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Original article

Syntheses and evaluation of the antioxidant activity of novel methoxypsoralen derivatives

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ABSTRACT

A series of 5- and 8-methoxypsoralen (MOP) analogs, suitable for structure-antioxidative/anti-inflammatory activity relationship studies, were synthesized using as key-reactions the selective monobromination of MOPs with *N*-bromosaccharin and either a Heck reaction or a Suzuki coupling or a Suzuki coupling followed by a Wittig reaction to install side-chains of the acrylate- or benzoate- or cinnamate-type, respectively. The 8-MOP analogs **19** and **24**, incorporating at position 5 of the psoralen nucleus a butyl acrylate or a *tert*-butyl cinnamate moiety, were the most powerful inhibitors of soybean LOX and inhibited effectively lipid peroxidation. Analog **19** was a more potent anti-inflammatory agent than the reference compound indomethacin and of comparable cytocompatibility to 8-MOP whereas analog **24** was a weaker inhibitor of inflammation than indomethacin and significantly more cytotoxic than 8-MOP. The results of the biological tests are discussed in terms of structural characteristics.

1. Introduction

Several natural products with a coumarinic moiety have been reported to have multiple biological activities [1]. Coumarins might affect the formation and scavenging of reactive oxygen species (ROS) and influence processes involving free radical-mediated injury. ROS, constantly generated in normal condition by aerobic metabolism, include free radicals. In normal biological systems, redox homeostasis is maintained by controlling the balance between ROS production and various types of scavengers called antioxidants. The persistent production of abnormally large amount of ROS, however, may lead to persistent changes in signal transduction and gene expression, which in turn may give rise to certain diseases. Psoralens belong to a family of linear furocoumarin derivatives that have various photobiological effects. Their main chemical characteristic is the presence of a furan ring fused to the coumarin basic structure. Natural isolated furocoumarins displayed potent antioxidant effects against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and against renal epithelial cell injury by using 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) to generate alkylperoxyl radicals *in vitro* [2]. It has been shown that UVA irradiation of psoralens in air generates a large amount of photoproducts, which are ROS [3]. It is well recognized that PUVA therapy is accompanied by toxic side effects; the role of ROS in PUVA-induced undesirable side effects has been postulated.

The synthetic 4,5′,8-trimethylpsoralen (trioxsalen, TRX, **1**) and the naturally occurring 8-methoxypsoralen (8-MOP, xanthotoxin, **11**) and 5-methoxypsoralen (5-MOP, bergapten, **25**) (Fig. 1) are commonly used in combination with UVA light radiation for the systemic treatment of skin hyperproliferative diseases like psoriasis [4]. We have recently reported the synthesis of a series of TRX (**1**)

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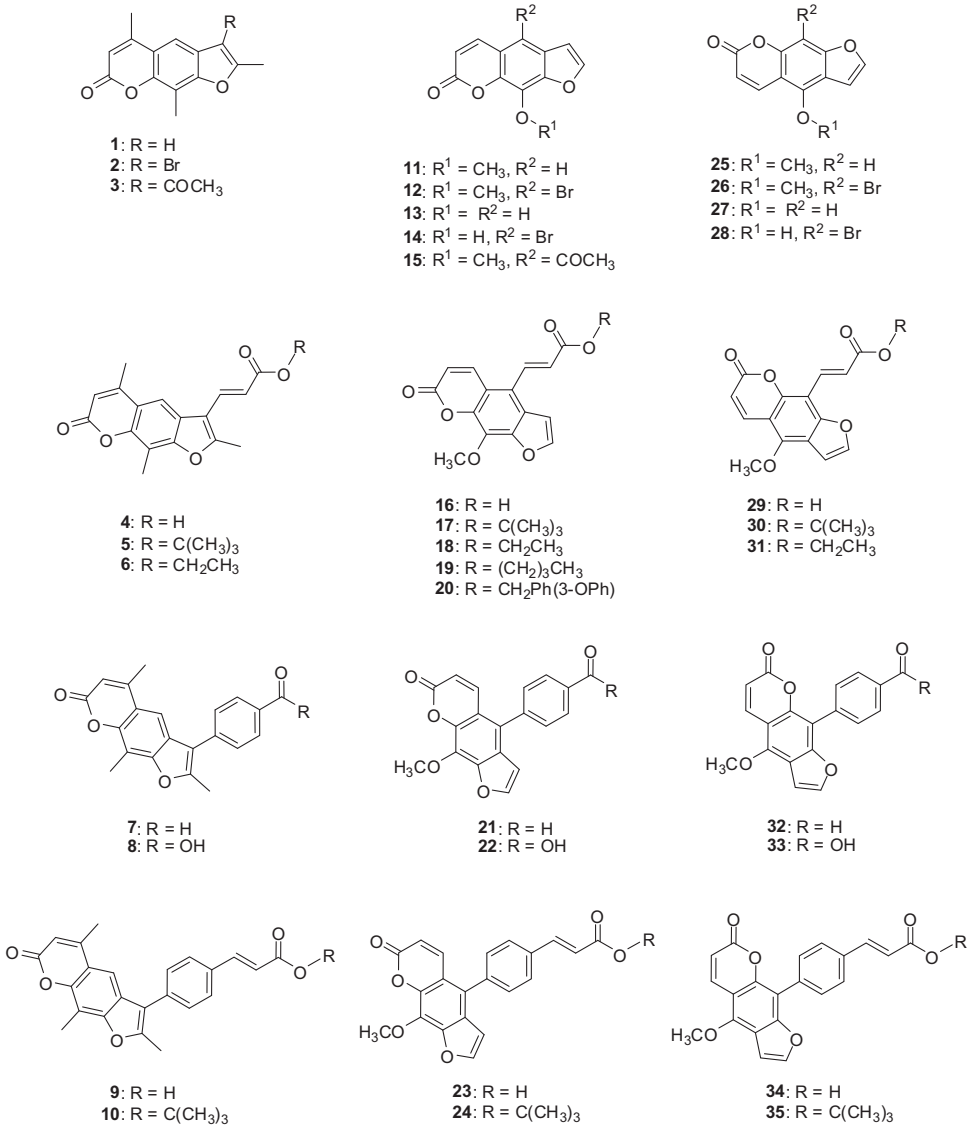


Fig. 1. Structures of compounds encountered in the present work.

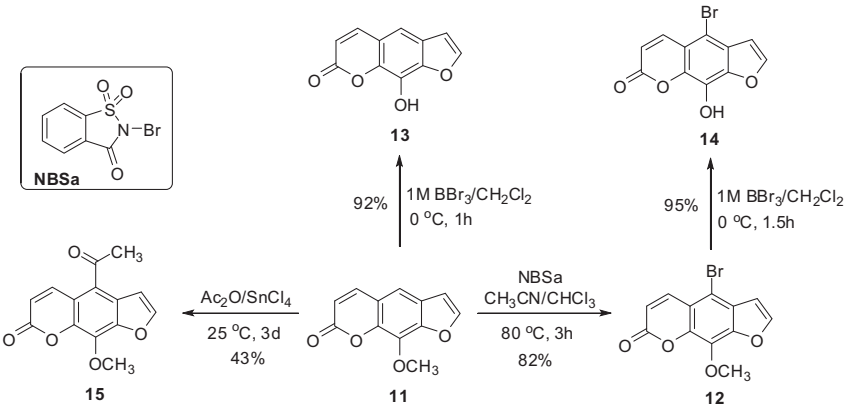
derivatives (see e.g. compounds **2**–**5** and **7**–**10**, Fig. 1) through its chemical modification [5,6] and evaluated their potential antioxidative and anti-inflammatory activities [6]. One of the synthesized compounds, namely *tert*-butyl ester **5**, was found to be a potent inhibitor of soybean LOX (IC_{50} 9.5 μM) exhibiting at the same time high anti-lipid peroxidation and anti-inflammatory activities.

We therefore considered of interest to synthesize the corresponding as well as other derivatives (see compounds **12**–**24** and **26**–**35** in Fig. 1) of the other two biologically active psoralens, that is 5- and 8-MOP, and compare their antioxidative and anti-inflammatory activities with those of the TRX derivatives, aiming at the development of more potent antioxidants/anti-inflammatory agents based on the psoralen nucleus. Through these compounds, we could hopefully determine the effect of the following structural elements on the biological activity: (a) the substituents on the psoralen nucleus, (b) the presence of a free hydroxyl function on the psoralen nucleus, (c) the presence of a free carboxyl group or an ester functionality in the side-chain (d) the nature of the alkyl group (steric bulk, lipophilicity) of the ester function in the side-chain, and (e) the nature of the side-chain.

2. Results and discussion

2.1. Chemistry

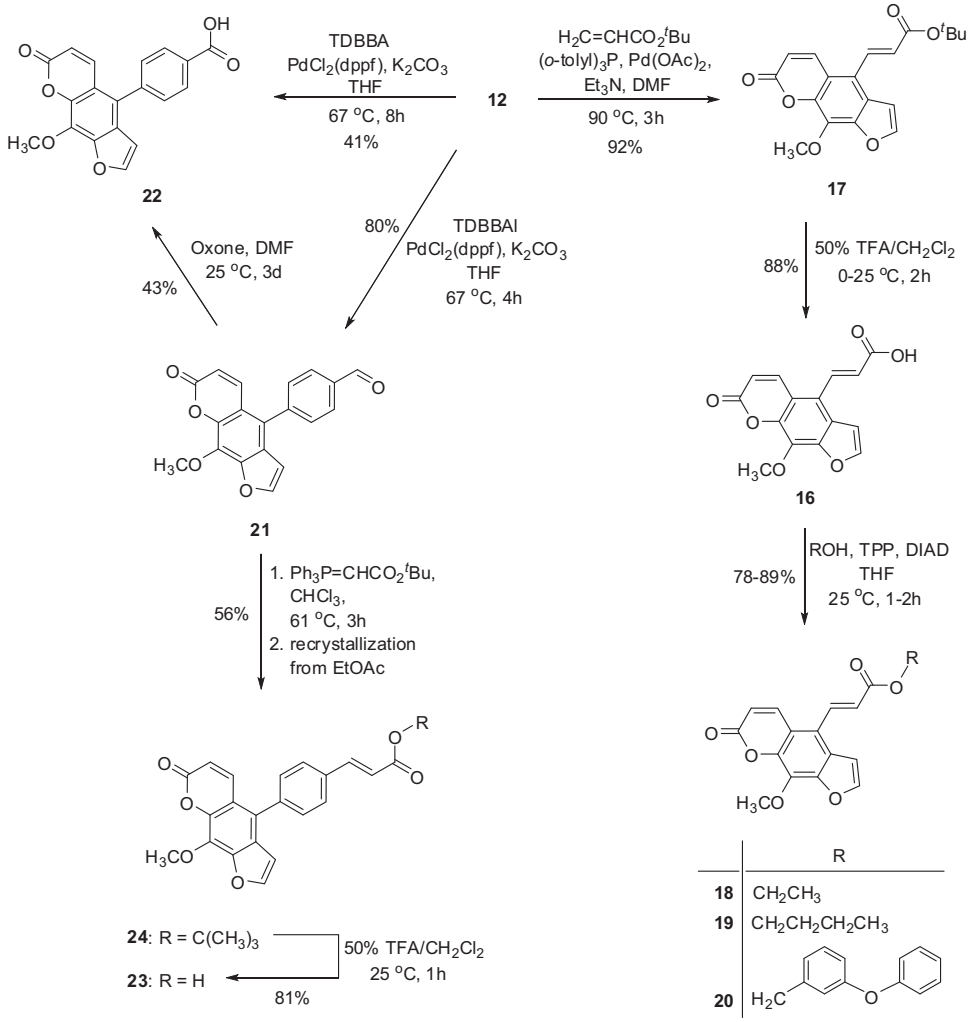
The synthesis of trioxsalen analogs **2**–**5** and **7**–**10** have been previously described [5]. The synthesis of 8-MOP analogs described herein started from the commercially available 8-MOP. Demethylation of 8-MOP was routinely effected by 1 M BBr_3 in CH_2Cl_2 to give 8-hydroxypsoralen (8-HOP, xanthotoxol, **13**) in 92% yield [7]. On the other hand, treatment of 8-MOP with acetic anhydride in the presence of $SnCl_4$ at room temperature (RT) for 3 d gave unexceptionally the 5-acetyl derivative **15** (Scheme 1) of 8-MOP in 43% yield. Direct bromination of 5- or 8-MOP with Br_2 or *N*-bromosuccinimide (NBS) has been described to lead to polybrominated derivatives [8]. Selective bromination at position 5 of 8-MOP has been also effected in a multi-step synthesis involving nitration, reduction, diazotation and finally treatment with CuBr to give bromide **12** in 33% total yield [9]. We decided to investigate the use of the mild brominating agent *N*-bromosaccharin (NBSa), known to brominate electron-rich aromatic systems at the *p* position [10], to effect the direct selective bromination of MOPs. Indeed, treatment of 8-MOP with NBSa in $CH_3CN/CHCl_3$ at 80 °C for 3 h provided the



Scheme 1.

desired 5-bromo-8-MOP (**12**) in 82% yield. BBr_3-mediated demethylation of bromide **12**, as for 8-MOP, provided the corresponding hydroxy analog **14** in 95% yield.

