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Graphical Abstract

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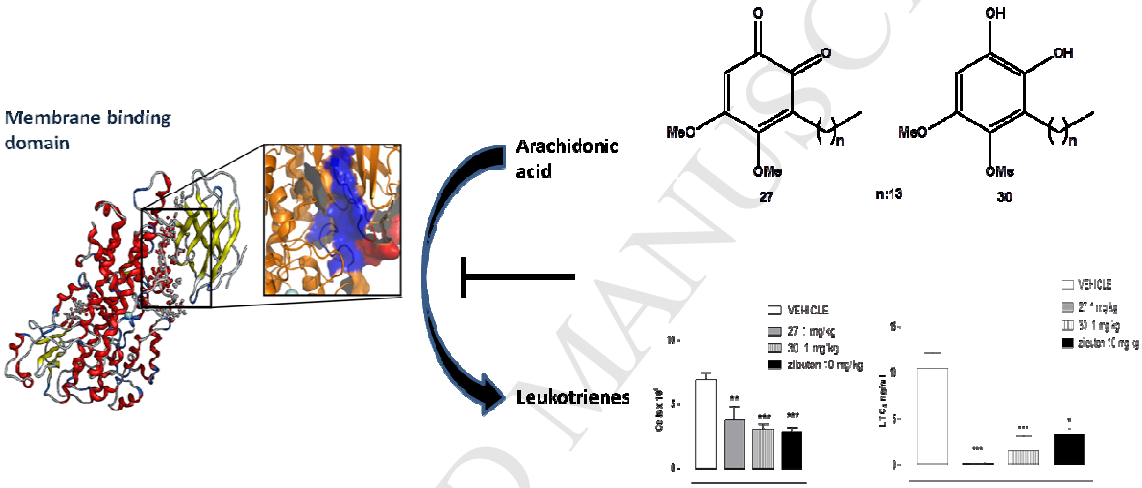

Optimization of Benzoquinone and Hydroquinone Derivatives as Potent Inhibitors of Human 5-Lipoxygenase

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Abstract

Aiming to assess the biological activities of synthetic 1,4-benzoquinones, we previously synthesized different libraries of benzoquinones with lipophilic and bulky alkyl- or aryl-substituents that inhibited 5-lipoxygenase (5-LO). The high potency of 4,5-dimethoxy-3-alkyl-1,2-benzoquinones on 5-LO led to the idea to further modify the structures and thus to improve the inhibitory potential *in vitro* and *in vivo* as well as to investigate SARs. Systematic structural optimization through accurate structure-based design resulted in compound **30** (3-tridecyl-4,5-dimethoxybenzene-1,2-diol), an ubiquinol derivative that exhibited the strongest anti-inflammatory effect, with a 10-fold improved 5-LO inhibitory activity (IC_{50} = 28 nM) in activated neutrophils. Moreover, **30** significantly reduced inflammatory reactions in the carrageenan-induced mouse paw oedema and in zymosan-induced peritonitis in mice. Compound **30** (1 mg/kg, i.p.) potently suppressed the levels of cysteinyl-LTs 30 min after zymosan, outperforming zileuton at a dose of 10 mg/kg. The binding patterns of the quinone- and hydroquinone-based 5-LO inhibitors were analyzed by molecular docking. Together, we elucidated the optimal alkyl chain pattern of quinones and corresponding hydroquinones and reveal a series of highly potent 5-LO inhibitors with effectiveness *in vivo* that might be useful as anti-inflammatory drugs.

1. Introduction

Leukotrienes (LTs) are inflammatory mediators produced via the 5-lipoxygenase (5-LO) pathway and are linked to diverse inflammatory disorders. Intervention with LTs represents a pertinent pharmacological approach against inflammatory diseases, and anti-LT therapy has been validated in clinical trials of asthma and allergic rhinitis, with potential in other respiratory and allergic disorders [1], as well as in cardiovascular diseases such as atherosclerosis, myocardial infarction, stroke and abdominal aortic aneurysm [2]. In addition, such inhibitors may be tools for the elucidation of the involvement of 5-LO and LTs in diverse biological processes.

Over the last two decades, several studies have shown that quinone derivatives possess a number of biological and pharmacological applications, and hydroquinones are of considerable scientific interest because of their versatile biological activities, where also the corresponding (hydro) quinone may display multiple actions [3]. Our groups have been interested for a long time in the synthesis and the biological evaluation of anti-cancer and anti-inflammatory agents including quinone-based compounds [4-15]. Within the context of our investigations towards the synthesis of quinone derivatives with prospects for therapeutic use, we recently studied the natural compound embelin and RF-Id, a synthetic derivative of bolinaquinone (**Figure 1**) [7-8].

The high potency against 5-LO and the promising *in vivo* efficacy of the corresponding synthesized compounds stimulated us to further modify the structures and thus, to improve the inhibitory potential as well as to investigate SARs of this class of compounds. Starting from embelin, the alkyl chain length in position 3 (*n*-undecyl residue) was varied by introducing saturated linear *n*-alkyl residues or isoprenoid side chains. Next, one or two hydroxyl groups were methylated in 2- and 5-position, respectively, and finally, the 2,5-dimethoxy-1,4-benzoquinone core was replaced by a 4,5-methoxy-1,2-benzoquinones backbone (*ortho*-

quinone structures) leading to compounds decorated with the same linear and prenylated chains as for the other groups [10-11].

We have shown that the increase of the *n*-alkyl side-chain length determines the 5-LO inhibitory activity in neutrophils stimulated with the Ca^{2+}-mobilizing agent A23187. Thus, *ortho*-quinones with simple *n*-undecyl (**25)** or *n*-dodecyl (**26**) and *n*-tridecyl (**27)** residues were most potent in intact human neutrophils with IC_{50} values in the submicromolar range (approx. 50-100 nM) [10-11]. Starting from this basis and considering that modifications on the quinone scaffold did not affect the polarity of the linear chain as reported in the literature so far, we were interested, whether the presence of a polar group (i.e. hydroxyl) in the *n*-alkyl chain would affect the potency against 5-LO. Thus, we took into account the marked anti-inflammatory properties of Idebenone (2,3-dimethoxy-5-methyl-6(10-hydroxydecyl)-1,4-benzoquinone) [16-18], a synthetic analogue of coenzyme Q10 (CoQ10), with potent antioxidant activity, able to inhibit the enzymatic metabolism of arachidonic acid by cyclooxygenase and lipoxygenases, with potential anti-inflammatory activity in the central nervous system [18]. Notably, introduction of a polar group into the lipophilic *n*-alkyl chain may also improve water solubility and thus bioavailability. We therefore envisioned the preparation of quinone derivatives bearing a long (i.e., > C8) hydrophilic chain in order to evaluate the potential of such modification for interference with 5-LO activity. Furthermore, we aimed to obtain broader insights into the relationship between the molecular structure and the biological activity against 5-LO of the most active quinone derivatives (**25**, **26**, **27**) and their reduced hydroquinone forms (**28**, **29**, **30**)**.** Finally we decided to synthesize different hydroquinones with the same alkyl chain (**33**, **34**), to evaluate if different positions of the hydroxyl group would allow for better interference with 5-LO's active site.

Results and discussion

2.1 Chemistry

Hydroxylated *ortho*-quinones **10-12** and *para*-quinones **13-15** were synthesized starting from 1,2,4,5-tetramethoxybenzene, which was subjected to an *ortho*-metalation reaction in the presence of n-BuLi and tetramethylethylenediamine (TMEDA). The lithium derivative was reacted with different alkyl bromides **4-6** giving intermediates **7-9**. Silylation of bromo-alcohols **1-3** was performed with tert-butyl dimethyl silyl chloride furnishing desired compounds **4-6** with good yields. Cerium ammonium nitrate (CAN)-mediated oxidative cleavage provided a mixture of 4,5-dimethoxy-1,2-benzoquinones (**10-12**) and 2,5-dimethoxy-1,4-benzoquinones (**13-15**) following a synthetic procedure previously described by us [10] (**Scheme 1**). Removal of the protecting group from tetramethoxy intermediates **7, 8,** and **9** by acidic cleavage furnished the desired compounds **16**, **17,** and **18** in good yields (**Scheme 1**). The synthesis of compounds **21** and **23** started from 10-tert-butyl dimethyl silanyloxy decanal (**19**) which was reacted with 1,2,4,5-tetramethoxybenzene following the same experimental conditions as described above, furnishing compound **20** with 28% yield. Subsequent CAN-mediated oxidative cleavage at -10°C for 10 min allowed removing the TBDMS group and oxidizing to obtain *para*-quinone derivative **21**. Under these conditions, the *ortho*-quinone analog rapidly decomposed. Acetylation of **20** with acetic anhydride and further treatment with CAN provided compound **23** with 20% yield (**Scheme 2**). 4,5-Dimethoxybenzene-1,2-diols (**28, 29** and **30**) were obtained from the corresponding *ortho*-quinones after treatment with $NaBH_4$ in ethanol (**Scheme 3**), with the same reaction conditions we designed and synthesized compounds **33** and **34** to test the influence of hydroxyl group.

2.2. Evaluation of structure-activity relationships and molecular docking studies

In order to assess the effects of the synthesized compounds on 5-LO product synthesis, a cell-free assay using isolated human recombinant 5-LO and a cell-based assay using human

neutrophils were applied. The cell-free assay identifies compounds that directly interfere with 5-LO catalytic activity, whereas the cell-based test system considers cellular regulatory aspects of 5-LO product synthesis as well. As such, the cell-based assay offers several possible points of attack of a given compound (e.g., inhibition of 5-LO-activating protein (FLAP) or coactosine-like protein (CLP), interference with lipid hydroperoxides, protein kinases, Ca^{+2} mobilization, and 5-LO translocation) [4]. The reference 5-LO inhibitor N-[1-(1-benzothien-2-yl)ethyl]-N-hydroxyurea (zileuton) was used to control the 5-LO activity assays.

