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Author manuscript

*Bioorg Med Chem Lett.* Author manuscript; available in PMC 2021 September 15.

Published in final edited form as:

*Bioorg Med Chem Lett.* 2020 September 15; 30(18): 127430. doi:10.1016/j.bmcl.2020.127430.

## Bioisosteric substitution of adamantane with bicyclic lipophilic groups improves water solubility of human soluble epoxide hydrolase inhibitors.

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### Abstract

A series of inhibitors of the soluble epoxide hydrolase (sEH) containing lipophilic groups of natural origin (camphanyl, norcamphanyl, furan-2-yl) were developed. Inhibitory potency ranging from 0.4 nM to 2.16  $\mu$ M were obtained. While having the same level of inhibitory activity bicyclic ureas are up to 10-fold more soluble than the corresponding ureas containing adamantyl or 4-trifluoromethoxyphenyl substituents. This makes them easier to formulate, more bioavailable and thus more promising as therapeutic sEH inhibitors. *Endo/exo*-form of compound **2b** derived from L-camphor is 14-fold more potent than the corresponding analogue derived from D-camphor (IC<sub>50</sub> = 3.7 nM vs. 50.6 nM) indicating enantiomeric preference.

### Keywords

soluble epoxide hydrolase; inhibitor; adamantane; urea; camphor; norcamphane

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The human soluble epoxide hydrolase (sEH) is involved in metabolism of epoxides derived from arachidonic acid and other natural epoxy-fatty acids,<sup>1</sup> which have multiple, largely beneficial, physiological activities.<sup>2</sup> sEH converts epoxides into the corresponding vicinal diols through the addition of a water molecule, thus affecting pain, inflammation and other pathological states.<sup>2</sup> Thereby inhibition of sEH could be beneficial in treatment of numerous cardiovascular, neuronal and renal diseases.<sup>3,4</sup>

Although, thousands of various sEH inhibitors (sEHI) have been designed and synthesized over the last decades<sup>5-7</sup>, they are characterized by low water-solubility, which makes them hard to formulate, as well diminishes their bioavailability and *in vivo* efficacy. Good solubility appears critical to their success as a potential medicines for the treatment of neurological diseases.<sup>8</sup> Hundreds of sEH inhibitors featuring a common structure of R-Ad-X-NH-C(O)-NH-R', where Ad is adamantan-1-yl or adamantan-2-yl, X is an alkyl or aryl linker and R and R' are alkyl, aryl or heterocyclic groups, have been synthesized and evaluated *in vitro* and in several *in vivo* models.<sup>9-12</sup> However, poor metabolic stability of adamantane containing ureas limits their usefulness and application in some cases.

Recently, ureas containing polycycles such as bisnoradamantane and diamantane were investigated as sEH inhibitors.<sup>13</sup> The replacement of adamantane with larger lipophilic groups led to increase of inhibitory activity but was accompanied by tremendous decrease in metabolic stability and water solubility. In attempt to improve water solubility and metabolic stability, herein, we changed one of the most common sEHI lipophilic fragment, an adamantane group, with natural occurring cyclic and bicyclic groups such as furan-2-yl, camphanyl and norcamphanyl and investigated the effects of such substitution on the potency and properties of the resulting compounds.

Reaction of either bicyclic isocyanates<sup>14</sup> with amines of bicyclic amines<sup>15</sup> with isocyanates were used to synthesize ureas **1a**, **1b**, **2a**, **2b**, **2d**, **2e** and **3a-c** (Scheme 1). Furan-2-ylmethanamine was used for compound **2c**.

Moreover, ureas containing both adamantyl and bicyclic (**4a**, **4b**) or furan-2-yl (**4c**) moieties were synthesized (Scheme 2).

Diureas containing two adamantyl fragments with two urea groups linked with aliphatic spacer are also potent sEHI.<sup>10</sup> High potency of these compounds is supposed to be due to the binding of second urea group with Ser374 of the sEH active site. In this case it is of interest to evaluate the effect of bioisosteric substitution in diureas (Scheme 3).