Attachment of the acrylate unit on the 8-MOP nucleus by a Heck reaction between the bromide **12** and *tert*-butyl acrylate was mediated by $Pd(OAc)_2$ and (*o*-tolyl)$_3$P in the presence of triethylamine (TEA) in DMF at 90 °C for 3 h. Following routine flash column chromatography (FCC), the anticipated ester **17** (Scheme 2) was obtained in 92% yield. The *tert*-butyl group was routinely removed by 50% trifluoroacetic acid (TFA) in CH_2Cl_2 at 0–25 °C for 2 h to give the unsaturated acid **16** in 88% yield. From this acid, the corresponding esters **18**–**20** were obtained in 78–89% yield by



Scheme 2.

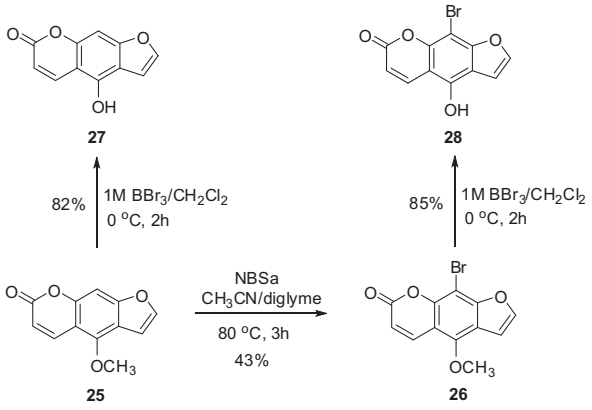
Mitsunobu esterification with ethanol, *n*-butanol and 3-phenoxyphenylmethanol in tetrahydrofuran (THF), in the presence of triphenylphosphine (TPP) and diisopropyl azodicarboxylate (DIAD) at room temperature (RT) for 1–2 h. Using identical reaction conditions, the ethyl ester **6** of the TRX-derived acid **4** was also obtained in 92% yield.

On the other hand, Suzuki coupling between bromide **12** and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (TDBBAl) in the presence of $PdCl_2$(dppf) and K_2CO_3 in THF at 67 °C for 4 h gave the anticipated aromatic aldehyde **21** in 80% yield. The latter could be oxidized to the corresponding acid **22** in 43% yield using Oxone® in DMF at RT for 3 d. The same acid could be also obtained by the direct Suzuki coupling of bromide **12** with 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid (TDBBA) in 41% yield. Finally, Wittig reaction of the thus obtained aldehyde **21** with the stabilized ylide $Ph_3P{=}CO_2^tBu$ in $CHCl_3$, at 61 °C for 3 h, gave the *trans tert*-butyl ester **24** in 56% yield, following recrystallization from ethyl acetate. From this ester, acid **23** was obtained in 81% yield by routine TFA-mediated deprotection (Scheme 2).

Similarly, the synthesis of 5-MOP analogs described herein started from the also commercially available 5-MOP. Demethylation of 5-MOP was also effected by 1 M BBr_3 in CH_2Cl_2 to give 5-hydroxypsoralen (5-HOP, bergaptol, **27**) in 82% yield [11]. On the other hand, treatment of 5-MOP with NBSa in CH_3CN/diglyme at 80 °C for 3 h provided the desired 8-bromo-5-MOP (**26**) in 43% yield. BBr_3-mediated demethylation of bromide **26**, as for 5-MOP, provided the corresponding hydroxy analog **28** in 85% yield (Scheme 3). Attempted preparation of 8-acetyl-5-MOP under a variety of conditions was unsuccessful.

Attachment of the acrylate unit on the 5-MOP nucleus by a Heck reaction between the bromide **26** and *tert*-butyl acrylate was mediated by $Pd(OAc)_2$ and (*o*-tolyl)$_3$P in the presence of TEA in DMF at 90 °C for 12 h. Following routine FCC, the anticipated ester **30** (Scheme 4) was obtained in 51% yield. The *tert*-butyl group was routinely removed by 50% TFA in CH_2Cl_2 at 0–25 °C for 2 h to give the unsaturated acid **29** in 90% yield. From this acid, the corresponding ethyl ester **31** was obtained in 88% yield by Mitsunobu esterification with ethanol in the presence of TPP and DIAD at RT for 1.5 h.

On the other hand, Suzuki coupling between bromide **26** and TDBBAl in the presence of $PdCl_2$(dppf) and K_2CO_3 in THF at 67 °C for 4 h gave the anticipated aromatic aldehyde **32** in 60% yield. The latter could be oxidized to the corresponding acid **33** in 38% yield using Oxone® in DMF at 60 °C for 2 d. Finally, Wittig reaction of the thus obtained aldehyde **21** with the stabilized ylide $Ph_3P{=}CO_2^tBu$ in $CHCl_3$, at 61 °C for 3 h, gave the *trans tert*-butyl ester **35** in 64% yield, following recrystallization from ethyl acetate. From this ester, acid **34** was obtained in 82% yield by routine TFA-mediated deprotection (Scheme 4).



Scheme 3.

2.2. Biological evaluation

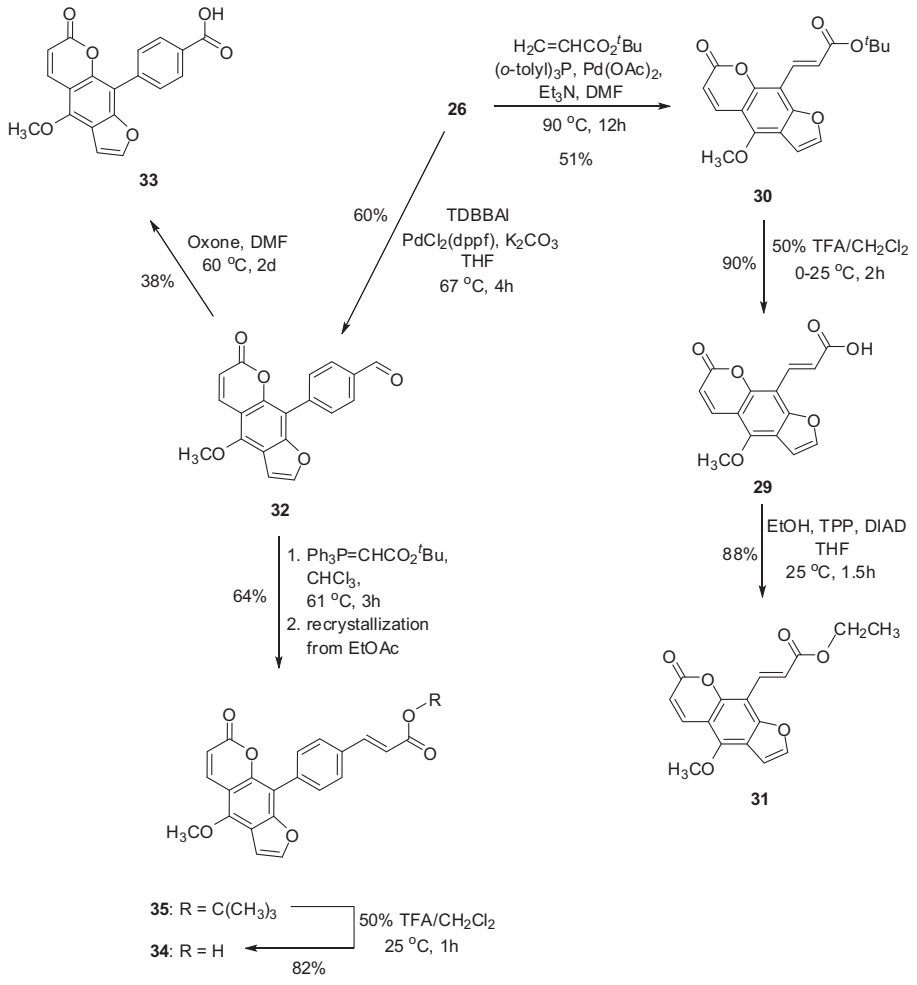
2.2.1. Antioxidant and anti-inflammatory activity – in vitro antioxidant activity studies

The antioxidant activity, the inhibitory activity on soybean lipoxygenase (LOX) and lipid peroxidation, as well as the anti-inflammatory activity *in vivo* on rat paw edema induced by carageenan, of a series of TRX derivatives including compounds **1**–**5** and **7**–**10** have been evaluated and reported recently [6]. Among these compounds, the *tert*-butyl ester **5** was identified as a very potent anti-LOX agent with potent anti-lipid peroxidation activity and anti-inflammatory potency almost identical to the reference compound, namely the non-steroidal anti-inflammatory drug (NSAID) indomethacin. It is well known that free radicals play an important role in the inflammatory process [12]. Many NSAIDs have been reported to act either as inhibitors of free radical production or as radical scavengers [13]. Consequently, compounds with antioxidant properties could be expected to offer protection in rheumatoid arthritis and inflammation and to lead to potentially effective drugs.

In the present investigation, the MOPs **11** and **25** as well as a series of analogs, including compounds **12**–**20**, **22**–**24**, **26**–**31** and **33**–**35**, were studied with regard to their antioxidant ability as well as to their ability to inhibit soybean LOX, in comparison to well known antioxidant agents, e.g. the nordihydroguaiaretic acid (NDGA) and the 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), in the anticipation of identifying more potent antioxidants. For the sake of comparison the ethyl ester **6**, an analog of TRX, was also synthesized. The design of the synthesized compound was made in such a way in order to also identify meaningful structure–biological activity relationships. Taking into account the multifactorial character of oxidative stress and inflammation, we decided to evaluate the *in vitro* antioxidant activity of the synthesized molecules using two different antioxidant assays: a) interaction with the stable free radical DPPH, b) interaction with the water-soluble azo compound AAPH.

Both require a spectrophotometric measurement and a certain reaction time in order to obtain reproducible results [14]. In its oxidized form, the DPPH radical has an absorbance maximum centered at about 517 nm [15]. The DPPH method is described as a simple, rapid and convenient method independent of sample polarity for screening many samples for radical scavenging activity [16]. These advantages made the DPPH method interesting for testing our compounds. The use of the free radical reactions initiator AAPH is recommended as more appropriate for measuring radical-scavenging activity *in vitro*, because the activity of the alkylperoxyl radicals produced by the action of AAPH shows a greater similarity to cellular activities such as lipid peroxidation [17].

The interaction/reducing activity (RA) of the examined compounds with the stable free radical DPPH is shown in Tables 1–3 (full structures of compounds are provided in Table S1 of the Electronic supplementary information). For the sake of comparison, already reported RA% values for TRX and corresponding analogs were also included in the table. This interaction, indicating their radical scavenging ability in an iron-free system, was measured at 100 μM (20/60 min). In the DPPH assay, the dominant chemical reaction involved is the reduction of the DPPH radical by an electron transfer (ET) from the antioxidant. Particularly effective such antioxidants are the phenoxide anions from phenolic compounds like catechol and derivatives, such as NDGA. As it is apparent from the data of Table 1, the mother compounds TRX, 8-MOP and 5-MOP



Scheme 4.

showed very low interaction values 0–16%. Bromination of the psoralen nucleus generally caused lower interaction with DPPH with the noticeable exception of bromide **2**, which was the most potent inhibitor (100% after 60 min) of all compounds examined. On the other hand, acetylation of the psoralen nucleus caused generally small changes in the interaction, higher in the case of TRX and lower

Table 1
Antioxidative activity data for bromo- and acetyl-substituted psoralens. Interaction% with DPPH (RA%); *in vitro* inhibition of soybean lipoxygenase (LOX) (IC_{50}) or %LOX inh.; %inhibition of lipid peroxidation (AAPH%).

