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Imidazolidine-2,4,5- and pirimidine-2,4,6-triones – New primary pharmacophore for soluble epoxide hydrolase inhibitors with enhanced water solubility

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ABSTRACT

A series of inhibitors of the soluble epoxide hydrolase (sEH) containing imidazolidine-2,4,5-trione or pirimidine-2,4,6-trione has been synthesized. Inhibition potency of the described compounds ranges from 8.4 μM to 0.4 nM. The tested compounds possess higher water solubility than their preceding ureas. Molecular docking indicates new bond between the triones and the active site of sEH that in part explain the observed potency of the new pharmacophores. While less potent than the corresponding ureas, the modifications of urea group reported herein yield compounds with higher water solubility, thus permitting easier formulation.

The human soluble epoxide hydrolase (sEH) is involved in the metabolism of arachidonic acid epoxides and other natural epoxy-fatty acids,[1] which have numerous, largely beneficial, biological activities.[2] Through the addition of a water molecule, sEH converts epoxides into corresponding vicinal diols thus affecting inflammatory processes, pain and other disease states.[2] Thereby inhibition of sEH could be beneficial in treatment of many renal, cardiovascular and neuronal diseases.[3,4] Although thousands of various sEH inhibitors (sEHI) were synthesized over the last two decades,[5–7] they have limited solubility, making them hard to formulate, as well as limited bioavailability, especially toward the CNS where sEH is emerging as a potential target for neurological diseases.[8] Toward improving water solubility and metabolic stability, herein, we changed the most common sEHI primary pharmacophore, an urea group, with imidazolidine-2,4,5-trione or pirimidine-2,4,6-trione groups and investigate the effects of such substitution on the potency and properties of the resulting compounds.

Urea-type sEH inhibitors bearing either adamantyl or aromatic moiety as lipophilic group both possess poor water solubility,[9,10] that could be explained by the intermolecular interactions between urea molecules.[11,12] Unfortunately the same hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs) from the urea group are essential for sEH inhibition.[13] However, we proposed that certain changes in the urea-group can improve water solubility while sustaining potency on a decent (nanomolar) level. Thus, we converted urea group in known sEH inhibitors into imidazolidine-2,4,5-trione or pirimidine-2,4,6-trione, because additional HBAs should enhance water solubility and prevent intermolecular interactions between urea molecules.

To synthesize imidazolidine-2,4,5-triones, the reaction of ureas with oxalyl chloride. Reaction was carried out in anhydrous THF for 2 h with reflux (Scheme 1).[14,15]

In most cases imidazolidine-2,4,5-triones are significantly less active than the corresponding ureas, with loss of potency between 2.3 and 6000 folds, except for compound **1c**, which is as potent as the corresponding urea (Table 1). Imidazolidine-2,4,5-trione **1k** (8.4 μM, 700-folds less active than the preceding urea) is the least active among

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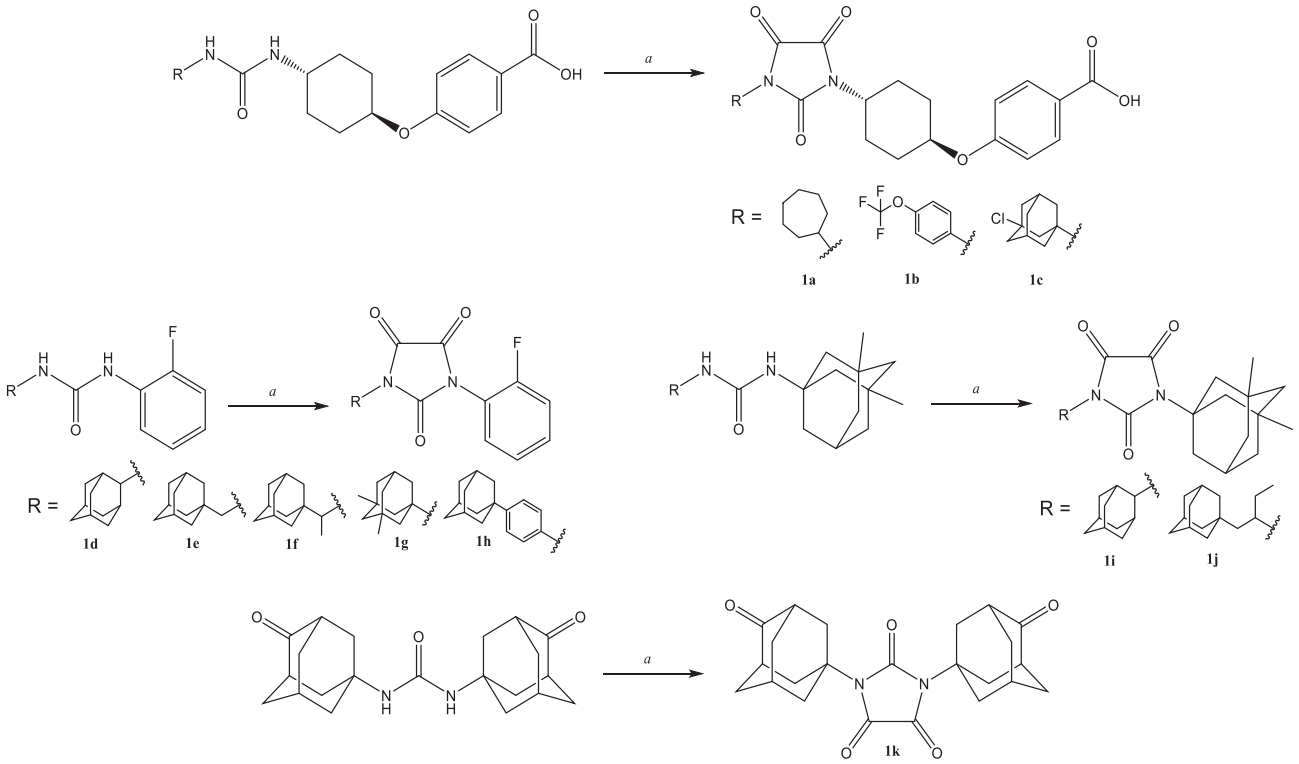