For ureas containing camphanyl moiety (**2b**, **2e**, **3b**, **3c** and **5c-f**) starting camphaniline (1,7,7-trimethylbicyclo[2.2.1]heptan-2-amine) is a mixture of four stereoisomers: *S* and *R* enantiomers, each containing both *exo* and *endo* isomers derived from L-camphor and D-camphor respectively. To test the impact of each enantiomer on inhibition potency pure *S* and *R* forms of compound **2b** were synthesized. *Exo* and *endo* diastereomers were not separated. To our knowledge, no studies showing the effect of stereochemistry on sEH inhibitory activity has been performed before. Potency of the compounds was measured against the human sEH, and their solubility determined in phosphate buffer (Table 1).

A vast majority of synthesized ureas showed high potency, inhibiting sEH in concentrations as low as 0.4 nM. The only exceptions are compounds **3a** (~1.58  $\mu$ M), **3b** (2.17  $\mu$ M) and **5g** (1.80  $\mu$ M). Relatively low activity of compounds **3a** and **3b** correlates with previous results on adamantane ureas and thioureas with the same lipophilic part.<sup>17</sup> In all series of diureas **5a-i**, elongation of the aliphatic chain between the two urea fragments led to a remarkable increase in potency. *Endo/exo-S*-form of compound **2b** is 14-fold more active than the corresponding *R*-form, meaning that more active species of camphene-containing compounds **2e**, **3c**, **5e** and **5f** could be synthesized.

Solubility in sodium phosphate buffer for the most of synthesized compounds lays in a range (100–300  $\mu$ M) much higher than the original adamantane derivatives. For example, for ureas containing *trans*-4-[(4-aminocyclohexyl)oxy]benzoic acid part, compound **1a** (norcamphane lipophilic group, IC<sub>50</sub> = 1.5 nM) is 2-fold more soluble than **t-AUCB** (adamantane lipophilic group, IC<sub>50</sub> = 2.0 nM) and significantly more soluble than **t-TUCB** (4-(trifluoromethoxy)phenyl lipophilic group, IC<sub>50</sub> = 2.0 nM).

To understand the effect of inhibitor spatial configuration on potency, all ureas containing camphane-2-amine isomers (**2b**) were docked in similar poses with 2-fluorophenyl moiety of ligand maintaining  $\pi$ - $\pi$  stacking interactions with W336 and urea fragment interacting with catalytic residues Y466, Y383 and D335 of the active site (Fig. 1).

The analysis of the molecular dynamics simulation results demonstrated low variation among the calculated binding energy results. Also, in general, the *S*-isomers are better binders in terms of calculated values which are consistent with experimental results. The internal dielectric constant (*indi*) was varied to probe the protein environment as it may depend on amino acid composition of the binding site. Per-residue binding decomposition energy was performed for MM-PBSA (Table 2), results obtained with *indi* equals to 4 are the more consistent with experimental results, and in some cases worked better.<sup>18</sup> The configurational change in the camphanyl scaffold leads to redistribution of the energy contributions between two main catalytic residues (D335, Y466) which structurally is interpreted by differences in hydrogen bond stability between catalytic residues and the isomers of *N*-(camphan-2-yl)-*N'*-(2-fluorophenyl)urea.

In conclusion, the replacement of adamantyl and 4-(trifluoromethoxy)phenyl groups with natural bicyclic lipophilic groups yielded a series of sEHI with similar potency as the original compounds. However, the new compounds are up to 10-fold more soluble than the corresponding ureas containing adamantyl or 4-trifluoromethoxyphenyl substituents, which makes them easier to formulate, more bioavailable and thus more promising as therapeutic sEH inhibitors. Interestingly, the *endo/exo-(S)*-form (3.7 nM) of camphanyl-containing compound **2b** is 14-fold more active than the corresponding *endo/exo-(R)*-form (50.6 nM), demonstrating enantiomeric preference in inhibitor binding.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

This work was partially supported by grant of the Russian Science Foundation project no. 19-73-10002 (synthesis of compounds 1a, 1b, 2b-e and 5a-i), Russian Fund for Basic Research grant number 19-33-60024 (synthesis of compounds 2a, 3a-c, 4a-c), National Institute of Environmental Health Sciences (NIEHS) grant R35 ES030443.

