"Novel 1,4-benzoquinones derivatives, able to inhibit the enzyme 5-lipoxygenase"

Research activity that I have carried out thanks to STRAIN project, involved the synthesis and biological characterization of novel 1,4-benzoquinones derivatives, able to inhibit the enzyme 5-lipoxygenase known by the acronym 5-LO.

Leukotrienes (LTs) are bioactive lipid mediators produced from arachidonic acid (AA) with pivotal roles in immune reactions, inflammation, and allergy. 5-Lipoxygenase (5-LO), a nonheme iron-containing dioxygenase, is the key enzyme in the biosynthesis of LTs that catalyzes the incorporation of molecular oxygen into AA yielding the intermediate 5(S)-hydroperoxyeicosatetraenoic acid (5-HPETE), and subsequently dehydrates 5-HPETE to LTA_4. The latter is either converted by LTA_4 hydrolase to LTB_4 or by LTC_4 synthases to the glutathione conjugate LTC_4 that is further degraded to LTD_4 and LTE_4. The biological effects that are evoked by LTs are mediated by different G protein-coupled receptors, specific for LTB_4 ($BLT_{1/2}$) or LTC_4, D_4 and E_4 ($cysLT_{1/2}$), as well as by additional receptors. While LTB_4 causes chemotaxis towards various types of leukocytes and activates phagocytes, LTC_4, D_4 and E_4 are potent bronchoconstrictors, cause mucus secretion in the lung, and increase vasopermeability of postcapillary venules. Accordingly, LTs are implicated as mediators in a variety of inflammatory and allergic diseases including asthma, allergic rhinitis,and autoimmune disorders, as well as cardiovascular diseases. Moreover, due to stimulation of survival, proliferation and migration of cancer cells by LTs, a role in cancer became apparent. Based on its key function in the initiation of LT-related diseases, 5-LO has long been considered as a promising target for therapeutic intervention with those disorders. In fact, numerous series of synthetic agents as well as natural products have been identified as 5-LO inhibitors but most of these compounds exhibited only limited potency, were rather unspecific for 5-LO with potential side effects, and/or lacked efficiency in vivo (O.Werz et al.*Expert Opin.Ther.Pat*. 2010). Moreover, the characterization of the cellular and molecular mechanism of 5-LO inhibition by the inhibitor was often neglected and detailed follow-up studies were not consequently performed or pursued. For example, the 1,4-benzoquinone **A** (2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone, AA-861, Fig. 1) was initially presented in 1982 as one of the first 5-LO inhibitors and many subsequent pharmacological studies confirmed the efficiency and anti-inflammatory efficacy in vivo, and **A** even succeeded in aclinical trial for prevention of seasonal allergic rhinitis. Nevertheless,the precise mechanisms of inhibition of 5-LO has remained elusive until recently.

In the course of previous biological investigations of simplified derivatives of the marine hydroxyquinone bolinaquinone with antitumour activity, compound **B** (2,5-dihydroxy-3-undecyl-1,4-benzoquinone, embelin, Figure. 1) was identified, a naturally occurring 1,4-benzoquinone from Embelia ribes, that dually inhibits 5-LO as well as microsomal prostaglandin E_2 synthase (mPGES)-1 but does not interfere with 12/15-LOs, cytosolic phospholipase (PL)A_2 or cyclooxygenase (COX) enzymes (R.Filosa et al. *Biochem.Pharmacol.* 2013). In a parallel study was synthesized and characterized a series of related 2,5-dihydroxylated 1,4-benzoquinones with various lipophilic and bulky alkyl- or aryl-substituents in 3-position (R.Filosa et al. *Eur.J.Med.Chem* 2015), exemplified by 3-((decahydronaphthalen-6-yl)methyl)-2,5-dihydroxycyclohexa-2,5-diene-1,4-dione (compound **C**, Figure. 1), as 5-LO inhibitors with anti-inflammatory activity in vivo. The simple structure and good potency of **B** and **C** with IC_{50} values in the submicromolar range stimulated us to systematically modify the structure of **B** and thus, to improve the inhibitory potential. Here I report the synthesis and the biological evaluation of 14 different 3-mono-alkyl-substituted 1,4-benzoquinones or 1,2-benzoquinones Table 1.

