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The Structure Activity Relationship of Urea Derivatives as Anti-Tuberculosis Agents

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Abstract

The treatment of tuberculosis is becoming more difficult due to the ever increasing prevalence of drug resistance. Thus, it is imperative that novel anti-tuberculosis agents, with unique mechanisms of action, be discovered and developed. The direct anti-tubercular testing of a small compound library led to discovery of adamantyl urea hit compound **1**. In this study, the hit was followed up through the synthesis of an optimization library. This library was generated by systematically replacing each section of the molecule with a similar moiety until a clear structure activity relationship was obtained with respect to anti-tubercular activity. The best compounds in this series contained a 1-adamantyl-3-phenyl urea core and had potent activity against *Mycobacterium tuberculosis* plus an acceptable therapeutic index. It was noted that the compounds identified and the pharmacophore developed is consistent with inhibitors of epoxide hydrolase family of enzymes. Consequently, the compounds were tested for inhibition of representative epoxide hydrolases: *M. tuberculosis* EphB and EphE; and human soluble epoxide hydrolase. Many of the optimized inhibitors showed both potent EphB and EphE inhibition suggesting the antitubercular activity is through inhibition of multiple epoxide hydrolyase enzymes. The inhibitors also showed potent inhibition of humans soluble expoxide hydrolyase, but limited cytotoxicity suggesting that future studies must be towards increasing the selectivity of epoxide hydrolyase inhibition towards the *M. tuberculosis* enzymes.

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Supporting Information
RP-HPLC purity and retention times and MIC data for non-tubercular antibacterial testing.

Keywords

Urea; Tuberculosis; Epoxide Hydrolase

1. Introduction

Increasing drug resistance and poor activity of existing therapies towards the latent stage of *Mycobacterium tuberculosis* infection has produced a clear need to develop novel therapeutics to treat tuberculosis.[1] Thus fast-acting drugs with novel mechanisms of action that are not cross resistant to existing drugs are being sought actively. To tackle this problem two primary screening strategies are being applied in tuberculosis drug discovery - target based high throughput screening and phenotypic minimum inhibitory concentration (MIC) based screening of whole cell bacteria. Although target/enzyme based high throughput screening for new tuberculosis therapeutics has been widely adopted, this strategy has not produced many notable successes, an experience that mirrors the success of this approach in other antibacterial drug discovery programs.[2] On the contrary, direct phenotypic based MIC screening of commercial and proprietary libraries has recently produced a number of interesting clinical candidates including the diaryl quinolone, TMC207, and benzothiazinone, BTZ043,[3–4] for which the ultimate enzymatic target and antitubercular mode of action for these compounds was derived after potent inhibitors were identified.

We have previously adopted an analogous approach to screen various available chemical libraries directly for anti-tuberculosis activity. After the identification of selective hits, mode of action studies are performed producing novel validated anti-tubercular drug candidates.[5] In this study a ~12,000 compound library from LeadScreen (Tripos) was screened for anti-tuberculosis activity by microbroth dilution in Middlebrook 7H9 media. Three hundred and eight compounds initially showed activity at 10 μM. One of the best of these hits was the urea hit **1**, which upon resynthesis and retesting displayed a confirmed anti-tuberculosis MIC of 0.03 μM (0.01 μg/ml) (Fig. 1). Interestingly, the structure of the urea compound **1** was found to be very similar to inhibitors of mammalian soluble epoxide hydrolase (sEH[6–7] and the recently reported inhibitors of the *M. tuberculosis* epoxide hydrolase (EH) enzyme B (EphB).[6] Figure 2 shows the general structure and specific examples for the urea-based sEH and *M. tuberculosis* EphB inhibitors. This lead us to hypothesize that hit **1** targets the Eph enzymes of *M. tuberculosis.* Initial analysis of the tuberculosis genome showed it contained at least six putative EH enzymes.[8] This unusually large number of EHs compared to other bacteria, suggests these enzymes play important roles in the physiology of *M. tuberculosis*; notably, lipid metabolism and detoxification of reactive oxygen species derived from the host's immune system. In this regard, Eph enzymes represent promising targets for anti-tubercular drug discovery. Moreover, they represent potentially druggable targets with an active site suitable for small molecule therapeutic intervention.[6, 9] Since *M. tuberculosis* contains several EH enzymes with similar active sites and perhaps redundancy in function this presents a considerable challenge to obtain compounds with anti-tubercular activity through target based discovery. We therefore adopted a direct MIC approach for rapidly determining the initial therapeutic potential of these inhibitors. Currently, there are no reports on the whole cell anti-tubercular activity of EH inhibitors, though molecules with similar structures have recently been described with good inhibition of *M. tuberculosis* EphB[6] or antitubercular MICs.[10–11] In this study, the three sections – the aryl ring, the alkyl ring and urea – of hit **1** were systematically modified to develop a detailed anti-tubercular structure activity relationship for this series of compounds and the results were rationalized with respect to the binding site of EphB.

2. Chemistry

The optimization of compound **1** began by modifying each side of the urea moiety with a selection of substituents to probe anti-tubercular structure activity relationships (SAR) for potentcy and selectivity. These positions are shown as R (aryl) and R (adamantyl) in Scheme 1. The synthesis of these derivatives was carried out by reacting the desired amine with the corresponding isocyanate in dichloromethane in the presence of triethylamine (Scheme 1). Using this facile chemistry, an array of compounds (**1–30**) was rapidly synthesized using parallel synthesis.

The second series of compounds was synthesized focusing on modifications of the urea moiety. The first compound targeted was the thiourea derivative **31** to explore the structural similarity to already known thiourea anti-tubercular drugs such as thiocarlide and thiacetazone.[12–13] As shown in Scheme 2A, this synthesis was identical to the urea synthesis with the exception that the isothiocyanate was used in place of the isocyanate to give **31** in good yield.

Scheme 2B shows the synthesis of the carbamate derivative **32**. This synthesis coupled the adamantyl alcohol with the phenyl isocyanate in order to form the desired product again in high yield. Further modification of the urea moiety is shown by the synthesis of the mono- and dimethylated derivatives **33** and **34** shown in Scheme 2C. The synthesis of the **33** started from **1**, which was selectively deprotonated at the more acidic urea nitrogen adjacent to the phenyl ring with *n*-butyl lithium at low temperature. The anion was then reacted with iodomethane to form compound **33** in good yield. DiN-methylated urea **34** was obtained in an analogous fashion using increased equivalents of *n*-butyl lithium and iodomethane.

The final series of compounds synthesized aimed to decrease the lipophilicity by the introduction of oxygen containing substituents, as solubility issues were noted for some of the most promising compounds in preliminary testing of the first two series. The first compound targeted was a methyl *para*-amino salicylic ester substituted urea **36**, a known inhibitor of human sEH and EphB,[6] which has been shown to be orally bioavailable. Compound **36** was synthesized according to the published synthesis of T. Kasagami et al. [14–15]

Other oxygenated and potentially more soluble urea analogs **37–41** were synthesized according to Scheme 1. To complete the series we were interested in generating and testing compound **42,** the urea derivative and metabolite of the diphenylurea tuberculosis drug isoxyl. Compound **42** was synthesized by alkylating 4-nitrophenol with 1-bromo-4-methylpentane to produce the O-alkylated intermediate (Scheme 3). This nitro intermediate was then reduced by hydrogenolysis to produce the aniline intermediate. This compound was then reacted with triphosgene or thiophosgene to produce the **42** and **43** (Isoxyl) in acceptable yields.