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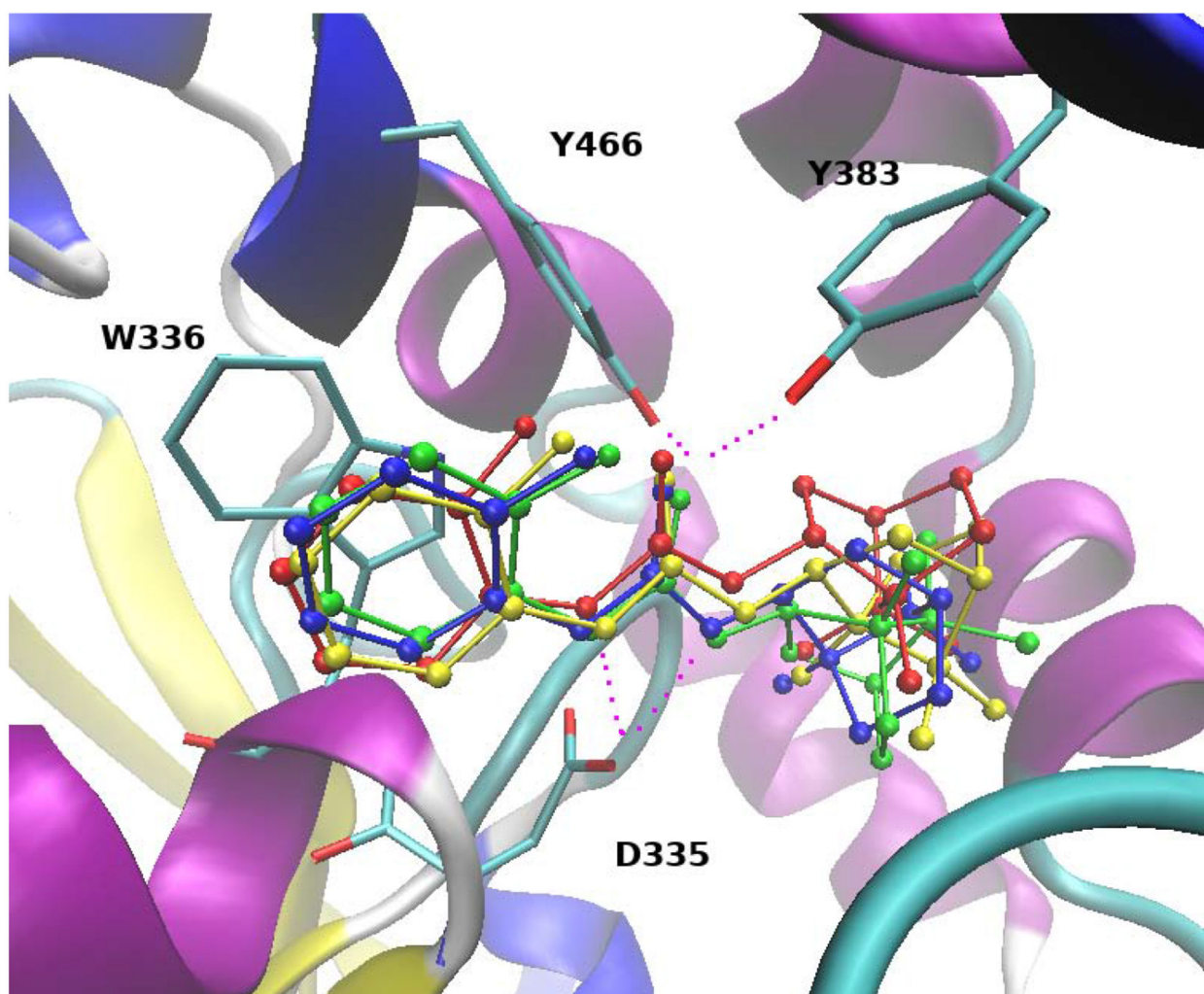
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Ureas with bicyclic lipophilic groups were studied as sEH inhibitors.

