time while similar work with carbamate and urea based inhibitors was reported earlier.[59,60] As detailed in the experimental, samples of purified ΔTM rFAAH were incubated for 60 min with a 2-fold molar excess of **21d** in DMSO or with DMSO vehicle only, after which incubation samples were desalted and analyzed by MS to determine the intact mass of each. The spectrum of the DMSO treated sample showed a prominent peak with an average mass of 64390.7 Da for the ΔTM rFAAH. The average intact mass of **21d**-treated ΔTM rFAAH was 226.6 Da greater than that of the unmodified enzyme (Figure 3), a result consistent with enzyme sulfonylation by **21d** and the consequent calculated mass increase of 226.2 Da. Together, our experiments indicate that compound **21d** acts as an irreversible inhibitor that covalently modifies the enzyme. Most probably, this involves an S_N2 reaction resulting in sulfonylation of ΔTM rFAAH at the catalytic Ser241 (Figure 4). It should be noted that this potential pathway is congruent with the reported mechanism of inactivation of serine esterases by sulfonyl fluorides.[61,62]

Molecular modeling

To gain insight into the features required for potent FAAH inhibition, three sulfonyl fluoride analogs, **11c**, **20d** and **21d**, were covalently docked into rFAAH as detailed in the experimental. Our docking experiments are based on enzyme sulfonylation at the catalytic Ser241 (Figure 4). In each docked pose (Figure 5), the ligand is found in the acyl binding channel of rFAAH, with one oxygen of the sulfonyl moiety involved in extensive hydrogen bonding with the oxyanion hole (formed by Ile238, Gly239, Gly240 and Ser241). Addition of a hydroxyl group in the phenyl ring allows for the formation of a hydrogen bond between the ligand and Thr488, possibly increasing the potency of **21d**. Similarly, introduction of a benzyloxy substituent produces analog **20d**, whose enhanced potency is probably due to an increase in hydrophobic contacts within the enzyme's binding channel.

Conclusions

In summary, our SAR study includes sulfonyl fluoride derivatives where the sulfonyl fluoride group is connected to a substituted or non-substituted phenyl ring through a linear alkyl linker. Bio-data suggest that: 1) a five to seven carbon long linker is optimal for FAAH deactivation, 2) addition of a hydroxy or benzyloxy group in the phenyl ring enhances inhibitory activity, 3) replacement of the linker's benzylic methylene group with an oxygen atom is well tolerated, and 4) all new analogs show a significant degree of selectivity for FAAH over MGL. Studies with recombinant human FAAH revealed that **21d** has similar inhibitory potency for both the rat and the human enzyme while experiments using rapid dilution assays and mass spectrometric analysis suggested that the compound is a covalent modifier for the enzyme and inhibits its action in an irreversible manner. Cannabinoid receptor binding studies of the most successful analogs indicate that they had low to moderate binding affinities for CB1 and CB2. Docking experiments have identified key pharmacophoric features for potent FAAH inhibition.

Experimental section

Materials

All reagents and solvents were purchased from Aldrich Chemical Company, unless otherwise specified, and used without further purification. All anhydrous reactions were performed under a static argon atmosphere in flame-dried glassware using scrupulously dry solvents. Flash column chromatography employed silica gel 60 (230-400 mesh). All compounds were demonstrated to be homogeneous by analytical TLC on pre-coated silica gel TLC plates (Merck, 60 F_{245} on glass, layer thickness 250 μm), and chromatograms were visualized by phosphomolybdic acid staining. Melting points were determined on a micro-melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer. NMR spectra were recorded in $CDCl_3$, unless otherwise stated, on a Bruker AC 300 (^1H at 300 MHz, ^{13}C at 75 MHz) or on a Bruker Ultra Shield 400 WB plus (^1H at 400 MHz, ^{13}C at 100 MHz) or on a Varian INOVA-500 (^1H at 500 MHz, ^{13}C at 125 MHz) spectrometers and chemical shifts are reported in units of δ relative to internal TMS. Multiplicities are indicated as br (broadened), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and coupling constants (J) are reported in hertz (Hz). Low and high-resolution mass spectra were performed in School of Chemical Sciences, University of Illinois at Urbana-Champaign. Mass spectral data are reported in the form of *m/z* (intensity relative to base = 100). Elemental analyses were obtained in Baron Consulting Co, Milford, CT, and were within ± 0.4% of the theoretical values (see supporting information). Purities of the tested compounds were determined by elemental analysis and were > 95%.

General procedure for the synthesis of phenylalkyl iodides (9b-9f)

A round bottom flask was charged with phenylalkyl alcohol **8** (1 equiv.), acetonitrile/diethyl ether mixture (1:2), triphenyl phosphine (1.3 equiv.), imidazole (1.3 equiv.), and iodine (1.3 equiv.). The solution was blanketed with argon and capped, and the reaction stirred for 4-5 hours at room temperature. The resulting mixture diluted with diethyl ether, washed with water, aqueous sodium thiosulfate, and brine, dried ($MgSO_4$) and evaporated. Purification by flash column chromatography on silica gel (10% diethyl ether-hexane) gave phenylalkyl iodide **9** in 72-85% yield.

4-Phenylbutyl iodide[63] **(9b)**: colorless oil, yield: 83%. ^1H NMR (300 MHz, $CDCl_3$) δ 7.40-7.11 (m, 5H, ArH), 3.20 (t, J = 7.2 Hz, 2H, -CH_2I), 2.61 (t, J = 7.0 Hz, 2H, -CH_2-Ph), 1.93-1.59 (m, 4H).

5-Phenylpentyl iodide[64] **(9c)**: colorless oil, yield: 85%. ^1H NMR (300 MHz, $CDCl_3$) δ 7.49-7.11 (m, 5H, ArH), 3.18 (t, J = 7.3 Hz, 2H, -CH_2I), 2.62 (t, J = 7.1 Hz, 2H, -CH_2-Ph), 1.98-1.32 (m, 6H).

6-Phenylhexyl iodide[64] **(9d)**: colorless oil, yield: 81%. ^1H NMR (300 MHz, $CDCl_3$) δ 7.44-7.08 (m, 5H, ArH), 3.17 (t, J = 7.2 Hz, 2H, -CH_2I), 2.60 (t, J = 7.0 Hz, 2H, -CH_2-Ph), 1.81 (m, 2H), 1.68-1.06 (m, 6H); mass spectrum *m/z* (relative intensity) 288 (M^+, 5), 117 (7), 105 (13), 91 (100), 77 (18), 65 (32).

7-Phenylheptyl iodide[65] **(9e)**: colorless oil, yield: 76%. ^1H NMR (300 MHz, $CDCl_3$) δ 7.40-7.05 (m, 5H, ArH), 3.19 (t, J = 7.3 Hz, 2H, -CH_2I), 2.60 (t, J = 7.1 Hz, 2H, -CH_2-Ph), 1.80 (m, 2H), 1.71-1.02 (m, 8H).

8-Phenyloctyl iodide[64] **(9f)**: colorless oil, yield: 72%. ^1H NMR (300 MHz, $CDCl_3$) δ 7.41-7.01 (m, 5H, ArH), 3.18 (t, J = 7.3 Hz, 2H, -CH_2I), 2.59 (t, J = 7.1 Hz, 2H, -CH_2-Ph), 1.81 (m, 2H), 1.70-1.10 (m, 10H).

General procedure for the synthesis of phenylalkylsulfonyl chlorides (10a-10f)

A solution of phenylalkyl iodide **9** (1 equiv.) in a mixture of dry *n*-pentane/diethyl ether (3:2) was cooled to −78°C under argon, and *t*-BuLi (2.2 equiv., using a 1.7 M solution of *t*-BuLi in hexane) was added dropwise over a 2 min period. The mixture was stirred for 10 min at −78°C and then was transferred by cannula to a cooled (−78°C) and dry solution of SO_2Cl_2 in *n*-pentane over a 20 min period. Following the addition, the reaction mixture was stirred for 1 hour at −78°C and then allowed to warm to room temperature over a 3 hours period. The reaction mixture was quenched by dropwise addition of water, then diluted with diethyl ether and the organic phase was separated. The aqueous phase was extracted with diethyl ether, the combined organic layer was dried ($MgSO_4$) and the solvent was evaporated. Purification by flash column chromatography on silica gel gave phenylalkylsulfonyl chloride **10** in 19-23% yield.

3-Phenylpropane-1-sulfonyl chloride[66] **(10a)**: colorless viscous oil, yield: 19%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50-7.03 (m, 5H, ArH), 3.66 (m as t, *J* = 7.7 Hz, 2H, -CH_2SO_2Cl), 2.63 (t, *J* = 7.2 Hz, 2H, -C*H*$_2$-Ph), 2.22 (qt, *J* = 7.4 Hz, 2H); mass spectrum *m/z* (relative intensity) 220 (M^++2, 3), 218 (M^+, 9), 183 (5), 117 (69), 91 (100). Exact mass calculated for $C_9H_{11}ClO_2S$, 218.0168; found, 218.0165.

4-Phenylbutane-1-sulfonyl chloride[67] **(10b)**: colorless viscous oil (lit.[67] m p = 41-41.5°C), yield: 22%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40-7.08 (m, 5H, ArH), 3.65 (m as t, *J* = 7.6 Hz, 2H, -CH_2SO_2Cl), 2.70 (t, *J* = 7.1 Hz, 2H, -C*H*$_2$-Ph), 2.12 (qt, *J* = 7.2 Hz, 2H), 1.82 (qt, *J* = 6.9 Hz, 2H).

5-Phenylpentane-1-sulfonyl chloride[68] **(10c)**: colorless viscous oil, yield: 23%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41-7.09 (m, 5H, ArH), 3.65 (m as t, *J* = 7.7 Hz, 2H, -CH_2SO_2Cl), 2.65 (t, *J* = 7.3 Hz, 2H, -C*H*$_2$-Ph), 2.07 (qt, *J* = 7.2 Hz, 2H), 1.82-1.40 (m, 4H); mass spectrum *m/z* (relative intensity) 248 (M^++2, 2), 246 (M^+, 6), 117 (55), 91 (100).

6-Phenylhexane-1-sulfonyl chloride (10d): colorless viscous oil, yield: 22%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39-6.98 (m, 5H, ArH), 3.63 (m as t, *J* = 7.6 Hz, 2H, -CH_2SO_2Cl), 2.62 (t, *J* = 7.2 Hz, 2H, -C*H*$_2$-Ph), 1.97 (qt, *J* = 7.6 Hz, 2H), 1.72-1.19 (m, 6H); mass spectrum *m/z* (relative intensity) 262 (M^++2, 3), 260 (M^+, 9), 91 (100). Exact mass calculated for $C_{12}H_{17}ClO_2S$, 260.0638; found, 260.0639.

7-Phenylheptane-1-sulfonyl chloride (10e): colorless viscous oil, yield: 20%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39-7.07 (m, 5H, ArH), 3.64 (m as t, *J* = 7.8 Hz, 2H, -CH_2SO_2Cl), 2.62 (t, *J* = 7.2 Hz, 2H, -C*H*$_2$-Ph), 2.11-1.75 (m, 2H), 1.72-1.13 (m, 8H); mass spectrum *m/z* (relative intensity) 276 (M^++2, 2), 274 (M^+, 6), 91 (100). Exact mass calculated for $C_{13}H_{19}ClO_2S$, 274.0794; found, 274.0790.

8-Phenyloctane-1-sulfonyl chloride (10f): colorless viscous oil, yield: 21%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42-7.08 (m, 5H, ArH), 3.64 (m as t, *J* = 7.8 Hz, 2H, -CH_2SO_2Cl), 2.61 (t, *J* = 7.1 Hz, 2H, -C*H*$_2$-Ph), 2.04 (m, 2H), 1.68-1.05 (m, 10H); mass spectrum *m/z* (relative intensity) 290 (M^++2, 3), 288 (M^+, 9), 91 (100). Exact mass calculated for $C_{14}H_{21}ClO_2S$, 288.0951; found, 288.0954.

Phenylalkylsulfonyl fluorides (11)

The synthesis was carried out as described for **20a** (see text below), using phenylalkylsulfonyl chlorides **10a-10f** (1 equiv.) and NH_4F (2 equiv.) in dry acetone. The crude products obtained after workup were purified by flash column chromatography on silica gel (diethyl ether in hexane) to give **11a-11f** as colorless viscous liquids in 91-93% yields.

3-Phenylpropane-1-sulfonyl fluoride (11a): Yield: 91%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46-7.15 (m, 5H, ArH), 3.33 (m as dt, J = 10.5 Hz, J = 4.4 Hz, 2H, -CH_2SO_2F), 2.82 (t, J = 7.3 Hz, 2H, -CH_2-Ph), 2.29 (qt, J = 7.5 Hz, 2H); mass spectrum *m/z* (relative intensity) 202 (M^+, 19), 117 (30), 91 (100). Exact mass calculated for $C_9H_{11}FO_2S$, 202.0464; found, 202.0465.

4-Phenylbutane-1-sulfonyl fluoride (11b): Yield: 92%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45-7.08 (m, 5H, ArH), 3.35 (m as dt, J = 10.5 Hz, J = 4.3 Hz, 2H, -CH_2SO_2F), 2.63 (t, J = 7.2 Hz, 2H, -CH_2-Ph), 2.10-1.52 (m, 4H); mass spectrum *m/z* (relative intensity) 216 (M^+, 39), 104 (7), 91 (100). Exact mass calculated for $C_{10}H_{13}FO_2S$, 216.0620; found, 216.0621.

5-Phenylpentane-1-sulfonyl fluoride (11c): Yield: 93%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41-7.08 (m, 5H, ArH), 3.34 (m as dt, J = 10.3 Hz, J = 4.2 Hz, 2H, -CH_2SO_2F), 2.63 (t, J = 7.3 Hz, 2H, -CH_2-Ph), 2.05 (qt, J = 7.0 Hz, 2H), 1.80-1.39 (m, 4H); mass spectrum *m/z* (relative intensity) 230 (M^+, 20), 117 (4), 105 (8), 91 (100). Exact mass calculated for $C_{11}H_{15}FO_2S$, 230.07768; found, 230.07763.

6-Phenylhexane-1-sulfonyl fluoride (11d): Yield: 93%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-7.09 (m, 5H, ArH), 3.33 (m as dt, J = 10.5 Hz, J = 4.3 Hz, 2H, -CH_2SO_2F), 2.62 (t, J = 7.3 Hz, 2H, -CH_2-Ph), 1.94 (qt, J = 7.6 Hz, 2H), 1.75-1.22 (m, 6H); mass spectrum *m/z* (relative intensity) 244 (M^+, 17), 117 (2), 105 (9), 91 (100). Exact mass calculated for $C_{12}H_{17}FO_2S$, 244.0933; found, 244.0936.

7-Phenylheptane-1-sulfonyl fluoride (11e): Yield: 91%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39-7.08 (m, 5H, ArH), 3.33 (m as dt, J = 10.5 Hz, J = 4.3 Hz, 2H, -CH_2SO_2F), 2.62 (t, J = 7.2 Hz, 2H, -CH_2-Ph), 2.09-1.74 (m, 2H), 1.71-1.12 (m, 8H); mass spectrum *m/z* (relative intensity) 258 (M^+, 18), 105 (11), 91 (100). Exact mass calculated for $C_{13}H_{19}FO_2S$, 258.1090; found, 258.1087.

8-Phenyloctane-1-sulfonyl fluoride (11f): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45-7.05 (m, 5H, ArH), 3.32 (m as dt, J = 10.3 Hz, J = 4.2 Hz, 2H, -CH_2SO_2F), 2.59 (t, J = 7.1 Hz, 2H, -CH_2-Ph), 2.10-1.72 (m, 2H), 1.70-1.13 (m, 10H). mass spectrum *m/z* (relative intensity) 272 (M^+, 19), 117 (2), 105 (13), 91 (100). Exact mass calculated for $C_{14}H_{21}FO_2S$, 272.1246; found, 272.1244.

