Soluble Epoxide Hydrolase Inhibitors: Design, Synthesis, *in vitro* Profiling and *in vivo* Evaluation in Murine Models of Pain

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INTRODUCTION

The inhibition of soluble epoxide hydrolase (sEH) has been suggested as a novel pharmacological approach for the treatment of pain-related disorders and various inflammatory diseases.[1]

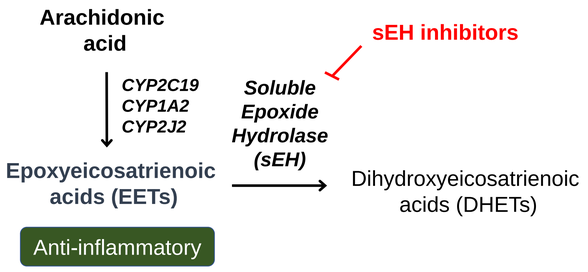
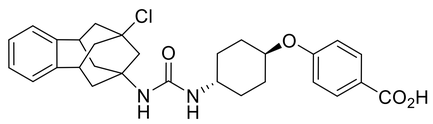


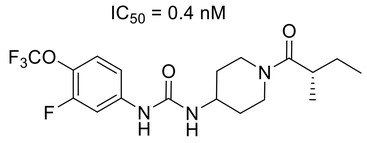
Figure 1. Metabolism pathway of arachidonic acid.

OBJECTIVE

Recently, we discovered that a selected member of a new family of benzohomoadamantane-based sEH inhibitors (sEHI), **1**, showed *in vivo* efficacy in a murine model of acute pancreatitis.[2] Taking into account the structure of the clinical candidate sEHI for neuropathic pain **EC5026**,[3] herein we report further structure-activity relationships within the series of benzohomoadamantane-derived sEHI with the aim to conduct a screening cascade and to perform an *in vivo* proof of concept in murine models of pain with the selected candidate.



1
IC_{50} = 0.4 nM



EC5026
Clinical candidate for Neuropathic Pain
IC_{50} = 0.4 nM

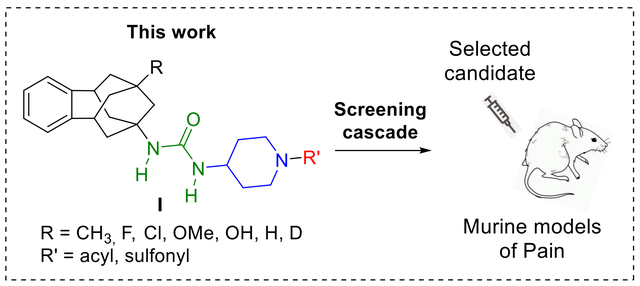


Figure 1. Objective of this work.

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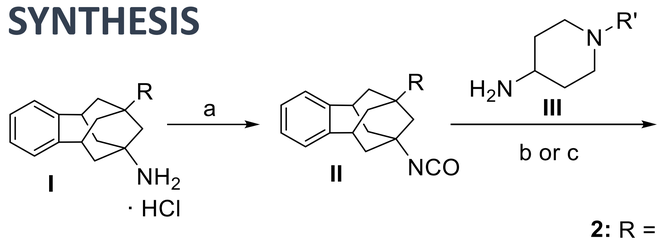
[1]Morisseau, C.; Hammock, B.D. *Annu. Rev. Pharmacol. Toxicol.* ***2013***, *53*, 37–58.

[2]Codony S.; Calvó-Tusell C.; Valverde E.; Osuna S.; Moriseau C.; Loza M.I.; Brea J.; Pérez C.; Rodríguez-Franco M.I.; Pizarro-Delgado J.; Corpas R.; Griñán-Ferré C.; Pallàs M.; Sanfeliu C.; Vázquez-Carrera M.; Hammock B. D.; Feixas F.; Vázquez S. *J. Med. Chem.*, **2021**, *64*, 5429–5446.

[3]Hammock, B. D.; McReynolds, C. B.; Wagner, K.; Buckpitt, A.; Cortes-Puch, I.; Croston, G.; Lee, K. S. S.; Yang, J.; Schmidt, W. K.; Hwang, S. H. *J. Med. Chem.* **2021**, 64, 1856-1872.

SYNTHESIS



2: R = CH_3, R' = propionyl
3: R = CH_3, R' = tetrahydro-2*H*-pyran-4 carbonyl
4: R = CH_3, R' = isopropylsufonyl
5: R = CH_3, R' = cyclopropanecarbonyl
6: R = Cl, R' = acetyl
7: R = Cl, R' = propionyl
8: R = Cl, R' = tetrahydro-2*H*-pyran-4 carbonyl
9: R = Cl, R' = isopropylsulfonyl
10: R = Cl, R' = cyclopropanecarbonyl
11: R = Cl, R' = 2,2,2-trifluoroacetyl
12: R = Cl, R' = 1-fluorocyclopropane-1-carbonyl
13: R = F, R' = acetyl
14: R = F, R' = tetrahydro-2*H*-pyran-4 carbonyl
15: R = F, R' = cyclopropanecarbonyl
16: R = OH, R' = acetyl
17: R = OMe, R' = acetyl
18: R = D, R' = tetrahydro-2*H*-pyran-4 carbonyl

Scheme 1. Synthesis of the new sEHIs. [a]Reagents and conditions: a) triphosgene, $NaHCO_3$, DCM, 30 min; b) DCM, overnight; c) *n*-BuLi, anh. THF, anh. DCM, overnight.

SCREENING CASCADE

Further *in vitro* profiling (human and murine sEH inhibition, human and mice microsomal stability, solubility, cytotoxicity, cytochromes inhibition, Caco-2 permeability, selectivity and hERG inhibition) allowed us to select compounds **8**, **14** and **15** for *in vivo* studies.