Results for inhibition of 5-LO in cell-free assays and in intact cells by the test compounds, as well as more detailed concentration-response curves are presented in **Tables 1** to **3** and in **Figures 2** and **3**. Of note, the *ortho*-quinone **27** and its reduced form **30**, equipped with a C13 *n*-alkyl chain lacking hydroxyl groups, were revealed as most potent direct 5-LO inhibitors with IC_{50} values in cell-free assays of 10 and 60 nM, respectively. Interestingly, variation of the substrate concentration in cell-free assays revealed no competition of **27** or **30** with AA, instead inhibition of 5-LO by the test compounds was even enhanced at higher AA concentrations ($\geq$ 20 µM) especially for the potent *ortho*-quinone **27** (**Figure 4**), suggesting a non- or uncompetitive rather than a competitive mode of action. From this result, we concluded that the compounds do not bind to the AA binding site but interfere at a different point of attack with 5-LO activity.

To locate the alternative binding site for the quinones in 5-LO, we performed a pocket finder calculation implemented in MOE [19]. The pocket finder identified two alternative cavities that could act as binding sites for the quinones: a wide cavity on the opposite side of the substrate channel around and a canyon-like site situated between the membrane binding C2 domain and the catalytic domain (**Figure 5**). The latter is positioned near a binding site suggested for pirinixic acid derivatives [20] and ideally shaped to accommodate the long lipophilic *n*-alkyl chains with polar groups at the ω position to form hydrogen bond

interactions at the end. In the docking, the most common interaction partners are Arg101 and Gln141 on one side of the channel and Tyr383, Tyr81, Trp102, and Glu622 on the other (**Figure 6A**). Typically, two or more of these residues form hydrogen bonds with the oxygen atoms from the substituted quinone ring, as shown in **Figure 6** for compounds **15**, **23**, and **27**. The additional hydroxyl group at the ω-end of the *n*-alkyl chain in *para*-quinones (**15** and **23**) forms hydrogen bonds with either Arg101 or Gln141 in the docking simulation, but apparently does not improve the 5-LO inhibitory activity in cell-free assays (**Table 2**). As shown in **Table 1**, also introduction of the hydroxyl group in the ω-position of the *n*-alkyl chain of *ortho*-quinones resulted in a decrease of potency (for example, **26** *vs* **12** and **25** vs **11**). Of interest, however, is compound **23** that in addition to the ω-hydroxyl contains an acetyl group at C-1 of the *n*-alkyl chain which confers potent inhibition of 5-LO in cell-free assays (**Table 2**). It is interesting to note that the 5-LO inhibitory activity of the compounds in the cell-based test system was not diminished by hydroxyl substitution, in particular, efficient 5-LO inhibitory activity was evident for compound **12** (IC_{50} = 120 nM).

The *ortho*-quinones were sometimes considerably more active than the *para*-quinone derivatives (i.e., **10** *vs* **13**, **11** *vs* **14**). In the docking simulation the *ortho*-quinones formed interactions with Tyr383, which were not predicted for the *para*-quinones, due to a slightly different tilt of the ring. This confirms our previous finding that more favorable interactions of the *ortho*-quinone versus the *para*-quinone scaffold in the binding of 5-LO may exist [10]. On the other hand, additional interactions of the *ortho*-quinones may contribute to inhibition of 5-LO product synthesis, for example interaction with other targets (i.e. FLAP, CLP, p38MAPK, ERK or CaMKII).

The 5-LO inhibitory potencies of the compounds are in line with computational predictions, as shown in **Figure 6**. The docking simulation suggests that the substituted ring of the quinones is usually positioned at the polar solvent accessible region of the binding pocket, forming hydrogen bonds with Tyr383, Tyr81, Trp102, and Glu622 (**Figure 6B**). The

lipophilic *n*-alkyl chain fills the hydrophobic grove that leads to the other opening. Arg101 and Gln141 serve as anchor points for the hydroxyl group present in some of the derivatives (**Figure 6C**). The carboxylate group added in compound **23** does not form additional hydrogen bonds, however both the hydroxyl group at the alkyl chain and the ring seem to be ideally positioned for interaction with Gln141, Tyr81, and Glu622 (**Figure 6D**).

With the accepted general concept that quinones are reduced *in situ* in the intracellular environment, where high concentration of GSH create a reducing milieu, the formed active hydroquinones are able to scavenge radicals and therefore act also as antioxidants [21-22]. In the case of the hydroquinones **28**, **29**, and **30**, they showed a comparable activity against isolated 5-LO versus the corresponding quinones (**Figures 2**, **3**); under cell-based conditions, **30** was highly active with an IC_{50} of 28 nM. Because both the quinone core and the *n*-alkyl chain play an important role in 5-LO inhibition, we were interested in derivatives of **30** in order to explore the influence of the hydroquinone nucleus. Compounds **33** and **34**, with the optimal length of the *n*-alkyl chain (i.e. 13 carbons) but altered methylation pattern of the hydroxyl groups, demonstrated a clear decrease of activity, especially in cell-based assays (IC_{50} = 1600 nM for **33** and 310 nM for **34,** versus **30** with IC_{50} = 28 nM; **Table 3** and **Figure 3**). Molecular docking studies support a similar binding mode for **30**, **33** and **34**, which explore equivalent spaces of the binding cavity (**Fig. 7**).

*2.3 Anti-inflammatory effectiveness of derivatives **27**, **30**, in carrageenan-induced paw edema*

The *ortho*-quinone **27** and its reduced form **30** were the most potent inhibitors of 5-LO in cell-based systems within this series. Thus, we investigated their effectiveness in two different *in vivo* models of acute inflammation. First, we evaluated their effect on carrageenan-induced paw oedema, which represents a well-established model of acute inflammation [23]. Intraplantar injection of carrageenan led to an increase in oedema formation, expressed as increase in hind paw volume, with a maximum reached at 4 hrs post-carrageenan application, a second peak at 48 hrs, and remaining elevated up to 72 hrs (**Figure 8**). Compound **27** (1

mg/kg, i.p.) significantly reduced paw oedema during the early (4 hrs) and late (48 hrs) time points. Furthermore, in agreement with the results from intact cells, the corresponding hydroquinone **30** was efficient in the *in vivo* study, in particular at the later phase. Thus, treatment of mice with 1 mg/kg **27** or **30** reduced paw volume at 4 hrs post carrageenan equally well (by 75% and 77.4%, respectively), while **30** was more efficient in repressing oedema formation at 48 and 72 hrs versus **27**.

*2.4 Anti-inflammatory effectiveness of derivatives **27** and **30** in zymosan-induced peritonitis*

We investigated the effectiveness of **27** and **30** in another model of acute inflammation, namely zymosan-induced mouse peritonitis, which is strongly related to the formation of LTs. Compounds **27** and **30** (1 mg/kg, i.p.) potently suppressed the levels of cysteinyl-LTs 30 min after zymosan, outperforming zileuton at a dose of 10 mg/kg, i.p. (**Table 4**). After 4 hrs post zymosan **27** and **30** (1 mg/kg, i.p.) prevented cell infiltration about equally well to zileuton at 10 mg/kg, i.p (**Table 4**).

3. Conclusions

In this report we present the synthesis, structure determination, and the *in vitro* and *in vivo* evaluation of a series of new quinone analogues aiming to find novel lead structures for the development of 5-LO inhibitors. Our findings support that also the hydroquinone scaffold can interact with high-affinity with 5-LO in a specific manner exhibiting high effectiveness in inflammatory models *in vivo*. We suggest that these compounds might represent privileged structures for the selective modulation of LT biosynthesis as well as for obtaining more potent and target-focused anti-inflammatory drugs.

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). ^{1}H 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.

The purity (95% or higher) of all final products that were evaluated for bioactivity was assessed by HPLC. The analytical HPLC analyses were carried out on Beckman Coulter 125 S, equipped with two high pressure binary gradient delivery systems, a System Gold 166 variable-wavelength UV e vis detector and a Rheodyne 7725i injector (Rheodyne, Inc., Cotati, CA, USA) with a 20-mL stainless steel loop.

For the analytical tests, compounds were prepared dissolving in methanol (0.5 mg/mL). Each solution (20 mL) was injected in a Jupiter Phenomenex RP 18 (4.5 _ 250 mm) analytical column. The mobile phase was a combination mixture of H_2O + 0.1% TFA (solvent A) and CH_3CN + 0.1% TFA (solvent B). The elution was made in gradient from 5% of B to 71% in 35 min, the flow rate was 1.0 mL/min.

*4.2 General procedure for synthesis of compounds **4**, **5** and **6***

To a solution of compounds**1, 2** or **3** (4.0 mmol) in CH_2Cl_2 (12 mL), stirred at 0 °C, imidazole (1.5 eq.), DMAP (0.1 eq.) and TBDMSCl (1.2 eq.) were added. The mixture was stirred over night at room temperature, washed with water and brine. Organic layer was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. Crude mixture was purified through

flash chromatography eluting with hexane/ethyl acetate 8:2, affording desired product as colorless oil.

*4.2.1 (8-bromooctyloxy)(tert-butyl)dimethylsilane (**4**)*

Yield: 76%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 0.07 (s, 6H); 0.92 (s, 9H); 1.3 (s, 6H); 1.41-1.60 (m, 4H); 1.87 (m, 2H); 3.43 (t, 2H, J=6Hz); 3.62 (t, 2H, J=6Hz).

*4.2.2 (10-bromodecyloxy)(tert-butyl)dimethylsilane (**5**)*

Yield: 85%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 0.06 (s, 6H); 0.89 (s, 9H); 1.3 (s, 10H); 1.41-1.60 (m, 4H); 1.85 (m, 2H); 3.43 (t, 2H, J=6Hz); 3.62 (t, 2H, J=6Hz).

*4.2.3 (11-bromoundecyloxy)(tert-butyl)dimethylsilane (**6**)*

Yield: 57%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 0.07 (s, 6H); 0.92 (s, 9H); 1.3 (s, 12H); 1.41-1.60 (m, 4H); 1.87 (m, 2H); 3.43 (t, 2H, J=6Hz); 3.62 (t, 2H, J=6Hz).

*4.3 General procedure for synthesis of compounds **7**, **8** and **9***

To a solution of tetramethoxybenzene (350 mg, 1.7 mmol, 1 eq.) [23]. and TMEDA (1.1 eq.) in anhydrous THF (7.5 mL) was added dropwise a solution of n-BuLi (1.2 eq.) at -20°C. The reaction was stirred for 30 min and then was warmed to -10 °C and stirred at this temperature for 30 min. The alkyl halide **4-6** (1.05 eq) was added dropwise, and the reaction was stirred overnight at room temperature. Saturated NH_4Cl was added to the reaction, and the aqueous phase was extracted with diethyl ether. The organic phases were combined and dried over MgSO4. Removal of the organic solvent under reduced pressure afforded crude mixture that was purified through flash chromatography eluting with hexane/ethyl acetate 95:5.