3. Results and Discussion

Table 1 shows the *M. tuberculosis* MIC activity for the first series of compounds. In addition to whole cell phenotypic MIC screening we tested these compounds against pure recombinant EphB (Rv1938) and EphE (Rv3670). For the EphB and EphE enzyme inhibition assays, urea inhibitors were tested in duplicates at 10 nM against the fluorescent EphB and EphE substrate cyano(2-methoxynaphthalen-6-yl)methyl trans-(3-phenyl-oxyran-2-yl) methyl carbonate (Epoxy Fluor 7, Cayman Chemical, Ann Arbor, MI), at a substrate final concentration of 5 μM. The tables above show the results expressed as percent inhibition of the control reaction (containing solely EphB and substrate or EphE and substrate). IC_{50} values were determined against the mammalian sEH also using Cyano(2-

methoxynaphthalen-6-yl)methyl trans-(3-phenyl-oxyran-2-yl) methyl carbonate as the fluorescent substrate.[16]

Overall, these compounds show good activity primarily against *M. tuberculosis* (H37Rv). In this series, compounds **1**, **3**, **11**, **20**, **24** showed the best activity with an MIC value of 0.01 μg/mL that is comparable to the anti-tuberculosis drug isoniazid (MIC 0.01 μg/mL), which was used as a control. Interestingly, of these five compounds, only **1** and **24** inhibited EphB greater than 50% but all (**1**, **3**, **11**, **20**, **24)** showed >70% inhibition of EphE. This suggests that the potent *M. tuberculosis* MIC values are more closely correlated to EphE inhibition rather than EphB inhibition and that inhibition of multiple Eph enzyme isoforms is likely a contributing factor to the potent anti-tubercular activity of compounds in this series.

Some significant SAR emerged from this data. The substituent at the one position has a strong preference for the bulky aliphatic ring system such as adamantyl with the other urea substituent at the three position favoring an aryl ring. Substituting adamantyl of **1** for a cyclohexyl **8** or cyclopentyl **9** considerably decreased the activity, while a cyclooctyl **7** ring system only minimally decreased the activity to a level comparable to the drug ethambutol (0.8 μg/mL). Substitution in the aryl ring at the meta and/or para positions (Compounds **10–14**) were more tolerated with those substitutions not having a major impact on the anti-tuberculosis activity. In the group of compounds which contain the trifluorophenyl moiety (Compounds **1–9**), the adamantyl compounds **1** and **3** exhibit the best activity with potent MIC values of 0.01 μg/mL. With the exception of compounds **3** and **6**, all compounds **1–9** inhibited EphB greater than 50%. Within this series the bulky hydrophobic adamantyl group is preferred over a straight-chain alkyl group **4**. With regard to compounds that only contain aliphatic groups (Compounds **15–21**), the best MIC value is displayed by the compound with the n-hexyl alkyl chain **20**. Interestingly, compound **20** has a relatively poor EphB inhibition percentage at 32% but potent EphE inhibition at 100%. When comparing the compounds with the 3-chloro-4-methylphenyl substitution (Compounds **22–26**), compounds with a bulky alkyl rings on the opposite side (adamantylmethylene and cyclooctyl) result in the most active chemicals (**22** and **24**). This trend continues in the compounds in the 2-fluoro-3-(trifluoromethyl)phenyl arylurea series **27–30** further suggesting a reproducible SAR that favors bulky alkyl and aryl substituted ureas for maximal antituberculosis activity.

Table 2 shows the MIC data for the second series of compounds in which the central urea moiety was modified. In this series, it is clearly seen that the unmodified urea moiety is preferred for best anti-tuberculosis activity, human sEH and Eph inhibition. Introduction of a thiourea **31** produces a 80-fold decrease in MIC activity. Replacing the urea with a carbamate **32** produces an additional 10-fold larger decrease in anti-*M. tuberculosis* potency. Mono N-methylation of the urea **33** shows a similar decrease in activity and di-N-methylation **34** produces an even greater decrease in MIC activity. In this small library there is a clear correlation between anti-tuberculosis activity and Eph inhibition, strongly supporting our hypothesis that Eph enzymes are the anti-tubercular target of these inhibitors.

Table 3 shows the *M. tuberculosis* MIC and Eph data for the third series of oxygenated ureas designed in part to increase the solubility of this series and to expand the SAR. Compounds **35** and **36** showed good antitubercular activity while the remaining compounds **37**–**40** had much poorer activity. It is of interest to note that **35** and **36** were the only two in this series which contained the preferred SAR arrangement of having a bulky aliphatic ring on one side of the molecule and having an aromatic ring on the other. When comparing compounds **37** and **38** to aliphatic analog **19**, placing the oxygen in the alkyl chain dramatically decreases antitubercular activity. Similar trends were observed for EphB and EphE inhibition. Compound **41,** the urea analog and metabolite of thiourea tuberculosis drug thiocarlide **42**, was found to be inactive. This is in contrast to the comparison of compounds **1** and **31**,

where replacing the urea moiety with a thiourea decreased but did not eliminate all antitubercular activity demonstrating the contribution of the adamantyl and phenyl groups to the overall activity. This also suggests that thiocarlide and its metabolites act on a different target than the compounds selected here, in agreement with prior mode of action studies for thiocarlide.[12]

In this study, clear structure activity relationships (SAR) for anti-tubercular activity of urea-based inhibitors has emerged with the best compounds containing a 1-cycloalkyl-3-phenyl disubstituted urea structure. The 1–3 disubstituted urea moiety was shown to be most active over other urea substitution patterns and urea bioisosteres. For the cycloalkyl ring substituent, a strong preference for bulky groups such as adamantyl was preferred for anti-tubercular activity. This is consistent with previous findings by Biswal *et al*,[6] which indicates the importance of the adamantyl substituent in inhibiting *M. tuberculosis* EphB. Therefore, bulky groups such as adamantyl appear relevant for both enzyme and whole cell activities. This is noteworthy as other adamantyl analogs have been shown to have good anti-tubercular activity; most notably the clinical candidate adamantyl diamine SQ109 and adamantyl amides discovered through high throughput screening by the NIH sponsored TAACF program.[10, 17–18] Substitutions to the phenyl ring were well tolerated; suggesting further analogs of this position may be worthwhile.

Most compounds had some activity against EphB and EphE with some being so potent that they are close to the accurate limit of detection for our assay, further supporting our hypothesis that these urea-based compounds are readily recognized by EH enzymes. Fourteen compounds inhibited EphB >50% and twenty eight compounds inhibited EphE >50% at 10 nM. In general, compounds containing adamantane and the 2,3,4-trifluorophenyl were more active against EphB and EphE than the other compounds. Compounds **3**, **10**, **11**, **20**, **22** have good MIC values good EphE activity but relatively poor EphB inhibition suggesting that EphE inhibition is the major contributor to MIC activity. However, there are many putative EH enzymes encoded in the tuberculosis genome, suggesting that the highly potent phenotypic MIC values may be attributed to these compounds hitting multiple EH targets. Compounds in this series displayed varying cytotoxicity, which did not correlate with either antitubercular MIC or Eph inhibition. However, the most potent antitubercular compounds all had acceptable therapeutic indexes (Cytoxicity/MIC >100). Twenty-three of the forty-two compounds tested had significant inhibition of human sEH (<10nM), suggesting the need for the identification of new urea-based inhibitors possessing selectivity for *M. tuberculosis* EH enzymes over sEH enzymes in future studies.

The SAR and Eph inhibition data provide support that α/β-hydrolase fold EHs may be the actual targets of these ureas in *M. tuberculosis* due to the structural similarities of these compounds to human sEH inhibitors.[19] It therefore appears that urea hit **1** and derivatives competitively bind to the active site of EH enzymes to cause inhibition. By interacting with this conserved catalytic domain it is highly likely that these compounds inhibit multiple EH targets by binding in similar ways, which may explain their potent MIC activity. Future ligand design using the active site of model EH EphB may therefore act as a platform for future discoveries against the multiple EHs found in *M. tuberculosis*. This may lead to compounds with potent MIC activity that likely results from binding of the inhibitors to multiple related members of α/β-hydrolase fold family producing an additive lethal effect.[20]

Evidence for a targeted mode of action is bolstered by a lack of a pure correlation between lipophilicity and MIC against *M. tuberculosis*, suggesting that activity was not due to disruption of the lipophilic tubercular cell wall, though it does appear that the compounds do need to have adequate lipophilicity to enter the bacteria. Specificity of this series is also

supported by the most active compounds also exhibiting good activity against *Mycobacterium smegmatis* and the general lack of activity of most analogs against other gram-positive and –negative bacterial pathogens including *Staphylococcus aureus*, *Bacillus anthracis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (See Supplementary data). In general all urea-based compounds with potent MICs that were comparable to isoniazid and ethambutol (0.01–1.6 μg/mL), showed good therapeutic selectivity for *M. tuberculosis* with selectivity indices of 16.5->21,900. Importantly, this indicates that phenotypically selective antitubercular ureas may be obtained. Increasing solubility, metabolic stability and selectivity away from inhibition of human sEH are currently the principal design drivers that are being used to further optimize this series.