Cpd	sEH IC_{50} [a] (nM)		Microsomal stability[b] (%)		Cytochrome inhibition (% at 10 µM)	Solubility[c] (µM)	PAMPA-BBB	Cytotoxicity LD_{50} (µM)	
	Human	Murine	Human	Mouse	CYP 2C19			PI[d]	MTT[e]
8	0.4	1.0	47	64	38 ± 4	57	CNS +	>100	>100
14	0.4	0.5	66	84	32 ± 4	95	CNS +/-	>100	>100
15	0.4	0.4	58	60	26 ± 5	92	CNS +	>100	>100

Table 1. *In vitro* profiling of selected sEHIs. [a]Reported IC_{50} values are the average of three replicates. [b]Percentage of remaining compound after 60 min of incubation with pooled human and mouse microsomes in the presence of NADPH at 37 °C. [c]Solubility measured in a 1% DMSO: 99% PBS buffer solution. [d]Cytotoxicity tested by propidium iodide (PI) staining after 24h incubation in SH-SY5Y cells. [e]Cytotoxicity tested by 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay after 24h incubation in SH-SY5Y cells.

PHARMACOKINETIC STUDIES IN MICE (5 mg/Kg, S.C.)

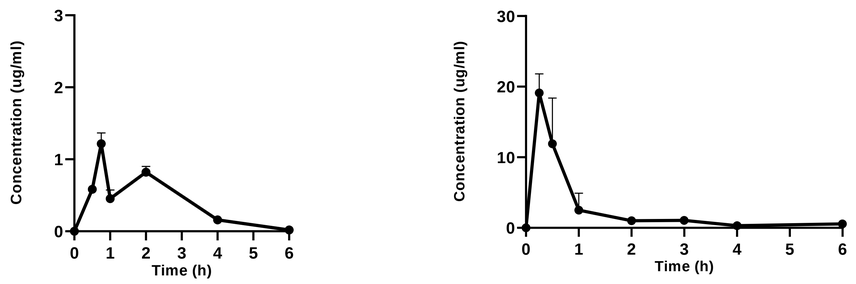


Figure 2. Plasma concentration *vs* time for compounds **8** and **14**, respectively.

Cpd	HL (h)	Tmax (h)	Cmax (µg/mL)	AUClast (µg*h/mL)	AUCINF (µg*h/mL)
8	3.42	0.75	1.2	2.4	2.5
14	0.70	0.25	19.1	13.5	13.6

Table 2. Pharmacokinetic parameters for compounds **8** and **14**.

IN VIVO EFFICACY STUDIES

A first *in vivo* efficacy study was performed in the capsaicin-induced secondary mechanical hypersensitivity (allodynia) model in mice.

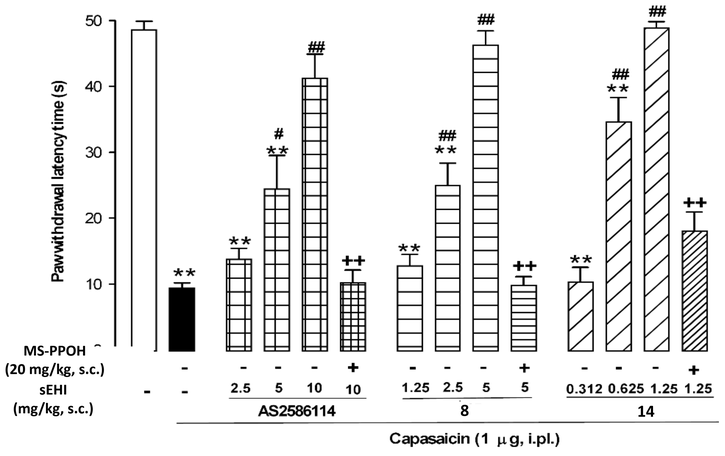


Figure 2. Reduction of capsaicin-induced secondary mechanical hypersensitivity in mice by the systemic administration of AS2586114, and compounds **8** and **14**, is due to sEH inhibition. The data shown represent the effect of the subcutaneous (s.c.) administration of AS2586114, **8** and **14** administered alone or associated to the CYP450 epoxidase inhibitor MS-PPOH (s.c.) on paw withdrawal latency in mice treated intraplantarly (i.pl.) with capsaicin. **p < 0.01 between nonsensitized mice (open bar) and the other experimental groups; #p < 0.05, ##p < 0.01 between capsaicin-treated mice injected with the sEHI or their solvent (black bar); ††p < 0.01 sEHI-treated mice associated or not with MS-PPOH.

CONCLUSIONS

- We have further explored medicinal chemistry around new benzohomoadamantane-based piperidine derivatives, analogs of the clinical candidate EC5026.
- An in *vitro* screening cascade and pharmacokinetic studies allowed us to select two candidates for *in vivo* efficacy studies.
- The administration of compounds **8** and **14** reduced pain in the capsaicin-induced murine model of allodynia in a dose-dependent manner and outperformed AS2586114, a standard sEHI.
- Hence, this study opens a whole range of applications of the benzohomoadamantane-based sEHIs in the pain field.

ACKNOWLEDGEMENTS

This research by the Grant PID2020-118127RB-I00 funded by MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe" to S.V. Financial support from *Fundació Bosch i Gimpera, Universitat de Barcelona* (F2I grant), to S.V., and from the Xunta de Galicia (ED431G 2019/02 and ED431C 2018/21) to M.I.L. are acknowledged. Partial support was provided by NIH-NIEHS River Award R35 ES03443, NIH-NIEHS Superfund Program P42 ES004699, NINDS R01 DK107767, and NIDDK R01 DK103616 to B.D.H. S.C. acknowledges a PhD fellowship from the Universitat de Barcelona (APIF grant).

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