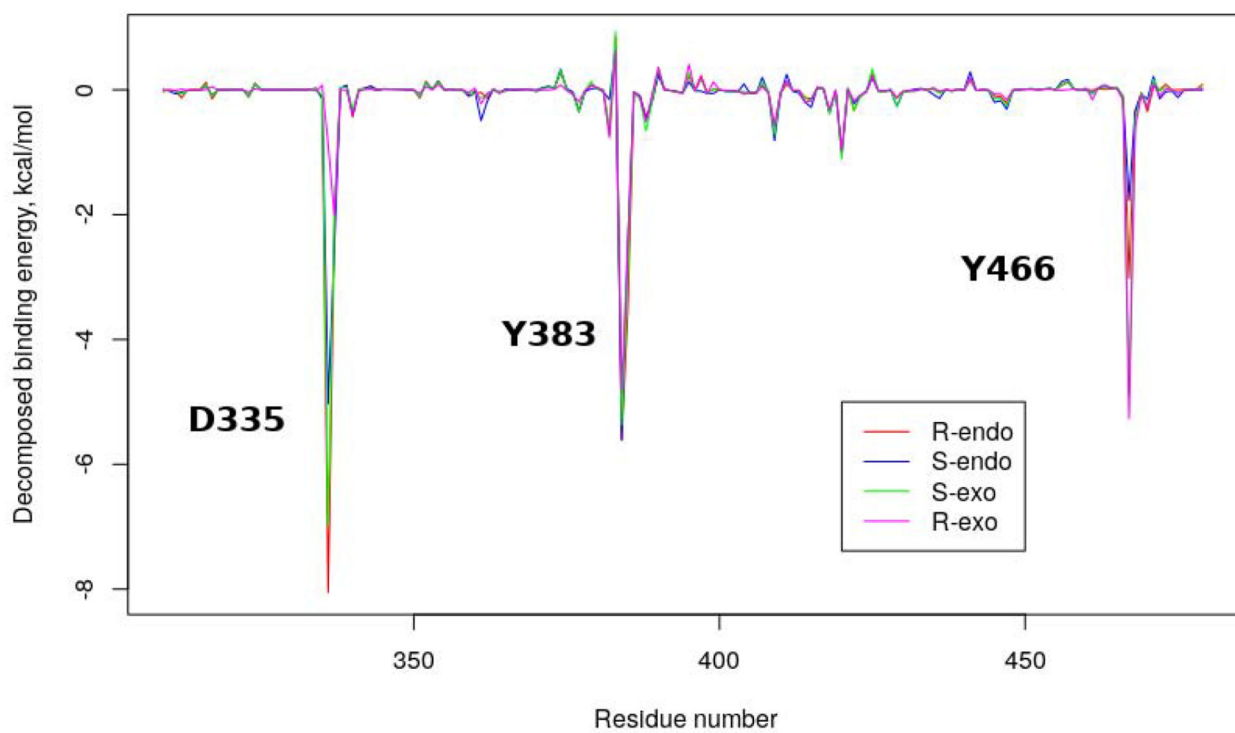
24 ureas with bicyclic lipophilic groups were synthesized.

Bicyclic ureas 10-fold more soluble than the ureas containing adamantyl or aromatic groups.

