European Journal of Medicinal Chemistry 108 (2016) 466–475

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Exploring the role of chloro and methyl substitutions in 2-phenylthiomethyl-benzoindole derivatives for 5-LOX enzyme inhibition

Antonella Peduto [a], Verena Krauth [b], Selene Collarile [a], Fiederike Dehm [b], Marika Ambruosi [a], Carmela Belardo [a], Francesca Guida [a], Antonio Massa [c], Veronica Esposito [d], Sabatino Maione [a], Mario de Rosa [a], Oliver Werz [b], Rosanna Filosa [a, *]

[a] Department of Experimental Medicine, Second University of Naples, Via Costantinopoli, 16 – 80138 Naples, Italy
[b] Chair of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, University of Jena, Philosophenweg 14, D-07743 Jena, Germany
[c] Department of Chemistry, University of Salerno, Via Ponte don Melillo, 84084 Fisciano, SA, Italy
[d] Dipartimento di Farmacia, Università degli Studi di Napoli Federico II, Via D. Montesano 4980131 Napoli, Italy

ARTICLE INFO

Article history:
Received 16 September 2015
Received in revised form
26 November 2015
Accepted 27 November 2015
Available online 7 December 2015

Keywords:
5-lipoxygenase
Leukotriene
Indoles

ABSTRACT

Following the results we previously reported on a series of ethyl 2-phenylthiomethyl 5-hydroxyindole-3-carboxylate derivatives as 5-lipoxygenase (5-LOX) inhibitors, in order to obtain a more selective compound with respect to the previous generation of derivatives, we decided to modify the structure of the core ligand.

The first level of structural modification involved the annelation of benzene to the indole, yielding corresponding benzo[*g*]indole derivatives, systematic optimization of methyl or chlorine groups in meta-, ortho- and ortho/para-position of 2-phenylthiomethyl moiety were applied. The reported results show that extension of the aromatic core led to a great enhancement of activity, especially in cell-free assay, and the accurate structure-based design provided compounds **6f**, **6g** and **6l** that block 5-LOX activity in cell-free assays with IC_{50} ranging from 0.17 to 0.22 μM, and suppress 5-LOX product synthesis in polymorphonuclear leukocytes with IC_{50} ranging from 0.19 to 0.37 μM. Moreover we have identified **6f** and **6l** as dual 5-lipoxygenase (5-LO) and microsomal prostaglandin E_2 synthase-1 (mPGES-1) inhibitors and compound **6l** significantly reduces inflammatory reactions in the carrageenan-induced mouse paw oedema. The reported *in vivo* analysis, together with the accessible synthetic procedure, stimulate for the generation of further potent antinflammatory benzoindoles-based agents.

1. Introduction

In the last decades, several studies have demonstrated the key role played by 5-lipoxygenase (5-LOX) in inflammation-related disorders. Leukotrienes (LT), formed from AA by catalysis of 5-LOX, are fast reacting pro-inflammatory mediators of the immune system [1,2]; besides their physiological roles, they primarily mediate inflammatory and allergic reactions [3] and are involved in the onset of inflammatory diseases such as asthma, allergic rhinitis, rheumatoid arthritis and also cardiovascular disorder [4]. However, numerous studies have demonstrated the overexpression of 5-LOX in tissue samples of primary tumor cells as well as in established cancer cell lines [5]. An increasing body of evidence suggests a crucial role for 5-LOX products in the early stage of pancreatic, prostate and colorectal carcinogenesis [6] and glioma cell lines [7]. Recent studies showed that 5-LOX expression appears to be upregulated in patients with neurodegenerative disease like AD, demonstrating the key role of leukotrienes promoting Aβ generation [8–10]. In other reports, the potent and selective 5-LOX inhibitor zileuton reduced the amyloid and tau pathology as well as memory impairments in different mouse models of AD [11,12].

Continuing our studies on small molecules able to block 5-LOX activity [13–18], we recently reported the design and synthesis of a small collection of differently decorated 5-hydroxyindole-3-carboxylates derivatives able to interact with 5-LOX at nanomolar

* Corresponding author. Department of Experimental Medicine, Second University of Naples, Via Costantinopoli, 16 – 80138 Naples, Italy.
E-mail address: rosanna.filosa@unina2.it (R. Filosa).

http://dx.doi.org/10.1016/j.ejmech.2015.11.048
0223-5234/

concentration [14]. Our previous investigations have disclosed the influence of the substitution pattern on the phenylthiomethyl moiety, the simultaneous presence of chlorine in ortho-positions of the thiophenol ring as well as the introduction of three methyl groups (ortho, para) that led to compound with marked increase of 5-LOX inhibitory activity [14]. Among all tested compounds, ethyl 5-hydroxy-2-(mesitylthiomethyl)-1-methyl-1H-indole-3-carboxylate (**1**) showed good potency in inhibiting 5-LOX activity in cell-free assays with IC_{50} = 0.7 μM and suppressed 5-LOX product synthesis in polymorphonuclear leukocytes with IC_{50} = 0.23 μM, being equally potent to the well-recognized reference inhibitor zileuton, used as antiasthmatic drug in the clinics. With regard to other AA-metabolizing enzymes like COX-1, COX-2 and 12-LO, compound **1** was rather selective for 5-LOX with minor effects also on 15-LOX. Moreover, our previous works reported that the annelation of a [g]benzene ring to the indole led to more potent inhibitors, exemplified by compounds **2a** and **2b** with IC_{50} of 0.086 and 0.097 μM in cell-free and 0.23 and 1.2 μM in cell-based assays, respectively [19,20] (Fig. 1). We showed also that benzo[g]indol-3-carboxylates potently inhibit mPGES-1 and thus represent a novel class of dual 5-LO/mPGES-1 inhibitors.

With the goal of increasing potency and establishing a better understanding of the pharmacophore for 5-LO inhibition, we focused our investigation on the effects of introduction one or more chlorine and methyl substituents on the benzoindole ring. Moreover, introduction of a methoxy or ethoxy group at C5 position and N alkylation of the indole nitrogen atom (**7a-b**, **8a-b** and **11**) were also carried out in order to investigate the effect of OH and NH on the potency against 5-LOX.

Here we report the synthesis and the pharmacological profile of this second generation of benzoindole derivatives with anti-inflammatory effectiveness *in vivo*. While further studies are certainly needed to better describe the exact anti-inflammatory mode of action, this novel focused small molecules library exerts new interesting hits shedding further light on the structural requirements needed for an optimal ligand–enzyme interaction.

2. Results and discussion

2.1. Chemistry

The synthesis of desired compounds **6a-l** (Scheme 1) has been accomplished in three steps of reaction. The final Nenitzescu reaction has proven to be the simplest entry into 5-hydroxy-1*H*-benzo[*g*]indoles. Essentially, stirring of the enaminoesters **5a-l** with 1.0 equiv of 1,4-naphthoquinone in presence of catalytic amount of ZnI_2 at room temperature led to the expected 5-hydroxy-1*H*-benzo[*g*]indoles. Nenitzescu reactants **5a-l** were prepared starting from thiophenols **3a-l**, which were first reacted with ethyl 4-chloroacetoacetate to give the *β*-ketoesters **4a-l**. *β*-Ketoesters **4a-l** were converted into their appropriate enaminoesters **5a-l** by refluxing them in toluene with an excess of ammonium acetate. **7a-b** and **8a-b** were prepared by alkylation of **6b** with iodoalcanes

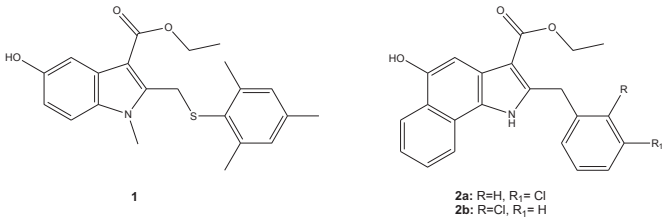
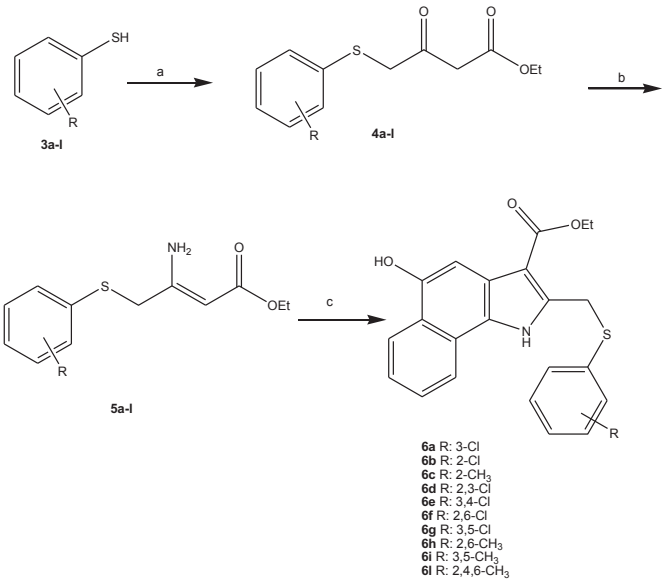


Fig. 1. Chemical structure of compounds **1, 2a** and **2b.**

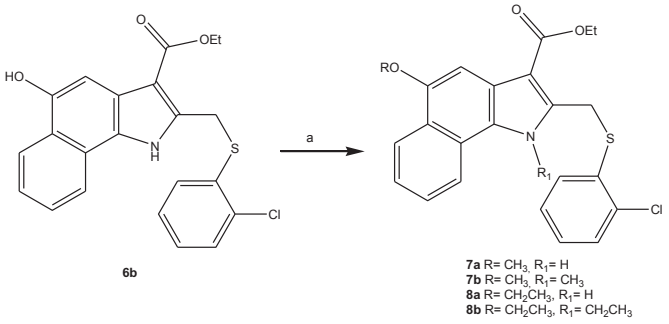


Scheme 1. Synthesis of compounds **6a-l**. Reagents and conditions: a) 4-chloroacetoacetate, NEt_3, CH_2Cl_2, 0 °C, 30 min; b) NH_4OAc, HOAc toluene, reflux, 6h; c) 1,4-naphtoquinone, ZnI_2, CH_2Cl_2, reflux, 40 min.