Scheme 1. Reagents and conditions: *a*. Oxalyl chloride (1.2 eq.), THF, 66 °C, 2 h.

compounds **1a-k**. Presence of 5 HBAs in the molecule probably prevents it from entering the active site of the sEH. However, for compound **1c** its activity equals activity of corresponding urea.

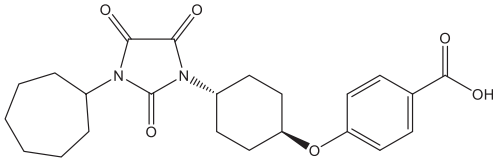
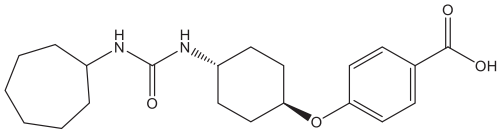
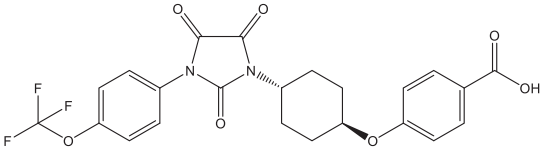
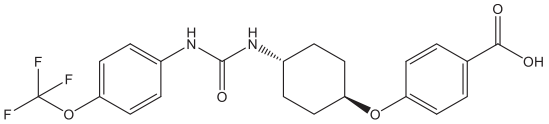
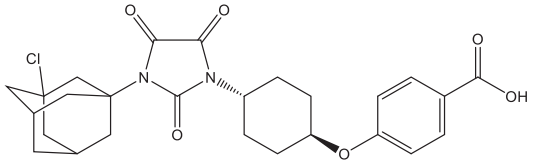
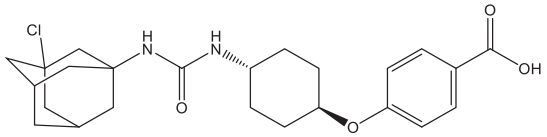
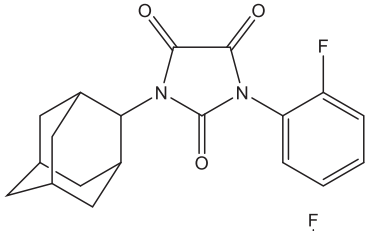
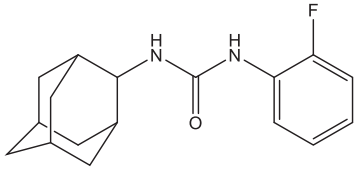
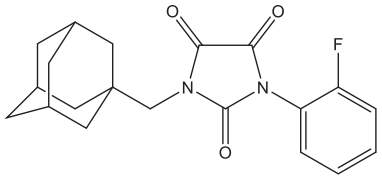
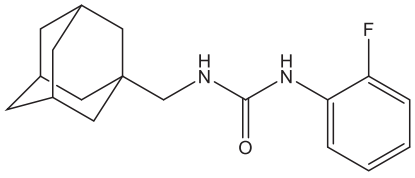
As expected, in most cases, the triones yielded more water soluble than the corresponding urea. Compound **1b** is 40-fold more soluble than its corresponding urea (*t*-TUCB). However, in some cases (**1c** and **1k**) triones were less soluble than its preceding ureas. In addition, melting points of triones were up to 101 °C less than those for the corresponding ureas except for compounds **1a** and **1b**. Because in some conditions, triones can degrade back to the original ureas, the better physical properties of triones can enhanced their formulation as prodrugs of urea-based sEHI.

It was previously showed that diureas, which contain two urea groups linked with aliphatic spacer are very potent sEHI.[9] Molecular docking suggests that the high potency of these compounds is due to the binding of the second urea group with Ser374 of the sEH. We used diureas with various linkers between adamantane and urea fragments to synthesize corresponding di-imidazolidine-2,4,5-triones **2a-g** and tri-imidazolidine-2,4,5-trione **2h** (Scheme 2).

Data (Table 2) shows that the di-imidazolidine-2,4,5-triones **2a-h** possess significantly lower melting points than those for corresponding ureas. Compounds **2f** and **2g** are liquid at room temperature, which suggests that their melting points are at least 193 and 167 °C lower. The ureas are known to form intermolecular hydrogen bonds resulting in a brick wall fashion crystalline lattice. Thus, the strong decreased in melting point observed, coupled with the higher molecular weight of the imidazolidine-2,4,5-triones, illustrates a lower number of intermolecular interactions in the trione crystals. Therefore, the up to 115-fold decrease in inhibitory activity (except of compound **2d** which is 2.2-fold more active than its preceding urea) of imidazolidine-2,4,5-triones is somewhat not surprising. As observed for the single triones (Table 1), the di-triones are also 16- and 9-fold more soluble in water than their corresponding diureas.

