SYNTHESIS, IN VITRO PROFILING AND IN VIVO EVALUATION OF SOLUBLE EPOXIDE HYDROLASE INHIBITORS FOR VISCERAL PAIN

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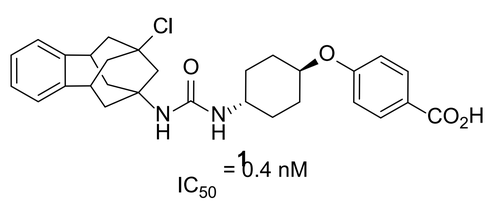
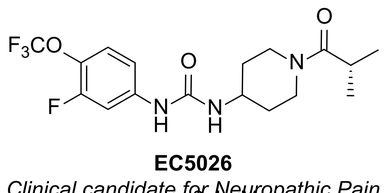
INTRODUCTION AND OBJECTIVE

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]

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.[4]



1
IC_{50} = 0.4 nM



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

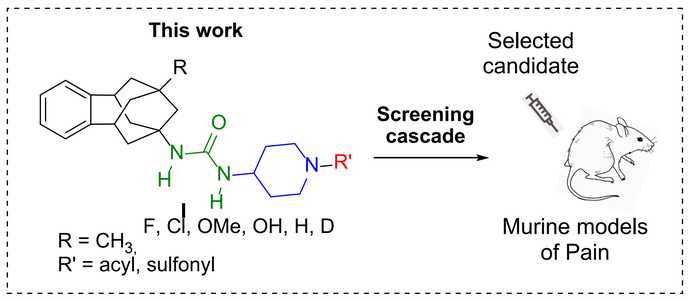


Figure 2. Objective of this work.

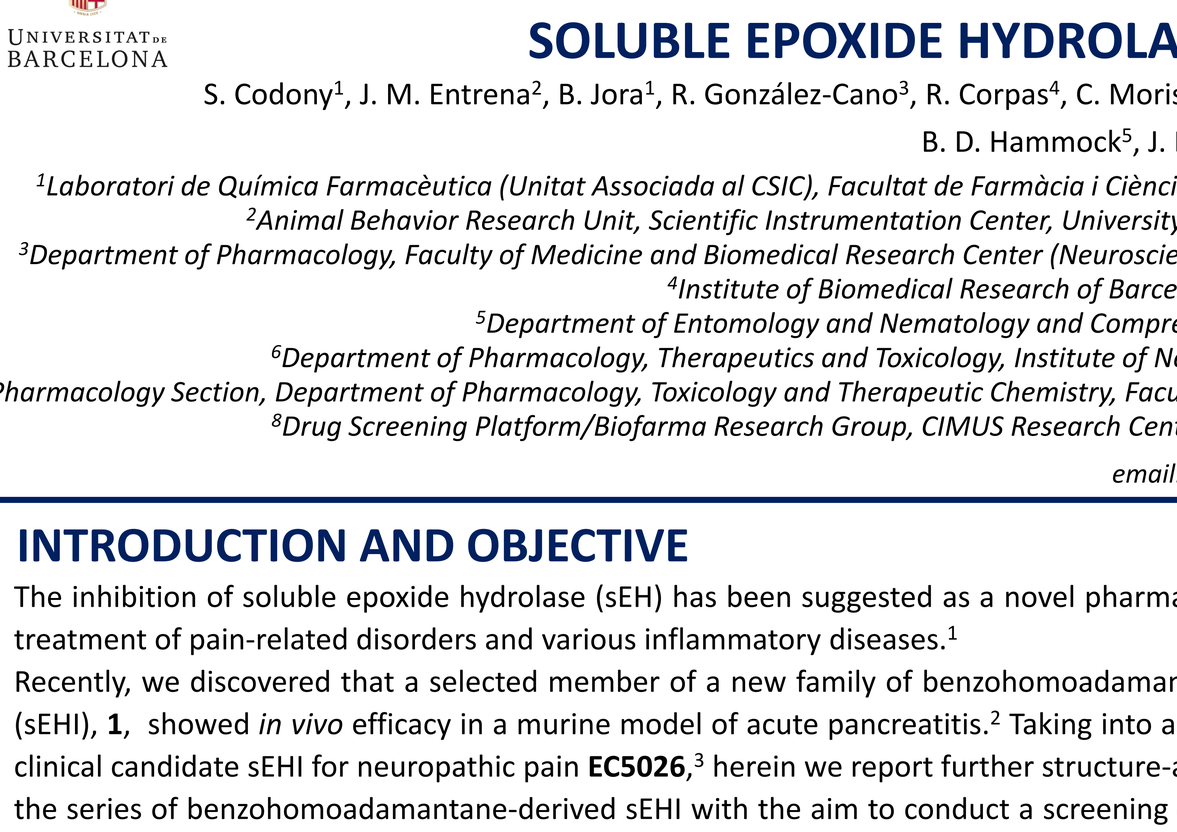
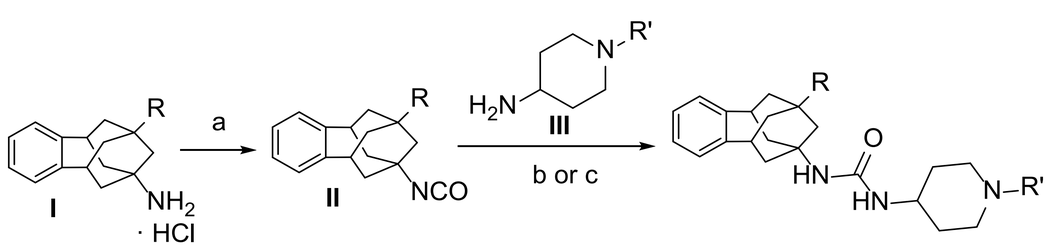


