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Exploring the size of the lipophilic unit of the soluble epoxide hydrolase inhibitors

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Abstract

Soluble epoxide hydrolase (sEH) inhibitors are potential drugs for several diseases. Adamantyl ureas are excellent sEH inhibitors but have limited metabolic stability. Herein, we report the effect of replacing the adamantane group by alternative polycyclic hydrocarbons on sEH inhibition, solubility, permeability and metabolic stability. Compounds bearing smaller or larger polycyclic hydrocarbons than adamantane yielded all good inhibition potency of the human sEH (0.4–21.7 nM), indicating that sEH is able to accommodate inhibitors of very different size. Human liver microsomal stability of adamantane containing inhibitors is lower than that of their corresponding adamantane counterparts.

Keywords

Adamantane; Inhibitor; Isocyanate; Soluble epoxide hydrolase; Urea

1. Introduction

Arachidonic acid (AA), a polyunsaturated fatty acid, plays important roles in cellular signaling as a second messenger and is also a precursor for a wide variety of lipid mediators that are involved in many physiological and pathophysiological processes. The first step in the biosynthesis of these mediators, known as eicosanoids or oxylipins, is an oxidation, which can be catalyzed by cyclooxygenases (COX), lipoxygenases (LOX), and cytochrome

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Appendix A. Supplementary data

Supplementary data (¹H and ¹³C NMR spectra of the compounds) to this article can be found online at <https://doi.org/10.1016/j.bmc.2019.115078>.

P450 enzymes.¹ Most research on AA derivatives has focused on prostaglandins, processed by COX, and leukotrienes, originated from LOX. Both types of metabolites are potent inflammatory mediators and, consequently, several pharmaceuticals have been produced to alleviate inflammatory conditions. These included non-selective COX-1 and COX-2 inhibitors (e.g., ibuprofen, indomethacin), selective COX-2 inhibitors (e.g., celecoxib, etoricoxib), and 5-LOX inhibitors (e.g., zileuton).²⁻⁴

Comparatively, the third pathway remains relatively unexplored. Cytochromes P450 enzymes transform AA to various biologically active compounds, including epoxyeicosatrienoic acids (EETs).^{5,6} EETs are reported to exhibit anti-inflammatory and anti-nociceptive properties and are involved in the regulation of blood pressure and cellular stress.⁷⁻¹¹ Soluble epoxide hydrolase (sEH, *EPHX2*, E.C. 3.3.2.3), a member of the α/β -hydrolase fold family of enzymes, catalyzes the hydrolysis of EETs to the corresponding dihydroxyeicosatrienoic acids (DHETs), reducing the beneficial activities of EETs.¹²⁻¹⁵ The inhibition of sEH *in vivo* by potent, selective inhibitors results in an increase of the concentration of the EETs, reducing blood pressure and inflammatory and pain states, thereby suggesting that sEH inhibitors may serve as novel agents for treating hypertension, inflammatory diseases, pain and, more recently, neurodegenerative diseases.¹⁶⁻²¹

X-ray crystallographic studies revealed that sEH has an active site with a catalytic triad at the corner of an L-shaped hydrophobic pocket. The triad includes a nucleophilic aspartic acid, which attacks the epoxide carbon-highly polarized by hydrogen bonds with two tyrosine residues, and a histidine-aspartic acid pair, which activates the hydrolysis of the acyl-enzyme intermediate.²² Therefore, lipophilic groups such as cyclohexyl or adamantyl are commonly present in potent sEH inhibitors in order to establish hydrophobic interactions with the pocket. In fact, the first sEH inhibitor to enter in clinical trials was AR9281, an adamantyl urea (Fig. 1).²³ Specifically, hundreds of sEH inhibitors featuring a common structure of Ad-NH-C(O)-NH-R, where Ad is adamantan-1-yl and R is alkyl, aryl or heterocyclyl groups, have been synthesized and, subsequently, evaluated in several *in vivo* models (Fig. 1).²³⁻³⁵ However, the poor metabolic stability of some adamantane containing ureas could limit their usefulness to treat patients.³⁶ Notwithstanding the high potency generally associated to adamantane-derived sEH inhibitors, alternative polycyclic hydrocarbons have been scarcely evaluated.

In this work, a series of ring-contracted and ring-expanded analogs of three potent adamantane sEH inhibitors, AR9281 ($IC_{50} = 7.0$ nM),²³ *t*-AUCB ($IC_{50} = 0.5$ nM),³⁷ and **1** ($IC_{50} = 0.4$ nM),³⁸ were synthesized and pharmacologically evaluated in order to test if alterations in the size of the lipophilic unit attached to the urea significantly impact its potency toward the human sEH (Figs. 1 and 2) as well as influencing solubility, permeability and metabolic stability.

2. Results and discussion

2.1. Chemistry

Adamantyl ureas are typically synthesized by the reaction of adamantyl isocyanates with a primary amine. Alternatively, the reaction of amantadine (1-adamantylamine) with an

isocyanate also furnishes adamantyl ureas. Taking into account that 2,3,4-trifluorophenylisocyanate is a commercially available compound, for the preparation of the analogs of urea **1**, we reacted this isocyanate with four different amines, **4–7**, featuring smaller polycyclic rings than adamantane. Bisnoradamantane amines **4**, **5** and **6** were synthesized following reported procedures,^{39,40} while noramantadine **7** is commercially available. For comparative purposes, we also synthesized, using the same reaction, urea **1** and its isomer **10** (Scheme 1).

In order to obtain the ring-expanded analog **15**, we started from diamantanamine **8**, which was synthesized in two steps from commercially available diamantane, **16**. Oxidation of **16** with sulfuric acid followed by a reductive amination of ketone **17** by ammonium acetate and NaCNBH₃ led to amine **8** (Scheme 2). For the synthesis of the ring-contracted and ring-expanded analogs of *t*-AUCB and AR9281, we first prepared the required isocyanate by the reaction of the corresponding polycyclic amine with triphosgene. The reaction of these isocyanates with either *t*-4-[(4-aminocyclohexyl)oxy]benzoic acid (Scheme 3) or *N*-acetyl-4-aminopiperidine, **22** (Scheme 4), furnished the desired compounds. An alternative procedure was employed for the synthesis of urea **24**, involving the activation of **22** with 1,1'-carbonyldiimidazole (Scheme 4).

2.2. sEH inhibition and structure-activity relationships

The potency of the new compounds as human soluble epoxide hydrolase inhibitors was tested using a previously reported sensitive fluorescent-based assay (Table 1).⁴¹

Within the series of the 2,3,4-trifluorophenyl inhibitors, sequential ring contraction from adamantanes **1** or **10** to noradamantane **14** and to bisnoradamantane **13** resulted in a decrease of the inhibitory potency (compare entries 1 and 2 vs 5 and 6, Table 1), likely because of the reduction of hydrophobic interactions between the ring-contracted moiety and the lipophilic pocket of the enzyme and the increase of desolvation energy to transfer the molecule from the solution state to the receptor cavity. This reduction in potency was also observed in the other two series of sEH inhibitors (compare entry 11 vs 13, and entry 8 vs 9, Table 1).

Nevertheless, the inhibitory potency was restored by the introduction of two methyl groups in the bridgehead positions of the bisnoradamantane moiety (compare entries 1 and 2 vs entries 3 and 4, and entry 11 vs entry 12, Table 1), probably because the addition of the methyl groups compensates the reduction in size from the adamantane to the bisnoradamantane scaffold. Furthermore, the results showed that the introduction of a methylene unit between the hydrophobic moiety and the urea does not affect the potency of the compounds (compare entry 3 vs 4, Table 1).

Taking into account that the reduction of the polycyclic moiety from adamantane to bisnoradamantane led, within the three series of inhibitors, to a reduction of the potency, we wondered if the opposite was true. That is, whether an increase in the size of the lipophilic unit of the inhibitor would lead to more potent compounds.

With the aim of exploring the ring-expanded analogs, the adamantane ring was replaced by the much larger diamantane moiety. Somehow surprisingly, considering the substantial increase in size and previous consideration of the adamantyl group as the marginal biggest group as the *N*-substituent for sEH inhibitors,⁴² diamantane ureas **26** and **28** showed IC₅₀ values in the same range as that of AR9281 (compare entry 11 vs entries 14 and 15). Considering that **26** was slightly more potent than its isomer **28**, we synthesized two further analogs derived from diamantane **8**, i.e., the new ureas **15** and **21**, analogs of inhibitors **1** and *t*-AUCB, respectively. In line with the aforementioned results, diamantane derivative **21** showed to be as potent as *t*-AUCB (compare entry 8 vs 10, Table 1). However, within the trifluorophenyl series, the diamantane derivative **15** was considerably less potent than adamantane derivatives **1** or **10** (compare entries 1 and 2 vs 7, Table 1). The dissimilar behavior of **15** compared with **21** and **26** could be due to an opposite binding orientation of **15** compared to that of **21** and **26**, as observed previously for a different series of sEH inhibitors.⁴³

Typically, steric parameters have stronger effects on the potency of inhibitors against murine sEH rather than on the human sEH.^{32,41,43,44} For example, it has recently been reported that the progressive introduction of one, two or three methyl groups in the bridgehead positions of the adamantane unit of *t*-AUCB did not lead to significant changes in the IC₅₀ values against the human enzyme, while leading to a gradual increase in the IC₅₀ values against the murine enzyme.³² However, when we tested the inhibition of the murine sEH by AR9281 and three analogs (**24**, **26** and **28**), we did not find significant differences between their activities in human and murine species (Table 1).