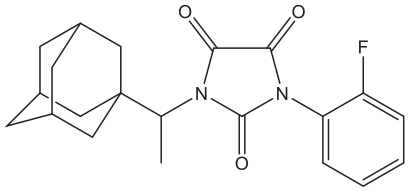
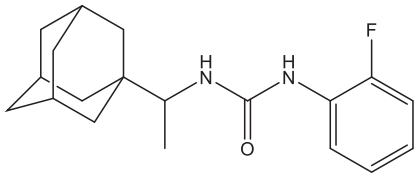
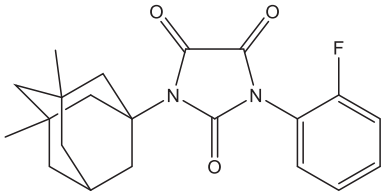
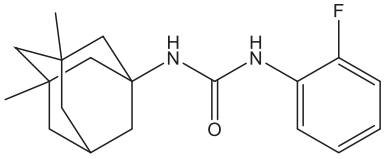
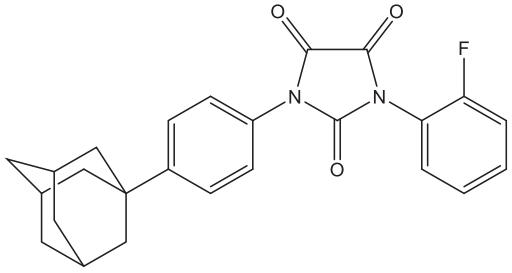
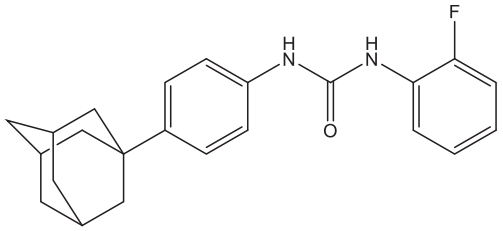
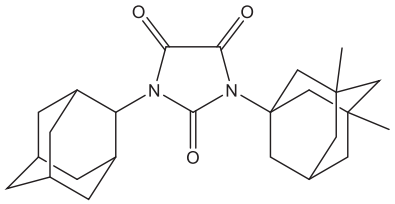
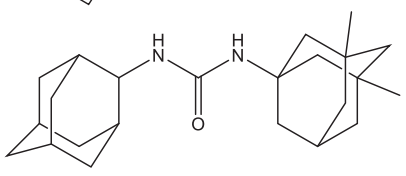
To rationalize the activity and solubility trends in the current data sets of sEH inhibitors we performed a classic QSAR study based on fragment descriptors and regularized linear regression. The obtained model is satisfactory and can explain some features of the data set and the calculated F-statistics values confirmed the significance of the constructed models and the quality of the constructed models is satisfactory. The fragment descriptors which showed non-zero coefficients in the descriptor selection procedure are shown at Fig. 1(A) (activity). The only descriptors which demonstrates suitable significance level in a *t*-test are *frag7* ($p = 0.07$) and *frag10* ($p = 0.002$). Their influence may be interpreted in the following way: *frag7* can be found in compounds which contain *N*-adamantylurea fragments which is easily accommodated in the sEH active site and form hydrogen bonds with Asp335 and Tyr383, while unsubstituted *N*-adamantyl-imidazolidine-trione containing compounds contain *frag10* and, according to the docking results, have an alternative binding mode to the enzyme. Asp335 can exist in two different conformations in sEH active site: the most stable is highlighted by red color (Fig. 2(A)) it forms two hydrogen bonds with the backbone amides of Trp336 and Gly266 and the other one is shown at Fig. 2(A) with ordinary color scheme. The latter one is observed for the binding mode of compounds **2a** where one of carbonyl oxygens forms hydrogen bonds with the backbone amides of Trp336 and Gly266 substituting Asp335 side chain. Thus, the number of hydrogen bonds between the enzyme and the ligand are roughly the same for **2a** and its urea derivative **2a***. The urea derivative forms two hydrogen bonds with Asp335 while one of the carbonyl oxygens of **2a** forms two hydrogen bonds with the backbone amide groups of Trp336

Table 1
IC_{50} values and some physicochemical properties for imidazolidine-2,4,5-triones **1a-k** and their corresponding ureas.

