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Effects of adamantane alterations on soluble epoxide hydrolase inhibition potency, physical properties and metabolic stability

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Abstract

Adamantyl groups are widely used in medicinal chemistry. However, metabolism limits their usage. Herein, we report the first systematic study of adamantyl ureas and diureas bearing substituents in bridgehead positions of adamantane and/or spacers between urea groups and adamantane group, and tested their effects on soluble epoxide hydrolase inhibitor potency and metabolic stability. Interestingly, the effect on activity against human and murine sEH varied in opposite ways with each new methyl group introduced into the molecule. Compounds with three methyl substituents in adamantane were very poor inhibitors of murine sEH while still very potent against human sEH. In addition, diureas with terminal groups bigger than sEH catalytic tunnel diameter were still good inhibitors suggesting that the active site of sEH opens to capture the substrate or inhibitor molecule. The introduction of one methyl group leads to 4-fold increase in potency without noticeable loss of metabolic stability compared to the unsubstituted adamantane. However, introduction of two or three methyl groups leads to 8-fold and 98-fold decrease in stability in human liver microsomes for the corresponding compounds.

Graphical abstract

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Supplementary Material

Supplementary data containing detailed NMR spectra on synthesized compounds can be found in online version.

Keywords

soluble epoxide hydrolase; inhibitor; adamantane; isocyanate; urea

1. Introduction

The mammalian soluble epoxide hydrolase (sEH, E.C. 3.3.2.10) is involved in the metabolism of epoxy-fatty acids to vicinal diols through a catalytic addition of a water molecule.[1, 2] Endogenous substrates for the sEH include epoxides of arachidonic acid (epoxyeicosatrienoic acids, or EETs), docosahexaenoic acid (EpDPEs), and other epoxides of fatty acids (EpFAs).[3, 4] EETs and other EpFAs are important lipid mediators that have key roles in blood pressure regulation by exercising vasodilatory effects through the activation of the Ca^{2+}-activated K^+ channels in endothelial cells, which are beneficial in many renal and cardiovascular diseases.[5, 6] Furthermore, the EETs also have anti-inflammatory and analgesic properties.[7] Recently, EETs but not EpDPEs have been reported to promote angiogenesis in humans possibly due to their cyclooxygenase metabolites.[8, 9] Conversion of EET to DHET by sEH produces a molecule that is more polar, readily conjugated and removed from the site of action. The inhibition of sEH *in vivo* by highly selective inhibitors results in an increase in the concentration of EETs, EpDPEs and other EpFAs and is accompanied by a reduction in angiotensin driven blood pressure, but also reduction of inflammation and pain, thereby suggesting that sEH is a promising target for the treatment of hypertension, inflammatory diseases and pain.[10–12]

Early on, small *N,N'*-disubstituted symmetric ureas, such as 1,3-dicyclohexyl urea (DCU) or 1,3-diadamantyl urea (DAU), were found to be very potent inhibitors of sEH.[13–17] However, such compounds have poor solubility in many solvents. To improve solubility, asymmetric ureas with a flexible side chain, such as AUDA (12-(3-adamantylureido)-dodecanoic acid), were developed. While this class of sEH inhibitor shows biological effects when tested *in vivo*, they are rapidly metabolized, limiting their utility.[18, 19] Therefore, to improve the metabolic stability, a third class of conformationally restricted inhibitors, such as TPPU 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea[31] or *t*-AUCB (*trans*-4-((4-(3-adamantylureido)-cyclohexyl)oxy)-benzoic acid)[19], were designed. This latest series includes very potent and more metabolically stable sEH inhibitors that permit *in vivo* studies. However, many of these compounds have in general poor water solubility and have a high melting point, making formulation difficult. Finally, a promising candidate produced by GlaxoSmithKlein, GSK2256294, has gone through phase I trials and phase II vascular trial and trial for the treatment of chronic obstructive pulmonary disorder (COPD) have been announced.[20]

Interestingly, existing adamantyl sEH inhibitors contain an unsubstituted adamantane in their structure. Compounds containing substituents in the adamantane part have never been systematically tested as sEH inhibitors. Also compounds having spacers between adamantane part and urea group were tested sporadically. Herein, we are testing the hypothesis that alterations in the adamantane part could regulate metabolic stability and

water solubility of such inhibitors while maintaining good potency toward the target enzyme.

2. Results and Discussion

2.1 Synthesis of isocyanates

A convenient and efficient way used to synthesize unsymmetric ureas is the reaction of an isocyanate with an amine. Therefore, using synthetic scheme 1, we prepared a series of nine adamantyl containing isocyanates **1–9**. The well-known Curtius rearrangement reaction was improved and applied to the corresponding acid chlorides.[21] An improvement in the reaction led to the corresponding isocyanates through the domino reaction directly from their acid chlorides without isolation of the azide intermediates, which are potentially explosive.[22] Adamantyl carboxylic acids with one to three methyl, ethyl or chlorine substituents in bridgehead positions as well as adamantyl acetic acids were chosen as starting materials for preparation of substituted adamantyl-isocyanates.

2.2 Synthesis and evaluation of symmetrical diadamantyl ureas

The main route of *in vivo* metabolism of adamantyl containing sEH inhibitors is hydroxylation of the bridge and bridgehead positions of adamantane shell.[23] Thus we hypothesized that introduction of substituents into the bridgehead positions of adamantane will help to directly protect the bridgehead position and increased polarity and steric hindrance will make it more difficult for P450's to oxidize the bridge positions as well. Another benefit obtained from the substituents is increased solubility for oxygenated compounds and lower melting points. Adamantane melting point is 269.6–270.8 °C,[24] 1-methyladamantane – 100–101 °C,[25] 1,3-dimethyladamantane and 1,3,5-trimethyladamantane are liquid compounds with boiling points of 203.5 and 210.3 °C respectively.[26] The same effect should be applied to the adamantane derivatives. Introduction of spacers between adamantane and functional groups also decreases melting points. For example, 1-isocyanatoadamantane melting point is 143–144 °C[27] while 1-isocyanatomethyladamantane is a liquid compound. As predicted, compounds **1–7** appear as liquids and compound **9** has very low melting point of 29–30 °C.

It should be noted that introduction of HBDs or HBAs into adamantyl part of sEHI could lead to the significant loss of inhibitory activity. It was reported that bridgehead- and bridge-hydroxy analogs of 1-adamantan-1-yl-3-(5-(2-(2-ethoxyethoxy)ethoxy)pentyl)urea possess 62- and 34-fold lower activity.[36] To improve water solubility of corresponding ureas compound **9**, which contains a chlorine atom on a bridgehead position, was synthesized. Compound **9** has a lower clogP (1.53) compared 1-isocyanatoadamantane (2.99) and as it will be shown below, allows us to develop significantly more water soluble sEH inhibitors. With the new adamantyl containing isocyanates, we developed several series of disubstituted ureas, and diureas.

Symmetric ureas **1–4a, 9a** were prepared (Scheme 2) as basic reference compounds. Symmetric ureas show high inhibitory activity and low water solubility (Table 1), and thus, such an impurity can noticeably affect properties of corresponding compounds.

Introduction of alkyl groups into bridgehead positions of adamantane surprisingly led to the 2–3 fold increase in water solubility of ureas **1–4a** (Table 1). This could be explained by the decrease of intermolecular interaction between urea groups of such molecules due to the more bulky nature and less homogenous surface of substituted adamantane radicals. The difference in melting points corresponds to the difference between adamantane and its homologues. 1-Methyl substituent slightly lowers the melting point, a second methyl lowers it more and the melting point of an adamantane with three methyl substituents tends to rise. We found that inhibitory activity had no obvious correspondence to the physical properties, and for compound **1a** the enzyme inhibition is 2-fold higher than basic diadamantyl urea (**DAU**, Table 1). Compound **2a** and **3a** have the same IC_{50} and **4a** has a 4-fold lower activity. Introduction of ethyl group (**4a**, Table 1) if compared to adamantane with two methyl groups gives lower water solubility (which is still 2-fold better than **DAU**) and melting point along with 4-fold decrease in inhibitory activity on the recombinant human sEH.

Interestingly, introduction of chlorine into bridgehead position of adamantane compound **9a** increased the activity (2-fold) but more importantly led to the more than 2-fold decrease in melting point and around 20-fold increase in water solubility. This makes isocyanate **9** a very promising reactant for the synthesis of urea-type sEH inhibitors.

2.3 Inhibitory activity of 1471 and 1163 homologues

The next step was to investigate how adamantyl substituents affect the properties of known adamantyl containing sEH inhibitors. Compounds **1471** and **1163** were used for reference as two of the most promising among inhibitors bearing adamantane fragment and compound **1770** as their non-adamantane rference. *trans*-4-((4-aminocyclohexyl)oxy)benzoic acid was used as a starting amine to synthesize compounds **1–5b, 8b** and **9b**.

While, compared to the unsubstituted adamantane, the introduction of one and two methyl groups led to the slightly increased water solubility and decreased melting points, the presence of a third methyl substitution on the adamantane **3b** yields physical properties similar to the ones of the unsubstituted compound (Table 1). Introduction of a methylene spacer between the adamantane and the urea group in the compound **5b** gave the most significant decrease (70 °C) in melting point, probably due to the increased flexibility, accompanied with a 2-fold decrease in water solubility. Interestingly, compound **8b** showed more than a 6-fold increase in water solubility while maintaining the same melting point. In terms of inhibitory potency, the same pattern is observed for the two series: **1471, 1–3b** and **DAU 1–3a** studied (Table 1). Compounds with one methyl group yield the highest inhibitory potency (lowest IC_{50}); the presence of a second methyl gives a 2-fold decrease in human sEH inhibitory activity yielding IC_{50}s similar to the unsubstituted compounds, and the potency is decreased even more by the addition of a third methyl. Interestingly, the presence of the chloride atom yields a significantly higher water solubility (5–10-folds). Surprisingly, the analogs of compound **1163** (Table 1) did not follow the pattern described above (less difference in potency and water solubility). Compounds **2c** and **4c** possess human sEH inhibitory activity and water solubility similar to the **1163** but have moderately lower melting points. But compounds **5c** and **7b** differing only by the methylene spacer show 4-fold and 2-fold higher activity respectively. However, while existence of such spacer leads to

certain melting point decrease it also decreases the water solubility up to 1.5-fold. Thus, introduction of methylene spacer between urea group and adamantane (or other bulky group) part could be used to increase sEH inhibitory activity for compounds which already possess moderate water solubility.

2.4 Synthesis and inhibitory activity of non-symmetric diadamantyl ureas

Next, to enlarge our dataset on the effects of substitution on the adamantane, a variety of non-symmetric diadamantyl ureas were synthesized (Scheme 5). Isocyanates **2–5, 7–9** were introduced into the reaction with adamantyl amine in order to synthesize this diverse series of ureas. In this series a 1,4-phenylene spacer between adamantane and urea group was used as well as spacers containing a secondary amino group (**2e, 2f, 5e, 5f**).

Compared to **2a** or **5a**, the introduction of spacers containing secondary amino groups (NH-$(CH_2)_2$ and NH-$(CH_2)_3$) in **2e, 2f, 5e** and **5f** leads to a marked decrease in activity accompanied by a significant increase in water solubility (Table 2). In the **5a** series, replacement of one methylene spacer by 1,4-phenylene (**5g**) gives a roughly 2-fold lower inhibitory potency while maintaining melting point and water solubility. Further elongation of spacer from one to two methylene groups (**5d**) leads to further gain in potency and small decrease of melting point without a noticeable change in water solubility. Separately, introduction of larger branched spacers (**2d, 3d, 3g**, and **7d**) usually led to a severe decrease in inhibitory activity and water solubility and increase in melting point when compared to the corresponding compounds with one methylene spacer. Attaching the urea on position 2 of the adamantane yielded compounds with higher melting point than the corresponding ureas at the 1-position. However, water solubility of both types of compounds is similar. As expected, changing one adamantane by 1,3,5-triazaadamantan-7-amine (**2h, 4d** and **7g**) increased significantly the water solubility; however, it was accompanied by over 25-fold decrease in inhibitory activity rending such alteration impractical. Finally, compound **8c** shows that adamantanone could be very promising alternative to adamantane since **8c** possess high activity of 0.4 nM while having approximately 10-fold higher water solubility than most of diadamantyl ureas.