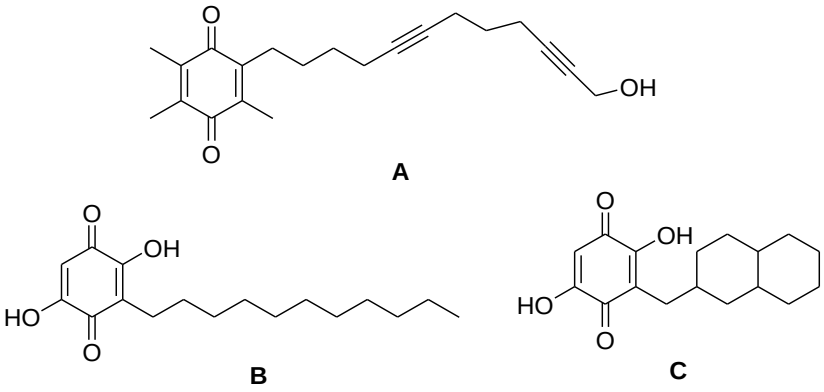
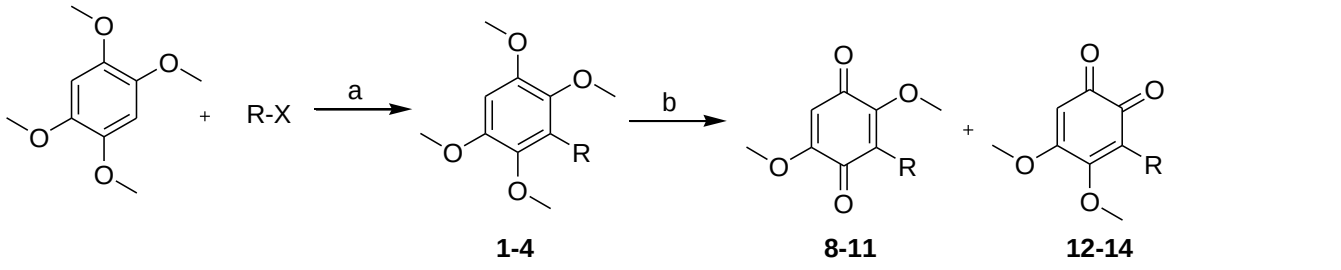


Figure. 1. Chemical structures of compound **A**, **B** and **C** that inhibit 5-LO.

The desired compounds were synthesized starting from 1,2,4,5-tetramethoxybenzene which was subjected to an orthoemetalation reaction in the presence of n-BuLi and hexamethylphosphoramide (HMPA) (Scheme 1). The lithium derivative was reacted with different alkyl halides giving intermediates 1-4 in good yields. Cerium ammonium nitrate (CAN)-mediated oxidative reaction provided a mixture of 2,5-dimethoxy-1,4-benzoquinones (8-11) and 4,5-dimethoxy-1,2-benzoquinones (12-14). To a solution of compounds 1-4 (1 mmol) in CH_3CN, was added a solution of CAN (2.5 mmol) in CH_3CN e H_2O (7:3) dropwise at 7° C (salt-ice bath). The reaction was allowed to stir at rt for 2 h, and diluted with ether. The organic layer was washed with distilled water, brine (20 ml), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified over silica gel using hexane:EtOAc (8:2 e 7:3) as eluent.



1: R=hexadecacyl
2: R=decyl
3: R=decanoyl
4: R=octanoyl

8: R=1,10-dihydroxydecyl
9: R=undecanoyl
10: R=octanoyl
11: R=10-hydroxydecyl acetate
12: R=decanoyl
13: R=undecanoyl
14 R=octanoyl

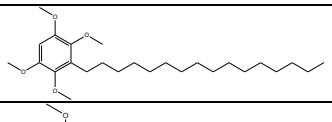
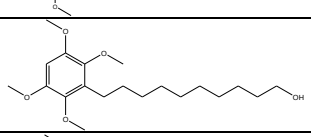
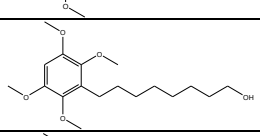
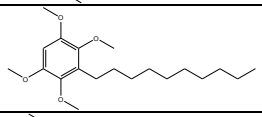
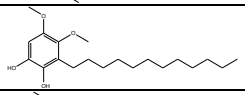
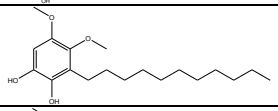
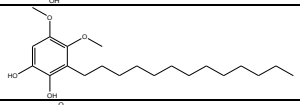
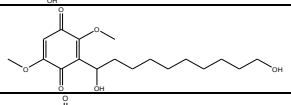
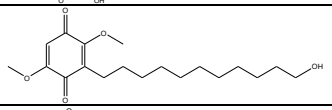
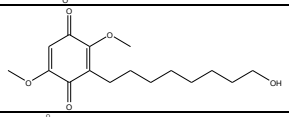
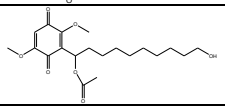
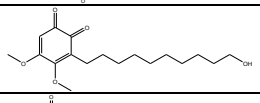
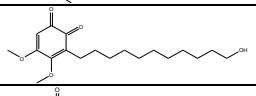
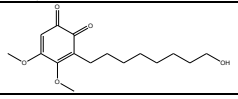
Scheme 1. Ortho-metalation of 1, 2, 4, 5-tetramethoxybenzene and synthesis of 2,5-dimethoxy- 1,4-benzoquinones and 4,5-dimethoxy-1,2-benzoquinones. Reagents and conditions: a) n-BuLi, HMPA, THF, from 40° C to room temperature, 12 h. b) CAN, CH_3CN:H_2O 7:3, -7 °C, 10 min.