2.3. Microsomal stability

It is known that the adamantane nucleus is prone to rapid metabolism *in vivo* giving rise to a variety of inactive hydroxylated derivatives. This results in low drug concentrations in blood and short *in vivo* half-life. Metabolism studies have shown that the bridgehead hydroxylation (tertiary carbon) is favored over the secondary carbon positions, producing water-soluble hydroxyadamantane derivatives in the liver, which are then easily excreted.⁴⁵ Additionally, metabolic studies showed that liver microsomes from phenobarbital-treated rats readily metabolize diamantane to mono-, di- and possibly tri-hydroxy derivatives.^{46,47} It is also known that several diamantanes are cytochromes P450 inhibitors.^{48,49}

Considering the aforementioned metabolism liability of the adamantane and diamantane scaffolds, we assessed the *in vitro* stability of some representative new ureas in human microsomes in order to examine the impact of the different hydrophobic units in their metabolic stabilities.

As anticipated, diamantane derivatives were extremely labile compounds, with their adamantane counterparts being considerably more stable (compare entries 1 vs 6, 7 vs 9, and 10 vs 13 and 14, Table 2). These results are in the line with what was expected, since diamantane moiety features more tertiary carbon atoms than the adamantane ring, which are prone to be hydroxylated.

Finally, the bisnoradamantane and the noradamantane units seem to have similar (compare entries 1 vs 3 and 4, and 1 vs 5 and 10 vs 12, Table 2) or somehow reduced (compare entries 7 vs 8 and 10 vs 11, Table 2) microsomal stability than adamantane.

2.4. Solubility and lipophilicity

In order to assess the impact of the polycyclic scaffold in the solubility of the inhibitors, we experimentally determined their solubility in a 1% DMSO: 99% PBS buffer solution.

As expected, within the trifluorophenylurea series, the solubility highly increases from diamantane **15** to adamantane **1** (18 and 57 μM , respectively, Table 2) and then, slightly further increases to the noradamantane **14** and to the bisnoradamantane **13** (65 and 82 μM , respectively, Table 2). In fact, the diamantane derivatives were dramatically less soluble than their adamantane, noradamantane or bisnoradamantane counterparts (compare entries 6 vs 1, 3, 4 and 5, entries 9 vs 7 and 8 and entries 13 and 14 vs 10 and 11, Table 2). Finally, considering the right-hand side of the inhibitors, the acetylpiperidine derivatives were the more soluble compounds, with the two other series having similar solubility.

Of note, the experimental solubility values showed a good correlation with the calculated lipophilicity values (see Table 2), the more soluble AR9281 analogs being the compounds with the lowest lipophilicity. As expected, for any given right-hand side unit, the diamantane derivatives showed always the higher lipophilicity.

2.5. Permeability

In order to evaluate the permeability of selected inhibitors, the Caco-2 cell permeability model was used in this study. Apparent permeability values (P_{app}) were determined from the amount permeated through the Caco-2 cell membranes at both apical-basolateral (A-B) and basolateral-apical (B-A) direction (Table 3).

Of note, the size of the lipophilic unit of the sEH inhibitors seems to be of little relevance regarding permeability, as evidenced through the comparison within the three series of inhibitors: the trifluorophenyl derivatives (compare entries 1 and 2 vs 3, Table 3), the benzoic acid derivatives (compare entries 4 vs 5, Table 3) and the acetylpiperidine derivatives (compare entries 6–8, Table 3). Regarding the right-hand side of the ureas, acetylpiperidine derivatives were endowed with the best permeability, while the trifluorophenyl compounds **1**, **10** and **15** displayed much lower permeability. As expected, benzoic acid derivatives *t*-AUCB and **21** were the less permeable compounds (Table 3).

3. Conclusions

Overall, it seems clear that the catalytic center of the sEH enzyme can accommodate polycycles of different sizes, ranging from the small bisnoradamantane moiety to the very large diamantane group. Notwithstanding this, it appears, particularly within the *t*-AUCB and AR9281 derivatives, that the replacement of the adamantane by larger polycyclic rings, such as the diamantanes, is better than the replacement by smaller ones.

Of note, although the present results highlight the interest of diamondoids as tools for investigating the size-limit of inhibitors,⁵¹ the low solubility and the high metabolic lability of these derivatives severely limits their potential use in medicinal chemistry.⁵²

4. Experimental section

4.1. Chemistry

4.1.1. General—Commercially available reagents and solvents were used without further purification unless stated otherwise. Preparative normal phase chromatography was performed on a CombiFlash Rf 150 (Teledyne Isco) with pre-packed RediSep Rf silica gel cartridges. Thin-layer chromatography was performed with aluminum-backed sheets with silica gel 60 F₂₅₄ (Merck, ref. 1.05554), and spots were visualized with UV light and 1% aqueous solution of KMnO₄. Melting points were determined in open capillary tubes with a MFB 595010M Gallenkamp. 400 MHz ¹H and 100.6 MHz ¹³C NMR spectra were recorded on a Varian Mercury 400 or on a Bruker 400 Avance III spectrometers. 500 MHz ¹H NMR spectra were recorded on a Varian Inova 500 spectrometer. The chemical shifts are reported in ppm (δscale) relative to internal tetramethylsilane, and coupling constants are reported in Hertz (Hz). Assignments given for the NMR spectra of selected new compounds have been carried out on the basis of DEPT, COSY ¹H/¹H (standard procedures), and COSY ¹H/¹³C (gHSQC and gHMBC sequences) experiments. IR spectra were run on Perkin-Elmer Spectrum RX I, Perkin-Elmer Spectrum TWO or Nicolet Avatar 320 FT-IR spectrophotometers. Absorption values are expressed as wave-numbers (cm⁻¹); only significant absorption bands are given. High-resolution mass spectrometry (HRMS) analyses were performed with an LC/MSD TOF Agilent Technologies spectrometer. The elemental analyses were carried out in a Flash 1112 series Thermofinnigan elemental microanalyzer (A5) to determine C, H, N and S. The structure of all new compounds was confirmed by elemental analysis and/or accurate mass measurement, IR, ¹H NMR and ¹³C NMR. The analytical samples of all the new compounds, which were subjected to pharmacological evaluation, possessed purity 95% as evidenced by their elemental analyses.

4.1.2. Diamantan-3-one (17)—Diamantane, **16** (600 mg, 3.19 mmol), was suspended in conc. H₂SO₄ 96% (5 mL). The mixture was stirred at 75 °C for 10 h. The reaction mixture was cooled to room temperature and poured on ice. This aqueous solution was extracted with diethyl ether (3 × 50 mL). The combined organic phases were dried over anh. Na₂SO₄ and filtered. Evaporation of the organics gave a white solid (494 mg). This residue was dissolved in DCM (20 mL) 25 g of neutrum alumina were added and the solvent was evaporated obtaining a white solid. Then, hexane (25 mL) was added, the suspension was stirred for 5 min and was filtrated (×2). Diethyl ether (50 mL) was added, the suspension was stirred for 5 min and it was filtrated. Evaporation of the organics gave **17** as a white solid (440 mg, 69% yield). The spectroscopic data coincide with those described in the bibliography.^{53,54}

4.1.3. Diamantane-3-amine (8)—Diamantan-3-one, **17** (583 mg, 2.88 mmol) was dissolved in IPA (6 mL), followed by the addition of AcONH₄ (3.33 g, 43.22 mmol). The mixture was stirred at reflux for 1 h. Then, NaCNBH₃ (1.26 g, 20.17 mmol) was added. The

reaction mixture was stirred at reflux for 24 h. The dark solution was cooled down to room temperature and 10 N NaOH was added until basic pH to quench the reaction. This mixture was extracted with DCM (3 × 50 mL) and the combined organic phases were dried over anh. Na₂SO₄ and filtered. Evaporation of the organics gave a white solid (580 mg) which was dissolved in EtOAc and extracted with 2 N HCl. The aqueous layer was basified with 5 N NaOH until basic pH and extracted with EtOAc. The combined organic phases were dried over anh. Na₂SO₄ and filtered. Evaporation of the organics *in vacuo* gave **8** as a white solid (390 mg, 66% yield). ¹H NMR (400 MHz, CDCl₃) δ: 1.50 (dt, *J* = 12.8 Hz; *J'* = 3.2 Hz, 1H), 1.61–1.83 (complex signal, 15H), 1.92–1.98 (complex signal, 2H), 2.91 (t, *J* = 2.8 Hz, 1H, 3-H). ¹³C NMR (100.6 MHz, CDCl₃) δ: 26.4 (CH), 31.1 (CH), 31.7 (CH₂), 32.4 (CH), 36.6 (CH), 37.0 (CH), 37.6, 37.7, 37.8, 38.83 and 38.0 (1 CH and 4 CH₂), 43.9 (CH), 56.4 (CH). HRMS-ESI⁺ *m/z* [M+H]⁺ calcd for [C₁₄H₂₁N+H]⁺: 204.1747, found: 204.1753.