2.5 Inhibitory activity of diadamantyl diureas

It was previously shown[32] that symmetric diadamantyl diureas are potent sEH inhibitors with low metabolic stability and higher water solubility than corresponding symmetric diadamantyl ureas. High inhibitory activity of diureas was thought to result from additional hydrogen bond formation between oxygen atom of the second urea group and Ser 374 in sEH active site pocket.[32] This assumption was later investigated by the thorough docking of various diureas.[33] Another possibility is the presence of two reaction centers in one symmetric molecule which removes the need of proper orientation prior to the entering of sEH domain. In this case it is interesting to test the effect of substituents in adamantane, new spacers between adamantane and urea group and new linkers between urea groups on the properties of corresponding diureas. Two approaches were used to synthesize diadamantyl diureas. First approach (Scheme 6) is based on the reaction of adamantyl isocyanates **2, 4–6, 9** with diamines while the second (Scheme 7) is the opposite with adamantyl amines reacting with diisocyanates.

We decided to find if there is a minimal length of the linker between two urea groups which allows them to reach Ser 374 or CSN 384 and thus increase the activity. To optimize the length of the linker, compounds with 2 to 10 methylene groups without branching **5j–q** and **9d–k** were prepared. All of these compounds possess approximately the same IC_{50} except of compound **5j** and **9d**, which are much less active, suggesting that three methylene groups are the minimum for the best interaction with sEH domain. Docking of compounds **5l, 5n** and **5p** shows that there is enough space at the sEH domain even for C_8 aliphatic chain between two urea groups (Fig. 1).

Spacers between adamantane and the urea group in diureas usually follow the same pattern as observed with simple ureas (**DAU** and **5a, 3e** and **3f**), a methylene spacer (**2i 5l, 5n, 5p, 6c**) leads to an increase in inhibitory activity (Table 3) accompanied by the better water solubility and lower (around 20–30°C) melting points which probably resulted from increased flexibility around the adamantane. Further increase of spacer to 1,2-ethylenes in compounds **10–12c** results in a 2-fold decrease of activity while maintaining water solubility and melting points on the same level. Separately, a phenylene group introduced either between adamantane and urea group or between two urea groups (Table 3) leads to the significant decrease in human sEH inhibitory activity. In case of compounds **2k, 5r, 5s, 9l** low activity could be the result of rotation restrictions. As for compounds **10–12d** it could be attributed by the lack of space in the hydrophobic pocket of sEH and 6-fold increase of activity along with the extension of linker from four methylene groups (**10d**) to eight (**12d**) is a result of better ability of this diurea to bind to the Ser 374. Diureas **10e, 11h** and **12f** represent the biggest branched aliphatic spacer between adamantane and urea group. This 1,2-pentylene spacer apparently is too big or poorly orientated to fit into the sEH pocket. Compound **11f** with a 1,2-butylene spacer has good inhibitory activity suggesting that it is the biggest branched spacer which allows to maintain a high level inhibitory activity. Finally, introduction of oxygen containing spacer $–O–CH_2–CH_2–CH_2–$ in compounds **11g** and **12e** although it leads to $IC_{50} < 10$ nM it have no positive impact on water solubility.

To obtain additional data on the benefit of substitution on the adamantane but away from the urea center, a series of compound containing 1,3,3-trisubstituted urea groups were developed (Scheme 8). Compounds **2l, 3h** and **5t** contain one disubstituted and one trisubstituted urea groups while compounds **5u** and **10f** have two trisubstituted urea groups.

Compounds **2l, 3h** and **5t** have good inhibition potency due to the presence of one disubstituted urea group in its structure however their melting points are very high. The lower activity of **5u** and **10f** probably resulted from a less than optimal orientation in the active pocket.

2.6 Comparison of activity against human, murine and rat sEH

While human health application is the ultimate objective of this work, research work is conducted first in rodent animal models. Thus, to test if our observations above with the human sEH could extend to sEH enzymes from other species, a series of 18 compounds which could visualize the impact of methyl substituents in adamantane were selected and

tested against the human, rat and mouse sEHs (Table 5). Compounds **10–12c** were also tested as mouse sEH inhibitors to test the effect of additional methylene groups in linkers

Using the Spearman's ranking coefficient calculation, we observed a reasonable similarity in the pattern of inhibition between the human and the rat sEH (rho = 0.69) as well as with the mouse sEH (rho = 0.81). It has been reported that steric parameters have stronger effects on the potency of inhibitors against murine sEH rather than the human sEH.[34] Beside comparable ranking, a similar steric effect was observed here, as the number of substitutions increased the effect on decreasing the inhibitory potency against the murine sEH was much stronger than against the human and rat sEH.

The significant difference in potency between the human and mouse sEH for compounds with trimethyladamantane moiety revealed by the fluorescent assay was confirmed by radioactive assay with [^{3}H]*trans*-diphenyl-propene oxide (*t*-DPPO) as a substrate.[35] This completely different assay shows the same differences in potency against human, mouse and rat enzymes. This gives us reason to believe that all other IC_{50} acquired by fluorescent method are reliable.

To understand the difference of selectivity between the human and murine enzyme, we compared the protein structures (Figure 2). When overlying the C-terminal active site of both enzymes, the only significant difference is that ILE 363 in the human sEH is substituted by MET 361 in the mouse sEH, which perturbs inside the catalytic tunnel and limits the size of the ligand that can fit into the murine enzyme.[33] In addition, if the catalytic site of the sEH is a true tunnel, this residue can stop a ligand too big to reach catalytic residues. To test this later hypothesis, we used the available crystal structures to measure the diameter of the catalytic tunnel. Then, compounds **13a-c** bearing terminal substituents bigger than sEH tunnel size evaluated by the X-Ray crystallography were synthesized (Scheme 9).

If the sEH binding site is a stable tunnel, such compounds would be unable to get into it and its IC_{50} should be extremely high. However, IC_{50}s for compounds **13a-c** are below 1 μM for human and rat and below 4 μM for mouse sEH (Table 7). The fact that compounds with such large terminal groups possess such good inhibition potency, for example **13b** has an IC_{50} < 100 nM human sEH, supports the ability of sEH to open in active site to capture the molecule of substrate or inhibitor.

2.7 Metabolic stability study

Finally, we tested the effect of methyl substituents in bridgehead positions of adamantane (**1b, 2b** and **3b**) on metabolic stability (Figure 3). The intrinsic clearance was then calculated and compared to the unsubstituted compound **1471** (Table 8).

Because hydroxylation of bridge and bridgehead positions in adamantane is the main P450 dependent metabolism,[36] and because bridgehead positions are also the most sensitive for the chemical reactivity, one would expect that the introduction of methyl groups into bridgehead position should protect the inhibitor from oxidation and reactivity. Surprisingly, the results obtained (Table 8) were the inverse of our initial assumption. There is almost no difference between **1471** and compound **1b** bearing one methyl group. Introduction of a

second methyl groups leads to 8-fold decrease of stability for compound **2b**. Finally compound **3b** in which all of the bridgehead carbons are tertiary and linked with methyl substituents is 98-fold less stable than parental **1471** (Figure 3). These results suggest a particular sensibility of the bridgehead methyl to enzymatic oxidation. This agrees with the metabolism of memantine (1-amino-3,5-dimethyladamantane),[38] for which the main metabolic pathway is the hydroxylation of adamantane cage and methyl substituents while the amino group remains intact. Not only are the methyl groups likely to be good sites for hydroxylation, but in general as a compound overall becomes more hydrophobic its sensitivity to oxidation by cytochrome P450 enzyme increases.

3. Conclusions

In conclusion, substituted adamantyl ureas were synthesized via new isocyanates. Using sEH as a model system, we observed that the substitutions influence greatly the potency of the resulting ureas and diureas, as well as physical properties and metabolic stability. Interestingly, the best compromise is the presence of only one substitution, with either a methyl or chloride, on the bridgehead of the adamantane. In addition, the presence of a methylene between the adamantane and the urea function has positive effect on potency and physical properties. These results have implication for the design of further sEH inhibitors but also toward other targets because of the ubiquitous use of adamantane in drug design.

4. Experimental

4.1 Chemistry

4.1.1 General experimental details—All reagents and solvent were purchased from commercial suppliers and were used directly without further purifications unless otherwise specified. All syntheses were carried out in a dry nitrogen atmosphere unless otherwise specified.

^{1}H NMR spectra were recorded on a Bruker DRX-500 spectrometer with deuterated chloroform ($CDCl_3$; δ = 7.24 ppm) or deuterated dimethyl sulfoxide (DMSO-d_6) containing TMS an internal standard. ^{13}C NMR spectra were recorded on a Bruker DRX-500 spectrometer at 125 MHz. Mass spectra were registered on Agilent GC 5975/MSD 7820 or Finnigan MAT INCOS 50. The purity of the inhibitors reported in this manuscript was determined by elemental analysis using Perkin-Elmer Series II 2400.

4.1.2 General Procedure for the synthesis of isocyanates 1–9—*First step*. To 1 equiv. of corresponding carboxylic acid was added 1.2 equiv. of thionyl chloride at room temperature. The reaction mass was refluxed for 1.5 h. After cooling, thionyl chloride was removed *in vacuo*. The residue was weighted and dissolved in anhydrous toluene and was used in the next step without further purification. *Second step*. Solution of acid chloride from first step was added dropwise for 1 h to the suspension of 1.1 equiv. of sodium azide in 20 equiv. of anhydrous toluene. The reaction mass was refluxed for 1 h. After cooling, reaction mass was filtered through the sodium chloride and toluene was removed *in vacuo*. The residue was purified by distillation *in vacuo* or crystallization from ethanol.

4.1.3 General Procedure for the synthesis of symmetric ureas 1–4a and 9a—To 1 equiv. of corresponding isocyanate in 40 equiv. of THF was added 0.05 equiv. of 1,8-Diazabicyclo[5.4.0]undec-7-ene at room temperature. Reaction mass was stirred at room temperature overnight. After adding 1N HCl and water, the resulting white precipitates were collected by suction filtration.

4.1.4 General Procedure for the synthesis of ureas 1–5b, 8b and 9b—To 1 equiv. of corresponding isocyanate in 40 equiv. of DMF was added 1.5 equiv. of *trans*-4-((4-aminocyclohexyl)oxy)benzoic acid and 1.5 equiv. of Et_3N at 0 °C. Reaction mass was stirred at room temperature overnight. After adding 1N HCl and water, the resulting white precipitates were collected by suction filtration. Other ureas obtained by the methods similar to the above described methods and described in detail in supplementary materials.

4.2 Determination of inhibitory potency (IC_{50})

The IC_{50} values reported herein were determined using either a fluorescent based assay[28] or a radioactive based assay.[34]

Fluorescent assay—Enzymes (~2nM mouse sEH, ~1nM rat sEH, or ~1 nM human sEH) were incubated at 30 °C with inhibitors ($[I]_{final}$ = 0.4 – 100,000 nM) for 5 min in 100 mM sodium phosphate buffer (200 μL, pH 7.4) containing 0.1 mg/mL of BSA and 1% of DMSO. The substrate (cyano(2-methoxynaphthalen-6-yl)methyl trans-(3-phenyloxyran-2-yl)methylcarbonate, CMNPC) was then added ($[S]_{final}$ = 5 μM). Activity was assessed by measuring the appearance of the fluorescent 6-methoxynaphthaldehyde product (λ_{em} = 330 nm, λ_{ex} = 465 nm) at 30 °C during a 10 min incubation (Spectramax M2; Molecular Device, Inc., Sunnyvale, CA). The IC_{50} values that are the concentrations of inhibitors that reduce activity by 50% were calculated from at least five different concentrations, each in triplicate, with at least 2 on either side of 50% activity mark.