4. Experimental

4.1. Reagents and Instrumentation

All anhydrous solvents and starting materials were purchased from Aldrich Chemical Co. (Milwaukee, WI). All reagent grade solvents used from chromatography were purchased from Fisher Scientific (Suwanee, GA) and flash column chromatography silica cartridges were obtained from Biotage Inc. (Lake Forest, VA). The reactions were monitored by thin-layer chromatography (TLC) on pre-coated Merch 60 F_{254} silica gel plates and visualized using UV light (254 nm). A Biotage FLASH column chromatography system was used to purify mixtures. All ^{1}H NMR spectra were recorded on a Varian INOVA-500 spectrometer. Chemical shifts (δ) are reported in parts per million relative to the residual solvent peak or internal standard (tetramethylsilane), and coupling constants (*J*) are reported in hertz (Hz). High resolution mass spectra were recorded on a Waters Xevo G2 QTOF LCMS using ESI. For compounds 32 and 42 mass spectra were recorded on a Bruker Esquire LCMS using ESI. Purity of the products was confirmed before testing by analytical RP-HPLC on a Shimadzu HPLC system, and all final compounds had a purity of 95% or greater as determined by RP-HPLC. *Gradient Conditions M1*: solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in MeOH): 0–1.00 min 95% A, 1.00–6.00 min 0–95% B (linear gradient), 6.00–9.50 min 100% B, 9.50–9.75 min 0–95% A, 9.75–10.0 min 95% A, detection by UV at 254 nm and by ELSD. *Gradient Conditions M2*: same as M1 except solvent B is (0.1% formic acid in acetonitrile). Melting points were obtained on the OptiMelt MPA100 Automated Melting Point System.

4.2. Synthesis of Urea Compounds. General Method

In a round bottom flask equipped with a stir bar, 1.2 mmol of the appropriate amine was added to 10 mL of anhydrous dichloromethane. To this solution, 1.0 mmol of the appropriate isocyanate followed by 3.6 mmol of triethylamine was added. The reaction was stirred at room temperature overnight. The solvent was removed by rotary evaporation. The resulting residue was purified by flash chromatography using a petroleum ether to ethyl acetate gradient to elute the final compound.

4.2.1. 1-(2-Adamantyl)-3-(2,3,4-trifluorophenyl)urea (1)—Yield: 303 mg (93%); Mp: 229–230 °C; ^{1}H NMR (DMSO-d_6): δ 1.56 (1H, s), 1.59 (1H, s), 1.69–1.90 (12H, m), 3.78 (1H, d, J = 8.0 Hz), 6.96 (1H, d, J = 8.0 Hz), 7.16–7.24 (1H, m), 7.96 (1H, m), 8.51 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 325.1528, found: 325.1533.

4.2.2. 1-(1-Adamantyl)-3-(2,3,4-trifluorophenyl)urea (2)—Yield: 326 mg (81%); Mp: 216–219 °C; ^{1}H NMR (DMSO-d_6): δ 1.63 (6H, s), 1.93 (6H, s), 2.03 (3H, s), 6.44 (1H, s), 7.13–7.21 (1H, m), 7.84–7.92 (1H, m), 8.26 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 325.1528, found: 325.1533.

4.2.3. 1-(1-(1-Adamantyl)methyl)-3-(2,3,4-trifluorophenyl)urea (3)—Yield: 297 mg (88%); Mp: 171–173 °C; ^{1}H NMR (DMSO-d_6): δ 1.55–1.74 (12H, m), 1.90–1.95 (3H, m), 2.81 (2H, d, J = 6.0 Hz), 6.60 (1H, t, J = 5.5 Hz), 7.15–7.23 (1H, m), 7.88–7.96 (1H, m), 8.44 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 339.1685, found: 339.1670.

4.2.4. 1-Heptyl-3-(2,3,4-trifluorophenyl)urea (4)—Yield: 350 mg (98%); Mp: 96–98 °C; ^{1}H NMR (DMSO-d_6): δ 0.87 (3H, t, J = 6.5 Hz), 1.24 (8H, s), 1.36–1.46 (2H, m), 3.08 (2H, q, J = 6.5 Hz), 6.57 (1H, t, J = 5.0 Hz), 7.15–7.23 (1H, m), 7.81–7.88 (1H, m), 8.40 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 289.1528, found: 289.1545.

4.2.5. 1-(2,3,4-Trifluorophenyl)-3-(2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)urea (5)—Yield: 399 mg (99%); Mp: 156–159 °C; ^{1}H NMR (DMSO-d_6): δ 0.90 (1H, d, J = 9.5 Hz), 1.00 (3H, s), 1.06 (3H, d, J = 7.0 Hz), 1.20 (3H, s), 1.49–1.56 (1H, m), 1.75–1.82 (2H, m), 1.88–1.94 (1H, m), 2.34–2.49 (2H, m), 3.97 (1H, quintuplet, J = 8.0 Hz), 6.70 (1H, d, J = 8.0 Hz), 7.14–7.22 (1H, m), 7.84–7.92 (1H, m), 8.26 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 327.1685, found: 327.1694.

4.2.6. 1-((6,6-Dimethylbicyclo[3.1.1]heptan-2-yl)methyl)-3-(2,3,4-trifluorophenyl)urea (6)—Yield: 336 mg (83%); Mp: 105–107 °C; ^{1}H NMR (DMSO-d_6): δ 0.86 (1H, d, J = 9.5 Hz), 1.00 (3H, s), 1.17 (3H, s), 1.39–1.49 (1H, m), 1.78–1.94 (5H, m), 2.06–2.15 (1H, m), 2.29–2.36 (1H, m), 3.02–3.14 (2H, m), 6.62 (1H, t, J = 5.5 Hz), 7.14–7.22 (1H, m), 7.82–7.90 (1H, m), 8.38 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 327.1685, found: 327.1694.

4.2.7. 1-Cyclooctyl-3-(2,3,4-trifluorophenyl)urea (7)—Yield: 371 mg (99%); Mp: 160–163 °C; ^{1}H NMR (DMSO-d_6): δ 1.42–1.66 (13H, m), 1.76 (2H, t, J = 9.5 Hz), 6.63 (1H, d, J = 8.0 Hz), 7.14–7.22 (1H, m), 7.84–7.93 (1H, m), 8.31 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 301.1528, found: 301.1541.

4.2.8. 1-Cyclohexyl-3-(2,3,4-trifluorophenyl)urea (8)—Yield: 247 mg (63%); Mp: 188–191 °C; ^{1}H NMR (DMSO-d_6): δ 1.30–1.28 (3H, m), 1.28–1.37 (2H, m), 1.48–1.57 (1H, m), 1.59–1.70 (2H, m), 1.74–1.84 (2H, m), 3.42–3.52 (1H, m), 6.60 (1H, d, J = 9.0 Hz), 7.15–7.23 (1H, m), 7.85–7.93 (1H, m), 8.33 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 273.1215, found: 273.1203.

4.2.9. 1-Cyclopentyl-3-(2,3,4-trifluorophenyl)urea (9)—Yield: 265 mg (71%); Mp: 184–186 °C; ^{1}H NMR (DMSO-d_6): δ 1.30–1.43 (2H, m), 1.47–1.68 (4H, m), 1.77–1.89 (2H, m), 3.94 (1H, q, J = 6.5 Hz), 6.66 (1H, d, J = 7.5 Hz), 7.14–7.23 (1H, m), 7.84–7.94 (1H, m), 8.27 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 259.1059, found: 259.1046.

4.2.10. 1-(2-Adamantyl)-3-(4-cyanophenyl)urea (10)—Yield: 265 mg (90%); Mp: 217–219 °C; ^{1}H NMR (DMSO-d_6): δ 1.57 (1H, s), 1.60 (1H, s), 1.68–1.89 (12H, m), 3.78 (1H, d, J = 7.5 Hz), 6.67 (1H, d, J = 7.5 Hz), 7.55 (2H, d, J = 8.5 Hz), 7.67 (2H, d, J = 8.5 Hz), 8.92 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 296.1764, found: 296.1769.