#	Structure	mp (°C)	Solubility (μM)[a]	Human sEH IC_{50} (nM)[b]
1a		212–214	280 ± 10	86.8
1a*		194–195[19]	100 ± 5	4.5
1b		295–296	210 ± 10	7.7
1b*t-TUCB		244–273[5]	5[16]	1 ± 0.1[17]
1c		205–207	250 ± 10	0.4
1c*		241–243[7]	500 ± 25[7]	0.4[7]
1d		155–158	250 ± 10	6127
1d*		196–197[11]	85 ± 5[11]	1.0[11]
1e		90–91	350 ± 10	310.6
1e*		191–192[11]	65 ± 5[11]	0.7[11]

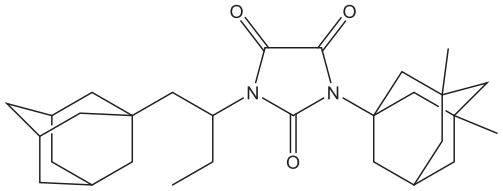
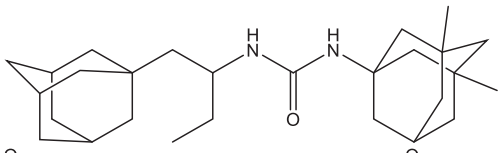
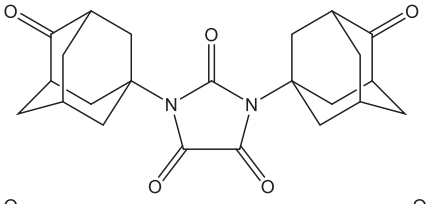
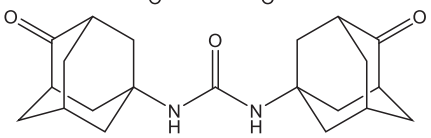
(*continued on next page*)

Table 1 (*continued*)

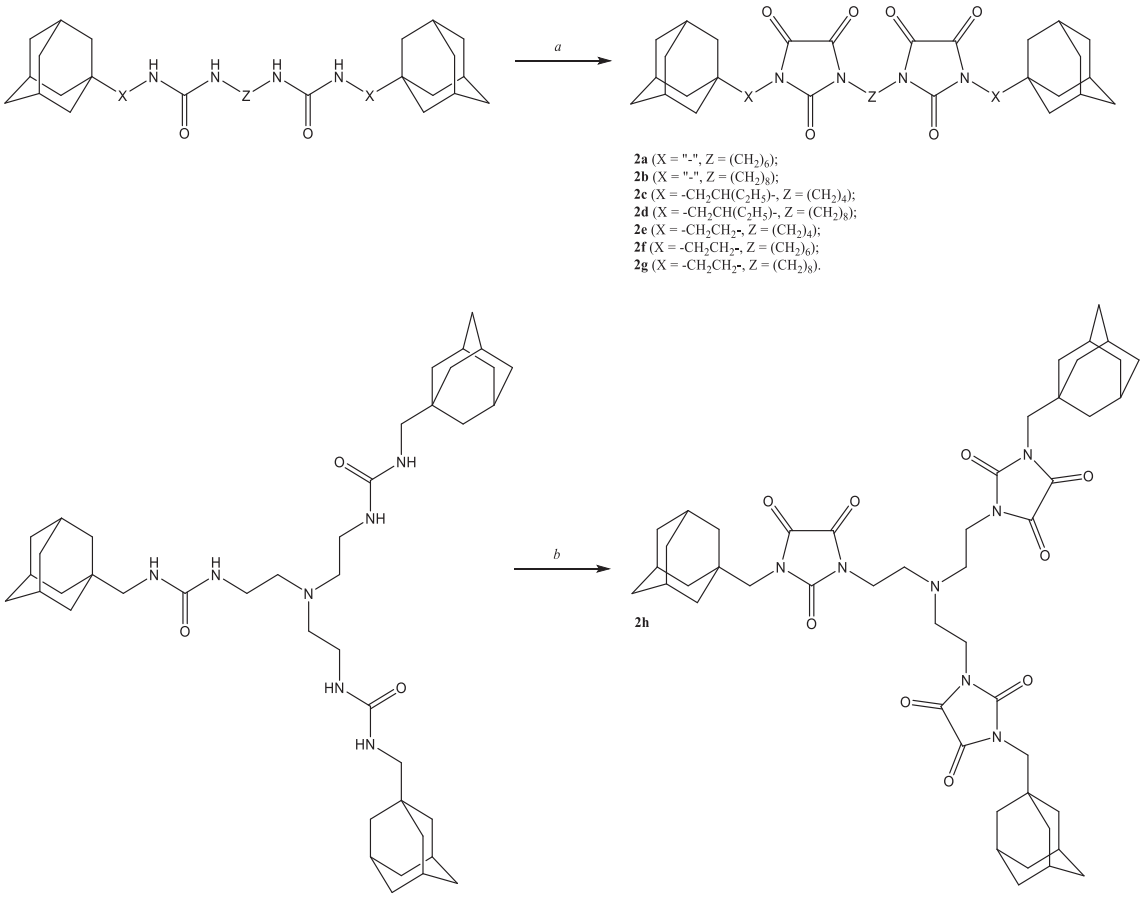
#	Structure	mp (°C)	Solubility (μM)[a]	Human sEH IC_{50} (nM)[b]
1f		159–160	900 ± 25	466.4
1f*		172–173[11]	55 ± 5[11]	55.6[11]
1g		129–130	200 ± 10	370.7
1g*		181–182[11]	65 ± 5[11]	3.6[11]
1h		144–145	250 ± 10	160.4
1h*		183–184[11]	85 ± 5[11]	94.2[11]
1i		260–263	100 ± 5	22.8
1i*		266–268[7]	20 ± 5[7]	9.6[7]