(6-Phenoxyhexyl)triphenylphosphonium bromide (13a)

The synthesis was carried out as described for **13b** (see text below), using 6-phenoxyhexyl bromide (**12a**) (2.8 g, 10.9 mmol) and triphenylphosphine (3.14 g, 12 mmol) in anhydrous benzene (11 mL), and gave **13a** as a white solid in 84% yield (4.75 g). m p 143-145°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (dd, J = 12 Hz, J = 8.0 Hz, 6H, -PPh_3), 7.78 (td, J = 8.0 Hz, J = 1.5 Hz, 3H, -PPh_3), 7.69 (td, J = 8.0 Hz, J = 3.5 Hz, 6H, -PPh_3), 7.25 (t, J = 7.7 Hz, 2H, 3-H, 5-H, -OPh), 6.91 (t, J = 7.7 Hz, 1H, 4-H, -OPh), 6.84 (d, J = 7.7 Hz, 2H, 2-H, 6-H, -OPh), 3.95–3.87 (m and t overlapping, 4H, -CH_2OPh and -C$H_2$$PPh_3$, especially 3.90, t, J = 6.3 Hz, -CH_2OPh), 1.80-1.60 (m, 6H), 1.49 (quintet, J = 7.7 Hz, 2H).

(4-Phenoxybutyl)triphenylphosphonium bromide (13b)

A mixture of 4-phenoxybutyl bromide (**12b**) (22.0 g, 96.1 mmol) and triphenylphosphine (27.6 g, 105.3 mmol) in anhydrous benzene (96 mL), was refluxed for two days under argon. The reaction mixture was cooled to room temperature, and the precipitating product (**13b**) was isolated by filtration under reduced pressure and washed with anhydrous diethyl ether. Yield: 85% (40.0 g); white solid, m p 185-186°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (dd, J = 12 Hz, J = 8.0 Hz, 6H, -PPh_3), 7.88 (td, J = 8.0 Hz, J = 1.5 Hz, 3H, -PPh_3), 7.80 (td,

J = 8.0 Hz, *J* = 3.5 Hz, 6H, -PPh_3), 7.25 (t, *J* = 7.9 Hz, 2H, 3-H, 5-H, -OPh), 6.92 (t, *J* = 7.9 Hz, 1H, 4-H, -OPh), 6.82 (d, *J* = 7.9 Hz, 2H, 2-H, 6-H, -OPh), 4.09 (t, *J* = 5.5 Hz, 2H, -C*H*$_2$OPh), 4.04-3.98 (m, 2H, -C*H*$_2$$PPh_3$), 2.25 (qt, *J* = 6.4 Hz, 2H), 1.93-1.85 (m, 2H).

1-(4-Methoxyphenyl)-7-phenoxyhept-1-ene (14a)

To a stirred suspension of (6-phenoxyhexyl)triphenylphosphonium bromide (**13a**) (4.60 g, 8.86 mmol) in dry THF (80 mL) at 0°C, under an argon atmosphere, was added potassium bis(trimethylsilyl)amide (1.76 g, 8.84 mmol). The resulting slurry was stirred for 5 min and then a solution of 4-methoxybenzaldehyde (0.61 g, 4.49 mmol) in dry THF (10 mL) was added dropwise. The reaction was stirred for an additional 10 min and upon completion was quenched by the addition of saturated aqueous NH_4Cl solution. The mixture was warmed to room temperature, the organic layer was separated, and the aqueous phase was extracted with diethyl ether. The combined organic layer was washed with brine, dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was purified through a short column of silica gel (5% diethyl ether in hexane) to give **14a** as a colorless liquid in 91% yield (1.21 g). On the basis of ^{1}H NMR analysis the product is a mixture of *cis* and *trans* isomers in a ratio 94:6 respectively. *cis* Isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (t, *J* = 7.2 Hz, 2H, 3-H, 5-H, -OPh), 7.21 (d, *J* = 8.7 Hz, 2H, 2-H, 6-H, -*Ph*-OMe), 6.92 (t, *J* = 7.2 Hz, 1H, 4-H, -OPh), 6.88 (d, *J* = 7.2 Hz, 2H, 2-H, 6-H, -OPh), 6.86 (d, *J* = 8.7 Hz, 2H, 3-H, 5-H, -*Ph*-OMe), 6.35 (d, *J* = 11.5 Hz, 1H, 1′-H), 5.57 (dt, *J* = 11.5 Hz, *J* = 7.5 Hz, 1H, 2′-H), 3.94 (t, *J* = 7.3 Hz, 2H, -C*H*$_2$-OPh), 3.81 (s, 3H, -OMe), 2.41-2.20 (m as q, *J* = 7.5 Hz, 2H, 3′-H), 1.78 (qt, *J* = 7.2 Hz, 2H), 1.58-1.48 (m, 4H); mass spectrum *m/z* (relative intensity) 296 (M^+, 12), 203 (17), 173 (8), 159 (8), 147 (78), 134 (18), 121 (100), 107 (6), 91 (19), 77 (8); Exact mass calculated for $C_{20}H_{24}O_2$, 296.1776; found, 296.1774; *trans* Isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.33 (d, *J* = 15.6 Hz, 1H, 1′-H), 6.08 (dt, *J* = 15.6 Hz, *J* = 7.4 Hz, 1H, 2′-H).

1-(3-Methoxyphenyl)-7-phenoxyhept-1-ene (14b)

The synthesis was carried out as described for **14a**, starting from **13a** (3.20 g, 6.17 mmol), potassium bis(trimethylsilyl)amide (1.22 g, 6.15 mmol) in dry THF (55 mL), and a solution of 3-methoxybenzaldehyde (0.28 g, 2.06 mmol) dry THF (5 mL). The crude product obtained after workup was purified by flash column chromatography on silica gel (5% diethyl ether in hexane) to give **14b** as a colorless liquid in 92% yield (564 mg). On the basis of ^{1}H NMR analysis the product is a mixture of *cis* and *trans* isomers in a ratio 92:8 respectively. *cis* Isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27-7.21 (t and t overlapping, 3H, 3-H, 5-H, -OPh and 5-H, -*Ph*-OMe), 6.92 (t, *J* = 7.3 Hz, 1H, 4-H, -OPh), 6.88 (d, *J* = 7.3 Hz, 2H, 2-H, 6-H, -OPh), 6.87 (d, *J* = 8.5 Hz, 1H, 6-H, -*Ph*-OMe), 6.82 (d, *J* = 2.7 Hz, 1H, 2-H, -*Ph*-OMe), 6.77 (dd, *J* = 8.5 Hz, *J* = 2.7 Hz, 1H, 4-H, -*Ph*-OMe), 6.39 (d, *J* = 11.7 Hz, 1H, 1′-H), 5.67 (dt, *J* = 11.7 Hz, *J* = 7.5 Hz, 1H, 2′-H), 3.94 (t, *J* = 6.5 Hz, 2H, -C*H*$_2$OPh), 3.80 (s, 3H, OMe), 2.38 (q, *J* = 6.5, 2H, 3′-H), 1.78 (qt, *J* = 6.5 Hz, 2H), 1.56-1.46 (m, 4H); mass spectrum *m/z* (relative intensity) 296 (M^+, 43), 203 (39), 173 (9), 159 (20), 147 (42), 134 (28), 121 (100), 107 (8), 91 (23), 77 (16); Exact mass calculated for $C_{20}H_{24}O_2$, 296.1776; found, 296.1776; *trans* Isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.36 (d, *J* = 15.6 Hz, 1H, 1′-H), 6.22 (dt, *J* = 15.6 Hz, *J* = 7.4 Hz, 1H, 2′-H).

1-(2-Methoxyphenyl)-7-phenoxyhept-1-ene (14c)

The synthesis was carried out as described for **14a**, starting from **13a** (2.0 g, 3.85 mmol), potassium bis(trimethylsilyl)amide (0.762 g, 3.83 mmol) in dry THF (30 mL), and a solution of 2-methoxybenzaldehyde (0.20 g, 1.47 mmol) in dry THF (5 mL). The crude product obtained after workup was purified by flash column chromatography on silica gel (5% diethyl ether in hexane) to give **14c** as a colorless liquid in 91% yield (396 mg). On the basis

of ^{1}H NMR analysis the product is a mixture of *cis* and *trans* isomers in a ratio 93:7 respectively. *cis* Isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29-7.21 (m, 4H, 3-H, 5-H, -OPh and 4-H, 6-H, -*Ph*-OMe, overlapping), 6.95-6.86 (m, 5H, 2-H, 4-H, 6-H, -OPh and 3-H, 5-H, -*Ph*-OMe, overlapping), 6.52 (d, *J* = 11.5 Hz, 1H, 1′-H), 5.73 (dt, *J* = 11.5 Hz, *J* = 7.5 Hz, 1H, 2′-H), 3.93 (t, *J* = 6.7 Hz, 2H, -C*H*$_2$-OPh), 3.83 (s, 3H, -OMe), 2.28 (m as q, *J* = 7.2 Hz, 2H, 3′-H), 1.76 (qt, *J* = 7.3 Hz, 2H), 1.53-1.46 (m, 4H); mass spectrum *m/z* (relative intensity) 296 (M^+, 4), 203 (6), 173 (4), 159 (7), 147 (34), 134 (7), 121 (100), 107 (8), 91 (32), 77 (11); Exact mass calculated for $C_{20}H_{24}O_2$, 296.1776; found, 296.1775; *trans Isomer*: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.49 (d, *J* = 15.6 Hz, 1H, 1′-H), 6.22 (dt, *J* = 15.6 Hz, *J* = 7.4 Hz, 1H, 2′-H).

1-(4-Methoxyphenyl)-5-phenoxypent-1-ene (14d)

The synthesis was carried out as described for **14a**, starting from **13b** (29.0 g, 59.1 mmol), potassium bis(trimethylsilyl)amide (11.7 g, 58.8 mmol) in dry THF (200 mL), and a solution of 4-methoxybenzaldehyde (2.9 g, 21.3 mmol) in dry THF (10 mL). The crude product obtained after workup was purified by flash column chromatography on silica gel (5% diethyl ether in hexane) to give **14d** as a colorless liquid in 93% yield (5.31 g). On the basis of ^{1}H NMR analysis the product is a mixture of *cis* and *trans* isomers in a ratio 95:5 respectively. *cis* Isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 (t, *J* = 7.3 Hz, 2H, 3-H, 5-H, -OPh), 7.22 (d, *J* = 8.7 Hz, 2H, 2-H, 6-H, -*Ph*-OMe), 6.92 (t, *J* = 7.3 Hz, 1H, 4-H, -OPh), 6.87 (d, *J* = 7.3 Hz, 2H, 2-H, 6-H, -OPh), 6.85 (d, *J* = 8.7 Hz, 2H, 3-H, 5-H, -*Ph*-OMe), 6.39 (d, *J* = 11.5 Hz, 1H, 1′-H), 5.60 (dt, *J* = 11.5 Hz, *J* = 7.5 Hz, 1H, 2′-H), 3.98 (t, *J* = 6.5 Hz, 2H, -C*H*$_2$-OPh), 3.80 (s, 3H, -OMe), 2.51 (m as qd, *J* = 7.5 Hz, *J* = 2.1 Hz, 2H, 3′-H), 1.94 (qt, *J* = 7.2 Hz, 2H, 4′-H); mass spectrum *m/z* (relative intensity) 268 (M^+, 2), 149 (16), 107 (52), 91 (100); Exact mass calculated for $C_{18}H_{20}O_2$, 268.1463; found, 268.1466; *trans* Isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.37 (d, *J* = 15.6 Hz, 1H, 1′-H), 6.10 (dt, *J* = 15.6 Hz, *J* = 7.4 Hz, 1H, 2′-H).

1-(4-Methoxyphenyl)-7-phenoxyheptane (15a)

A mixture of **14a** (1.19 g, 4.02 mmol) and 10% Pd/C (0.18 g, 15% w/w) in ethyl acetate (40 mL) was placed in a Parr apparatus (Parr Instrument Co, Moline, IL) and treated with hydrogen at 30 psi for 6 hours. The catalyst was removed by filtration through a pad of celite and the filtrate was evaporated under reduced pressure to give **15a** as a white solid (1.14 g, 95% yield) which was used in the next step without further purification. m p 32-34°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (t, *J* = 8.5 Hz, 2H, 3-H, 5-H, -OPh), 7.11 (d, *J* = 8.2 Hz, 2H, 2-H, 6-H, -*Ph*-OMe), 6.95 (t, *J* = 8.5 Hz, 1H, 4-H, -OPh), 6.92 (d, *J* = 8.5 Hz, 2H, 2-H, 6-H, -OPh), 6.84 (d, *J* = 8.2 Hz, 2H, 3-H, 5-H, -*Ph*-OMe), 3.97 (t, *J* = 6.7 Hz, 2H, -C*H*$_2$-OPh), 3.81,(s, 3H, OMe) 2.57 (t, *J* = 7.5 Hz, 2H, -C*H*$_2$-Ph-OMe), 1.78 (qt, *J* = 6.7 Hz, 2H of the 7-phenoxyheptyl group), 1.62 (qt, *J* = 7.5 Hz, 2H of the 7-phenoxyheptyl group), 1.48 (qt, *J* = 7.5 Hz, 2H of the 7-phenoxyheptyl group), 1.44-1.34 (m, 4H of the 7-phenoxyheptyl group); mass spectrum *m/z* (relative intensity) 298 (M^+, 17), 204 (5), 147 (11), 134 (7), 121 (100), 107 (3), 94 (7), 77 (8); Exact mass calculated for $C_{20}H_{26}O_2$, 298.1933; found, 298.1933.

1-(3-Methoxyphenyl)-7-phenoxyheptane (15b)

The synthesis was carried out as described for **15a**, using **14b** (0.55 g, 1.86 mmol), and 10% Pd/C (0.080 g, 15% w/w) in AcOEt (20 mL), and gave **15b** as a colorless viscous liquid in 96% yield (530 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (t, *J* = 7.7 Hz, 2H, 3-H, 5-H, -OPh), 7.19 (t, *J* = 8.0 Hz, 1H, 5-H, -*Ph*-OMe), 6.92 (t, *J* = 7.7 Hz, 1H, 4-H, -OPh), 6.89 (d, *J* = 7.7 Hz, 2H, 2-H, 6-H, -OPh), 6.77 (d, *J* = 8.0 Hz, 1H, 6-H, -*Ph*-OMe), 6.74-6.70 (d and d overlapping, 2H, 2-H, 4-H, -*Ph*-OMe), 3.94 (t, *J* = 6.5Hz, 2H, -C*H*$_2$OPh), 3.79 (s, 3H,

OMe), 2.58 (t, *J* = 7.5Hz, 2H, -C*H*$_2$-Ph-OMe), 1.77 (qt, *J* = 7.0 Hz, 2H of the 7-phenoxyheptyl group), 1.62 (qt, *J* = 7.0 Hz, 2H of the 7-phenoxyheptyl group), 1.50-1.42 (m, 2H of the 7-phenoxyheptyl group), 1.42-1.34 (m, 4H of the 7-phenoxyheptyl group); mass spectrum *m/z* (relative intensity) 298 (M^+, 45), 204 (6), 147 (7), 134 (9), 121 (100), 107 (6), 94 (14), 77 (9); Exact mass calculated for $C_{20}H_{26}O_2$, 298.1933; found, 298.1934.