4.2.11. 1-(2-Adamantyl)-3-phenethylurea (11)—Yield: 229 mg (77%); Mp: 115–117 °C; ^{1}H NMR (DMSO-d_6): δ 1.49 (1H, s), 1.51 (1H, s), 1.65–1.86 (12H, m), 2.68 (2H, t, J = 7.0 Hz), 3.24 (2H, q, J = 7.0 Hz), 3.67 (1 H, d, J = 8.0 Hz), 5.82 (1H, t, J = 5.5 Hz), 6.11 (1H, d, J = 8.5 Hz), 7.21 (3H, d, J = 6.0 Hz), 7.30 (2H, t, J = 7.5 Hz); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 299.2124, found: 299.2128.

4.2.12. 1-(3-Acetylphenyl)-3-(2-adamantyl)urea (12)—Yield: 212 mg (72%); Mp: 213–215 °C; ^{1}H NMR (DMSO-d_6): δ 1.57 (1H, s), 1.60 (1H, s), 1.69–1.91 (13H, m), 2.55 (3H, s), 3.79 (1H, d, J = 7.5 Hz), 6.50 (1H, d, J = 7.5 Hz), 7.38 (1H, t, J = 8.0 Hz), 7.50 (1H, d, J = 8.0 Hz), 7.58 (1H, d, J = 8.0 Hz), 8.00 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 313.1917, found: 313.1909.

4.2.13. 1-(2-Adamantyl)-3-benzylurea (13)—Yield: 234 mg (81%); Mp: 205–207 °C; ^{1}H NMR (DMSO-d_6): δ 1.51 (1H, s), 1.53 (1H, s), 1.66–1.88 (12H, m), 3.70 (1H, d, J = 8.0 Hz), 4.21 (2H, d, J = 6.0 Hz), 6.18 (1H, d, J = 8.0 Hz), 6.27 (1H, t, J = 6.0 Hz), 7.24 (3H, q, J = 7.5 Hz), 7.33 (2H, t, J = 7.5 Hz); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 285.1968, found: 285.1974.

4.2.14. 1-(2-Adamantyl)-3-(3-chloro-2-methylphenyl)urea (14)—Yield: 261 mg (82%); Mp: 254–257 °C; ^{1}H NMR (DMSO-d_6): δ 1.57 (1H, s), 1.59 (1H, s), 1.70–1.86 (10H, m), 1.91 (2H, d, J = 12.5 Hz), 2.26 (3H, s), 3.79 (1H, d, J = 7.5 Hz), 6.91 (1H, d, J = 8.0 Hz), 7.03 (1H, d, J = 7.5 Hz), 7.10 (1H, t, J = 8.0 Hz), 7.84–7.91 (2H, m); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 319.1578, found: 319.1569.

4.2.15. 1-(2-Adamantyl)-3-isopropylurea (15)—Yield: 162 mg (69%); Mp: 240–242 °C; ^{1}H NMR (DMSO-d_6): δ 1.02 (6H, d, J = 6.5 Hz), 1.49 (1H, s), 1.52 (1H, s), 1.66–1.85 (12H, m), 3.59–3.68 (2H, m), 5.66 (1H, d, J = 7.5 Hz), 5.95 (1H, d, J = 8.0 Hz); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 237.1968, found: 237.1964.

4.2.16. 1-(2-Adamantyl)-3-*tert*-butylurea (16)—Yield: 185 mg (74%); Mp: >300 °C; ^{1}H NMR (DMSO-d_6): δ 1.22 (9H, s), 1.49 (1H, s), 1.52 (1H, s), 1.65–1.86 (12H, m), 3.64 (1H, d, J = 8.0 Hz), 5.70 (1H, s), 5.95 (1H, d, J = 8.5 Hz); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 251.2124, found: 251.2121.

4.2.17. 1-(2-Adamantyl)-3-propylurea (17)—Yield: 199 mg (84%); Mp: 194–196 °C; ^{1}H NMR (DMSO-d_6): δ 0.84 (3H, t, J = 7.0 Hz), 1.37 (2H, sextuplet, J = 7.5 Hz), 1.49 (1H, s), 1.52 (1H, s), 1.66–1.86 (12H, m), 2.94 (2H, q, J = 7.0 Hz), 3.66 (1H, d, J = 8.0 Hz), 5.81 (1H, t, J = 5.5 Hz), 6.02 (1H, d, J = 8.0 Hz); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 237.1968, found: 237.1964.

4.2.18. 1-(2-Adamantyl)-3-cyclohexylurea (18)—Yield: 234 mg (85%); Mp: >300 °C; ^{1}H NMR (DMSO-d_6): δ 1.01–1.32 (5H, m), 1.49 (1H, s), 1.52 (1H, s), 1.58–1.86 (17H, m), 3.65 (1H, d, J = 7.5 Hz), 5.75 (1H, d, J = 8.0 Hz), 5.98 (1H, d, J = 8.5 Hz); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 277.2281, found: 277.2284.

4.2.19. 1-(2-Adamantyl)-3-pentylurea (19)—Yield: 246 mg (93%); Mp: 151–153 °C; ^{1}H NMR ($CDCl_3$): δ 0.93 (3H, t, J = 6.5 Hz), 1.30–1.39 (4H, m), 1.53 (2H, quintet, J = 7.5 Hz), 1.62 (2H, s), 1.76 (2H, s), 1.80 (1H, s), 1.86 (7H, t, J = 11.0 Hz), 1.93 (2H, s), 3.19 (2H, q, J = 7.0 Hz), 3.81 (1H, s), 4.26 (1H, s), 4.64 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 265.2281, found: 265.2297.

4.2.20. 1-(2-Adamantyl)-3-hexylurea (20)—Yield: 200 mg (71%); Mp: 95–98 °C; ^{1}H NMR (DMSO-d_6): δ 0.87 (3H, t, J = 6.5 Hz), 1.21–1.39 (8H, m), 1.49 (1H, s), 1.52 (1H, s), 1.66–1.86 (12H, m), 2.97 (2H, q, J = 6.5 Hz), 3.65 (1H, d, J = 8.0 Hz), 5.78 (1H, t, J = 5.5 Hz), 6.01 (1H, d, J = 8.5 Hz); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 279.2437, found: 279.2443.

4.2.21. 1-(2-Adamantyl)-3-heptylurea (21)—Yield: 292 mg (85%); Mp: 83–86 °C; ^{1}H NMR ($CDCl_3$): δ 0.90 (3H, t, *J* = 6.0 Hz), 1.24–1.38 (8H, m), 1.52 (2H, t, *J* = 6.5 Hz), 1.48–1.56 (2H, m), 1.75 (2H, s), 1.77 (1H, s), 1.86 (7H, t, *J* = 11.0 Hz), 1.93 (2H, s), 3.18 (2H, q, *J* = 6.5 Hz), 3.81 (1H, s), 4.34 (1H, s), 4.70 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 293.2594, found: 293.2582.

4.2.22. 1-(1-(1-Adamantyl)methyl)-3-(3-chloro-4-methylphenyl)urea (22)—Yield: 301 mg (91%); Mp: 183–185 °C; ^{1}H NMR (DMSO-d_6): δ 1.45 (5H, s), 1.56–1.76 (7H, m), 1.95 (3H, s), 2.23 (3H, s), 2.79 (2H, d, *J* = 6.0 Hz), 6.30–6.38 (1H, m), 7.04–7.10 (1H, m), 7.17 (1H, d, *J* = 8.0 Hz), 7.65–7.71 (1H, m), 8.46 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 333.1734, found: 333.1730.

4.2.23. 1-(3-Chloro-4-methylphenyl)-3-heptylurea (23)—Yield: 266 mg (90%); Mp: 99–101 °C; ^{1}H NMR (CD_3OD): δ 0.93 (3H, t, *J* = 6.5 Hz), 1.29–1.42 (8H, m), 1.50–1.58 (2H, m), 2.30 (3H, s), 3.19 (2H, t, *J* = 6.5 Hz), 7.09–7.17 (2H, m), 7.53 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 283.1578, found: 283.1566.

4.2.24. 1-(3-Chloro-4-methylphenyl)-3-cyclooctylurea (24)—Yield: 277 mg (94%); Mp: 179–182 °C; ^{1}H NMR (DMSO-d_6): δ 1.42–1.68 (14H, m), 2.23 (3H, s), 3.65–3.73 (1H, m), 6.13 (1H, d, *J* = 8.0 Hz), 7.05 (1H, d, *J* = 8.0 Hz), 7.16 (1H, d, *J* = 8.0 Hz), 7.65 (1H, s), 8.35 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 295.1578, found: 295.1555.