Radioactive assay—Enzymes (2 nM mouse sEH or 3 nM human sEH 5nM rat sEH) were incubated with inhibitors ($[I]_{final}$ = 1 – 100,000 nM) at 30° C for 5 min in sodium phosphate buffer (pH 7.4) containing 0.1 mg/mL of BSA and 1% of DMSO, prior to substrate introduction ($[S]_{final}$: 50 μM; ~12,000 dpm/assay). The enzymes were incubated at 30°C for 10 min, and the reaction was quenched by the addition of 60 μL methanol and 200 μL isooctane, which extracts the remaining epoxide from the aqueous phase. The activity was followed by measuring the quantity of radioactive diol formed in the aqueous phase using a liquid scintillation counter (Tri-Carb 2810TR, Perkin Elmer, Waltham, MA). Under the conditions used, rates were linear with both time and enzyme concentration and resulted in at least 5% but not more than 30% hydrolysis of the substrate. Assays were performed in triplicate. The IC_{50} was determined by regression of at least five data points with a minimum of two points in the linear region of the curve on either side of the IC_{50}.

4.3 Microsomal stability

The stability of the sEH inhibitors was determined using human liver microsomes as described.[9] The inhibitors ($[I]_{final}$ = 1 uM) to a suspension of human liver microsomes ($[E]_{final}$= 1 mg/mL) in potassium phosphate buffer (0.1M pH7.4) containing 3mM $MgCl_2$

and 1 mM EDTA. After 5 minutes incubation at 37 °C, the reaction was started by the addition of NADPH generating system (buffer was added in the control tubes). After 30 minutes at 37 °C, the reaction was stopped by the addition of one volume of methanol containing CUDA (200 nM) as surrogate. The amount of remaining inhibitors were determine by LC/MS/MS. The stability is reported as a percentage of compound remaining after 30 minutes in these conditions. Results are triplicate average.

Mass spectrometry analysis were performed using a Waters Quattro Premier triple quadrupole tandem mass spectrometer (Micromass, Manchester, UK) interfaced to an electrospray ionization (ESI) source. The MS was coupled with a Waters Acquity UPLC (Waters, Milford, MA, USA). A Varian Pursuit5 C18 RP HPLC column (150 mm × 2.1 mm, particle size 5 μm) was used to separate the analytes. The ESI was performed following HPLC in the positive mode at 2.51 kV capillary voltage. The source and the desolvation temperatures were set at 120 and 300°C, respectively. Cone gas (N_2) and desolvation gas (N_2) were maintained at flow rates of 10 and 700 L/h, respectively. Dwell time was set to 0.1 s. A regression curve for each compound was obtained from at least six different concentrations of standard stock solutions ($R^2 > 0.99$).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- Ureas with alteration in adamantane fragment were systematically studied as sEH inhibitors.
- 94 adamantyl ureas with substituents in bridge and bridgehead positions were synthesized.
- Difference of activity against human, rat and murine sEH was investigated.
- Dependence of substituents number in adamantane on metabolic stability was studied.

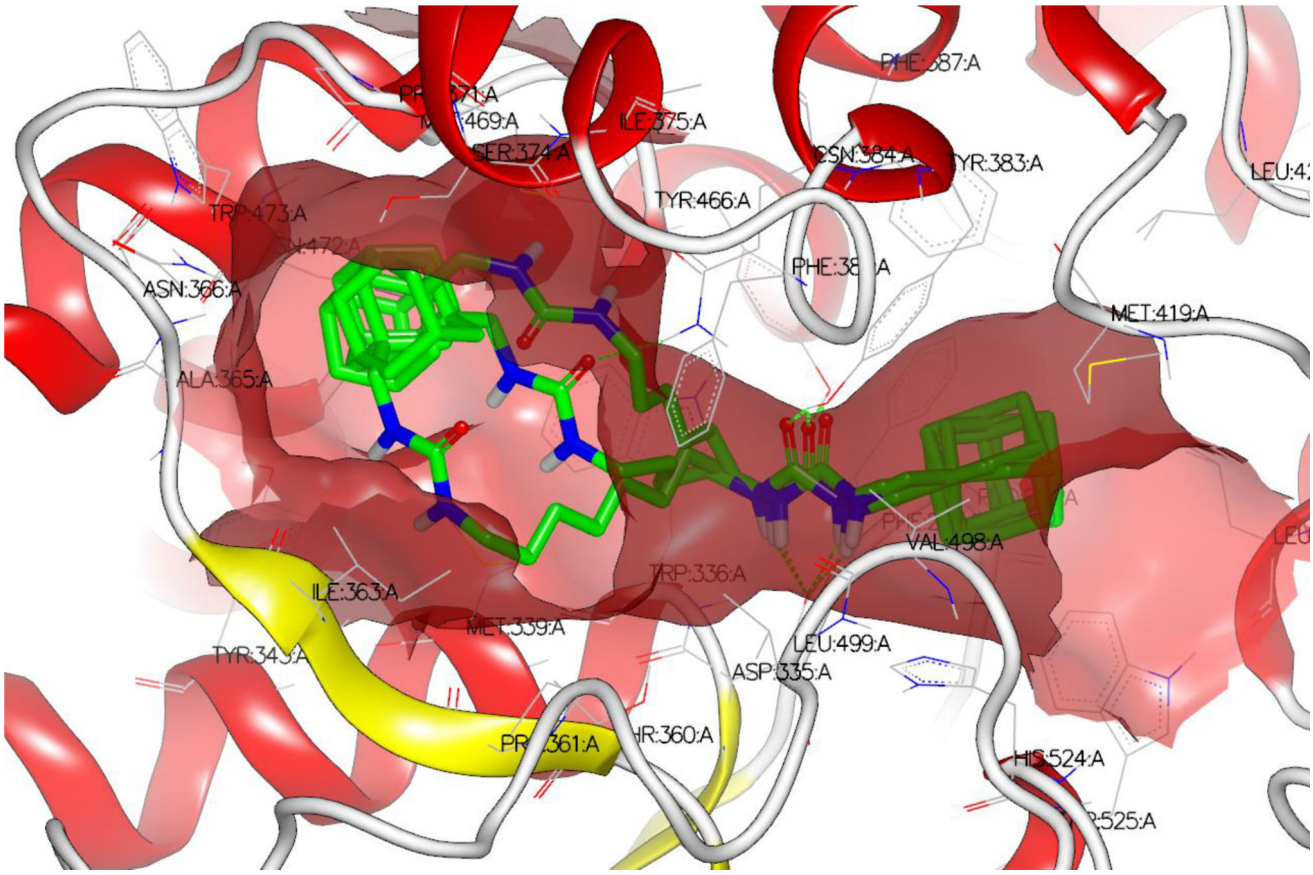


Figure 1.
Docking of compounds **5l, 5n** and **5p** shows the space capacity of sEH domain.

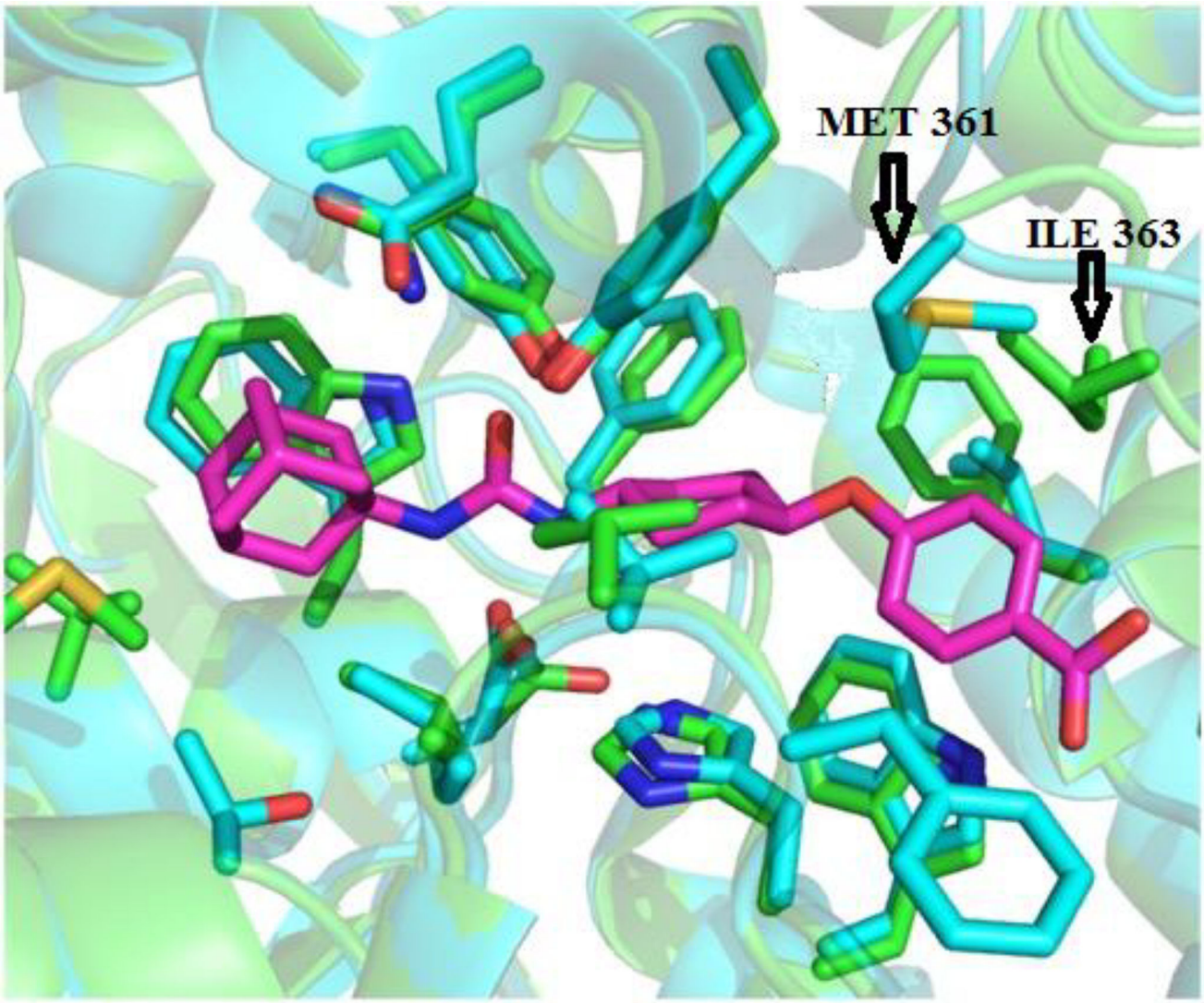


Figure 2.
Compound **1471** (red) docked into human (cyan) and mouse (green) sEH.