Figure 1. Metabolism pathway of Arachidonic acid.

SYNTHESIS



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.

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

PHARMACOKINETIC STUDIES IN MICE (5 mg/kg, s.c.)

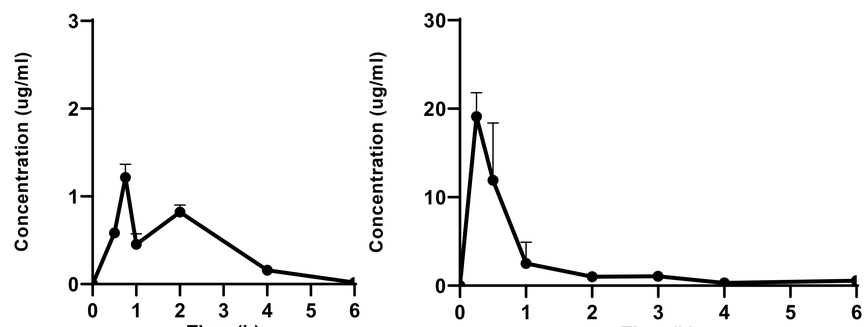


Figure 3. Plasma concentration *vs* time for compounds **8** (left) and **14** (right), respectively.

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.[4]

IN VIVO EFFICACY STUDIES IN MICE

Capsaicin-induced secondary mechanical hypersensitivity (allodynia) model.