(*continued on next page*)

Table 1 (*continued*)

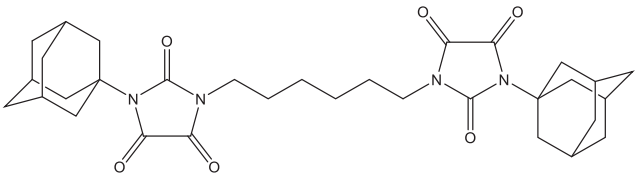
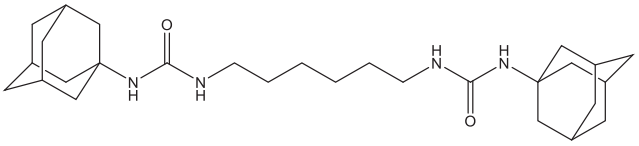
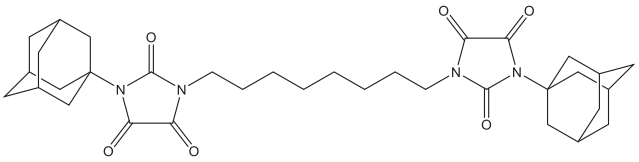
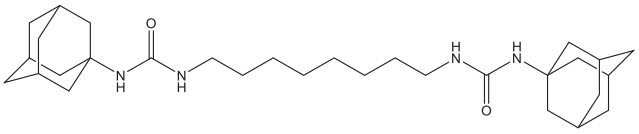
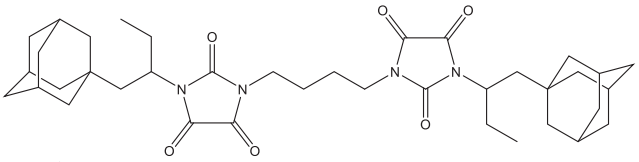
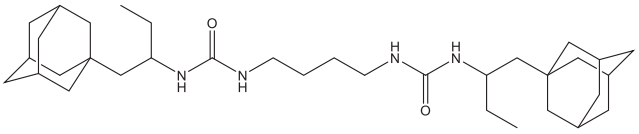
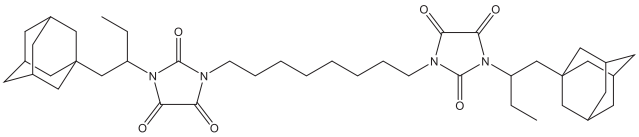
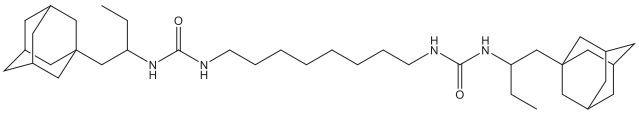
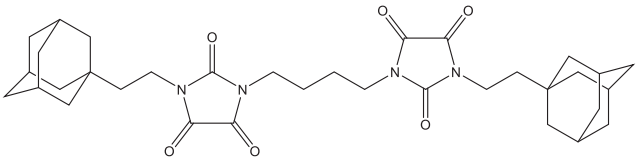
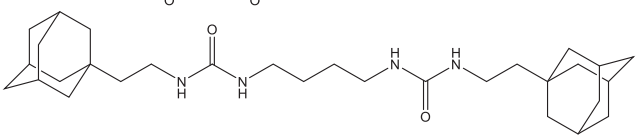
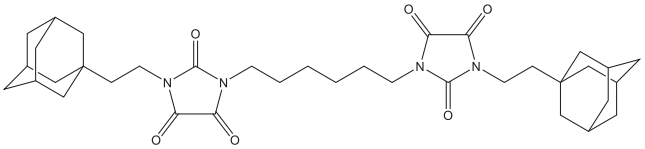
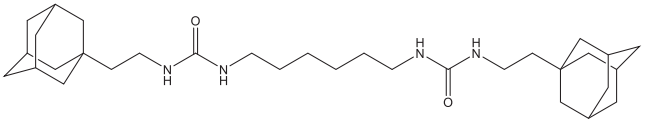
#	Structure	mp (°C)	Solubility (μM)[a]	Human sEH IC_{50} (nM)[b]
1j		153–155	100 ± 5	216.1
1j*		194–196[7]	40 ± 5[7]	13.8[7]
1k		185–186	150 ± 10	8417
1k*		273–274	1750 ± 25	12.0

[a] Solubilities were measured in sodium phosphate buffer (pH 7.4, 0.1 M) containing 1% of DMSO.
[b] Determined via a kinetic fluorescent assay. Results are means of three separate experiments.[18]