4.2.25. 1-(3-Chloro-4-methylphenyl)-3-(3-fluorobenzyl)urea (25)—Yield: 235 mg (80%); Mp: 146–148 °C; ^{1}H NMR (CD_3OD): δ 2.30 (3H, s), 4.40 (2H, s), 7.00 (1H, t, *J* = 8.5 Hz), 7.08 (1H, d, *J* = 10.0 Hz), 7.12–7.19 (3H, m), 7.35 (1H, q, *J* = 6.5 Hz), 7.54 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 293.0858, found: 293.0864.

4.2.26. 1-(3-Chloro-4-methylphenyl)-3-(4-phenylbutan-2-yl)urea (26)—Yield: 245 mg (78%); Mp: 111–113 °C; ^{1}H NMR (CD_3OD): δ 1.20 (3H, d, *J* = 6.5 Hz), 1.77 (2H, q, *J* = 7.0 Hz), 2.29 (3H, s), 2.62–2.76 (2H, m), 3.78–3.86 (1H, m), 7.08–7.31 (7H, m), 7.54 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 317.1421, found: 317.1401.

4.2.27. 1-(2-Fluoro-3-(trifluoromethyl)phenyl)-3-heptylurea (27)—Yield: 385 mg (87%); Mp: 77–79 °C; ^{1}H NMR (DMSO-d_6): δ 0.86 (3H, t, *J* = 6.5 Hz), 1.20–1.33 (8H, m), 1.43 (2H, t, *J* = 6.5 Hz), 3.10 (2H, q, *J* = 6.5 Hz), 6.67 (1H, t, *J* = 5.5 Hz), 7.23–7.32 (2H, m), 8.42 (1H, t, *J* = 7.0 Hz), 8.55 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 321.1591, found: 321.1581.

4.2.28. 1-Cyclooctyl-3-(2-fluoro-3-(trifluoromethyl)phenyl)urea (28)—Yield: 442 mg (96%); Mp: 127–129 °C; ^{1}H NMR ($CDCl_3$): δ 1.54–1.79 (14H, m), 1.85–1.94 (2H, m), 3.82–3.90 (1H, m), 7.25 (2H, d), 8.33–8.40 (1H, m); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 333.1591, found: 333.1580.

4.2.29. 1-(2-Fluoro-3-(trifluoromethyl)phenyl)-3-(4-phenylbutan-2-yl)urea (29)—Yield: 463 mg (95%); Mp: 145–148 °C; ^{1}H NMR (DMSO-d_6): δ 1.13 (3H, d, *J* = 6.5 Hz), 1.71 (2H, d, *J* = 7.0 Hz), 2.62 (2H, d, *J* = 7.5 Hz), 3.64–3.74 (1H, m), 6.68 (1H, d, *J* = 7.0 Hz), 7.13–7.32 (7H, m), 8.40–8.52 (2H, m); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 355.1434, found: 355.1419.

4.2.30. 1-(3-Chlorobenzyl)-3-(2-fluoro-3-(trifluoromethyl)phenyl)urea (30)—Yield: 451 mg (94%); Mp: 152–153°C; ^{1}H NMR (DMSO-d_6): δ 4.35 (2H, d, *J* = 6.0 Hz),

7.22 (1H, t, *J* = 5.5 Hz), 7.26–7.35 (4H, m), 7.36–7.41 (2H, m), 8.40–8.46 (1H, m), 8.76 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 347.0575, found: 347.0589.

4.2.31. 1-(2-Adamantyl)-3-(4-methoxyphenyl)urea (35)—Yield: 250 mg (83%); Mp: 125–128 °C; ^{1}H NMR (DMSO-d_6): δ 1.56 (1H, s), 1.58 (1H, s), 1.68–1.90 (12H, m), 3.70 (3H, s), 3.76 (1H, d, *J* = 8.0 Hz), 6.34 (1H, d, *J* = 8.0 Hz), 6.82 (2H, d, *J* = 8.5 Hz), 7.28 (2H, d, *J* = 9.0 Hz), 8.20 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 301.1917, found: 301.1932.

4.2.32. Methyl 4-(3-(1-adamantyl)ureido)-2-hydroxybenzoate (36)—Yield: 850 mg (41%); Mp: 207–210 °C; ^{1}H NMR ($CDCl_3$): δ 1.68 (6H, s), 2.02–2.16 (9H, m), 3.90 (3H, s), 4.73 (1H, s), 6.62 (1H, s), 6.83 (1H, s), 6.98 (1H, d, *J* = 9 Hz), 7.70 (1H, d, *J* = 8.5 Hz), 10.83 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 345.1815, found: 345.1801.

4.2.33. 1-(1-Adamantyl)-3-(2-ethoxyethyl)urea (37)—Yield: 249 mg (94%); Mp: 116–118 °C; ^{1}H NMR ($CDCl_3$): δ 1.23 (3H, t, *J* = 7.0 Hz), 1.69 (6H, s), 1.98 (6H, s), 2.09 (3H, s), 3.33 (2H, q, *J* = 5.0 Hz), 3.49–3.56 (4H, m), 4.41 (1H, s), 4.56 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 267.2073, found: 267.2083.

4.2.34. 1-(1-Adamantyl)-3-(3-methoxypropyl)urea (38)—Yield: 251 mg (94%); Mp: 135–137 °C; ^{1}H NMR ($CDCl_3$): δ 1.69 (6H, s), 1.77 (2H, quintet, *J* = 6.0 Hz), 1.98 (6H, s), 2.09 (3H, s), 3.25 (2H, q, *J* = 6.0 Hz), 3.36 (3H, s), 3.49 (2H, t, *J* = 5.5 Hz); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 267.2073, found: 267.2083.

4.2.35. 1-(1-Adamantyl)-3-(2-isopropoxyethyl)urea (39)—Yield: 244 mg (87%); Mp: 92–95 °C; ^{1}H NMR ($CDCl_3$): δ 1.18 (6H, d, *J* = 6.0 Hz), 1.68 (6H, s), 1.98 (6H, s), 2.08 (3H, s), 3.30 (2H, q, *J* = 5.5 Hz), 3.50 (2H, t, *J* = 5 Hz), 3.61 (1H, m), 4.50 (1H, s), 4.61 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 281.2230, found: 281.2228.

4.2.36. 1-(1-Adamantyl)-3-(2-propoxyethyl)urea (40)—Yield: 271 mg (97%); Mp: 87–90 °C; ^{1}H NMR ($CDCl_3$): δ 0.95 (3H, t, *J* = 7.5 Hz), 1.58–1.66 (2H, m), 1.69 (6H, s), 1.98 (6H, s), 2.09 (3H, s), 3.33 (2H, q, *J* = 5.0 Hz), 3.42 (2H, t, *J* = 6.5 Hz), 3.50 (2H, t, *J* = 5.0 Hz), 4.39 (1H, s), 4.54 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 281.2230, found: 281.2228.

4.3. 1-(2-Adamantyl)-3-(2,3,4-trifluorophenyl)thiourea (31)

In a round bottom flask equipped with a stir bar, 2-adamantanamine (0.24 g, 1.3 mmol) was dissolved in 10 mL of anhydrous dichloromethane. To this solution, 2,3,4-trifluorophenyl isothiocyanate (0.14 mL, 1.1 mmol) followed by triethylamine (0.53 mL, 3.8 mmol) was added. The reaction was stirred at room temperature overnight. The solvent was removed by rotary evaporation. The resulting residue was purified by flash chromatography using a petroleum ether to ethyl acetate gradient to elute the final compound (330 mg, 92%). Mp: 176–179 °C; ^{1}H NMR (DMSO-d_6): δ 1.61 (1H, s), 1.64 (1H, s), 1.67–1.83 (10H, m), 2.01 (2H, s), 4.33 (1H, s), 7.23–7.31 (1H, m), 7.66 (1H, s), 8.19 (1H, s), 9.30 (1H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 341.1300, found: 341.1314.