*4.3.1 (8-(2,3,5,6-tetramethoxyphenyl)octyloxy)(tert-butyl)dimethylsilane (**7**)*

Yield: 59%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 0.06 (s, 6H); 0.89 (s, 9H); 1.3 (s, 6H); 1.41-1.60 (m, 4H); 1.65 (m, 2H); 2.61 (t, 2H, J=8Hz); 3.61 (t, 2H, J=6Hz); 3.79 (s, 6H); 3.87 (s, 6H); 6.43 (s, 1H).

*4.3.2 (10-(2,3,5,6-tetramethoxyphenyl)decyloxy)(tert-butyl)dimethylsilane (**8**)*

Yield: 37%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 0.06 (s, 6H); 0.89 (s, 9H); 1.3 (s, 10H); 1.41-1.60 (m, 4H); 1.65 (m, 2H); 2.63 (t, 2H, J=8Hz); 3.62 (t, 2H, J=6Hz); 3.79 (s, 6H); 3.87 (s, 6H); 6.43 (s, 1H).

4.3.3 (11-(2,3,5,6-tetramethoxyphenyl)undecyloxy)(tert-butyl)dimethylsilane (9)

Yield: 63%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 0.06 (s, 6H); 0.90 (s, 9H); 1.3 (s, 12H); 1.41-1.60 (m, 4H); 1.63 (m, 2H); 2.61 (t, 2H, J=8Hz); 3.62 (t, 2H, J=6Hz); 3.79 (s, 6H); 3.87 (s, 6H); 6.43 (s, 1H).

*4.4 General procedure for synthesis of compounds **10-15***

To a solution of compounds **7-9** (0.5 mmol) in CH_3CN (6 mL), was added a solution of CAN (2.5 mmol) in CH_3CN-H_2O (11 mL, 7:3) dropwise at -10 °C (salt-ice bath). The reaction was stirred for 10 minutes at -10 °C and diluted with water and extracted with ethyl acetate. The organic layer was washed two times with distilled water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified over silica gel using hexane:EtOAc (50:50) as eluent to give desired compounds.

*4.4.1 3-(8-hydroxyoctyl)-4,5-dimethoxycyclohexa-3,5-diene-1,2-dione (**10**)*

Yield: 36%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 1.26 (s, 10H); 1.54-1.58 (m, 2H); 2.41 (t, 2H, J=8Hz); 3.64 (t, 2H, J=6Hz); 3.92 (s, 6H); 5.70 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 23.4, 25.7, 29.3, 32.7, 56.8, 62.0, 63.0, 101.3, 102.2, 130.5, 159.0, 166.4, 178.7, 180.7. ESI(m/z): 297.3 [M^+ + 1].

*4.4.2 3-(10-hydroxydecyl)-4,5-dimethoxycyclohexa-3,5-diene-1,2-dione (**11**)*

Yield: 45%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 1.28 (s, 14H); 1.54-1.58 (m, 2H); 2.41 (t, 2H, J=8Hz); 3.64 (t, 2H, J=6Hz); 3.91 (s, 6H); 5.69 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 22.2, 25.8, 27.9, 32.9, 56.2, 61.6, 69.7, 103.0, 105.4, 128.1, 160.0, 160.8, 181.1. ESI(m/z): 325.4 [M^+ + 1].

*4.4.3 3-(11-hydroxyundecyl)-4,5-dimethoxycyclohexa-3,5-diene-1,2-dione (**12**)*

Yield: 37%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 1.26 (s, 16H); 1.54-1.58 (m, 2H); 2.41 (t, 2H, J=8Hz); 3.64 (t, 2H, J=6Hz); 3.91 (s, 6H); 5.69 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 22.6, 25.7, 28.0, 29.4, 32.7, 50.8, 56.7, 63.1, 102.2, 119.3, 151.7, 161.1, 181.7, 182.9. ESI(m/z): 361.3 [M^+ + 23].

*4.4.4 3-(8-hydroxyoctyl)-2,5-dimethoxycyclohexa-2,5-diene-1,4-dione (**13**)*

Yield: 36%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 1.30 (s, 10H); 1.54-1.58 (m, 2H); 2.44 (t, 2H, J=8Hz); 3.66 (t, 2H, J=6Hz); 3.80 (s, 3H); 4.04 (s, 3H); 5.73 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 23.0, 25.7, 28.6, 29.4, 32.7, 56.4, 61.4, 63.0, 105.4, 130.6, 155.9, 158.7, 182.5, 183.6. ESI(m/z): 319.6 [M^+ + 23].

*4.4.5 3-(10-hydroxydecyl)-2,5-dimethoxycyclohexa-2,5-diene-1,4-dione (**14**)*

Yield: 28%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 1.28 (s, 14H); 1.54-1.58 (m, 2H); 2.44 (t, 2H, J=8Hz); 3.64 (t, 2H, J=6Hz); 3.82 (s, 3H); 4.05 (s, 3H); 5.74 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 22.9, 25.7, 28.6, 29.4, 33.2, 56.4, 61.4, 63.0, 104.9, 130.6, 155.9, 158.7, 182.5, 183.6. ESI(m/z): 325.4 [M^+ + 1].

*4.4.6 3-(11-hydroxyundecyl)-2,5-dimethoxycyclohexa-2,5-diene-1,4-dione (**15**)*

Yield: 26%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 1.29 (s, 16H); 1.54-1.58 (m, 2H); 2.44 (t, 2H, J=8Hz); 3.66 (t, 2H, J=6Hz); 3.82 (s, 3H); 4.07 (s, 3H); 5.75 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 23.1, 25.7, 28.6, 29.4, 32.8, 56.4, 61.4, 63.0, 105.4, 130.7, 155.9, 158.7, 182.4, 183.6. ESI(m/z): 339.5 [M^+ + 1].

*4.5 General procedure for synthesis of compounds **16-18***

A solution of appropriate tert-butyl-dimethylsilane **7-9** (0.3 mmol) in a mixture of THF (5mL), H_2O (1.4 mL) and HCOOH (4.2 mL) was stirred at room temperature for 30 min. Saturated $NaHCO_3$ was added and the aqueous phase was extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The

residue was purified over silica gel using hexane:EtOAc (80:20-50:50) as eluent to give desired compounds colorless oils.

*4.5.1 8-(2,3,5,6-tetramethoxyphenyl)octan-1-ol (**16**)*

Yield: 51%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 1.3 (s, 8H); 1.41-1.60 (m, 4H); 2.61 (t, 2H, J=8Hz); 3.62 (t, 2H, J=6Hz); 3.79 (s, 6H); 3.85 (s, 6H); 6.42 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ:24.6, 25.7 (2C), 29.3 (2C), 29.4, 30.7, 32.7, 32.8 (2C), 56.2, 60.9, 63.0, 96.5, 131.0, 140.9 (2C), 148.8 (2C). ESI(m/z): 327.8 [M^+ + 1].

*4.5.2 10-(2,3,5,6-tetramethoxyphenyl) decan-1-ol (**17**)*

Yield: 56%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 1.3 (s, 12H); 1.41-1.60 (m, 4H); 2.61 (t, 2H, J=8Hz); 3.62 (t, 2H, J=6Hz); 3.79 (s, 6H); 3.85 (s, 6H); 6.42 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ:24.6, 25.7 (2C), 29.3 (4C), 29.4, 30.7, 32.7, 32.8 (2C), 56.2, 60.9, 63.0, 96.5, 131.0, 140.9 (2C), 148.8 (2C) ESI(m/z): 377.5 [M^+ + 23].

*4.5.3 11-(2,3,5,6-tetramethoxyphenyl)undecan-1-ol (**18**)*

Yield: 53%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 1.3 (s, 14H); 1.41-1.60 (m, 4H); 2.61 (t, 2H, J=8Hz); 3.62 (t, 2H, J=6Hz); 3.79 (s, 6H); 3.85 (s, 6H); 6.42 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ:24.6, 25.7 (3C), 29.3 (4C), 29.4, 30.7, 32.7, 32.8 (2C), 56.2, 60.9, 63.0, 96.5, 131.0, 140.9 (2C), 148.8 (2C) ESI(m/z): 391.4 [M^+ + 23].

*4.5.4 (10-hydroxy-10-(2,3,5,6-tetramethoxyphenyl)decyloxy)(tert-butyl)dimethylsilane (**20**)*

To a solution of tetramethoxybenzene (210mg, 1.03 mmol, 1 eq.). [24] and TMEDA (1.1 eq.) in anhydrous THF (7.5 mL) was added dropwise a solution of n-BuLi (1.05 eq.) at -50 °C.

The reaction was stirred for 30 min and then was warmed to -10 °C and stirred at this temperature for 30 min. A solution of 10-tert-butyl dimethyl silanyloxy decanal (**19**) [25] (1.05 eq) in 5 mL of dry THF was added dropwise, and the reaction was stirred overnight at room temperature. Saturated NH_4Cl was added to the reaction, and the aqueous phase was extracted with diethyl ether. The organic phases were combined and dried over $MgSO_4$. Removal of the organic solvent under reduced pressure afforded crude mixture that was

purified through flash chromatography eluting with hexane/ethyl acetate 90:10. Desired compound was obtained as a colorless oil (Yield: 28%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 0.06 (s, 6H); 0.89 (s, 9H); 1.3 (s, 10H); 1.41-1.60 (m, 4H); 1.65 (m, 2H); 3.62 (t, 2H, J=6Hz); 3.79 (s, 6H); 3.87 (s, 6H); 4.91 (m, 1H); 6.43 (s, 1H).

*4.5.5 3-(1,10-dihydroxydecyl)-2,5-dimethoxycyclohexa-2,5-diene-1,4-dione (**21**)*

To a solution of compounds **20** (60 mg, 0.125 mmol) in CH_3CN (1.4 mL), was added a solution of CAN (2.5 mmol) in CH_3CN-H_2O (2.6 mL, 7:3) dropwise at -10 °C (salt-ice bath). The reaction was stirred for 10 minutes at -10 °C and diluted with water and extracted with ethyl acetate. The organic layer was washed two times with distilled water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified over silica gel using petroleum ether:EtOAc (15:85) as eluent to give desired compound as orange oil (Yield: 21%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 1.3 (s, 10H); 1.41-1.60 (m, 4H); 1.65 (m, 2H); 3.62 (t, 2H, J=6Hz); 3.79 (s, 3H); 4.12 (s, 3H); 4.78 (m, 1H); 5.76 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 23.2, 25.7, 29.0, 30.8, 32.8, 58.3, 62.2, 105.5, 131.6, 155.9, 158.7, 182.1, 183.0. ESI(m/z): 341.5 [M^+ + 1].

