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Short communication

2-Arylalkyl-substituted anthracenones as inhibitors of 12-lipoxygenase enzymes. 2. Structure–activity relationships of the linker chain

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Abstract

A series of 2-arylalkyl-substituted anthracenones were tested as inhibitors of three types of 12-lipoxygenase isoforms in epidermal homogenate of mice, bovine platelets and porcine leukocytes. Their inhibitory activities were compared with those to inhibit the 5-lipoxygenase enzyme in bovine leukocytes. The compounds were synthesised by Marschalk, Wittig or Horner–Emmons reaction at the anthracenedione stage and then reduced to the anthracenones. Structure–activity relationship for the chain linking the anthracenone nucleus and the phenyl ring terminus was investigated. The 2-phenylethyl analogues were among the most potent inhibitors, and 3,4-dimethoxy-substituted **10f** was identified as a selective inhibitor of the 12-LO enzymes over 5-LO. Selectivity for 12-LO isoforms was observed with an increase in the overall lipophilicity of the inhibitors. However, none of the linker chains of the 2-substituted anthracenones provided inhibitors that were able to discriminate between the 12-LO isoforms.

Keywords: anthracenone; 12(*S*)-HETE; lipophilicity; 5-lipoxygenase; 12-lipoxygenase

1. Introduction

12-Hydroxyeicosatetraenoic acid (12-HETE) is a hydroxylated lipid formed as a product of the 12-lipoxygenases, a class of dioxygenase enzymes containing a non-heme iron [1]. Lipoxygenases (LO) catalyze the stereospecific insertion of molecular oxygen into arachidonic acid, and the products formed are optically active (*S*)- or (*R*)-enantiomers of hydroperoxy fatty acids, which are further metabolized to hydroxy derivatives as end products [1]. According to their positional specificity with respect to arachidonic acid, mammalian LO are categorized into different groups predominantly expressed in hematopoietic cell types, as well as in epidermal cells [2].

The 5-LO pathway has been the major focus of study due to the pronounced pro-inflammatory role of leukotrienes and the approval of 5-LO inhibitors for the clinical treatment of asthma [3]. Although less well characterized, the 12-LO pathway may also play an important role in the progression of human diseases such as cancer [4] and psoriasis [5].

Three isoforms of 12-LO have been characterized. They are platelet-type (*p*12-LO), leukocyte-type (*l*12-LO) and the epidermal-type (*e*12-LO) 12-lipoxyenase [2,6,7]. The 12(*S*)-HETE enantiomer can arise from these isoforms [8–11]. In the mouse and in human skin also, a cDNA was shown to encode a 12(*R*)-lipoxygenase [12,13].

Even though many inhibitors of LO are known, very few with selectivity against 12-LO have been published

Abbreviations: 12(*S*)-HETE, 12(*S*)-hydroxyeicosatetraenoic acid; LO, lipoxygenase; SAR, structure–activity relationships.

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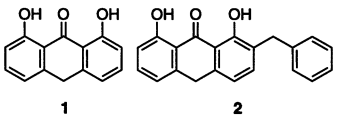


Fig. 1. Structures of anthralin (**1**) and a 2-phenylmethyl-substituted analogue (**2**).

[14]. A selective inhibitor of 12(*S*)-HETE biosynthesis would greatly aid in the evaluation of the role of this 12-LO metabolite in human disease. Such an agent might provide a new therapeutic approach to diseases such as psoriasis and cancer. During the course of our search for antipsoriatic agents, we have established 10-substituted derivatives of anthralin (**1**, Fig. 1) as inhibitors of 5-lipoxygenase [15–17]. Furthermore, a series of derivatives with lipophilic side chains at the 2-position was synthesized and found to selectively inhibit the 12-LO isoforms as opposed to 5-LO. Structure–activity relationships (SAR) were studied for these compounds to determine which structural elements were required for activity and to improve their selectivities. Our recent paper discussed SAR for modifications of the terminal aryl ring of 2-phenylmethyl-substituted anthracenone **2** [18]. This paper will discuss SAR involving structural modifications of the chain linking the anthracenone nucleus and the phenyl ring terminus.