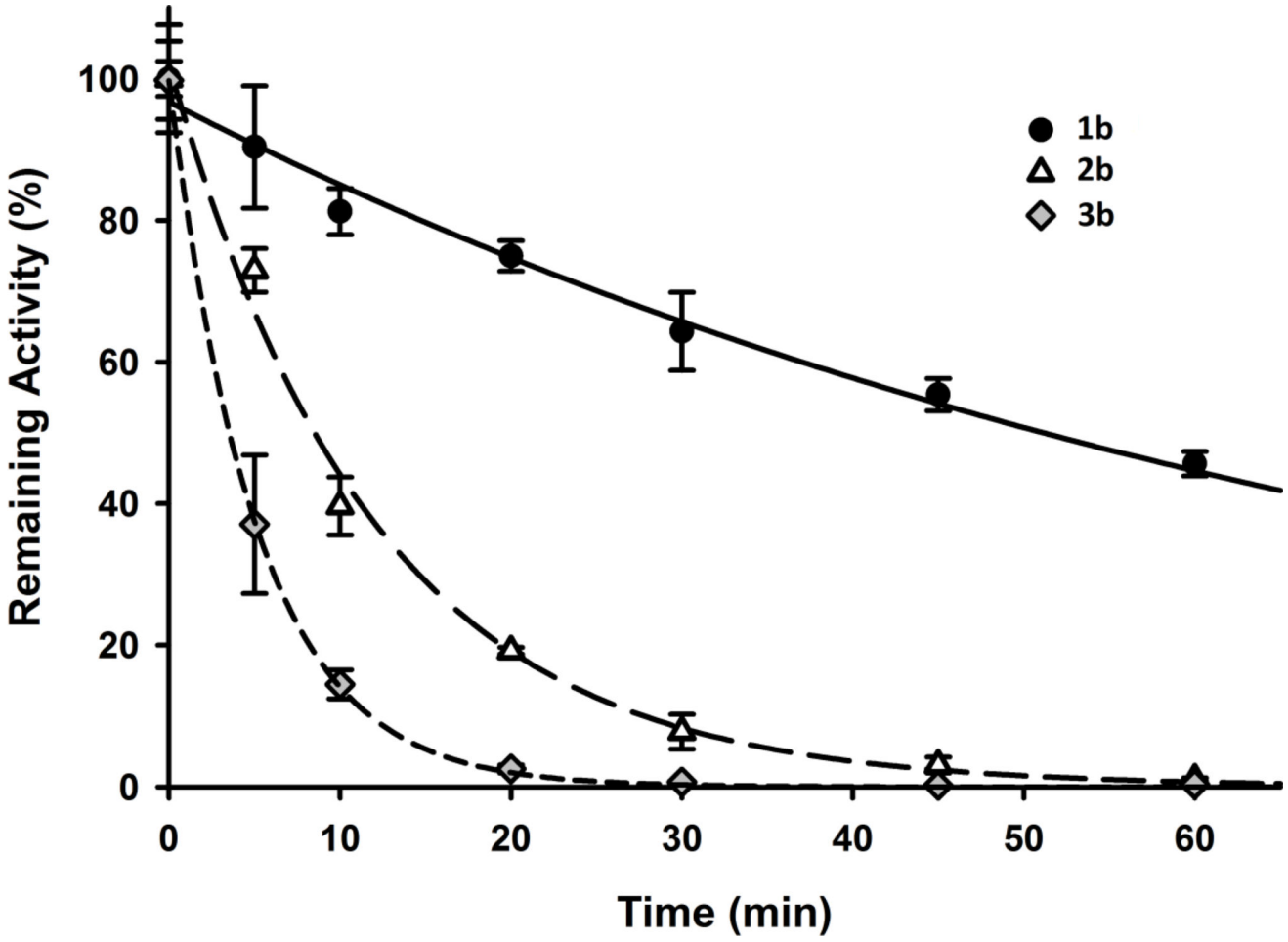
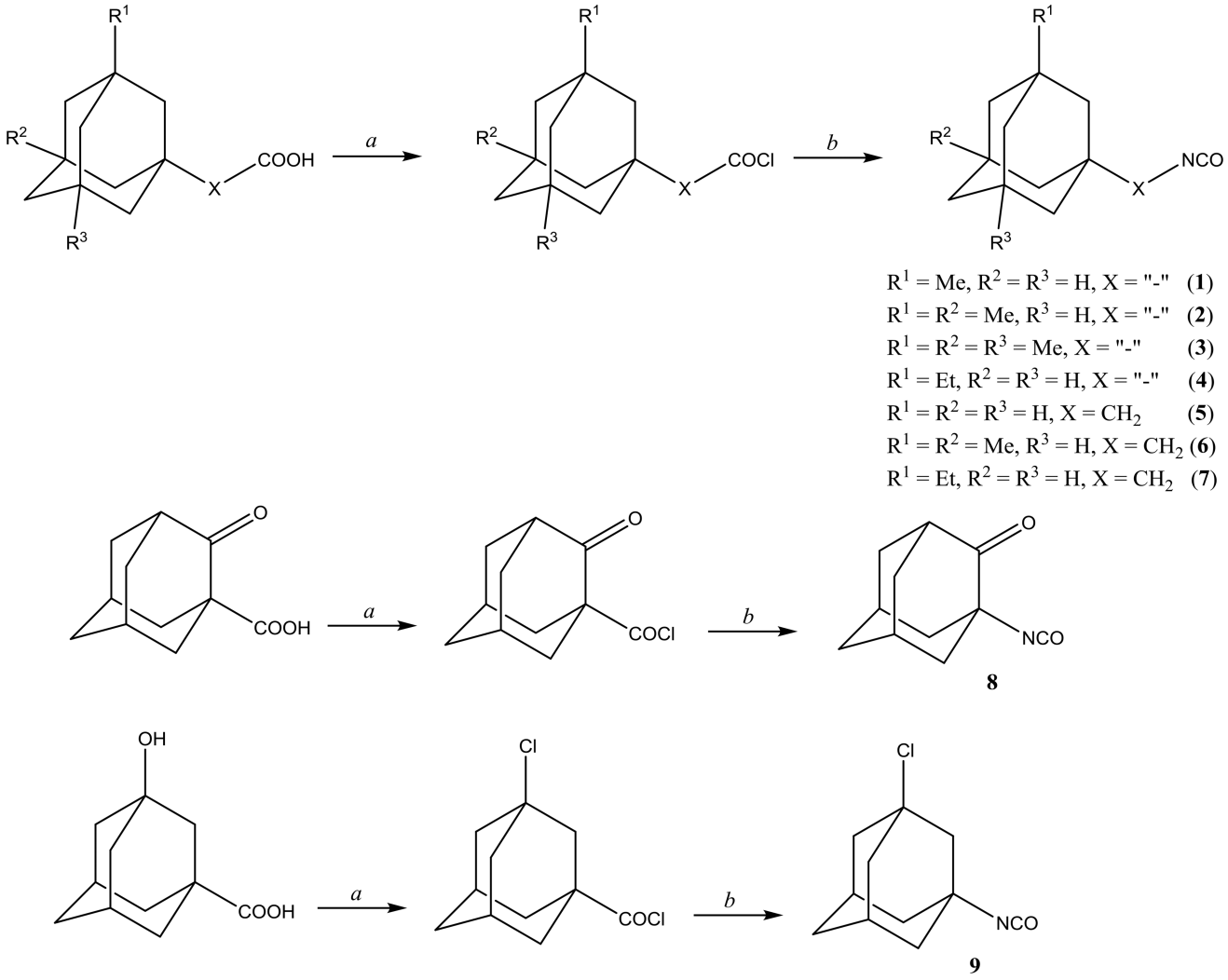
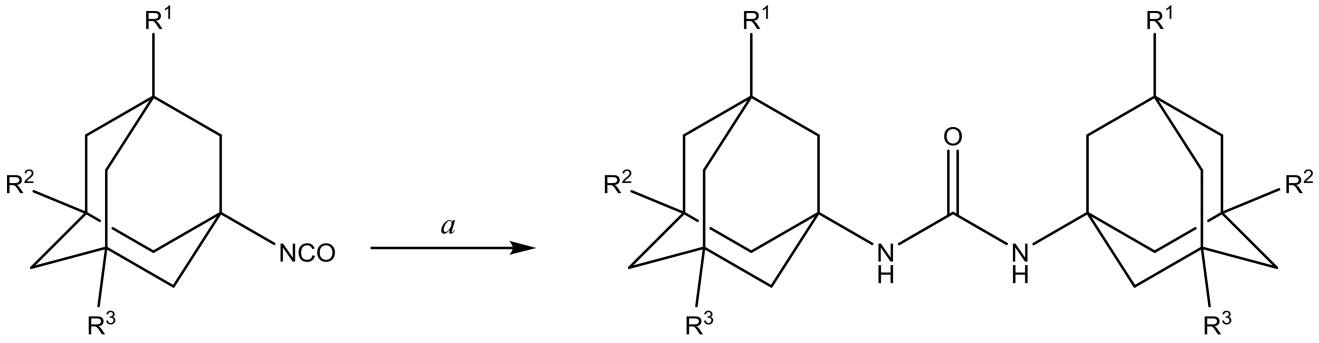


Figure 3.
Two and three methyl substituted **2b** and **3b** almost fully cleared after 60 min.



R^1 = Me, R^2 = R^3 = H, X = "-" (**1**)
R^1 = R^2 = Me, R^3 = H, X = "-" (**2**)
R^1 = R^2 = R^3 = Me, X = "-" (**3**)
R^1 = Et, R^2 = R^3 = H, X = "-" (**4**)
R^1 = R^2 = R^3 = H, X = CH_2 (**5**)
R^1 = R^2 = Me, R^3 = H, X = CH_2 (**6**)
R^1 = Et, R^2 = R^3 = H, X = CH_2 (**7**)

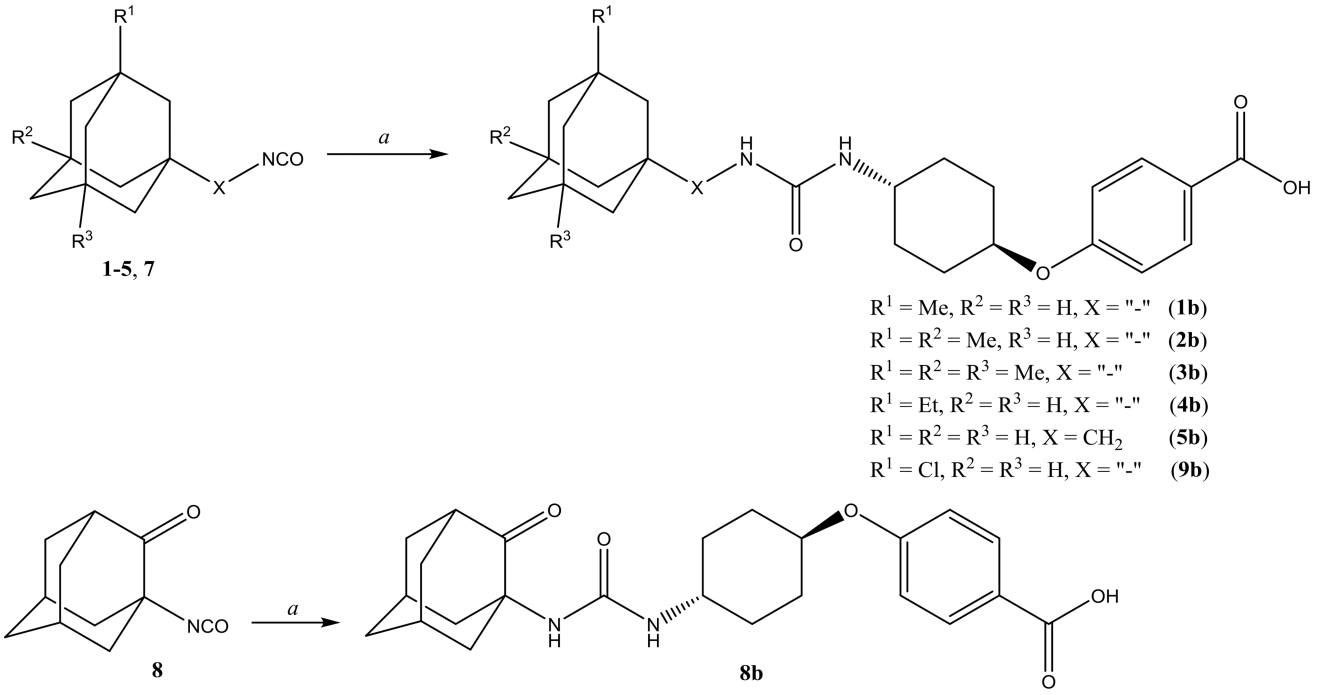
Scheme 1.
Synthesis of isocyanates **1–9**. *a.* $SOCl_2$, reflux, 1h; *b.* NaN_3, toluene, acetonitrile, reflux, 2h.