Compds	Clog *P* [27]	DPPH% 100 μM 20/60 min	IC_{50} μM or % LOX inh. @ 100 μM	AAPH% @ 100 μM
1[6]	3.47	na/na	40	na
2[6]	4.33	na/100	na	35
3[6]	2.98	14/14	79 μM	67
11	2.31	16/16	50 μM	5
12	3.19	8/8	40 μM	62
13	2.18	25/22	89 μM	5
14	3.08	33/43	50 μM	83
15	1.80	8/8	100 μM	85
25	2.31	14/14	na	49
26	3.19	7/7	90 μM	83
27	2.18	34/36	41.5 μM	95
28	3.08	20/56	30.5 μM	81
NDGA	3.92	81/83	28 μM	
Trolox				63

na, no activity.

Table 2
Antioxidative activity data for psoralens substituted by an acrylate moiety. Interaction% with DPPH (RA%); *in vitro* inhibition of soybean lipoxygenase (LOX) (IC_{50}) or %LOX inh.; %inhibition of lipid peroxidation (AAPH%); inhibition% of carrageenin-induced rat paw edema (ICPE%).

Compds	Clog *P* [27]	DPPH% 100 μM 20/60 min	IC_{50} μM or % LOX inh. @ 100 μM	AAPH% @ 100 μM	ICPE% 0.01 mmol/kg
4[6]	3.57	11/11	9	73	nt
5[6]	5.03	11/11	9.4 μM	80	46**
6	4.32	8/60	na	77	nt
16	2.90	7/7	50 μM	62	nt
17	4.36	3/3	na	89	nt
18	3.66	5/6	75 μM	83	nt
19	4.77	5/7	6 μM	79	57**
20	6.99	7/9	55 μM	89	nt
29	2.40	14/14	83 μM	55	nt
30	3.87	na/na	na	79	nt
31	3.16	na/na	na	8	nt
NDGA	3.92	81/83	28 μM		
Trolox				63	
Indomethacin					47**

The effect on edema is expressed as percent of weight increase of hind paw (and as percent of inhibition of edema) in comparison to controls. Each value represents the mean obtained from 6 to 15 animals in two independent experiments. In all cases, significant difference from control: $^{**}p < 0.01$ (Student's *t* test); na, no activity; nt, not tested.

Table 3
Antioxidative activity data for psoralens substituted by a benzoate or a cinnamate moiety. Interaction% with DPPH (RA %); *in vitro* inhibition of soybean lipoxygenase (LOX) (IC_{50}) or %LOX inh.; %inhibition of lipid peroxidation (AAPH%); inhibition% of carrageenin-induced rat paw edema (ICPE%).

Compds	Clog *P* [27]	DPPH% 100 μM 20/60 min	IC_{50} μM or %LOX inh. @ 100 μM	AAPH% @ 100 μM	ICPE% 0.01 mmol/kg
8[6]	4.80	na/na	na	80	nt
9[6]	5.15	na/na	13	84	nt
10[6]	6.62	na/na	33	41	nt
22	3.94	11/15	na	100	nt
23	4.29	41/44	62 μM	12	nt
24	5.75	15/18	7.5 μM	72	26.2**
33	3.95	22/19	60 μM	66	nt
34	4.29	22/28	24 μM	55	nt
35	5.75	8/12	100 μM	77	
NDGA	3.92	81/83	28 μM		
Trolox				63	
Indomethacin					47**

The effect on edema is expressed as percent of weight increase of hind paw (and as percent of inhibition of edema) in comparison to controls. Each value represents the mean obtained from 6 to 15 animals in two independent experiments. In all cases, significant difference from control: $^{**}p < 0.01$ (Student's *t* test); na, no activity; nt, not tested.

in the case of 8-MOP. As expected, demethylation of parent compounds MOPs caused 3–4 fold increase of the interaction as this is evidence from the values of 8-HOP (25/22%) and 5-HOP (34/36%). The presence of a Br atom in position *p* to the hydroxyl group had an opposite effect in the two molecules after 20 min, increase of interaction (33%) for the 8-MOP analog and decrease (20%) for the 5-HOP analog. However, increase for both analogs was observed after 60 min (43% for the former and 56% for the latter).

From the data presented in Table 2, it is apparent that introduction of an acrylate unit in the psoralen nucleus is accompanied by a noticeable increase in the case of TRX (8–11% after 20 min; 11–60% after 60 min), a rather small decrease (3–9%) in the case of 8-MOP, and an abolishment of activity in the case of 5-MOP with the exception of acid **29** which was equally active (14%) to 5-MOP. It is interesting to note that free acids show similar activities to corresponding esters with the exception of the 5-MOP case where acid **29** (14%) was significantly more active than the esters **30** and **31**, which were inactive. Also the structure of the alcohol part of the ester (esters **17**–**20**) seemed not to cause significant change in the activity (3–9%).

From the date presented in Table 3, it is apparent that introduction of a benzoate substructure in the TRX nucleus causes no change (inactive compound) in the activity of parent molecule and only a slight increase in the activity of the 8-MOP case, that is 11/15% (compound **22**) as compared to 16/16% for 8-MOP. However, in the case of 5-MOP (14/14%) the opposite effect was observed for the carboxylic acid **33** (22/19%). Similarly, introduction of a cinnamate unit in the TRX molecule caused no change, both molecules examined (acid **9** and *tert*-butyl ester **10**) being inactive. On the other hand in the case of 8-MOP, introduction of a cinnamate unit was accompanied by essentially no change in antioxidative activity in the case of *tert*-butyl ester **24** (15/18%) but a considerable increase in the case of the acid **23** (41/44%). Finally, in the case of 5-MOP (14/14%) the introduction of a cinnamate unit had an opposite effect dependent on whether an acid or an ester unit was present. Thus, acid **34** was more active (22/28%) than 5-MOP, whereas *tert*-butyl ester **35** was less active (8/12%). The low interaction values generally observed with all psoralen analogs examined can be attributed to the absence of easily oxidizable functionalities like the ones (two catechol subunits) present in NDGA, which is used herein as a reference compound, and to the bulk of the molecules. Thus, these derivatives cannot reach and interact with DPPH.

In our studies, AAPH was used as a free radical initiator to follow oxidative changes of linoleic acid to conjugated diene hydroperoxide. Azo compounds generating free radicals through spontaneous thermal decomposition are useful for free radical production studies *in vitro* [17]. The water-soluble azo compound AAPH has been extensively used as a clean and controllable source of thermally produced alkylperoxyl free radicals. In the AAPH assay, the highly reactive alkylperoxyl free radicals are intercepted mainly by a hydrogen atom transfer (HAT) from the antioxidant [18]. Therefore, particularly effective HAT agents are compounds with high hydrogen atom donating ability, that is compounds with low heteroatom–H bond dissociation energies and/or compounds from which hydrogen abstraction leads to sterically hindered radicals as well as compounds from which abstraction of hydrogen leads to C-centered radicals stabilized by resonance.

The inhibitory activity of the examined compounds is also shown in Tables 1–3. From the data of Table 1, it is apparent the mother compounds TRX, 8-MOP and 5-MOP showed very low interaction values 0–5% whereas 5-MOP presented an inhibitory value of 49%, however substantially lower than the reference compound Trolox (63%). Bromination of the psoralen nucleus generally caused significant increase (35–83%) of the inhibitory potency of the compounds with the most potent (bromide **26**) also coming from the 5-MOP series. Even higher increase (67–85%) in the anti-lipid peroxidation potential was observed upon acetylating the psoralen nucleus with the most potent compound **15** coming from the 8-MOP series. Although demethylation of parent compound 8-MOP had no effect on the inhibitory activity (compound **11**, 5%; compound **13**, 5%), demethylation of 5-MOP lead to the compound **27** (95%) with almost twice the activity of parent molecule (49%). Compound **27** was actually the second best anti-lipid peroxidation inhibitor from all compounds examined. The presence of a bromine atom in a position *p* to the hydroxyl group had an opposite effect in the two molecules, a large increase of inhibition (83%) for the 8-HOP analog and a small decrease (81%) for the 5-HOP analog.

From the data presented in Table 2, it is apparent that introduction of an acrylate unit in the psoralen nucleus (compounds **4**–**6**) is accompanied by a large increase (73–80%) in the case of TRX (not active) and also a large increase (compounds **16**–**20**; 62–89%) in the case of 8-MOP (5%) but only a small increase (55–79%) in the case of 5-MOP (49%). However, in the case of ethyl ester **31** a serious decrease (8%) was observed. It is interesting to note that free acids generally show somewhat lower activities than the corresponding esters with the exception of the couple **29** (55%) and **31** (8%). Also, the structure of the alcohol part of the ester (esters **5**–**6** and **17**–**20**) seemed not to cause significant change in the activity (only 4–10%), with the exception of the couple **30** (79%) and **31** (8%). However in all three subfamilies, the *tert*-butyl esters presented the higher inhibition.

From the data presented in Table 3, it is apparent that introduction of a benzoate substructure in the psoralen nucleus causes a large increase (80%) in the inhibitory activity of TRX (inactive) and also a large increase (100%) in the inhibitory activity of 8-MOP (5%), whereas in the case of 5-MOP (49%) had only a small effect (66%). Actually, analog **22** of 8-MOP was the most potent inhibitor (100%) of all compounds examined in the course of the present work. Similarly, introduction of a cinnamate unit in the TRX molecule caused a significant increase in the inhibitory activity (41–84%). In the cases of the other two parent compounds an increase in the inhibitory activity was observed which was not so pronounced compared to the TRX analogs. Actually, acids **23** (12%) and **34** (55%) were of comparable activity to the parent molecules 8-MOP (5%) and 5-MOP (49%) respectively, whereas the corresponding esters **24** (72%) and **35** (77%) were much more active. On the contrary, in

the TRX case the psoralen-substituted cinnamic acid **9** (84%) was twice as inhibitory as its corresponding *tert*-butyl ester **10** (41%).

In general, modification of TRX leads invariably to an improvement of its anti-lipid peroxidation capacity. TRX analogs with substantially higher inhibitory activities (e.g. 73–84%) than the reference compound Trolox (63%) include side-chains like acetyl, acrylate, benzoic acid and cinnamic acid, which would readily stabilize C-centered free radicals upon abstraction of a H atom by the alkylperoxyl radicals, or may intercept alkylperoxyl radicals by their unsaturated side-chain. 8-MOP analogs with similar or even higher inhibitory activities (e.g. 79–100%) include generally same side-chains like the ones described for the TRX analogs. 5-MOP analogs with slightly lower activities (77–95%) than the 8-MOP analogs include side-chains like *tert*-butyl acrylate and cinnamate. It is apparent that the MOP nucleus stabilizes better than the TRX nucleus radicals created by a HAT mechanism. Interestingly, potent inhibitors of lipid peroxidation are observed for 5-MOP upon demethylation and/or bromination (analogs **26**–**28**; 81–95%) and for 8-MOP upon bromination and demethylation (analog **14**; 83%). It is obvious that in this case, the alkylperoxyl radicals remove a H atom from the hydroxyl function and the thus obtained phenoxy-type radical is stabilized by resonance, especially when in the *p* position there are two adjacent O atoms present. Indeed, analog **27** is the second most potent (95%) anti-lipid peroxidation agent amongst all compounds examined.