4.4. 2-Adamantyl 2,3,4-trifluorophenylcarbamate (32)

In a round bottom flask equipped with a stir bar, 2-adamantanol (0.42 g, 2.8 mmol) was dissolved in 10 mL of anhydrous dichloromethane. To this solution, 2,3,4-trifluorophenyl isocyanate (2.8 mL, 2.3 mmol) followed by triethylamine (1.2 mL, 8.3 mmol) was added. The reaction was stirred at room temperature overnight. The solvent was removed by rotary evaporation. The resulting residue was purified by flash chromatography using a petroleum

ether to ethyl acetate gradient to elute the final compound (727 mg, 97%). Mp: 114–116 °C; ^{1}H NMR (DMSO-d_6): δ 1.52 (1H, s), 1.56 (1H, s), 1.67–1.90 (10H, m), 2.00 (1H, s), 4.78 (1H, s), 7.22–7.35 (1H, m), 7.38–7.51 (1H, m), 9.46 (1H, s); ESI-MS *m/z*: 348.1 $[M + Na]^+$.

4.5. 1-(2-Adamantyl)-3-methyl-3-(2,3,4-trifluorophenyl)urea (33)

To a round bottom flask containing **1** (0.20 g, 0.62 mmol), anhydrous THF (10 mL) was added under argon. The solution was then cooled to −78°C. To the cold solution, *n*-butyl lithium (0.39 mL, 0.62 mmol) was added drop wise and allowed to stir at −78°C for 10 min. To this solution, iodomethane (0.038 mL, 0.62 mmol) was added drop wise at −78°C. The solution was then allowed to warm to room temperature and then refluxed for 24 hrs. The solvent was then removed by rotary evaporation. The resulting residue was purified by flash chromatography using a petroleum ether to ethyl acetate gradient to elute the final compound as an oil (192 mg, 92%). ^{1}H NMR (DMSO-d_6): δ 1.44 (1H, s), 1.47 (1H, s), 1.65–1.84 (10H, m), 1.86 (2H, s), 3.14 (3H, s), 370 (1H, s), 5.62 (1H, d, J = 5.5 Hz), 7.26–7.31 (1H, m), 7.31–7.38 (1H, m); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 339.1685, found: 339.1670.

4.6. 1-(2-Adamantyl)-1,3-dimethyl-3-(2,3,4-trifluorophenyl)urea (34)

To a round bottom flask containing **1** (0.40 g, 1.2 mmol), anhydrous THF (25 mL) was added under argon. The solution was then cooled to −78°C. To the cold solution, *n*-butyl lithium (1.5 mL, 2.5 mmol) was added drop wise and allowed to stir at −78°C for 10 min. To this solution, iodomethane (0.15 mL, 2.5 mmol) was added drop wise at −78°C. The solution was then allowed to warm to room temperature and then refluxed for 24 hrs. The solvent was then removed by rotary evaporation. The resulting residue was purified by flash chromatography using a petroleum ether to ethyl acetate gradient to elute the final compound (190 mg, 44%). Mp: 71–74 °C; ^{1}H NMR (DMSO-d_6): δ 1.50 (1H, s), 1.52 (1H, s), 1.66–1.84 (10 H, m), 2.18 (2H, s), 2.66 (3H, s), 3.11 (3H, s), 3.61 (1H, s), 7.20–7.26 (1H, m), 7.34–7.41 (1H, m); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 353.1841, found: 353.1837.

4.7. 1,3-Bis(4-(isopentyloxy)phenyl)urea (41)

To a round-bottom flask, 4-nitrophenol (2.0 g, 14 mmol) and sodium hydroxide (1.7 g, 43 mmol), was dissolved in N,N-dimethylformamide (50 mL). The solution was stirred at room temperature for 15 min. After stirring, 1-bromo-4-methylpentane (5.4 mL, 43 mmol) was added to the solution and refluxed for 3 h. The resulting solution was washed with 0.7 N HCl (3×20 mL) then extracted with diethyl ether (3×20 mL). The combined organic fractions were then washed with brine (3×20 mL) and dried over magnesium sulfate. The solution was then filtered and the solvent by using rotary evaporation. The crude mixture was then purified by flash chromatography using a petroleum ether to ethyl acetate gradient to obtain pure intermediate (2.69 g, 90%). The intermediate nitro compound was then converted to an aniline by hydrogenation using Pd/C catalyst overnight. The aniline was used in the next step without further purification (1.85 g, 80%). In a round bottom flask, the aniline (1.27 g, 7.08 mmol) was dissolved in dichloromethane and then triethylamine (2.0 mL, 14 mmol) was added and the solution was allowed to stir at room temperature for 15 min. In a separate round bottom flask, triphosgene (0.70 g, 2.4 mmol) was dissolved in dichloromethane. Then the solution of the aniline was slowly added into the solution of thiophosgene under argon and allowed to stir overnight at room temperature. The product (301 mg, 33%) crashed out of solution and was filtered off and washed with dichloromethane. Mp: 173–175 °C; ^{1}H NMR (DMSO-d_6): δ 0.93 (12H, d, J = 6.5 Hz), 1.59 (4H, q, J = 6.5 Hz), 1.72–1.82 (2H, m), 3.94 (4H, t, J = 6.5 Hz), 6.85 (4H, d, J = 9.0 Hz), 7.31 (4H, d, J = 9.0 Hz), 8.35 (2H, s); ESI-HRMS *m/z*: $[M + H]^+$ calculated: 385.2492, found: 385.2501.

4.8. 1,3-Bis(4-(isopentyloxy)phenyl)thiourea (Isoxyl) (42)

To a round-bottom flask, 4-nitrophenol (2.0 g, 14 mmol) and sodium hydroxide (1.7 g, 43 mmol), was dissolved in N,N-dimethylformamide (50 mL). The solution was stirred at room temperature for 15 min. After stirring, 1-bromo-4-methylpentane (5.4 mL, 43 mmol) was added to the solution and refluxed for 3 h. The resulting solution was washed with 0.7 N HCl (3×20 mL) then extracted with diethyl ether (3×20 mL). The combined organic fractions were then washed with brine (3×20 mL) and dried over magnesium sulfate. The solution was then filtered and the solvent by using rotary evaporation. The crude mixture was then purified by flash chromatography using a petroleum ether to ethyl acetate gradient to obtain pure intermediate (2.69 g, 90%). The intermediate nitro compound was then converted to an aniline by hydrogenation using Pd/C catalyst overnight. The aniline was used in the next step without further purification (1.85 g, 80%). In a round bottom flask, the aniline (1.9 g, 11 mmol) was dissolved in dichloromethane and then triethylamine (2.9 mL, 21 mmol) was added and the solution was allowed to stir at room temperature for 15 min. In a separate round bottom flask, thiophosgene (0.26 mL, 3.4 mmol) was dissolved in dichloromethane. Then the solution of the aniline was slowly added into the solution of thiophosgene under argon and allowed to stir overnight at room temperature. The product (683 mg, 50%) crashed out of solution and was filtered off and washed with dichloromethane. Mp: 139–141 °C; ^{1}H NMR (DMSO-d_6): δ 0.94 (12H, d, J = 7.0 Hz), 1.61 (4H, q, J = 7.0 Hz), 1.74–1.84 (2H, m), 3.98 (4H, t, J = 7.0 Hz), 6.90 (4H, d, J = 9.0 Hz), 7.10 (4H, d, J = 9.0 Hz), 9.45 (2H, s); ESI-MS *m/z*: 423.2 $[M + Na]^+$.

4.9. MIC determination

MIC values were determined against *M. tuberculosis* (H37Rv) and other bacteria by the microbroth dilution method.[21] A broth culture of *M. tuberculosis* or *M. smegmatis* mc^{2}155 was grown in Middlebrook 7H9 medium with 10% ADC supplement to an OD_{600} of 0.4–0.6. The culture was diluted with 7H9 medium to an OD_{600} of 0.01, and 100μL was added to a microtiter plate containing two-fold serial dilutions of the tested compounds for a final volume of 200μL. The plates were incubated at 37 C for 7 days. The MIC_{90} was determined by visual inspection and defined as the concentration that inhibited 90% of growth. MICs against other bacteria were performed according to the CLSI method and also read by visual inspection.[21–22]

4.10. Cytotoxicity study

Cytotoxicity assays were performed using the Vero monkey kidney cell line (CCL-81) obtained from the American Type Culture Collection (ATCC, Manassas, VA). Vero cells were propagated in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum and maintained in a humidified incubator (37°C, 5% CO_2). After dislodging cells with a cell scraper, they were collected by centrifugation, resuspended in fresh medium at ~10^6 cells/mL, dispensed into 96-well microtiter plates (100 μl/well) and incubated for 18 h at 37°C before being used for cytotoxicity assays. Test compounds were subsequently added at concentrations ranging from 400-0.2 μg/mL and incubation continued for another 72 h before the cytopathic effects of compounds was determined using the MTT Cell Proliferation Assay (ATCC). The cytotoxic IC_{50} defined as the concentration causing 50% reduction in Vero cell viability, was obtained from a dose response curve plotted from percentage activity versus $\log_{10}$ concentration. Therapeutic selectivity was then determined as the Cytotoxic IC_{50} divided by the MIC against *M. tuberculosis*.