1-(2-Methoxyphenyl)-7-phenoxyheptane (15c)

The synthesis was carried out as described for **15a**, using **14c** (0.35 g, 1.18 mmol), and 10% Pd/C (0.050 g, 14% w/w) in AcOEt (20 mL), and gave **15c** as a colorless viscous liquid in 94% yield (330 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (t, *J* = 7.5 Hz, 2H, 3-H, 5-H, -OPh), 7.16 (t, *J* = 7.5 Hz, 1H, 4-H, -*Ph*-OMe), 7.12 (d, *J* = 7.5 Hz, 1H, 6-H, -*Ph*-OMe), 6.95-6.81 (m, 5H, 2-H, 4-H, 6-H, -OPh and 3-H, 5-H, -*Ph*-OMe, overlapping), 3.95 (t, *J* = 6.7 Hz, 2H, -C*H*$_2$-OPh), 3.81 (s, 3H, OMe), 2.60 (t, *J* = 7.7, 2H, -C*H*$_2$-Ph-OMe), 1.78 (qt, *J* = 7.0 Hz, 2H of the 7-phenoxyheptyl group), 1.59 (qt, *J* = 6.9 Hz, 2H of the 7-phenoxyheptyl group), 1.48-1.43 (m, 2H of the 7-phenoxyheptyl group), 1.42-1.35 (m, 4H of the 7-phenoxyheptyl group); mass spectrum *m/z* (relative intensity) 298 (M^+, 19), 204 (7), 147 (13), 134 (11), 121 (100), 107 (8), 94 (43), 77 (10); Exact mass calculated for $C_{20}H_{26}O_2$, 298.1933; found, 298.1935.

1-(4-Methoxyphenyl)-5-phenoxypentane (15d)

The synthesis was carried out as described for **15a**, using **14d** (3.67 g, 13.59 mmol) and 10% Pd/C (0.55 g, 15% w/w) in AcOEt (100 mL), and gave **15d** as a white solid in 95% yield (3.52 g). m p 32-34°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (t, *J* = 7.5 Hz, 2H, 3-H, 5-H, -OPh), 7.09 (d, *J* = 8.5 Hz, 2H, 2-H, 6-H, -*Ph*-OMe), 6.92 (t, *J* = 7.5 Hz, 1H, 4-H, -OPh), 6.88 (d, *J* = 7.5 Hz, 2H, 2-H, 6-H, -OPh), 6.82 (d, *J* = 8.5 Hz, 2H, 3-H, 5-H, - *Ph*-OMe), 3.94 (t, *J* = 6.5 Hz, 2H, -C*H*$_2$-OPh), 3.78 (s, 3H, OMe), 2.58 (t, *J* = 7.5 Hz, 2H, -C*H*$_2$-Ph-OMe), 1.80 (qt, *J* = 6.7 Hz, 2H of the 5-phenoxypentyl group), 1.66 (qt, *J* = 7.0 Hz, 2H of the 5-phenoxypentyl group), 1.49 (qt, *J* = 7.5 Hz, 2H of the 5-phenoxypentyl group); mass spectrum *m/z* (relative intensity) 270 (M^+, 18), 177 (7), 147 (18), 134 (6), 121 (100), 94 (6), 77 (8); Exact mass calculated for $C_{18}H_{22}O_2$, 270.1620; found, 270.1620.

7-Bromo-1-(4-hydroxyphenyl)heptane (16a)

To a stirred solution of **15a** (1.1 g, 3.69 mmol) in anhydrous CH_2Cl_2, (40 mL), at −30°C, under an argon atmosphere, was added boron tribromide (8 mL, 1M solution in CH_2Cl_2). The mixture was gradually warmed to room temperature and stirred for an additional 2 hours. Unreacted boron tribromide was destroyed by the addition of aqueous saturated $NaHCO_3$ solution at 0°C. The resulting mixture was warmed to room temperature and volatiles were removed in vacuo. The residue was diluted with ethyl acetate and washed with saturated $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$ and the solvent evaporated under reduced pressure. Purification by flash column chromatography on silica gel (20% diethyl ether in petroleum ether) gave **16a** as a viscous liquid in 93% yield (930 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.03 (d, *J* = 8.5 Hz, 2H, 2-H, 6-H, -*Ph*-OH), 6.74 (d, *J* = 8.5 Hz, 2H, 3-H, 5-H, -*Ph*-OH), 4.59 (br s, 1H, OH), 3.40 (t, *J* = 6.7 Hz, 2H, -C*H*$_2$Br), 2.53 (t, *J* =7.7 Hz, 2H, -C*H*$_2$-Ph-OH), 1.84 (qt, *J* = 7.0 Hz, 2H of the 7-bromoheptyl group), 1.57 (qt, *J* = 7.5 Hz, 2H of the 7-bromoheptyl group), 1.46-1.38 (m, 2H of the 7-bromoheptyl group), 1.36–1.31 (m, 4H of the 7-bromoheptyl group); mass spectrum *m/z* (relative intensity) 272 (M^++2, 7), 270 (M^+, 7), 147 (2), 133 (4), 120 (3), 107 (100), 91 (2), 77 (5); Exact mass calculated for $C_{13}H_{19}BrO$, 270.0619; found, 270.0620.

7-Bromo-1-(3-hydroxyphenyl)heptane (16b)

The synthesis was carried out as described for **16a**, using **15b** (500 mg, 1.68 mmol) and BBr_3 (1M solution in CH_2Cl_2, 3.7 mL, 3.7 mmol) in anhydrous CH_2Cl_2 (16 mL). The crude product obtained after workup was purified by flash column chromatography on silica gel (20% diethyl ether in hexane) to give **16b** as a viscous liquid in 92% yield (420 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (t, *J* = 7.7 Hz, 1H, 5-H, -*Ph*-OH), 6.75 (d, *J* = 7.7 Hz, 1H, 6-H, -*Ph*-OH), 6.68-6.63 (d and d overlapping, 2H, 2-H, 4-H, -*Ph*-OH), 4.70 (br s, 1H, OH), 3.40 (t, *J* = 6.7 Hz, 2H, -C*H*$_2$Br), 2.56 (t, *J* = 7.7 Hz, 2H, -C*H*$_2$-Ph-OH), 1.85 (qt, *J* = 7.0 Hz, 2H of the 7-bromoheptyl group), 1.60 (qt, *J* = 7.3 Hz, 2H of the 7-bromoheptyl group), 1.46–1.38 (m, 2H of the 7-bromoheptyl group), 1.36–1.30 (m, 4H of the 7-bromoheptyl group); mass spectrum *m/z* (relative intensity) 272 (M^++2, 8), 270 (M^+, 8), 149 (4), 147 (3), 133 (3), 121 (13), 108 (100), 91 (4), 77 (10); Exact mass calculated for $C_{13}H_{19}BrO$, 270.0619; found, 270.0618.

7-Bromo-1-(2-hydroxyphenyl)heptane (16c)

The synthesis was carried out as described for **16a**, using **15c** (300 mg, 1.01 mmol) and BBr_3 (1M solution in CH_2Cl_2, 2.2 mL, 2.2 mmol) in anhydrous CH_2Cl_2 (10 mL). The crude product obtained after workup was purified by flash column chromatography on silica gel (20% diethyl ether in hexane) to give **16c** as a viscous liquid in 90% yield (247 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11 (dd, *J* = 7.5 Hz, *J* = 2.0 Hz, 1H, 6-H, -*Ph*-OH), 7.07 (td, *J* = 7.5 Hz, *J* = 2.0 Hz, 1H, 4-H, -*Ph*-OH), 6.87 (td, *J* = 7.5 Hz, *J* = 1.5 Hz, 1H, 5-H, -*Ph*-OH), 6.75 (dd, *J* = 7.5 Hz, *J* = 1.5 Hz, 1H, 3-H, -*Ph*-OH), 4.62 (br s, 1H, OH), 3.40 (t, *J* = 7.0 Hz, 2H, -CH_2Br), 2.60 (t, *J* = 8.0 Hz, 2H, -C*H*$_2$-Ph-OH), 1.85 (qt, *J* = 6.7 Hz, 2H of the 7-bromoheptyl group), 1.62 (qt, *J* = 7.2 Hz, 2H of the 7-bromoheptyl group), 1.4 (qt, *J* = 7.5 Hz, 2H of the 7-bromoheptyl group), 1.40–1.35 (m, 4H of the 7-bromoheptyl group); mass spectrum *m/z* (relative intensity) 272 (M^++2, 9), 270 (M^+, 9), 147 (2), 133 (4), 120 (5), 107 (100), 91 (4), 77 (9); Exact mass calculated for $C_{13}H_{19}BrO$, 270.0619; found, 270.0621.

5-Bromo-1-(4-hydroxyphenyl)pentane (16d)

The synthesis was carried out as described for **16a**, using **15d** (3.43 g, 12.7 mmol) and BBr_3 (1M solution in CH_2Cl_2, 32 mL, 32 mmol) in anhydrous CH_2Cl_2 (120 mL). The crude product obtained after workup was purified by flash column chromatography on silica gel (20% diethyl ether in hexane) to give **16d** as a viscous liquid in 92% yield (2.84 g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.04 (d, *J* = 8.5 Hz, 2H, 2-H, 6-H, -*Ph*-OH), 6.75 (d, *J* = 8.5 Hz, 2H, 3-H, 5-H, -*Ph*-OH), 4.55 (br s, 1H, OH), 3.34 (t, *J* = 6.7 Hz, 2H, -C*H*$_2$Br), 2.55 (t, *J* =7.7 Hz, 2H, -C*H*$_2$-Ph-OH), 1.88 (qt, *J* = 7.5 Hz, 2H of the 5-bromopentyl group), 1.60 (qt, *J* = 7.5 Hz, 2H of the 5-bromopentyl group), 1.46 (qt, *J* = 7.4 Hz, 2H of the 5-bromopentyl group); mass spectrum *m/z* (relative intensity) 244 (M^++2, 9), 242 (M^+, 9), 120 (2), 107 (100), 91 (4), 77 (7); Exact mass calculated for $C_{11}H_{15}BrO$, 242.0306; found, 242.0303.

7-Bromo-1-(4-benzyloxyphenyl)heptane (17a)

To a stirred solution of **16a** (900 mg, 3.32 mmol) in anhydrous acetone (40 mL), was added anhydrous K_2CO_3 (1.38 g, 10 mmol) and benzyl bromide (624 mg, 3.65 mmol) and the mixture was refluxed for 6 hours under argon. The reaction mixture was cooled to room temperature, diluted with acetone and insoluble materials were filtered off. The filtrate was evaporated under reduced pressure and the residue was dissolved in diethyl ether (50 mL). The ethereal solution was washed with water and brine, dried ($MgSO_4$) and evaporated. Purification by flash column chromatography on silica gel (5% diethyl ether in hexane) afforded **17a** as a white solid in 78% yield (938 mg). m p 32-34°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, *J* = 7.0 Hz, 2H, 2-H, 6-H, -OCH_2*Ph*), 7.38 (t, *J* = 7.0 Hz, 2H, 3-H, 5-H, -OCH_2*Ph*), 7.32 (t, *J* = 7.0 Hz, 1H, -OCH_2*Ph*), 7.08 (d, *J* = 8.5 Hz, 2H, 2-H, 6-H, -*Ph*-OBn),

6.90 (d, *J*= 8.5 Hz, 2H, 3-H, 5-H, -*Ph*-OBn), 5.04 (s, 2H, -OC*H*$_2$Ph), 3.34 (t, *J* = 7.2 Hz, 2H, -CH$_2$Br), 2.54 (t, *J* = 7.5 Hz, 2H, -C*H*$_2$-Ph-OBn), 1.85 (qt, *J* = 7.7 Hz, 2H of the 7-bromoheptyl group), 1.58 (qt, *J* = 7.5 Hz, 2H of the 7-bromoheptyl group), 1.46-1.38 (m, 2H of the 7-bromoheptyl group), 1.37-1.30 (m, 4H of the 7-bromoheptyl group); mass spectrum *m/z* (relative intensity) 362 (M$^+$+2, 6), 360 (M$^+$, 6), 184 (7), 91 (100), 65 (9); Exact mass calculated for $C_{20}H_{25}BrO$, 360.1089; found, 360.1088.

7-Bromo-1-(3-benzyloxyphenyl)heptane (17b)

The synthesis was carried out as described for **17a**, using **16b** (400 mg, 1.48 mmol), K_2CO_3 (612 mg, 4.44 mmol) and benzyl bromide (278 mg, 1.63 mmol) in anhydrous acetone. The crude product obtained after workup was purified by flash column chromatography on silica gel (10% diethyl ether in hexane) to give **17b** as a viscous liquid in 77% yield (411 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, *J* = 7.7 Hz, 2H, 2-H. 6-H, -OCH$_2$*Ph*), 7.39 (t, *J* = 7.7 Hz, 2H, 3-H, 5-H, -OCH$_2$*Ph*), 7.32 (t, *J* = 7.7 Hz 1H, 1H, 4-H, -OCH$_2$*Ph*), 7.19 (t, *J* = 7.9 Hz, 1H, 5-H, -*Ph*-OBn), 6.83-6.76 (m, 3H, 2-H, 4-H, 6-H, -*Ph*-OBn), 5.05 (s, 2H, -OC*H*$_2$Ph), 3.40 (t, *J* = 6.6 Hz, 2H, -C*H*$_2$Br), 2.58 (t, *J* = 7.7 Hz, 2H, -C*H*$_2$-Ph-OBn), 1.84 (qt, *J* = 7.1 Hz, 2H of the 7-bromoheptyl group), 1.61 (qt, *J* = 7.7 Hz, 2H of the 7-bromoheptyl group), 1.42 (qt, *J* = 7.0 Hz, 2H of the 7-bromoheptyl group), 1.35–1.31 (m, 4H of the 7-bromoheptyl group); mass spectrum *m/z* (relative intensity) 362 (M$^+$+2, 13), 360 (M$^+$, 13), 183 (3), 91 (100), 65 (6); Exact mass calculated for $C_{20}H_{25}BrO$, 360.1089; found, 360.1090.

7-Bromo-1-(2-benzyloxyphenyl)heptane (17c)

The synthesis was carried out as described for **17a**, using **16c** (230 mg, 0.85 mmol), K_2CO_3 (352 mg, 2.55 mmol) and benzyl bromide (160 mg, 0.936 mmol) in anhydrous acetone. The crude product obtained after workup was purified by flash column chromatography on silica gel (10% diethyl ether in hexane) to give **17c** as a viscous liquid in 78% yield (240 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, *J* = 7.5 Hz, 2H, 2-H. 6-H, -OCH$_2$*Ph*), 7.39 (t, *J* = 7.5 Hz, 2H, 3-H, 5-H, -OCH$_2$*Ph*), 7.32 (t, *J* = 7.5 Hz, 1H, 4-H, -OCH$_2$*Ph*), 7.18-7.13 (m, 2H, 4-H, 6-H, -*Ph*-OBn), 6.92-6.88 (m, 2H, 3-H, 5-H, -*Ph*-OBn), 5.08 (s, 2H, -OC*H*$_2$Ph), 3.37 (t, *J* = 7.0 Hz, 2H, -CH$_2$Br), 2.67 (t, *J* = 7.5 Hz, 2H, -C*H*$_2$-Ph-OBn), 1.82 (qt, *J* = 7.7 Hz, 2H of the 7-bromoheptyl group), 1.62 (qt, *J* = 7.5 Hz, 2H of the 7-bromoheptyl group), 1.39 (qt, *J* = 7.2 Hz, 2H of the 7-bromoheptyl group), 1.36–1.31 (m, 4H of the 7-bromoheptyl group); mass spectrum *m/z* (relative intensity) 362 (M$^+$+2, 10), 360 (M$^+$, 10), 190 (4), 91 (100), 77 (3), 65 (6); Exact mass calculated for $C_{20}H_{25}BrO$, 360.1089; found, 360.1089.