An examination of the data for the antioxidant effect of the tested MOP analogs in the two assays used reveals that compounds with high interactions with AAPH generally show very low interactions with DPPH. Representative examples of this notion (DPPH % = 0–9; AAPH% = 77–100) are the compounds **15**, **17**–**20**, **22**, **26**, **30** and **35**. Inverse behavior is presented in few cases only, for example in the compounds **13** and **23**, and also in very few instances, such as the compounds **11** and **31** comparable behavior (very low activity) in the two assays is observed. These results show that lipid peroxidation activity is not always accompanied by DPPH radical scavenging ability and *vice versa* [19,20]. This can be attributed to the different chemical reactions involved in the two assays [18]. In the DPPH assay, a reduction of the long-lived N-centered radical mainly takes place through ET from the antioxidant. Relatively effective such antioxidants in the MOP analogs examined are for example the phenoxide anions from the phenolic compounds **13**, **14** and **27**, **28** (DPPH% = 22–56 after 60 min). On the other hand in the AAPH assay, the highly reactive alkylperoxyl radical is neutralized mainly by HAT from the antioxidant. Obviously, compounds acting as particularly effective HAT agents are the phenolic compound **27** and the 8-MOP-derived analogs **17**, **20** and **22** (AAPH% = 89–100). The antioxidant activity with either DPPH or AAPH varies and depends on the structure of the analog, with interesting examples being the couples of the phenolic compounds **13** (xanthotoxol) and **27** (bergaptol) and of the 8-MOP derived analogs **23** and **24**. In the former case although both compounds had comparable interaction values with DPPH, they presented a huge difference in their interaction values with APPH. On the other hand, acid **23** interacted more significantly with DPPH than the corresponding *tert*-butyl ester **24** (41% compared to 15%) whereas the opposite was observed for the interaction with AAPH (12% compared to 72%).

Eicosanoids are oxygenated metabolites of arachidonic acid with a broad implication in a diversity of diseases. The 5-lipoxygenase (5-LOX) catalyzes the first two steps in the metabolism of arachidonic acid to leukotrienes (LTs) which are considered to be important in the pathogenesis of neutrophil-mediated inflammatory diseases [21] with a marked relation to the severity of cardiovascular diseases, asthma and cancer. Inhibitors of LOX have attracted attention initially as potential agents for the treatment of inflammatory and allergic diseases, asthma, certain types of cancer and cardiovascular diseases [22,23].

In this context, we decided to further evaluate the parent molecules 8- and 5-MOP and the synthesized compounds **6**, **12**–**20**, **22**–**24**, **26**–**31** and **33**–**35** for their ability to inhibit soybean LOX by the UV absorbance based enzyme assay [24]. Most of the LOX inhibitors are antioxidants or free radical scavengers [25]. LOXs contain a “non-heme” iron per molecule in the enzyme active site as high-spin Fe^{2+} in the native state and the high spin Fe^{3+} in the activated state. Some studies suggest a relationship between LOX inhibition and the ability of the inhibitors to reduce Fe^{3+} at the active site to the catalytically inactive Fe^{2+}. This inhibition is related to their ability to reduce the iron species in the active site to the catalytically inactive ferrous form [25], whereas several LOX inhibitors are excellent ligands for Fe^{3+}. NDGA, a known inhibitor of soybean LOX, was used as a reference compound (IC_{50} 28 μM).

The values for the inhibitory activity of the examined compounds are tabulated in Tables 1–3. From the data of Table 1, it is apparent that the parent molecule TRX presented a low inhibitory potency (40%) whereas 5-MOP was found inactive. In sharp contrast, 8-MOP was quite active with an IC_{50} value 50 μM. Bromination of TRX resulted in complete abolishment of activity. On the contrary, bromination of 8- and 5-MOP resulted in an increase of the inhibitory potency more pronounced in the case of 5-MOP. Thus, bromides **12** and **26** had IC_{50} 40 and 90 μM, respectively. On the other hand, acetylation of the psoralen nucleus had an opposite effect on the inhibitory potency of TRX and 8-MOP, that is increase (IC_{50} 79 μM) in the former and decrease (IC_{50} 100 μM) in the latter. Although demethylation of parent compound 8-MOP and the corresponding bromide **12** caused a reduction (IC_{50} 89 and 50 μM, respectively) in the inhibitory activity, the demethylation of 5-MOP and its corresponding bromide **26** had the opposite and more pronounced effect (IC_{50} 41.5 and 30.5 μM, respectively). Compound **26** was actually the most potent inhibitor in this set of compounds and of comparable activity to NDGA (IC_{50} 28 μM). A comparison of the couples **13** and **14** and **27** and **28** shows that that insertion of a Br atom in position *p* to the hydroxyl group leads to an increase of inhibition for both analogs 8- and 5-HOP.

From the data presented in Table 2, it is apparent that introduction of an acrylic acid unit in the psoralen nucleus is accompanied by a large decrease (9%) in the case of TRX (40%) and large increase (IC_{50} 83 μM) in the case of 5-MOP (inactive). However, it had no effect (IC_{50} 50 μM) in the case of 8-MOP (IC_{50} 50 μM). Interestingly, the corresponding *tert*-butyl esters **17** and **30** were totally inactive. On the contrary, the *tert*-butyl ester **5** was extremely potent (IC_{50} 9.4 μM), being the second most potent inhibitor of LOX in this series of compounds. A comparison of the IC_{50} values of the corresponding ethyl esters **6**, **18** and **31** shows that with the exception of ester **18** (IC_{50} 75 μM) the other two esters are totally inactive. Finally, the structure of the alcohol part of the ester (in esters **5**–**6** and **17**–**20** but not in esters **30**–**31**) seems to have a significant effect on the inhibitory activity, with the butyl esters (*tert*-butyl in the TRX subset and *n*-butyl in the 8-MOP subset) being the most potent. Actually, *n*-butyl ester **19** was the most potent (IC_{50} 6 μM) LOX inhibitor amongst all the examined compounds.

From the data presented in Table 3, it is apparent that introduction of a benzoate substructure in the psoralen nucleus has a varying effect on the inhibitory activity of the parent molecules. Thus, in the case of TRX (40%) a serious reduction is observed (acid **8**, inactive). Reduction in the inhibitory potency (acid **22**, inactive) is also observed in the case of 8-MOP (IC_{50} 50 μM). On the contrary, a serious increase is observed (acid **33**; IC_{50} 60 μM) in the case of 5-MOP (inactive). Introduction of a cinnamate unit in the TRX molecule caused a decrease in the inhibitory activity (13–33%). In

the case of 8-MOP (IC_{50} 50 μM), a slight decrease was observed for acid **23** (IC_{50} 62 μM) and a large increase for *tert*-butyl ester **24** (IC_{50} 7.5 μM). Actually, ester **24** was the second most active LOX inhibitor amongst all compounds examined. On the other hand in the case of 5-MOP (inactive), an increase in the inhibitory activity (IC_{50} 24–100 μM) was observed for both analogs, that is the acid **34** and the *tert*-butyl ester **35**. With the exception of *tert*-butyl ester **35**, *tert*-butyl cinnamates are better LOX inhibitors than their corresponding free acids. Most active inhibitors are obtained when the *tert*-butyl cinnamate is substituted at position *p* with the 8-MOP unit (IC_{50} 7.5 μM).

Lipophilicity is referred as an important physicochemical property for LOX inhibitors [26]. Herein, the theoretically calculated Clog *P* [27] values generally support this statement. Notable exceptions from Table 1 are the couple of compounds **25** (Clog $P = 2.31$; inactive) and **27** (Clog $P = 2.18$; $IC_{50} = 41.5$ μM) and the bromides **26** (Clog $P = 3.19$; $IC_{50} = 90$ μM) and **28** (Clog $P = 3.08$; $IC_{50} = 30.5$ μM). From Table 2, notable exceptions are the couples of compounds **19** (Clog $P = 4.77$; $IC_{50} = 6$ μM) and **20** (Clog $P = 6.99$; $IC_{50} = 55$ μM), and **29** (Clog $P = 2.40$; $IC_{50} = 83$ μM) and **30** (Clog $P = 3.87$; IC_{50} = inactive). Finally from Table 3, notable exceptions are the couple of compounds **34** (Clog $P = 4.29$; $IC_{50} = 24$ μM) and **35** (Clog $P = 5.75$; $IC_{50} = 100$ μM). Interestingly, the most potent inhibitors ($IC_{50} = 6–9.4$ μM) within this series of psoralen analogs, i.e. compounds **5**, **19** and **24**, are characterized by relatively high lipophilicity values (Clog $P = 4.77–5.75$).

In general, the present studies indicate that the 8-MOP-derived *n*-butyl ester **19** ($IC_{50} = 6$ μM) and *tert*-butyl ester **24** ($IC_{50} = 7.5$ μM) can be considered as lead-compounds for the development of more active LOX inhibitors.

2.2.2. Anti-inflammatory activity in vivo

In acute toxicity experiments, the *in vivo* examined compounds did not present toxic effects in doses up to 0.1 mmol/kg body weight. For the anti-inflammatory assay, we used a dose of 0.01 mmol/kg equimolar to the administered standard drug indomethacin for the sake of comparison.

We have recently reported that the TRX-derived *tert*-butyl ester **5** ($IC_{50} = 9.4$ μM; AAPH% = 80) presented essentially an equipotent anti-inflammatory activity (46%) with the reference compound indomethacin (47%) [6], thus indicating that psoralen derivatives might be possibly used as anti-inflammatory compounds. Herein, for the *in vivo* anti-inflammatory screening, the selection of the compounds was based on their potent inhibitory activity on LOX and their good anti-lipid peroxidation activity. The *in vivo* examination of the anti-inflammatory activity of selected compounds was performed by using the functional model of carrageenin-induced rat paw edema. Carrageenin-induced edema is a non-specific inflammation resulting from a complex of diverse mediators [28].

The most potent compounds in the present series of MOP analogs, that are the 8-MOP-derived *n*-butyl ester **19** ($IC_{50} = 6$ μM; AAPH% = 79) and the 8-MOP-derived *tert*-butyl ester **24** ($IC_{50} = 7.5$ μM; AAPH% = 72%), were selected for *in vivo* studies. As shown in Tables 2 and 3, the most potent inhibitor was analog **19**, with an inhibitory potency (57%) higher than indomethacin (47%), followed by analog **24** which was a weaker inhibitor of inflammation (26.2%) compared to indomethacin.

2.2.3. Cytotoxicity

Taking into consideration the potential applicability of members of the present family of psoralen analogs for biomedical applications, we decided to evaluate the cytocompatibility of the most potent LOX inhibitors **5**, **19**, **24**, **28** and **34** with IC_{50} values in the range 6–30.5 μM, with IC_{50} for the reference compound NDGA being 28 μM. Three of these inhibitors, namely psoralen analogs **5**, **19** and **24** had been also examined *in vivo* for their anti-inflammatory activity. For the sake of comparison, the corresponding parent molecules TRX, 8-MOP and 5-MOP were also included in the study. The cytocompatibility was determined by the viability of 1BR3 human skin fibroblast cells in the presence of different concentrations of these compounds using the MTT test essentially as described in ref 31. The cytocompatibility of these compounds was evaluated in the presence of different concentrations of the compounds (1–100 μM) and the results are presented in Fig. 2 in the form of a bar-graph of the % cell survival values for the examined compounds (see also Table S2 in the Electronic supplementary information).

At the lowest concentration examined (1 μM), the parent compounds presented no cytotoxicity (101–107% survival) and the analogs **19**, **24** and **28**, were essentially no cytotoxic (96–99%). Notable exceptions were the analogs **5** (68%) and **34** (65.6%) with almost equipotent cytotoxicity. Although the parent compounds and analogs **19** and **28** presented low, if any, cytotoxicities (88–102% survival) in the whole area of concentrations examined (up to 100 μM), the analogs **5**, **24** and **34** presented steadily increasing cytotoxicities whose values followed the increase of concentration. Actually, at the highest concentration examined (100 μM), these compounds showed high cytotoxicities (13–46%) with the most cytotoxic compounds being the analogs **5** (22.2% survival) and **34** (13.3% survival).

From the cytocompatibility data presented above, it can be assumed that attachment of a cinnamate unit on methoxypsoralens results in analogs (**24** and **34**) with significant cytotoxicity as does the inclusion of an acrylate unit on TRX (analog **5**). On the contrary, attachment of a *n*-butyl acrylate unit on 8-MOP (analog **19**) essentially does not change the high cytocompatibility of the parent molecule. Similarly, demethylation of a MOP and introduction of a Br atom in position *p* to the hydroxyl group seems to have no significant effect on the high cytocompatibility of the parent molecule.