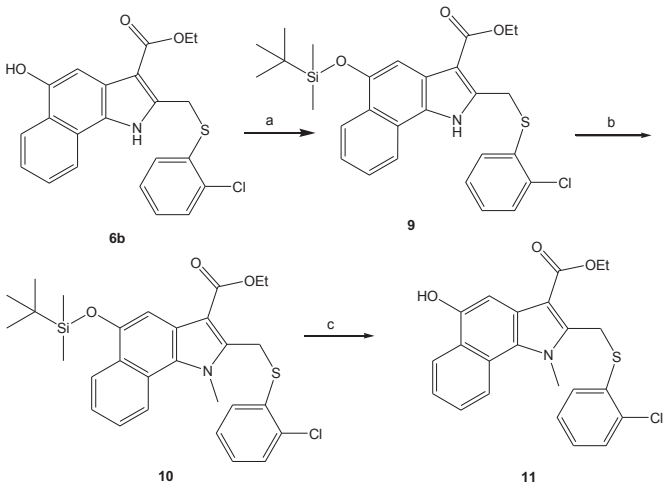
under basic conditions using microwave irradiation. Protection of the hydroxy group of **6b** with tertbutyl dimethyl silyl chloride (TBDSCl), alkylation with iodomethane and final deprotection with TBAF in THF allowed to obtain desired compound **11** in good yield (Scheme 2) (see Scheme 3)

2.2. Evaluation of 5-LOX activity and structure-activity Relationships

In order to assess the effects of the synthesized compounds on 5-LOX product synthesis, a cell-free assay using isolated human recombinant 5-LOX and a cell-based assay using human neutrophils was applied. The cell-free assay allows identifying compounds that directly interfere with 5-LOX catalytic activity, whereas the cell-based test system considers cellular regulatory aspects of 5-LOX product synthesis, and as such offers several points of attack of a given compound (e.g., inhibition of FLAP or coactosine-like protein (CLP), interference with 5-LOX-activating lipid hydroperoxides, protein kinases or Ca^{2+} mobilization, and 5-LOX translocation/membrane association) [21]. The reference 5-LOX inhibitor N-[1-(1-benzothien-2-yl)ethyl]-N-hydroxyurea (zileuton) was used to control the 5-LOX activity assays. As shown in Table 1, all tested compounds (**6a-l**) which maintain OH and NH groups in



Scheme 2. Synthesis of compounds **7a-b** and **8a-b**. Reagents and conditions: a) Iodomethane or iodoethane K_2CO_3, Acetone, MW, 30 min, 130 °C.



Scheme 3. Synthesis of compound **11**. Reagents and conditions: a) Imidazole, TBDSCl, CH_2Cl_2, 3h, rt; b) NaH, dry THF, 2h, 0 °C; c) TBAF 1M in THF, THF, 10 min, 0 °C.

their structure showed a significant activity in inhibiting 5-LOX in cell-based assay with IC_{50} values in the low micromolar range while in cell-free test, the substituent plays a key role in the activity.

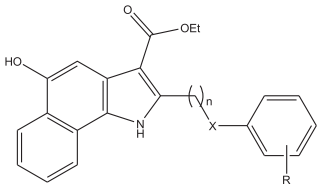
The corresponding 2-phenylthiomethyl benzoindoles of derivatives **2a** and **2b**, **6a** and **6b** retain a good activity in cell-based assay, especially for derivative **6b** which is 6-fold more active than **2b** but they showed a decrease of potency in cell-free test. The replacement of chlorine in position 2 with a methyl (**6c** *vs* **6b**) does not significantly alter the efficiency in intact cells (IC_{50} = 0.18 μM and 0.26 μM respectively) while an improvement of activity is observed in the cell-free assay (0.33 *vs* 0.82 μM). With regard to polychlorinated derivatives, the position of the substituent is fundamental for the activity, especially in the cell-free assay. In fact, compounds **6f** (2,6-Cl) and **6g** (3,5-Cl) showed the best activity in inhibiting 5-LOX, with IC_{50} values of 0.17 and 0.22 μM, respectively, while the activity of compounds **6d** (2,3 Cl) and **6e** (3,4 Cl) is very low, in particular for **6d** with an IC_{50} > 1 μM. These results could imply the presence of a bulky hydrophobic pocket in the binding site of enzyme which allows the arrangement of two chlorines. Considering IC_{50} values in cell-based assay, we observed a similar activity for all polychlorinated derivatives especially for **6e, 6f** and **6g**, while **6d** is slightly less active. This could be due a supplementary mechanism of inhibition of 5-LOX product synthesis, by interaction with other targets (i.e. FLAP or PLA_2).

In the case of polymethylated molecules **6h**, **6i** and **6l,** they showed a comparable activity in cell-based conditions with IC_{50} of 0.17, 0.29 and 0.37 μM, respectively; otherwise only compound **6l** was active against isolated 5-LOX with an IC_{50} of 0.2 μM. The alkylation of the hydroxy group of compound **6b** led to compounds **7a** and **8a** which demonstrated a significant decrease of activity, especially in cell-based assays (IC_{50} = 1.15 μM for **7a** and 1.26 μM for **8b** vs 0.182 μM for **6b**). The di-alkylation of OH and NH groups is detrimental for the activity, in fact, compounds **7b** and **8b** showed an IC_{50} > 10 μM. The N-methyl derivative of **6b**, i.e. compound **11**, is completely inactive with IC_{50} values > 10 μM in both assays (Table 2). Unlike for indoles studied in our previous work [14], these data demonstrate that in benzoindole derivatives the substitution of hydrogen of the indole nitrogen with an alkyl residue is not tolerated, maybe for a different positioning of molecule in the active site.

2.3. *Anti-inflammatory effectiveness of derivatives **1**, **6f, 6g, 6l** in carrageenan-induced paw oedema*

In order to verify the anti-inflammatory efficacy of the most interesting compounds of the series, we evaluated their effect on carrageenan-induced paw oedema, which represents a well-established model of acute inflammation [22]. Many 5-LOX and FLAP inhibitors that potently repressed 5-LOX product synthesis in isolated cells failed to do so in carrageenan-induced acute inflammation known as accompanied by elevated LT levels. Intraplantar injection of carrageenan led to an increase in hind paw volume, expressed as oedema, monitored for a period of 6 h. The increase in paw volume (vehicle, i.p. treatment) reached a maximum at 4 h post-carrageenan application (Fig. 2). Indole derivative **1** that previously showed potent inhibition of 5-LO in cell-based

Table 1
Inhibition of 5-LOX activity of compounds **6a-l**, **2a-b** and zileuton in a cell-based assay (intact PMNL) and in a cell-free assay. Data are given as mean ± S.E.M., n = 3–4.



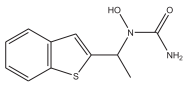
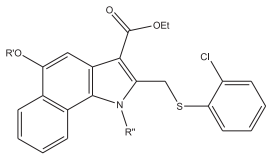
				5-LOX activity; cell-free		5-LOX activity; intact PMNL	
Cpd	R	n	X	IC_{50} (μM)	Remaining activity at 1 μM (%)	IC_{50} (μM)	Remaining activity at 1 μM (%)
6a	3-Cl	1	S	>1	63.8 ± 5	0.257 ± 0.1	
6b	2-Cl	1	S	0.82 ± 0.03		0.182 ± 0.09	
6c	2-CH_3	1	S	0.33 ± 0.15		0.255 ± 0.05	
6d	2,3-Cl	1	S	>1	72 ± 14.8	0.657 ± 0.07	
6e	3,4-Cl	1	S	0.93 ± 0.03		0.237 ± 0.09	
6f	2,6-Cl	1	S	0.17 ± 0.11		0.19 ± 0.09	
6g	3,5-Cl	1	S	0.22 ± 0.07		0.24 ± 0.12	
6h	2,6-CH_3	1	S	>1	65.9 ± 5.7	0.17 ± 0.12	
6i	3,5-CH_3	1	S	>1	63 ± 1.7	0.29 ± 0.07	
6l	2,4,6-CH_3	1	S	0.2 ± 0.06		0.37 ± 0.07	
2a	3-Cl	0	CH_2	0.086 ± 0.02[19]		0.23 ± 0.07[19]	
2b	2-Cl	0	CH_2	0.097 ± 0.09[19]		1.2 ± 0.07[19]	
Zileuton				0.6[14]		0.7[14]	

Table 2
Inhibition of 5-LOX activity of compounds **7a-b**, **8a-b, 11** vs **6b** in a cell-based assay (intact PMNL) and in a cell-free assay. Data are given as mean ± S.E.M., n = 3–4.