*4.5.6 10-acetoxy-10-(2,3,5,6-tetramethoxyphenyl)decyloxy)(tert-butyl)dimethylsilane (**22**)*

Acetic anhydride (0.119 mL, 1.24 mmol, 4.0 eq.) TEA (0.087 mL, 0.622 mmol, 2.0 eq.) and DMAP (8mg, 0.065 mmol, 0.21 eq.) were added to a stirred solution of **20** (148mg, 0.311 mmol) in anhydrous diethyl ether (2.0 mL) under nitrogen atmosphere. The reaction mixture was stirred for 2 h at room temperature. Saturated $NaHCO_3$ was added and the aqueous phase was extracted with diethyl ether. The organic phases were combined and dried over $MgSO_4$. Removal of the organic solvent under reduced pressure afforded crude mixture that was purified through flash chromatography eluting with hexane/ethyl acetate 80:20. Desired compound was obtained as a colorless oil (Yield: 71%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 0.06 (s, 6H); 0.89 (s, 9H); 1.3 (s, 10H); 1.41-1.60 (m, 4H); 1.65 (m, 2H); 2.09 (s, 3H); 3.62 (t, 2H, J=6Hz); 3.85 (s, 6H); 3.87 (s, 6H); 6.27 (m, 1H); 6.50 (s, 1H).

*4.5.7 3-(1-acetoxy-10-hydroxydecyl)-2,5-dimethoxycyclohexa-2,5-diene-1,4-dione (**23**)*

To a solution of compounds **22** (114 mg, 0.218 mmol, 1eq.) in CH_3CN (2 mL), was added a solution of CAN (2.5 mmol) in CH_3CN-H_2O (3.7 mL, 7:3) dropwise at -10 °C (salt-ice bath). The reaction was stirred for 10 minutes at -10 °C and diluted with water and extracted with ethyl acetate. The organic layer was washed two times with distilled water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified over silica gel using petroleum ether:EtOAc (15:85) as eluent to give desired compound a yellow oil (Yield: 20%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 1.3 (s, 10H); 1.41-1.60 (m, 4H); 1.65 (m, 2H); 2.09 (s, 3H); 3.62 (t, 2H, J=6Hz); 3.81 (s, 3H); 4.11 (s, 3H); 5.77 (s, 1H); 5.82 (m, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 19.9, 23.2, 25.7, 29.0, 30.8, 32.8, 58.3, 63.5, 105.5, 131.6, 155.9, 158.7, 170.8, 182.1, 183.0. ESI(m/z): 383.6 [M^++1].

*4.6 General procedure for synthesis of compounds **28**, **29**, **30***

To a suspension of starting (0.092 mmol) in ethanol (3.0 mL), cooled at 0°C, $NaBH_4$ was slowly added (2.5 eq.). The mixture was reacted for 1 h at the same temperature. The excess of $NaBH_4$ was neutralized with HCl 1M, the mixture was diluted with water and extracted three times with diethyl ether. The organic phases were combined and dried over $MgSO_4$. Removal of the organic solvent under reduced pressure afforded crude mixture that was purified through flash chromatography eluting with hexane/ethyl acetate 70:30.

*4.6.1 3-undecyl-4,5-dimethoxybenzene-1,2-diol (**28**)*

Yield: 83%. ^{1}H NMR ($CDCl_3$). δ 0.90 (t, 3H, J = 8 Hz); 1.37 (s, 16H); 1.60 (m, 2H); 2.68 (t, 2H, J= 8 Hz); 3.81 (s, 6H); 4.58 (OH); 5.36 (OH), 6.42 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 14.1, 23.1, 24.8, 28.1, 28.3, 29.4, 29.5, 29.7, 30.0, 32.1 (2C), 56.1, 60.1, 98.7, 124.3, 135.2, 139.5, 140.2, 147.8. ESI(m/z): 347.1 [M^++23].

*4.6.2 3-dodecyl-4,5-dimethoxybenzene-1,2-diol (**29**)*

Yield: 63%. ^{1}H NMR ($CDCl_3$). δ 0.90 (t, 3H, J = 8 Hz); 1.37 (s, 18H); 1.52 (m, 2H); 2.62 (t, 2H, J= 8 Hz); 3.78 (s, 6H); 4.83 (OH); 5.62 (OH), 6.42 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz):

δ: 14.1, 23.1, 24.8, 28.1, 28.3, 29.4, 29.5, 29.7 (2C), 30.0, 32.1 (2C), 56.1, 60.1, 98.7, 124.3, 135.2, 139.5, 140.2, 147.8. ESI(m/z): 361.3 [M^++23].

*4.6.3 3-tridecyl-4,5-dimethoxybenzene-1,2-diol (**30**)*

Yield: 40%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.90 (t, 3H, J = 8 Hz); 1.37 (s, 20H); 1.52 (m, 2H); 2.62 (t, 2H, J= 8 Hz); 3.78 (s, 6H); 4.73 (OH); 5.80 (OH), 6.42 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 14.1, 23.1, 24.8, 28.1, 28.3 (2C),, 29.4 (2C), 29.5, 29.7, 30.0, 32.1 (2C), 56.1, 60.1, 98.7, 124.3, 135.2, 139.5, 140.2, 147.8. ESI(m/z): 353.4 [M^++1].

*4.6.4 5-methoxy-3-tridecylbenzene-1,2,4-triol (**33**)*

Yield: 80%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.90 (t, 3H); 1.28 (s, 20H); 1.52 (m, 2H); 2.67 (t, 2H Hz); 3.8 (s, 3H); 5.34 (OH), 6.42 (s, 1H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ: 14.1, 22.7, 23.9, 28.1, 29.5, 32.0, 56.6, 102.2, 117.0, 136.0, 137.7, 139.9, 161.1. ESI(m/z): 339.6 [M^++1].

*4.6.5 2,5-dimethoxy-3-tridecylbenzene-1,4-diol (**34**)*

Yield: 70%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.92 (t, 3H); 1.31 (s, 20H); 1.56 (m, 2H); 2.62 (t, 2H); 3.72 (s, 3H); 3.79 (s, 3H); 6.37 (s, 1H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ 14.1, 22.7, 24.5, 29.6, 31.9, 56.2, 61.8, 96.7, 122.4, 137.1, 138.9, 141.5, 143.0 ESI(m/z): 375.2 [M^++23].

4.7 Biological evaluation and assay systems

4.7.1. Materials

Zileuton was purchased from Sequoia Research Products (Oxford, UK), zymosan from Sigma (Milan, Italy) and LTC_4 enzyme immunoassay (EIA) from Cayman Chemical, (Inalco, Milan, Italy).

4.7.2. Cells and isolation

Neutrophils were freshly isolated from leukocyte concentrates obtained from the Institute of Transfusion Medicine, University Hospital Jena as described [26]. Briefly, human peripheral blood was collected in heparinized tubes (16 I.E. heparin/ml blood) by venipuncture from fasted (12 h) adult healthy volunteers, with consent, and leukocyte concentrates were prepared by centrifugation (4000 x g, 20 min, 20 °C). The subjects had no apparent inflammatory conditions and had not taken anti-inflammatory drugs for at least ten days prior to blood collection. Neutrophils were immediately isolated by dextran sedimentation and centrifugation on Nycoprep cushions (PAA Laboratories, Linz, Austria) and hypotonic lysis of erythrocytes was performed as described [26]. Neutrophils were finally resuspended in PBS pH 7.4 containing 1 mg/ml glucose and 1 mM $CaCl_2$ (PGC buffer) (purity > 96-97%).

4.7.3 Animals

Male CD-1 mice (35–40 g; Charles River) (30 rats, 50 mice) were housed at the Department of Pharmacy (Naples, Italy) in a controlled environment (23 °C, humidity range of 40–70% and 12 h light/dark cycles) and provided with standard rodent chow and water. Animals were allowed to acclimatize for 4 days before the experiments and were subjected to a 12 h light–12 h dark schedule. Experiments were conducted during the light phase. Animal care was in compliance with Italian regulations on protection of animals used for experimental and other scientific purpose (Ministerial Decree 116/92) as well as with the European Economic Community regulations (Official Journal of the European Community L 358/1 12/18/1986).

4.7.4 Mouse paw oedema

Mice were lightly anaesthetized by inhalation of enflurane and depth of anesthesia was assessed by checking both abdominal and pedal withdrawal reflex. They were then given a subplantar injection of 50 µL of carrageenan 1% (w v^{-1}). Paw volume was measured using a hydroplethismometer specially modified for small volumes (Ugo Basile, Milan, Italy) immediately before subplantar injection (basal value) and 2, 4, 6, 24, 48 and 72 h thereafter. Mice were divided into seven groups (n = 6) and received an i.p. injection of compounds or vehicle (DMSO), 30 min before the carrageenan injection.

4.7.5 Zymosan-Induced Peritonitis in Mice

For zymosan-induced peritonitis in mice, **27**, **30** or zileuton at the indicated dose or vehicle (0.5 mL of 0.9% saline solution containing 2% DMSO) was given i.p. 30 min before zymosan i.p. injection (0.5 mL of suspension of 2 mg/mL in 0.9% w/v saline). Mice were killed by inhalation of CO_2 at 30 min and 4 h after zymosan injection, for LTC_4 evaluation and cell infiltration, respectively [27] Exudates were collected by peritoneal lavage with 2 mL of cold PBS, the cells in the exudates were counted and LTC_4 was measured by EIA.

4.7.6 5-LO activity assays

For analysis of 5-LO products in intact cells, neutrophils (5×10^6) were resuspended in 1 ml PGC buffer, preincubated for 15 min at 37 °C with test compounds or vehicle (0.1% DMSO) and Ca^{2+}-ionophore A23187 (2.5 µM) plus 20 µM arachidonic acid was added. After 10 min at 37 °C the reaction was stopped on ice by addition of 1 ml of methanol. 30 µl 1 N HCl and 500 µl PBS, and 200 ng prostaglandin (PG)B_1 were added and the samples were subjected to solid phase extraction on C18-columns (100 mg, UCT, Bristol, PA, USA). 5-LO products (LTB_4 and its trans-isomers, and 5-H(P)ETE) were analyzed by RP-HPLC and quantities calculated on the basis of the internal standard PGB_1. Cys-LTs C_4, D_4 and E_4 were not detected (amounts were below detection limit), and oxidation products of LTB_4 were not determined.