The results from the cytotoxicity studies taking together with the results from the anti-inflammatory, antioxidative and LOX inhibition studies indicate that the TRX analog **5**, the 8-MOP analog **24** and the 5-MOP analog **34** may be used in biomedical applications with caution whereas the 8-MOP analog **19** is a compound deserving further development as an antioxidant/anti-inflammatory agent.

2.3. Molecular docking studies – docking studies on soybean lipoxygenase

We thought of interest to examine whether the possible mechanism of action as well as the differences in the inhibitory

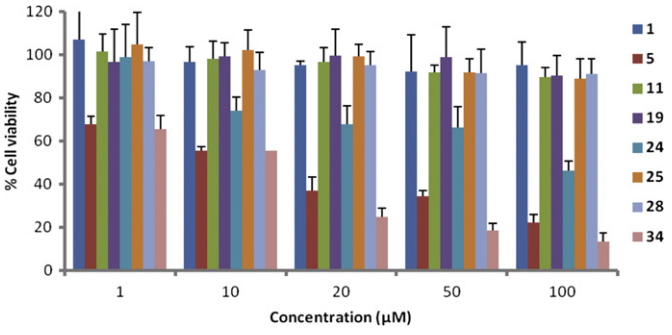


Fig. 2. Cytotoxicity of psoralen analogs on 1BR3 cells (24 h incubation).

activity toward soybean LOX of the most potent analogs **5**, **19** and **24** could be explained by docking calculations. For the docking studies of LOX we have used the 1RRH (soybean LOX) available from the Protein Data Bank (PDB) with a resolution of 2 Å [29]. The lack of structural data for human LOX, lead us to model human LOX using soybean enzyme because of its availability and highly characterized structure [30]. We used the 1RRH (soybean LOX) from PDB with Fe^{+3} running blind docking. Two soybean LOX models were derived from 1RRH. One with a ligand taken from 1IK3 (ligand ID: 9OH) for the docking studies into the catalytic cavity and another one without any ligand in the catalytic cavity for the blind docking simulation. The metal center in both models has been considered as a cation, with charge $q = +3$ and no bond restraint was applied between the iron and the ligands. Lennard–Jones parameters for Fe (III) force field can be resumed as: $\sigma_{vdw} = 2.138157\ e^{-01}$ nm, $\varepsilon_{vdw} = 2.092\ e^{-1}$ kJ/mol. The validation of our docking study was made with the ligands crystallized in the protein of complex 1RRH, where our docking results were in good agreement with the structure of the molecules in the active site of the protein.

The docking orientations of the most potent inhibitors **5**, **19** and **24** of LOX are given in Fig. 3. It is important to mention that compound **5** presented significant binding energy to the protein. A number of H-bonds have been observed in the cavity of lipoxygenase. H-bonds were developed: a) with Leu766 from the oxygen of the furan ring of compound **5** and b) with His511 and Arg719 from both the carbonyl oxygen and the oxygen of the ester group. Hydrophobic interactions were developed from the aromatic part of compound **5**.

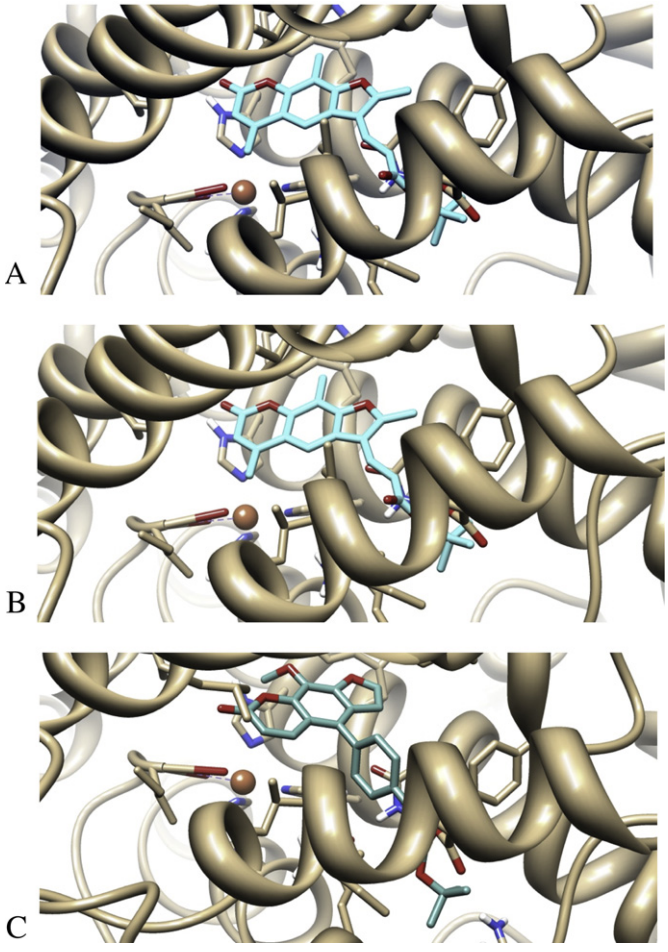


Fig. 3. Docked poses of compound **5** (A) (Aquamarine), compound **19** (B) (Sky blue) and compound **24** (C) (Turquoise) in the soybean LOX binding site. Side-chains of relevant binding site residues are rendered as ball and stick models. The iron ion is rendered as an orange sphere. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

As it is shown in Fig. 3C, compound **24** is well fitted in the cavity of lipoxygenase nearby the Fe atom. Strong hydrogen bonds were developed with Arg719 and Ser503 from the carbonyl oxygen of the ester group. The enzyme–inhibitor complex is further stabilized by hydrophobic interactions among the aromatic moieties.

Finally, compound **19** was also found to formulate a complex with lipoxygenase 1RRH. H-bonds are presented with many aminoacids like: Thr568 from the oxygen of furan ring and Ala517, Thr515 and His516 with the carbonyl oxygen. Strong lipophilic interactions were also observed.

All the compounds seem to take similar conformation in the active site of lipoxygenase. Especially compounds **5** and **24** seem to take approximately the same orientation into the active site while compound **19** is presenting a slightly different orientation. Blockage of one of the cavity entrances might disturb the access to the catalytic site. From the above, the possibility of an allosteric effect of the compounds might be considered. The docking study suggests that the biological response of the examined compounds could be explained by the proper geometry of the predicted conformations at the active site.

3. Conclusion

Direct bromination of 5- and 8-MOP with NBSa allows for the selective introduction of a Br atom at position 8 and 5, respectively. The thus obtained key-intermediates were employed for the efficient preparation of a series of MOP analogs bearing an acrylate or a benzoate or a cinnamate unit. The reducing ability of the resulting MOP analogs was determined using two assays. The examined compounds generally presented low interaction values with DPPH but generally high interaction values with AAPH, thus indicating their ability to act as efficient inhibitors of lipid peroxidation. In addition, members of this family of compounds, the 8-MOP analogs **19** and **24**, inhibited strongly the enzyme soybean LOX, *ca*. 4 times more than the reference compound NDGA. Molecular docking studies showed that these inhibitors fitted well into the cavity of soybean LOX and held firmly through the development hydrogen bonds and lipophilic interactions. Modeling studies were found to be in accordance with our experimental biological results. The anti-inflammatory activity of analog **19** was higher than the reference compound indomethacin, whereas that of **24** was weaker.

On the other hand, the cytotoxicity studies performed indicate that inclusion of a cinnamate moiety into the MOP nucleus (analogs **24** and **34**) seems to lead to a significant increase in cytotoxicity in particular in the case of 5-MOP (analog **34**), whereas the inclusion of an acrylate unit (TRX analog **5**) leads to increased toxicity of the TRX system but not of the 8-MOP system (analog **19**). These observations make the *n*-butyl ester **19** the best candidate for further studies and possible biomedical applications, which are actually currently in progress.

4. Experimental section

4.1. Chemistry

4.1.1. General synthetic

Melting points were determined with a Buchi SMP-20 apparatus and are uncorrected. IR spectra were recorded for KBr pellets unless otherwise stated on a Perkin Elmer 16 PC FT-IR spectrophotometer. ^{1}H NMR spectra were obtained at 400.13 MHz and ^{13}C NMR spectra at 100.62 MHz, using a Bruker DPX spectrometer. Chemical shifts

(δ) are in parts per million (ppm) downfield from TMS used as the internal standard. The assignments of the ^{1}H NMR spectra (see Supplementary material) were based on chemical shift arguments, analysis of coupling patterns and signal intensities. Electron-spray ionization (ESI) mass spectra were obtained on a WATERS Micromass ZQ spectrometer, equipped with a quantropole detector, using MeOH as solvent. GC analyses were performed on an Agilent Technologies 6890 N gas chromatograph fitted with a capillary column (30.0 m; 250 mm; 0.25 mm nominal) having as stationary phase HP-5MS 5% Phenyl Methyl Siloxane. Carrier gas flow-rate: 26.4 mL/min He; injection port temperature 300 °C; program: 70–300 °C at 20.58 min. Electron Impact mass spectra were recorded at 20 eV on an Agilent Technologies 5975B instrument, tandem to the above mentioned GC spectrometer. High-resolution ESI-MS spectra were recorded on a Bruker APEX II FT-ICR spectrometer. Microanalyses were performed on a Carlo Erba EA 1108 CHNS elemental analyzer in the Laboratory of Instrumental Analysis of the University of Patras. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values. FCC was performed on Merck silica gel 60 (230–400 mesh) and TLC on 60 Merck 60F$_{254}$ films (0.2 mm) precoated on aluminum foil. Spots were visualized with UV light at 254 nm and charring agents. Unless otherwise stated, the solvent systems used for the FCC purifications are identical to those indicated in the R_f values of the purified compounds. The eluant systems used were: (A) PhMe:EtOAc (95:5), (B) PhMe:EtOAc (9:1), (C) PhMe:EtOAc (7:3), (D) $CHCl_3$:MeOH (95:5), (E) $CHCl_3$:MeOH (9:1). All solvents (Merck) were dried and/or purified according to standard procedures prior to use. Anhydrous Na_2SO_4 was used for drying solutions and subsequently solvents were routinely removed at *ca.* 40 °C under reduced pressure (water aspirator). All reagents employed in the present work were purchased from either Aldrich or Fluka and used without further purification. All reactions described below were conducted under dim light.

Purity determination. Analytical reversed phase high-performance liquid chromatography was performed on a Waters Alliance 2695 HPLC system equipped with an autosampler, a high-pressure pump and a photodiode array detector (Waters 2998). The purity of the final compounds used in the *in vivo* experiments was determined using UV detection ($\lambda = 254$ nm). The chromatographic method employed the following: column, Merck PuroSpher® C-8 (125.0 cm × 4.5 mm, 5 μm); mobile phase A, 0.08% TFA in water; mobile phase B, acetonitrile with 0.08% TFA; flow rate, 1.0 mL/min; elution profile, gradient elution from 50 to 60 to 100% B over 20–30 min. According to these analyses, purities for all *in vivo* tested compounds were >97.4% (see Electronic supplementary information).

4.1.2. Syntheses of psoralen analogs

*4.1.2.1. 5-Bromo-8-methoxypsoralen (**12**).* A solution of 8-methoxypsoralen (**11**, 0.48 g, 2.21 mmol) in CH_3CN (3.7 mL) and $CHCl_3$ (2.3 mL), was heated to 80 °C with stirring for 20 min. Then, *N*-bromosaccharin (0.79 g, 3 mmol) was added in one portion and the resulting orange solution was stirred at this temperature for additional 2.5 h. After completion of the reaction (TLC monitoring), the resulting mixture was cooled at room temperature, diluted with Et_2O and refrigerated overnight. The precipitated product was filtered, washed with cold Et_2O, and dried in vacuo. The crude material was recrystallized from CH_3CN to give pure bromide **12** (0.53 g, 82%) as a pale yellow solid; mp 183–185 °C (lit. [9] 185–186 °C); $R_f = 0.39$ (B); IR (KBr, cm^{-1}): ν 1732 (d), 1590, 1330, 1152, 1106, 750; ^{1}H NMR ($CDCl_3$, δ): 8.14 (1H, d, $J = 10.0$ Hz), 7.73 (1H, d, $J = 2.2$ Hz), 6.89 (1H, d, $J = 2.2$ Hz), 6.46 (1H, d, $J = 10.0$ Hz), 4.29 (3H, s); ^{13}C NMR ($CDCl_3$, δ): 160.0, 147.1, 144.0, 142.9, 134.7, 128.2 (2C), 121.4, 116.1, 107.7, 105.8, 61.9; GC–MS (EI, 20 eV): $t_R = 13.56$ min, *m/z* 296 and 294 (M$^+$, 100%), 281 and 279 (M–CH_3, 14), 253 and 251 ([(M–CH_3)–CO], 13), 215 (M–Br, 36).