Cpd	R′	R″	5-LOX activity; Cell-free IC_{50} (μM)	Remaining activity at 10 μM (%)	5-LOX activity; Intact PMNL IC_{50} (μM)	Remaining activity at 10 μM (%)
6b	H	H	0.82 ± 0.03		0.182 ± 0.09	
7a	CH_3	H	1.74 ± 0.13		1.15 ± 0.12	
7b	CH_3	CH_3	>10	98.7 ± 2.3	>10	97.6 ± 1.5
8a	CH_2CH_3	H	2.04 ± 0.139		1.26 ± 0.084	
8b	CH_2CH_3	CH_2CH_3	>10	99.4 ± 9.3	>10	95.5 ± 12.2
11	H	CH_3	>10	81.4 ± 13.2	>10	61.9 ± 12.3

(IC_{50} = 0.23 μM) and cell-free assays (IC_{50} = 0.7 μM) was used as lead structure [14]. We observed that **1** (1, 2 and 4 mg/kg, i.p.) significantly reduced paw oedema during the 2–4 h period in a dose-dependent manner (Fig. 2). Futhermore, in agreement with the results from intact cells, the corresponding benzoindole derivative of **1**, compound **6l** was more efficient in the *in vivo* test (Fig. 3). Among the benzo[g]indol-3-carboxylates tested (see Table 1), **6f** and **6g** were the most efficient to inhibit LTB_4 formation in cell-free assays and maintained their potency in intact PMNL. In vivo experiment **6f** failed to evoke significant changes, while **6g** (1, 2 and 4 mg/kg, i.p.) decreased oedema, as compared with vehicle-treated animals (Fig. 4). In fact, in mice treated with 4 mg/kg **1**, **6f**, **6g** or **6l**, the peak of the response to carrageenan at 4 h was reduced by 60.9%, 9.1%, 60%, and 77.4%, respectively, while zileuton (20 mg/kg, i.p.), used as reference, caused only 38.2% inhibition.

2.4. Screening of indole-3-carboxylates 1, 6f, 6g, 6l as direct mPGES-1inhibitors

Based on the potent anti-inflammatory efficacy of **6l** *in vivo*, the compound may also interfere with other enzymes involved in the generation of pro-inflammatory eicosanoids. Indeed, it may suppress other pro-inflammatory events such as the biosynthesis of PGE_2 as demonstrated for the related benzoindoles [20]. To confirm this hypothesis it was analyzed for inhibition of PGE_2 formation in the cell-free assay together with the test compounds(Table 3); derivatives that achieved more than 50% inhibition at 10 μM, IC_{50} values were assessed.

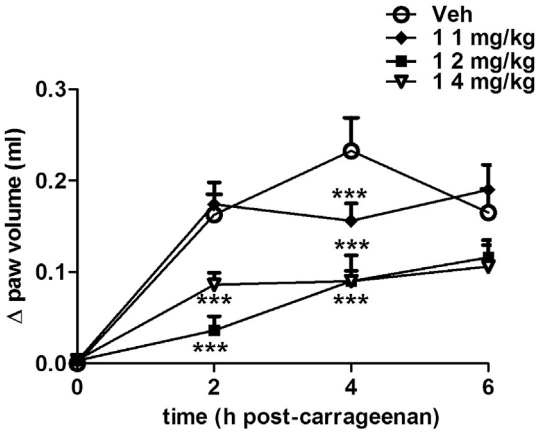


Fig. 2. Effect of compounds **1** (1, 2, 4 mg/kg, i.p.) on carrageenan-induced paw oedema 0–6 h after carrageenan injection. Data are expressed as mean ± SEM (n = 6–8 for each group). * or *** indicate significant (P < 0.05 or P < 0.001, respectively) differences vs carrageenan + vehicle-treated animals.

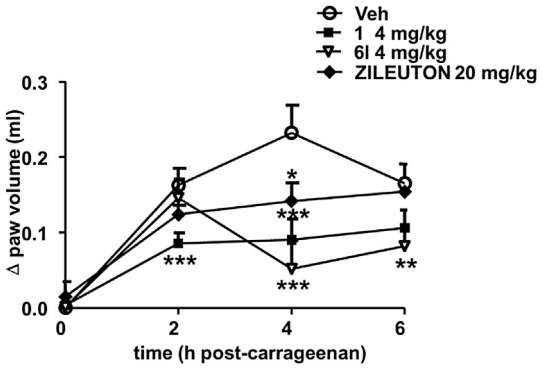


Fig. 3. Effect of compounds **1** (4 mg/kg, i.p.), **6l** (4 mg/kg, i.p.) and zileuton (20 mg/kg, i.p.) on carrageenan-induced paw oedema 0–6 h after carrageenan injection. Data are expressed as mean ± SEM (n = 6–8 for each group). * or *** indicate significant (P < 0.05 or P < 0.001, respectively) differences vs carrageenan + vehicle-treated animals.

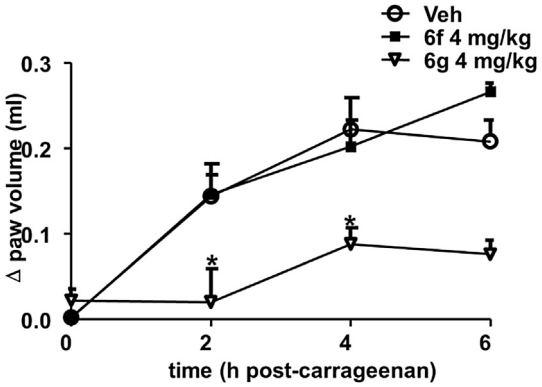
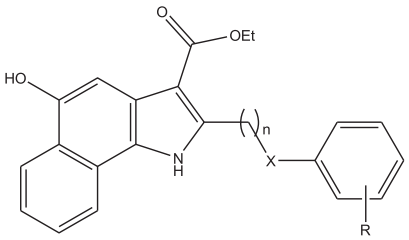
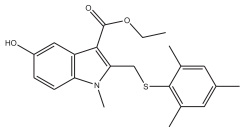


Fig. 4. Effect of compounds **6f** (4 mg/kg, i.p.) and **6g** (4 mg/kg, i.p.) on carrageenan-induced paw oedema 0–6 h after carrageenan injection. Data are expressed as mean ± SEM (n = 6–8 for each group). * or *** indicate significant (P < 0.05 or P < 0.001, respectively) differences vs carrageenan + vehicle-treated animals.

Only a moderate inhibition of mPGES-1 activity was observed at 10 μM (27.8 ± 0.79%) for compound **1,** enlargement of the hydrophobic core structure by annelation of benzene to the indole of **1**, yielding the corresponding benzo[g]indole derivate **6l**, in accordance with our previous results, led to an IC_{50} of 1.9 μM. Exchange

Table 3
Inhibition of m-PGES-1 activity of compounds **l**, **2a, 2b, 6f, 6g 6l**. Data are given as mean ± S.E.M., n = 3–4.



Cpd	R	n	X	IC_{50} (μM) mPGES-1	Remaining activity a μM (%)
6f	2,6-Cl	1	S	1.33 ± 0.10	
6g	3,5-Cl	1	S	13.84 ± 0.27	
6l	2,4,6-CH_3	1	S	1.93 ± 0.07	
2a	3-Cl	0	CH_2		57 ± 6[20]
1					87.33 ± 3.88

of the methyl by a chlorine substituents in o-position slightly improved the potency (IC_{50} = 1.33), while variation of the positioning of the chlorine in the thiol ring of **6g** to meta (compound **6f**) was detrimental and led to an IC_{50} of 13.8 μM.

Although potent inhibition of mPGES-1 under cell-free conditions must not necessarily result in efficient suppression of *in vivo* prostanoid formation the high potency on isolated mPGES-1well correlate the peak of the response to carrageenan at 4 h *in vivo* of **6l.**

3. Conclusion

In this paper a series of new ethyl benzo[*g*]indole-3-carboxylate was prepared and tested to evaluate their ability to inhibit 5-LOX activity. Considering the *in vitro* biological data we suggest that the ability to inhibit 5-LOX is closely related to the chemical structures of the molecules.

Our studies allowed us to draw a tentative SAR profile and to optimize this series, in particular:

1. Alkylation of the indole nitrogen causes loss of activity (**7b**, **8b** and **11**).
2. Substitution of hydroxyl group in position 5 of benzoindole ring with methoxy or ethoxy residues decreases the potency (**7a** and **8a**).
3. Substitutions on the thiophenol ring influences 5-LOX activity. The simultaneous presence of chlorine in ortho or meta-positions of the thiophenol ring (**6f** and **6g**), as well as the introduction of three methyl groups (**6l**) (ortho, para), causes a marked increase of 5-LOX inhibitory activity in cell-free assays.

Moreover **6g** and **6l** are able to inhibit mPGES-1 activity in cell-free and exhibit IC_{50} values of 1.33 μM and 1.93 μM respectively.

The antiflammatory properties of selected compounds (**6f**, **6g** and **6l**) was confirmed *in vivo* evaluating their ability to reduce carrageenan-induced paw oedema. The most interesting compound **6l** caused a reduction of oedema of 77.4%, which is superior over zileuton and indole **1** used as reference compounds.

Future studies addressing their anti-inflammatory effectiveness in other animal models will reveal the therapeutic potential of these new benzo[*g*]indole-3-carboxylates.

4. Experimental section

4.1. Chemistry

All reagents were analytical grade and purchased from Sigma–Aldrich (Milano, Italy). Flash chromatography was performed on Carlo Erba silica gel 60 (230–400 mesh; CarloErba, Milan, Italy). TLC was carried out using plates coated with silica gel 60F 254 nm purchased from Merck (Darmstadt, Germany). ^1H and ^{13}C NMR spectra were registered on a Brucker AC 300. Chemical shifts are reported in ppm. The abbreviations used are follows: s, singlet; d, doublet; dd double doublet; bs, broad signal. MS spectrometry analysis ESI-MS was carried out on a Finnigan LCQ Deca ion trap instrument. Microanalyses were carried out on a Carlo Erba 1106 elemental analyzer. Melting points were performed by Stuart melting point SMP30 and are uncorrected. All microwave experiments were performed in a self-tuning single mode Biotage Initiator Microwave.

4.2. General procedure for the synthesis of Ethyl 4-(thioaryloxy) acetoacetates (*4a-l)*

A solution of 4-chloroacetoacetate (500 mg, 3.28 mmol, 1 eq.), NEt_3 (348 mg, 3.44 mmol, 1.05 eq.) and appropriate thiophenols **3a-l** (1.03 eq.) in CH_2Cl_2 (6.5 mL) was stirred at 0 °C for 30 min. The reaction mixture was diluted with EtOAc and washed with an aqueous solution of NaOH (1 M), hydrochloric acid (1 M) and brine. The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (silica gel, hexane/EtOAc 9:1).