For analysis of 5-LO activity in cell-free assays, E. coli BL21 cells were transformed with pT3-5-LO plasmid (provided by Dr. Olof Radmark, Karolinska Institute, Stockholm, Sweden), recombinant 5-LO protein was expressed and purified on an ATP-agarose column as described previously [28]. Aliquots of semi-purified 5-LO (0.5 µg) were diluted with ice-cold PBS containing 1 mM EDTA, and 1 mM ATP was added (final volume was 1 ml). Samples were pre-incubated with the test compounds or vehicle (0.1% DMSO) as indicated. After 15 min at 4 °C, samples were pre-warmed for 30 sec at 37 °C, and 2 mM $CaCl_2$ plus the indicated concentrations of arachidonic acid were added to initiate 5-LO product formation. After 10 min at 37 °C, the reaction was stopped by addition of 1 ml ice-cold methanol, and the formed metabolites were analyzed by RP-HPLC as described [29] 5-LO products include the all-trans isomers of LTB_4 and 5-H(P)ETE.

4.8 Docking protocol

The crystal structure 3o8y was prepared by correcting four virtual mutations (Glu13Trp, His14Phe, Gly75Trp, and Ser76Leu) to represent the wild type enzyme rather than the crystallized stable 5-LO [30]It was then energetically minimized in Discovery Studio (version 3.5, Biovia Inc. www.biovia.com), and 52 hydrogen atoms were added for docking. The docking experiments were performed using GOLD version 5.2 [31] with GoldPLP as scoring function. The binding site was located using the Pocketfinder tool within MOE[32] For the docking, the binding site was defined around Ile167 in a 10 Å radius. All other settings were kept default. Protein-ligand interactions were visualized using LigandScout 3.1[33]

4.9 Statistical analysis

Data obtained are expressed as mean ± S.E. of single determinations performed in three or four independent experiments at different days. IC_{50} values were graphically calculated from averaged measurements at 4-5 different concentrations of the compounds using SigmaPlot 12.0 (Systat Software Inc., San Jose, USA). Statistical evaluation of the data was performed

by one-way ANOVA followed by a Bonferroni or TukeyeKramer post-hoc test for multiple comparisons respectively. A p value <0.05 (*) was considered significant.

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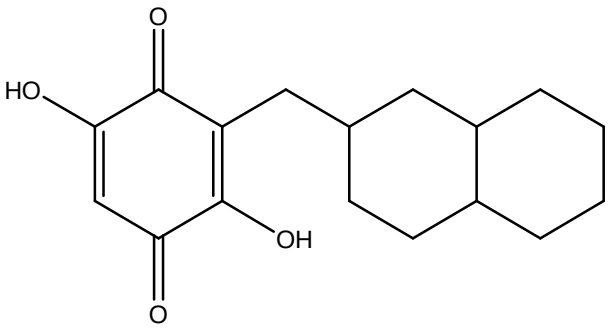

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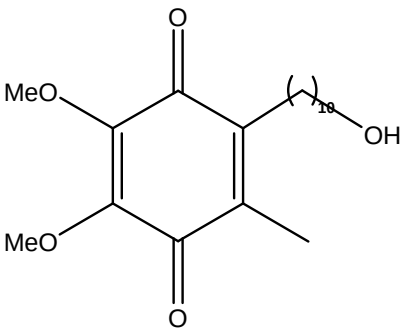
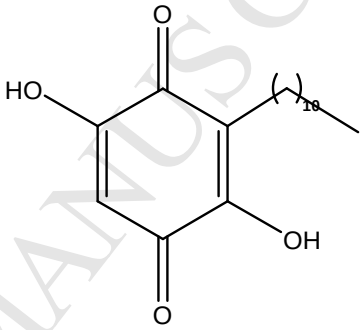