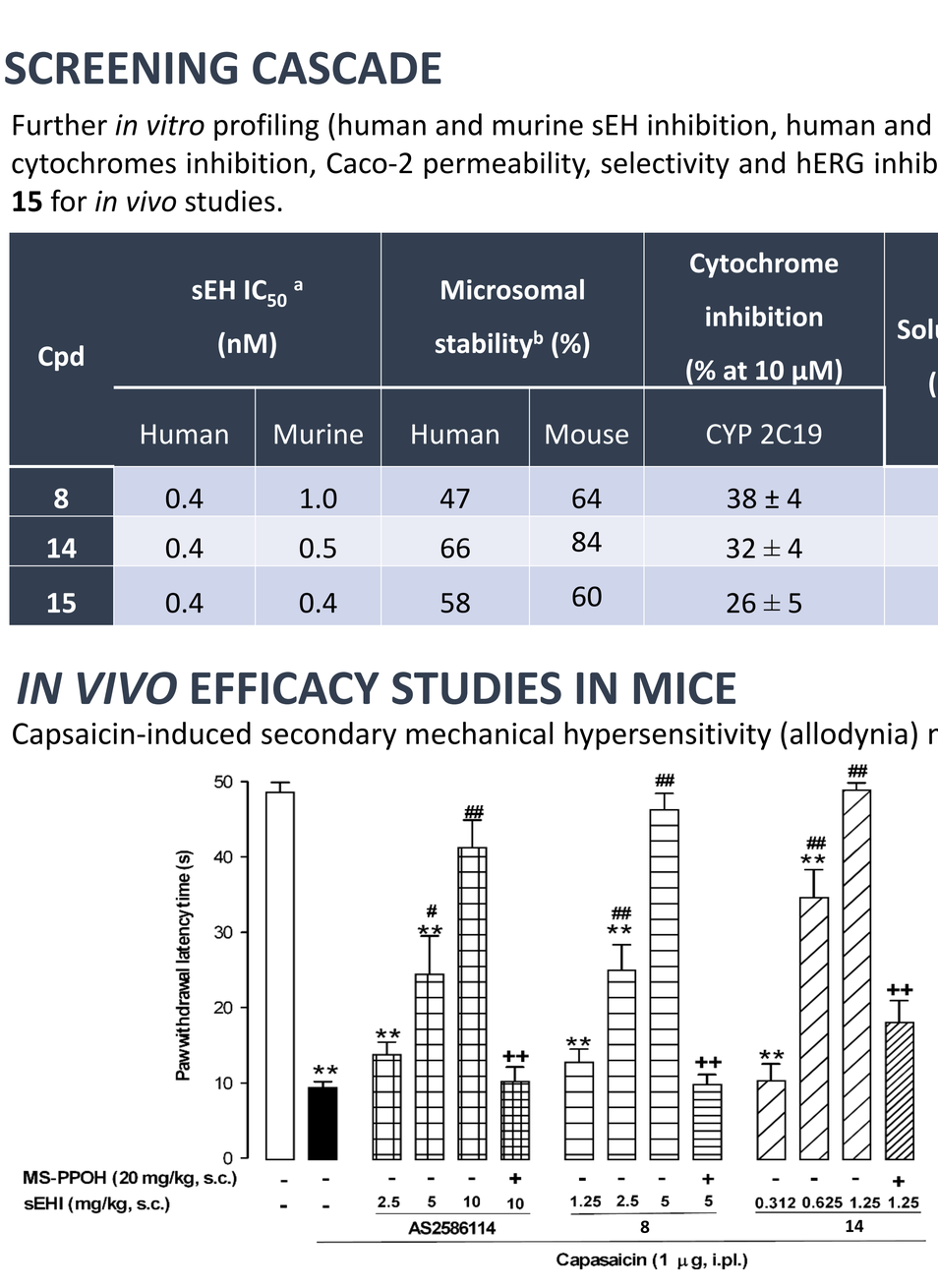


Figure 4. Reduction of capsaicin-induced secondary mechanical hypersensitivity in mice by the systemic administration of known AS2586114,[5] and compounds **8** and **14**, is due to sEH inhibition.[4]

Cyclophosphamide(CYP)-induced cystitis (visceral pain).

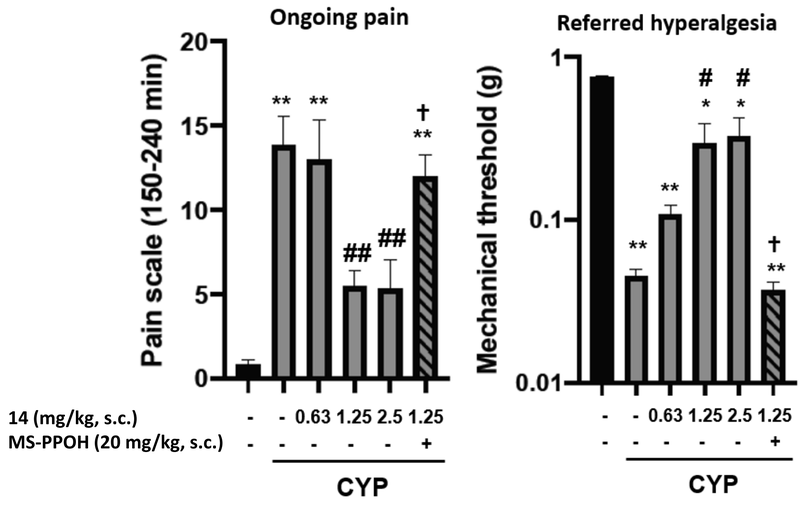


Figure 5. Effects of compound **14** on pain-related behaviors and referred mechanical hyperalgesia induced by CYP.[4]

CONCLUSIONS

- We have further explored medicinal chemistry around new benzohomoadamantane-based piperidine derivatives.
- 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 models of allodynia and visceral pain.[4]
- This study opens a whole range of applications of the benzohomoadamantane-based sEHIs in the pain field.

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