1-4, **9**

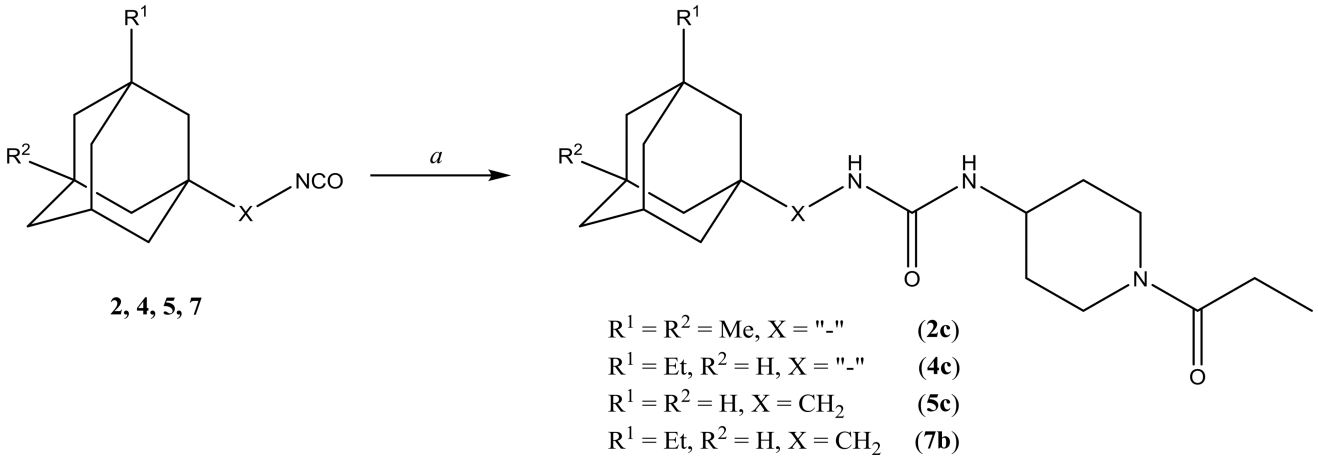
R^1 = Me, R^2 = R^3 = H (**1a**)
R^1 = R^2 = Me, R^3 = H (**2a**)
R^1 = R^2 = R^3 = Me (**3a**)
R^1 = Et, R^2 = R^3 = H (**4a**)
R^1 = Cl, R^2 = R^3 = H (**9a**)

Scheme 2.
Preparation of symmetric disubstituted ureas **1–4a, 9a**. *a*. THF, DBU, 3h



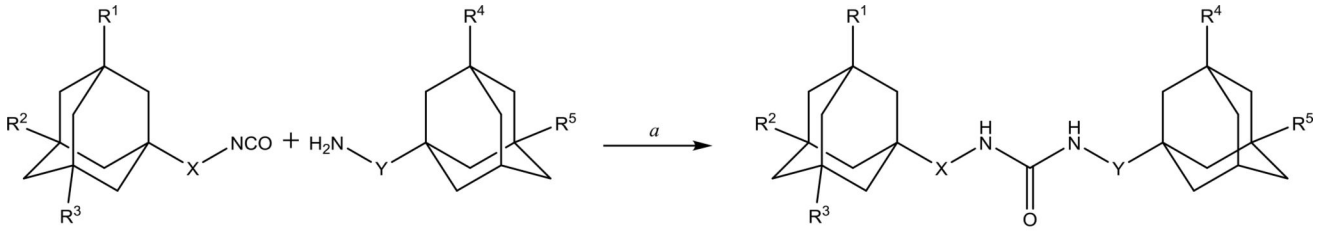
Scheme 3.

Preparation of disubstituted ureas **1–5b, 8b, 9b**. *a*. 4-((4-aminocyclohexyl)oxy) benzoic acid, DMF, Et_3N, 12h.

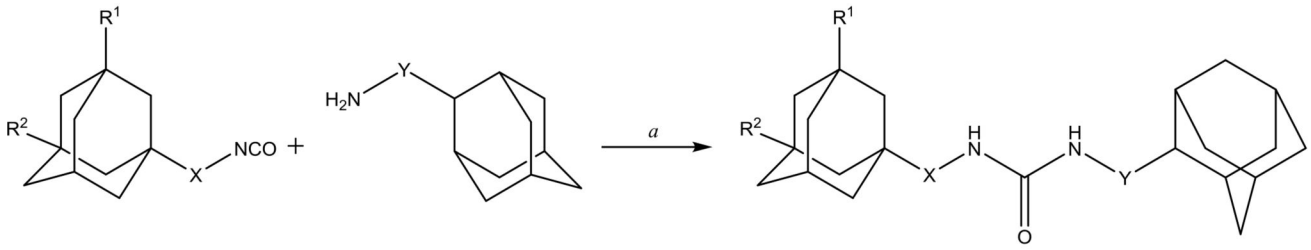


Scheme 4.

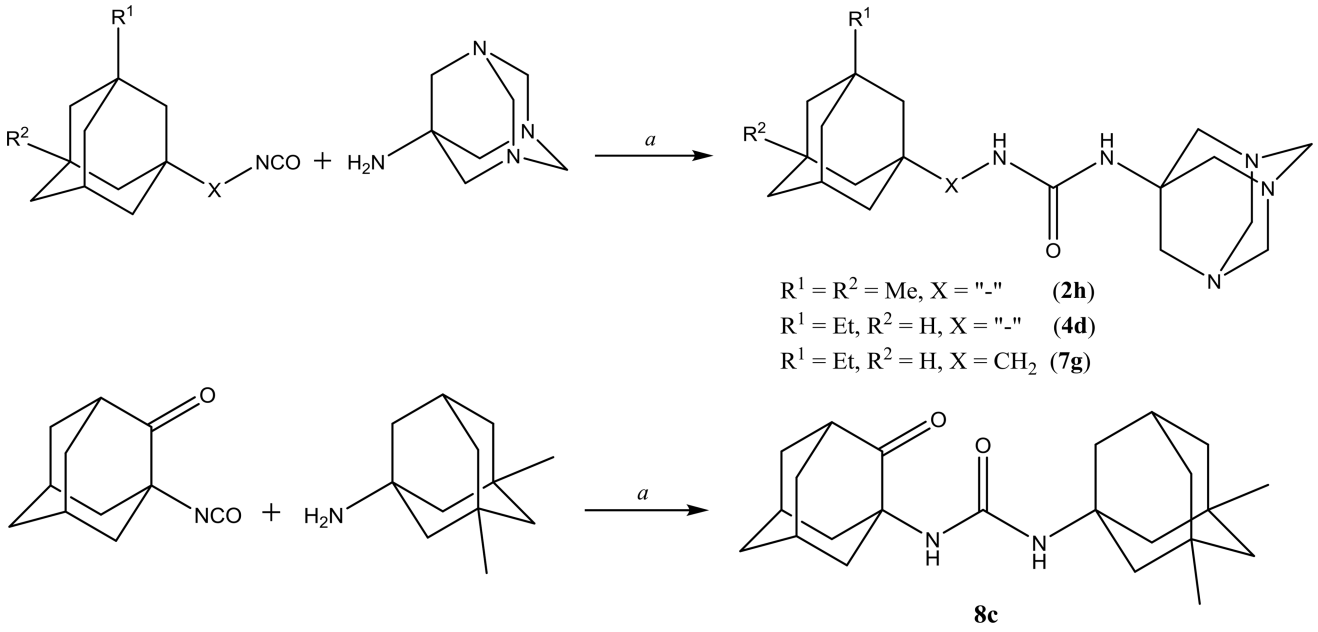
Preparation of disubstituted ureas **2c, 4c, 5c, 7b**. *a.* 1-(4-aminopiperidin-1-yl)propan-1-one, DMF, Et_3N, 12h.



$R^1 = R^2 = Me, R^3 = H, X = "-", R^4 = R^5 = H, Y = CH_2\text{-}CH(Et)$ **(2d)**
$R^1 = R^2 = Me, R^3 = H, X = "-", R^4 = R^5 = H, Y = NH\text{-}(CH_2)_2$ **(2e)**
$R^1 = R^2 = Me, R^3 = H, X = "-", R^4 = R^5 = H, Y = NH\text{-}(CH_2)_3$ **(2f)**
$R^1 = R^2 = R^3 = Me, X = "-", R^4 = R^5 = H, Y = "-"$ **(3c)**
$R^1 = R^2 = R^3 = Me, X = "-", R^4 = R^5 = H, Y = CH(CH_3)$ **(3d)**
$R^1 = R^2 = R^3 = Me, X = "-", R^4 = R^5 = Me, Y = "-"$ **(3e)**
$R^1 = R^2 = R^3 = Me, X = "-", R^4 = R^5 = Me, Y = CH_2$ **(3f)**
$R^1 = R^2 = R^3 = Me, X = "-", R^4 = R^5 = H, Y = CH_2\text{-}CH(Et)$ **(3g)**
$R^1 = R^2 = R^3 = H, X = CH_2, R^4 = R^5 = H, Y = CH_2$ **(5a)**
$R^1 = R^2 = R^3 = H, X = CH_2, R^4 = R^5 = H, Y = (CH_2)_2$ **(5d)**
$R^1 = R^2 = R^3 = H, X = CH_2, R^4 = R^5 = H, Y = NH\text{-}(CH_2)_2$ **(5e)**
$R^1 = R^2 = R^3 = H, X = CH_2, R^4 = R^5 = H, Y = NH\text{-}(CH_2)_3$ **(5f)**
$R^1 = R^2 = R^3 = H, X = CH_2, R^4 = R^5 = H, Y = 1,4\text{-}Ph$ **(5g)**
$R^1 = R^2 = R^3 = H, X = CH_2, R^4 = O\text{-}C(O)\text{-}CF_3, R^5 = H, Y = "-"$ **(5h)**
$R^1 = Et\ R^2 = R^3 = H, X = CH_2, R^4 = R^5 = H, Y = "-"$ **(7c)**
$R^1 = Et\ R^2 = R^3 = H, X = CH_2, R^4 = R^5 = H, Y = CH(CH_3)$ **(7d)**
$R^1 = Et\ R^2 = R^3 = H, X = CH_2, R^4 = R^5 = Me, Y = "-"$ **(7e)**

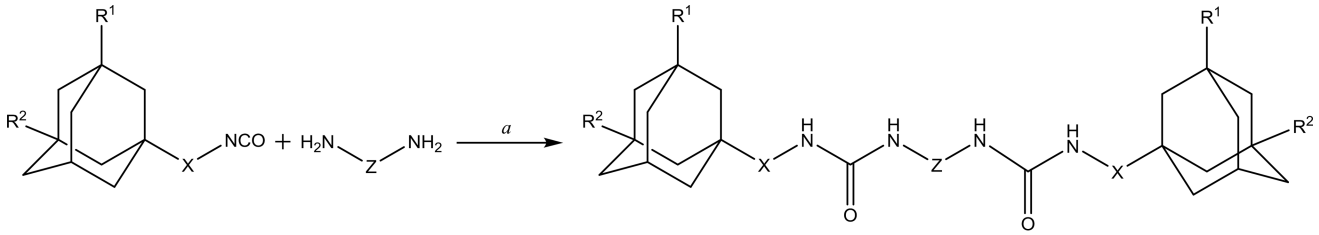


$R^1 = R^2 = Me, X = "-", Y = "-"$ **(2g)**
$R^1 = R^2 = H, X = CH_2, Y = CH_2\text{-}CH(Pr)$ **(5i)**
$R^1 = Et, R^2 = H, X = CH_2, Y = "-"$ **(7f)**
$R^1 = Cl, R^2 = H, X = "-", Y = = CH_2\text{-}CH(Pr)$ **(9c)**



Scheme 5.