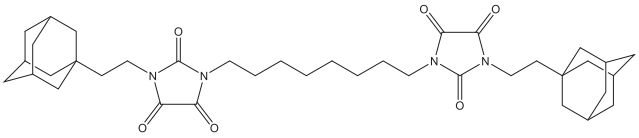
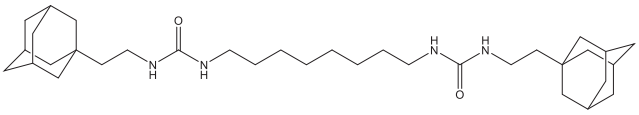
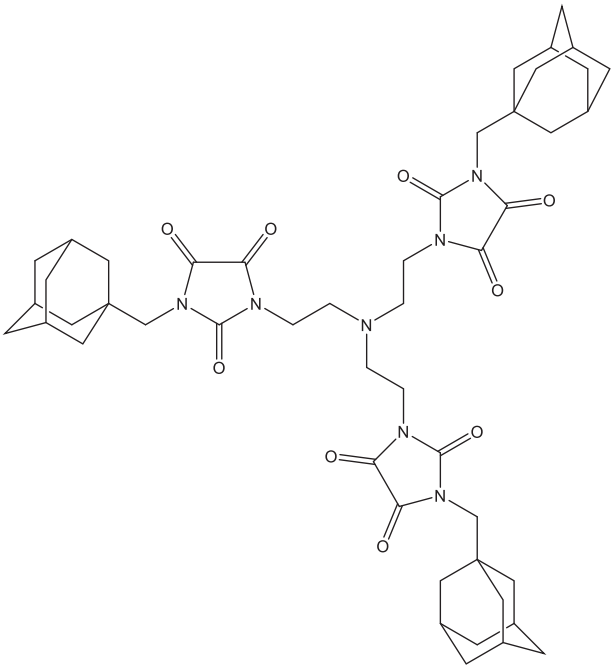
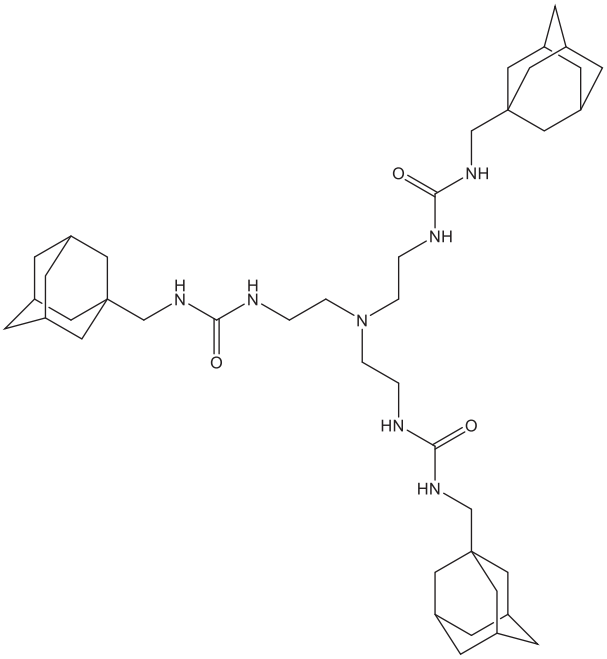
Scheme 2. Reagents and conditions: *a*. Oxalyl chloride (2.5 eq.), THF, reflux, 2 h. b. Oxalyl chloride (3.5 eq.), THF, reflux, 2 h.

Table 2
IC_{50} values and some physicochemical properties for imidazolidine-2,4,5-triones **2a-h** and its corresponding preceding ureas.

#	Structure	mp (°C)	Solubility (μM)[a]	Human sEH IC_{50} (nM)[b]
2a		217–220	120 ± 5	1.6
2a*		228–230[9]	25 ± 5[9]	0.6[9]
2b		127–130	110 ± 5	3.8
2b*		256–258[9]	30 ± 5[9]	0.9[9]
2c		118–121	90 ± 5	8.4
2c*		210–213[9]	n/a	2.3[9]
2d		122–125	100 ± 5	649.6
2d*		130–131[9]	n/a	1442.6[9]
2e		39–40	900 ± 25	294.4
2e*		229–230[7]	100 ± 10[7]	3.4[7]
2f		n/a[c]	1300 ± 25	92.0
2f*		192–193[7]	80 ± 10[7]	0.8[7]

(*continued on next page*)

Table 2 (*continued*)

#	Structure	mp (°C)	Solubility (μM)[a]	Human sEH IC_{50} (nM)[b]
2g		n/a[c]	600 ± 10	438.1
2g*		166–167[7]	75 ± 5[7]	1.5[7]
2h		167–168	650 ± 25	80.3
2h*		222–223	70 ± 5	6.7

[a] Solubilities were measured in sodium phosphate buffer (pH 7.4, 0.1 M) containing 1% of DMSO.
[b] Determined via a kinetic fluorescent assay. Results are means of three separate experiments.[18]
[c] Liquid compounds. Do not crystallize at −20 °C.