*4.1.2.2. 8-Bromo-5-methoxypsoralen (**26**).* A solution of 5-methoxypsoralen (**25**, 0.65 g, 3 mmol) in CH_3CN (3 mL) and diglyme (6.6 mL) was heated at 80 °C with stirring for 20 min. Then, *N*-bromosaccharin (1.18 g, 4.5 mmol) was added in one portion and the resulting yellow solution was stirred at this temperature for additional 3 h. After completion of the reaction (TLC monitoring), the resulting mixture was cooled at room temperature, diluted with Et_2O and refrigerated overnight. The precipitated product was filtered, washed with cold Et_2O, and dried in vacuo. The crude material was recrystallized from diglyme to give pure bromide **26** (0.38 g, 43%) as a pale yellow solid; mp 223–225 °C; $R_f = 0.35$ (B); IR (KBr, cm^{-1}): ν 1737, 1588, 1330, 1152, 1106, 750; found: C, 48.68; H, 2.53. Calc. for $C_{12}H_7BrO_4$: C, 48.84; H, 2.39%; ^{1}H NMR ($CDCl_3$, δ): 8.12 (1H, d, $J = 9.6$ Hz), 7.67 (1H, d, $J = 2.2$ Hz), 7.11 (1H, d, $J = 2.2$ Hz), 6.29 (1H, d, $J = 9.6$ Hz), 4.27 (3H, s); ^{13}C NMR ($CDCl_3$, δ): 160.3, 147.4, 144.3, 143.2, 135.0, 128.5, 127.0, 121.7, 116.4, 108.0, 106.1, 62.2; GC–MS (EI, 20 eV): $t_R = 14.39$ min, *m/z* 296 and 294 (M$^+$, 100%), 281 and 279 (M–CH_3, 27), 253 and 251 ([(M–CH_3)–CO], 10).

*4.1.2.3. 5-Acetyl-8-methoxypsoralen (**15**).* To a suspension of 8-MOP (**11**, 0.65 g, 3 mmol) in Ac_2O (8 mL), $SnCl_4$ (0.26 mL, 2.2 mmol) was added. The resulting mixture was stirred at room temperature for 3 d. After the consumption of the starting material, the reaction mixture was treated with ice-cold H_2O and finally extracted with $CHCl_3$. The organic layer was washed with H_2O, dried over Na_2SO_4 and evaporated to leave a residue, which was crystallized (EtOAc) to give pure **15** (0.34 g, 43%) as a white solid; mp 131–133 °C; $R_f = 0.22$ (C); IR (KBr, cm^{-1}): ν 1734, 1664, 1576, 1162, 1106, 754; found: C, 64.91; H, 4.05. Calc. for $C_{14}H_{10}O_5$: C, 65.12; H, 3.90%; ^{1}H NMR (DMSO-d_6, δ): 8.30 (1H, d, $J = 2.4$ Hz), 8.29 (1H, d, $J = 10.0$ Hz), 7.35 (1H, d, $J = 2.4$ Hz), 6.54 (1H, d, $J = 10.0$ Hz), 4.28 (3H, s), 2.74 (3H, s); ^{13}C NMR (DMSO-d_6, δ): 200.2, 159.3, 149.6, 146.2, 143.0, 142.6, 134.5, 125.4, 123.6, 116.3, 114.5, 107.3, 61.6, 32.5; GC–MS (EI, 20 eV): t_R = 11.36 min, *m/z* 258 (M$^+$, 100%), 243 (M–CH_3, 68), 216 (M–CH_2CO, 57), 201 (216-CH_3, 26).

*4.1.2.4. General procedure for the preparation of analogs **13**, **14**, **27** and **28**.* To an ice-cold solution (0 °C) of 8- or 5-MOP (1 mmol) or the corresponding bromides **12** or **26** (1 mmol) in CH_2Cl_2 (3.5 mL), a 1 M solution of BBr_3 (4 mmol) in CH_2Cl_2 was added dropwise through a glass syringe. The resulting suspension was stirred at 0 °C for 1–2 h. After the completion of the reaction, ice-chips were added and the resulting mixture was extracted with CH_2Cl_2 and washed several times with H_2O. The combined organic layers were dried with anhydrous Na_2SO_4 and evaporated to dryness to obtain the anticipated phenolic compounds.

*4.1.2.4.1. Xanthotoxol (**13**).* Reaction time: 1 h; yield: 92% (0.18 g of white solid); mp 245–248 °C (lit. [7] 244–246 °C); $R_f = 0.10$ (B).

*4.1.2.4.2. Bergaptol (**27**).* Reaction time: 2 h; yield: 82% (0.16 g of white solid); mp 285–288 °C (lit. [11] 283–284 °C); $R_f = 0.17$ (C).

*4.1.2.4.3. 5-Bromoxanthotoxol (**14**).* Reaction time: 1.5 h; yield: 95% (0.27 g of white solid); mp 289 °C (dec.); $R_f = 0.30$ (C); IR (KBr, cm^{-1}): ν 3324, 1756 (d), 1596, 1430, 1162, 1106, 746; found C, 47.23; H, 1.62. Calc. for $C_{11}H_5BrO_4$: C, 47.01; H, 1.79%; ^{1}H NMR (DMSO-d_6, δ): 10.99 (1H, s), 8.20 (1H, d, $J = 2.4$ Hz), 8.15 (1H, d, $J = 10.0$ Hz), 6.99 (1H, d, $J = 2.4$ Hz), 6.53 (1H, d, $J = 10.0$ Hz); ^{13}C NMR (DMSO-d_6, δ): 160.3, 149.4, 145.4, 143.7, 141.5, 131.1. 127.8, 116.7, 116.0, 108.0, 101.6; GC–MS (EI, 20 eV): t_R = 15.31 min, *m/z* 282 and 280 (M$^+$, 65%) 254 and 252 (M–CO, 10) 201 (M–Br, 100).

*4.1.2.4.4. 8-Bromobergaptol (**28**).* Reaction time: 2 h; yield: 85% (0.24 g of white solid); mp 302 °C (dec.); R_f = 0.28 (C); IR (KBr, cm^{-1}): ν 3320, 1758 (d), 1594, 1430, 1162, 746, 1106; found C, 47.27;

H, 1.59. Calc. for $C_{11}H_5BrO_4$: C, 47.01; H, 1.79%; ^{1}H NMR (DMSO-d_6, δ): 10.82 (1H, s), 8.09 (1H, d, J = 10.0 Hz), 7.64 (1H, d, J = 2.4 Hz), 7.08 (1H, d, J = 2.4 Hz), 6.26 (1H, d, J = 10.0 Hz); ^{13}C NMR (DMSO-d_6, δ): 160.4, 149.5 (2C), 143.8, 141.6, 131.2. 127.8, 116.8 (2C), 108.1, 101.7; GC–MS (EI, 20 eV): t_R = 16.73 min, m/z: 282 and 280 (M^+, 72), 201 (M–Br, 100).

*4.1.2.5. General procedure for the synthesis of tert-butyl esters **17** and **30**.* A mixture of bromide **12** or **26** (0.4 g, 1.35 mmol), *tert*-butyl acrylate [0.4 mL (2.7 mmol) and 1 mL (6.8 mmol), respectively], (*o*-tolyl)$_3$P (0.2 g, 0.67 mmol) and TEA (2.1 mL, 15 mmol) in DMF (11 mL) was degassed at −10 °C. Then, $Pd(AcO)_2$ (0.052 g, 0.23 mmol) was added and the resulting mixture was redegassed at the same temperature. The resulting reaction mixture was heated at 90 °C and vigorously stirred until the consumption of the bromide. After the completion of the reaction, the mixture was passed through a short celite pad. The precipitate on the filter was washed with a small volume of DMF and the filtrates were combined and diluted with $CHCl_3$. The organic phase was washed with H_2O, dried over Na_2SO_4, and evaporated to a small volume. This was then directly applied on a flash column to give, following elution, pure esters **17** and **30**.

*4.1.2.5.1. tert-Butyl E-3-(8-methoxypsoralen-5-yl)propenoate (**17**).* Reaction time: 3 h; yield: 92% (0.42 g of pale yellow solid); mp 120–123 °C; R_f = 0.12 (A); IR (KBr, cm^{-1}): ν 1729, 1569, 1278, 1144, 1044; found C, 66.42; H, 5.56. Calc. for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30%; ^{1}H NMR ($CDCl_3$, δ): 8.14 (1H, d, J = 10.0 Hz), 8.04 (1H, d, J = 16.0), 7.75 (1H, d, J = 2.4 Hz), 7.07 (1H, d, J = 2.4 Hz), 6.44 (1H, d, J = 10.0 Hz), 6.36 (1H, d, J = 16.0 Hz), 4.33 (3H, s), 1.57 (9H, s); ^{13}C NMR ($CDCl_3$, δ): 165.8, 160.0, 147.5, 147.2, 143.6, 140.6, 136.8, 133.9, 126.5, 125.4, 118.8, 115.4, 115.2, 106.7, 81.5, 61.6, 28.4 (3C); MS (ESI, 30 eV): m/z 723.2 (2M + K)$^+$, 707.3 (2M + Na)$^+$, 381.2 (M + K)$^+$, 365.2 (M + Na)$^+$, 343.0 (M + H)$^+$.

*4.1.2.5.2. tert-Butyl E-3-(5-methoxypsoralen-8-yl)propenoate (**30**).* Reaction time 12 h; yield: 51% (0.24 g of pale yellow solid); mp 172–175 °C; R_f = 0.18 (A); IR (KBr, cm^{-1}): ν 1724, 1572, 1278, 1144, 1044; found C, 66.48; H, 5.61. Calc. for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30%; ^{1}H NMR ($CDCl_3$, δ): 8.12 (1H, d, J = 16.4 Hz), 8.11 (1H, d, J = 9.8 Hz), 7.68 (1H, d, J = 2.4 Hz), 7.07 (1H, d, J = 2.4 Hz), 7.03 (1H, d, J = 16.4 Hz), 6.27 (1H, d, J = 9.8 Hz), 4.31 (3H, s), 1.53 (9H, s); ^{13}C NMR ($CDCl_3$, δ): 166.4, 160.3, 147.4 (2C), 144.3, 143.2, 133.1, 135.0, 128.5, 121.7, 116.4 (2C), 108.0, 106.1, 81.8, 62.2, 28.2 (3C); MS (ESI, 30 eV): m/z 381.0 (M + K)$^+$, 365.0 (M + Na)$^+$, 343.2 (M + H)$^+$.

*4.1.2.6. General procedure for the synthesis of aldehydes **21** and **32**.* To a solution (−10 °C) of boronate, TDBBAl (0.16 g, 0.7 mmol) and bromide **12** or **26** (0.17 g, 0.56 mmol) in anhydrous THF (5 mL), K_2CO_3 (0.23 g, 1.7 mmol) was added and the resulting mixture was degassed. Then $PdCl_2$(dppf) (0.033 g, 0.04 mmol) was added and the resulting mixture was redegassed. The resulting reaction mixture was refluxed until the consumption of the starting material (TLC monitoring). Then, the obtained mixture was passed through a short pad of celite. The filtrate was evaporated to dryness and the residue was subjected to flash column chromatography to give pure product, which crystallized upon addition of Et_2O. The crystalline product was filtered, washed on the filter, and dried under reduced pressure to give pure aldehyde.