5-Bromo-1-(4-benzyloxyphenyl)pentane (17d)

The synthesis was carried out as described for **17a**, using **16d** (2.79 g, 11.5 mmol), K_2CO_3 (4.24 g, 30.75 mmol) and benzyl bromide (2.31 g, 13.51 mmol) in anhydrous acetone. The crude product obtained after workup was purified by flash column chromatography on silica gel (5% diethyl ether in hexane) to give **17d** as a semisolid material in 81% yield (3.11 g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (d, *J* = 7.5 Hz, 2H, 2-H, 6-H, -OCH$_2$*Ph*), 7.37 (t, *J* = 7.5 Hz, 2H, 3-H, 5-H, -OCH$_2$*Ph*), 7.31 (t, *J* = 7.5 Hz, 1H, -OCH$_2$*Ph*), 7.08 (d, *J* = 8.5 Hz, 2H, 2-H, 6-H, -*Ph*-OBn), 6.90 (d, *J*= 8.5 Hz, 2H, 3-H, 5-H, -*Ph*-OBn), 5.03 (s, 2H, -OC*H*$_2$Ph), 3.39 (t, *J* = 6.7 Hz, 2H, -CH$_2$Br), 2.56 (t, *J* = 7.7 Hz, 2H, -C*H*$_2$-Ph-OBn), 1.87 (qt, *J* = 6.7 Hz, 2H of the 5-bromopentyl group), 1.61 (qt *J* = 7.7 Hz, 2H of the 5-bromopentyl group), 1.47 (qt *J* = 6.7 Hz, 2H of the 5-bromopentyl group); mass spectrum *m/z* (relative intensity) 334 (M$^+$+2, 15), 332 (M$^+$, 15), 197 (2), 91 (100), 65 (7); Exact mass calculated for $C_{18}H_{21}BrO$, 332.0776; found, 332.0776.

7-(4-Benzyloxyphenyl)heptanesulfonic acid sodium salt (18a)

A stirred mixture of **17a** (900 mg, 2.49 mmol) and Na_2SO_3 (423 mg, 3.36 mmol) in EtOH (20 mL)/H_2O (10 ml) was heated under reflux for 24 hours. The reaction mixture was cooled to room temperature and the solvent evaporated under reduced pressure. The residue was scrupulously dried under high vacuum and the crude product **18a**, (pale yellow solid) was used in the next step without further purification.

7-(3-Benzyloxyphenyl)heptanesulfonic acid sodium salt (18b)

The synthesis was carried out as described for **18a**, using **17b** (400 mg, 1.11 mmol), Na_2SO_3 (190 mg, 1.5 mmol) and EtOH (8 mL)/H_2O (4 ml) mixture. The crude product **18b** was used in the next step without further purification.

7-(2-Benzyloxyphenyl)heptanesulfonic acid sodium salt (18c)

The synthesis was carried out as described for **18a**, using **17c** (0.231 g, 0.64 mmol), Na_2SO_3 (0.11 g, 0.89 mmol) and EtOH (8 mL)/H_2O (4 ml) mixture. The crude product **18c** was used in the next step without further purification.

5-(4-Benzyloxyphenyl)pentanesulfonic acid sodium salt (18d)

Method A—The procedure described for **18a** was followed, using **17d** (0.95 g, 2.85 mmol), Na_2SO_3 (0.5 g, 4.0 mmol) and EtOH (20 mL)/H_2O (10 ml) mixture. The crude product **18d** was used in the next step without further purification.

Method B—A mixture of **17d** (0.95 g, 2.85 mmol) and Na_2SO_3 (0.5 g, 4.0 mmol) in THF/EtOH/H_2O (1:2:2 mixture, 15 mL) was heated for 15 minutes at 160°C under microwave irradiation (300 W) using a CEM Discover system. The reaction mixture was cooled to room temperature and volatiles were removed under reduced pressure. The residue was scrupulously dried under high vacuo and the crude product **18d**, (a pale yellow solid) was used in the next step without further purification.

7-(4-Benzyloxyphenyl)heptanesulfonyl chloride (19a)

To a stirred suspension of **18a** in anhydrous benzene (20 mL)/DMF (0.2 ml), was added thionyl chloride (890 mg, 7.49 mmol) and the mixture was heated at 50°C for 3 hours under argon. The reaction mixture was quenched by dropwise addition of water at room temperature and extracted with diethyl ether. The organic layer was washed with brine, dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. Purification by flash column chromatography on silica gel (20% diethyl ether in hexane) gave **19a** as a white solid in 40% yield from **17a** (380 mg). m p 33-35°C; IR (neat) 3033, 2945, 2923, 1610, 1513, 1371 (s), 1244, 1164 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, *J* = 7.5 Hz, 2H, 2-H, 6-H, -OCH_2*Ph*), 7.38 (t, *J* = 7.5 Hz, 2H, 3-H, 5-H, -OCH_2*Ph*), 7.32 (t, *J* = 7.5 Hz 1H, 4-H, -OCH_2*Ph*), 7.08 (d, *J* = 8.5 Hz, 2H, 2-H, 6-H, -*Ph*-OBn), 6.90 (d, *J* = 8.5 Hz, 2H, 3-H, 5-H, -*Ph*-OBn), 5.04 (s, 2H, -O*C*H_2Ph), 3.64 (m as t, *J* = 8.0 Hz, half of an AA'XX' system, 2H, -CH_2SO_2Cl), 2.55 (t, *J* = 7.5 Hz, 2H, -CH_2-Ph-OBn), 2.03 (m as qt, *J* = 7.7 Hz, 2H, -C$H_2CH_2SO_2$Cl), 1.62-1.54 (m, 2H), 1.52-1.46 (m, 2H), 1.40-1.30 (m, 4H); mass spectrum *m/z* (relative intensity) 382 (M^++2, 2), 380 (M^+, 6), 282 (7), 149 (8), 107 (7), 91 (100); Exact mass calculated for $C_{20}H_{25}ClO_3S$, 380.1213; found, 380.1211.

7-(3-Benzyloxyphenyl)heptanesulfonyl chloride (19b)

The synthesis was carried out as described for **19a**, using **18b** and thionyl chloride (360 mg, 3.03 mmol) in benzene (9 mL)/DMF (0.1 mL). The crude product obtained after workup was purified by flash column chromatography on silica gel (20% diethyl ether in hexane) to

give **19b** as a viscous liquid in 39% yield from **17b** (163 mg). IR (neat) 3033, 2945, 2920, 1610, 1513, 1371 (s), 1242, 1160 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, *J* =7.5 Hz, 2H, 2-H. 6-H, -OCH_2*Ph*), 7.39 (t, *J* =7.5 Hz, 2H, 3-H, 5-H, -OCH_2*Ph*), 7.32 (t, *J* = 7.5 Hz, 1H, 4-H, -OCH_2*Ph*), 7.20 (t, *J* = 7.6 Hz, 1H, 5-H, -*Ph*-OBn), 6.83-6.76 (m, 3H, 2-H, 4-H, 6-H, -*Ph*-OBn), 5.05 (s, 2H, -OC*H*$_2$Ph), 3.64 (m as t, *J* = 8.0 Hz, half of an AA'XX' system, 2H, -CH_2SO_2Cl), 2.58 (t, *J* = 7.6 Hz, 2H, -C*H*$_2$-Ph-OBn), 2.03 (m as qt, *J* = 7.7 Hz, half of an AA'XX' system, 2H, -C*H*$_2CH_2SO_2Cl$), 1.62 (qt, *J* = 7.5 Hz, 2H), 1.48 (qt, *J* = 7.5 Hz, 2H), 1.42-1.30 (m, 4H); mass spectrum *m/z* (relative intensity) 382 (M^++2, 1), 380 (M^+, 3), 149 (8), 107 (7), 91 (100); Exact mass calculated for $C_{20}H_{25}ClO_3S$, 380.1213; found, 380.1215.

7-(2-Benzyloxyphenyl)heptanesulfonyl chloride (19c)

The synthesis was carried out as described for **19a**, using **18c** and thionyl chloride (228 mg, 1.92 mmol) in benzene (9 mL)/DMF (0.1 mL). The crude product obtained after workup was purified by flash column chromatography on silica gel (20% diethyl ether in hexane) to give **19c** as a viscous liquid in 38% yield from **17c** (92 mg). IR (neat) 3028, 2941, 2921, 1611, 1517, 1371 (s), 1244, 1164 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, *J* = 8.0 Hz, 2H, 2-H. 6-H, -OCH_2*Ph*), 7.39 (t, *J* = 8.0 Hz, 2H, 3-H, 5-H, -OCH_2*Ph*), 7.33 (t, *J* = 8.0 Hz, 1H, 4-H, -OCH_2*Ph*), 7.18-7.13 (t and d overlapping, 2H, 4-H, 6-H, -*Ph*-OBn), 6.91 (d, *J* = 8.0 Hz, 1H, 3-H, -*Ph*-OBn), 6.90 (t, *J* = 8.0 Hz, 1H, 5-H, -*Ph*-OBn), 5.08 (s, 2H, -OC*H*$_2$Ph), 3.58 (m as t, *J* = 8.0 Hz, half of an AA'XX' system, 2H, -CH_2SO_2Cl), 2.67 (t, *J* = 7.7 Hz, 2H, -C*H*$_2$-Ph-OBn), 1.99 (m as qt, *J* = 7.7 Hz, half of an AA'XX' system, 2H, -C*H*$_2CH_2SO_2Cl$), 1.62 (qt, *J* = 7.5 Hz, 2H), 1.46-1.40 (m, 2H), 1.39-1.33 (m, 4H); mass spectrum *m/z* (relative intensity) 382 (M^++2, 1), 380 (M^+, 3), 149 (21), 107 (9), 91 (100); Exact mass calculated for $C_{20}H_{25}ClO_3S$, 380.1213; found, 380.1211.

5-(4-Benzyloxyphenyl)pentanesulfonyl chloride (19d)

The synthesis was carried out as described for **19a**, using **18d** and thionyl chloride (1.0 g, 8.40 mmol) in benzene (27 mL)/DMF (0.3 mL). The crude product obtained after workup was purified by flash column chromatography on silica gel (20% diethyl ether in hexane) to give **19d** as a white solid in 37% yield from **17d** (372 mg). Using **18d** derived from **Method B** and thionyl chloride (1.0 g, 7.41 mmol) in benzene (27 mL)/DMF (0.3 mL), the title compound was obtained in 50% yield from **17d** (501 mg). m p 58-60°C; IR (neat) 3031, 2944, 2923, 1610, 1512, 1372 (s), 1244, 1164 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, *J* = 7.5 Hz, 2H, 2-H, 6-H, -OCH_2*Ph*), 7.38 (t, *J* = 7.5 Hz, 2H, 3-H, 5-H, -OCH_2*Ph*), 7.32 (t, *J* = 7.5 Hz 1H, 4-H, -OCH_2*Ph*), 7.07 (d, *J* = 8.5 Hz, 2H, 2-H, 6-H, -*Ph*-OBn), 6.90 (d, *J* = 8.5 Hz, 2H, 3-H, 5-H, -*Ph*-OBn), 5.03 (s, 2H, -OC*H*$_2$Ph), 3.63 (m as t, *J* = 8.0 Hz, half of an AA'XX' system, 2H, -CH_2SO_2Cl), 2.59 (t, *J* = 7.5 Hz, 2H, -C*H*$_2$-Ph-OBn), 2.06 (m as qt, *J* = 7.7 Hz, 2H, -C*H*$_2CH_2SO_2Cl$), 1.66 (qt, *J* = 7.5 Hz, 2H), 1.51 (qt, *J* = 7.4 Hz, 2H); mass spectrum *m/z* (relative intensity) 354 (M^++2, 4), 352 (M^+, 15), 256 (39), 238 (37), 196 (41), 168 (33), 107 (38), 91 (100), 77 (79); Exact mass calculated for $C_{18}H_{21}ClO_3S$, 352.0900; found, 352.0900.

7-(4-Benzyloxyphenyl)heptanesulfonyl fluoride (20a)

To a stirred solution of **19a** (300 mg, 0.79 mmol) in dry acetone (20 mL), was added anhydrous NH_4F (66 mg, 1.8 mmol) and the mixture refluxed for 2 hours under argon. The reaction mixture was cooled to room temperature, and the solvent evaporated under reduced pressure. The residue was dissolved in diethyl ether and the ethereal solution was successively washed with water and brine, dried ($MgSO_4$) and evaporated in vacuo. Purification by flash column chromatography on silica gel (20% diethyl ether in hexane) gave **20a** as a white solid in 93% yield (267 mg). m p 35-38°C; IR (neat) 3043, 2955, 1512, 1397 (s), 1235, 1194 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, *J* = 7.5 Hz, 2H, 2-H, 6-H, -

OCH_2*Ph*), 7.38 (t, *J* = 7.5 Hz, 2H, 3-H, 5-H, -OCH_2*Ph*), 7.32 (t, *J* = 7.5 Hz, 1H, 4-H, -OCH_2*Ph*), 7.08 (d, *J* = 8.7 Hz, 2H, 2-H, 6-H, -*Ph*-OBn), 6.90 (d, *J*= 8.7 Hz, 2H, 3-H, 5-H, -*Ph*-OBn), 5.04 (s, 2H, -O*C*H_2Ph), 3.34 (m as dt, *J* = 11.0 Hz, *J* = 4.5 Hz, 2H, -CH_2SO_2F), 2.55 (t, *J* = 7.5 Hz, 2H, -CH_2-Ph-OBn), 1.94 (m as qt, *J* = 8.0 Hz, 2H, -C$H_2CH_2SO_2F$), 1.59 (qt, *J* = 7.0 Hz, 2H), 1.47 (qt, *J* = 7.4 Hz, 2H), 1.40-1.31 (m, 4H); mass spectrum *m/z* (relative intensity) 364 (M^+, 10), 149 (2), 107 (6), 91 (100), 65 (6); Exact mass calculated for $C_{20}H_{25}FO_3S$, 364.1508; found, 364.1512.

7-(3-Benzyloxyphenyl)heptanesulfonyl fluoride (20b)

The synthesis was carried out as described for **20a**, using **19b** (149 mg, 0.39 mmol) and NH_4F (29 mg, 0.78 mmol) in dry acetone (10 mL). The crude product obtained after workup was purified by flash column chromatography on silica gel (20% diethyl ether in hexane) to give **20b** as a viscous liquid in 90% yield (129 mg). IR (neat) 3051, 2952, 1512, 1397 (s), 1234, 1194 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, *J* = 7.5 Hz, 2H, 2-H. 6-H, -OCH_2*Ph*), 7.39 (t, *J* =7.5 Hz, 2H, 3-H, 5-H, -OCH_2*Ph*), 7.32 (t, *J* =7.5 Hz, 1H, 4-H, -OCH_2*Ph*), 7.19 (t, *J* = 7.7 Hz, 1H, 5-H, -*Ph*-OBn), 6.82-6.76 (m, 3H, 2-H, 4-H, 6-H, -*Ph*-OBn), 5.05 (s, 2H, -O*C*H_2Ph), 3.34 (m as dt, *J* = 11.0 Hz, *J* = 4.5 Hz, 2H, -CH_2SO_2F), 2.58 (t, *J* = 7.5 Hz, 2H, -CH_2-Ph-OBn), 1.93 (m as qt, *J* = 7.8 Hz, 2H, -C$H_2CH_2SO_2F$), 1.61 (qt, *J* = 7.5 Hz, 2H), 1.47 (qt, *J* = 7.2 Hz, 2H), 1.40-1.29 (m, 4H); mass spectrum *m/z* (relative intensity) 364 (M^+, 13), 149 (3), 107 (2), 91 (100), 77 (3), 65 (6); Exact mass calculated for $C_{20}H_{25}FO_3S$, 364.1508; found, 364.1507.