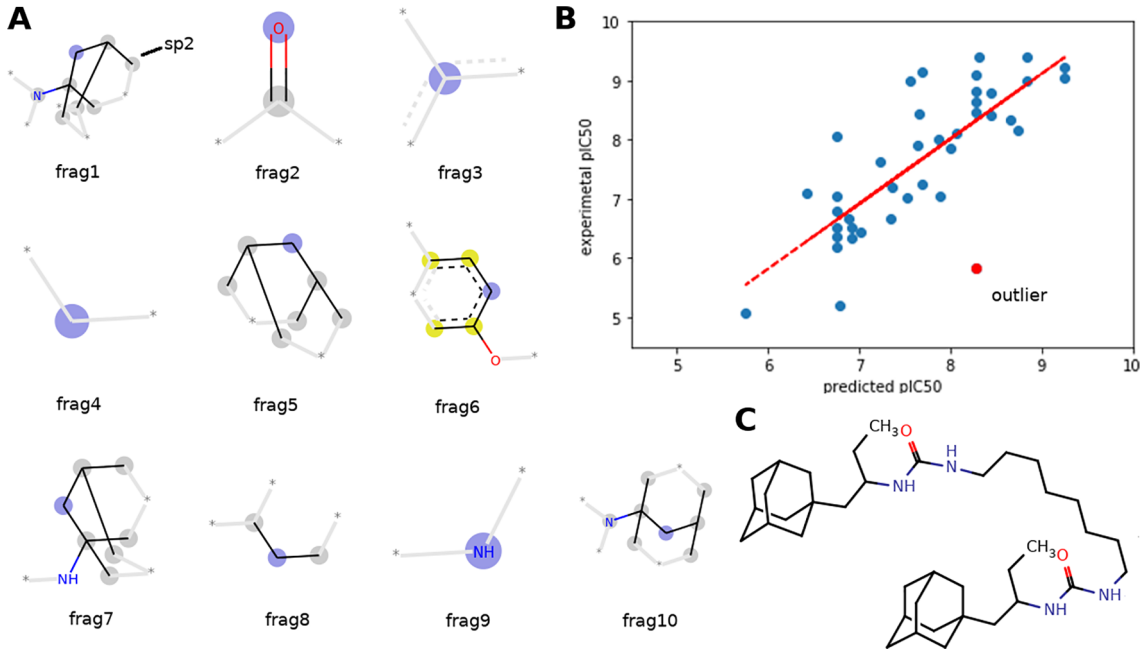


Fig. 1. (A) Molecular fragments have non-zero coefficients in the final regression equation for pIC_{50}. Central atom of each fragment is highlighted by blue color, the aliphatic carbon atoms are labeled by grey color while the aromatic ones are colored with yellow; (B) The final prediction results of the final model, the possible outlier is shown in red color; (C) The structural formula of the possible outlier. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

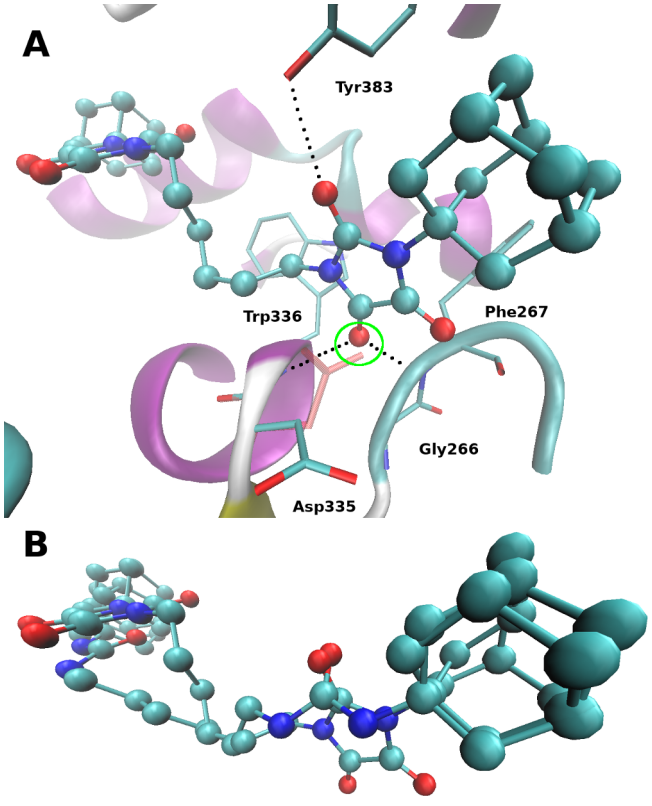


Fig. 2. (A) The binding mode of the compound **2a**. Black points show the hydrogen bonds which compound **2a** maintain with protein residues. The green circle labels atoms which are in close proximity to each other. (B) The superposition of the docked structured of **2a** and its urea derivative in the binding site. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and Phe267.

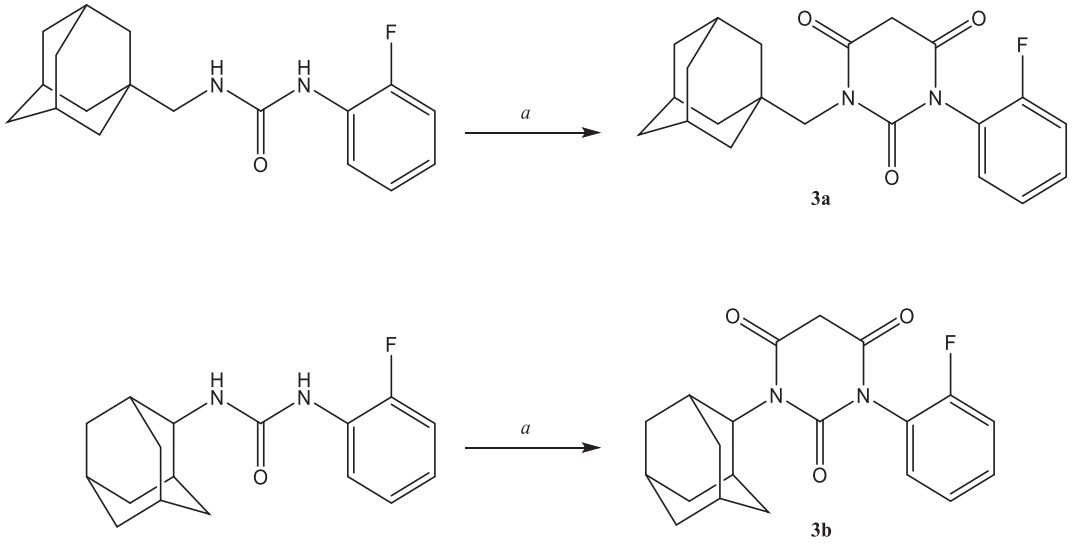
Finally, we synthesized two pirimidine-2,4,6-triones **3a** and **3b** (Scheme 3) by substituting oxalyl chloride with malonyl chloride in the above described reaction.