2. Chemistry

The 2-phenylethyl analogues were prepared by one of the three synthetic routes shown in Fig. 2. In the first approach, Marschalk reaction [19] of 1-hydroxy-8-methoxy-9,10-anthracenedione (**3**) [20] with 4-chlorophenylacetaldehyde provided anthracenedione **4b**. In the second approach, the bromide **5** [21] was converted into the phosphonium bromide (**6**) with triphenylphosphine in *n*-butyl acetate, in analogy to a literature method [22]. In a Wittig-type reaction, the yield of **6**, which was formed in the presence of sodium hydride, reacted with appropriate benzaldehydes to yield the olefins **7c** and **7f**–**7h**. In the third approach, olefins **7d** and **7e** were obtained from the Horner–Emmons reaction [23] of carbaldehyde **8** [24] and diethyl benzylphosphonates in the presence of sodium *tert*-butoxide. Olefins **7c**–**7h** were catalytically hydrogenated to provide the 2-phenylethyl-substituted anthracenediones **9c**, **9d**, **9f**–**9i**, in the course of which 3,4-bis-benzyloxy analogue **7e** was hydrogenolytically cleaved to the catechol analogue **9i**. Subsequent reduction of the anthracenediones to the desired anthracenones **10b**–**10d** and **10f**–**10i** with $SnCl_2$–HCl proceeded with concomitant ether cleavage of the methoxy groups at position 1 and 8 of the anthracenedione nucleus. This was selective for those compounds having an additional methoxy group in the terminal phenyl ring of the 2-substituent

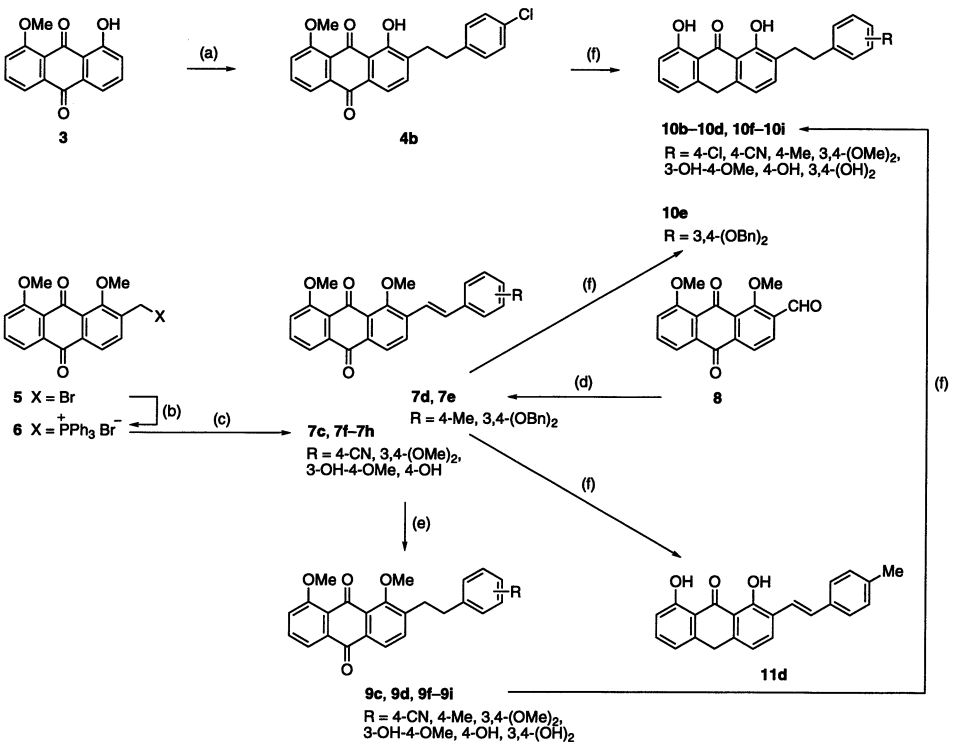


Fig. 2. Reagents: (a) $Na_2S_2O_4$, NaOH, 4-chlorophenylacetaldehyde, N_2, 90 °C; (b) PPh_3, *n*-BuOAc, 100 °C; (c) NaH, DMSO, *R*-PhCHO, N_2, 60 °C; (d) *R*-BnPO(OEt)$_2$, DMF, Me_3COK, N_2, 0 °C; (e) H_2, Pd–C, EtOH, RT, (f) $SnCl_2$, HCl, glacial HOAc, 118 °C.

(**10f**, **10g**). Anthracenone **10e** was directly obtained from anthracenedione **7e** under identical conditions.

Unfortunately, reduction of anthracenediones having olefin linker chains (**7c**–**7e**) to the corresponding anthracenones under the conditions described failed. Also, other methods such as metal hydride reagents, Clemmensen (zinc amalgam in HCl) or Wolff–Kishner (hydrazine and KOH) reduction were not successful. In no case could the desired structures with the stilbene skeleton be isolated, but large amounts of **1**, derived by cleavage of the 2-phenylethenyl chain, together with the 2-phenylethyl-substituted anthracenones were observed. The exception was 2-phenylethenyl analogue **11d** with a 4-methyl group. The ^{1}H-NMR of this compound showed the characteristic coupling pattern of an ethenyl spacer, and coupling constants of the olefinic protons ($J = 16.5$ Hz) indicated compound **11d** was in the *E*-configuration.