*4.1.2.6.1. 4-(8-Methoxypsoralen-5-yl)benzaldehyde (**21**).* Reaction time: 4 h; yield: 80% (0.14 g of pale yellow solid); mp 207–210 °C; R_f = 0.31 (B); IR (KBr, cm^{-1}): ν 1766, 1712, 1604; found C, 71.04; H, 3.57. Calc. for $C_{19}H_{12}O_5$: C, 71.25; H, 3.78%; ^{1}H NMR ($CDCl_3$, δ): 10.14 (1H, s), 8.06 (2H, d, J = 8.4 Hz), 7.73 (1H, d, J = 9.8 Hz), 7.71 (d, 1H, J = 2.4 Hz), 7.58 (2H, d, J = 8.4 Hz), 6.63 (1H, d, J = 2.4 Hz), 6.34 (1H, d, J = 9.8 Hz), 4.35 (3H, s); ^{13}C NMR ($CDCl_3$, δ): 191.8, 160.2, 147.3 (2C), 143.8, 142.1, 142.0, 136.2, 133.0, 131.3 (2C), 130.3 (2C), 126.0, 125.1, 115.2, 114.4, 106.4, 61.7; MS (ESI, 30 eV): m/z 679.0 (2M + K)$^+$, 663.1 (2M + Na)$^+$, 343.1 (M + Na)$^+$, 321.3 (M + H)$^+$.

*4.1.2.6.2. 4-(5-Methoxypsoralen-8-yl)benzaldehyde (**32**).* Reaction time: 4 h; yield: 92% (0.11 g of white solid); mp 230–233 °C; R_f = 0.25 (B); IR (KBr, cm^{-1}): ν 1738, 1708, 1604, 1397, 1108; found C, 71.10; H, 3.52. Calc. for $C_{19}H_{12}O_5$: C, 71.25; H, 3.78%; ^{1}H NMR ($CDCl_3$, δ): 10.05 (1H, s), 8.24 (1H, d, J = 9.8 Hz), 7.96 (2H, d, J = 8.0 Hz), 7.86 (2H, d, J = 8.0 Hz), 7.62 (1H, d, J = 2.2 Hz), 7.11 (1H, d, J = 2.2 Hz), 4.31 (3H, s); ^{13}C NMR ($CDCl_3$, δ): 192.8, 160.7, 145.3, 139.6, 138.3, 137.0, 135.8, 135.4 (2C), 131.3, 130.0 (2C), 129.0 (2C), 128.9 (2C), 113.0, 105.5, 61.7; MS (ESI, 30 eV): m/z 663.1 (2M + Na)$^+$, 343.1 (M + Na)$^+$, 321.2 (M + H)$^+$.

*4.1.2.7. General procedure for the syntheses of tert-butyl esters **24** and **35**.* To a solution of aldehyde **21** or **32** (0.23 g, 0.7 mmol) in $CHCl_3$ (1.5 mL), the stabilized phosphorane $Ph_3P{=}CHCO_2^tBu$ (0.24 g, 0.9 mmol) was added. The resulting reaction mixture was stirred at room temperature until the completion of the reaction. Then, the reaction mixture was evaporated and the residue was subjected to flash column chromatography to give the anticipated ester.

*4.1.2.7.1. tert-Butyl E-3-[4-(8-methoxypsoralen-5-yl)phenyl]propenoate (**24**).* Ester **24** was obtained as an inseparable mixture of the two isomers [*E*:*Z* = 9:1 (based on ^{1}H NMR)]. Recrystallization from AcOEt gave pure ester *E*-**24** as white solid. Reaction time: 3 h; yield: 56% (0.16 g); mp 112–114 °C; R_f = 0.44 (B); IR (KBr, cm^{-1}): ν 1734, 1690, 1636, 1586, 1322, 1148, 1092, 762; found C, 71.52; H, 5.41. Calc. for $C_{25}H_{22}O_6$: C, 71.76; H, 5.30; ^{1}H NMR ($CDCl_3$, δ): 7.78 (1H, d, J = 10.0 Hz), 7.68 (1H, d, J = 2.0 Hz), 7.67 (2H, d, J = 8.4 Hz), 7.66 (1H, d, J = 15.0 Hz), 7.40 (1H, d, J = 8.4 Hz), 6.66 (1H, d, J = 2.0 Hz), 6.46 (1H, d, J = 16.0 Hz), 6.32 (1H, d, J = 10.0 Hz), 4.34 (3H, s), 1.56 (9H, s); ^{13}C NMR ($CDCl_3$, δ): 166.3, 160.4, 147.2, 147.0, 144.0, 142.7, 142.5, 137.4, 134.9, 132.7, 131.1 (2C), 128.5 (2C), 126.0, 121.5, 114.8, 114.5 (2C), 106.4, 81.1, 61.7, 28.4 (3C); MS (ESI, 30 eV): m/z 441.3 (M + Na)$^+$, 419.1 (M + H)$^+$.

*4.1.2.7.2. tert-Butyl E-3-[4-(5-methoxypsoralen-8-yl)phenyl]propenoate (**35**).* Reaction time: 3 h; yield: 64% (0.19 g of a white solid); mp 120–123 °C; R_f = 0.43 (B); IR (KBr, cm^{-1}): ν 1739, 1692, 1636, 1586, 1148, 1092, 762; found C, 71.48; H, 5.47. Calc. for $C_{25}H_{22}O_6$: C, 71.76; H, 5.30%; ^{1}H NMR ($CDCl_3$, δ): 8.22 (1H, d, J = 9.8 Hz), 7.71 (2H, d, J = 8.2 Hz), 7.65 (1H, d, J = 15.8 Hz), 7.64 (2H, d, J = 8.2 Hz) 7.62 (1H, d, J = 2.4 Hz), 7.08 (1H, d, J = 2.4 Hz), 6.42 (1H, d, J = 15.8 Hz), 6.31 (1H, d, J = 9.8 Hz), 4.31 (3H, s), 1.56 (9H, s); ^{13}C NMR ($CDCl_3$, δ): 166.4, 160.8, 156.1, 148.8, 145.0, 143.7 (2C), 139.4, 134.2 (2C), 131.9, 130.9 (2C), 128.0 (2C), 120.5, 113.8 (3C), 105.2, 80.6, 60.3, 28.3 (3C); MS (ESI, 30 eV): m/z 858.9 (2M + Na)$^+$, 441.3 (M + Na)$^+$, 419.2 (M + H)$^+$.

*4.1.2.8. General procedure for the acidolysis of esters **17**, **24**, **30** and **35**. Preparation of carboxylic acids **16**, **23**, **29** and **34**.* An ice-cold solution (0 °C) of esters **17**, **24**, **30** or **35** (0.1 g, 0.24 mmol) in TFA and CH_2Cl_2 (1:1, 2 mL) was kept at this temperature for 30 min. Then, acidolysis was continued at room temperature until the completion of the reaction. Evaporation of the volatile components under reduced pressure left a residue, which was triturated with Et_2O. Overnight cooling produced product as solid, which was filtered, washed (ice-cold Et_2O) and dried under vacuo to give the anticipated pure acids.

*4.1.2.8.1. E-3-(8-Methoxypsoralen-5-yl)propenoic acid (**16**).* Reaction time 2 h; yield: 88% (0.06 g of white); mp 232 °C (dec.); R_f = 0.34 (E); IR (KBr, cm^{-1}): ν 3700–3250, 1732, 1694, 1574; found C, 62.83; H, 3.67. Calc. for $C_{15}H_{10}O_6$: C, 62.94; H, 3.52%; ^{1}H NMR (DMSO-d_6, δ): 12.71 (1H, br s), 8.36 (1H, d, J = 10.2 Hz), 8.23 (1H, d, J = 2.0 Hz), 8.11 (1H, d, J = 16.0 Hz), 7.33 (1H, d, J = 2.0 Hz), 6.48 (1H, d, J = 10.2 Hz), 6.45 (1H, d, J = 16.0 Hz), 4.23 (3H, s); ^{13}C NMR (DMSO-d_6, δ): 167.0, 159.2, 149.0, 146.4, 142.9, 141.4, 136.9, 132.9,

125.4, 124.8, 118.6, 114.9 (2C), 106.6, 61.2; MS (ESI, 30 eV): *m*/*z* 309.5 $(M + Na)^+$, 287.1 $(M + H)^+$.

*4.1.2.8.2. E-3-(5-Methoxypsoralen-8-yl)propenoic acid (**23**).* Reaction time 2 h; yield: 90% (0.062 g of white solid); mp 229 °C (dec.); $R_f = 0.27$ (E); IR (KBr, cm^{-1}): ν 3700–3250, 1738, 1694, 1622, 1574; found C, 62.78; H, 3.63. Calc. for $C_{15}H_{10}O_6$: C, 62.94; H, 3.52%; ^{1}H NMR (DMSO-d_6, δ): 8.24 (1H, d, J = 9.8 Hz), 8.21 (1H, d, J = 2.4 Hz), 8.01 (1H, d, J = 16.2 Hz), 7.65 (1H, d, J = 2.4 Hz), 6.96 (1H, d, J = 16.2 Hz), 6.41 (1H, d, J = 9.8 Hz), 4.34 (3H, s); ^{13}C NMR (DMSO-d_6, δ): 166.5, 158.6, 148.5 (2C), 142.4, 140.9, 136.4, 132.4, 124.9 (2C), 118.0, 114.4 (2C), 106.2, 60.6; MS (ESI, 30 eV): *m*/*z* 309.5 $(M + Na)^+$, 287.1 $(M + H)^+$.

*4.1.2.8.3. E-3-[4-(8-Methoxypsoralen-5-yl)phenyl]propenoic acid (**29**).* Reaction time 1 h; yield: 81% (0.07 g of white solid); mp 213 °C (dec.); R_f = 0.31 (E); IR (KBr, cm^{-1}): ν 3600–3250, 1707, 1685, 1626, 1557; found C, 69.49; H, 4.01. Calc. for $C_{21}H_{14}O_6$: C, 69.61; H, 3.89%; ^{1}H NMR (DMSO-d_6, δ): 12.49 (1H, br s), 8.16 (1H, d, J = 2.4 Hz), 7.90 (2H, d, J = 8.0 Hz), 7.82 (1H, d, J = 9.8 Hz), 7.71 (1H, d, J = 16.0 Hz), 7.49 (2H, d, J = 8.0 Hz), 6.81 (1H, d, J = 2.4 Hz), 6.65 (1H, d, J = 16.0 Hz), 6.41 (1H, d, J = 9.8 Hz), 4.23 (3H, s); ^{13}C NMR (DMSO-d_6, δ): 167.9, 159.8, 148.8, 146.8, 143.6, 143.5, 143.0, 137.1, 134.6, 132.0, 131.3 (2C), 129.1 (2C), 126.2, 125.8, 120.5, 114.9, 114.3, 106.7, 61.6; MS (ESI, 30 eV): *m*/*z* 363.6 $(M + H)^+$.

*4.1.2.8.4. E-3-[4-(5-Methoxypsoralen-8-yl)phenyl]propenoic acid (**34**).* Reaction time 1 h; yield: 82% (0.07 g of white solid); mp 238 °C (dec.); R_f = 0.34 (E); IR (KBr, cm^{-1}): ν 3600–3400, 1739, 1693, 16.22, 1579; found C, 69.53; H, 3.99. $C_{21}H_{14}O_6$: C, 69.61; H, 3.89%; ^{1}H NMR (DMSO-d_6, δ): 12.44 (1H, br s), 8.11 (1H, d, J = 2.4 Hz), 7.94 (1H, d, J = 9.8 Hz), 7.84 (2H, d, J = 8.0 Hz), 7.65 (1H, d, J = 16.0 Hz), 7.44 (2H, d, J = 8.0 Hz), 6.76 (1H, d, J = 2.4 Hz), 6.60 (1H, d, J = 16.0 Hz), 6.36 (1H, d, J = 9.8 Hz), 4.18 (3H, s); ^{13}C NMR (DMSO-d_6, δ): 167.4, 161.8, 157.1, 150.0, 149.9, 146.0, 144.2 (2C), 140.4, 135.2 (2C), 131.9 (2C), 129.0 (2C), 125.2, 121.5, 113.9, 113.7, 106.2, 61.4; MS (ESI, 30 eV): 762.9 $(2M + K)^+$, 746.9 $(2M + Na)^+$, 400.9 $(M + K)^+$, 385.2 $(M + Na)^+$.