Embelin



Idebenone

Fig.1

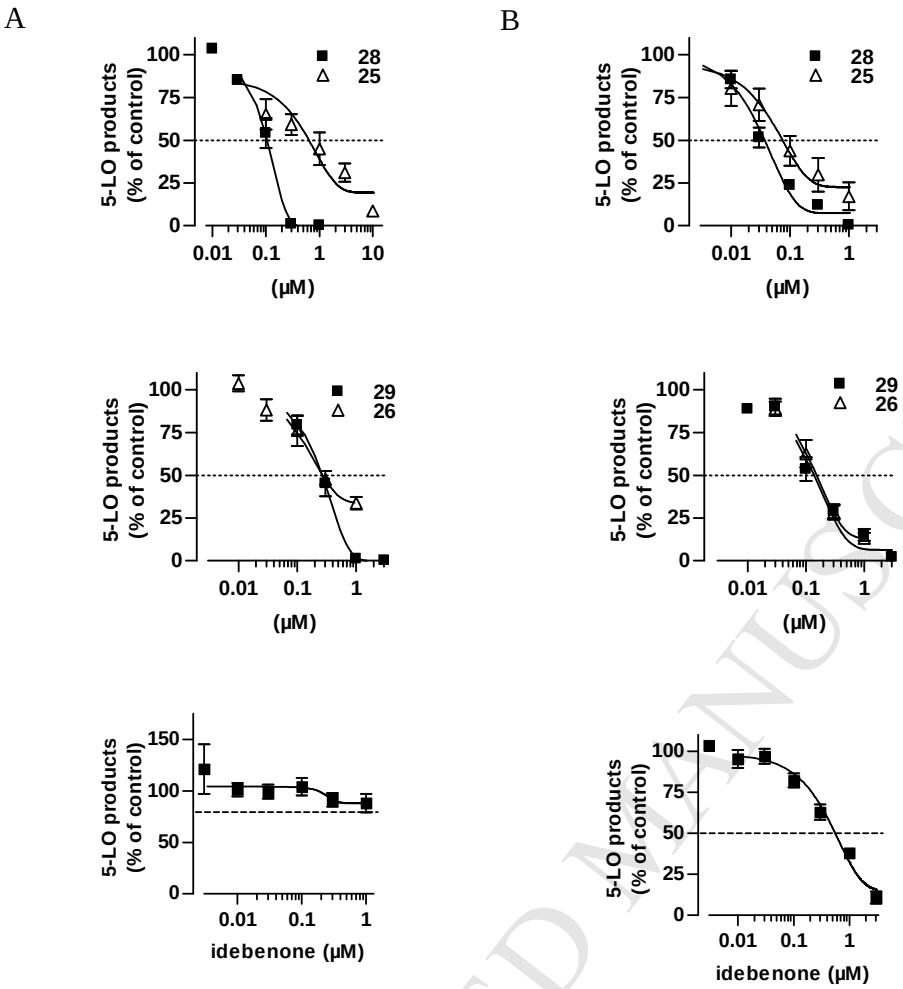


Fig. 2

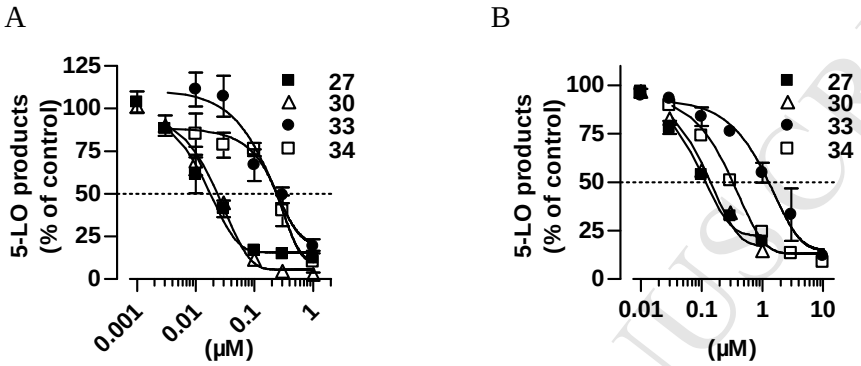


Fig. 3

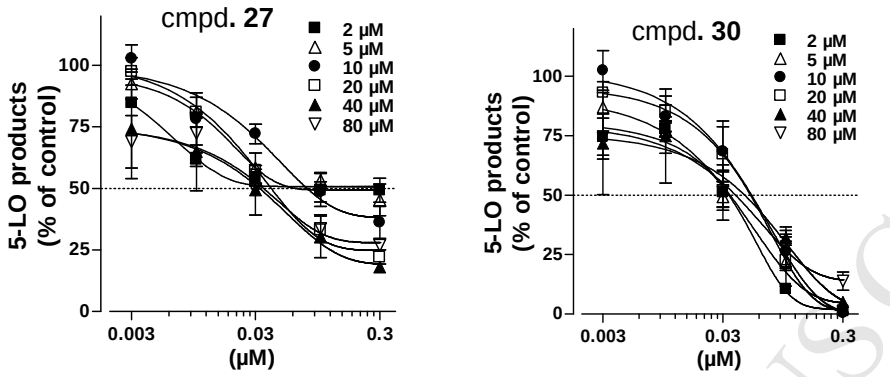


Fig. 4

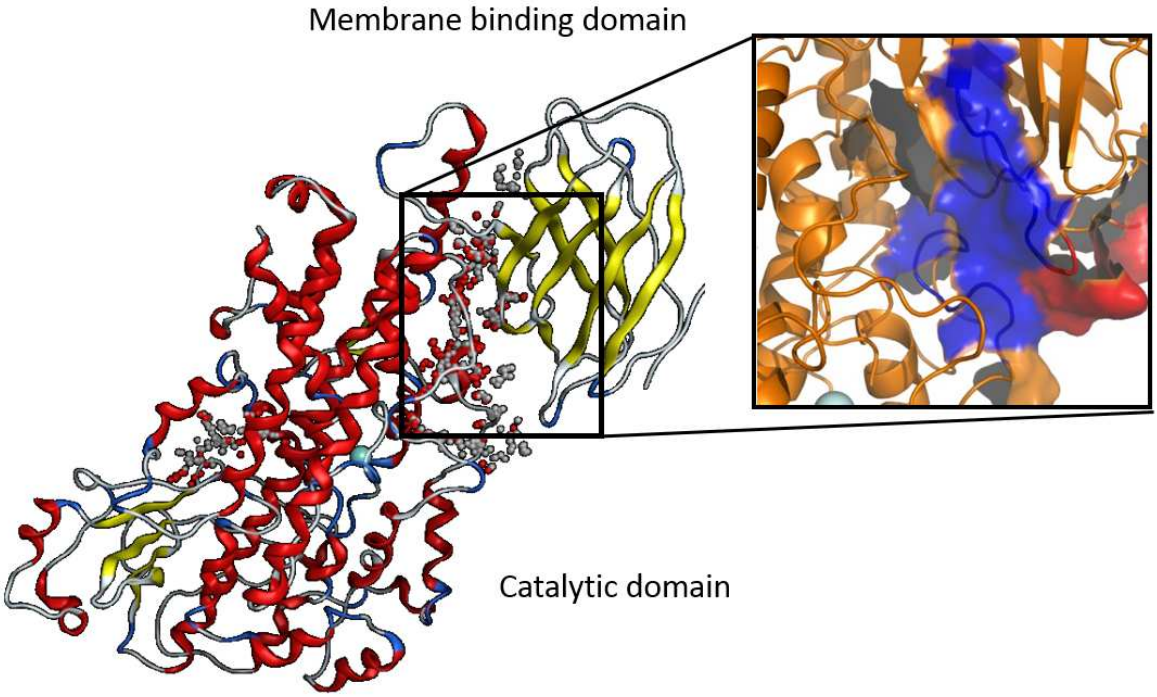


Fig. 5

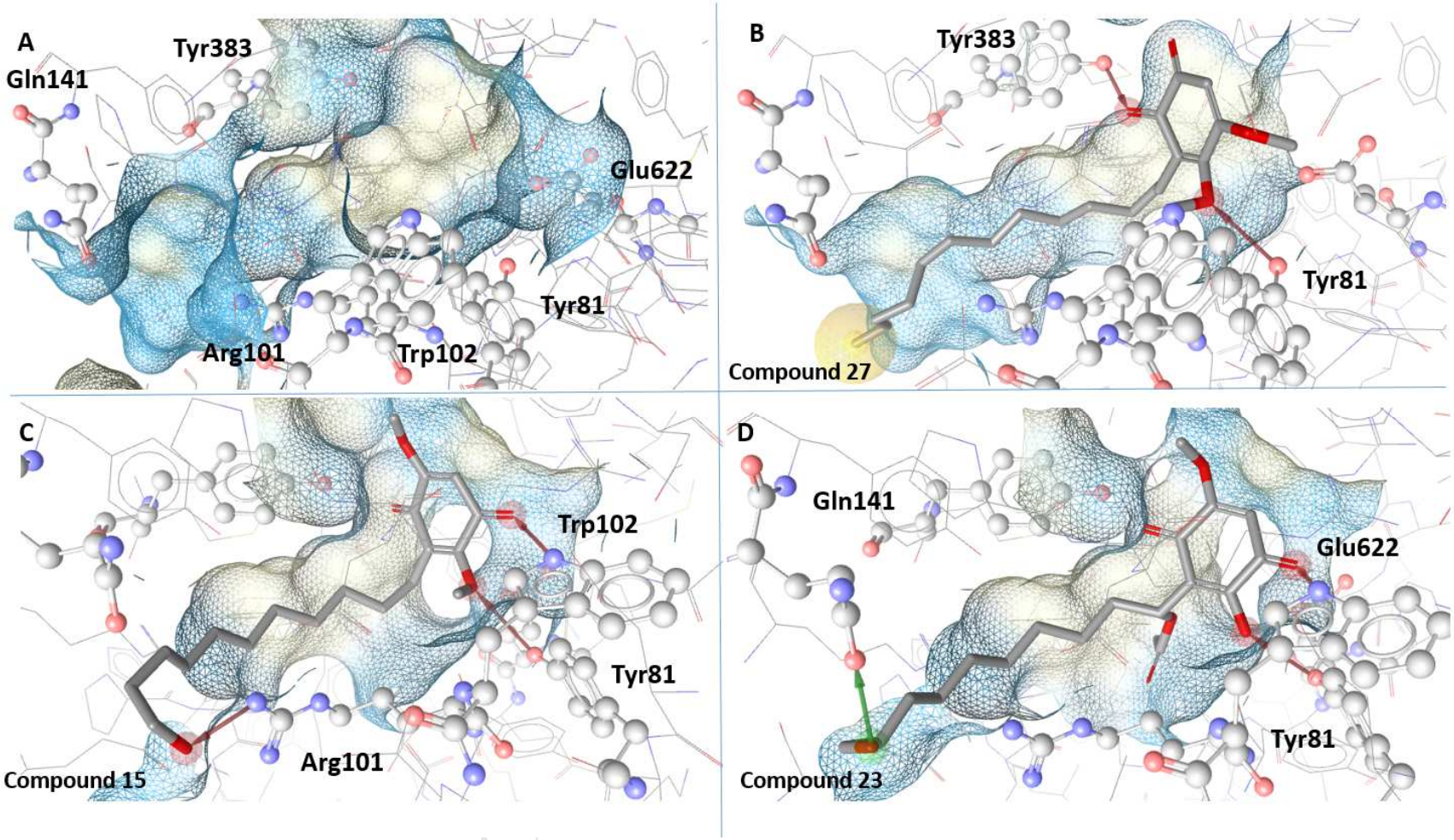

A
Gln141
Tyr383
Glu622
Tyr81
Arg101
Trp102
B
Tyr383
Tyr81
Compound 27
C
Trp102
Tyr81
Arg101
Compound 15
D
Gln141
Glu622
Tyr81
Compound 23

Fig. 6

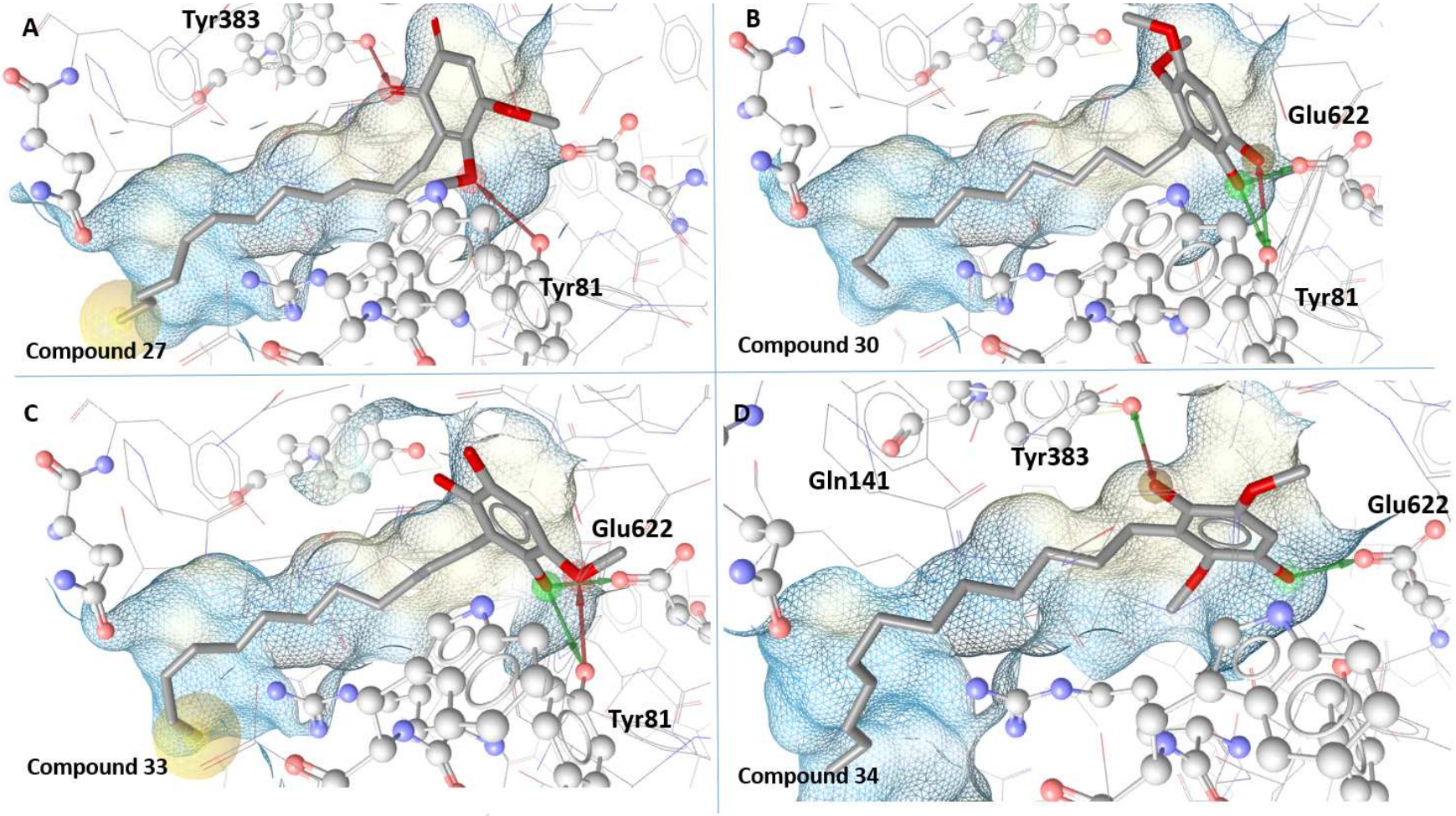

A
Tyr383
Tyr81
Compound 27
B
Glu622
Tyr81
Compound 30
C
Glu622
Tyr81
Compound 33
D
Gln141
Tyr383
Glu622
Compound 34