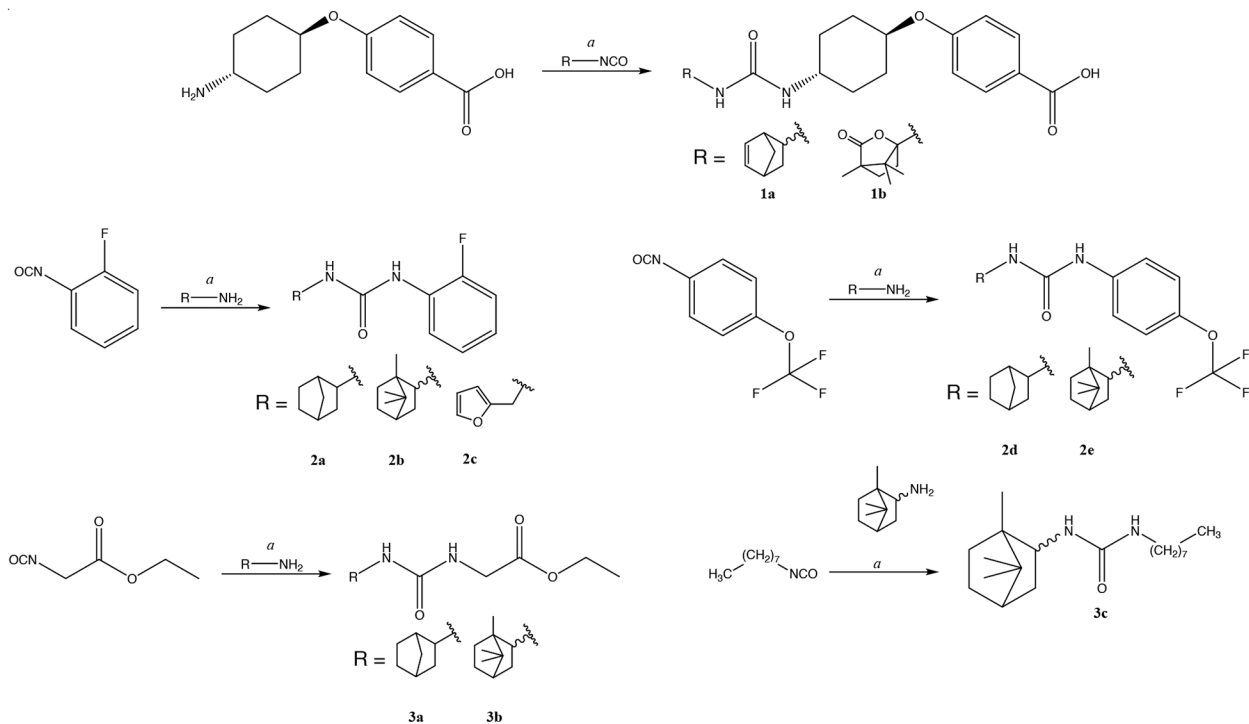
Effect of stereochemistry on sEH inhibitory activity was shown.



**Figure 1.**  
The docking results of the isomers of *N*-(camphan-2-yl)-*N'*-2-(fluorophenyl)urea (*endo*-(*R*) - blue, *exo*-(*R*) - red, *endo*-(*S*) - green, *exo*-(*S*) - yellow). Hydrogen bonds are marked with dashed lines. Hydrogen atoms are omitted for clarity.