4.1.4. General procedure for the synthesis of the ureas 1 and 10–15—In a round-bottom flask equipped with a stir bar under nitrogen atmosphere the appropriate amine hydrochloride (1.2 mmol) was added to anh. DCM (~110 mM). To this suspension 2,3,4-trifluorophenyl isocyanate (1 mmol) followed by triethylamine (7 mmol) was added. The reaction mixture was stirred at room temperature overnight. Then the solvent was removed *in vacuo* and the resulting crude was purified by column chromatography.

4.1.5. 1-(1-Adamantyl)-3-(2,3,4-trifluorophenyl)urea (1)—From adamantan-1-amine hydrochloride (**2·HCl**) (162 mg) and following the general procedure a crude was obtained. Column chromatography (SiO₂, Hexane/Ethyl Acetate mixture) followed by evaporation *in vacuo* of the appropriate fractions gave the urea **1** (280 mg, quantitative yield). The analytical sample was obtained by crystallization from methanol. The spectroscopic data coincide with those described in the bibliography.³⁷

4.1.6. 1-(2-Adamantyl)-3-(2,3,4-trifluorophenyl)urea (10)—From adamantan-2-amine hydrochloride (**3·HCl**) (166 mg) and following the general procedure a crude was obtained. Column chromatography (SiO₂, Hexane/Ethyl Acetate mixture) followed by evaporation *in vacuo* of the appropriate fractions gave the urea **10** (270 mg, 94% yield). The analytical sample was obtained by crystallization from EtOAc/pentane. The spectroscopic data were identical to those previously published.³⁷

4.1.7. 1-[(3,7-Dimethyl(tricyclo[3.3.0.0^{3,7}])oct-1-yl)methyl]-3-(2,3,4-trifluorophenyl)urea (11)—From (3,7-dimethyltricyclo[3.3.0.0^{3,7}])octan-1-yl)methanamine hydrochloride (**6·HCl**) (50 mg) and following the general procedure a crude was obtained. Column chromatography (SiO₂, Hexane/Ethyl Acetate mixture) followed by concentration *in vacuo* of the appropriate fractions gave the urea **11** (82 mg, 98% yield) as a white solid, mp 133–134 °C. IR (ATR) ν: 3323, 2952, 2881, 1637, 1621, 1570, 1510, 1474, 1292, 1244, 1177, 1045, 1001, 985, 809, 794, 755, 682, 653 cm⁻¹. ¹H NMR (500 MHz, CD₃OD) δ: 1.17 (s, 6H, C3(7)-CH₃), 1.32–1.38 (complex signal, 4H, 4(6)-H_a and 2(8)-H_a), 1.42 (d, *J* = 7.5 Hz, 2H, 2(8)-H_b), 1.59 (dd, *J* = 8 Hz, *J'* = 3 Hz, 2H, 4(6)-H_b), 2.10 (t, *J* = 3 Hz, 1H, 5-H), 3.42 (s, 2H, CH₂-N), 7.01 (m, 1H, 5'-H), 7.75 (m, 1H, 6'-H). ¹³C NMR (125.7 MHz, CD₃OD) δ: 17.1 [CH₃, C3(7)-CH₃], 42.7 (CH, C5), 43.8 [CH₂, CH₂-N], 48.5

[C, C3(7)], 52.3 (C,C1), 54.9 [CH₂, C4(6)], 57.2 [CH₂, C2(8)], 112.3 (CH, dd, ²J_{C-F} = 17.7 Hz, ³J_{C-F} = 3.9 Hz, C5'), 116.7 (CH, C6'), 127.0 (C, d, ²J_{C-F} = 6 Hz, C1'), 141.5 (C, dt, ¹J_{C-F} = 247 Hz, ²J_{C-F} = 15 Hz, C3'), 143.6 (C, dd, ¹J_{C-F} = 246 Hz, ²J_{C-F} = 12 Hz, C4'), 147.5 (C, dd, ¹J_{C-F} = 243 Hz, ²J_{C-F} = 9 Hz, C2'), 157.8 (C, CO). MS (DIP), *m/z* (%); significant ions: 338 (M⁺, 1), 149 [(C₁₁H₁₇)⁺, 43], 148 (86), 147 [(C₆H₄F₃N)⁺, 42], 136 (19), 135 [(C₁₀H₁₅)⁺, 100], 119 (18), 107 (56), 106 (15), 105 (15), 93 (42), 91 (28), 79 (16), 77 (16). Elemental analysis: Calculated for C₁₈H₂₁F₃N₂O: C 63.89, H 6.26, N 8.28. Found: C 63.83, H 6.52, N 8.26.

4.1.8. 1-(3,7-Dimethyl(tricyclo[3.3.0.0^{3,7}]oct-1-yl))-3-(2,3,4-trifluorophenyl)urea (12)—From 3,7-dimethyltricyclo[3.3.0.0^{3,7}]octan-1-amine hydrochloride (**5·HCl**) (61 mg) and following the general procedure a crude was obtained. Column chromatography (SiO₂, Hexane/Ethyl Acetate mixture) followed by evaporation *in vacuo* of the appropriate fractions gave the urea **12** (50 mg, 47% yield) as a white solid, mp 174–175 °C. IR (ATR) ν : 3335, 2957, 2930, 2882, 2158, 2005, 1686, 1656, 1637, 1621, 1565, 1509, 1471, 1308, 1289, 1242, 1204, 1165, 1154, 1118, 1081, 1064, 1020, 1009, 964, 946, 816, 796, 719, 694, 678, 657 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ : 1.15 [s, 6H, 3(7)-CH₃], 1.39 [dd, *J* = 8.4 Hz, *J*' = 3.6 Hz, 2H, 4(6)-H_a], 1.64 [dd, *J* = 7.6 Hz, *J*' = 3.6 Hz, 2H, 2(8)-H_a], 1.76 [dd, *J* = 8.4 Hz, *J*' = 3.0 Hz, 2H, 4(6)-H_b], 1.82 [d, *J* = 7.6 Hz, 2H, 2(8)-H_b], 2.38 (t, *J* = 3.0 Hz, 1H, 5-H), 5.47 (broad s, 1H, 1-NH), 6.75 (broad s, 1H, 3-NH), 6.89 (m, 1H, 5'-H), 7.80 (m, 1H, 6'-H). ¹³C NMR (100.6 MHz, CDCl₃) δ : 16.5 [CH₃, C3(7)-CH₃], 44.8 (CH, C5), 46.2 [C, C3(7)], 53.1 [CH₂, C4(6)], 57.5 [CH₂, C2(8)], 61.8 (C, C1), 111.4 (CH, dd, ²J_{C-F} = 17.7 Hz, ³J_{C-F} = 3.8 Hz, C5'), 115.0 (CH, t, ³J_{C-F} = 5 Hz, C6'), 124.8 (C, dd, ²J_{C-F} = 8 Hz, ³J_{C-F} = 3.4 Hz, C1'), 139.7 (C, ddd, ¹J_{C-F} = 249 Hz, ²J_{C-F} = 16.3 Hz, ²J_{C-F} = 13.7 Hz, C3'), 142.1 (C, ddd, ¹J_{C-F} = 244 Hz, ²J_{C-F} = 11.9 Hz, ³J_{C-F} = 3.2 Hz, C4'), 141.4 (C, ddd, ¹J_{C-F} = 245 Hz, ²J_{C-F} = 10 Hz, ³J_{C-F} = 2.8 Hz, C2'), 154.6 (C, CO). MS (EI), *m/z* (%); significant ions: 324 (M⁺, 8), 268 (15), 148 (44), 147 [(C₆H₄F₃N)⁺, 100], 146 (23), 136 (17), 134 (84), 122 (89), 121 (54), 120 (40), 119 (81), 110 (20), 109(51), 108 (50), 107 (28), 106 (16), 105 (21), 96 (17), 95 (70), 94 (54), 93 (52), 92 (18), 91 (44), 81 (17), 80 (18), 79 (37), 77 (33), 67 (24), 55 (16), 41 (23). Elemental analysis: Calculated for C₁₇H₁₉F₃N₂O: C 62.95, H 5.90, N 8.64. Found: C 63.12, H 6.17, N 8.48.