Fig. 7

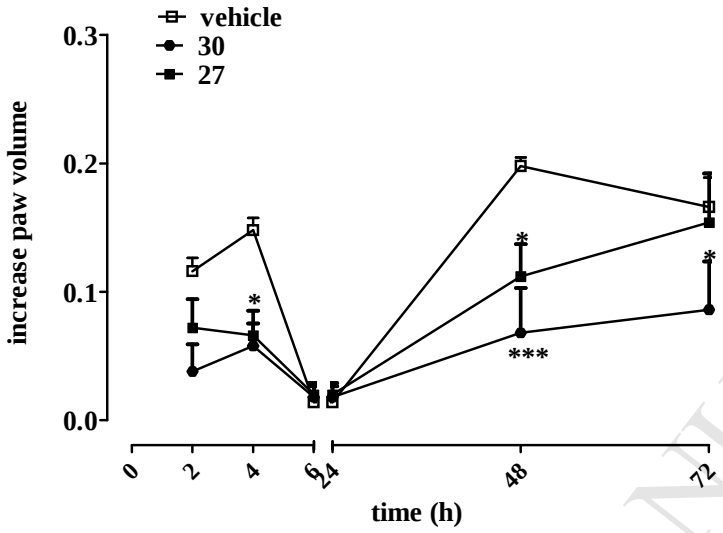


Fig. 8

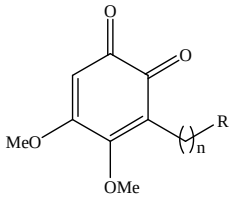
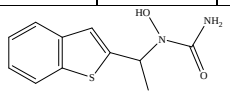
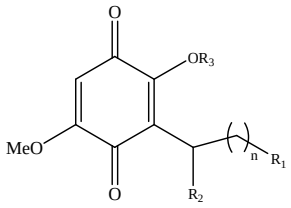
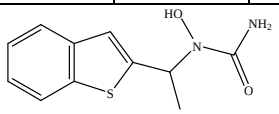
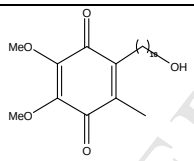
			**5-LO activity, IC_{50} [µM]**	
			Cell-free	**Cell-based A23187+AA**
	n	**R**		
10	8	-OH	0.22 ± 0.06	0.15 ± 0.04
11	10	-OH	1.08 ± 0.03	0.36 ± 0.03
12	11	-OH	1.96 ± 0.10	0.12 ± 0.04
25[a]	10	$-CH_3$	0.09±0.04	0.10±0.03
26[a]	11	$-CH_3$	0.13±0.12	0.15±0.03
27[a]	12	$-CH_3$	0.08±0.01	0.05±0.02
 **zileuton**			0.59± 0.10	2.5± 1.2

Table 1

a = Data from Filosa R. et al. *European Journal of Medicinal Chemistry* 94 (**2015**) 132-139

					**5-LO activity, IC_{50} [µM]**	
					Cell-free	**Cell-based A23187+AA**
	n	**R_1**	**R_2**	**R_3**		
13	8	-OH	-H	$-CH_3$	1.84 ± 0.07	0.33 ± 0.05
14	10	-OH	-H	$-CH_3$	1.75± 0.03	0.55± 0.02
15	11	OH	-H	$-CH_3$	1.51± 0.07	0.20 ± 0.04
21	9	-OH	-OH	-OH	1.46 ± 0.06	0.66 ± 0.06
23	9	-OH	-OAc	-OH	0.49 ± 0.05	1.62 ± 0.05
31[a]	11	$-CH_3$	-H	-H	0.92± 0.47	2.30±1.1
32[a]	11	$-CH_3$	-H	$-CH_3$	0.31± 0.05	0.26± 0.01
 **Zileuton**					0.59± 0.10	2.5± 1.2
 **Idebenone**					>10	0.55 ± 0.08

a = Data from Filosa R. et al. *European Journal of Medicinal Chemistry* 94 (**2015**) 132-139

Table 2

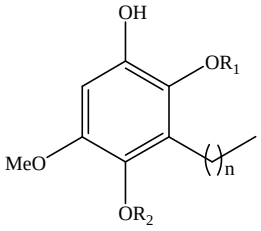
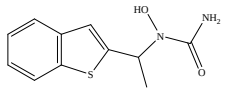
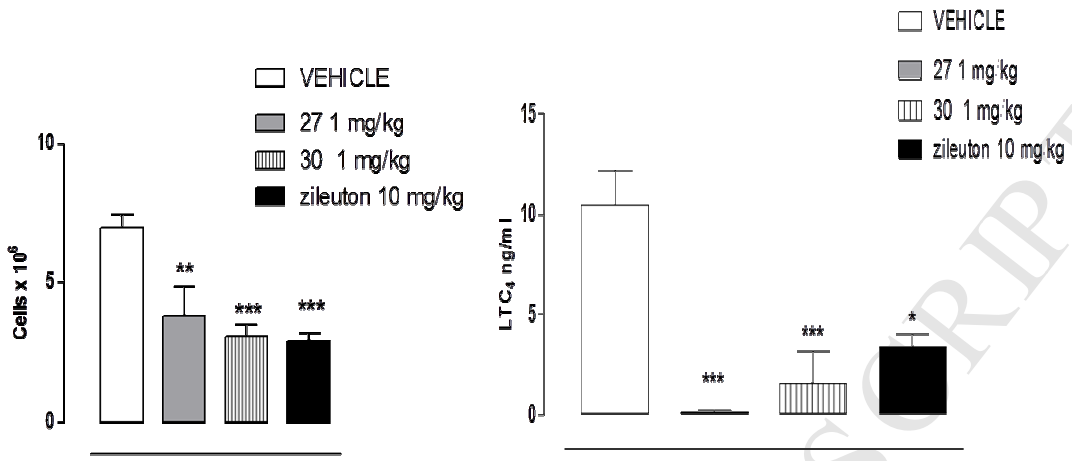
				**5-LO activity, IC_{50} [μM]**	
				Cell-free	**Cell-based A23187+AA**
	n	**R_1**	**R_2**		
28	10	-H	$-CH_3$	0.10 ± 0.05	0.03 ± 0.04
29	11	-H	$-CH_3$	0.24 ± 0.03	0.09 ± 0.06
30	12	-H	$-CH_3$	0.06 ± 0.02	0.028 ± 0.02
33	12	-H	-H	0.29± 0.05	1.60± 0.58
34	12	$-CH_3$	-H	0.26± 0.07	0.31± 0.03
 **Zileuton**				0.59± 0.10	2.5± 1.2