4.11. EphB and EphE inhibition determination

The fluorescent assay used to measure the inhibition of EphB (Rv1938) and EphE (Rv3670) by urea derivatives was similar to that described by Biswal and colleagues and used the

fluorescent substrate cyano(6-methoxy-2-napthalenyl)methyl[(2,3)-3-phenyloxiranyl]methyl ester carbonate (Epoxy Fluor 7 from Cayman Chemical, Ann Arbor, MI).[6] Briefly, the purified EphB protein (0.8 μg per well; [E] = 106 nM)[6] or crude extracts prepared from *E. coli* BL21AI/pGEFII-EphE expressing *ephE* (a kind gift from Drs. M. Arand and A. Cronin, University of Zurich, Switzerland) (10 μg; estimated [E] = 10 to 15 nM) were pre-incubated with inhibitors ([I] = 10 nM in 1% DMSO final concentration) for 10 min in Bis-Tris-HCl buffer (25 mM, pH 7.0, containing 0.1 mg/ml of bovine serum albumin) at 30°C prior to substrate introduction ([S] = 5 μM). All assays were run for 30 min at 30°C and performed in duplicate. Hydrolysis of the substrate epoxide was monitored at excitation and emission wavelengths of 320 and 460 nm, respectively. Results are expressed as percentage inhibition of the control reaction containing no inhibitor. *E. coli* BL21AI extracts not expressing EphE showed no activity under the assay conditions used here.

4.12. sHE inhibition study

IC50 values were determined using a sensitive fluorescent based assay.[16] Cyano(2-methoxynaphthalen-6-yl)methyl trans-(3-phenyl-oxyran-2-yl) methyl carbonate (CMNPC) was used as the fluorescent substrate. Purified recombinant Human sEH (1nM)[23] was incubated with the inhibitor for 5 min in pH 7.4 sodioum phosphate buffer (100mM) containing 0.1mg/mL of BSA at 30°C prior to substrate introduction ([S] = 5μM). Activity was determined by monitoring the appearance of 6-methoxy-2-naphthaldehyde over 10 minutes by fluorescence detection with an excitation wavelength of 330 nm and an emission wavelength of 465 nm. Reported IC50 values are the average of three replicates with at least two datum points above and at least two below the IC50. The fluorescent assay as performed here has a standard error between 10 and 20%, suggesting that differences of two-fold or greater are significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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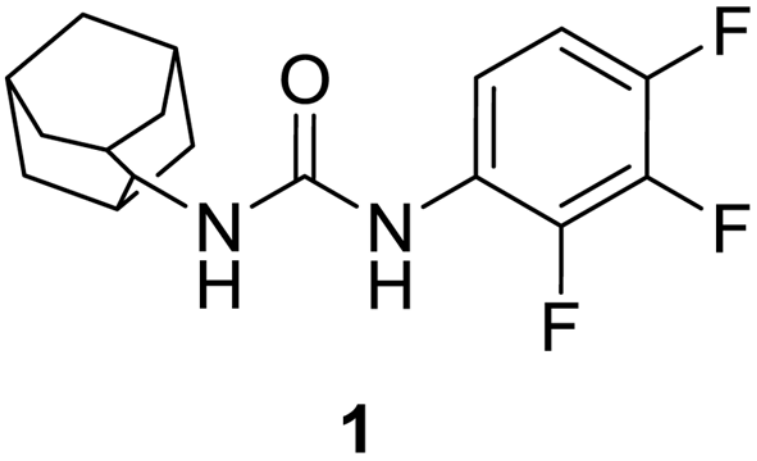
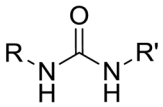


1

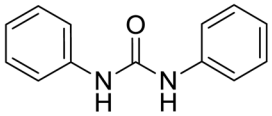
Figure 1.
Lead Compound **1**.

A.

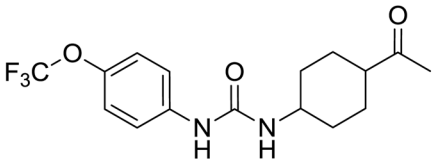


R and R' = Alkyl (linear and cyclic) and Aryl (substituted and non-substituted).

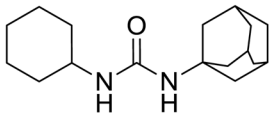
B.



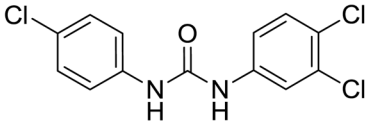
EphB = 19 nM
sEH = 390 nM



EphB = 31,600 nM
sEH = 12 nM



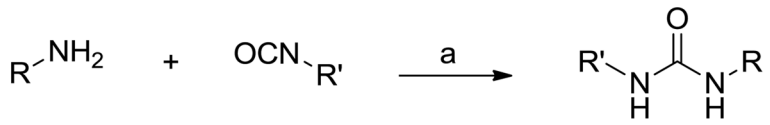
EphB = 130 nM
sEH = 2 nM



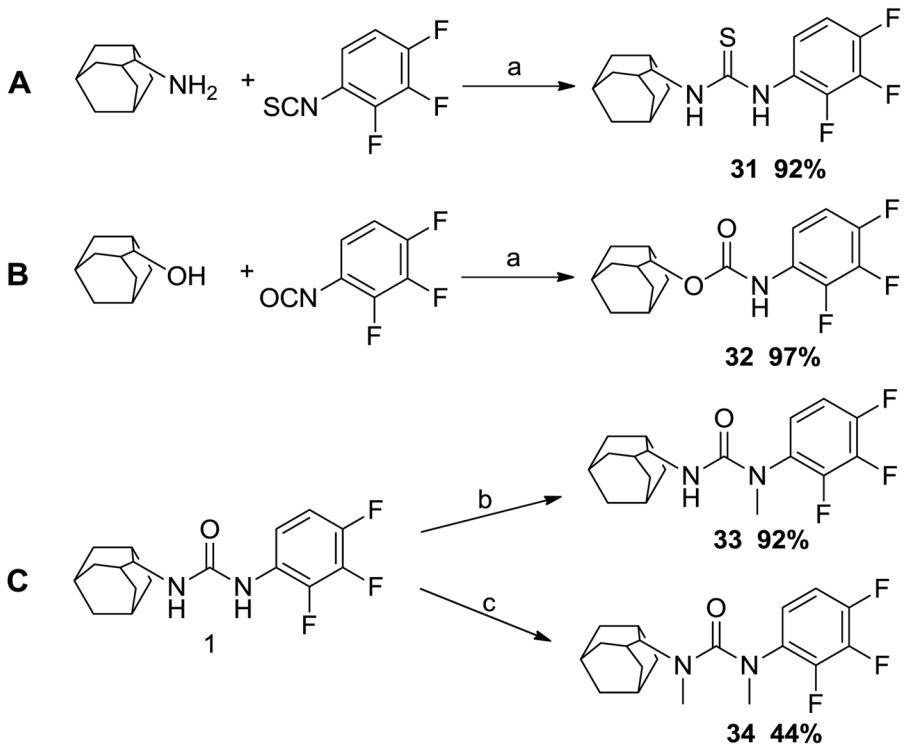
EphB = 220 nM
sEH = 13 nM

Figure 2.