4.1.9. 1-(Tricyclo[3.3.0.0^{3,7}]oct-1-yl)-3-(2,3,4-trifluorophenyl)urea (13)—From tricyclo[3.3.0.0^{3,7}]octan-1-amine hydrochloride (**4·HCl**) (19 mg) and following the general procedure a crude was obtained. Column chromatography (SiO₂, Hexane/Ethyl Acetate mixture) followed by evaporation *in vacuo* of the appropriate fractions gave the urea **13** (24 mg, 66% yield) as a white solid, mp 185–186 °C. IR (ATR) ν : 3331, 3105, 2970, 2943, 2894, 2159, 1656, 1640, 1620, 1563, 1510, 1467, 1318, 1288, 1244, 1204, 1171, 1107, 1076, 1065, 1017, 979, 823, 800, 764, 723, 710, 690, 668, 645, 620 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ : 1.51 [d, *J* = 8.8 Hz, 2H, 4(6)-H_a], 1.74 [complex signal, 6H, 4(6)-H_a, 2(8)-H₂], 2.34 [broad s, 2H, 3(7)-H], 2.42 (m, 1H, 5-H), 5.42 (broad s, 1H, 1-NH), 6.67 (broad s, 1H, 3-NH), 6.90 (m, 1H, 5'-H), 7.82 (m, 1H, 6'-H). ¹³C NMR (100.6 MHz, CDCl₃) δ : 32.8 [CH, C3(7)], 43.0 (CH, C5), 46.6 [CH₂, C4(6)], 51.1 [CH₂, C2(8)], 61.6 (C, C1), 111.4 (CH, dd, ²J_{C-F} = 17.7 Hz, ³J_{C-F} = 3.7 Hz, C5'), 115.1 (CH, t, ³J_{C-F} = 5.6 Hz, C6'), 124.8 (C, dd, ²J_{C-F} = 8 Hz, ³J_{C-F} = 3.5 Hz, C1'), 139.7 (C, dt, ¹J_{C-F} = 245 Hz, ²J_{C-F} = 15 Hz, C3'), 142.2

(C, dd, $^1J_{C-F} = 225$ Hz, $^2J_{C-F} = 12$ Hz, C4'), 146.5 (C, dd, $^1J_{C-F} = 246$ Hz, $^2J_{C-F} = 10$ Hz, Ar-C2'), 154.6 (C, CO). MS (DIP), m/z (%); significant ions: 296 (M⁺, 34), 268 (14), 267 (24), 254 (22), 147 [(C₆H₄F₃N)⁺, 77], 146 (23), 119 (14), 95 (29), 94 (1 0 0), 82 (29), 81 (62), 80(20), 79(18). Elemental analysis: Calculated for C₁₅H₁₅F₃N₂O: C 60.81, H 5.10, N 9.45. Found: C 60.87, H 5.34, N 9.19.

4.1.10. 1-(Tricyclo[3.3.1.0^{3,7}]non-3-yl)-3-(2,3,4-trifluorophenyl)urea (14)—From tricyclo[3.3.1.0^{3,7}]nonyl-3-amine hydrochloride (**7·HCl**) (100 mg) and following the general procedure, a yellow solid was obtained (191 mg). Column chromatography (Hexane/Ethyl Acetate mixture) gave urea **14** as a white solid (52 mg, 57% yield), mp 192–193 °C. IR (ATR) ν : 661, 757, 798, 956, 1005, 1021, 1052, 1083, 1101, 1155, 1176, 1248, 1287, 1325, 1382, 1429, 1470, 1509, 1563, 1633, 1656, 2346, 2852, 2925, 3343 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ : 1.54–1.69 [complex signal, 4H, 9-H₂ and 6(8)-H_{ax}], 1.92 [dd, $J = 10.0$ Hz, $J' = 2.8$ Hz, 2H, 2(4)-H_{ax}], 2.01–2.10 [complex signal, 4H, 6(8)-H_{eq} and 2(4)-H_{eq}], 2.25 [broad singlet, 2H, 1(5)-H], 2.40 [tt, $J = 6.8$ Hz, $J' = 2.5$ Hz, 1(7)-H], 7.00 (m, 1H, 5'-H), 7.69 (m, 1H, 6'-H). ¹³C NMR (100.6 MHz, CDCl₃) δ : 35.9 (CH₂, C9), 38.8 (CH, C1 and C5), 44.3 (CH₂, C6 and C6), 44.8 (CH, C7), 49.9 (CH₂, C2 and C4), 65.3 (C, C3), 112.2 (CH, dd, $^2J_{C-F} = 18$ Hz, $^3J_{C-F} = 4$ Hz, C5'), 116.8 (CH, C6'), 126.9 (C, dd, $J = 3$ Hz, $J' = 8$ Hz, C1'), 139.9 (C, dt, $^1J_{C-F} = 247.4$, $^2J_{C-F} = 14$, C3'), 146–148 (complex signal, C4' and C2'), 156.8 (C, CO). Elemental analysis: Calcd for C₁₆H₁₇F₃N₂O·0.25 MeOH: C 61.31, H 5.70, N 8.80. Found C 61.51, H 5.94, N 8.55.

4.1.11. 1-(Diamant-3-yl)-3-(2,3,4-trifluorophenyl)urea (15)—From diamantane-3-amine (**8**) (160 mg) and following the general procedure, a solid was obtained (291 mg). Column chromatography (Hexane/Dichloromethane mixture) gave the urea **15** (72 mg, 24% yield) as a white solid, mp 199–200 °C. IR (ATR) ν : 678, 806, 1002, 1025, 1088, 1210, 1249, 1289, 1467, 1513, 1564, 1623, 1671, 1862, 1933, 1997, 2107, 2198, 2357, 2413, 2903 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ : 1.55–1.84 (complex signal, 17 diamantane-H), 3.87 (m, 1H, 3 diamantane-H), 5.57 (broad s, 1H, 1-NH), 6.90 (m, 1H, 5'-H), 7.09 (broad s, 1H, 3-NH), 7.74 (m, 1H, 6'-H). ¹³C NMR (100.6 MHz, CDCl₃) δ : 26.1 (CH), 30.1 (CH), 32.3 (CH), 32.7 (CH₂), 36.3 (CH), 36.6 (CH), 37.2, 37.3, 37.35, 37.4 and 37.5 (1 CH and 4 CH₂), 37.7 (CH₂) 41.5 (CH, C4), 55.2 (CH, C3), 111.5 (CH, dd, $^2J_{C-F} = 18$ Hz, $^3J_{C-F} = 4$ Hz, C5'), 115.1 (CH, C6'), 125.0 (C, dd, $^2J_{C-F} = 8$ Hz, $^3J_{C-F} = 3$ Hz, C1'), 141.1 (C, dt, $^1J_{C-F} = 247$, $^2J_{C-F} = 16$, C3'), 146.5 (C, dm, $^1J_{C-F} = 246$ Hz, C4'), 146.9 (C, dm, $^1J_{C-F} = 248$ Hz, C2'), 154.6 (C, CO). Elemental analysis: Calcd for C₂₁H₂₃F₃N₂O·0.25 CH₂Cl₂: C 64.18, H 5.96, N 7.04. Found: C 64.40, H 6.35, N 6.71.

4.1.12. Tricyclo[3.3.1.0^{3,7}]nonyl-3-isocyanate (18)—Tricyclo[3.3.1.0^{3,7}]nonyl-3-amine hydrochloride (**7·HCl**) (750 mg, 4.33 mmol) was suspended in DCM (52 mL) and aq. NaHCO₃ (22 mL) was added. Under argon atmosphere, the mixture was stirred and cooled to 4 °C on an ice bath. Immediately, trisphosgene (642 mg, 2.16 mmol) was added. The mixture was stirred at 4 °C for 30 min. The 2 phases were separated and the organic layer was washed with brine (2 × 30 mL). The organic phase was dried over anh. Na₂SO₄ and filtered. Evaporation *in vacuo* of the organics gave **18** as a yellowish oil (360 mg, 51% yield) which was used in the next step without further purification.

4.1.13. 4-((Trans-4-(3-(tricyclo[3.3.1.0^{3,7}]non-3-yl)ureido)cyclohexyl)oxy)benzoic acid (19)—Under argon atmosphere, tricyclo[3.3.1.0^{3,7}]nonyl-3-isocyanate (**18**) (250 mg, 1.53 mmol) was dissolved in anh. DCM (16 mL). 4-[(*trans*-4-aminocyclohexyl)oxy]benzoic acid⁵⁵ hydrochloride (497 mg, 1.83 mmol) and Et₃N (619 mg, 6.12 mmol) were added. The reaction mixture was stirred at 30 °C overnight. Water (60 mL) was added to the resulting mixture and two phases were separated. The aqueous phase was washed with DCM (2 × 50 mL) and acidified until pH = 2 with 5 N HCl. This acid solution was extracted with DCM (5 × 30 mL) and the organic layer was dried over anh. Na₂SO₄, filtered and evaporated. The residue was dissolved in EtOAc, washed with 2 N HCl, dried over anh. Na₂SO₄, filtered and evaporated. The organics were evaporated to afford a yellowish oil (70 mg, 12% yield). Urea **19** was obtained by crystallization from hot MeOH as white solid, mp 262–263 °C. IR (ATR) ν : 3377, 3334, 2926, 2862, 1683, 1628, 1605, 1556, 1508, 1456, 1421, 1382, 1326, 1304, 1248, 1163, 1129, 1119, 1096, 1008, 953, 902, 847, 775, 698, 636, 599 cm⁻¹. ¹H NMR (400 MHz, CD₃OD) δ : 1.36 (dq, J = 3.2 Hz, J' = 13.2 Hz, 2H, 3'(5')-H_{ax}], 1.51–1.66 [complex signal, 6H, 2'(6')-H_{ax}, 9''-H₂ and 6''(8'')-H_{ax}], 1.86 [dd, J = 10.0 Hz, J' = 2.8 Hz, 2H, 2''(4'')-H_{ax}], 1.98–2.05 [complex signal, 6H, 6''(8'')-H_{eq} and 3'(5')-H_{eq} and 2''(4'')-H_{eq}], 2.13 [dd, J = 4 Hz, J' = 12.8 Hz, 2H, 2'(6')-H_{eq}], 2.22 [broad s, 2H, 1''(5'')-H], 2.34 (t, J = 6.8 Hz, 1H, 7''-H), 4.39 (m, 1H, 1'-H), 6.96 [d, J = 9.2 Hz, 2H, 3(5)-H], 7.93 [d, J = 8.8 Hz, 2H, 2(6)-H]. ¹³C NMR (100.6 MHz, CD₃OD) δ : 29.1 [CH₂, C2'(6')], 29.6 [CH₂, C3'(5')], 33.9 (CH₂, C9''), 36.7 [CH, C1''(5'')], 42.3 [CH₂, C6''(8'')], 42.9 (CH, C7''), 46.8 (CH, C4'), 48.2 [CH₂, C2''(4'')], 63.3 (C, C3''), 74.0 (CH, C1'), 114.2 [CH, C3(5)], 121.2 (C, C1), 130.6 [CH, C2(6)], 157.9 (C, CO), 161.3 (C, C4), 166.5 (CO₂H). HRMS-ESI⁻ m/z [M-H]⁻ calcd for [C₂₃H₃₀N₂O₄-H]⁻: 397.2133, found: 397.2147.