Compound **3a** is more active than compound **3b** (Table 3), confirming that a single methylene spacer between the adamantane and the primary pharmacophore lead to increase of the inhibitory activity.[9] Both pirimidine-2,4,6-triones **3a** and **3b** show higher inhibitory activity against sEH when compared to the corresponding imidazolidine-2,4,5-triones **1e** and **1d** (Table 1), but they are up to 100-fold less active than the corresponding ureas. Pirimidine-2,4,6-triones **3a** and **3b** are 21 and 10-fold more soluble than the ureas and approximately 4-fold more soluble than the corresponding imidazolidine-2,4,5-triones **1d** and **1e**. Because, like the imidazolidine-2,4,5-triones in some conditions, the pirimidine-2,4,6-triones can degrade back to the original ureas, the better physical properties of the triones can enhanced their formulation as pro-drugs of urea-based sEHI (Table 4).

Series of imidazolidine-2,4,5-triones and primidine-2,4,6-triones were synthesized and investigated for their inhibitory activity against sEH as well as their physical properties. Synthesized compounds are less active than its preceding ureas but possess higher water solubility and lower melting point. Molecular docking with the new compounds highlights possible new bonds with the enzyme that could be used to increase potency. In addition, the new pharmacophore yield molecules that should easier to formulate, and that could be used as pro-drugs for urea-based inhibitor of sEH.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

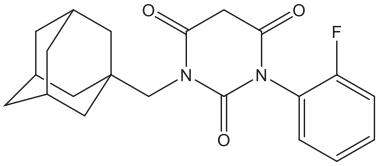
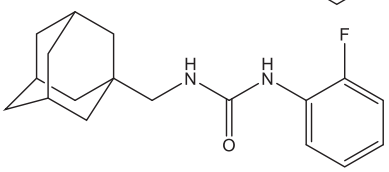


Scheme 3. Reagents and conditions: *a*. Malonyl chloride (1.2 eq.), THF, 66 °C, 2 h.

Table 3
The obtained regression equations for activity and solubility.

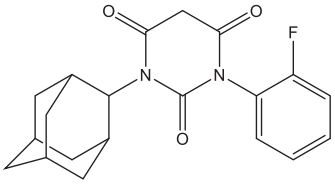
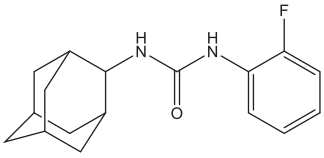
Outcome type	Equation	Q^2	R^2	$RMSE_{cv}$	F-value
Activity (pIC_{50})	pIC_{50} = 7.386–0.174616 * counts_frag1 − 0.069048 * counts_frag2 − 0.043058 * counts_frag3 + 0.002409 * counts_frag4 + 0.015568 * counts_frag5 + 0.106981 * counts_frag6 + 0.107737 * counts_frag7 + 0.113848 * counts_frag8 + 0.177734 * counts_frag9 + 0.196090 * counts_frag10	0.46	0.67	0.85	5.72
	descriptor	Std. error	*t*-value	p-value	
	intercept	0.81	9.01	$< 10^{-6}$	
	frag1	0.19	−1.5565	0.12	
	frag2	0.16	−0.6346	0.53	
	frag3	0.09	−0.7240	0.47	
	frag4	0.03	0.2218	0.83	
	frag5	0.07	0.8327	0.41	
	frag6	0.11	0.9326	0.36	
	frag7	0.08	1.8443	0.07	
	frag8	0.22	1.1164	0.27	
	frag9	0.20	0.6367	0.53	
	frag10	0.08	3.2223	0.002	

Table 4
IC_{50} values and some physicochemical properties for pirimidine-2,4,6-triones **3a** and **3b** and its corresponding preceding ureas.

#	Structure	mp (°C)	Solubility (μM)[a]	Human sEH IC_{50} (nM)[b]
3a		90–91	1400 ± 50	61.0
1a*		191–192[11]	65 ± 5[11]	0.7[11]

(*continued on next page*)

Table 4 (*continued*)

#	Structure	mp (°C)	Solubility (μM)[a]	Human sEH IC_{50} (nM)[b]
3b		115–116	900 ± 50	210.4
1b*		196–197[11]	85 ± 5[11]	1.0[11]

[a] Solubilities were measured in sodium phosphate buffer (pH 7.4, 0.1 M) containing 1% of DMSO.
[b] Determined via a kinetic fluorescent assay. Results are means of three separate experiments.[18]

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.126908.

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