Other 2-phenylalkyl (**10a**, **12a**–**12e**, **13**) analogues and the 2-benzoyl (**14a**–**14d**) analogues were available from previous work [25,26].

3. Lipoxygenase inhibition

Although the products of 5-LO have attracted much attention and the development of 5-LO inhibitors has been extensively described [3,27], not many inhibitors of 12-LO are available. However, specific inhibitors of 12-LO would be useful tools for characterising the different physiological roles of the two enzymes. Particularly, their metabolites have been suspected to play an important role in the pathogenesis of psoriasis, because a number of phenomena observed in this skin disease can be explained, at least in part, by the action of leukotrienes and 12-HETE [5]. Therefore, we have evaluated the effects of the 2-substituted anthracenones on 12-LO activity in epidermal homogenate of mice [28]. Further, we also evaluated their inhibitory action against 12-LO isoenzymes in bovine platelets and porcine leukocytes as well as their ability to inhibit 5-LO in bovine leukocytes, in order to investigate a SAR and to explore their selectivity.

4. Results and discussion

The IC_{50} values for the inhibitory actions of the novel anthracenones against LO enzymes, together with their structures, are listed in Table 1. The general LO inhibitor nordihydroguaiaretic acid (NDGA) was used as a positive control and this compound appeared to be a more selective inhibitor of 5-LO. However, it should be noted that the IC_{50} values against the 12-LO enzymes reported here are not as low as those reported in other studies [29,30], although the values are not strictly comparable because of differences in the assay conditions. In particular, IC_{50} values for LO inhibition are dependent on cell density [31] and may thus vary with the assay performed.

The parent anthracenone anthralin (**1**) was only a moderate inhibitor of 5-LO in bovine leukocytes and an equal potent inhibitor of the 12-LO isoforms, whereas the phenylmethyl analogue **2** was a selective inhibitor of *p*12-LO. In the following, SAR of compounds with connecting chains other than methyl were explored. The higher homologue of **2**, phenylethyl analogue **10a**, was a somewhat more potent inhibitor, but exhibited activity against all 12-LO isoforms. Expanding the phenylethyl to a phenylpropyl chain (**12a**) provided a non-selective LO inhibitor, whereas phenylbutyl analogue **13** was a moderate inhibitor with selectivity for the 12-LO enzymes.

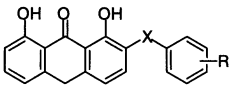
Introduction of a phenolic group (**10g**, **10h**, **12d**) resulted in activity against 5-LO and therefore produced non-selective inhibitors. The 5-LO inhibitory potency was greatly enhanced by the addition of further hydroxyl groups (**10i**, **12e**). Although catechol analogue **10i** and pyrogallol analogue **12e** were also among the most potent inhibitors of the 12-LO enzymes, these agents would be poor choices as potentially selective inhibitors.

Exchanging the methyl linker of **2** with a carbonyl linker, as in **14a**, was detrimental for selectivity. This was due to a strong decrease in lipophilicity. Even though the benzonitrile **14b** and the benzoic acid **14c** were devoid of phenolic groups in the terminal phenyl ring, they also inhibited the 5-LO enzyme, as these analogues exhibited the lowest $\log P$ values of the compounds assayed in this study. In general, selectivity for 12-LO inhibition was largely dependent on the overall lipophilicity of the compounds, as already observed with arylmethyl analogues [18]. Except for the phenylpropyl analogues, inhibitors having a $\log P$ value of higher than 5 were not active against 5-LO.

Among the phenylethyl analogues (**10a**–**10i**) with a substituted phenyl ring, **10f**, which has a 3,4-dimethoxy substitution pattern, was the most selective inhibitor of the 12-LO enzymes as opposed to 5-LO. Unfortunately, **10f** did not exhibit specificity for a single 12-LO isoform. Exchanging merely one of the methoxy groups of **10f** with a hydroxyl group (**10g**) destroyed selectivity.