4.1.14. Diamantane-3-isocyanate (20)—Triphosgene (110 mg, 0.368 mmol) was added in a single portion to a solution diamantane-3-amine (**8**) (150 mg, 0.73 mmol) in DCM (10.5 mL) and saturated NaHCO₃ solution (4.5 mL). The resulting biphasic mixture was stirred at room temperature for 30 min. Then, the two phases were separated and the organic layer was washed with brine, dried over anh. Na₂SO₄ and filtered. Evaporation *in vacuo* provided the isocyanate **20** as a white solid (152 mg, 90% yield), which was used in the next step without further purification.

4.1.15. 4-((Trans-4-(3-(diamantan-3-yl)ureido)cyclohexyl)oxy)benzoic acid (21)—4-[(*Trans*-4-aminocyclohexyl)oxy]benzoic acid hydrochloride⁵⁵ (196 mg, 0.720 mmol) is dissolved in DMF (5 mL) and diamantane-3-isocyanate (**20**) (150 mg, 0.65 mmol) was added followed by Et₃N (145 mg, 1.44 mmol). The reaction mixture was stirred at 50 °C for 3 days. The suspension was filtrated and the solvent was evaporated to obtain a brown solid (315 mg) which was dissolved in DCM and washed with 2 N HCl (2 × 20 mL). The organic layer was dried over anh. Na₂SO₄, filtered and evaporated. The resulting residue was crystallized from hot DCM affording the urea **21** (111 mg, 37% yield) as a white solid, mp 229–230 °C. IR (ATR) ν : 633, 695, 770, 845, 1031, 1052, 1088, 1163, 1243, 1312, 1504, 1568, 1599, 1710, 1956, 2020, 2237, 2346, 2496, 2868, 2904 cm⁻¹. ¹H NMR (400 MHz, CD₃OD) δ : 1.36 (dq, J = 3.2 Hz, J' = 13.2 Hz, 2H, 3'(5')-H_{ax}], 1.52–1.63 [complex signal, 3H, 2'(6')-H_{ax} and 1 diamantane-H], 1.67–1.91 (complex signal, 17 diamantane-H),

2.02 [dd, $J = 4.4$ Hz, $J' = 13.2$ Hz, 2H, 3'(5')-H_{eq}], 2.13 [dd, $J = 3.6$ Hz, $J' = 13.2$ Hz, 2H, 2'(6')-H_{eq}], 3.58 (m, 1H, 4-H'), 3.73 (t, $J = 2.8$ Hz, 1H, 3''-H), 4.39 (m, 1H, 1'-H), 6.95 [d, $J = 8.8$ Hz, 2H, 3(5)-H], 7.94 [d, $J = 8.8$ Hz, 2H, 2(6)-H]. ¹³C NMR (100.6 MHz, CD₃OD) δ : 27.7 (CH), 31.1 [CH₂, C2'(6')], 31.7 [CH₂, C3'(5')], 31.8 (CH), 33.4 (CH), 33.6 (CH₂), 38.0 (CH), 38.2, 38.6, 38.74, 38.77, 38.93, 38.95, 39.0, 43.3 (CH), 48.9 (CH, C4'), 55.2 (CH, C3''), 76.0 (CH, C1'), 111.4 (C, C1), 116.1 [CH, C3(5)], 132.8 [CH, C2(6)], 159.9, (C, CO), 163.0 (C, C4), 170.3 (CO₂H). Elemental analysis: Calcd for C₂₈H₃₆N₂O₄·0.1 CH₂Cl₂: C 71.34, H 7.71, N 5.92. Found: C 71.36, H 7.85, N 5.70.

4.1.16. N-(1-Acetylpiperidin-4-yl)-1H-imidazole-1-carboxamide (23)—*N,N'*-carbonyldiimidazole (400 mg, 2.46 mmol) was suspended in anh. 1,2-dichloroethane (15 mL) under nitrogen. Then 1-acetyl-4-aminopiperidine (**22**) (250 mg, 1.76 mmol) was added and the reaction mixture was heated to 50 °C for 21 h. With an external ice bath, the mixture was cooled down for 30 min. The resulting solid was collected by filtration *in vacuo* and washed with 1,2-DCE (20 mL) affording **23** (312 mg, 75% yield) as a white solid, mp 191–193 °C. IR (ATR) ν : 3216, 3118, 3038, 2918, 2342, 2074, 1709, 1613, 1542, 1479, 1463, 1441, 1369, 1358, 1320, 1281, 1272, 1233, 1195, 1137, 1111, 1090, 1068, 1053, 1001, 984, 974, 916, 902, 859, 799, 748, 652 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ : 1.37 (complex signal, 2H, 3-H_{ax}, 5-H_{ax}), 1.99 (dm, $J = 12.8$ Hz, $J' = 4$ Hz, 1H) and 2.21 (dm, $J = 12.8$ Hz, $J' = 4$ Hz, 1H) (3'-H_{eq} and 5'-H_{eq}), 2.09 (s, 3H, COCH₃), 2.69 (ddd, $J = 13$ Hz, $J' = 2.6$ Hz, 1H) and 3.21 (ddd, $J = 13$ Hz, $J' = 2.6$ Hz, 1H) (2'-H_{ax} and 6'-H_{ax}), 3.86 (dm, $J = 13.6$ Hz, 1H) and 4.67 (dm, $J = 13.6$ Hz, 1H) (2'-H_{eq} and 6'-H_{eq}), 4.10 (m, 1H, 4'-H), 7.06 (dd, $J = 1.6$ Hz, $J' = 0.8$ Hz, 1H, 4-H), 7.29 (broad d, $J = 7.6$ Hz, 1H, NH), 7.60 (dd, $J = 1.6$ Hz, $J' = 1.2$ Hz, 1H, 5-H), 8.29 (dd, $J = 1.2$ Hz, $J' = 0.8$ Hz, 1H, 2-H). ¹³C NMR (100.6 MHz, CDCl₃) δ : 21.5 (CH₃, COCH₃), 31.4 and 33.1 (CH₂, C3' and C5'), 40.9 and 45.6 (CH₂, C2' and C6'), 48.2 (CH, C4'), 116.2 (CH, C5), 130.3 (CH, C4), 136.2 (CH, C2), 148.5 (C, NHCNH), 169.2 (C, COCH₃). MS (DIP), m/z (%); significant ions: 169 (10), 168 (100), 153 (19), 126 (53), 125 (31), 85 (19), 84 (42), 83 (20), 82 (23), 81 (21), 68 (98), 57 (40), 56 (56), 55 (16). HRMS-ESI⁺ m/z [$M+H$]⁺ calcd for [C₁₁H₁₆N₄O₂+H]⁺: 237.1346, found: 237.1345.

4.1.17. 1-(1-Acetylpiperidin-4-yl)-3-(3,7-dimethyl(tricyclo[3.3.0.0^{3,7}] octa-1-yl)urea (24)—In a round bottom flask equipped with a condenser apparatus and magnetic stirrer a solution of 3,7-dimethyltricyclo[3.3.0.0^{3,7}]octan-1-amine hydrochloride (**5·HCl**) (68 mg, 0.36 mmol) in chloroform (5 mL) was prepared, to which was added *N*-(1-acetylpiperidin-4-yl)-1*H*-imidazole-1-carboxamide (**23**) (172 mg, 0.73 mmol) followed by triethylamine (0.06 mL, 0.40 mmol). The solution was heated to 50 °C for 25 h, whereupon the reaction mixture was tempered to room temperature and evaporated *in vacuo* to dryness (384 mg). Purification by column chromatography (SiO₂, Dichloromethane/Methanol mixture) afforded **24** (90 mg, 77% yield) as a white solid, mp 165–167 °C. IR (ATR) ν : 3359, 3244, 2947, 2878, 2170, 2034, 1960, 1613, 1556, 1477, 1443, 1371, 1318, 1264, 1227, 1151, 1096, 1033, 978, 717, 639 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ : 1.11 [s, 6H, 3'(7')-CH₃], 1.22 [complex signal, 2H, 2(6)-H_a], 1.34 [dd, $J = 8.2$ Hz, $J' = 3.4$ Hz, 2H, 4'(6')-H_a], 1.54 [dd, $J = 7.4$ Hz, $J' = 3.4$ Hz, 2H, 2'(8')-H_a], 1.70 [dd, $J = 8.2$ Hz, $J' = 2.6$ Hz, 2H, 4'(6')-H_b], 1.75 [d, $J = 7.6$ Hz, 2H, 2'(8')-H_b], 1.90 and 2.03 (complex signal, 2H, 3-H_{ax} and 5-H_{ax}), 2.07 (s, 3H, COCH₃), 2.27 (t, $J = 2.6$ Hz, 1H, 5'-H), 2.75 (dt, $J = 14.0$ Hz,