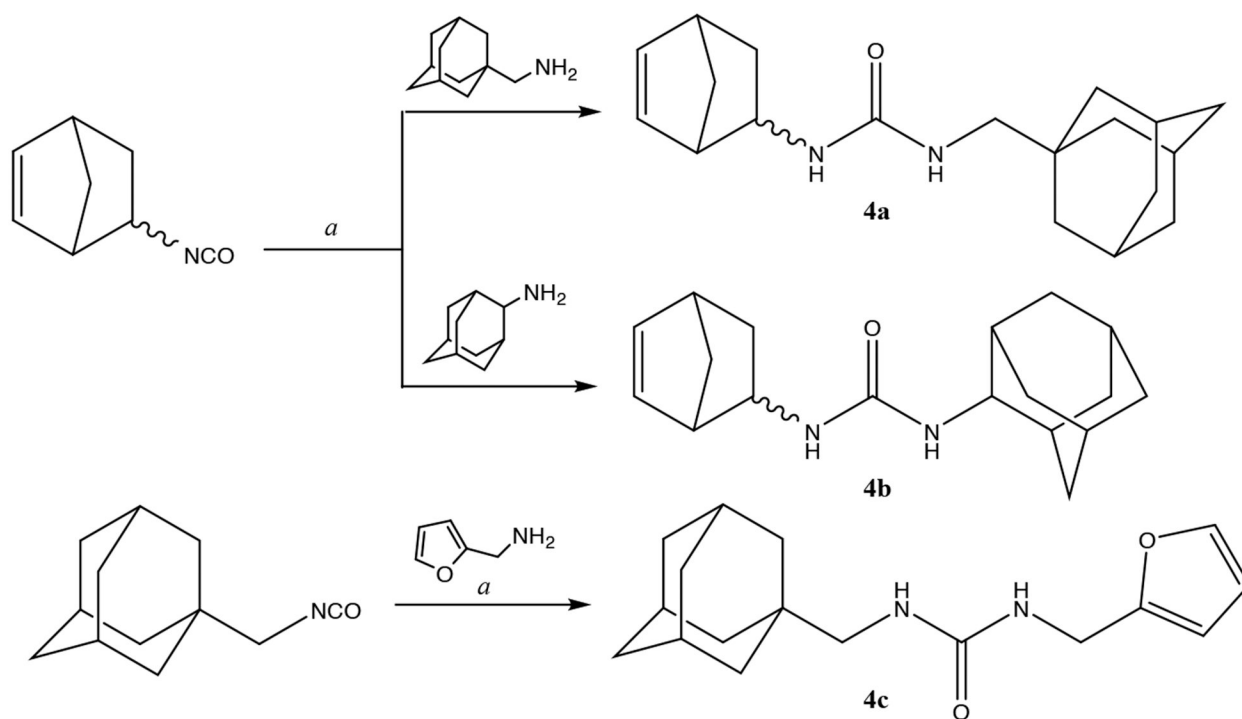


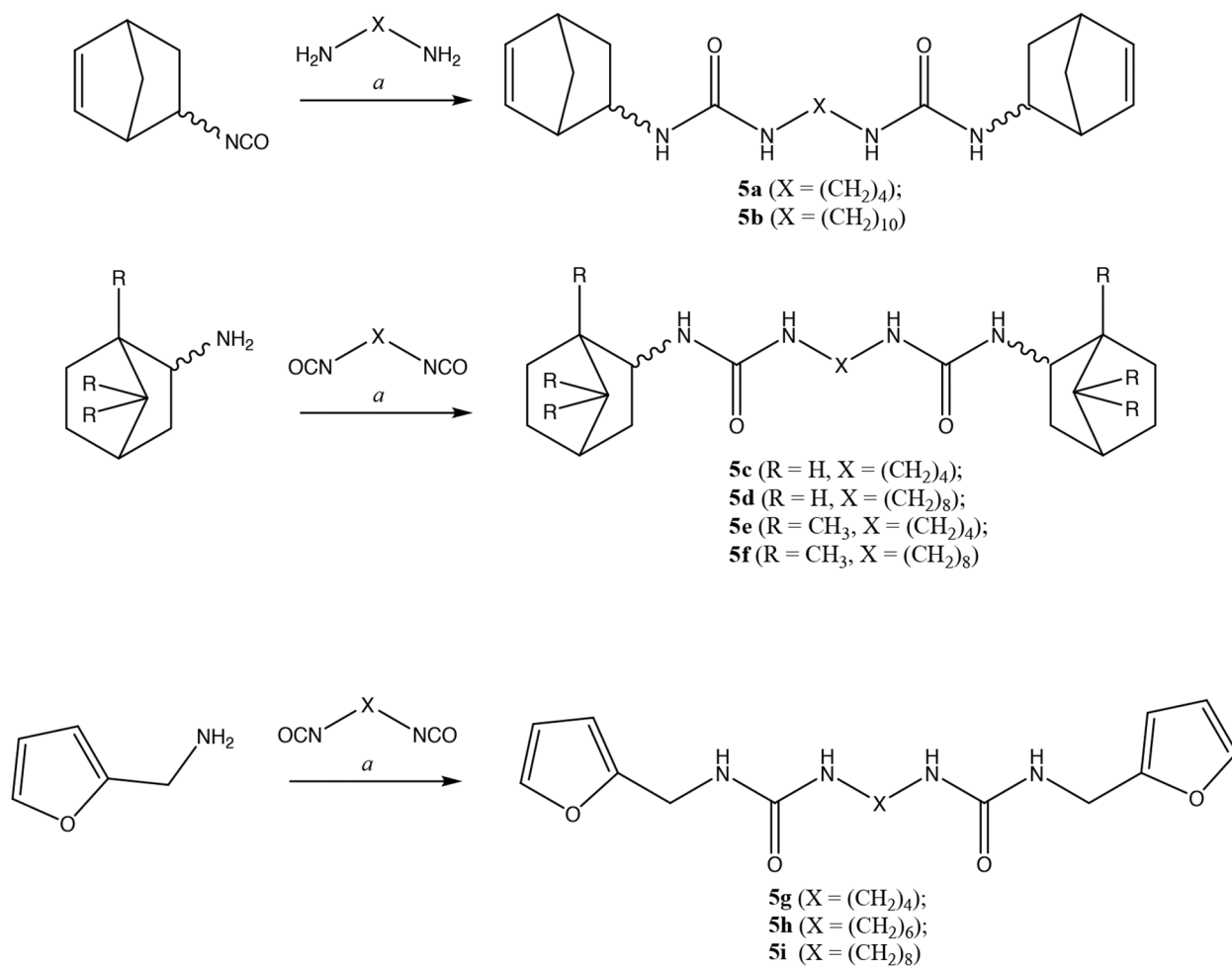
**Figure 2.** The results of binding energy decomposition for the isomers of *N*-(camphan-2-yl)-*N'*-(2-fluorophenyl)urea (MM-PBSA, *indi* = 4).

**Scheme 1.**

Reagents and conditions: *a*. DMF, Et<sub>3</sub>N, rt, 8h.



**Scheme 2.**Reagents and conditions: *a*. DMF, Et<sub>3</sub>N, rt, 8h.

**Scheme 3.**

Reagents and conditions: *a.* DMF, Et<sub>3</sub>N, rt, 8h.

**Table 1**IC<sub>50</sub> values and some physicochemical properties for ureas **1a**, **1b**, **2a-e**, **3a-c**, **4a-c** and **5a-i**.

| #             | Structure | mp (°C)              | logP <sup>a</sup> | Solubility (μM) <sup>b</sup> | Human sEH IC <sub>50</sub> (nM) <sup>c</sup> |
|---------------|-----------|----------------------|-------------------|------------------------------|--|
| <b>1a</b>     |           | 143–144              | 3.77              | 350±25                       | 1.5  |
| <b>1b</b>     |           | 324–325              | 4.20              | 300±25                       | 35.3   |
| <b>t-AUCB</b> |           | 250–255 <sup>5</sup> | 5.18              | 160 ±20 <sup>11</sup>        | 2.0 <sup>5</sup>                             |
| <b>t-TUCB</b> |           | 244–273 <sup>5</sup> | 4.92              | 5 <sup>19</sup>              | 1.0±0.1 <sup>20</sup>                        |
| <b>2a</b>     |           | 195–196              | 3.09              | 325±25                       | 6.8  |
| <b>2b</b>     |           | 238–239              | 4.23              | 225±25                       | 14.4   |
| <b>(S)-2b</b> |           | 241–242              | 4.23              | 225±25                       | 3.7  |
| <b>(R)-2b</b> |           | 235–236              | 4.23              | 225±25                       | 50.6   |
| <b>2c</b>     |           | 137–138              | 2.22              | 425±25                       | 635  |
| <b>2d</b>     |           | 150–151              | 3.95              | -                            | 0.4  |