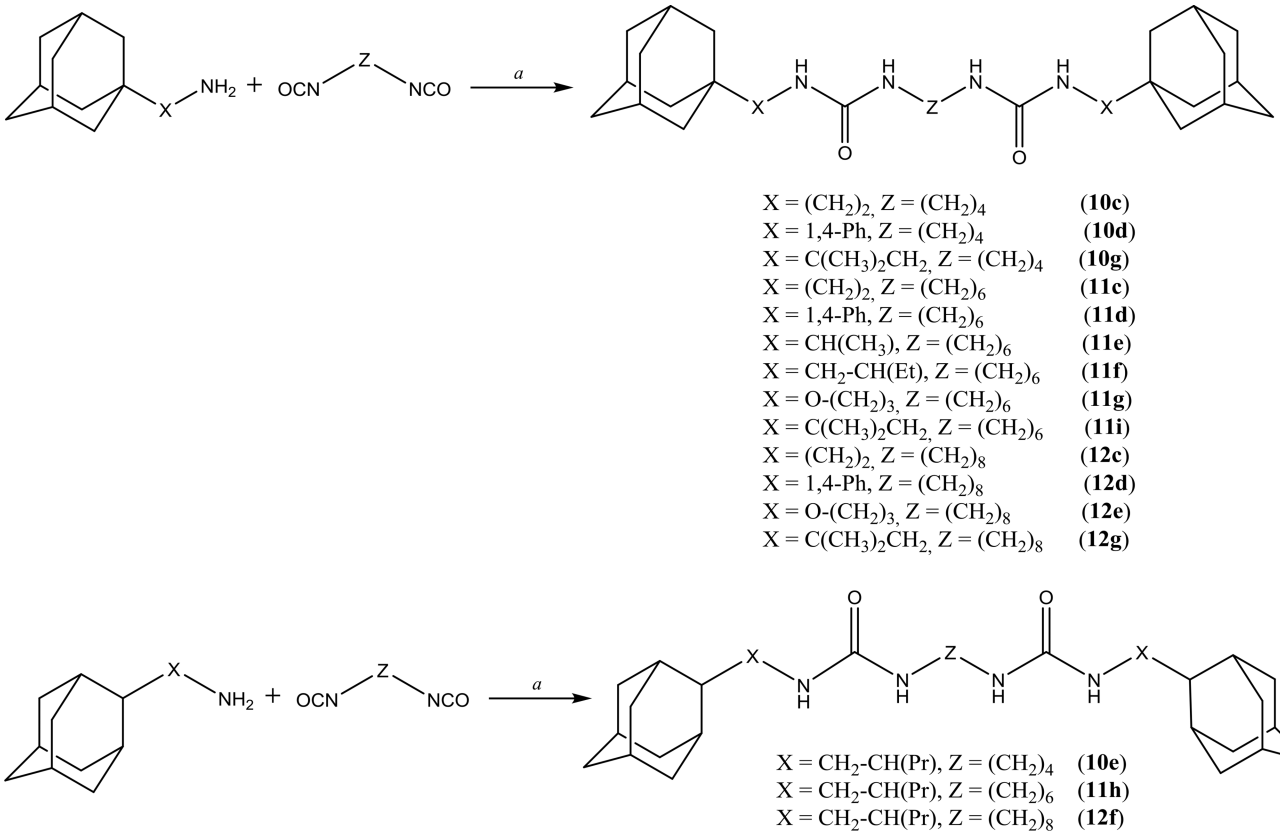
Preparation of non-symmetric diadamantyl disubstituted ureas. *a*. DMF, Et_3N, 12h, double excess of Et_3N used if initial amine used in form of hydrochloride.



$R^1 = R^2 = Me$, X = "-", Z = $(CH_2)_4$ (**2i**)
$R^1 = R^2 = Me$, X = "-", Z = $(CH_2)_8$ (**2j**)
$R^1 = R^2 = Me$, X = "-", Z = 1,4-Ph (**2k**)
$R^1 = Et$, $R^2 = H$, X = "-", Z = $(CH_2)_3$ (**4e**)
$R^1 = Et$, $R^2 = H$, X = "-", Z = $(CH_2)_4$ (**4f**)
$R^1 = Et$, $R^2 = H$, X = "-", Z = $(CH_2)_5$ (**4g**)
$R^1 = Et$, $R^2 = H$, X = "-", Z = $(CH_2)_6$ (**4h**)
$R^1 = R^2 = H$, X = CH_2, Z = $(CH_2)_2$ (**5j**)
$R^1 = R^2 = H$, X = CH_2, Z = $(CH_2)_3$ (**5k**)
$R^1 = R^2 = H$, X = CH_2, Z = $(CH_2)_4$ (**5l**)
$R^1 = R^2 = H$, X = CH_2, Z = $(CH_2)_5$ (**5m**)
$R^1 = R^2 = H$, X = CH_2, Z = $(CH_2)_6$ (**5m**)
$R^1 = R^2 = H$, X = CH_2, Z = $(CH_2)_7$ (**5o**)
$R^1 = R^2 = H$, X = CH_2, Z = $(CH_2)_8$ (**5p**)
$R^1 = R^2 = H$, X = CH_2, Z = $(CH_2)_{10}$ (**5q**)
$R^1 = R^2 = H$, X = CH_2, Z = 1,2-Ph (**5r**)
$R^1 = R^2 = H$, X = CH_2, Z = 1,4-Ph (**5s**)

$R^1 = R^2 = Me$, X = CH_2, Z = $(CH_2)_4$ (**6c**)
$R^1 = R^2 = Me$, X = CH_2, Z = $(CH_2)_6$ (**6d**)
$R^1 = R^2 = Me$, X = CH_2, Z = $(CH_2)_8$ (**6e**)
$R^1 = Cl$, $R^2 = H$, X = "-", Z = $(CH_2)_2$ (**9d**)
$R^1 = Cl$, $R^2 = H$, X = "-", Z = $(CH_2)_3$ (**9e**)
$R^1 = Cl$, $R^2 = H$, X = "-", Z = $(CH_2)_4$ (**9f**)
$R^1 = Cl$, $R^2 = H$, X = "-", Z = $(CH_2)_5$ (**9g**)
$R^1 = Cl$, $R^2 = H$, X = "-", Z = $(CH_2)_6$ (**9h**)
$R^1 = Cl$, $R^2 = H$, X = "-", Z = $(CH_2)_7$ (**9i**)
$R^1 = Cl$, $R^2 = H$, X = "-", Z = $(CH_2)_8$ (**9j**)
$R^1 = Cl$, $R^2 = H$, X = "-", Z = $(CH_2)_{10}$ (**9k**)
$R^1 = Cl$, $R^2 = H$, X = "-", Z = 1,2-Ph (**9l**)

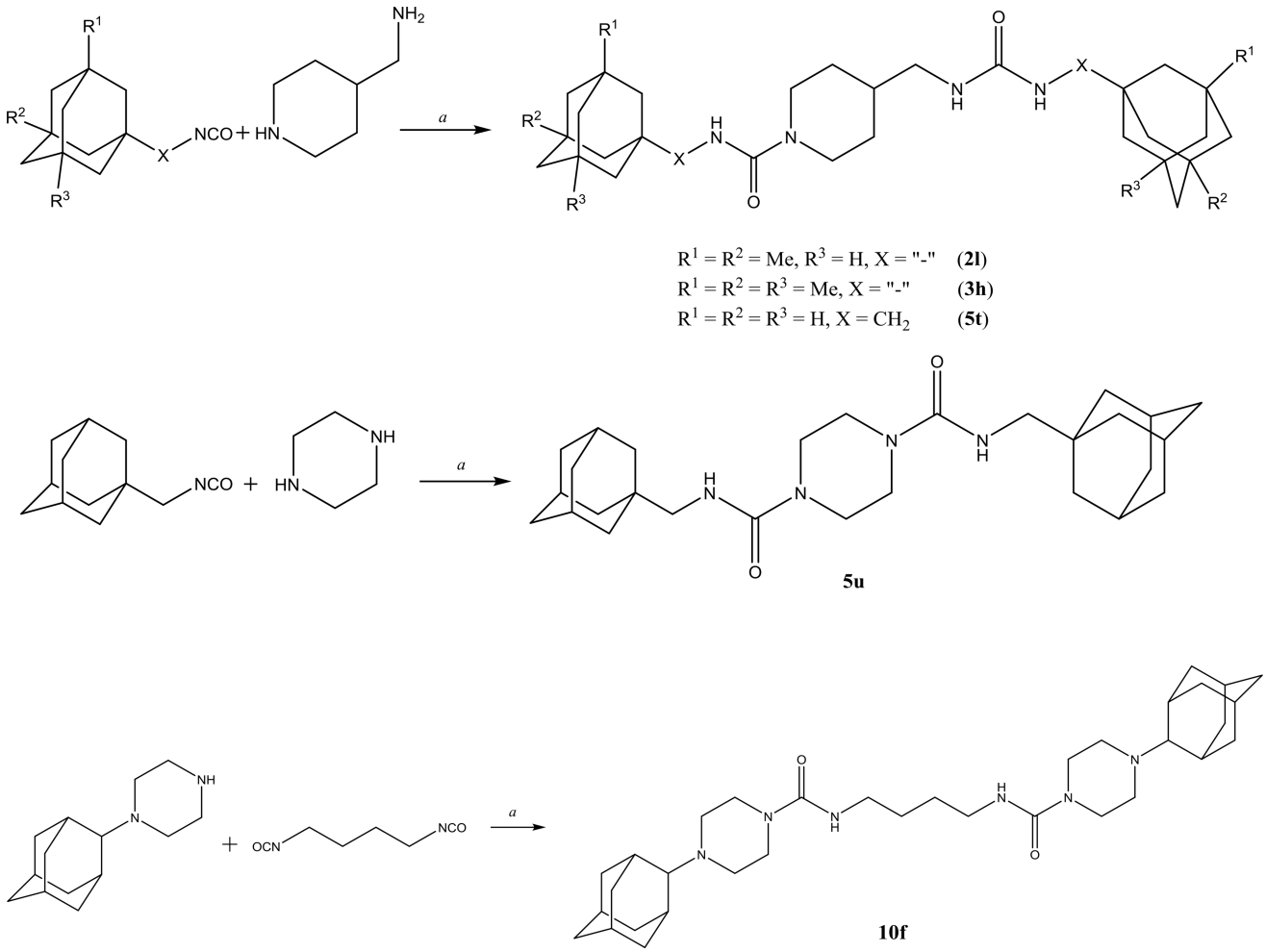
Scheme 6.
Preparation of symmetric diadamantyl disubstituted diureas. *a*. DMF, Et_3N, 12h.



X = $(CH_2)_2$, Z = $(CH_2)_4$ (**10c**)
X = 1,4-Ph, Z = $(CH_2)_4$ (**10d**)
X = $C(CH_3)_2CH_2$, Z = $(CH_2)_4$ (**10g**)
X = $(CH_2)_2$, Z = $(CH_2)_6$ (**11c**)
X = 1,4-Ph, Z = $(CH_2)_6$ (**11d**)
X = $CH(CH_3)$, Z = $(CH_2)_6$ (**11e**)
X = CH_2-CH(Et), Z = $(CH_2)_6$ (**11f**)
X = O-$(CH_2)_3$, Z = $(CH_2)_6$ (**11g**)
X = $C(CH_3)_2CH_2$, Z = $(CH_2)_6$ (**11i**)
X = $(CH_2)_2$, Z = $(CH_2)_8$ (**12c**)
X = 1,4-Ph, Z = $(CH_2)_8$ (**12d**)
X = O-$(CH_2)_3$, Z = $(CH_2)_8$ (**12e**)
X = $C(CH_3)_2CH_2$, Z = $(CH_2)_8$ (**12g**)

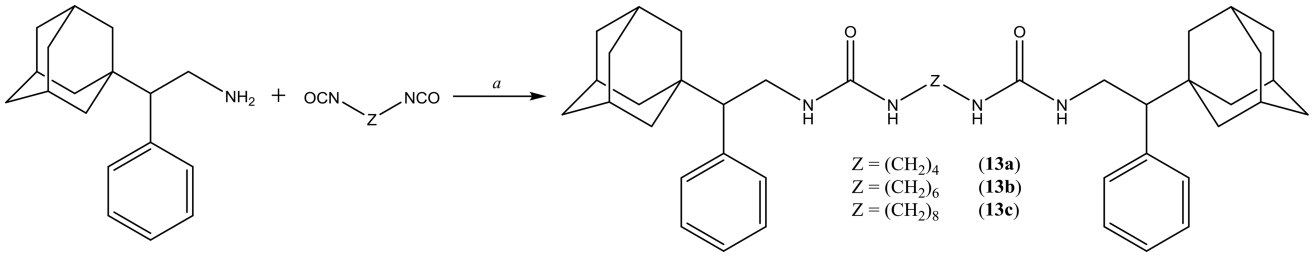
X = CH_2-CH(Pr), Z = $(CH_2)_4$ (**10e**)
X = CH_2-CH(Pr), Z = $(CH_2)_6$ (**11h**)
X = CH_2-CH(Pr), Z = $(CH_2)_8$ (**12f**)

Scheme 7.
Preparation of symmetric diadamantyl disubstituted diureas. *a*. DMF, Et_3N, 12h.



Scheme 8.

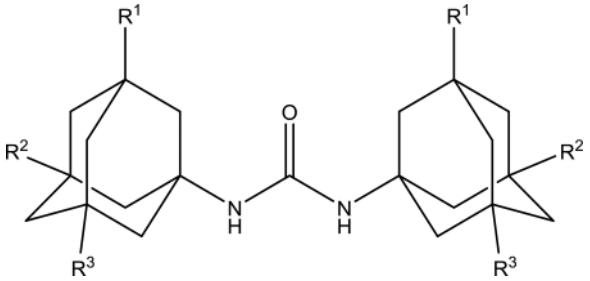
Preparation of compounds bearing trisubstituted urea group. *a*. DMF, Et_3N, 12h.