The phenylethyl analogues are flexible structures, which can interact with all 12-LO isoforms. However, specific 12-LO inhibitors would be useful tools for differentiating the physiological roles of the 12-LO isoenzymes in disease states such as psoriasis and cancer. To develop an isoform-selective inhibitor of the anthracenone class, the conformational mobility of

Table 1
Inhibition of mouse epidermal 12-LO, bovine platelet 12-LO, porcine leukocyte 12-LO, and bovine leukocyte 5-LO by 1,8-dihydroxy-2-phenylalkyl-9(10*H*)-anthracenones.



cpd	X	R	log *P* [a]	*e*12-LO [b] IC_{50} (μM)	*p*12-LO [c] IC_{50} (μM)	*l*12-LO [d] IC_{50} (μM)	5-LO [e] IC_{50} (μM)
2	CH_2	H	5.62	>30	13	>30	>30
10a	$(CH_2)_2$	H	6.23	7	7	9	>30
10b	$(CH_2)_2$	4-Cl	6.35	23	14	11	>30
10c	$(CH_2)_2$	4-CN	5.20	18	7	24	>30
10d	$(CH_2)_2$	4-Me	6.52	16	12	21	>30
10e	$(CH_2)_2$	3,4-$(OBn)_2$	7.78	30	>30	>30	>30
10f	$(CH_2)_2$	3,4-$(OMe)_2$	5.36	6	6	5	>30
10g	$(CH_2)_2$	3-OH-4-OMe	4.86	6	6	4	6
10h	$(CH_2)_2$	4-OH	4.81	12	8	16	2
10i	$(CH_2)_2$	3,4-$(OH)_2$	4.46	5	4	5	0.7
11d	*E*-CH=CH	4-Me	8.16	>30	>30	>30	>30
12a	$(CH_2)_3$	H	6.44	12	10	13	11
12b	$(CH_2)_3$	4-OMe	6.46	14	4	14	>30
12c	$(CH_2)_3$	3,4,5-$(OMe)_3$	5.64	>30	9	7	6
12d	$(CH_2)_3$	4-OH	5.27	11	8	6	2
12e	$(CH_2)_3$	3,4,5-$(OH)_3$	3.96	7	5	4	0.6
13	$(CH_2)_4$	H	6.81	19	7	16	>30
14a	CO	H	4.47	16	6	18	18
14b	CO	4-CN	3.32	8	4	11	2
14c	CO	4-CO_2H	2.92	7	11	14	2
14d	CO	4-CO_2Me	4.20	30	27	>30	>30
Anthralin			4.23	9	9	9	37
NDGA [f]				21	12	24	0.5

[a] Experimentally determined partition coefficient.
[b] Inhibition of 12(*S*)-HETE biosynthesis in mouse epidermal homogenates.
[c] Inhibition of 12(*S*)-HETE biosynthesis in bovine platelets.
[d] Inhibition of 12(*S*)-HETE biosynthesis in porcine leukocytes.
[e] Inhibition of LTB_4 biosynthesis in bovine leukocytes. For each value of the LO assays, inhibition was significantly different with respect to that of the control ($n = 3$ or more, S.D. $\leq 10\%$, $P < 0.01$).
[f] Nordihydroguaiaretic acid (NDGA) was used as the standard inhibitor.

analogues such as **10d** was restricted by introduction of a trans double bond. Thus, stilbene derivative **11d** maintained the electronic character of **10d**, but introduced additional structural rigidity and steric barriers, interactions potentially either beneficial or detrimental to the inhibition of the 12-LO isoenzymes. None of the LO, including 5-LO, were inhibited by **11d**, which seemed to indicate that introduction of this structural modification was unfavourable, however. It is not clear at the moment if this is simply related to the stilbene structure of **11d** or if the high overall lipophilicity of this compound prevents activity. In support of this, with a log *P* value of 8.16 the lipophilicity of **11d** is comparable to that of the inactive **10e** (log $P = 7.78$). Further work directed toward successful synthesis of less lipophilic compounds will be required to elucidate the nature of the interactions of stilbenes with the 12-LO isoenzymes.

5. Conclusions

Among the 2-phenylethyl analogues, **10f** was identified as a selective inhibitor of the 12-LO enzymes as compared with 5-LO. In general, selectivity for 12-LO isoforms was observed for the more lipophilic inhibitors. However, none of the linker chains of the 2-substituted anthracenones provided inhibitors that were able to discriminate between the 12-LO isoforms.

6. Experimental protocols

6.1. Chemistry

6.1.1. General

For analytical instruments and methods, see reference [15].