7-(2-Benzyloxyphenyl)heptanesulfonyl fluoride (20c)

The synthesis was carried out as described for **20a**, using **19c** (85 mg, 0.22 mmol) and NH_4F (18 mg, 0.486 mmol) in dry acetone (10 mL). The crude product obtained after workup was purified by flash column chromatography on silica gel (20% diethyl ether in hexane) to give **20c** as a viscous liquid in 90% yield (73 mg). IR (neat) 3037, 2940, 1513, 1398 (s), 1235, 1194 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, *J* = 7.5 Hz, 2H, 2-H. 6-H, -OCH_2*Ph*), 7.39 (t, *J* = 7.5 Hz, 2H, 3-H, 5-H, -OCH_2*Ph*), 7.34 (t, *J* = 7.5 Hz, 1H, 4-H, -OCH_2*Ph*), 7.18-7.12 (t and d overlapping, 2H, 4-H, 6-H, -*Ph*-OBn), 6.91 (d, *J* = 8.0 Hz, 1H, 3-H, -*Ph*-OBn), 6.90 (t, *J* = 8.0 Hz, 1H, 5-H, -*Ph*-OBn), 5.07 (s, 2H, -O*C*H_2Ph), 3.28 (m as dt, *J* = 11.0 Hz, *J* = 4.5 Hz, 2H, -CH_2SO_2F), 2.67 (t, *J* = 7.5 Hz, 2H, -CH_2-Ph-OBn), 1.89 (m as qt, *J* = 7.8 Hz, 2H, -C$H_2CH_2SO_2F$), 1.62 (qt, *J* = 7.5 Hz, 2H), 1.46-1.40 (m, 2H), 1.39-1.33 (m, 4H); mass spectrum *m/z* (relative intensity) 364 (M^+, 12), 107 (7), 91 (100), 65 (6); Exact mass calculated for $C_{20}H_{25}FO_3S$, 364.1508; found, 364.1506.

5-(4-Benzyloxyphenyl)pentanesulfonyl fluoride (20d)

The synthesis was carried out as described for **20a**, using **19d** (300 mg, 0.85 mmol) and NH_4F (60 mg, 1.64 mmol) in dry acetone (20 mL). The crude product obtained after workup was purified by flash column chromatography on silica gel (20% diethyl ether in hexane) to give **20d** as a white solid in 92% yield (263 mg). m p 66-68°C; IR (neat) 3042, 2961, 1511, 1397 (s), 1235, 1195 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, *J* = 7.5 Hz, 2H, 2-H, 6-H, -OCH_2*Ph*), 7.38 (t, *J* = 7.5 Hz, 2H, 3-H, 5-H, -OCH_2*Ph*), 7.32 (t, *J* = 7.5 Hz, 1H, 4-H, -OCH_2*Ph*), 7.08 (d, *J* = 8.5 Hz, 2H, 2-H, 6-H, -*Ph*-OBn), 6.90 (d, *J*= 8.5 Hz, 2H, 3-H, 5-H, -*Ph*-OBn), 5.04 (s, 2H, -O*C*H_2Ph), 3.34 (m as dt, *J* = 11.0 Hz, *J* = 4.5 Hz, 2H, -CH_2SO_2F), 2.58 (t, *J* = 7.3 Hz, 2H, -CH_2-Ph-OBn), 1.97 (m as qt, *J* = 7.7 Hz, 2H, -C$H_2CH_2SO_2F$), 1.65 (qt, *J* = 7.5 Hz, 2H), 1.50 (qt, *J* = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.14 (4-C, -CH_2-*Ph*-OBn), 137.17 (1-C, -O-CH_2-*Ph*), 134.01 (1-C, -CH_2-*Ph*-OBn), 129.25, 128.57, 127.92, 127.47, 114.82 (3-C, 5-C, -CH_2-*Ph*-OBn), 70.08 (-O-*C*H_2-Ph), 50.89(d, *J* = 16.1 Hz, -*C*H_2SO_2F), 34.47 (-*CH*$_2$-Ph-OBn), 30.77, 27.33, 23.32; mass spectrum *m/z* (relative

intensity) 336 (M^+, 17), 260 (3), 224 (14), 196 (9), 149 (3), 121 (9), 107 (4), 91 (100), 65 (9); Exact mass calculated for $C_{18}H_{21}FO_3S$, 336.1195; found, 336.1198.

7-(4-Hydroxyphenyl)heptanesulfonyl fluoride (21a)

To a solution of **20a** (182 mg, 0.5 mmol) in ethanedithiol (4 mL), at room temperature, under an argon atmosphere was added $BF_3{\cdot}Et_2O$ (282 mg, 2.0 mmol). The reaction mixture was stirred for 1 hour and then diluted with diethyl ether and water. The organic layer was separated and the aqueous phase extracted with diethyl ether. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (50% diethyl ether in hexane) to give **21a** as a white solid in 70% yield (96 mg). m p 47-51°C; IR (neat) 3396 (br, OH), 2923, 2851, 1611, 1513, 1394 (s), 1237, 1198 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.03 (d, *J* = 8.5 Hz, 2H, ArH), 6.75 (d, *J* = 8.5 Hz, 2H, ArH), 4.62 (br s, 1H, OH), 3.33 (m as dt, *J* = 11.0 Hz, *J* = 4.5 Hz, 2H, -CH_2SO_2F), 2.53 (t, *J* = 7.3 Hz, 2H, -C*H*$_2$-Ph-OH), 1.93 (m as qt, *J* = 8.0 Hz, 2H, -C*H*$_2$$CH_2SO_2F$), 1.58 (qt, *J* = 6.9 Hz, 2H), 1.47 (qt, *J* = 7.4 Hz, 2H), 1.40-1.30 (m, 4H); mass spectrum *m/z* (relative intensity) 274 (M^+, 18), 120 (3), 107 (100), 91 (2), 77 (6); Exact mass calculated for $C_{13}H_{19}FO_3S$, 274.1039; found, 274.1037.

7-(3-Hydroxyphenyl)heptanesulfonyl fluoride (21b)

The synthesis was carried out as described for **21a**, using **20b** (100 mg, 0.27 mmol) and $BF{\cdot}_3Et_2O$ (140 mg, 1.0 mmol) in ethanedithiol (4 mL). The residue obtained after work up was purified by flash column chromatography on silica gel (50% diethyl ether in hexane) to give **21b** as a viscous liquid in 68% yield (51 mg). IR (neat) 3380 (br, OH), 2935, 2854, 1612, 1513, 1394 (s), 1238, 1198 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (t, *J* = 7.5 Hz, 1H, 5-H, -*Ph*-OH), 6.74 (d, *J* = 7.5 Hz, 1H, 6-H, -*Ph*-OH), 6.66-6.63 (m, 2H, 2-H, 4-H, -*Ph*-OH), 4.74 (br s, 1H, OH), 3.35 (m as dt, *J* = 11.0 Hz, *J* = 4.5 Hz, 2H, -CH_2SO_2F), 2.56 (t, *J* = 7.7 Hz, 2H, -C*H*$_2$-Ph-OH), 1.94 (m as qt, *J* = 7.7 Hz, 2H, -C*H*$_2$$CH_2SO_2F$), 1.61 (qt, *J* = 7.5 Hz, 2H), 1.48 (qt, *J* = 7.2 Hz, 2H), 1.40-1.31 (m, 4H); mass spectrum *m/z* (relative intensity) 274 (M^+, 25), 121 (21), 108 (100), 91 (23), 77 (17); Exact mass calculated for $C_{13}H_{19}FO_3S$, 274.1039; found, 274.1039.

7-(2-Hydroxyphenyl)heptanesulfonyl fluoride (21c)

The synthesis was carried out as described for **21a**, using **20c** (65 mg, 0.18 mmol) and $BF_3{\cdot}Et_2O$ (92 mg, 0.65 mmol) in ethanedithiol (2mL). The residue obtained after work up was purified by flash column chromatography on silica gel (50% diethyl ether in hexane) to give **21c** as a viscous liquid in 69% yield (34 mg). IR (neat) 3395 (br, OH), 2927, 2854, 1612, 1513, 1395 (s), 1238, 1198 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.10 (dd, *J* = 7.5 Hz, *J* = 1.6 Hz, 1H, 6-H, -*Ph*-OH), 7.07 (td, *J* = 7.5 Hz, *J* = 1.6 Hz, 1H, 4-H, -*Ph*-OH), 6.87 (td, *J* = 7.5 Hz, *J* = 1.2 Hz, 1H, 5-H, -*Ph*-OH), 6.75 (dd, *J* = 7.5 Hz, *J* = 1.2 Hz, 1H, 3-H, -*Ph*-OH), 4.73 (br s, 1H, OH), 3.35 (m as dt, *J* = 11.0 Hz, *J* = 4.5 Hz, 2H, -CH_2SO_2F), 2.61 (t, *J* = 7.5 Hz, 2H, -C*H*$_2$-Ph-OH), 1.94 (m as qt, *J* = 7.8 Hz, 2H, -C*H*$_2$$CH_2SO_2F$), 1.62 (m, 2H), 1.52-1.44 (m, 2H), 1.42-1.34 (m, 4H); mass spectrum *m/z* (relative intensity) 274 (M^+, 16), 120 (3), 107 (100), 91 (7), 77 (8); Exact mass calculated for $C_{13}H_{19}FO_3S$, 274.1039; found, 274.1042.

5-(4-Hydroxyphenyl)pentanesulfonyl fluoride (21d)

The synthesis was carried out as described for **21a**, using **19d** (0.24 g, 0.71 mmol) and $BF_3{\cdot}Et_2O$ (0.47 g, 3.32 mmol) in ethanedithiol (7 mL). The residue obtained after workup was purified by flash column chromatography on silica gel (50% diethyl ether in hexane) to give **21d** as a white solid in 66% yield (117 mg). m p 32-35°C; IR (neat) 3387 (br, OH), 2920, 2851, 1611, 1514, 1394 (s), 1238, 1196 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.02 (d, *J*

= 8.2 Hz, 2H, ArH), 6.76 (d, *J* = 8.2 Hz, 2H, ArH), 4.65 (br s, 1H, OH), 3.34 (m as dt, *J* = 11.0 Hz, *J* = 4.5 Hz, 2H, -CH_2SO_2F), 2.57 (t, *J* = 7.0 Hz, 2H, -C*H*$_2$-Ph-OH), 1.96 (m as qt, *J* = 7.7 Hz, 2H, -C*H*$_2$$CH_2SO_2F$), 1.64 (qt, *J* = 7.5 Hz, 2H), 1.50 (qt, *J* = 7.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.45 (4-C, -CH_2-*Ph*-OH), 135.63 (1-C, -CH_2-*Ph*-OH), 131.22 (2-C, 6-C, -CH_2-*Ph*-OH), 116.35 (3-C, 5-C, -CH_2-*Ph*-OH), 50.92 (d, *J* = 16.1 Hz, -*C*H_2SO_2F), 35.72 (-*C*H_2-Ph-OH), 30.91, 27.43, 22.37; mass spectrum *m/z* (relative intensity) 246 (M^+, 14), 120 (3), 107 (100), 91 (4), 77 (7); Exact mass calculated for $C_{11}H_{15}FO_3S$, 246.0726; found, 246.0725.

4-Bromo-1-(4-benzyloxyphenoxy)butane (23)

A stirred mixture of 4-benzyloxyphenol (**22**, 400 mg, 2 mmol), anhydrous potassium carbonate (331 mg, 2.4 mmol), 18-crown-6 (580 mg, 2.2 mmol), and 1,4-dibromobutane (518 mg, 2.4 mmol) in anhydrous acetone (20 mL), was heated under reflux for 3 hours. The reaction mixture was cooled to room temperature, the solid materials were filtered off, and the solvent was removed in vacuo. The residue was dissolved in diethyl ether and the ethereal solution was washed sequentially with water and brine, dried ($MgSO_4$), and evaporated under reduced pressure. Purification by flash column chromatography on silica gel (20% diethyl ether in hexane) gave **23** as a white solid in 50% yield (337 mg). m p 68-69°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46-7.28 (m, 5H, 2-H, 3-H, 4-H, 5-H, 6-H, -O-CH_2-*Ph*), 6.90 (m as d, *J* = 8.7 Hz, 2H, -O-*Ph*-OBn), 6.81 (m as d, *J* = 8.7 Hz, 2H, -O-*Ph*-OBn), 5.01 (s, 2H, -OC*H*$_2$-Ph), 3.94 (t, *J* = 6.0 Hz, 2H, -C*H*$_2$-O-Ph-), 3.48 (t, *J* = 6.6 Hz, 2H, -CH_2Br), 2.58 (qt, *J* = 6.5 Hz, 2H of the 4-bromobutyl group), 1.91 (qt, *J* = 6.6 Hz, 2H of the 4-bromobutyl group).

4-(4-Benzyloxyphenoxy)butanesulfonic acid sodium salt (24)

The synthesis was carried out as described for **18d** (**Method B**) using 4-bromo-1-(4-benzyloxyphenoxy)butane (**23**) (250 mg, 0.75 mmol) and Na_2SO_3 (121 mg, 1.2 mmol) in THF/EtOH/H_2O (1:2:2, 5 mL). The crude product **24** (a pale yellow solid) was used in the next step without further purification.

4-(4-Benzyloxyphenoxy)butanesulfonyl chloride (25)

The synthesis was carried out as described for **19a**. Using **24** derived from **Method B** and thionyl chloride (180 mg, 1.51 mmol) in benzene (9 mL)/DMF (0.1 mL), the title compound was obtained in 51% yield (from **23**, 136 mg) as a viscous liquid, after purification by flash column chromatography on silica gel (20% diethyl ether in hexane). IR (neat) 3033, 2945, 2868, 1610, 1512, 1371 (s), 1164 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, *J* = 7.8 Hz, 2H, 2-H, 6-H, -OCH_2*Ph*), 7.40 (t, *J* = 7.8 Hz, 2H, 3-H, 5-H, -OCH_2*Ph*), 7.34 (t, *J* = 7.9 Hz, 1H, 4-H, -OCH_2*Ph*), 6.93 (d, *J* = 8.5 Hz, 2H, -O-*Ph*-OBn), 6.84 (d, *J* = 8.5 Hz, 2H, -O-*Ph*-OBn), 5.04 (s, 2H, -OC*H*$_2$Ph), 4.00 (t, *J* = 6.0 Hz, 2H, -C*H*$_2$-O-Ph-OBn), 3.81 (m as t, *J* = 7.2 Hz, half of an AA'XX' system, 2H, -CH_2SO_2Cl), 2.29 (m as qt, *J* = 7.1 Hz, 2H, -C*H*$_2$$CH_2SO_2Cl$), 2.00 (qt, *J* = 7.3 Hz, 2H, -C*H*$_2$$CH_2CH_2SO_2Cl$); mass spectrum *m/z* (relative intensity) 356 (M^++2, 3), 354 (M^+, 9), 246 (9), 200 (4), 139 (4), 128 (3), 107 (23), 91 (100), 65 (9); Exact mass calculated for $C_{17}H_{19}ClO_4S$, 354.0693; found, 354,0692.

4-(4-Benzyloxyphenoxy)butanesulfonyl fluoride (26)

The title compound was synthesized as described for **20a**, using **25** (100 mg, 0.3 mmol) and NH_4F (52 mg, 1.4 mmol) in dry acetone (4 mL). Purification by flash column chromatography on silica gel (20% diethyl ether in hexane) gave **26** as a white solid (m p 108-110°C) in 93% yield (88 mg). IR (neat) 3062, 2961, 2874, 1512, 1397 (s), 1235, 1194 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, *J* = 8.0 Hz, 2H, 2-H, 6-H, -OCH_2*Ph*), 7.40 (t, *J* = 8.0 Hz, 2H, 3-H, 5-H, -OCH_2*Ph*), 7.34 (t, *J* = 8.0 Hz, 1H, 4-H, -OCH_2*Ph*), 6.93 (d, *J* = 9.0

Hz, 2H, -O-*Ph*-OBn), 6.83 (d, *J*= 9.0 Hz, 2H, -O-*Ph*-OBn), 5.04 (s, 2H, -OCH_2Ph), 4.00 (t, *J* = 6.2 Hz, 2H, -CH_2-O-Ph-OBn), 3.51 (m as dt, *J* = 11.0 Hz, *J* = 4.5 Hz, 2H, -CH_2SO_2F), 2.20 (m as qt, *J* = 7.5 Hz, 2H, -C$H_2CH_2SO_2F$), 1.98 (qt, *J* = 7.5 Hz, 2H, -$CH_2CH_2CH_2SO_2F$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.75 (4-C, -O-*Ph*-OBn), 152.21 (1-C, -O-*Ph*-OBn), 137.22 (1-C, -O-CH_2-*Ph*), 129.27, 128.21, 127.73, 115.87, 115.23, 70.27 (-O-CH_2-Ph), 66.57 (-CH_2-O-Ph-), 50.82 (d, *J* = 16.2 Hz, -CH_2SO_2F), 27.48, 21.17; mass spectrum *m/z* (relative intensity) 338 (M^+, 15), 196 (3), 139 (6), 107 (8), 91 (100), 65 (7); Exact mass calculated for $C_{17}H_{19}FO_4S$, 338.0988; found, 338.0985.