$J' = 2.8$ Hz, 1H, 2- H_{ax} or 6- H_{ax}), 3.14 (dt, $J = 11.2$ Hz, $J' = 2.8$ Hz, 2H, 6- H_{ax} or 2- H_{ax}), 3.73 (broad d, $J = 13$ Hz, 2H, 6- H_{eq} or 2- H_{eq}), 3.83 (m, 1H, 4-H), 4.42 (broad d, $J = 13$ Hz, 2H, 2- H_{eq} or 6- H_{eq}), 4.79 (d, $J = 7.6$ Hz, 1H, 1-NH), 5.18 (broad s, 1H, 3-NH). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ : 16.5 [CH_3 , $C3'(7')$ - CH_3], 21.4 (CH_3 , $COCH_3$), 33.6 [CH_2 , $C3(5)$], 40.6 (CH_2 , $C2$), 44.7 (CH , $C5'$), 45.3 (CH_2 , $C6$), 46.1 [C , $C3'(7')$], 46.9 (CH , $C4$), 53.2 [CH_2 , $C4'(6')$], 57.6 [CH_2 , $C2'(8')$], 61.7 (C , $C1'$), 157.4 (C , CO urea), 169.0 (C , $COCH_3$). MS (DIP), m/z (%); significant ions: 278 (10), 277 (58), 263 (20), 178 (10), 169 (25), 151 (18), 150 (22), 148 (25), 143 (100), 136 (26), 135 (43), 134 (31), 127 (16), 126 (23), 125 (17), 123 (11), 122 (86), 121 (29), 119 (25), 110 (22), 109 (86), 108 (48), 96 (18), 95 (62), 94 (29), 93 (16), 91 (15), 84 (31), 83 (16), 82 (33), 80 (11), 79 (12), 77 (11), 67 (11), 57 (13), 56 (25), 55 (17). Elemental analysis: Calculated for $C_{18}H_{29}N_3O_2$: C 67.68, H 9.15, N 13.15. Calculated for $C_{18}H_{29}N_3O_2 \cdot 1.0 H_2O$: C 64.07, H 9.26, N 12.45. Found: C 64.00, H 9.31, N 12.40.

4.1.18. 1-(1-Acetylpiperidin-4-yl)-3-(tricyclo[3.3.1.0^{3,7}]non-3-yl)urea (25)—

Under argon atmosphere, *tricyclo[3.3.1.0^{3,7}]nonane-3-isocyanate* (**18**) (360 mg, 2.20 mmol) was dissolved in anh. DCM (10 mL). 1-acetyl-4-aminopiperidine (375 mg, 2.64 mmol) and Et_3N (445 mg, 4.40 mmol) were added. The reaction mixture was stirred at room temperature overnight. The solvent was removed *in vacuo* and the residue was dissolved in EtOAc and washed with 2 N HCl. The organics were dried over anh. Na_2SO_4 , filtered and evaporated *in vacuo* affording a white yellowish solid which was washed with acetone and EtOAc affording the urea **25** as a yellowish solid (240 mg, 36% yield), mp 164–165 °C. IR (ATR) ν : 638, 705, 783, 860, 904, 974, 992, 1059, 1139, 1230, 1269, 1318, 1361, 1429, 1555, 1620, 1659, 2351, 2919, 3328 cm^{-1} . 1H NMR (400 MHz, $CDCl_3$) δ : 1.16–1.29 (complex signal, 2H, 4- H_{ax} and 5- H_{ax}), 1.47–1.61 [complex signal, 4H, 9'- H_2 and 6'(8')- H_{ax}], 1.80 [dd, $J = 10.0$ Hz, $J' = 2.8$ Hz, 2H, 2'(4')- H_{ax}], 1.84–2.01 [complex signal, 6H, 6'(8')- H_{eq} , 2'(4')- H_{eq} , 4- H_{eq} and 5- H_{eq}], 2.07 (s, 3H, 8-H), 2.23 [broad singlet, 2H, 1'(5')-H], 2.34 (t, $J = 6.8$ Hz, 1H, 7'-H), 2.74 (dt, $J = 11.2$ Hz, $J' = 2.4$ Hz, 1H, 2- H_{ax} or 6- H_{ax}), 3.13 (dt, $J = 12$ Hz, $J' = 2.4$ Hz, 1H, 6- H_{ax} or 2- H_{ax}), 3.71–3.85 (complex signal, 2H, 2- H_{eq} or 6- H_{eq} and 4-H), 4.43 (d, $J = 13.6$ Hz, 1H, 6- H_{eq} or 2- H_{eq}), 4.89 (d, $J = 8.0$ Hz, 1H, NH), 5.11 (s, 1H, NH). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ : 21.4 (CH_3 , $COCH_3$), 32.5 (CH_2 , $C4$ or $C5$), 33.7 (CH_2 , $C5$ or $C4$), 34.8 (CH_2 , $C9'$), 37.3 [CH , $C1'(5')$], 40.7 (CH_2 , $C2$ or $C6$), 43.4 [CH_2 , $C6'(8')$], 43.7 (CH , $C7'$), 45.4 (CH_2 , $C6$ or $C2$), 46.7 (CH , $C4$), 49.30 and 49.32 (CH_2 , $C2'$ and $C4'$), 64.1 (C , $C3'$), 157.1 (CO , urea), 169.0 (CO , $COCH_3$). Elemental analysis: Calcd for $C_{17}H_{27}N_3O_2 \cdot 0.15C_5H_{12}$: C 67.42, H 9.18, N 13.29. Found: C 66.38, H 9.00, N 13.05.

4.1.19. 1-(1-Acetylpiperidin-4-yl)-3-(diamant-3-yl)urea (26)—

Diamantane-3-isocyanate (**20**) (155 mg, 0.67 mmol) was dissolved in DCM (3 mL) and 1-acetyl-4-aminopiperidine (115 mg, 0.811 mmol) dissolved in DCM (2 mL) was added. The mixture was stirred at room temperature overnight. Evaporation of the solvent gave a white solid (272 mg). Column chromatography (Dichloromethane/Methanol mixtures) gave the urea **26** (160 mg, 65% yield) as a white solid, mp 230–231 °C. IR (ATR) ν : 669, 727, 770, 808, 862, 917, 989, 1047, 1136, 1240, 1319, 1364, 1453, 1560, 1629, 1794, 1855, 1893, 1944, 1977, 2051, 2102, 2153, 2209, 2270, 2352, 2418, 2545, 2596, 2734, 2877, 3020, 3071, 3275,

3316, 3494, 3566, 3688 cm^{-1} . ^1H NMR (400 MHz, CD_3OD) δ : 1.32 [complex signal, 2H, 3(5)- H_{ax}], 1.66–1.82 (complex signal, 16H, diamantane-H), 1.85–1.98 (complex signal, 4H, 3- H_{eq} , 5- H_{eq} , 2 diamantane-H), 2.10 (s, 3H, COCH_3), 2.91 (dt, $J = 11.2$ Hz, $J' = 2.8$ Hz, 1H, 2- H_{ax} or 6- H_{ax}), 3.25 (dt, $J = 11.2$ Hz, $J' = 3.2$ Hz, 2H, 6- H_{ax} or 2- H_{ax}), 3.71–3.78 (complex signal, 2H, 4-H and 1'-H), 3.85 (dt, $J = 14$ Hz, $J' = 2.4$ Hz, 2H, 6- H_{eq} or 2- H_{eq}), 4.29 (dt, $J = 13$ Hz, $J' = 2.8$ Hz, 2H, 2- H_{eq} or 6- H_{eq}). ^{13}C NMR (100.6 MHz, CD_3OD) δ : 21.2 (CH_3 , COCH_3), 27.7 (CH), 31.8 (CH), 33.3 (CH_2 , C3 or C5), 33.4 (CH_2), 33.6 (CH_2), 34.1 (CH_2 , C5 or C3), 38.0, 38.1, 38.6, 38.7 (2 carbon), 38.91, 38.94 and 39.0 (3 CH_2 and 5 CH, diamantane signals), 41.6 (CH_2 , C2 or C6), 43.3 (CH, C4'), 46.3 (CH_2 , C6 or C2), 47.7 (CH, C4), 55.9 (CH, C3'), 159.7 (C, CO urea), 171.5 (C, COCH_3). HRMS-ESI⁺ m/z [$\text{M}+\text{H}$]⁺ calcd for $[\text{C}_{22}\text{H}_{33}\text{N}_3\text{O}_2+\text{H}]^+$: 372.2646, found: 372.2644.