6.1.2. General procedure for the preparation of 2-substituted anthracenediones by Marschalk alkylation

*6.1.2.1. 2-[2-(4-Chlorophenyl)ethyl]-1-hydroxy-8-methoxy-9,10-anthracenedione (**4b**).* To a solution of 1.5% NaOH in water (300 mL) was added **3** [20] (5.08 g, 20.0 mmol) and a solution of $Na_2S_2O_4$ (6.0 g, 34.5 mmol) in water (25 mL) at 40 °C under N_2. The solution was heated to 50 °C for 15 min. 4-Chlorophenylacetaldehyde [32] (15.5 g, 100 mmol) was added, dropwise over 30 min at 70 °C, and the reaction mixture was stirred for 2 h. It was stirred for an additional 12 h at 90 °C, then cooled to room temperature (r.t.), aerated for 30 min, and acidified with 6 N HCl. The orange suspension thus obtained was extracted with CH_2Cl_2 (3 × 200 mL), the organic phase was washed with water and dried over Na_2SO_4. The solvent was evaporated, and the residue was purified by column chromatography (SiO_2–CH_2Cl_2) to provide yellow crystals; 21% yield; melting point (m.p.) 135–136 °C; ^{1}H-NMR ($CDCl_3$) δ 13.42 (s, 1H), 7.92–7.24 (m, 9H), 3.99 (s, 3H), 2.95 (m, 4H); FTIR 3450 (OH), 1669 (CO), 1632 cm^{-1} (CO···OH). Anal. $C_{23}H_{17}ClO_4$ (C, H).

6.1.3. General procedure for the preparation of 2-substituted anthracenediones by Wittig reaction

*6.1.3.1. 2-(1,8-Dimethoxy-9,10-dioxo-2-anthracenyl)-methyl)triphenylphosphonium bromide (**6**).* To a solution of 2-bromomethyl-1,8-dimethoxy-9,10-anthracenedione [21] (**5**, 1.0 g, 2.76 mmol) in *n*-butylacetate (10 mL) at 100 °C was slowly added triphenylphosphine (1.25 g, 4.76 mmol). After 5 min, the reaction was cooled to r.t. and the precipitate filtered by suction. The crude product was dried and used in the subsequent Wittig reaction.

*6.1.3.2. 4-[2-(9,10-diHydro-1,8-dimethoxy-9,10-dioxo-2-anthracenyl)-(E)-ethenyl]benzonitrile (**7c**).* A suspension of NaH (0.1 g, 3.3 mmol) in DMSO (10 mL) was heated to 70 °C for 30 min under N_2 and then cooled. A solution of the phosphonium salt **6** (1.0 g, 1.9 mmol) in DMSO (10 mL) was added. After 10 min, a solution of 4-cyanobenzaldehyde (0.25 g, 1.9 mmol) in DMSO (10 mL) was added and the reaction mixture heated at 60 °C for 1 h. The cooled mixture was extracted with CH_2Cl_2 (3 × 100 mL), the organic phase was washed with a saturated solution of NaCl, then with water, and dried over Na_2SO_4. The solvent was evaporated, and the residue was purified by column chromatography (SiO_2; CH_2Cl_2–EtOAc, 95/5) to provide orange crystals; 45% yield; m.p. 255–256 °C; ^{1}H-NMR ($CDCl_3$) δ 8.00–6.75 (m, 11H), 4.00 (s, 6H); FTIR 2220 (CN), 1676 (CO) cm^{-1}. Anal. $C_{25}H_{17}NO_4$ (C, H, N).

*6.1.3.3. 1,8-diMethoxy-2-(E)-[2-(3,4-dimethoxyphenyl)-ethenyl]-9,10-anthracenedione (**7f**).* The title compound was obtained from **6** and 3,4-dimethoxybenzaldehyde as described for **7c** and chromatographed (CH_2Cl_2–Et_2O, 90/10) to provide orange–yellow crystals; 42% yield; m.p. 181–182 °C; ^{1}H-NMR ($CDCl_3$) δ 7.95–6.80 (m, 10H), 4.00 (s, 6H), 3.92 (s, 3H), 3.88 (s, 3H); FTIR 1680 (CO) cm^{-1}. Anal. $C_{26}H_{22}O_6$ (C, H).

*6.1.3.4. 2-(E)-[2-(3-Hydroxy-4-methoxyphenyl)ethenyl]-1,8-dimethoxy-9,10-anthracenedione (**7g**).* The title compound was obtained from **6** and 3-hydroxy-4-methoxybenzaldehyde as described for **7c** and chromatographed (CH_2Cl_2–Et_2O, 90/10) to provide orange crystals; 40% yield; m.p. 201–202 °C; ^{1}H-NMR ($CDCl_3$) δ 8.05–6.90 (m, 10H), 5.81 (s, 1H), 4.10 (s, 6H), 4.00 (s, 3H); FTIR 1667 (CO) cm^{-1}. Anal. $C_{25}H_{20}O_6$ (C, H).