4.2.1. Ethyl 4-(3-chlorophenylthio)-3-oxobutanoate *(4a)*

Yield: 81%. ^1H NMR data are in agreement with those reported in literature [23].

4.2.2. Ethyl 4-(2-chlorophenylthio)-3-oxobutanoate *(4b)*

Yield: 68%. ^1H NMR data are in agreement with those reported in literature [23].

4.2.3. Ethyl 4-(o-tolylthio)-3-oxobutanoate *(4c)*

Yield: 79%. ^1H NMR data are in agreement with those reported in literature [24].

*4.2.4. Ethyl 4-(2,3-dichlorophenylthio)-3-oxobutanoate **(4d)***

Yield: 86%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ1.29 (t, 3H, J = 7.34 Hz); 3.67 (s, 2H); 3.92 (s, 2H); 4.21 (q, 2H, J = 7.34 Hz); 7.19 (m, 2H); 7.34 (d, 1H, J = 7.88 Hz).

*4.2.5. Ethyl 4-(3,4-dichlorophenylthio)-3-oxobutanoate **(4e)***

Yield: 96%. ^{1}H NMR data are in agreement with those reported in literature [23].

*4.2.6. Ethyl 4-(2,6-dichlorophenylthio)-3-oxobutanoate **(4f)***

Yield: 88%. ^{1}H NMR data are in agreement with those reported in literature [23].

*4.2.7. Ethyl 4-(3,5-dichlorophenylthio)-3-oxobutanoate **(4g)***

Yield: 62%. ^{1}H NMR data are in agreement with those reported in literature [23].

*4.2.8. Ethyl 4-(2,6-dimethylphenylthio)-3-oxobutanoate **(4h)***

Yield: 85%.^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.29 (t, 3H, J = 7.34 Hz); 2.59 (s, 6H),; 3.53 (s, 2H); 3.55 (s, 2H); 4.18 (q, 2H, J = 7.34 Hz); 7.09–7.19 (m, 3H).

*4.2.9. Ethyl 4-(3,5-dimethylphenylthio)-3-oxobutanoate **(4i)***

Yield: 92%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.28 (t, 3H, J = 7.34 Hz); 2.29 (s, 6H); 3.65 (s, 2H); 3.80 (s, 2H); 4.20 (q, 2H, J = 7.34 Hz); 6.88 (s 1H); 6.98 (s, 2H).

*4.2.10. Ethyl 4-(mesitylthio)-3-oxobutanoate **(4l)***

Yield: 86%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.37 (t, 3H, J = 7.34 Hz); 2.20 (s, 3H); 2.45 (s, 6H); 3.58 (s, 2H); 3.60 (s, 2H); 4.17 (q, 2H, J = 7.34 Hz); 6.87 (s, 2H).

*4.3. General procedure for the synthesis of Ethyl 4-(thioaryloxy) enaminoesters (**5a-l**)*

The appropriate β-ketoester **4a-l** (3.0 mmol) and ammonium acetate (1.16 g, 15.0 mmol) were dissolved in dry toluene (10 mL). After adding four drops of acetic acid, the reaction mixture was refluxed for 6 h under azeotropic removal of water. After cooling down to RT, the mixture was washed with saturated aqueous $NaHCO_3$ solution. The resulting organic layer was dried over Na_2SO_4, filtered, and evaporated to give a residue, which was purified by flash chromatography on silica gel (hexane/Ethyl acetate 9:1).

*4.3.1. (Z)-ethyl 4-(3-chlorophenylthio)-3-aminobut-2-enoate **(5a)***

Yield: 45%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.28 (t, 3H, J = 7.34 Hz); 3.63 (s, 2H); 4.14 (q, 2H, J = 7.34 Hz); 4.67 (s, 1H); 7.20–7.25 (m, 3H); 7.34 (s, 1H).

*4.3.2. (Z)-ethyl 4-(2-chlorophenylthio)-3-aminobut-2-enoate **(5b)***

Yield: 67%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.27 (t, 3H, J = 7.34 Hz); 3.69 (s, 2H); 4.15 (q, 2H, J = 7.34 Hz); 4.70 (s, 1H); 7.18 (td, 1H, J = 1.5, 7.2 Hz); 7.24 (td, 1H, J = 1.5, 7.2 Hz); 7.33 (dd, 1H, J = 1.5, 7.2 Hz); 7.40 (dd, 1H, J = 1.5, 7.2 Hz).

*4.3.3. (Z)-ethyl 4-(o-tolylthio)-3-aminobut-2-enoate **(5c)***

Yield: 87%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.23 (t, 3H, J = 7.34 Hz); 2.38 (s, 3H); 3.67 (s, 2H); 4.12 (q, 2H, J = 7.34 Hz); 4.67 (s, 1H); 7.08–7.22 (m, 4H).

*4.3.4. (Z)-ethyl 4-(2,3-dichlorophenylthio)-3-aminobut-2-enoate **(5d)***

Yield:73%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.28 (t, 3H, J = 7.34 Hz); 3.70 (s, 2H); 4.13 (q, 2H, J = 7.34 Hz); 4.73 (s, 1H); 7.15–7.22 (m, 2H); 7.34 (dd, 1H, J = 2.4, 6.9 Hz).

*4.3.5. (Z)-ethyl 4-(3,4-dichlorophenylthio)-3-aminobut-2-enoate **(5e)***

Yield: 62%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.27 (t, 3H, J = 7.34 Hz); 3.59 (s, 2H); 4.14 (q, 2H, J = 7.34 Hz); 4.64 (s, 1H); 7.18 (dd, 1H J = 2.1, 8.4 Hz); 7.38 (d, 1H, J = 8.4 Hz); 7.45 (s, 1H).

*4.3.6. (Z)-ethyl 4-(2,6-dichlorophenylthio)-3-aminobut-2-enoate **(5f)***

Yield: 73%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.22 (t, 3H, J = 7.34 Hz); 3.63 (s, 2H); 4.08 (q, 2H, J = 7.34 Hz); 4.50 (s, 1H); 7.37 (t, 1H, J = 7.88 Hz); 7.48 (d, 2H, J = 7.88 Hz).

*4.3.7. (Z)-ethyl 4-(3,5-dichlorophenylthio)-3-aminobut-2-enoate **(5g)***

Yield: 59%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, 3H, J = 7.34 Hz); 3.62 (s, 2H); 4.13 (q, 2H, J = 7.34 Hz); 4.70 (s, 1H); 7.21 (d, 3H, J = 1.9 Hz).

*4.3.8. (Z)-ethyl 4-(2,6-dimethylphenylthio)-3-aminobut-2-enoate **(5h)***

Yield: 68%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.28 (t, 3H, J = 7.34 Hz); 2.55 (s, 6H); 3.28 (s, 2H); 4.14 (q, 2H, J = 7.34 Hz); 4.43 (s, 1H); 7.09–7.19 (m, 3H).

*4.3.9. (Z)-ethyl 4-(3,5-dimethylphenylthio)-3-aminobut-2-enoate **(5i)***

Yield: 53%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.27 (t, 3H, J = 7.34 Hz); 2.29 (s, 6H); 3.60 (s, 2H); 4.16 (q, 2H, J = 7.34 Hz); 4.65 (s, 1H); 6.87 (s, 1H); 6.98 (s, 2H).

*4.3.10. (Z)-ethyl 3-amino-4-(mesitylthio)but-2-enoate **(5l)***

Yield: 58%. ^{1}H NMR (CD_3OD-d_4, 300 MHz) δ 1.20 (t, 3H, J = 7.34 Hz); 2.28 (s, 3H); 2.52 (s, 6H); 3.22 (s, 2H); 4.05 (q, 2H, J = 7.34 Hz); 4.13 (s, 1H); 4.65 (br, NH_2); 6.98 (s, 2H).

*4.4. General procedure for the synthesis of 5-Hydroxy-1H-benzo[g] indole-3-carboxylate **(6a-l)***

To a solution of 1,4-naphthoquinone (1.0 mmol) in 3 mL CH_2Cl_2, ZnI_2 (32.0 mg, 0.1 mmol) was added and the resulting mixture was heated to boiling temperature. A solution of enaminoesters **5a-l**, respectively (1.0 mmol), in 2 mL of CH_2Cl_2 was added drop by drop under stirring for 5–10 min. After refluxing for additional 20 min, the mixture was cooled to 0–5 °C for 2–3 h. The precipitated crystals were filtered off and washed with CH_2Cl_2 and hexane.

*4.4.1. Ethyl 2-((3-chlorophenylthio)methyl)-5-hydroxy-1H-benzo [g]indole-3-carboxylate **(6a)***

Rf (hexane/ethyl acetate 8:2) = 0.28. Yield: 4%. M.p. 167.5 °C dec. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.35 (t, 3H, J = 7.34 Hz); 4.28 (q, 2H, J = 7.34 Hz); 4.77 (s, 2H); 7.25–732 (m, 3H); 7.40–747 (m, 2H); 7.51 (s, 1H); 7.55 (t, 1H, J = 6.61 Hz); 8.16 (d, 1H, J = 7.80 Hz); 8.27 (d, 1H, J = 7.80 Hz); 9.69 (s, 1H, OH); 12.39 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 14.4, (CH_3CH_2), 29.3 (CH_2S), 59.9, (CH_3CH_2), 100.1 (C-3), 121.4 (C-5a), 121.7 (C-9), 121.8 (C-4), 122.7 (C-3a), 125.1 (C-9b), 126.5 (C-9a), 126.8–127.6 (C-6, C-7, C-8, C-2′, C-4′, C-5′, C-6′), 131.5 (C-1′), 133.4 (C-5), 134.5 (C-3′), 143.8 (C-2), 165.5 (C=O). MS-ESI (m/z): 410.06 [M^-]. Anal. Calcd. for $C_{22}H_{18}ClNO_3S$ C, 64.15; H, 4.40; Cl, 8.61; N, 3.40; O, 11.65; S, 7.78. Found C, 63.10; H, 4.45; Cl, 8.53; N, 3.32; O, 11.54; S, 7.62.