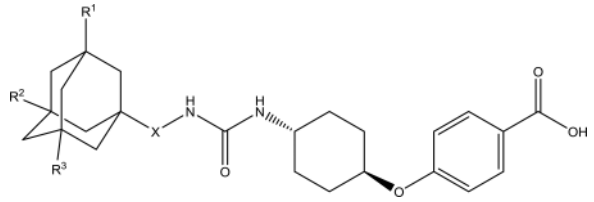
Scheme 9.
a. DMF, Et_3N, 12h

Table 1

IC_{50} values for adamantyl urea based sEH inhibitors **1–4a, 9a, 1–5b, 8b, 9b, 2c, 4c, 5c, 7b**



#	R^1	R^2	R^3	Human sEH IC_{50}[a] (nM)	Solubility[b] (μM)	Mp (°C)
1a	Me	H	H	0.5 ±0.1	20 ±5	290–292
2a	Me	Me	H	1.1 ±0.2	25 ±5	277–278
3a	Me	Me	Me	1.6 ±0.2	20 ±5	305–306
4a	Et	H	H	4.0 ±0.2	15 ±5	248–251
9a	Cl	H	H	0.6 ±0.1	140 ±10	133–135
DAU[c]	H	H	H	1.1[28]	7 ±3[28]	312[30]



#	R^1	R^2	R^3	X	Human sEH IC_{50}[a] (nM)	Solubility[b] (μM)	Mp (°C)
1b	Me	H	H	-	0.5 ±0.1	200 ±10	238–239
2b	Me	Me	H	-	0.8 ±0.1	180 ±10	231–232
3b	Me	Me	Me	-	1.7 ±0.3	160 ±10	260–262
4b	Et	H	H	-	1.8 ±0.1	50 ±10	218–219

#	R^1	R^2	R^3	X	Human sEH IC_{50} [a] (nM)	Solubility [b] (μM)	Mp (°C)
5b	H	H	H	$-CH_2-$	1.6 ±0.3	100 ±10	182–183
9b	Cl	H	H	-	1.4 ±0.2	500 ±25	241–243
1471 [d]	H	H	H	-	2.0[19]	160 ±20	250–255
8b					4.5 ±0.5	1000 ±50	252–254

#	R^1	R^2	X	Human she IC_{50} [a] (nM)	Solubility [b] (μM)	Mp (°C)
2c	Me	Me	-	3.3 ±0.3	210 ±10	181–182
4c	Et	H	-	3.8 ±0.5	100 ±20	187–188
5c	H	H	$-CH_2-$	0.8 ±0.1	120 ±10	173–174
7b	Et	H	$-CH_2-$	1.8 ±0.2	150 ±20	169–170
1163 [e]	H	H	-	3.2[31]	180 ±10	217–221[31]

#	R^1	R^2	X	Human sEH IC_{50}[a] (nM)	Solubility[b] (μM)	Mp (°C)
1770[f]				3.7[31]	167	198–201[31]

[a] As determined via a kinetic fluorescent assay. Results are means of three separate experiments.[29]

[b] Solubilities were measured in sodium phosphate buffer (pH 7.4, 0.1 M) containing 1% of DMSO.

[c] **DAU** – diadamantyl urea properties given for comparison.

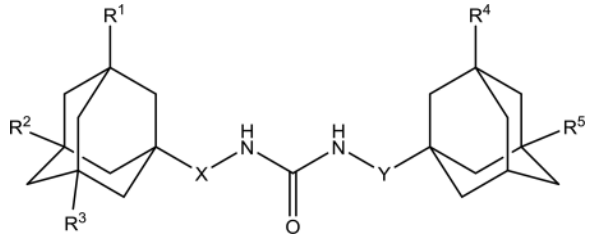
[d] **1471**– 4-((4-(3-(adamantan-1-yl)ureido)*trans*-cyclohexyl)oxy)benzoic acid properties given for comparison.

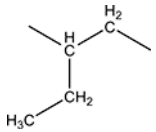
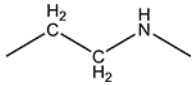
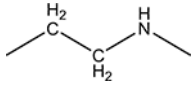
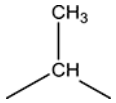
[e] **1163** – 1-(adamantan-1-yl)-3-(1-propionylpiperidin-4-yl)urea properties given for comparison.

[f] **1770** – 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea properties given for comparison.

Table 2

IC_{50} values for non-symmetric diadamantyl disubstituted ureas **2d-h, 3c-g, 4c, 5d-i, 7c-g, 8c, 9c**

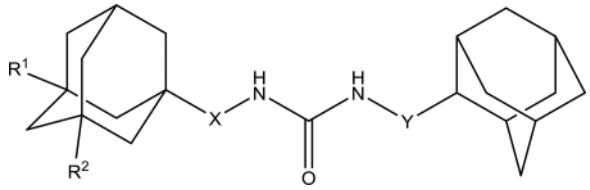


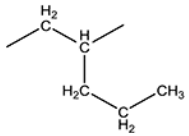
#	R^1	R^2	R^3	X	Y	R^4	R^5	Human sEH IC_{50}[a] (nM)	Solubility[b] (μM)	Mp (°C)
2d	Me	Me	H	-		H	H	13.8	40 ± 5	240–243
2e	Me	Me	H	-		H	H	119.9	210 ± 15	180–181
2f	Me	Me	H	-		H	H	79.6	1550 ± 50	174–175
3c	Me	Me	Me	--	-	H	H	6.1	35 ± 5	320–323
3d	Me	Me	Me	-		H	H	25.4	12 ± 2	229–231
3e	Me	Me	Me	--	-	Me	Me	160.0	25 ± 5	304–305
3f	Me	Me	Me	-	$-CH_2-$	Me	Me	2.8	25 ± 5	287–288

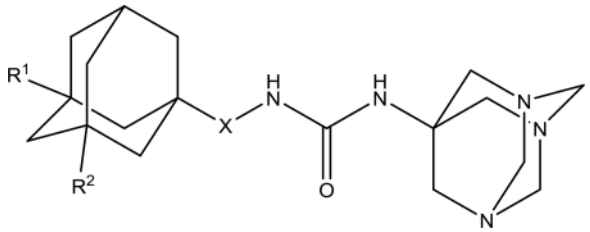
#	R1	R2	R3	X	Y	R4	R5	Human sEH IC50 a (nM)	Solubility b (μM)	Mp (°C)
3g	Me	Me	Me	-		H	H	22.9	20 ± 5	290–293
5a	H	H	H	-CH_2-	-CH_2-	H	H	5.4	50 ± 5	137–139
5d	H	H	H	-CH_2-		H	H	4.4	45 ± 5	121–123
5e	H	H	H	-CH_2-		H	H	28.5	375 ± 25	194–196
5f	H	H	H	-CH_2-		H	H	17.2	2050 ± 50	184–187
5g	H	H	H	-CH_2-		H	H	12.2	50 ± 10	128–130
5h	H	H	H	-CH_2-	-		H	0.4	50 ± 10	168–170
7c	Et	H	H	-CH_2-	-	H	H	2.4	12 ± 3	160–162

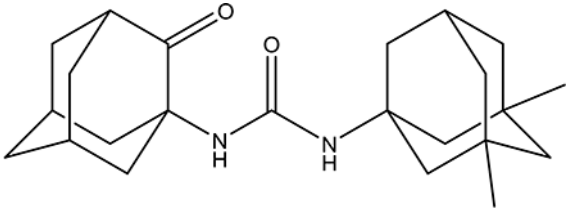
#	R^1	R^2	R^3	X	Y	R^4	R^5	Human sEH IC_{50} [a] (nM)	Solubility [b] (μM)	Mp (°C)
7d	Et	H	H	-CH_2-		H	H	12.8	17 ± 3	151–154
7e	Et	H	H	-CH_2-	-	Me	Me	1.5	8 ± 2	164–166

#	R^1	R^2	X	Y	Human sEH IC_{50} [a] (nM)	Solubility [b] (μM)	Mp (°C)
2g	Me	Me	-	-	9.6	20 ± 5	266–268
5i	H	H	-CH2-		1992	60 ± 10	196–199
7f	Et	H	-CH2-	-	2.7	16 ± 2	150–153



#	R^1	R^2	X	Y	Human sEH IC_{50}[a] (nM)	Solubility[b] (μM)	Mp (°C)
9c	Cl	H	-		3.1	45 ± 5	163–164



#	R^1	R^2	X	Human sEH IC_{50}[a] (nM)	Solubility[b] (μM)	Mp (°C)
2h	Me	Me	-	2894	110 ± 15	162–164
4d	Et	H	-	268.6	190 ± 15	172–175
7g	Et	H	$-CH_2-$	60.6	110 ± 15	131–132
8c				0.4	235 ± 15	202–204

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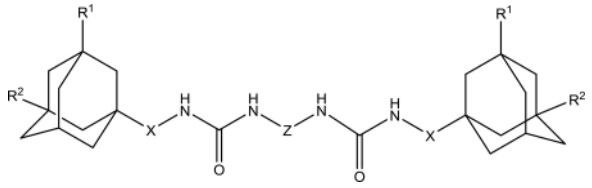
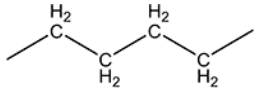
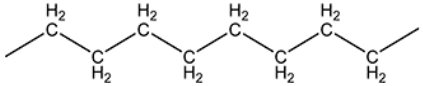
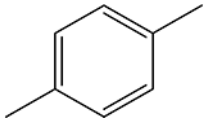
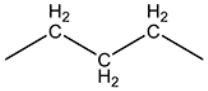
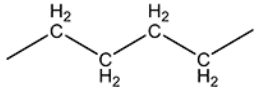
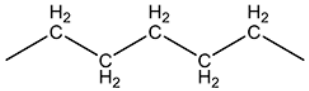
[a] As determined via a kinetic fluorescent assay. Results are means of three separate experiments. [29]

[b] Solubilities were measured in sodium phosphate buffer (pH 7.4, 0.1 M) containing 1% of DMSO.

Table 3

IC_{50} values for symmetric diadamantyl disubstituted diureas



#	R^1	R^2	X	Z	Human sEH IC_{50}[a] (nM)	Solubility[b] (μM)	Mp (°C)
2i	Me	Me	-		23.4	n/a	251–252
2j	Me	Me	-		3.7	n/a	180–181
2k	Me	Me	-		2475	n/a	327–329
4e	Et	H	-		1.7	10 ± 2	243–244
4f	Et	H	-		1.1	10 ± 2	260–262
4g	Et	H	-		1.2	6 ± 1	258–259

#	R^1	R^2	X	Z	Human sEH IC_{50} [a] (nM)	Solubility [b] (μM)	Mp (°C)
4h	Et	H	-		8.5	6 ± 1	247–249
5j	H	H	$-CH_2-$		179.2	90 ± 10	211–212
5k	H	H	$-CH_2-$		0.9	90 ± 10	225–226
5l	H	H	$-CH_2-$		0.9	80 ± 10	232–234
5m	H	H	$-CH_2-$		0.9	75 ± 5	243–245
5n	H	H	$-CH_2-$		0.4	65 ± 5	218–219
5o	H	H	$-CH_2-$		0.6	70 ± 10	165–166

#	R^1	R^2	X	Z	Human sEH IC_{50} [a] (nM)	Solubility [b] (μM)	Mp (°C)
5p	H	H	-CH_2-		0.7	55 ± 10	163–164
5q	H	H	-CH_2-		1.2	45 ± 5	154–155
5r	H	H	-CH_2-		1364	45 ± 5	249–250
5s	H	H	-CH_2-		779	50 ± 5	239–240
6c	Me	Me	-CH_2-		0.7	90 ± 5	244–246
6d	Me	Me	-CH_2-		1.0	75 ± 5	225–227
6e	Me	Me	-CH_2-		2.5	55 ± 5	188–190