*6.1.3.5. 2-(E)-[2-(4-Hydroxyphenyl)ethenyl]-1,8-dimethoxy-9,10-anthracenedione (**7h**).* The title compound was obtained from **6** and 4-hydroxybenzaldehyde as described for **7c** and chromatographed (CH_2Cl_2) to provide red crystals; 35% yield; m.p. 247–248 °C; ^{1}H-NMR ($CDCl_3$) δ 9.42 (s, 1H), 8.00–6.80 (m, 11H), 4.05 (s, 3H), 4.00 (s, 3H); FTIR 3384 (OH), 1663 (CO) cm^{-1}. Anal. $C_{24}H_{18}O_5$ (C, H).

6.1.4. diEthyl 4-methylbenzylphosphonate

Freshly distilled triethyl phosphite (19.9 g, 120 mmol) was added to 4-methylbenzylbromide (13.0 g, 70 mmol), and the mixture was heated at 200 °C for 1 h. Excess triethyl phosphite was removed at 60 °C in vacuo to yield a colourless oil; 82% yield; m.p. 247–248 °C; ^{1}H-NMR ($CDCl_3$) δ 7.27–7.09 (m, 4H), 4.01 (quint, $J = 7$ Hz, 4H), 3.11 (d, $J_{CHP} = 21$ Hz, 2H), 2.32 (s, 3H), 1.25 (m, $J = 7$ Hz, 6H).

The crude product was used in the subsequent Horner–Emmons reaction.

6.1.5. General procedure for the preparation of 2-substituted anthracenediones by Horner–Emmons reaction

*6.1.5.1. 1,8-diMethoxy-2-(E)-[2-(4-methylphenyl)-ethenyl]-9,10-anthracenedione (**7d**).* To a well-stirred solution of diethyl 4-methylbenzylphosphonate (0.242 g, 1.0 mmol) in absol. DMF (20 mL) was added to sodium *tert*-butoxide (0.11 g, 1.0 mmol) at 25 °C under N_2. The mixture was added dropwise to a solution of the carbaldehyde **8** [24] (0.30 g, 1.0 mmol) in DMF (10 mL), cooled to 0 °C. After 15 min, the product was precipitated with water–methanol (2/1, 20 mL) and filtered by suction. The residue was washed with water and dried. Purification by chromatography (SiO_2–CH_2Cl_2) provided yellow crystals; 34% yield; m.p. 181–183 °C; ^{1}H-NMR ($CDCl_3$) δ 8.05–7.20 (m, 9H), 7.55 (d, $J = 16$ Hz, 1H), 7.30 (d, $J = 16$ Hz, 1H), 4.04 (s, 3H), 4.01 (s, 3H), 2.39 (s, 3H); FTIR 1667 (CO) cm^{-1}; MS $m/z = 384$ (100, $[M]^+$). Anal. $C_{25}H_{20}O_4$ (C, H).

*6.1.5.2. 2-(E)-[2-(3,4-diBenzyloxyphenyl)ethenyl]-1,8-dimethoxy-9,10-anthracenedione (**7e**).* The title compound was obtained from **8** and diethyl 3,4-dibenzyloxybenzylphosphonate [33] as described for **7d** to provide yellow crystals; 56% yield; m.p. 149–152 °C; ^{1}H-NMR ($CDCl_3$) δ 8.02–6.93 (m, 20H), 5.24 (s, 2H), 5.21 (s, 2H), 4.03 (s, 3H), 3.97 (s, 3H); FTIR 1661 (CO) cm^{-1}; MS $m/z = 582$ (0.34, $[M]^+$), 91 (100). Anal. $C_{38}H_{30}O_6$ (C, H).

6.1.6. General procedure for hydrogenation

*6.1.6.1. 2-[2-(3,4-Dihydroxyphenyl)ethyl]-1,8-dimethoxy-9,10-anthracenedione (**9i**).* To a suspension of 5% Pd–C (500 mg) in absol. EtOH (35 mL), saturated with H_2, was added **7e** (1.17 g, 2.0 mmol). The mixture was hydrogenated at r.t. and atmospheric pressure, until the requisite amount of H_2 was taken up (12 h, TLC control). Then the catalyst was removed by filtration and the solvent evaporated in vacuo. Purification by chromatography (SiO_2; CH_2Cl_2–MeOH, 50/50) provided red–brown crystals; 55% yield; m.p. 212–214 °C; ^{1}H-NMR ($CDCl_3$) δ 7.86–6.62 (m, 8H), 5.49 (s, 1H), 5.30 (s, 1H), 4.03 (s, 3H), 3.97 (s, 3H), 2.90 (m, 4H); FTIR 1655 (CO) cm^{-1}. Anal. $C_{24}H_{20}O_6$ (C, H).