*4.4.2. Ethyl 2-((2-chlorophenylthio)methyl)-5-hydroxy-1H-benzo[g]indole-3-carboxylate **(6b)***

Rf (hexane/ethyl acetate 8:2) = 0.24. Yield: 21%. M.p. 209.0 °C dec. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.33 (t, 3H, J = 7.34 Hz); 4.26 (q, 2H, J = 7.34 Hz); 4.71 (s, 2H); 7.22 (t, 1H, J = 6.30 Hz); 7.35 (t, 1H, J = 6.30 Hz); 7.42 (d, 1H, J = 8.10 Hz); 7.49 (d, 1H, J = 8.10 Hz); 7.53 (s, 1H); 7.55–7.60 (m, 2H); 8.16 (d, 1H, J = 7.80 Hz); 8.27 (d, 1H, J = 7.80 Hz); 9.69 (s, 1H, OH); 12.39 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 14.4, (CH_3CH_2), 29.3 (CH_2S), 59.9, (CH_3CH_2), 100.1 (C-3), 121.4 (C-5a), 121.7 (C-9), 121.8 (C-4), 122.7 (C-3a), 125.1 (C-9b), 126.5 (C-9a), 126.8–127.6 (C-6, C-7, C-8, C-3′, C-4′, C-5′, C-6′), 131.5 (C-1′), 132.3 (C-2′), 133.4 (C-5), 143.8 (C-2), 165.5 (C=O). MS-ESI (m/z): 410.01 [M$^-$]. Anal. Calcd. for $C_{22}H_{18}ClNO_3S$ C, 64.15; H, 4.40; Cl, 8.61; N, 3.40; O, 11.65; S, 7.78 Found C, 63.75; H, 3.84; Cl, 8.15; N, 3.07; O, 11.25; S, 7.48.

*4.4.3. Ethyl 2-((o-tolylthio)methyl)-5-hydroxy-1H-benzo[g]indole-3-carboxylate **(6c)***

Rf (hexane/ethyl acetate 8:2) = 0.24. Yield: 56%. M.p. 184.5 °C dec. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.37 (t, 3H, J = 7.34 Hz); 2.22 (s, 3H); 4.26 (q, 2H, J = 7.34 Hz); 4.70 (s, 2H); 6.83–6.94 (m, 4H); 7.42 (t, 1H, J = 7.88 Hz); 7.52 (s, 1H); 7.57 (t, 1H, J = 7.88 Hz); 8.15 (d, 1H, J = 7.88 Hz); 8.30 (d, 1H, J = 7.88 Hz); 9.69 (s, 1H, OH); 12.39 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 14.4, (CH_3CH_2), 19.2, (o-CH_3), 29.3 (CH_2S), 59.9, (CH_3CH_2), 100.1 (C-3), 121.4 (C-5a), 121.7 (C-9), 121.8 (C-4), 122.7 (C-3a), 125.1 (C-9b), 126.5 (C-9a), 126.8–127.6 (C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′, C-6′), 131.5 (C-1′), 133.4 (C-5), 143.8 (C-2), 165.5 (C=O). MS-ESI (m/z): 390.13 [M$^-$]. Anal. Calcd. for $C_{23}H_{21}NO_3S$ C, 70.56; H, 5.41; N, 3.58; O, 12.26; S, 8.19 Found C, 71.48; H, 6.31; N, 4.23; O, 11.59; S, 7.37.

*4.4.4. Ethyl 2-((2,3-dichlorophenylthio)methyl)-5-hydroxy-1H-benzo[g]indole-3-carboxylate **(6d)***

Rf (hexane/ethyl acetate 8:2) = 0.22. Yield: 29%. M.p. 184.1 °C dec. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.33 (t, 3H, J = 7.34 Hz); 4.26 (q, 2H, J = 7.34 Hz); 4.71 (s, 2H); 7.38 (t, 1H, J = 6.30 Hz); 7.47 (t, 1H, J = 6.30 Hz); 7.53 (s, 1H); 7.55–7.60 (m, 3H); 8.16 (d, 1H, J = 7.80 Hz); 8.27 (d, 1H, J = 7.80 Hz); 9.69 (s, 1H, OH); 12.39 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 14.4, (CH_3CH_2), 29.3 (CH_2S), 59.9, (CH_3CH_2), 100.1 (C-3), 121.4 (C-5a), 121.7 (C-9), 121.8 (C-4), 122.7 (C-3a), 125.1 (C-9b), 126.5 (C-9a), 126.8–127.6 (C-6, C-7, C-8, C-4′, C-5′, C-6′), 137.5 (C-1′), 132.3 (C-2′-C-3′), 133.4 (C-5), 143.8 (C-2), 165.5 (C=O). MS-ESI (m/z): 446.05 [M$^-$]. Anal. Calcd. for $C_{22}H_{17}Cl_2NO_3S$ C, 59.20; H, 3.84; Cl, 15.89; N, 3.14; O, 10.75; S, 7.18 Found C, 58.73; H, 3.24; Cl, 15.19; N, 2.86; O, 10.05; S, 7.03.

*4.4.5. Ethyl 2-((3,4-dichlorophenylthio)methyl)-5-hydroxy-1H-benzo[g]indole-3-carboxylate **(6e)***

Rf (hexane/ethyl acetate 8:2) = 0.26. Yield: 16%. M.p. 183.5 °C dec. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.35 (t, 3H, J = 7.34 Hz); 4.28 (q, 2H, J = 7.34 Hz); 4.77 (s, 2H); 7.32 (d, 1H J = 6.63 Hz); 7.43 (t, 1H, J = 6.61 Hz); 7.48–7.52 (m, 3H); 7.64 (s, 1H); 8.16 (d, 1H, J = 7.80 Hz); 8.27 (d, 1H, J = 7.80 Hz); 9.69 (s, 1H, OH); 12.39 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 14.4, (CH_3CH_2), 29.3 (CH_2S), 59.9, (CH_3CH_2), 100.1 (C-3), 121.4 (C-5a), 121.7 (C-9), 121.8 (C-4), 122.7 (C-3a), 125.1 (C-9b), 126.5 (C-9a), 126.8–127.6 (C-6, C-7, C-8, C-2′, C-5′, C-6′), 129.8 (C-4′), 131.5 (C-1′), 133.4 (C-5), 133.6 (C-3′), 143.8 (C-2), 165.5 (C=O). MS-ESI (m/z): 445.94 [M$^-$]. Anal. Calcd. for $C_{22}H_{17}Cl_2NO_3S$ C, 59.20; H, 3.84; Cl, 15.89; N, 3.14; O, 10.75; S, 7.18 Found C, 58.70; H, 3.54; Cl, 15.37; N, 3.02; O, 10.25; S, 7.07.

*4.4.6. Ethyl 2-((2,6-dichlorophenylthio)methyl)-5-hydroxy-1H-benzo[g]indole-3-carboxylate **(6f)***

Rf (hexane/ethyl acetate 8:2) = 0.18. Yield: 35%. M.p. 191.8 °C dec. ^{1}H NMR (Acetone-d_6, 300 MHz) δ 1.33 (t, 3H, J = 7.34 Hz); 4.17 (q, 2H, J = 7.34 Hz); 4.70 (s, 2H); 7.32–7.55 (m, 4H); 7.64 (s, 1H); 8.16 (d, 1H, J = 7.88 Hz); 8.29 (d, 1H, J = 7.88 Hz); 8.64 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 14.4, (CH_3CH_2), 29.3 (CH_2S), 59.9, (CH_3CH_2), 100.1 (C-3), 121.4 (C-5a), 121.7 (C-9), 121.8 (C-4), 122.7 (C-3a), 125.1 (C-9b), 126.5 (C-9a), 126.8–127.6 (C-6, C-7, C-8, C-3′, C-4′, C-5′), 130.3 (C-2′-C-6′), 131.5 (C-1′), 133.4 (C-5), 143.8 (C-2), 165.5 (C=O). MS-ESI (m/z): 444.02 [M$^-$]. Anal. Calcd. for $C_{22}H_{17}Cl_2NO_3S$ C, 59.20; H, 3.84; Cl, 15.89; N, 3.14; O, 10.75; S, 7.18 Found C, 60.27; H, 3.59; Cl, 16.07; N, 3.82; O, 11.25; S, 7.87.

*4.4.7. Ethyl 2-((3,5-dichlorophenylthio)methyl)-5-hydroxy-1H-benzo[g]indole-3-carboxylate **(6g)***

Rf (hexane/ethyl acetate 8:2) = 0.26. Yield: 17%. M.p. 203.0 °C dec. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.37 (t, 3H, J = 7.34 Hz); 4.30 (q, 2H, J = 7.34 Hz); 4.82 (s, 2H); 7.41–7.47 (m, 3H); 7.52 (s, 1H); 7.57 (t, 1H, J = 7.88 Hz); 8.17 (d, 1H, J = 7.88 Hz); 8.28 (d, 1H, J = 7.88 Hz); 9.69 (s, 1H, OH); 12.39 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 14.4, (CH_3CH_2), 29.3 (CH_2S), 59.9, (CH_3CH_2), 100.1 (C-3), 121.4 (C-5a), 121.7 (C-9), 121.8 (C-4), 122.7 (C-3a), 125.1 (C-9b), 126.5 (C-9a), 126.8–127.6 (C-6, C-7, C-8, C-2′, C-4′, C-6′), 131.5 (C-1′), 133.4 (C-5), 135.6 (C-3′-C-5′), 143.8 (C-2), 165.5 (C=O). MS-ESI (m/z): 445.92 [M$^-$]. Anal. Calcd. for $C_{22}H_{17}Cl_2NO_3S$ C, 59.20; H, 3.84; Cl, 15.89; N, 3.14; O, 10.75; S, 7.18 Found C, 60.21; H, 4.03; Cl, 14.84; N, 2.89; O, 10.60; S, 7.23.