7-(4-Benzyloxyphenyl)heptane-1-sulfonic acid methyl ester (27)

A solution of **19a** (50 mg, 0.13 mmol) in MeOH (5 mL) was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and the residue was dissolved in diethyl ether. The ethereal solution was washed with water and brine, dried ($MgSO_4$), and evaporated under reduced pressure. Purification by flash column chromatography on silica gel (20% diethyl ether in hexane) gave **27** as a white solid in 84% yield (41 mg). m p 57-59°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, *J* = 7.5 Hz, 2H, 2-H, 6-H, -OCH_2*Ph*), 7.38 (t, *J* = 7.5 Hz, 2H, 3-H, 5-H, -OCH_2*Ph*), 7.32 (t, *J* = 7.5 Hz 1H, 4-H, -OCH_2*Ph*), 7.08 (d, *J* = 8.0 Hz, 2H, 2-H, 6-H, -*Ph*-OBn), 6.90 (d, *J* = 8.0 Hz, 2H, 3-H, 5-H, -*Ph*-OBn), 5.04 (s, 2H, -OCH_2Ph), 3.88 (s, 3H, OMe), 3.08 (m as t, *J* = 7.8 Hz, half of an AA'XX' system, 2H, -CH_2SO_2OMe), 2.54 (t, *J* = 7.2 Hz, 2H, -CH_2-Ph-OBn), 1.85 (m as qt, *J* = 8.0 Hz, 2H, -C$H_2CH_2SO_2OMe$), 1.58 (qt, *J* = 7.0 Hz, 2H), 1.43 (qt, *J* = 7.9 Hz, 2H), 1.38-1.30 (m, 4H).

Preparation of transmembrane domain(deleted rat FAAH (ΔTM rFAAH)

Rat ΔTM FAAH was expressed in *E. coli* cells and purified using the procedure disclosed by Patricelli et al.[53]

Preparation of human FAAH in fusion with N(terminal maltose binding tag (MBPΔTMhFAAH)

Human FAAH without putative transmembrane domain following maltose binding protein was expressed in *E. coli* cells using pMALcE4 vector (New England Biolabs) (unpublished data).

Fluorescent assay protocol for ΔTM rat FAAH

Compounds, N-arachidonoyl, 7-amino-4-methyl coumarin amide (AAMCA),[52] and arachidonoyl-methyl coumarin (AMC) were dissolved in DMSO (10 mM) and kept as stock solutions at −20 ° C before assay was performed. Compounds were diluted in 50:50 DMSO/assay buffer (50 mM HEPES, 1 mM EDTA, 0.1% BSA, pH 7.4) so as to have a final DMSO concentration below 8% in each reaction. For the initial screening assay, 3 concentrations (1 μM, 10 μM, and 100 μM) of test compounds, 15 μg of total protein in *E. coli* lysate containing ΔTM rFAAH, and assay buffer were pre-incubated for 15 min at 25°C. AAMCA (20 μM) was added prior to incubation at 25°C and kinetic fluorescence reading every 20 minutes (λ_{ex}= 360/λ_{em}= 460) for 4 hours on a BioTek Synergy HT Microplate Reader (BioTek Instruments, Winooski, VT). The fluorescence reading at the 3 hour time point (linear enzyme kinetics) was used to calculate percent inhibition based on control assays without inhibitor present. For the full dose response curves 8 concentrations of the test compounds were used and IC_{50} values were determined using Prizm software (GraphPad Software, Inc.).

Preparation of human MGL (hMGL)

Recombinant hexa-histidine-tagged human MGL (hMGL) was expressed in *E. coli* cells and purified following our recently reported procedures.[45,46]

Fluorescent assay protocol for hMGL

Compound inhibition of hMGL activity was assessed by a fluorometric assay recently developed in our laboratory.[43,45,46] This medium throughput assay involved a 96-well plate format in which hMGL activity was monitored by the hydrolysis of the substrate 7-hydroxy-6-methoxy-4-methyl coumarin ester (AHMMCE)[43,45,46] to form the fluorescent product, coumarin. In brief, various concentrations of each compound were pre-incubated with hMGL (175 ng of total protein in *E. coli* lysate containing hMGL) for 15 minutes at room temperature. Upon the addition of AHMMCE, the reaction was incubated at 25°C for 120 min; fluorescence readings were taken every 15 minutes at 360 nm/460 nm ($\lambda_{excitation}$/$\lambda_{emission}$) using a Synergy HT Plate Reader (Bio-Tek, Winooski, VT). Under these incubation conditions, negligible spontaneous AHMMCE hydrolysis was observed. External standards were used to convert relative fluorescence units to the amount of 4-methyl coumarin formed. All MGL assays were performed in triplicate for each inhibitor concentration, and IC_{50} values were calculated using Prizm software (GraphPad Software, Inc., San Diego, CA).

Radioligand binding assays

Rat brain CB1 and mouse CB2 assays. Compounds were tested for their affinities for the rat CB1 and mouse CB2 receptors using membrane preparations from rat brain or HEK 293 cells overexpressing mouse CB2, and [^{3}H]CP-55,940 as previously described.[50,56,69]

Rapid dilution assay

The assay was performed by following procedures similar to those described earlier.[58] Briefly, DMSO (2.5 μL; positive control of enzyme activity) and inhibitor **21d** in DMSO (2.5 μL, 2.35 μM; enzyme inhibition at a concentration 40 times higher its IC_{50} value) were added to two samples of 50 μL assay buffer (50 mM HEPES, 1mM EDTA 0.1% BSA, pH 7.4) containing 0.2 μg (3.1 pmol) of purified rat ΔTM FAAH respectively, and incubated at room temperature for 1 hour. A substrate self-hydrolysis control sample, containing 2.5 μL DMSO in 50 μL assay buffer (50 mM HEPES, 1mM EDTA 0.1% BSA, pH 7.4) without enzyme, was incubated at room temperature for 1 hour. All samples were diluted 100 times in assay buffer and aliquots of the resulting solutions (198 μL) were added in a 96-well assay plate (costar 3650) containing 2 μL of 1 mM DMSO solution of fluorogenic substrate, arachidonoyl 7-amino-4-methylcoumarin amide (AAMCA). Accumulation of the fluorescent product 7-amino-4-methylcoumarin (AMC) was measured at 360 nm/460 nm ($\lambda_{excitation}$/$\lambda_{emission}$) each 20 min up to 24 h using an EnVision Multilabel Reader at 25 °C (PerkinElmer Inc., Shelton, CT). To account for self-hydrolysis of the fluorogenic substrate, the average readings values of control without enzyme were systematically subtracted from samples with enzyme.

LC/MS Intact mass analysis of 21d-treated rat ΔTM FAAH

Two samples of purified rat δTM FAAH in 100 mM NaCl, 50 mM Tris, pH 8.0 buffer (50μL, 40μM) were incubated with 2.5 μL DMSO and 2.5 μL 1.68 mM **21d** (final inhibitor concentration was 80 μM) respectively at room temperature for 1 hr. To evaluate enzyme inhibition an aliquot of each mixture was analyzed in the fluorescent-based assay as previously described. The DMSO and inhibitor excess were removed from the samples on a Bio-Spin 6 column equilibrated with the same buffer. Samples were then taken for intact mass determination using a LCT-Premier^{XE} mass spectrometer (Waters). The instrument was calibrated with horse myoglobin (Sigma-Aldrich) and operated under the following conditions: source temperature, 80 °C; desolvation, 175°C; a capillary voltage of 3200 V, and cone voltage of 35 V. Samples (200 pmol) were injected onto a self-packed POROS 20 R2 protein trap column (Applied Biosystems) and desalted with 1.0 mL of Buffer A (0.05%

TFA in H_2O). The desalted protein was eluted from the trap column with a linear 15-75% acetonitrile B (acetonitrile, 0.05% TFA) gradient over 4 min at 50 μL/min.

Molecular modeling methods

The sulfonyl fluorides were covalently docked to the catalytic Ser241 of rFAAH (PDB ID: 1MT5)[2] with a covalent docking module in Prime.[70] This protocol forms the specified covalent bond and exhaustively samples the rotatable bonds of the ligand, producing a large number of potential poses. After clustering, the enzyme-ligand complexes are minimized and ranked by prime energy.

To validate the covalent docking protocol for FAAH, the methodology was tested on two covalent complexes: methyl arachidonyl fluorophosphonate (MAFP) bound to rFAAH (PDB ID: 1MT5),[2] and PF3845, a urea-based inhibitor, bound to the humanized variant of rFAAH (PDB ID: 3LJ6).[71] The ligands were extracted from the catalytic site and re-docked in a covalent binding mode. The root mean-square deviation (RMSD) between the lowest prime energy complexes and the crystal structures for MAFP and PF3845 were 1.15 and 0.31 Å respectively. These results indicate that the covalent docking module in Prime can provide a reliable ligand pose for FAAH, in terms of reproducing the experimental observed binding mode.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abreviations

AEA	N-arachidonoylethanolamine
2-AG	2-arachidonoyl glycerol
FAA	fatty acid amide
CB1	cannabinoid receptor 1
CB2	cannabinoid receptor 2
FAAH	fatty acid amide hydrolase
MGL	monoacylglycerol lipase
AS	amidase signature
(-)-Δ^9-THC	(-)-Δ^9-tetrahydrocannabinol
PMSF	phenylmethanesulfonyl fluoride
SAR	structure-activity relationship
DMF	dimethyl formamide
CHO	Chinese hamster ovary
AAMCA	arachidonyl-7-amino, 4-methyl coumarin amide
ΔTM-rFAAH	transmembrane-deleted rat FAAH

AHMMCE	arachidonoyl, 7-hydroxy-6-methoxy-4-methyl coumarin ester
HEK293	human embryonic kidney cell line
NMR	nuclear magnetic resonance
QTOF	quadrupole time of flight

References

(1). Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature. 1996; 384:83–87. [PubMed: 8900284]

(2). Bracey MH, Hanson MA, Masuda KR, Stevens RC, Cravatt BF. Structural adaptations in a membrane enzyme that terminates endocannabinoid signaling. Science. 2002; 298:1793–1796. [PubMed: 12459591]

(3). Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science. 1992; 258:1946–1949. [PubMed: 1470919]

(4). Devane WA, Dysarz FA 3rd, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Mol. Pharmacol. 1988; 34:605–613. [PubMed: 2848184]

(5). Cravatt BF, Prospero-Garcia O, Siuzdak G, Gilula NB, Henriksen SJ, Boger DL, Lerner RA. Chemical characterization of a family of brain lipids that induce sleep. Science. 1995; 268:1506–1509. [PubMed: 7770779]

(6). Boger DL, Patterson JE, Jin Q. Structural requirements for 5-HT2A and 5-HT1A serotonin receptor potentiation by the biologically active lipid oleamide. Proc. Natl. Acad. Sci. U. S. A. 1998; 95:4102–4107. [PubMed: 9539697]

(7). Yost CS, Hampson AJ, Leonoudakis D, Koblin DD, Bornheim LM, Gray AT. Oleamide potentiates benzodiazepine-sensitive gamma-aminobutyric acid receptor activity but does not alter minimum alveolar anesthetic concentration. Anesth. Analg. 1998; 86:1294–1300. [PubMed: 9620523]

(8). Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, Lichtman AH. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. Proc. Natl. Acad. Sci. U. S. A. 2001; 98:9371–9376. [PubMed: 11470906]

(9). Hwang J, Adamson C, Butler D, Janero DR, Makriyannis A, Bahr BA. Enhancement of endocannabinoid signaling by fatty acid amide hydrolase inhibition: a neuroprotective therapeutic modality. Life Sci. 2010; 86:615–623. [PubMed: 19527737]

(10). Ahn K, Johnson DS, Cravatt BF. Fatty acid amide hydrolase as a potential therapeutic target for the treatment of pain and CNS disorders. Expert Opin Drug Discov. 2009; 4:763–784. [PubMed: 20544003]

(11). Calignano A, La Rana G, Giuffrida A, Piomelli D. Control of pain initiation by endogenous cannabinoids. Nature. 1998; 394:277–281. [PubMed: 9685157]

(12). Ligresti A, Bisogno T, Matias I, De Petrocellis L, Cascio MG, Cosenza V, D'Argenio G, Scaglione G, Bifulco M, Sorrentini I, Di Marzo V. Possible endocannabinoid control of colorectal cancer growth. Gastroenterology. 2003; 125:677–687. [PubMed: 12949714]

(13). Melck D, Rueda D, Galve-Roperh I, De Petrocellis L, Guzman M, Di Marzo V. Involvement of the cAMP/protein kinase A pathway and of mitogen-activated protein kinase in the anti-proliferative effects of anandamide in human breast cancer cells. FEBS Lett. 1999; 463:235–240. [PubMed: 10606728]

(14). Mimeault M, Pommery N, Wattez N, Bailly C, Henichart JP. Anti-proliferative and apoptotic effects of anandamide in human prostatic cancer cell lines: implication of epidermal growth factor receptor down-regulation and ceramide production. Prostate. 2003; 56:1–12. [PubMed: 12746841]

(15). Castellano C, Cabib S, Palmisano A, Di Marzo V, Puglisi-Allegra S. The effects of anandamide on memory consolidation in mice involve both D1 and D2 dopamine receptors. Behav. Pharmacol. 1997; 8:707–712. [PubMed: 9832956]

(16). Mallet PE, Beninger RJ. The cannabinoid CB1 receptor antagonist SR141716A attenuates the memory impairment produced by delta-9-tetrahydrocannabinol or anandamide. Psychopharmacology (Berl). 1998; 140:11–19. [PubMed: 9862397]

(17). Naidoo V, Nikas SP, Karanian DA, Hwang J, Zhao J, Wood JT, Alapafuja SO, Vadivel SK, Butler D, Makriyannis A, Bahr BA. A new generation fatty acid amide hydrolase inhibitor protects against kainate-induced excitotoxicity. J. Mol. Neurosci. 2011; 43:493–502. [PubMed: 21069475]

(18). Karanian DA, Karim SL, Wood JT, Williams JS, Lin S, Makriyannis A, Bahr BA. Endocannabinoid enhancement protects against kainic acid-induced seizures and associated brain damage. J. Pharmacol. Exp. Ther. 2007; 322:1059–1066. [PubMed: 17545313]

(19). Karanian DA, Brown QB, Makriyannis A, Kosten TA, Bahr BA. Dual modulation of endocannabinoid transport and fatty acid amide hydrolase protects against excitotoxicity. J. Neurosci. 2005; 25:7813–7820. [PubMed: 16120783]

(20). Wallace MJ, Martin BR, DeLorenzo RJ. Evidence for a physiological role of endocannabinoids in the modulation of seizure threshold and severity. Eur. J. Pharmacol. 2002; 452:295–301. [PubMed: 12359270]

(21). Gomez R, Navarro M, Ferrer B, Trigo JM, Bilbao A, Del Arco I, Cippitelli A, Nava F, Piomelli D, Rodriguez de Fonseca F. A peripheral mechanism for CB1 cannabinoid receptor-dependent modulation of feeding. J. Neurosci. 2002; 22:9612–9617. [PubMed: 12417686]

(22). Milton NG. Anandamide and noladin ether prevent neurotoxicity of the human amyloid-beta peptide. Neurosci. Lett. 2002; 332:127–130. [PubMed: 12384227]

(23). Gobbi G, Bambico FR, Mangieri R, Bortolato M, Campolongo P, Solinas M, Cassano T, Morgese MG, Debonnel G, Duranti A, Tontini A, Tarzia G, Mor M, Trezza V, Goldberg SR, Cuomo V, Piomelli D. Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. Proc. Natl. Acad. Sci. U. S. A. 2005; 102:18620–18625. [PubMed: 16352709]

(24). Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A, Mor M, Tarzia G, La Rana G, Calignano A, Giustino A, Tattoli M, Palmery M, Cuomo V, Piomelli D. Modulation of anxiety through blockade of anandamide hydrolysis. Nat. Med. 2003; 9:76–81. [PubMed: 12461523]

(25). Godlewski G, Alapafuja SO, Batkai S, Nikas SP, Cinar R, Offertaler L, Osei-Hyiaman D, Liu J, Mukhopadhyay B, Harvey-White J, Tam J, Pacak K, Blankman JL, Cravatt BF, Makriyannis A, Kunos G. Inhibitor of fatty acid amide hydrolase normalizes cardiovascular function in hypertension without adverse metabolic effects. Chem. Biol. (Cambridge, MA, United States). 2010; 17:1256–1266.