Analogously, compounds **7c**, **7d**, **7f**–**h** were hydrogenated. The crude products were used in the subsequent reduction step.

6.1.7. General procedure for the reduction of 9,10-anthracenediones to 9(10H)-anthracenones [34]

*6.1.7.1. 2-[2-(4-Chlorophenyl)ethyl]-1,8-dihydroxy-9(10H)-anthracenone (**10b**).* To a solution of **4b** (0.40 g, 1.0 mmol) in glacial HOAc (20 mL) heated to reflux was added, dropwise over 3 h, a solution of 40% $SnCl_2$ in 37% HCl (10 mL). The solution was then cooled, and the resulting crystals were collected by filtration. Purification by chromatography (SiO_2–CH_2Cl_2) provided yellow crystals; 44% yield; m.p. 156–158 °C; ^{1}H-NMR ($CDCl_3$) δ 12.39 (s, 1H), 12.09 (s, 1H), 7.55–6.75 (m, 9H), 4.28 (s, 2H), 2.95 (m, 4H); FTIR 3442 (OH), 1618 cm^{-1} (CO···OH); MS $m/z = 364$ (12, $[M]^+$), 239 (100). Anal. $C_{22}H_{17}ClO_3$ (C, H).

*6.1.7.2. 4-[2-(9,10-Dihydro-1,8-dihydroxy-9-oxo-2-anthracenyl)ethyl]benzonitrile (**10c**).* The title compound was obtained from hydrogenation of **7c** as described for **9i** and subsequent reduction as described for **10b** to provide yellow crystals; 41% yield; m.p. 221–222 °C; ^{1}H-NMR ($CDCl_3$) δ 12.65 (s, 1H), 12.38 (s, 1H), 7.60–6.80 (m, 9H), 4.30 (s, 2H), 3.00 (m, 4H); FTIR 2226 (CN), 1618 cm^{-1} (CO···OH); MS $m/z = 355$ (21, $[M]^+$), 239 (100). Anal. $C_{23}H_{17}NO_3$ (C, H, N).

*6.1.7.3. 1,8-diHydroxy-2-[2-(4-methylphenyl)ethyl]-9(10H)-anthracenone (**10d**).* The title compound was directly obtained from **7d** as described for **10b** to provide yellow crystals; 52% yield; m.p. 126–128 °C; ^{1}H-NMR ($CDCl_3$) δ 12.64 (s, 1H), 12.28 (s, 1H), 8.05–7.20 (m, 9H), 4.32 (s, 2H), 2.93 (m, 4H), 2.37 (s, 3H); FTIR 3436 (OH), 1619 cm^{-1} (CO···OH); MS $m/z = 344$ (19, $[M]^+$), 239 (100). Anal. $C_{23}H_{20}O_3$ (C, H).

*6.1.7.4. 2-[2-(3,4-diBenzyloxyphenyl)ethyl]-1,8-dihydroxy-9(10H)-anthracenone (**10e**).* The title compound was directly obtained from **7e** as described for **10b** to provide yellow crystals; 39% yield; m.p. 141–144 °C; ^{1}H-NMR ($CDCl_3$) δ 12.64 (s, 1H), 12.35 (s, 1H), 8.02–6.93 (m, 18H), 5.24 (s, 2H), 5.17 (s, 2H), 4.31 (s, 2H), 2.93 (m, 4H); FTIR 3440 (OH), 1618 cm^{-1} (CO···OH). Anal. $C_{36}H_{30}O_5$ (C, H).

*6.1.7.5. 1,8-diHydroxy-2-[2-(3,4-dimethoxyphenyl)-ethyl]-9(10H)-anthracenone (**10f**).* The title compound was obtained from hydrogenation of **7f** as described for **9i** and subsequent reduction as described for **10b**. Purification by chromatography (CH_2Cl_2–Et_2O, 90/10) provided yellow crystals; 28% yield; m.p. 156–157 °C; ^{1}H-NMR ($CDCl_3$) δ 12.39 (s, 1H), 12.09 (s, 1H), 7.55–6.75 (m, 8H), 4.28 (s, 2H), 3.89 (s, 6H), 2.95 (m, 4H); FTIR 1618 cm^{-1} (CO···OH); MS $m/z = 390$ (35, $[M]^+$), 151 (100). Anal. $C_{24}H_{22}O_5$ (C, H).