*4.4.8. Ethyl 2-((2,6-dimethylphenylthio)methyl)-5-hydroxy-1H-benzo[g]indole-3-carboxylate **(6h)***

Rf (hexane/ethyl acetate 8:2) = 0.30. Yield: 37%. M.p. 197.5 °C dec.^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.26 (t, 3H, J = 7.34 Hz); 2.29 (s, 6H); 4.08 (q, 2H, J = 7.34 Hz); 4.40 (s, 2H); 7.02–7.18 (m, 3H); 7.42 (t, 1H, J = 6.61 Hz); 7.47 (s, 1H); 7.54 (t, 1H, J = 6.61 Hz); 8.16 (d, 1H, J = 7.80 Hz); 8.27 (d, 1H, J = 7.80 Hz); 9.64 (s, 1H, OH); 12.39 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 14.4, (CH_3CH_2), 19.2, (o,m-CH_3), 29.3 (CH_2S), 59.9, (CH_3CH_2), 100.1 (C-3), 121.4 (C-5a), 121.7 (C-9), 121.8 (C-4), 122.7 (C-3a), 125.1 (C-9b), 126.5 (C-9a), 126.8–127.6 (C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′, C-6′), 131.5 (C-1′), 133.4 (C-5), 143.8 (C-2), 165.5 (C=O). MS-ESI (m/z): 404.14 [M$^-$]. Anal. Calcd. for $C_{24}H_{23}NO_3S$ C, 71.09; H, 5.72; N, 3.45; O, 11.84; S, 7.91 Found C, 70.89; H, 5.22; N, 3.05; O, 10.82; S, 7.37.

*4.4.9. Ethyl 2-((3,5-dimethylphenylthio)methyl)-5-hydroxy-1H-benzo[g]indole-3-carboxylate **(6i)***

Rf (hexane/ethyl acetate 8:2) = 0.28. Yield: 37%. M.p. 158.3 °C dec. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.35 (t, 3H, J = 7.34 Hz); 2.20 (s, 6H); 4.26 (q, 2H, J = 7.34 Hz); 4.71 (s, 2H); 6.83 (s, 1H); 6.98 (s, 2H); 7.42 (t, 1H, J = 7.88 Hz); 7.52 (s, 1H); 7.57 (t, 1H, J = 7.88 Hz); 8.17 (d, 1H, J = 7.88 Hz); 8.31 (d, 1H, J = 7.88 Hz); 9.69 (s, 1H, OH); 12.39 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ14.4, (CH_3CH_2), 24.2 (m-CH_3), 29.3 (CH_2S), 59.9, (CH_3CH_2), 100.1 (C-3), 121.4 (C-5a), 121.7 (C-9), 121.8 (C-4), 122.7 (C-3a), 125.1 (C-9b), 126.5 (C-9a), 126.8–127.6 (C-6, C-7, C-8, C-2′, C-4′, C-6′), 131.5 (C-1′), 133.4 (C-5), 138.5 (C-3′,C-5′), 143.8 (C-2), 165.5 (C=O). MS-ESI (m/z): 404.10 [M$^-$]. Anal. Calcd. for $C_{24}H_{23}NO_3S$ C, 71.09; H, 5.72; N, 3.45; O, 11.84; S, 7.91 Found C, 70.89; H, 6.92; N, 2.98; O, 11.24; S, 7.37.

*4.4.10. Ethyl 5-hydroxy-2-((mesitylthio)methyl)-1H-benzo[g]indole-3-carboxylate **(6l)***

Rf (hexane/ethyl acetate 8:2) = 0.28. Yield: 27%. M.p. 178.5 °C dec. ^{1}H NMR (Acetone-d_6, 300 MHz) δ 1.32 (t, 3H, J = 7.34 Hz); 2.20 (s, 3H); 2.25 (s, 6H); 4.17 (q, 2H, J = 7.34 Hz); 4.44 (s, 2H); 6.87 (s, 2H); 7.45 (t, 1H, J = 7.88 Hz); 7.53 (t, 1H, J = 7.88 Hz); 7.65 (s, 1H); 8.15 (d, 1H, J = 7.88 Hz); 8.29 (d, 1H, J = 7.88 Hz); 8.67 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 14.4, (CH_3CH_2), 19.2, (o,m-CH_3), 24.9 (p-CH_3), 29.3 (CH_2S), 59.9, (CH_3CH_2), 100.1 (C-3), 121.4 (C-5a), 121.7 (C-9), 121.8 (C-4), 122.7 (C-3a), 125.1 (C-9b), 126.5 (C-9a), 126.8–127.6

(C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′, C-6′), 131.5 (C-1′), 133.4 (C-5), 143.8 (C-2), 165.5 (C=O). MS-ESI (*m*/*z*): 418.09 [M^-]. Anal. Calcd. for $C_{25}H_{25}NO_3S$ C, 71.57; H, 6.01; N, 3.34; O, 11.44; S, 7.64. Found C, 70.59; H, 6.12; N, 3.25; O, 11.37; S, 7.56.

4.5. General procedure for the synthesis of **7a-b** and **8a-b**

A mixture of **6b** (34 mg, 0.08 mmol), K_2CO_3 (57 mg, 0.40 mmol), and iodoalcane (3.75 eq.) in acetone (0.3 mL) was irradiated under MW, for 30 min at 130 °C. The mixture was filtered and evaporated under vacuum. The crude was purified by flash chromatography on silica gel (hexane/Ethyl acetate 9:1).

4.5.1. Ethyl 2-((2-chlorophenylthio)methyl)-5-methoxy-1H-benzo[g]indole-3-carboxylate (**7a**)

Rf (hexane/ethyl acetate 8:2) = 0.5. Yield: 27%. M.p. 188.5 °C dec. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.50 (t, 3H, J = 7.34 Hz); 4.15 (s, 3H); 4.26 (q, 2H, J = 7.34 Hz); 4.81 (s, 2H); 7.22 (t, 1H, J = 6.30 Hz); 7.35 (t, 1H, J = 6.30 Hz); 7.42 (d, 1H, J = 8.10 Hz); 7.49 (d, 1H, J = 8.10 Hz); 7.53 (s, 1H); 7.55–7.60 (m, 2H); 8.16 (d, 1H, J = 7.80 Hz); 8.27 (d, 1H, J = 7.80 Hz). Anal. Calcd. for $C_{23}H_{20}ClNO_3S$ C, 64.86; H, 4.73; Cl, 8.32; N, 3.29; O, 11.27; S, 7.53. Found C, 64.88; H, 4.76; Cl, 8.35; N, 3.32; O, 11.30; S, 7.50.

4.5.2. Ethyl 2-((2-chlorophenylthio)methyl)-5-methoxy-1-methyl-1H-benzo[g]indole-3-carboxylate (**7b**)

Rf (hexane/ethyl acetate 8:2) = 0.72. Yield: 32%. M.p. 198.5 °C dec. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.50 (t, 3H, J = 7.34 Hz); 4.10 (s, 3H); 4.15 (s, 3H); 4.26 (q, 2H, J = 7.34 Hz); 4.80 (s, 2H); 7.22 (t, 1H, J = 6.30 Hz); 7.35 (t, 1H, J = 6.30 Hz); 7.42 (d, 1H, J = 8.10 Hz); 7.49 (d, 1H, J = 8.10 Hz); 7.53 (s, 1H); 7.55–7.60 (m, 2H); 8.16 (d, 1H, J = 7.80 Hz); 8.27 (d, 1H, J = 7.80 Hz). Anal. Calcd. for $C_{24}H_{22}ClNO_3S$ C, 65.52; H, 5.04; Cl, 8.06; N, 3.18; O, 10.91; S, 7.29. Found C, 65.50; H, 5.08; Cl, 8.09; N, 3.21; O, 10.89; S, 7.32.

4.5.3. Ethyl 2-((2-chlorophenylthio)methyl)-5-ethoxy-1H-benzo[g]indole-3-carboxylate (**8a**)

Rf (hexane/ethyl acetate 8:2) = 0.5. Yield: 25%. M.p. 179.3 °C dec. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.53 (t, 3H, J = 7.34 Hz); 1.63 (t, 3H, J = 7.5 Hz); 4.26 (q, 2H, J = 7.34 Hz); 4.50 (q, 2H, J = 7.5 Hz); 4.80 (s, 2H); 7.22 (t, 1H, J = 6.30 Hz); 7.35 (t, 1H, J = 6.30 Hz); 7.42 (d, 1H, J = 8.10 Hz); 7.49 (d, 1H, J = 8.10 Hz); 7.53 (s, 1H), 7.55–7.60 (m, 2H); 8.16 (d, 1H, J = 7.80 Hz); 8.27 (d, 1H, J = 7.80 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.4, (CH_3CH_2), 19.2 (CH_3CH_2), 30.0 (CH_2S), 59.9 (CH_3CH_2), 63.9 (CH_3CH_2), 99.0 (C-3), 121.4 (C-5a), 121.7 (C-9), 121.8 (C-4), 122.7 (C-3a), 125.1 (C-9b), 126.5 (C-9a), 126.8–127.6 (C-6, C-7, C-8, C-2′, C-3′, C-4′, C-5′, C-6′), 131.5 (C-1′), 133.4 (C-5), 143.8 (C-2), 165.5 (C=O). Anal. Calcd. for $C_{24}H_{22}ClNO_3S$ C, 65.52; H, 5.04; Cl, 8.06; N, 3.18; O, 10.91; S, 7.29. Found C, 65.44; H, 5.09; Cl, 8.09; N, 3.20; O, 10.88; S, 7.32.