4.1.20. Diamantane-4-isocyanate (27)—Diamantane-4-amine hydrochloride (**9·HCl**) (110 mg, 0.458 mmol) was suspended in DCM (2 mL) and aq. NaHCO_3 was added, followed by triphosgene (68 mg, 0.23 mmol). The biphasic mixture was stirred at room temperature for 30 min. The two phases were separated and the organic layer was washed with brine. The organics were dried over anh. Na_2SO_4 , filtered and evaporated until 1 mL. The solution of isocyanate (**27**) in DCM was used in the next step without further purification.

4.1.21. 1-(1-Acetylpiperidin-4-yl)-3-(diamant-4-yl)urea (28)—To the solution of diamantane-4-isocyanate (**27**) (105 mg, 0.46 mmol) in DCM (1 mL) from the previous step is added 1-acetyl-4-aminopiperidine hydrochloride (**22·HCl**) (98 mg, 0.55 mmol) and DCM (1 mL), followed by Et_3N . The mixture was stirred at room temperature overnight. DCM was added to the mixture and it was washed with 2 N HCl (30 mL). The organics were dried over anh. Na_2SO_4 , filtered and evaporated to obtain a residue (48 mg). Column chromatography (Dichloromethane/Methanol mixture) gave the desired urea (**28**) (33 mg, 21% overall yield) as a beige solid, mp 195–196 °C. IR (ATR): ν : 3364, 2906, 2881, 2847, 1686, 1601, 1550, 1480, 1462, 1444, 1429, 1374, 1348, 1322, 1305, 1267, 1221, 1138, 1105, 1048, 1002, 986, 976, 918, 613, 597, 576 cm^{-1} . ^1H NMR (400 MHz, CDCl_3) δ : 1.19 (complex signal, 2H, 3- H_{ax} and 5- H_{ax}), 1.69–1.78 (complex signal, 10H, diamantane-H), 1.83–1.94 (complex signal, 11H, 3- H_{eq} , 5- H_{eq} , 9 diamantane-H), 2.08 (s, 3H, COCH_3), 2.73 (dt, $J = 11.6$ Hz, $J' = 3.2$ Hz, 1 H, 2- H_{ax} or 6- H_{ax}), 3.13 (dt, $J = 11.6$ Hz, $J' = 3.2$ Hz, 2H, 6- H_{ax} or 2- H_{ax}), 3.71–3.85 (complex signal, 2H, 4-H and 6- H_{eq} or 2- H_{eq}), 4.36 (broad s, NH, urea), 4.42–4.50 (complex signal, 2H, 2- H_{eq} or 6- H_{eq} and NH). ^{13}C NMR (100.6 MHz, CDCl_3) δ : 21.4 (CH_3 , COCH_3), 25.6 (CH, C9'), 32.4 (CH_2 , C3 or C5), 33.6 (CH_2 , C5 or C3), 36.6 (CH), 37.4 (CH_2), 38.7 (CH), 40.7 (CH_2 , C2 or C6), 43.0 (CH_2), 45.4 (CH_2 , C6 or C2), 46.9 (CH, C4), 49.8 (C, C4'), 156.6 (C, CO urea), 169.0 (C, COCH_3). HRMS-ESI⁺ m/z [$\text{M}+\text{H}$]⁺ calcd for $[\text{C}_{22}\text{H}_{33}\text{N}_3\text{O}_2+\text{H}]^+$: 372.2646, found: 372.2657.

4.2. Solubility

A 10 mM stock solution of the compound was serially diluted in 100% DMSO and 1 μL of this solution was added to a 384-well UV-transparent plate (Greiner) containing 99 μL of PBS. The plate was incubated at 37 °C for 2 h and the light scattering was measured in a

Nephelostar Plus reader (*BMG LABTECH*). The data was fitted to a segmented linear regression for measuring the compound solubility.

4.3. Microsomal stability

The human microsomes employed were purchased from Tebu-Xenotech. The compound was incubated at 37 °C with the microsomes in a 50 mM phosphate buffer (pH = 7.4) containing 3 mM MgCl₂, 1 mM NADP, 10 mM glucose-6-phosphate and 1 U/mL glucose-6-phosphate-dehydrogenase. Samples (75 µL) were taken from each well at 0, 10, 20, 40 and 60 min and transferred to a plate containing 4 °C 75 µL acetonitrile and 30 µL of 0.5% formic acid in water were added for improving the chromatographic conditions. The plate was centrifuged (46,000g, 30 min) and supernatants were taken and analyzed in a UPLC-MS/MS (Xevo-TQD, Waters) by employing a BEH C18 column and an isocratic gradient of 0.1% formic acid in water: 0.1% formic acid acetonitrile (60:40). The metabolic stability of the compounds was calculated from the logarithm of the remaining compounds at each of the time points studied.

4.4. Permeability

The Caco-2 cells were cultured to confluency, trypsinized and seeded onto a filter transwell inserted at a density of ~10,000 cells/well in DMEM cell culture medium. Confluent Caco-2 cells were subcultured at passages 58–62 and grown in a humidified atmosphere of 5% CO₂ at 37 °C. Following an overnight attachment period (24 h after seeding), the cell medium was replaced with fresh medium in both the apical and basolateral compartments every other day. The cell monolayers were used for transport studies 21 days post seeding. The monolayer integrity was checked by measuring the transepithelial electrical resistance (TEER) obtaining values ~500 Ω/cm². On the day of the study, after the TEER measurement, the medium was removed and the cells were washed twice with pre-warmed (37 °C) Hank's Balanced Salt Solution (HBSS) buffer to remove traces of medium. Stock solutions were made in dimethyl sulfoxide (DMSO), and further diluted in HBSS (final DMSO concentration 1%). Each compound and reference compounds (Colchicine, E3S) were all tested at a final concentration of 10 µM. For A → B directional transport, the donor working solution was added to the apical (A) compartment and the transport media as receiver working solution was added to the basolateral (B) compartment. For B → A directional transport, the donor working was added to the basolateral (B) compartment and transport media as receiver working solution was added to the apical (A) compartment. The cells were incubated at 37 °C for 2 h with gentle stirring.

At the end of the incubation, samples were taken from both donor and receiver compartments and transferred into 384-well plates and analyzed by UPLC-MS/MS. The detection was performed using an ACQUITY UPLC/Xevo TQD System. After the assay, Lucifer yellow was used to further validate the cell monolayer integrity, cells were incubated with LY 10 µM in HBSS for 1 h at 37 °C, obtaining permeability (Papp) values for LY of 10 nm/s confirming the well-established Caco-2 monolayer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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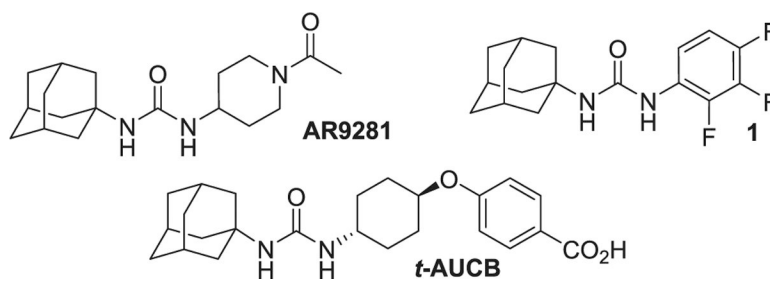


Fig. 1.
Adamantyl-based sEH inhibitors.