A) Summary of current urea-based sEH and *M. tuberculosis* EphB inhibitors.[6–7] B) Specific examples of urea-based sEH *M. tuberculosis* EphB inhibitors with IC_{50} values.[6–7]

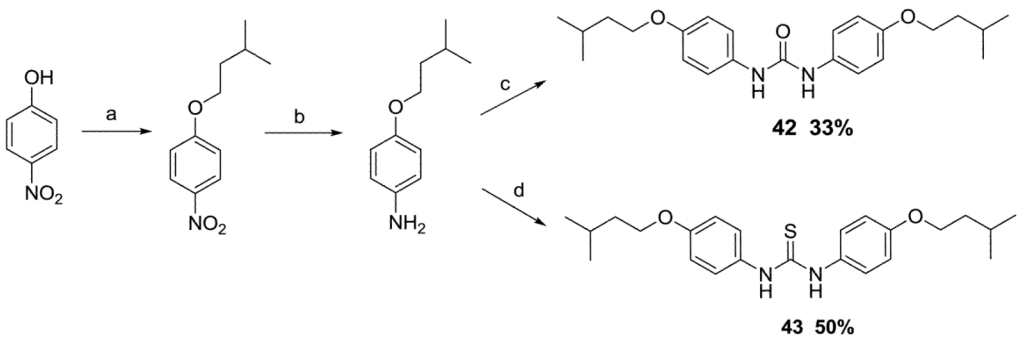


Scheme 1.
Reagents: (a) triethylamine, dry dichloromethane, room temperature, overnight.



Scheme 2.

Reagents: (a) TEA, dry DCM, room temperature, overnight; (b) (i) *n*-BuLi, dry THF, −78 C; (ii) iodomethane, reflux, 24 hr; (c) (i) *n*-BuLi, dry THF, −78 C; (ii) iodomethane, reflux, 24 hr.



Scheme 3.

Reagents: (a) 1-bromo-4-methylpentane, NaOH, DMF, 65 C, overnight; (b) Pd/C, H_2, EtOH, overnight; (c) triphosgene, TEA, DCM, room temperature, overnight; (d) thiophosgene, TEA, DCM, room temperature, overnight.

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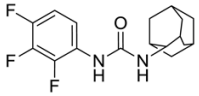
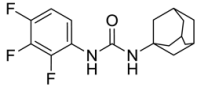
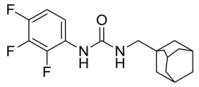
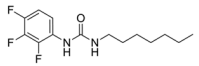
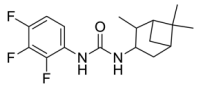
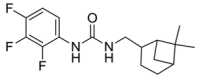
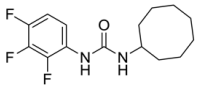
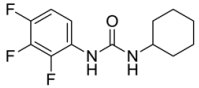
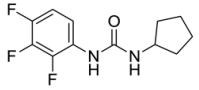
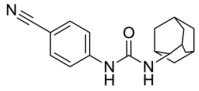
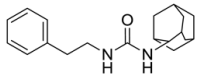
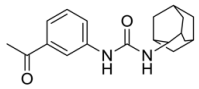
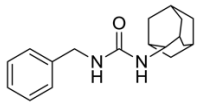
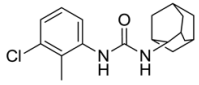
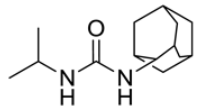
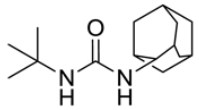
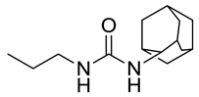
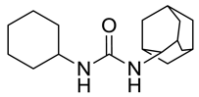
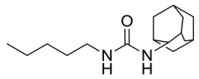
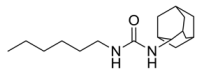
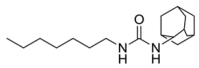
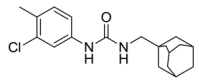
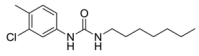
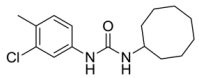
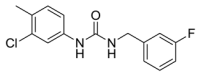
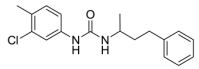
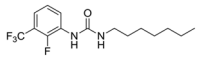
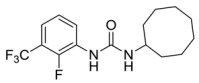
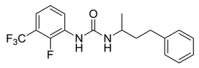
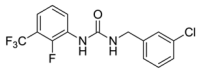
Table 1

No.	Structure	*M. tb* MIC (μg/mL)	Cytotox (μg/mL)	% EphB Inhibition at 10 nM	% EphE Inhibition at 10 nM	Human sEH IC_{50} (nM)
1		0.01	219	72.8	71.4	0.4
2		0.4	725.8	89.7	92.2	0.4
3		0.01	1.18	44.2	90.8	0.4
4		6	129.7	87.5	92.8	0.4
5		0.02	16	76.3	86.1	0.4
6		0.2	15.5	38.6	102.9	0.4
7		0.8	63.4	95.7	91.6	0.4
8		12.5	15.9	96.4	65.6	1.2
9		50	nd[c]	86.4	36.0	56.2
10		0.4	7.1	9.0	72.7	1.1
11		0.01	30.2	34.1	87.9	4.5

No.	Structure	*M. tb* MIC (μg/mL)	Cytotox (μg/mL)	% EphB Inhibition at 10 nM	% EphE Inhibition at 10 nM	Human sEH IC_{50} (nM)
12		0.1	9.7	80.6	31.4	0.5
13		3.1	4.2	ni[d]	34.1	14.8
14		1.6	26.4	9.8	58.3	4.5
15		50	23.4	27.2	57.3	84.4
16		12.5	371.9	20.7	55.4	24.2
17		12.5	170.9	51.9	73.6	61.4
18		10	25	11.6	70.2	0.4
19		0.39	11.6	58.6	90.5	6.4
20		0.01	174.8	32.3	100.4	1.0
21		0.2	20	37.1	99.9	0.5
22		0.02	4.34	33.9	94.1	0.4

No.	Structure	*M. tb* MIC (μg/mL)	Cytotox (μg/mL)	% EphB Inhibition at 10 nM	% EphE Inhibition at 10 nM	Human sEH IC_{50} (nM)
23		3.1	186.4	91.0	99.3	1.6
24		0.01	>400	83.6	98.2	0.4
25		6	342.3	18.6	19.0	16.6
26		6	>400	81.9	97.4	15.0
27		3	nd	5.5	46.8	0.4
28		0.8	25.3	17.0	56.3	0.4
29		1.6	38.5	ni	63.9	1.9
30		1.6	193.2	16.6	15.5	3.7

[a] In vitro antituberculosis activity of urea compounds against *M. tuberculosis* H37Rv;

[b] Cytotoxicity was determined against mammalian Vero cell line;

[c] nd = not determined;

[d] ni = no inhibition

Table 2

In vitro antitubercular activity of compounds bearing extra substitutions to the urea core.

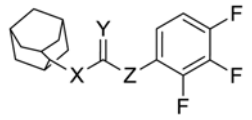
No.				*M. tb* MIC (μg/mL)	% EphB Inhibition at 10 nM	% EphE Inhibition at 10 nM	Human sEH IC_{50} (nM)
	X	**Y**	**Z**				
1	NH	O	NH	0.01	72.8	71.4	0.4
31	NH	S	NH	0.8	21.3	35.5	4.8
32	O	O	NH	6	10.2	21.4	463.8
33	NH	O	NMe	6	6.7	22.8	38.1
34	NMe	O	NMe	12	< 1	25.2	1838.1

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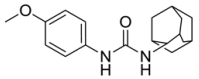
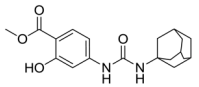
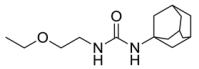
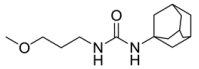
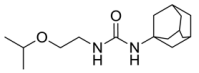
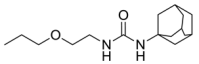
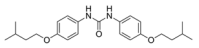
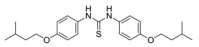
Table 3

In vitro Antituberculosis Activity and Cytotoxicities of Oxygenated Urea Compounds.

No.	Structure	M. tb MIC (μg/mL)	Cytotox (μg/mL)	% EphB Inh. at 10 nM	% EphE Inh. at 10 nM	Human sEH IC_{50} (nM)
35		0.4	13.4	95.1	83.1	14.3
36		0.4	30.3	26.9	79.5	1.2
37		50	nd[a]	8.7	17.6	524.8
38		100	20.4	23.7	35.9	386.1
39		25	6.4	11.9	14.7	260.4
40		25	264.8	16.6	35.0	242.6
41		200	nd	14.6	88.8	6.5
42* (Isoxyl)		2.5	nd	ni[b]	22	nd

* EphB and EphE inhibition data are IC_{50} values (nM);

a nd = not determined;

b ni = no inhibition