#	R^1	R^2	X	Z	Human sEH IC_{50} [a] (nM)	Solubility [b] (μM)	Mp (°C)
9d	Cl	H	-		144.5	55 ± 5	204–205
9e	Cl	H	-		1.7	75 ± 5	184–186
9f	Cl	H	-		1.3	55 ± 5	192–193
9g	Cl	H	-		1.8	60 ± 5	116–117
9h	Cl	H	-		1.6	45 ± 5	113–115
9i	Cl	H	-		1.4	75 ± 5	114–116
9j	Cl	H	-		1.2	75 ± 5	110–112

#	R^1	R^2	X	Z	Human sEH IC_{50} [a] (nM)	Solubility [b] (μM)	Mp (°C)
9k	Cl	H	-		0.9	75 ± 5	108–109
9l	Cl	H	-		408.6	65 ± 5	186–188
10c	H	H			3.4	100 ± 10	229–230
11c	H	H			0.8	80 ± 10	193–195
12c	H	H			1.5	75 ± 5	167–168
10d	H	H			1792	75 ± 5	158–160
11d	H	H			529.3	80 ± 5	183–185

#	R^1	R^2	X	Z	Human sEH IC_{50} [a] (nM)	Solubility [b] (μM)	Mp (°C)
12d	H	H			259.1	75 ± 5	167–168
11e	H	H			0.5	75 ± 5	164–165
11f	H	H			2.8	70 ± 5	175–176
11g	H	H			9.4	45 ± 5	139–140
12e	H	H			2.7	35 ± 5	224–225
10g	H	H			1.9	n/a	145–147

#	R^1	R^2	X	Z	Human sEH IC_{50} [a] (nM)	Solubility [b] (μM)	Mp (°C)
11i	H	H			4.3	n/a	115–117
12g	H	H			6.7	n/a	124–125

#	X	Z	Human she IC_{50} [a] (nM)	Solubility [b] (μM)	Mp (°C)
10e			3299	55 ± 5	134–136

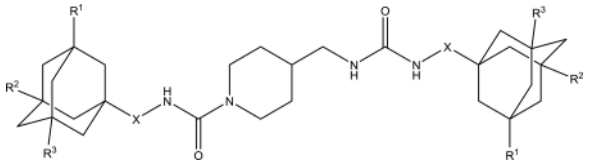
#	X	Z	Human she IC_{50} [a] (nM)	Solubility[b] (μM)	Mp (°C)
11h			35000	75 ± 5	65–67
12f			>100000	45 ± 5	74–75

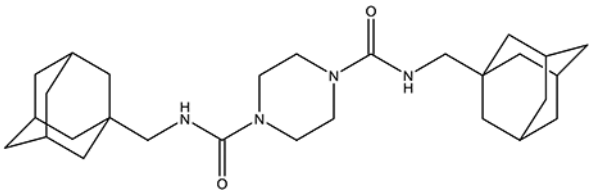
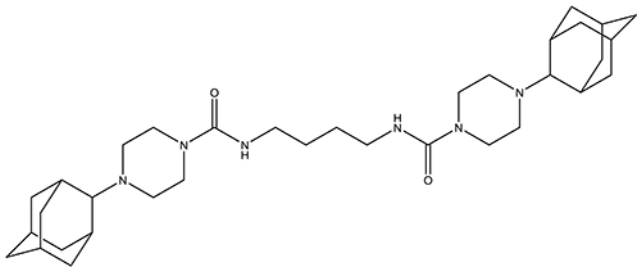
[a] As determined via a kinetic fluorescent assay. Results are means of three separate experiments.[29]

[b] Solubilities were measured in sodium phosphate buffer (pH 7.4, 0.1 M) containing 1% of DMSO.

Table 4

IC_{50} values for compounds **2l, 3h, 5t, 5u, 10f** bearing trisubstituted urea groups



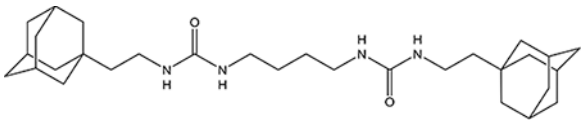
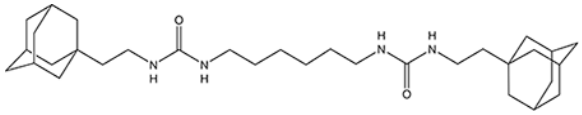
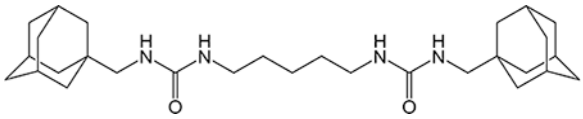
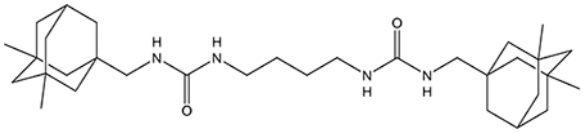
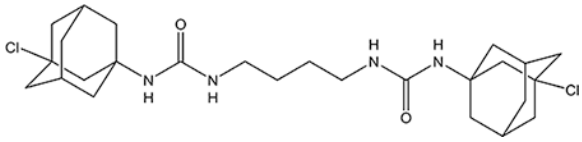
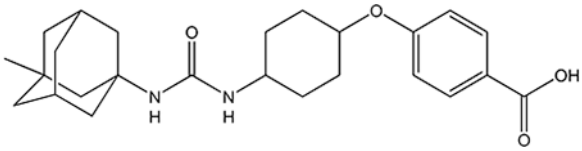
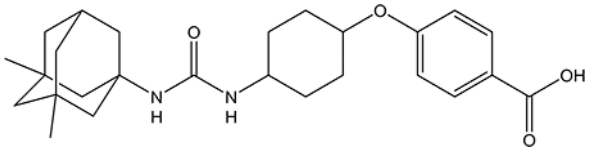
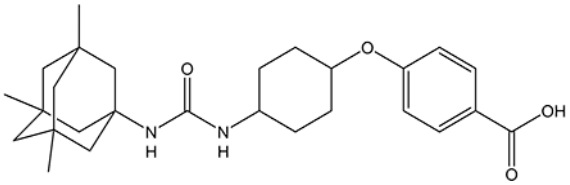
#	R^1	R^2	R^3	X	Human sEH IC_{50}[a] (nM)	Solubility[b] (μM)	Mp (°C)
2l	Me	Me	H	-	1.9	30 ± 5	232–234
3h	Me	Me	Me	-	6.5	20 ± 5	265–268
5t	H	H	H	$-CH_2-$	39.7	15 ± 5	127–128
5u					2006	90 ± 10	275–276
10f					16800	25 ± 5	195–197

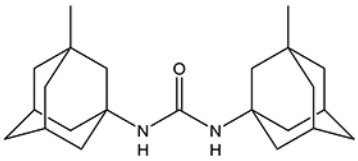
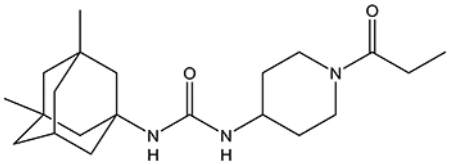
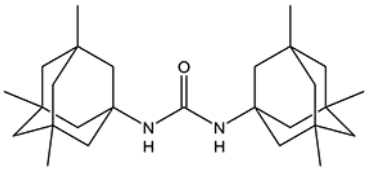
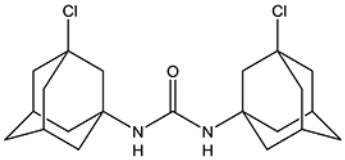
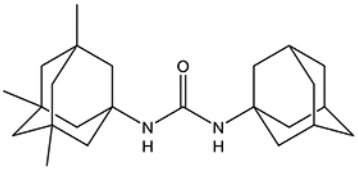
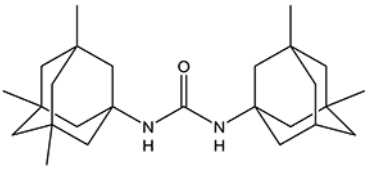
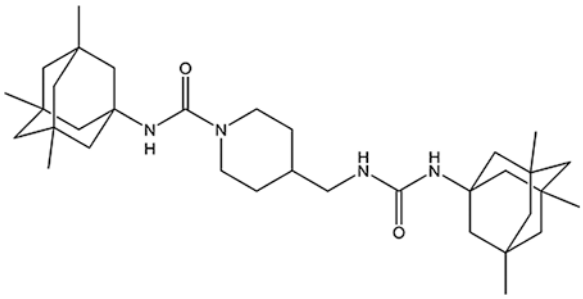
[a] As determined via a kinetic fluorescent assay. Results are means of three separate experiments. [29]

[b] Solubilities were measured in sodium phosphate buffer (pH 7.4, 0.1 M) containing 1% of DMSO.

Table 5

Comparison of potency against Human, Rat and Mouse sEH.

#	Structure	sEH IC_{50} (nM)[a]		
		Human	*Rat*	*Mouse*
10c		3.4	6.0	21.4
11c		0.8	2.1	5.8
5m		0.9	2.8	5.0
6c		0.7	2.8	11.0
9f		1.3	3.5	5.8
1b		0.5	1.6	2.0
2b		0.8	2.7	3.8
3b		1.7	11.0	34.5

#	Structure	sEH IC_{50} (nM)[a]		
		Human	*Rat*	*Mouse*
1a		0.5	3.1	5.5
2c		3.3	1.8	5.2
3a		1.6	25.3	66.2
9a		0.6	1.9	2.1
3c		6.1	5.1	106.9
3e		160.0	97.1	11376
3h		6.5	24.9	436

[a]As determined via a kinetic fluorescent assay. Results are means of three separate experiments. [29]

Table 6

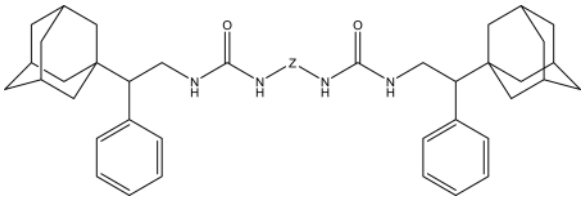
Difference between human and mouse sEH potency for compounds **3b, 3c, 3e** determined by fluorescent and radioactive assays.

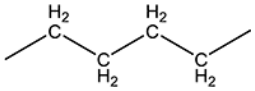
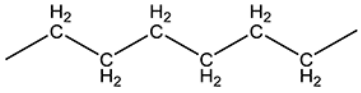
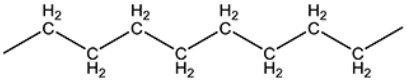
#	Human sEH IC_{50}		Rat sEH IC_{50}		Mouse sEH IC_{50}	
	Fluorescent	**Radioactive**	**Fluorescent**	**Radioactive**	**Fluorescent**	**Radioactive**
3b	1.7	8.8	11.0	28.1	34.5	363.0
3c	6.1	122.0	5.1	16.2	106.9	597.7
3e	160.0	5930	97.1	180.5	11379	>10000

IC_{50} values are dependent of the assay conditions. Because the substrate concentration in the radioactive assay ([S] = 50 μM) is 10-fold higher than in the fluorescent assay ([S] = 5 μM), one could expect that the inhibition potency will be larger with the radioactive assay than the fluorescent one.

Table 7

Human, rat and mouse sEH IC_{50} for compounds **13a–c**.



#	Z	sEH IC_{50} (nM)[a]		
		Human	***Rat***	***Mouse***
13a		605.0	951.3	3359.8
13b		91.5	1014.0	1785.0
13c		205.0	755.4	1866.1

[a]As determined via a kinetic fluorescent assay. Results are means of three separate experiments. [29]

Table 8

Metabolic stability of sEH inhibitors with substituents in bridgehead positions of adamantane against human microsomes (HLM).

#	Amount remaining after 30 minutes in contact with HLM (%)		T½ (min)	Cl_{int} * ($\mu L.min^{-1}.mg^{-1}$)
	− NADPH	+ NADPH		
1471[36]	97 ± 8	69 ± 2	56	12
1b	95 ± 3	64 ± 6	54 ± 3	13 ± 1
2b	100 ± 3	8 ± 2	8 ± 1	84 ± 6
3b	95 ± 4	0.7 ± 0.2	3.5 ± 0.3	196 ± 2

* Intrinsic clearance