4.5.4. Ethyl 2-((2-chlorophenylthio)methyl)-5-ethoxy-1-ethyl-1H-benzo[g]indole-3-carboxylate (**8b**)

Rf (hexane/ethyl acetate 8:2) = 0.68. Yield: 33%. M.p. 158.5 °C dec. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.53 (t, 3H, J = 7.34 Hz); 1.61 (m, 6H); 4.26 (q, 2H, J = 7.34 Hz); 4.50 (m, 4H); 4.80 (s, 2H); 7.22 (t, 1H, J = 6.30 Hz); 7.35 (t, 1H, J = 6.30 Hz); 7.42 (d, 1H, J = 8.10 Hz); 7.49 (d, 1H, J = 8.10 Hz); 7.53 (s, 1H); 7.55–7.60 (m, 2H); 8.16 (d, 1H, J = 7.80 Hz); 8.27 (d, 1H, J = 7.80 Hz). Anal. Calcd. for $C_{26}H_{26}ClNO_3S$ C, 66.73; H, 5.60; Cl, 7.58; N, 2.99; O, 10.26; S, 6.85. Found C, 66.77; H, 5.66; Cl, 7.55; N, 3.02; O, 10.23; S, 6.89.

4.6. Ethyl 2-((2-chlorophenylthio)methyl)-5-hydroxy-1-methyl-1H-benzo[g]indole-3-carboxylate (**11**)

A solution of **6b** (152 mg, 0.37 mmol), imidazole (93 mg, 1.48 mmol) and tert-butyl dimethyl silyl chloride (127 μL, 0.74 mmol) in CH_2Cl_2 was stirred for 3 h at room temperature. The mixture was washed with water and two times with brine. The organic layer, dried over anhydrous Na_2SO_4, was evaporated under reduced pressure and the crude was purified by flash chromatography (eluting system hexane/ethyl acetate 8:2) affording 185 mg of desired compound (**9**) as white cristals (yield: 95%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.18 (s, 6H); 1.01 (s, 9H); 1.34 (t, 3H, J = 7.34 Hz); 4.28 (q, 2H, J = 7.34 Hz); 4.70 (s, 2H); 7.22 (t, 1H, J = 6.30 Hz); 7.35 (t, 1H, J = 6.30 Hz); 7.42 (d, 1H, J = 8.10 Hz); 7.49 (d, 1H, J = 8.10 Hz); 7.53 (s, 1H); 7.55–7.60 (m, 2H); 8.16 (d, 1H, J = 7.80 Hz); 8.27 (d, 1H, J = 7.80 Hz).

A 25 mL three-necked round bottomed flask equipped with a stir bar was charged with 60% NaH (21 mg, 0.53 mmol) in mineral oil dissolved in 1 mL of dry THF, under nitrogen atmosphere. The solution was cooled at 0 °C and a solution of **9** (185 mg, 0.35 mmol) in 2 mL of dry THF was added. After 30 min iodomethane (26 μL, 0.42 mmol) was added and the mixture was stirred for 2 h. The mixture was diluted with water and extracted with CH_2Cl_2. Organic layer was washed with brine, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Desired compound (**10**) was obtained after purification with flash chromatography using hexane/ethyl acetate 9:1, as eluent mixture. (Yield: 50%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.18 (s, 6H); 1.01 (s, 9H); 1.33 (t, 3H, J = 7.34 Hz); 4.10 (s, 3H); 4.28 (q, 2H, J = 7.34 Hz); 4.70 (s, 2H); 7.22 (t, 1H, J = 6.30 Hz); 7.35 (t, 1H, J = 6.30 Hz); 7.42 (d, 1H, J = 8.10 Hz); 7.49 (d, 1H, J = 8.10 Hz); 7.53 (s, 1H); 7.55–7.60 (m, 2H); 8.16 (d, 1H, J = 7.80 Hz); 8.27 (d, 1H, J = 7.80 Hz).

A solution of TBAF 1M in THF (63 μL, 64 mmol) was added to a solution of **10** (35 mg, 64 mmol) in THF (420 μL) cooled at 0 °C. The mixture was stirred for 10 min at 0 °C and quenched with EtOAC and H_2O. Organic layer was washed with a saturated solution of $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* affording final compound **11** (Yield: 45%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) = 1.50 (t, 3H, J = 7.34 Hz); 4.15 (s, 3H); 4.26 (q, 2H, J = 7.34 Hz); 4.80 (s, 2H); 7.22 (t, 1H, J = 6.30 Hz); 7.35 (t, 1H, J = 6.30 Hz); 7.42 (d, 1H, J = 8.10 Hz); 7.49 (d, 1H, J = 8.10 Hz); 7.53 (s, 1H); 7.55–7.60 (m, 2H); 8.16 (d, 1H, J = 7.80 Hz); 8.27 (d, 1H, J = 7.80 Hz). Anal. Calcd. for $C_{23}H_{20}ClNO_3S$ C, 64.86; H, 4.73; Cl, 8.32; N, 3.29; O, 11.27; S, 7.53. Found C, 64.98; H, 4.82; Cl, 8.29; N, 3.21; O, 11.10; S, 7.44.

4.7. Biological evaluation and assay systems

4.7.1. Animals

Male ICR mice, 8 weeks old (Harlan, Italy), were housed 5 per cage under controlled illumination (12:12 h light:dark cycle; light on 06.00 h) and standard environmental conditions (room temperature 22 ± 1 °C, humidity 60 ± 10%) for at least 1 week before experimental use. Mouse chow and tap water were available ad libitum. The experimental procedures were in accordance with Italian and European regulations governing the care and treatment of laboratory animals (Permission no. 41/2007B). Animal care was in compliance with Ethical Guidelines of the IASP and European Community (E.C. L358/1 18/12/86) on the use and protection of animals in experimental research. All efforts were made to minimize animal suffering and to reduce the number of animals used.

4.7.2. Paw oedema

Oedema was induced by carrageenan injection (20 μL/paw, 2% w/v in saline) into the plantar surface of the right hind paw. All

derivatives were dissolved in DMSO (0.5% v/v in saline), Zileuton were dissolved in ethanol-cromophor-saline (1:1:18). The paw volume was measured using a hydroplethysmometer (Ugo Basile, Varese, Italy) at 2-4-6 h following carrageenan. Mice (n = 6–8 per group) were treated with **1** or **6f** (1–4 mg/kg, i.p.), **6g** and **6l** (1–4 mg/kg, i.p.) 30 min before the inflammatory insult. A separate experimental group received zileuton (20 mg/kg, i.p.) used as a reference drug before carrageenan.

4.7.3. Determination of 5-LOX product formation in cell-based assays

PMNL were isolated from human blood of adult healthy volunteers, with consent, obtained from the Institute of Transfusion Medicine, University Hospital Jena, as described [25]. PMNL (5×10^6 in 1 ml PG buffer) were preincubated with the test compounds (15 min, 37 °C) and 5-LOX product formation was started by addition of 1 mM $CaCl_2$ and 2.5 μM A23187. After 10 min at 37 °C, the reaction was stopped with 1 ml of methanol. Cells were placed on ice, centrifuged at 800 × g for 5 min at 4 °C and the supernatant was added to methanol. Formed 5-LOX metabolites were extracted and analyzed by HPLC as described [26]. 5-LOX product formation is expressed as ng of 5-LOX products per 10^6 cells, which includes LTB_4 and its all-trans isomers, 5(S),12(S)-di-hydroxy-6,10-trans-8,14-cis-eicosatetraenoic acid (5(S),12(S)-DiHETE), and 5(S)-hydro(pero)xy-6-trans-8,11,14-cis-eicosatetraenoic acid (5-H(p)ETE). Cysteinyl LTs C_4, D_4 and E_4 were not detected, and oxidation products of LTB_4 were not determined.

4.7.4. Expression and purification of human recombinant 5-LO from E. coli, and determination of 5-LOX activity in cell-free systems

E.coli BL21 was transformed with pT3-5LO plasmid, human recombinant 5-LOX protein was expressed at 37 °C, purified as described [27] and immediately used for 5-LOX activity assays. 5-LOX (0.5 μg) was diluted with PBS/EDTA and pre-incubated with test compounds. After 15 min at 4 °C, samples were pre-warmed for 30 s at 37 °C, and 2 mM $CaCl_2$ plus 20 μM AA were added to start 5-LO product formation. After 10 min at 37 °C formed metabolites were analyzed by HPLC as described for intact cells above.

4.7.5. Induction of mPGES-1 expression in A549 cells and isolation of microsomes

Preparation of A549 cells and determination of mPGES-1 activity was performed as described previously [20]. In brief, A549 cells (2 x10^6 cells in 20 ml medium) were plated in 175 cm^2 flasks and incubated for 16 h at 37 °C and 5% CO_2. Subsequently, the culture medium was replaced by fresh DMEM/high glucose (4.5 g/l) medium containing FCS (2%, v/v). In order to induce mPGES-1 expression, IL-1β (1 ng/ml) was added, and cells were incubated for another 72 h. Thereafter, cells were detached with trypsin/EDTA, washed with PBS, and frozen in liquid nitrogen. Ice-cold homogenization buffer (0.1 M potassium phosphate buffer pH 7.4, 1 mM phenylmethanesulfonylfluoride, 60 μg/ml soybean trypsin inhibitor, 1 μg/ml leupeptin, 2.5 mM glutathione, and 250 mM sucrose) was added, and after 15 min, cells were resuspended and sonicated on ice (3 × 20 s). The homogenate was subjected to differential centrifugation at 10,000 g for 10 min and 174,000 g for 1 h at 4 °C. The pellet (microsomal fraction) was resuspended in 1 ml homogenization buffer, and the total protein concentration was determined by Coomassie protein assay. Microsomal membrane fractions were stored at −80 °C for several weeks.