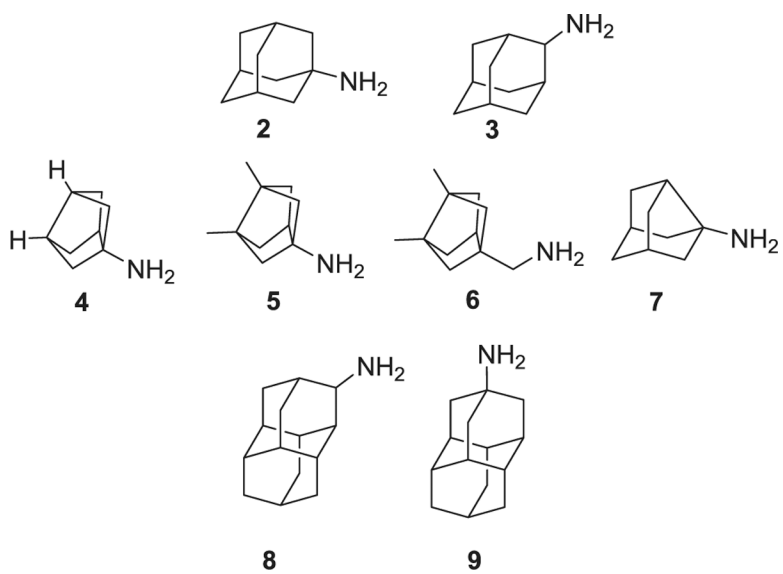
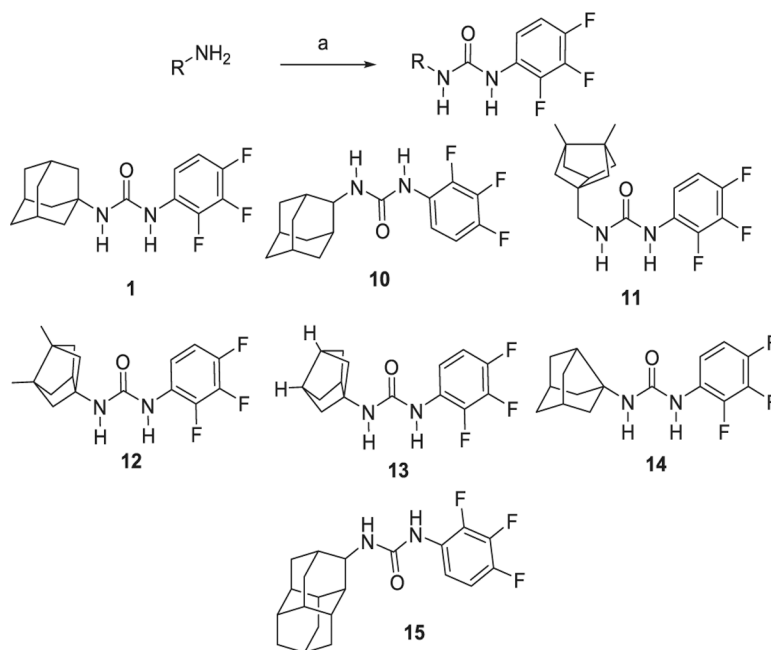
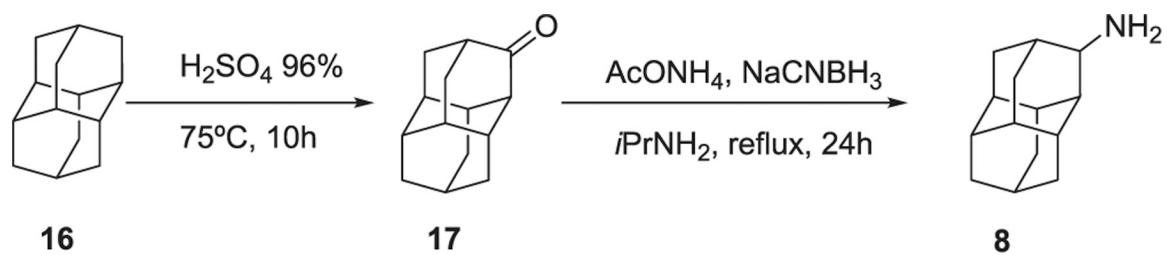


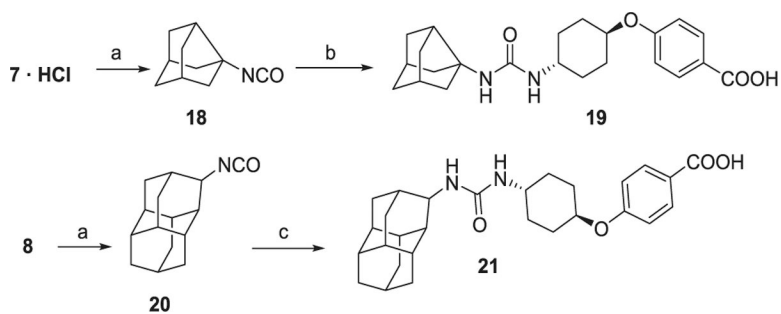
Fig. 2.
Polycyclic amines used in this study.

**Scheme 1.**

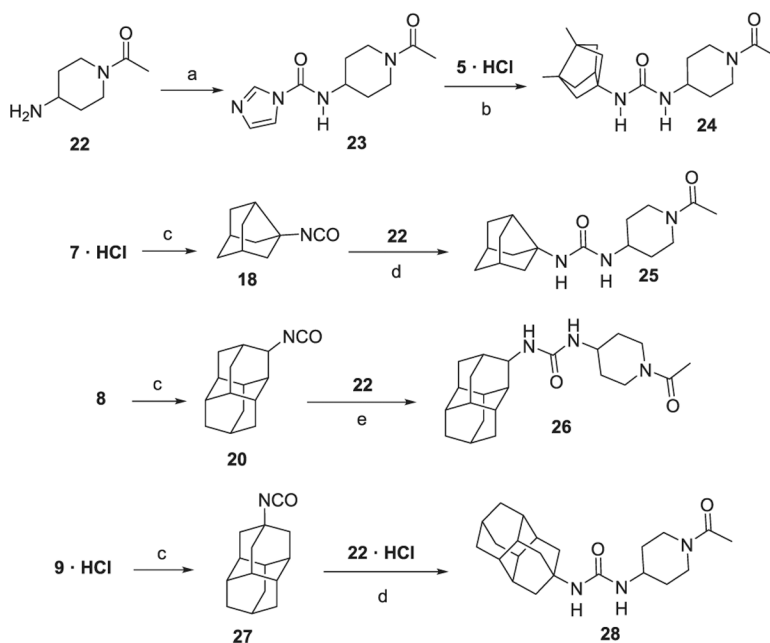
Synthesis of analogs containing a trifluorophenyl unit. Reagents and conditions: (a) 2,3,4-trifluorophenylisocyanate, Et_3N , anh. DCM, overnight.



Scheme 2.
Synthesis of diamantanamine **8**.

**Scheme 3.**

Synthesis of *t*-AUCB analogs. Reagents and conditions: (a) triphosgene, NaHCO₃, DCM, 30 min; (b) *t*-4-[(4-aminocyclohexyl)oxy]benzoic acid, Et₃N, DCM, 30 °C, overnight; (c) *t*-4-[(4-aminocyclohexyl)oxy]benzoic acid, Et₃N, DMF, 50 °C, 3 days.

**Scheme 4.**

Synthesis of AR9281 analogs. Reagents and conditions: (a) 1,1'-carbonyldiimidazole, 1,2-dichloroethane, 50 °C, 21 h; (b) Et₃N, CHCl₃, 50 °C, 24 h; (c) triphosgene, Et₃N, DCM, 30 min; (d) DCM, Et₃N, overnight; (e) DCM, overnight.

Table 1

Inhibitory activities against the human and murine sEH.

Entry	Compound	IC ₅₀ (nM) human sEH ^a	IC ₅₀ (nM) murine sEH ^a
1	1	0.4	ND ^b
2	10	0.4	ND
3	11	0.4	ND
4	12	0.5	ND
5	13	3.2	ND
6	14	3.3	ND
7	15	8.0	ND
8	<i>t</i> -AUCB	0.5	ND
9	19	8.6	ND
10	21	0.5	ND
11	AR9281	8.0	3.0
12	24	6.5	3.3
13	25	21.7	ND
14	26	3.4	5.0
15	28	7.2	1.8

^aReported IC₅₀ values are the average of three replicates. The fluorescent assay as performed here has a standard error between 10 and 20% suggesting that differences of two-fold or greater are significant. Because of the limitations of the assay it is difficult to distinguish among potencies < 0.5 nM.⁴¹

^bND: not determined.

Table 2

Solubility and microsomal stability of the new compounds.

Entry	Compound	Microsomal stability ^a	Solubility (μM) ^b	Lipophilicity ^c
1	1	34.3%	57	4.1
2	10	19.4%	ND ^d	3.9
3	12	30.0%	66	4.1
4	13	41.9%	82	3.5
5	14	30.2%	65	3.8
6	15	0.0%	18	4.5
7	<i>t</i> -AUCB	93.5%	60	3.7
8	19	64.6%	76	3.4
9	21	14.6%	7	3.9
10	AR9281	80.1%	> 100	2.1
11	24	59.3%	> 100	2.2
12	25	88.7%	ND	1.8
13	26	0.0%	86	2.5
14	28	2.6%	85	2.6

^aPercentage of remaining compound after 60 min of incubation with human microsomes obtained from Tebu-Xenotech in the presence of NADP at 37 °C. Metabolism of testosterone was used as a positive control for metabolism (22.4% remaining compound).

^bSolubility in a 1% DMSO: 99% PBS buffer solution, see experimental section for details.

^cLipophilicity refers to the consensus log $P_{0/w}$ value calculated using the SwissADME program⁵⁰ for five predictive log $P_{0/w}$ models (iLOGP, XLOGP3, WLOGP, MLOGP and SILICOS-IT).

^dND: not determined.

Table 3

Permeability in the Caco-2 cell line of selected sEH inhibitors.

Entry	Compound	P _{app} A→B (nm/s)	P _{app} B→A (nm/s)	ER ^a
1	1	11.8 ± 1.3	1.8 ± 0	0.2 ± 0
2	10	2.3 ± 0.1	7.3 ± 0.3	3.2 ± 0.2
3	15	16.2 ± 2.4	5.4 ± 0.1	0.3 ± 0.1
4	<i>t</i> -AUCB	1.9 ± 0.2	210.3 ± 53.7	111 ± 34.5
5	21	3.1 ± 0.3	67.5 ± 2.4	22.2 ± 1.9
6	24	159.2 ± 2.8	180.2 ± 31.6	1.1 ± 0.2
7	26	156.2 ± 13.6	146.9 ± 19.6	1.0 ± 0.2
8	28	208.3 ± 20.2	191.6 ± 38.5	0.9 ± 0.1

^aThe efflux ratio was calculated as $ER = (P_{app} B \rightarrow A) / (P_{app} A \rightarrow B)$. See the experimental section for further details. Permeability of estrone-3-sulfate and colchicine were used as references.