| #  | Structure | mp (°C) | logP <sup>a</sup> | Solubility (μM) <sup>b</sup> | Human sEH IC <sub>50</sub> (nM) <sup>c</sup> |
|----|-----------|---------|-------------------|------------------------------|--|
| 2e |           | 125–126 | 5.08              | -                            | 2.5  |
| 3a |           | 110–111 | 1.52              | >2000                        | 1,579  |
| 3b |           | 90–91   | 2.65              | >2000                        | 2,166  |
| 3c |           | 67–68   | 5.87              | 125±25                       | 6.5  |
| 4a |           | 223–224 | 4.20              | -                            | 0.7  |
| 4b |           | 263–264 | 4.03              | -                            | 0.9  |
| 4c |           | 135–136 | 3.34              | 300±25                       | 44.8   |
| 5a |           | 248–249 | 2.75              | -                            | 21.0   |
| 5b |           | 148–149 | 5.78              | -                            | 0.4  |
| 5c |           | 274–275 | 2.79              | 75±5                         | 11.8   |
| 5d |           | 215–216 | 4.81              | -                            | 0.4  |
| 5e |           | 308–309 | 5.05              | 10±2                         | 2.3  |

| #  | Structure | mp (°C) | logP <sup>a</sup> | Solubility (μM) <sup>b</sup> | Human sEH IC <sub>50</sub> (nM) <sup>c</sup> |
|----|-----------|---------|-------------------|------------------------------|--|
| 5f |           | 263–264 | 7.08              | 10±2                         | 0.4  |
| 5g |           | 225–226 | 1.04              | 225±25                       | 1,802  |
| 5h |           | 194–195 | 2.05              | 100±10                       | 86.3   |
| 5i |           | 190–191 | 3.06              | 50±10                        | 214  |

<sup>a</sup>Calculated using Molinspiration (<http://www.molinspiration.com>) © Molinspiration Cheminformatics.

<sup>b</sup>Solubilities were measured in sodium phosphate buffer (pH 7.4, 0.1 M) containing 1% of DMSO.

<sup>c</sup>Determined via a kinetic fluorescent assay. Results are means of three separate experiments.<sup>16</sup>

**Table 2**End-state free energy calculation results for the isomers of *N*-(camphan-2-yl)-*N'*-(2-fluorophenyl)urea

| Compound                  | MM-PBSA $\pm$ std. ( <i>indi</i> = 2), kcal/mol | MM-PBSA $\pm$ std. ( <i>indi</i> = 4), kcal/mol | MM-PBSA $\pm$ std. ( <i>indi</i> = 8), kcal/mol |
|---------------------------|---|---|---|
| <i>endo</i> -( <i>R</i> ) | -4.1 $\pm$ 3.2                                  | -10.4 $\pm$ 2.7                                 | -13.3 $\pm$ 2.7                                 |
| <i>exo</i> -( <i>R</i> )  | -5.1 $\pm$ 3.0                                  | -10.4 $\pm$ 2.5                                 | -13.1 $\pm$ 2.5                                 |
| <i>endo</i> -( <i>S</i> ) | -4.6 $\pm$ 3.5                                  | -11.2 $\pm$ 2.8                                 | -14.4 $\pm$ 2.7                                 |
| <i>exo</i> -( <i>S</i> )  | -6.2 $\pm$ 3.1                                  | -11.3 $\pm$ 2.5                                 | -13.8 $\pm$ 2.5                                 |

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