(26). Bashashati M, Storr MA, Nikas SP, Wood JT, Godlewski G, Liu J, Ho W, Keenan CM, Zhang H, Alapafuja SO, Cravatt BF, Lutz B, Mackie K, Kunos G, Patel KD, Makriyannis A, Davison JS, Sharkey KA. Inhibiting fatty acid amide hydrolase normalizes endotoxin-induced enhanced gastrointestinal motility in mice. Br. J. Pharmacol. 2012; 165:1556–1571. [PubMed: 21883147]

(27). Fowler CJ, Holt S, Nilsson O, Jonsson KO, Tiger G, Jacobsson SO. The endocannabinoid signaling system: pharmacological and therapeutic aspects. Pharmacol. Biochem. Behav. 2005; 81:248–262. [PubMed: 15935456]

(28). Minkkila A, Saario S, Nevalainen T. Discovery and development of endocannabinoid-hydrolyzing enzyme inhibitors. Curr. Top. Med. Chem. 2010; 10:828–858. [PubMed: 20370710]

(29). Deutsch DG, Lin S, Hill WA, Morse KL, Salehani D, Arreaza G, Omeir RL, Makriyannis A. Fatty acid sulfonyl fluorides inhibit anandamide metabolism and bind to the cannabinoid receptor. Biochem. Biophys. Res. Commun. 1997; 231:217–221. [PubMed: 9070252]

(30). Tarzia G, Duranti A, Tontini A, Piersanti G, Mor M, Rivara S, Plazzi PV, Park C, Kathuria S, Piomelli D. Design, synthesis, and structure-activity relationships of alkylcarbamic acid aryl esters, a new class of fatty acid amide hydrolase inhibitors. J. Med. Chem. 2003; 46:2352–2360. [PubMed: 12773040]

(31). Mor M, Rivara S, Lodola A, Plazzi PV, Tarzia G, Duranti A, Tontini A, Piersanti G, Kathuria S, Piomelli D. Cyclohexylcarbamic acid 3′- or 4′-substituted biphenyl-3-yl esters as fatty acid amide hydrolase inhibitors: synthesis, quantitative structure-activity relationships, and molecular modeling studies. J. Med. Chem. 2004; 47:4998–5008. [PubMed: 15456244]

(32). Johnson DS, Stiff C, Lazerwith SE, Kesten SR, Fay LK, Morris M, Beidler D, Liimatta MB, Smith SE, Dudley DT, Sadagopan N, Bhattachar SN, Kesten SJ, Nomanbhoy TK, Cravatt BF, Ahn K. Discovery of PF-04457845: A highly potent, orally bioavailable, and selective urea FAAH inhibitor. ACS Med. Chem. Lett. 2011; 2:91–96. [PubMed: 21666860]

(33). Boger DL, Sato H, Lerner AE, Austin BJ, Patterson JE, Patricelli MP, Cravatt BF. Trifluoromethyl ketone inhibitors of fatty acid amide hydrolase: a probe of structural and conformational features contributing to inhibition. Bioorg. Med. Chem. Lett. 1999; 9:265–270. [PubMed: 10021942]

(34). Boger DL, Miyauchi H, Du W, Hardouin C, Fecik RA, Cheng H, Hwang I, Hedrick MP, Leung D, Acevedo O, Guimaraes CR, Jorgensen WL, Cravatt BF. Discovery of a potent, selective, and efficacious class of reversible alpha-ketoheterocycle inhibitors of fatty acid amide hydrolase effective as analgesics. J. Med. Chem. 2005; 48:1849–1856. [PubMed: 15771430]

(35). Romero FA, Du W, Hwang I, Rayl TJ, Kimball FS, Leung D, Hoover HS, Apodaca RL, Breitenbucher JG, Cravatt BF, Boger DL. Potent and selective alpha-ketoheterocycle-based inhibitors of the anandamide and oleamide catabolizing enzyme, fatty acid amide hydrolase. J. Med. Chem. 2007; 50:1058–1068. [PubMed: 17279740]

(36). Kimball FS, Romero FA, Ezzili C, Garfunkle J, Rayl TJ, Hochstatter DG, Hwang I, Boger DL. Optimization of alpha-ketooxazole inhibitors of fatty acid amide hydrolase. J. Med. Chem. 2008; 51:937–947. [PubMed: 18247553]

(37). Feledziak M, Michaux C, Urbach A, Labar G, Muccioli GG, Lambert DM, Marchand-Brynaert J. beta-Lactams derived from a carbapenem chiron are selective inhibitors of human fatty acid amide hydrolase versus human monoacylglycerol lipase. J. Med. Chem. 2009; 52:7054–7068. [PubMed: 19877691]

(38). Muccioli GG, Fazio N, Scriba GK, Poppitz W, Cannata F, Poupaert JH, Wouters J, Lambert DM. Substituted 2-thioxoimidazolidin-4-ones and imidazolidine-2,4-diones as fatty acid amide hydrolase inhibitors templates. J. Med. Chem. 2006; 49:417–425. [PubMed: 16392827]

(39). Alapafuja SO, Nikas SP, Shukla VG, Papanastasiou I, Makriyannis A. Microwave assisted synthesis of sodium sulfonates precursors of sulfonyl chlorides and fluorides. Tetrahedron Lett. 2009; 50:7028–7031. [PubMed: 21132105]

(40). Gunduz-Cinar O, MacPherson KP, Cinar R, Gamble-George J, Sugden K, Williams B, Ramikie TS, Gorka AX, Alapafuja SO, Nikas SP, Makriyannis A, Poulton R, Patel S, Hariri AR, Caspi A, Moffitt TE, Kunos G, Holmes A. Convergent translational evidence of a role for anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity. Mol. Psychiatry. 2012 [Online early access] DOI:10.1038/mp.2012.1072. Published Online: June 1012, 2012.

(41). Makriyannis, A.; Nikas, SP.; Alapafuja, SO.; Shukla, VG. Fatty acid amide hydrolase inhibitors. WO Patent Application 2008/013963 A2. 2008. p. 123

(42). Makriyannis, A.; Nikas, SP.; Alapafuja, SO.; Shukla, VG. Monoacylgylcerol lipase inhibitors for modulation of cannabinoid activity. WO Patent Application 2009/052319 A1. 2009. p. 209

(43). Makriyannis, A.; Pandarinathan, L.; Zvonok, N.; Parkkari, T.; Chapman, L. Inhibitors of fatty acid amide hydrolase and monoacylglycerol lipase for modulation of cannabinoid receptors. WO Patent Application 2009/117444 A1. 2009. p. 109

(44). Naidoo V, Karanian DA, Vadivel SK, Locklear JR, Wood JT, Nasr M, Quizon PM, Graves EE, Shukla V, Makriyannis A, Bahr BA. Equipotent inhibition of fatty acid amide hydrolase and monoacylglycerol lipase - dual targets of the endocannabinoid system to protect against seizure pathology. Neurotherapeutics. 2012 [Online early access] DOI:10.1007/S13311-13011-10100-y. Published Online: Jannuary 13, 2012.

(45). Zvonok N, Pandarinathan L, Williams J, Johnston M, Karageorgos I, Janero DR, Krishnan SC, Makriyannis A. Covalent inhibitors of human monoacylglycerol lipase: ligand-assisted characterization of the catalytic site by mass spectrometry and mutational analysis. Chem. Biol. 2008; 15:854–862. [PubMed: 18721756]

(46). Zvonok N, Williams J, Johnston M, Pandarinathan L, Janero DR, Li J, Krishnan SC, Makriyannis A. Full mass spectrometric characterization of human monoacylglycerol lipase generated by large-scale expression and single-step purification. J. Proteome Res. 2008; 7:2158–2164. [PubMed: 18452279]

(47). Nikas SP, Grzybowska J, Papahatjis DP, Charalambous A, Banijamali AR, Chari R, Fan P, Kourouli T, Lin S, Nitowski AJ, Marciniak G, Guo Y, Li X, Wang CL, Makriyannis A. The role of halogen substitution in classical cannabinoids: a CB1 pharmacophore model. AAPS J. 2004; 6:e30. [PubMed: 15760095]

(48). Bailey WF, Punzalan ER. Convenient general method for the preparation of primary alkyllithiums by lithium-iodine exchange. J. Org. Chem. 1990; 55:5400–5406.

(49). Quast H, Kees F. Alkansulfonyl-chloride aus alkyllithium und sulforylchlorid. Synthesis. 1974; 7:489–490.

(50). Papahatjis DP, Nahmias VR, Nikas SP, Andreou T, Alapafuja SO, Tsotinis A, Guo J, Fan P, Makriyannis A. C1′-cycloalkyl side chain pharmacophore in tetrahydrocannabinols. J. Med. Chem. 2007; 50:4048–4060. [PubMed: 17672444]

(51). Bhatt MV, Kulkarni SU. Cleavage of ethers. Synthesis-Stuttgart. 1983:249–282.

(52). Ramarao MK, Murphy EA, Shen MW, Wang Y, Bushell KN, Huang N, Pan N, Williams C, Clark JD. A fluorescence-based assay for fatty acid amide hydrolase compatible with high-throughput screening. Anal. Biochem. 2005; 343:143–151. [PubMed: 16018870]

(53). Patricelli MP, Lashuel HA, Giang DK, Kelly JW, Cravatt BF. Comparative characterization of a wild type and transmembrane domain-deleted fatty acid amide hydrolase: identification of the transmembrane domain as a site for oligomerization. Biochemistry. 1998; 37:15177–15187. [PubMed: 9790682]

(54). Mileni M, Garfunkle J, DeMartino JK, Cravatt BF, Boger DL, Stevens RC. Binding and inactivation mechanism of a humanized fatty acid amide hydrolase by alpha-ketoheterocycle inhibitors revealed from cocrystal structures. J. Am. Chem. Soc. 2009; 131:10497–10506. [PubMed: 19722626]

(55). Mileni M, Garfunkle J, Ezzili C, Kimball FS, Cravatt BF, Stevens RC, Boger DL. X-ray crystallographic analysis of alpha-ketoheterocycle inhibitors bound to a humanized variant of fatty acid amide hydrolase. J. Med. Chem. 2010; 53:230–240. [PubMed: 19924997]

(56). Nikas SP, Alapafuja SO, Papanastasiou I, Paronis CA, Shukla VG, Papahatjis DP, Bowman AL, Halikhedkar A, Han X, Makriyannis A. Novel 1′,1′-chain substituted hexahydrocannabinols: 9beta-hydroxy-3 (1-hexyl-cyclobut-1-yl)-hexahydrocannabinol (AM2389) a highly potent cannabinoid receptor 1 (CB1) agonist. J. Med. Chem. 2010; 53:6996–7010. [PubMed: 20925434]

(57). Giang DK, Cravatt BF. Molecular characterization of human and mouse fatty acid amide hydrolases. Proc. Natl. Acad. Sci. U. S. A. 1997; 94:2238–2242. [PubMed: 9122178]

(58). Copeland, RA. Lead optimization and SAR for reversible inhibitors. In: Copeland, RA., editor. Evaluation of enzyme inhibitors in drug discovery. John Wiley and Sons Inc.; Hoboken, NJ: 2005. p. 125-128.

(59). Ahn K, Johnson DS, Fitzgerald LR, Liimatta M, Arendse A, Stevenson T, Lund ET, Nugent RA, Nomanbhoy TK, Alexander JP, Cravatt BF. Novel mechanistic class of fatty acid amide hydrolase inhibitors with remarkable selectivity. Biochemistry. 2007; 46:13019–13030. [PubMed: 17949010]

(60). Alexander JP, Cravatt BF. Mechanism of carbamate inactivation of FAAH: implications for the design of covalent inhibitors and in vivo functional probes for enzymes. Chem. Biol. 2005; 12:1179–1187. [PubMed: 16298297]

(61). Gold AM. Sulfonyl fluorides as inhibitors of esterases. 3. Identification of serine as the site of sulfonylation in phenylmethanesulfonyl alpha-chymotrypsin. Biochemistry. 1965; 4:897–901. [PubMed: 14337707]

(62). Gold AM, Fahrney D. Sulfonyl fluorides as inhibitors of esterases. Ii. Formation and reactions of phenylmethanesulfonyl alpha-chymotrypsin. Biochemistry. 1964; 3:783–791. [PubMed: 14211616]

(63). Yoshida T, Murai M, Abe M, Ichimaru N, Harada T, Nishioka T, Miyoshi H. Crucial structural factors and mode of action of polyene amides as inhibitors for mitochondrial NADH-ubiquinone oxidoreductase (complex I). Biochemistry. 2007; 46:10365–10372. [PubMed: 17685634]

(64). Hahn RC, Tompkins J. One-pot, one- and multi-carbon homologation of alkyl halides: reaction of Grignard reagents with chloroiodomethane. Tetrahedron Lett. 1990; 31:937–940.

(65). Ichimaru N, Murai M, Kakutani N, Kako J, Ishihara A, Nakagawa Y, Nishioka T, Yagi T, Miyoshi H. Synthesis and characterization of new piperazine-type inhibitors for mitochondrial NADH-ubiquinone oxidoreductase (complex I). Biochemistry. 2008; 47:10816–10826. [PubMed: 18781777]

(66). Castang S, Chantegrel B, Deshayes C, Dolmazon R, Gouet P, Haser R, Reverchon S, Nasser W, Hugouvieux-Cotte-Pattat N, Doutheau A. N-Sulfonyl homoserine lactones as antagonists of bacterial quorum sensing. Bioorg. Med. Chem. Lett. 2004; 14:5145–5149. [PubMed: 15380216]

(67). McManus SP, Smith MR, Abramovitch RA, Offor MN. Thermolysis of sulfonyl azides bearing nucleophilic neighboring groups. A search for anchimeric assistance. J. Org. Chem. 1984; 49:683–687.

(68). Truce WE, Milionis JP. Friedel-Crafts cyclization of ω-phenylalkanesulfonyl chlorides. J. Am. Chem. Soc. 1952; 74:974–977.

(69). Papahatjis DP, Nikas SP, Kourouli T, Chari R, Xu W, Pertwee RG, Makriyannis A. Pharmacophoric requirements for the cannabinoid side chain. Probing the cannabinoid receptor subsite at C1′. J. Med. Chem. 2003; 46:3221–3229. [PubMed: 12852753]

(70). Prime. Schrödinger, LLC; New York, NY: 2012.