Subsequently I conducted the biological evaluation of the compounds synthesized in the laboratories of Prof. Oliver Werz, of the 'Institute of Pharmacy, Friedrich-Schiller-University, Jena, Germany. To study the ability of the test compounds for direct inhibition of 5-LO, I applied a cell-free assay using purified human recombinant 5-LO enzyme. Aliquots of semi-purified 5-LO were diluted with ice-cold PBS containing EDTA, and ATP was added. Samples were pre-incubated with the test compounds or vehicle (0.1% DMSO). After 10 min at 4°C, samples were pre-warmed for 30s at 37 °C, and 2 mM $CaCl_2$ plus 20 mM AA was added to start 5-LO product formation. The reaction was stopped after 10 min at 37 °C by addition of ice-cold methanol, and the formed metabolites were analyzed by RP-HPLC. 5-LO products include the all-trans isomers of LTB_4 and 5(S)-hydro(pero)xy- 6-trans-8,11,14-cis-eicosatetraenoic acid.

To study the inhibitory potency on 5-LO product formation in intact cells, I used human PMNL that are a major source for LT biosynthesis. For determination of LO products in intact cells, PMNL ($5x10^6$) were resuspended in PGC buffer, preincubated for 15 min at 37 °C with test compounds or vehicle (0.1% DMSO), and incubated for 10 min at 37 °C with the indicated stimuli. Ca^{2+} ionophore A23187 was added with or without AA. 10 min later the reaction was stopped on ice by addition of methanol. A solution 1 N HCl and PBS, and 200 ng prostaglandin (PG)B_1 were added and the samples were subjected to solid phase extraction on C18-columns. 5-LO products (LTB_4, trans-isomers, 5-H(P)ETE), and the 12- and 15-LO products 12-HETE and 15-HETE, respectively, were analyzed by RP-HPLC and quantities calculated on the basis of the internal standard PGB1. Cys-$LTsC_4$, D_4 and E_4 were not detected, and oxidation products of LTB_4 were not determined.

The obtained results are reported in the Table 1.

Table 1.

NAME	STRUCTURE	**5-L0 activity; cell-free (n=2-3)**	**5-LO activity; cell-based (n=2-3)**	
			Ionophor + AA (n=3)	Ionophor (n=2-3)
		IC_{50} (µM)	IC_{50} (µM)	IC_{50} (µM)
1		95.59 ± 3.8[a]	108.52 ± 6.78[a]	118.62 ± 7.56[a]
2		95.6 ± 8.27[a]	1.653 ± 0.061	2.694 ± 0.044
3		7.065 ± 0.134	2.704 ± 0.037	3.206 ± 0.093
4		134.64 ± 9.5[a]	1.065 ± 0.047	2.0 ± 0.056
5		0.246 ± 0.036	0.093 ± 0.062	0.180 ± 0.026
6		0.107 ± 0.051	0.036 ± 0.043	0.105 ± 0.065
7		0.066 ± 0.063	0.028 ± 0.074	0.091 ± 0.055
8		1.461 ± 0.069	0.666 ± 0.069	1.859 ± 0.073
9		1.513± 0.07	0.206 ± 0.041	0.399 ± 0.044
10		1.842 ± 0.07	0.335 ± 0.051	1.091 ± 0.059
11		0.493 ± 0.057	1.628 ± 0.058	1.291 ± 0.068
12		1.078 ± 0.03	0.356 ± 0.026	2.289 ± 0.14
13		10.964 ± 0.104	0.124 ± 0.04	0.257 ± 0.043
14		0.217 ± 0.056	0.153 ± 0.036	0.297 ± 0.066

[a] Remaining activity at 10 µM (%)