4.7.6. Determination of PGE_2 synthase activity in microsomes of A549 cells

Microsomal membranes were diluted in potassium phosphate buffer (0.1 M, pH 7.4) containing 2.5 mM glutathione to give a final concentration of 50 μg/ml. Test compounds or vehicle (DMSO at a final concentration of 1%) were added, and after 15 min at 4 °C, the reaction (100 μl total volume) was initiated by addition of PGH_2 (20 μM, final concentration). After 1 min at 4 °C, the reaction was terminated using stop solution (100 μl; 40 mM $FeCl_2$, 80 mM citric acid, and 10 μM of 11β-PGE_2). PGE_2 was separated by solid phase extraction on reversed phase (RP)-C18 material using acetonitrile (200 μl) as eluent, and analyzed by RP-HPLC (30% acetonitrile aq + 0.007% TFA (v/v), Nova-Pak® C18 column, 5 × 100 mm, 4 μm particle size, flow rate 1 ml/min) with UV detection at 195 nm.11β-PGE_2 was used as internal standard to quantify PGE_2 product formation by integration of the area under the peaks. IC_{50} values were assessed by three determinations at compound concentrations of 0.1, 1, 10 μM.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.11.048.

References

[1] P.V. Afonso, M. Janka-Junttila, Y.J. Lee, C.P. McCann, C.M. Oliver, K.A. Aamer, W. Losert, M.T. Cicerone, C.A. Parent, LTB4 is a signal relay molecule during neutrophil chemotaxis, Dev. Cell. 22 (2012) .1079–91.
[2] I. Muller, M. Munder, P. Kropf, G.M. Hansch, Polymorphonuclear neutrophils and T lymphocytes: strange bedfellows or brothers in arms? Trends Immunol. 30 (2009) 522–530.
[3] C.D. Funk, Prostaglandins and leukotrienes: advances in eicosanoid biology, Science 294 (2001) 1871–1875.
[4] J.Z. Haeggström, A. Rinaldo-Matthis, C.E. Wheelock, A. Wetterholm, Advances in eicosanoid research, novel therapeutic implications, Bio-chemical Biophysical Res. Commun. 396 (2010) 135–139.
[5] X. Chen, S. Sood, C.S. Yang, N. Li, Z. Sun, Five-lipoxygenase pathway of arachidonic acid metabolism in carcino-genesis and cancer chemoprevention, Curr. Cancer Drug Targets 6 (2006) 613–622.
[6] O. Werz, D. Steinhilber, Selenium-dependent peroxidases suppress 5-lipoxygenase activity in B-lymphocytes and immature myeloid cells. The presence of peroxidase-insensitive 5-lipoxygenase activity in differentiated myeloid cells, Eur. J. Biochem. 242 (1996) 90–97.
[7] K. Ishii, M. Zaitsu, N. Yonemitsu, Y. Kan, Y. Hamasaki, M. Matsuo, 5-lipoxygenase pathway promotes cell proliferation in human glioma cell lines, Clin. Neuropathol. 28 (2009) 445–452.
[8] O. Firuzi, J. Zhuo, C.M. Chinnici, T. Wisniewski, D. Praticò, 5-Lipoxygenase gene disruption reduces amyloid-beta pathology in a mouse model of Alzheimer's disease, FASEB J. 22 (2008) 1169.
[9] M.D. Ikonomovic, E.E. Abrahamson, T. Uz, H. Manev, S.T. Dekosky, Increased 5-lipoxygenase immunoreactivity in the hippocampus of patients with Alzheimer's disease, J. Histochem. Cytochem 56 (2008) 1065.
[10] J. Chu, D. Praticò, 5-Lipoxygenase as an endogenous modulator of amyloid beta formation in vivo, Ann. Neurol. 69 (2011) 34–46.
[11] J. Chu, D. Praticò, Pharmacological blockade of 5-Lipoxygenase improves the amyloidotic phenotype of an Alzheimer's disease transgenic mouse model, Am. J. Pathol. 178 (2011) 1762–1769.
[12] A. Di Meco, E. Lauretti, A.N. Vagnozzi, D. Praticò, Zileuton restores memory impairments and reverses amyloid and tau pathology in aged Alzheimer's disease mice, Neurobiol. Aging 35 (2014) 2458.
[13] R. Filosa, A. Peduto, A.M. Schaible, V. Krauth, C. Weinigel, D. Barz, C. Petronzi, F. Bruno, F. Roviezzo, G. Spaziano, B. D'Agostino, M. De Rosa, O. Werz, Novel series of benzoquinones with high potency against 5-lipoxygenase in human polymorphonuclear leukocytes, Eur. J. Med. Chem. 94 (2015) 132–139.
[14] A. Peduto, F. Bruno, F. Dehm, V. Krauth, P. de Caprariis, C. Weinigel, D. Barz, A. Massa, M. De Rosa, O. Werz, R. Filosa, Further studies on ethyl 5-hydroxy-indole-3-carboxylate scaffold: design, synthesis and evaluation of 2-phenylthiomethyl-indole derivatives as efficient inhibitors of human 5-lipoxygenase, Eur. J. Med. Chem. 81 (2014) 492–498.
[15] R. Filosa, A. Peduto, P. Aparoy, A.M. Schaible, S. Luderer, V. Krauth, C. Petronzi, A. Massa, M. De Rosa, P. Reddana, O. Werz, Discovery and biological evaluation of novel 1,4-benzoquinone and related resorcinol derivatives that inhibit 5-lipoxygenase, Eur. J. Med. Chem. 67 (2013) 269–279.
[16] C. Petronzi, M. Festa, A. Peduto, M. Castellano, J. Marinello, A. Massa, A. Capasso, G. Capranico, A. La Gatta, M. De Rosa, M. Caraglia, R. Filosa, Cyclohexa-2,5-diene-1,4-dione-based antiproliferative agents: design, synthesis, and cytotoxic evaluation, J. Exp. Clin. Cancer Res. 30 (2013) 32–34.
[17] A.M. Schaible, H. Traber, V. Temml, S.M. Noha, R. Filosa, A. Peduto, C. Weinigel, D. Barz, D. Schuster, O. Werz, Potent inhibition of human 5-lipoxygenase and microsomal prostaglandCE$_2$ synthase-1 by the anti-carcinogenic and anti-inflammatory agent embelin, Biochem. Pharmacol. 86 (2013) 476–486.

[18] C. Petronzi, R. Filosa, A. Peduto, M.C. Monti, L. Margarucci, A. Massa, S.F. Ercolino, V. Bizzarro, L. Parente, R. Riccio, P. de Caprariis, Structure-based design, synthesis and preliminary anti-inflammatory activity of bolinaquinone analogues, Eur. J. Med. Chem. 46 (2011) 488–496.
[19] E.M. Karg, S. Luderer, C. Pergola, U. Bühring, A. Rossi, H. Northoff, L. Sautebin, R. Troschütz, O. Werz, Structural optimization and biological evaluation of 2-substituted 5-hydroxyindole-3-carboxylates as potent inhibitors of human 5-lipoxygenase, J. Med. Chem. 52 (2009) 3474–3483.
[20] A. Koeberle, E.M. Haberl, A. Rossi, C. Pergola, F. Dehm, H. Northoff, R. Troschuetz, L. Sautebin, O. Werz, Discovery of benzo[g]indol-3-carboxylates as potent inhibitors of microsomal prostaglandin E(2) synthase-1, Bioorg Med. Chem. 23 (2009) 7924–7932.
[21] O. Rådmark, O. Werz, D. Steinhilber, B. Samuelsson, 5-Lipoxygenase, a key enzyme for leukotriene biosynthesis in health and disease, Biochim. Biophys. Acta 1851 (2015) 331–339.
[22] C.J. Morris, Carrageenan-induced paw edema in the rat and mouse, Meth Mol. Biol. 225 (2003) 115–121.
[23] T. Chen, R. Benmohamed, A.C. Arvanites, H.R. Ranaivo, R.I. Morimoto, R.J. Ferrante, D.M. Watterson, D.R. Kirsch, R.B. Silverman, Arylsulfanyl pyrazolones block mutant SOD1-G93A aggregation. Potential application for the treatment of amyotrophic lateral sclerosis, Bioorg. Med. Chem. 19 (2011) 613–622.
[24] Y. Tominaga, R. Pratap, R.N. Castle, M.L. Lee, The synthesis of the monomethyl isomers of benzo[b]naphth[2,1-d]thiophene, J. Heterocycl. Chem. 19 (1982) 859–863.
[25] C. Pergola, J. Gerstmeier, B. Mönch, B. Çalışkan, S. Luderer, C. Weinigel, D. Barz, J. Maczewsky, S. Pace, A. Rossi, L. Sautebin, E. Banoglu, O. Werz, The novel benzimidazole derivative BRP-7 inhibits leukotriene biosynthesis in vitro and in vivo by targeting 5-lipoxygenase-activating protein (FLAP), Br. J. Pharmacol. 171 (2014) 3051–3064.
[26] C. Pergola, A. Rogge, D. Dodt, H. Northoff, C. Weinigel, D. Barz, O. Rådmark, L. Sautebin, O. Werz, Testosterone suppresses phospholipase D causing sex differences in leukotriene biosynthesis in human monocytes, FASEB J. 25 (2011) 3377–3387.
[27] C. Pergola, B. Jazzar, A. Rossi, H. Northoff, M. Hamburger, L. Sautebin, O. Werz, On the inhibition of 5-lipoxygenase product formation by tryptanthrin: mechanistic studies and efficiency in vivo. Br, J. Pharmacol. 165 (2011) 765–776.