(71). Mileni M, Kamtekar S, Wood DC, Benson TE, Cravatt BF, Stevens RC. Crystal structure of fatty acid amide hydrolase bound to the carbamate inhibitor URB597: discovery of a deacylating water molecule and insight into enzyme inactivation. J. Mol. Biol. 2010; 400:743–754. [PubMed: 20493882]

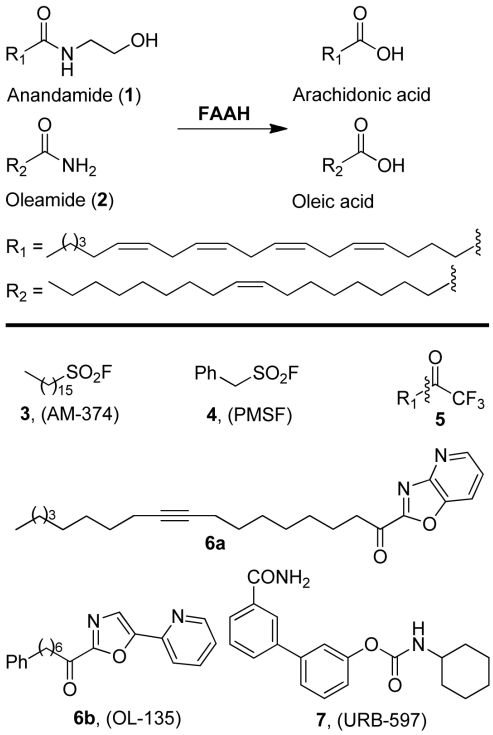


Figure 1.
Representative substrates (**1**, **2**) and inhibitors (**3**-**7**) of fatty acid amide hydrolase (FAAH).

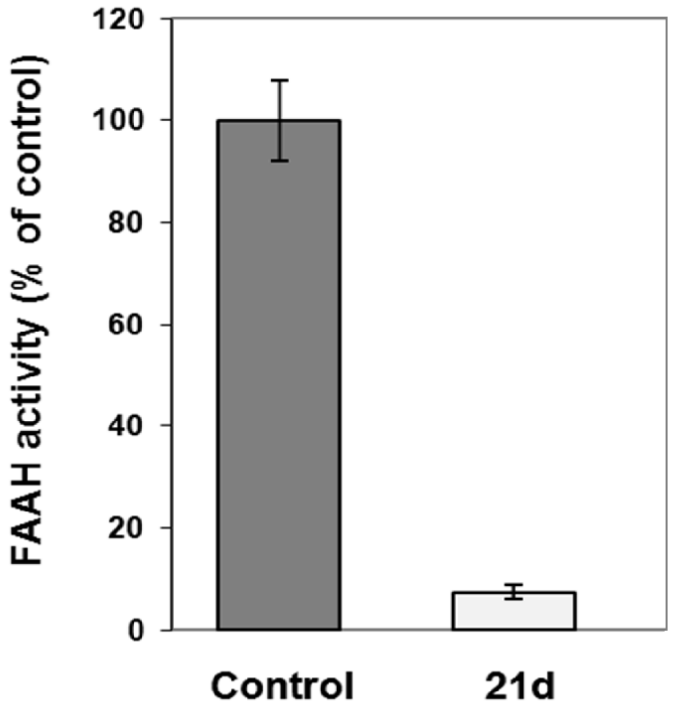


Figure 2.
Irreversible inhibition of purified ΔTM rFAAH with **21d**. Ordinate, enzymatic activity after rapid dilution of untreated (control) and **21d** treated FAAH. A negligible enzymatic activity (8%) was observed with **21d**.

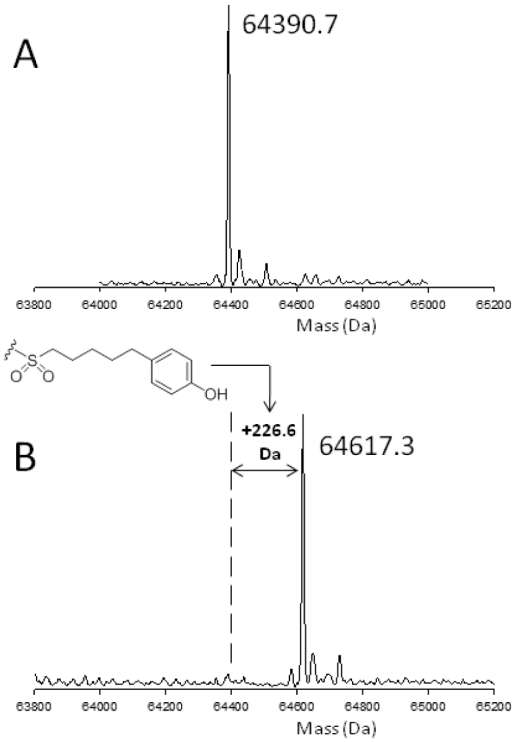


Figure 3.
MS analysis of ΔTM rFAAH enzyme masses. Intact masses of unmodified ΔTM rFAAH (A) and **21d**-modified ΔTM rFAAH (B). The increase in mass is consistent with enzyme sulfonylation by **21d**.

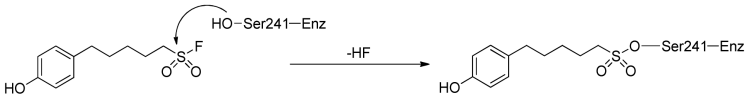


Figure 4.
Probable mechanism of ΔTM rFAAH sulfonylation by **21d**.

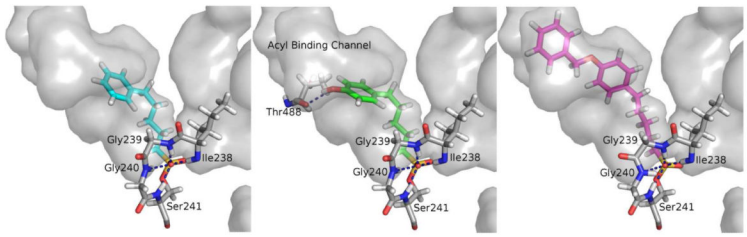
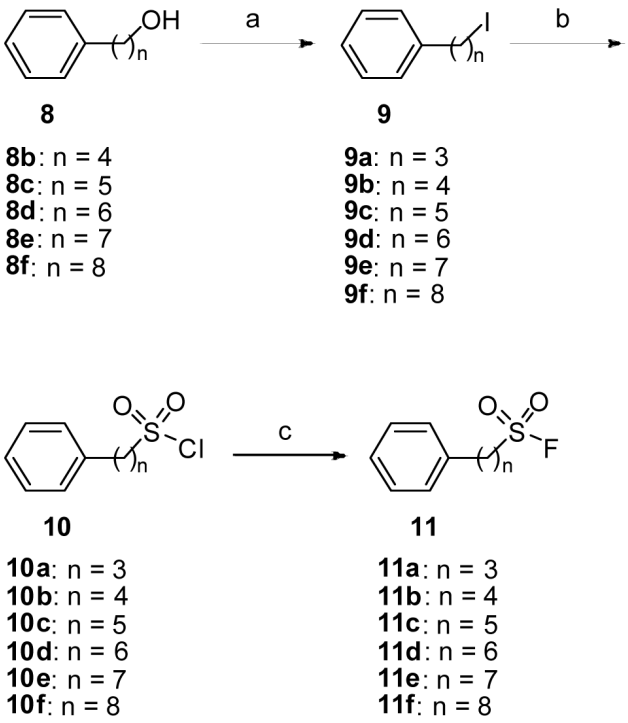


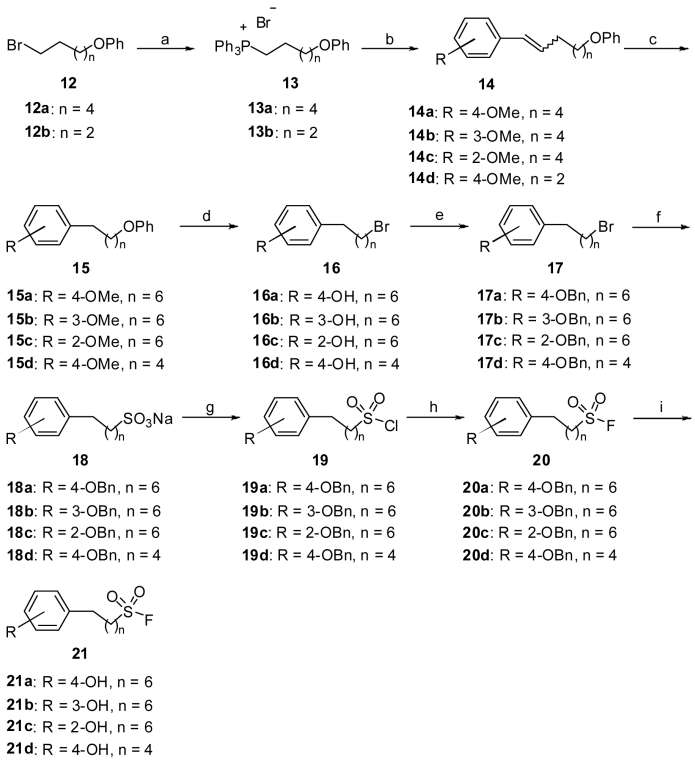
Figure 5.

11c (Cyan carbons), **21d** (green carbons) and **20d** (magenta carbons) covalently docked to the catalytic Ser241 of rFAAH in the acyl chain binding channel. There is significant hydrogen bonding of all ligands with the oxyanion hole (formed by the backbone of Ile238, Gly239, Gly240 and Ser241). **21d** also forms a hydrogen bond with the backbone carbonyl of Thr488. Hydrogen bonds are denoted by a blue dashed line and the surface of the binding channels is shown in grey.



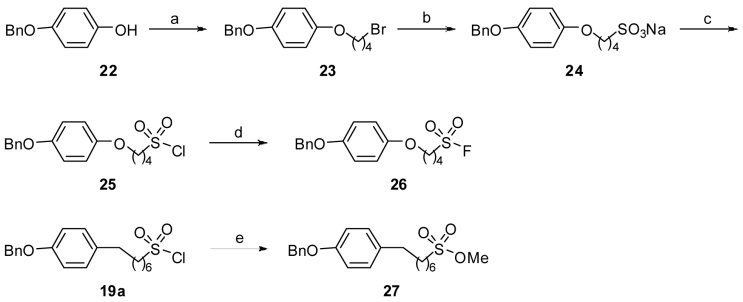
Scheme 1.

Reagents and conditions: (a) PPh_3, imidazole, I_2, MeCN/Et_2O, 0°C to r t, 72-85%; (b) (i) *t*-BuLi, Et_2O/pentane, −78°C, (ii) SO_2Cl_2, −78°C, 19-23%; (c) NH_4F, acetone, reflux, 91-93%.



Scheme 2.

Reagents and conditions: (a) Ph_3P, benzene, reflux, 2 days, 84-85%; (b) $(Me_3Si)_2N^-K^+$, THF, 0°C, 5 min, then 4- or 3- or 2-anisaldehyde, 10 min, 91-93%; (c) H_2, Pd/C, AcOEt, 30 psi, r t, 6 h, 94-96% (d) BBr_3, CH_2Cl_2, −30°C to r t, 2 h, 90-93%; (e) K_2CO_3, acetone, BnBr, reflux, 6 h, 77-81%; (f) Na_2SO_3, EtOH/H_2O, reflux, 24 h; or Na_2SO_3, THF/EtOH/H_2O, M.W, 160°C, 15 min; (g) $SOCl_2$, benzene/cat. DMF, 50°C, 3 h, 37-50% from **17a-17d**; (h) NH_4F, acetone, reflux, 2 h, 90-93%; (i) $BF_3{\cdot}OEt_2$, $HS(CH_2)_2SH$, rt, 1 h, 66-70%.

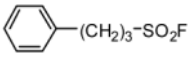
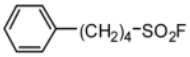
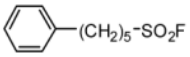
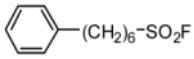
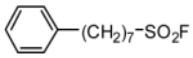
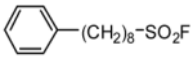
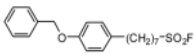
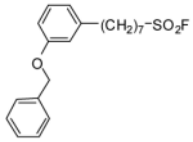
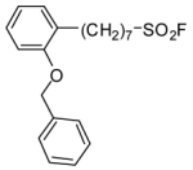
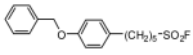
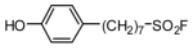
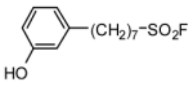
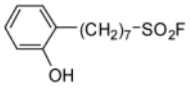


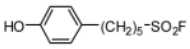
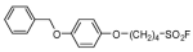
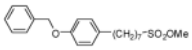
Scheme 3.

Reagents and conditions: (a) K_2CO_3, 18-crown-6, 1,4-dibromobutane, acetone, reflux, 3 h, 50%; (b) Na_2SO_3, THF/EtOH/H_2O, M.W, 160°C, 15 min; (c) $SOCl_2$, benzene/cat. DMF, 50°C, 3 h, 51% from **23**; (d) NH_4F, acetone, reflux, 2 h, 93%; (3) MeOH, rt, overnight, 84%.

Table 1

Compound inhibition data results[a] for rat FAAH and human MGL.

compd	Structure	rFAAH (IC_{50}, nM)[b]	hMGL (IC_{50}, nM)[b]	hMGL/rFAAH
11a		204.8 ± 21.3	N.D.	-
11b		88.2 ± 9.1	N.D.	-
11c		50.4 ± 5.8	N.D.	-
11d		37.7 ± 4.1	N.D.	-
11e		37.0 ± 4.2	N.D.	-
11f		176.3 ± 19.8	N.D.	-
20a		3.7 ± 0.6	248.9 ± 27.3	67
20b		3.6 ± 0.7	752.1 ± 57.2	209
20c		4.3 ± 0.6	116.1 ± 18.2	27
20d		1.5 ± 0.3	201.3 ± 26.8	134
21a		2.2 ± 0.4	427.1 ± 45.2	194
21b		2.5 ± 0.3	190.3 ± 20.1	76
21c		1.2 ± 0.2	56.1 ± 7.1	47

compd	Structure	rFAAH (IC_{50}, nM)[b]	hMGL (IC_{50}, nM)[b]	hMGL/rFAAH
21d (AM3506)		2.8 ± 0.3	383.1 ± 42.3	137
26		1.2 ± 0.2	156.2 ± 15.2	130
27		269.1 ± 28.2	>5,000	-

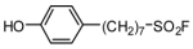
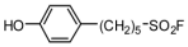
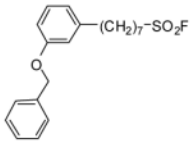
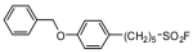
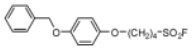
[a] Inhibition data for rFAAH and hMGL were determined using medium throughput fluorescent assays as described under experimental.

[b] IC_{50} values were determined from three independent experiments and are expressed as the mean of three values.

N.D.: Not Determined.

Table 2

Affinities (Ki) of selected analogs for rat CB1 and mouse CB2 cannabinoid receptors (95% confidence limits).

compd	Structure	rCB1 (Ki, nM)[a]	mCB2 (Ki, nM)[a]
21a		163 ± 34	698 ± 261
21d		192 ± 57	577 ± 145
20b		335 ± 88	99 ± 29
20d		118 ± 34	194 ± 45
26		104 ± 24	203 ± 52

[a]Affinities for CB1 and CB2 were determined using rat brain (CB1) or membranes from HEK293 cells expressing mouse CB2, and [^{3}H]CP-55,940 as the radioligand following previously described procedures.[50,56] Data were analyzed using nonlinear regression analysis. Ki values were obtained from three independent experiments run in duplicate and are expressed as the mean of the three values.

Table 3

Inhibition of rat and human FAAH[a] by **21d**.

compd	rFAAH (IC_{50}, nM)[b]	hFAAH (IC_{50}, nM)[b]
21d	2.8 ± 0.3	5.1 ± 0.5

[a] Inhibition for hFAAH was determined using medium through-put fluorescent assay as described for rFAAH.

[b] IC_{50} values were determined from three independent experiments and are expressed as the mean of three values.