*6.1.7.6. 1,8-diHydroxy-2-[2-(3-hydroxy-4-methoxyphenyl)ethyl]-9(10H)-anthracenone (**10g**).* The title compound was obtained from hydrogenation of **7g** as described for **9i** and subsequent reduction as described for **10b**. Purification by chromatography (CH_2Cl_2–Et_2O, 95/5) provided yellow crystals; 36% yield; m.p. 177–178 °C; ^{1}H-NMR ($CDCl_3$) δ 12.50 (s, 1H), 12.18 (s, 1H), 8.35 (s, 1H), 7.50–6.65 (m, 8H), 4.32 (s, 2H), 3.78 (s, 3H), 2.95 (m, 4H); FTIR 3382 (OH), 1611 cm^{-1} (CO···OH); MS $m/z = 376$ (29, $[M]^+$), 137 (100). Anal. $C_{23}H_{20}O_5$ (C, H).

*6.1.7.7. 1,8-diHydroxy-2-[2-(4-hydroxyphenyl)ethyl]-9(10H)-anthracenone (**10h**).* The title compound was obtained from hydrogenation of **7h** as described for **9i** and subsequent reduction as described for **10b**. Purification by chromatography (CH_2Cl_2–Et_2O, 90/10) provided orange–yellow crystals; 31% yield; m.p. 156–157 °C; ^{1}H-NMR ($CDCl_3$) δ 12.49 (s, 1H), 12.16 (s, 1H), 8.85 (s, 1H), 7.48–6.58 (m, 9H), 4.25 (s, 2H), 2.95 (m, 4H); FTIR 3314 (OH), 1622 cm^{-1} (CO···OH); MS $m/z = 346$ (29, $[M]^+$), 239 (100). Anal. $C_{22}H_{18}O_4$ (C, H).

*6.1.7.8. 1,8-diHydroxy-2-[2-(3,4-dihydroxyphenyl)-ethyl]-9(10H)-anthracenone (**10i**).* The title compound was obtained from **9i** as described for **10b** and

chromatographed (CH_2Cl_2–MeOH, 50/50) to provide brown–yellow crystals; 62% yield; m.p. 183–185 °C; ^{1}H-NMR ($CDCl_3$) δ 12.61 (s, 1H), 12.35 (s, 1H), 7.85–6.60 (m, 8H), 5.06 (s, 1H), 4.95 (s, 1H), 4.31 (s, 2H), 2.99–2.80 (m, 4H); FTIR 3342 (OH), 1616 cm^{-1} (CO···OH); MS $m/z = 362$ (24, $[M]^+$), 123 (100). Anal. $C_{22}H_{18}O_5$ (C, H).

*6.1.7.9. 1,8-diHydroxy-2-(E)-[2-(4-methylphenyl)-ethenyl]-9(10H)-anthracenone (**11d**).* The title compound was obtained from **7d**, together with **10d**, as described for **10b** to provide brown–yellow crystals; 24% yield; m.p. 170–174 °C; ^{1}H-NMR ($CDCl_3$) δ 12.91 (s, 1H), 12.29 (s, 1H), 7.83–6.90 (m, 9H), 7.49 (d, $J = 16.5$ Hz, 1H), 7.17 (d, $J = 16.5$ Hz, 1H), 4.37 (s, 2H), 2.32 (s, 3H); FTIR 3450 (OH), 1615 cm^{-1} (CO···OH); MS $m/z = 342$ (23, $[M]^+$), 239 (100), 117 (1.35), 115 (6), 105 (43). Anal. $C_{23}H_{18}O_3$ (C, H).

Anthracenones **2**, **10a**, **12a**–**e**, **13**, **14a** [25] and **14b**–**d** [26] were prepared as described.

6.2. Biological assay methods

Epidermal homogenates from NMRI mice were prepared as described [28], platelets were obtained from sodium EDTA-anticoagulated bovine blood and were suspended at a concentration of 4×10^7 cells per mL, porcine leukocytes were prepared from porcine blood in a similar fashion as described [35] and were suspended at a concentration of 1×10^6 cells per mL. The 12-LO [28] and the 5-LO [35] assays were performed essentially as described previously in full detail. 12(*S*)-HETE was analysed by chiral phase chromatography as described [28]. Inhibition was calculated by the comparison of the mean values of test compound ($n = 3$) with control ($n = 6$–8) activity: (one-test compound/control) $\times$ 100; S.D. $\leq$ 10%. Inhibition was statistically significant compared with that of the control (Student's *t*-test; $P < 0.01$). Each IC_{50} value was derived by interpolation of a log inhibitor concentration versus response plot using four or more concentrations of the compound, spanning the 50% inhibition point.

6.3. Lipophilicity

Determination of log *P* values was performed by a standard reversed-phase HPLC procedure as described [35].

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