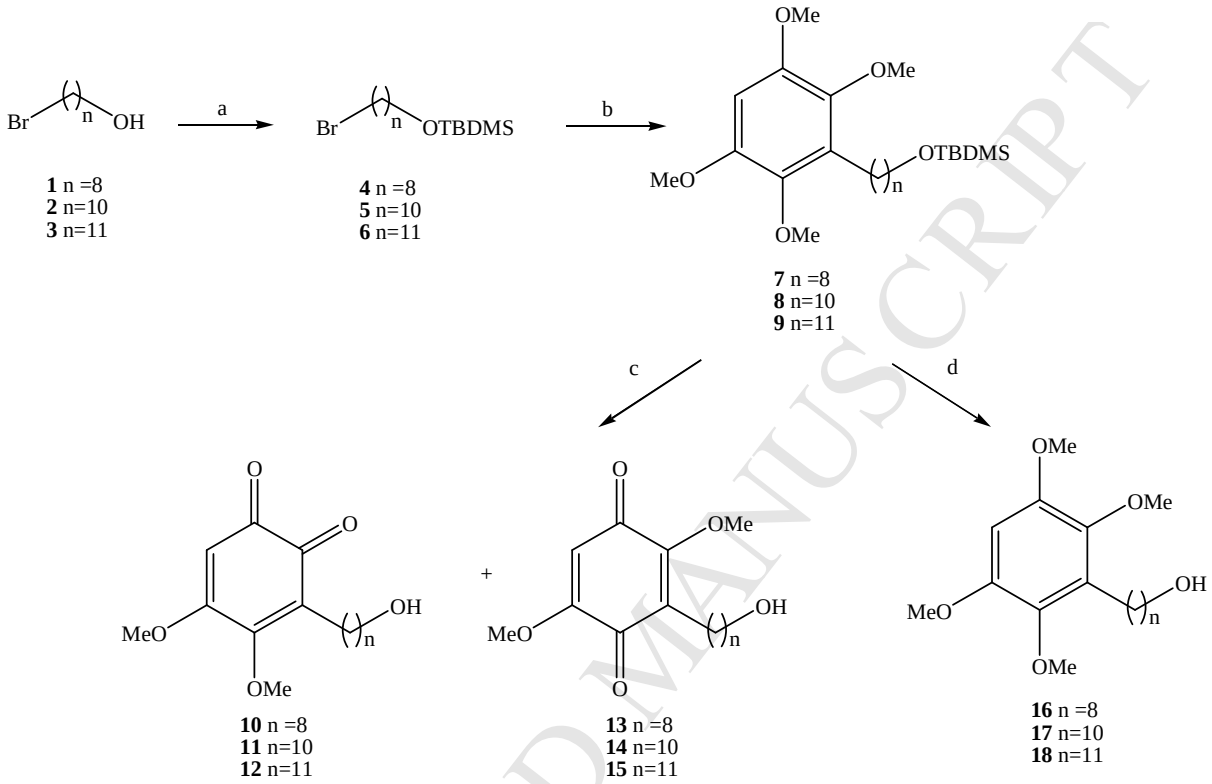
Table 3



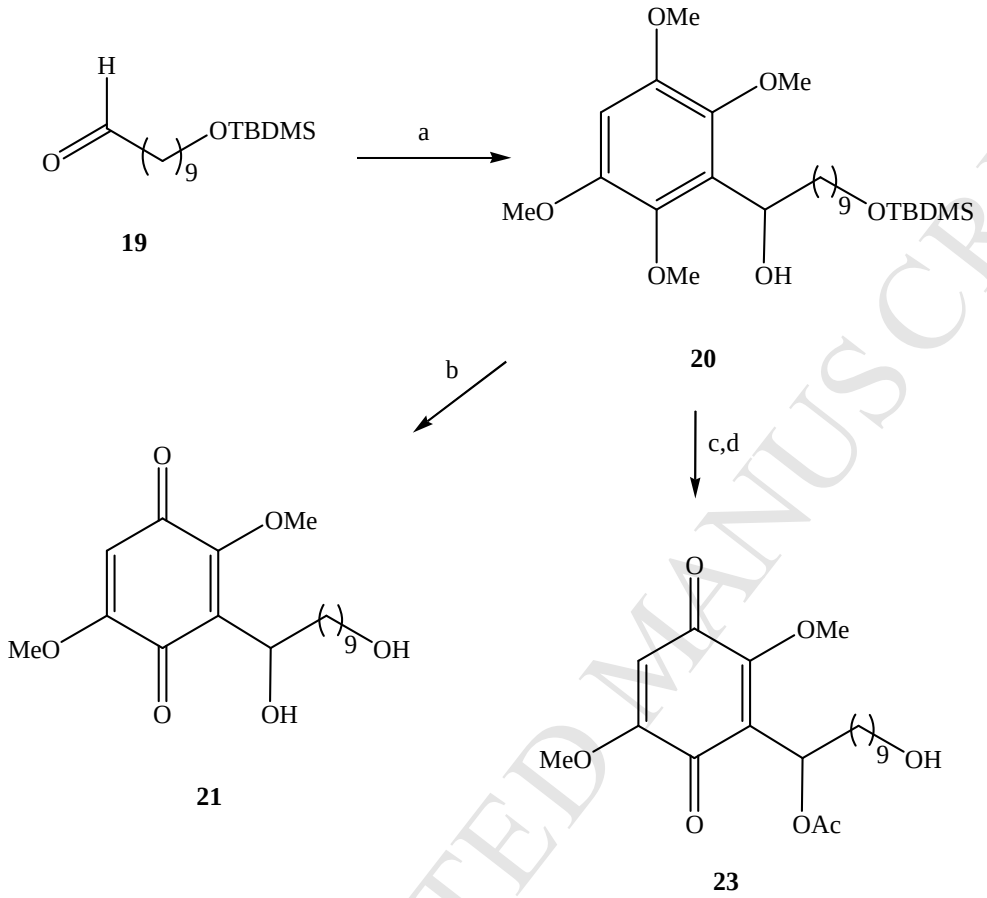
Treatment	LTC_4 (ng/mL)[a]	Inflammatory cells (× 10^6)[b]
Vehicle	10.48±1.72	7.0±0.47
compound 27, 1 mg/kg	0.14±0.05***	3.78±0.48**
compound 30, 1 mg/kg	1.58±0.66***	3.08±0.19***
zileuton, 10 mg/kg	3.35±0.7*	2.88±0.31***

Effect of **27** and **30** on zymosan-induced peritonitis in mice. Data are means ± SEM, n = 5-6. [a]Analysis was performed 30 min after zymosan injection. [b]Analysis was performed 4 h after zymosan injection. *P < 0.05; **P < 0.01; ***P < 0.001 versus vehicle (Student's t-test).

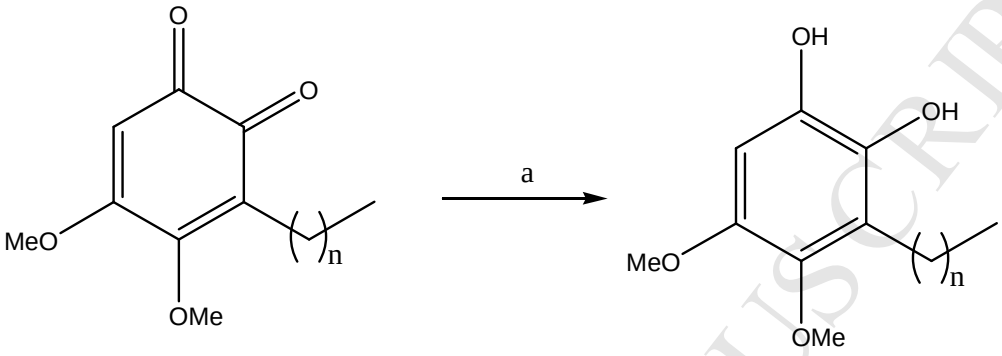
Table 4



Scheme 1



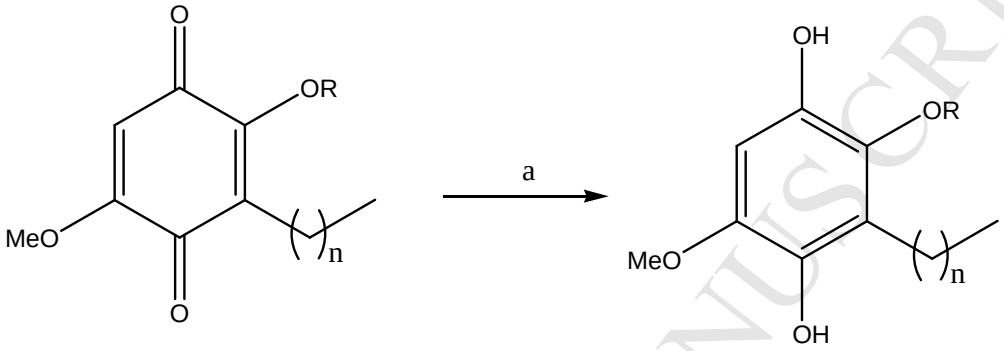
Scheme 2



25 n=10
26 n=11
27 n=12

28 n=10
29 n=11
30 n=12

Scheme 3



31 R= -H n=12
32 R= -CH_3 n=12

33 R= -H n=12
34 R= -CH_3 n=12

Scheme 4

Legends:

Fig.1 Structures of Id, Idebenone and Embelin.

Fig. 2 Effect of hydroquinones **28** and **29**, their corresponding quinones **25** and **26**, and Idebenone, respectively, on 5-LO product formation in cell-free and cell-based assays. (A) Purified 5-LO was incubated with compounds or vehicle (DMSO, 0.1%) at 4 °C for 15 min. Samples were pre-warmed for 30 s at 37 °C, 2 mM $CaCl_2$ and 20 µM AA were added, and after 10 min 5-LO product formation was determined. Data are expressed as percentage of control (100%), mean ± S.E.M., n = 3-4. (B) Human neutrophils (5×10^6) were pre-incubated with compounds or 0.1 % DMSO as vehicle for 15 min at 37 °C and stimulated with 2.5 µM A23187 and 20 µM AA for 10 min. 5-LO products were analyzed by HPLC. Data are expressed as percentage of control (100%), mean ± S.E.M., n = 3-4. Data are expressed as percentage of control (100%), mean ± S.E.M., n = 3-4.

Fig. 3 Effect of the quinone **27** and the reduced hydroquinone forms **30**, **33** and **34** on 5-LO product formation in cell-free assays and in human leukocytes. (A) Purified 5-LO was incubated with compounds or vehicle (DMSO, 0.1%) at 4 °C for 15 min. Samples were pre-warmed for 30 s at 37 °C, 2 mM $CaCl_2$ and 20 µM AA were added, and after 10 min 5-LO product formation was determined by HPLC. (B) Human neutrophils (5×10^6) were pre-incubated with compounds or 0.1 % DMSO as vehicle for 15 min at 37 °C and stimulated with 2.5 µM A23187 and 20 µM AA for 10 min. 5-LO products were analyzed by HPLC. Data are expressed as percentage of control (100%), mean ± S.E.M., n = 3-4.

Fig. 4 Inhibition of 5-LO activity in a cell-free assay by **27** and **30** at varying concentrations of arachidonic acid (AA) as substrate. Purified 5-LO was incubated with compounds or vehicle (DMSO, 0.1%) at 4 °C for 15 min. Samples were pre-warmed for 30 s at 37 °C, 2 mM $CaCl_2$ and the indicated concentrations of AA were added, and after 10 min 5-LO product formation was determined by HPLC. Data are expressed as percentage of control (100%), mean ± S.E.M., n = 3.

Fig. 5 The structure of 5-LO. The balls represent the cavities, as detected by Pocketfinder. The enlarged section marked in blue depicts the cavity located between the membrane binding domain and the catalytic domain of the enzyme. This is the cavity that was used in the docking simulation.

Fig. 6 Outline of the 5-LO binding site indicating amino acids typically observed forming hydrogen bonds with the active compounds (A). Compound **30** fitted into the 5-LO binding site. Hydrogen bonds are shown as arrows, hydrophobic contacts as yellow spheres (B). Compounds **15** (C) and **23** (D) fitted into the binding site of 5-LO. For more clarity, only hydrogen bonds are shown. The binding pocket surface is colored by aggregated hydrophobicity (grey) / hydrophilicity (blue).

Fig. 7 Docked binding modes of compounds **27**, **30**, **33**, and **34**. All compounds are anchored via hydrogen bonds (arrows) at the binding site opening marked by Tyr383, Tyr81, Trp102 and Glu622 The alkyl chain of all four compounds fills the hydrophobic grove between the catalytic and the membrane binding domain.

Fig. 8 Effect of compounds **30** (1 mg/kg, i.p.) **27** (1 mg/kg, i.p.) on carrageenan-induced paw oedema after carrageenan injection. Data are expressed as mean ± SEM (n ¼ 6e8 for each group). **P* < 0.05; ****P* < 0.001 versus vehicle (two way ANOVA and Bonferroni post test).

Table 1. Effects of 4,5-dimethoxy-1,2-benzoquinones on 5-LO activity. Data are expressed as means ± S.E. of single determinations obtained in three to four independent experiments.

Table 2. Effects of 1,4-benzoquinones on 5-LO activity. Data are expressed as means ± S.E. of single determinations obtained in three to four independent experiments.

Table 3. Effects of hydroquinone derivatives on 5-LO activity. Data are expressed as means ± S.E. of single determinations obtained in three to four independent experiments.

Scheme 1. Reagents and conditions: a) TBDMSCl, Imidazole, DMAP, CH_2Cl_2, rt, o.n.; b) 1,2,4,5-tetramethoxybenzene, BuLi 2.5M in hexane, TMEDA, dry THF, from -20 °C to rt, o.n.; c) CAN, CH_3CN/H_2O 7:3, -10 °C, 10 min; d) THF, H_2O, HCOOH, 30 min, rt.

Scheme 2. Reagents and conditions: a) 1,2,4,5-tetramethoxybenzene, BuLi 2.5M in hexane, TMEDA, dry THF, from -40 °C to rt, o.n.; b) CAN, CH_3CN/H_2O 7:3, -10 °C, 10 min; c) acetic anhydride, TEA, DMAP, Et_2O, 2h, rt; d) CAN, CH_3CN/H_2O 7:3, -10 °C, 10 min.

Scheme 3. Reagents and conditions: a) $NaBH_4$, EtOH, 0 °C, 1h.

Scheme 4. Reagents and conditions: a) $NaBH_4$, $ET_2O/THF/H_2O$, R.T, 2h.

Highlights

Molecular modification and optimitation process aiming to improve 5-LO inhibition. Our study described a new series of quinone compounds designed as candidate for antinflammatory therapy.