*4.1.2.9. General procedure for the preparation of acids **22** and **33***

*4.1.2.9.1. From aldehydes **21** and **32**.* To a suspension of aldehyde **21** or **32** (0.04 g, 0.11 mmol) in DMF (1 mL), Oxone® (0.21 g, 0.33 mmol) was added and the resulting mixtures were stirred at 25 °C (**21**) or at 60 °C (**32**) for 2–3 d. After completion of the reaction, the resulting mixtures were subjected to FCC to give the corresponding acids.

*4.1.2.9.1.1. 4-(8-Methoxypsoralen-5-yl)benzoic acid (**22**).* Reaction time 3 d; yield: 43% (0.016 g of white solid); mp 220 °C (dec.); R_f = 0.12 (D); IR (KBr, cm^{-1}): ν 3180–2500, 1735, 1698; found C, 67.92; H, 3.41. $C_{19}H_{12}O_6$: C, 67.86; H, 3.60%; ^{1}H NMR (DMSO-d_6, δ): 12.70 (1H, br s), 8.17 (1H, d, J = 2.4 Hz), 8.12 (2H, d, J = 8.2 Hz), 7.80 (1H, d, J = 9.8 Hz), 7.57 (2H, d, J = 8.2 Hz), 6.81 (1H, d, J = 2.4 Hz), 6.42 (1H, d, J = 9.8 Hz), 4.23 (3H, s); ^{13}C NMR (DMSO-d_6, δ): 168.9, 160.8, 149.8, 147.8, 144.6, 144.5, 144.0, 138.1, 135.6, 133.0, 132.3 (2C), 130.1 (2C), 126.8, 121.5, 115.9, 107.7, 62.6; MS (ESI, 30 eV): *m*/*z* 711.1 $(2M + K)^+$, 695.4 (2M + Na), 375.5 $(M + K)^+$, 359.6 $(M + Na)^+$.

*4.1.2.9.1.2. 4-(5-Methoxypsoralen-8-yl)benzoic acid (**33**).* Reaction time 2 d; yield: 38% (0.014 g of white solid); mp 228 °C (dec.); R_f = 0.33 (E); IR (KBr, cm^{-1}): ν 3600–3380, 1737, 1691, 1610, 1594, 1104; found C, 67.83; H, 3.48. $C_{19}H_{12}O_6$: C, 67.86; H, 3.60%; ^{1}H NMR (DMSO-d_6, δ): 7.78 (1H, d, J = 9.8 Hz), 7.68 (1H, d, J = 2.0 Hz), 7.62 (2H, d, J = 8.0 Hz), 7.40 (2H, d, J = 8.0 Hz), 6.66 (1H, d, J = 2.0 Hz), 6.32 (1H, d, J = 9.8 Hz), 4.34 (3H, s); ^{13}C NMR (DMSO-d_6, δ): 167.6, 162.0, 157.3, 146.2 (2C), 144.4 (2C), 140.6 (2C), 135.4 (2C), 132.1 (2C), 129.2 (2C), 121.7, 113.9, 106.4, 61.5; MS (ESI, 30 eV): *m*/*z* 375.5 $(M + K)^+$, 359.5 $(M + Na)^+$, 291.7 $(M-COOH)^+$.

*4.1.2.9.2. From bromide **12**.* To a solution (−10 °C) of boronate TDBBA (0.11 g, 0.44 mmol) and bromide **12** (0.1 g, 0.34 mmol) in anhydrous THF (3 mL), K_2CO_3 (0.09 g, 0.68 mmol) was added and the resulting mixture was degassed. Then, $PdCl_2$(dppf) (0.02 g, 0.02 mmol) was added and the resulting mixture was redegassed. The resulting reaction mixture was heated at 67 °C for 1 d. After the completion of the reaction, the mixture was acidified with 2 N aq. HCl solution to pH 1 and passed through a short pad of celite. The filtrate was evaporated to dryness and the residue was subjected to flash column chromatography to give pure product, which was crystallized upon addition of Et_2O. The thus obtained acid **22** had identical physical data with the sample obtained from **21**.

*4.1.2.10. General procedure for the preparation of esters **6**, **18**–**20** and **34**.* To an ice-cold (0 °C) suspension or solution of acids **4**, **16** or **31** (0.1 mmol), the corresponding alcohol (0.15 mmol) and TPP (0.032 g, 0.12 mmol) in freshly distilled THF (0.34 mL), DIAD (24 μL, 0.12 mmol) was added. The reaction mixtures were stirred at room temperature for 1–2 h. The anticipated esters **6**, **18**–**20** and **34** were obtained pure after FCC purification.

*4.1.2.10.1. Ethyl (E)-3-(trioxsalen-4′-yl)propenoate (**6**).* Reaction time: 2 h; yield: 92% (0.05 g of white solid); mp 191–192 °C; R_f = 0.31 (B); IR (KBr, cm^{-1}): ν 1738, 1712, 1636, 1570, 1160, 752; found C, 69.81; H, 5.68. Calc. for $C_{19}H_{18}O_5$: C, 69.93; H, 5.56%; ^{1}H NMR (DMSO-d_6, δ): 7.92 (1H, d, J = 16.0 Hz), 7.89 (1H, s), 6.87 (1H, d, J = 16.0 Hz), 6.29 (1H, s), 4.24 (2H, q, J = 7.2 Hz), 2.63 (3H, s), 2.46 (3H, s), 2.37 (3H, s), 1.29 (3H, t, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6, δ); 164.4, 163.9, 163.4, 155.2, 154.8, 149.9, 140.2, 121.9, 117.6, 115.0, 113.6, 113.2, 111.5, 109.4, 61.6, 19.9, 14.5, 13.7, 9.1; GC–MS (EI, 20 eV): t_R = 13.95 min, *m*/*z* 326 (M^+, 100%), 297 ($M-C_2H_5$, 13), 281 ($M-OC_2H_5$, 10), 252 ($M-HCO_2C_2H_5$, 19).

*4.1.2.10.2. Ethyl E-3-(8-methoxypsoralen-5-yl)propenoate (**18**).* Reaction time: 1.5 h; yield: 89% (0.028 g of white solid); mp 129–132 °C; R_f = 0.26 (B); IR (KBr, cm^{-1}): ν 1728, 1636, 1576, 1164, 752; found C, 64.82; H, 4.56. Calc. for $C_{17}H_{14}O_6$: C, 64.97; H, 4.49%; ^{1}H NMR (DMSO-d_6, δ): 8.38 (1H, d, J = 10.0 Hz), 8.23 (1H, d, J = 2.4 Hz), 8.16 (1H, d, J = 16.0 Hz), 7.36 (1H, d, J = 2.4 Hz), 6.51 (1H, d, J = 16.0 Hz), 6.49 (1H, d, J = 10.0 Hz), 4.25 (2H, q, J = 7.2 Hz), 4.24 (3H, s), 1.30 (3H, t, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6, δ): 165.8, 159.1, 149.0, 146.3, 142.9, 141.4, 137.4, 133.1, 125.0, 124.2, 118.3, 115.0 (2C), 106.6, 61.2, 60.5, 14.2; MS (ESI, 30 eV): *m*/*z* 667.1 $(2M + K)^+$, 651.1 $(2M + Na)^+$, 353.2 $(M + K)^+$, 337.2 $(M + Na)^+$.

*4.1.2.10.3. n-Butyl E-3-(8-methoxypsoralen-5-yl)propenoate (**19**).* Reaction time: 1 h; yield: 85% (0.03 g of white solid); mp 120–123 °C; R_f = 0.34 (B); IR (KBr, cm^{-1}): ν 1724, 1630, 1572, 1144, 1044, 750; found C, 66.85; H, 5.14. Calc. for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30%; ^{1}H NMR ($CDCl_3$, δ): 8.15 (1H, d, J = 16.0 Hz), 8.14 (1H, d, J = 10.0 Hz), 7.76 (1H, d, J = 2.4 Hz), 7.07 (1H, d, J = 2.4 Hz), 6.45 (1H, d, J = 10.0 Hz), 6.44 (1H, d, J = 16.0 Hz), 4.34 (3H, s), 4.26 (2H, t, J = 6.4 Hz), 1.72 (2H, quint., J = 7.2 Hz), 1.45 (2H, sext., J = 7.6 Hz), 0.98 (3H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, δ): 166.1, 159.9, 147.7 (2C), 147.2, 143.6, 140.5, 134.1, 125.5, 124.6, 118.5, 115.6, 115.4, 106.6, 65.1, 61.6, 31.0, 19.4, 14.0; MS (ESI, 30 eV): *m*/*z* 723.1 $(2M + K)^+$, 707.1 $(2M + Na)^+$.

*4.1.2.10.4. 3-Phenoxybenzyl E-3-(8-methoxypsoralen-5-yl)propenoate (**20**).* Reaction time: 2 h; yield: 92% (0.042 g of colorless oil); R_f = 0.48 (B); IR (neat, cm^{-1}): ν 1724, 1630, 1572, 744; found C, 71.69; H, 4.50. Calc. for $C_{28}H_{20}O_7$: C, 71.79; H, 4.30%; ^{1}H NMR ($CDCl_3$, δ): 8.20 (1H, d, J = 15.8 Hz), 8.13 (1H, d, J = 10.0 Hz), 7.76 (1H, d, J = 2.2 Hz), 7.35–7.29 (4H, m), 7.14–7.08 (3H, m), 7.06 (1H, d, J = 2.2 Hz), 7.03–6.98 (2H, m), 6.49 (1H, d, J = 15.8 Hz), 6.45 (1H, d, J = 10.0 Hz), 5.27 (2H, s), 4.36 (3H, s); ^{13}C NMR ($CDCl_3$, δ): 157.8, 157.2, 147.7 (2C), 143.1 (2C), 140.4, 138.6, 137.9, 130.2, 130.1, 130.0, 129.9 (2C), 123.9, 123.8, 123.6, 123.1, 121.7, 119.3 (2C), 118.1, 117.3, 115.7, 115.5, 106.6, 66.5, 61.6; HRMS-ESI (*m*/*z*): $[M + Na]^+$. Calc. for $C_{28}H_{20}NaO_7$, 491.11012; found, 491.11058.

*4.1.2.10.5. Ethyl E-3-(5-methoxypsoralen-8-yl)propenoate (**31**).* Reaction time: 1.5 h; yield: 88% (0.027 g of white solid); mp 164–167 °C; R_f = 0.31 (B); IR (KBr, cm^{-1}): ν 1733, 1633, 1571, 1164, 1034, 752; found C, 64.79; H, 4.52. Calc. for $C_{17}H_{14}O_6$: C, 64.97; H, 4.49%; ^{1}H NMR (DMSO-d_6, δ): 8.22 (1H, d, J = 9.6 Hz), 8.19 (1H, d, J = 2.4 Hz), 8.03 (1H, d, J = 16.4 Hz), 7.53 (1H, d, J = 2.4 Hz), 6.99 (1H, d, J = 16.4 Hz), 6.40 (1H, d, J = 9.6 Hz), 4.34 (3H, s), 4.24 (2H, q, J = 7.2 Hz), 1.29 (3H, t, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6, δ): 165.9, 159.4, 149.2 (2C), 141.6 (2C), 137.6, 133.3, 124.4 (2C), 118.5, 115.1 (2C), 106.8, 61.4, 60.6, 14.4; MS (ESI, 30 eV): m/z 651.2 $(2M + Na)^+$, 337.3 $(M + Na)^+$.

4.2. Biological evaluation

4.2.1. General biological

AAPH, DPPH, NDGA and Trolox were purchased from the Aldrich Chemical Co. Milwaukee, WI, (USA). Soybean LOX, linoleic acid sodium salt and indomethacin were obtained from Sigma Chemical, Co. (St. Louis, MO, USA) and carrageenin, type K, was commercially available. For the *in vivo* experiments, male and female Fischer-344 rats (180–240 g) were used. For the *in vitro* tests a Lambda 20 (Perkin–Elmer) UV–vis double beam spectrophotometer was used. Each *in vitro* experiment was performed at least in triplicate and the standard deviation of absorbance was less than 10% of the mean.

4.2.2. In vitro assays

4.2.2.1. Determination of the reducing activity of the stable radical DPPH. To an ethanolic solution of DPPH (0.05 mM) in absolute ethanol an equal volume of the compounds dissolved in DMSO was added. The mixture was shaken vigorously and allowed to stand for 20 min or 60 min; absorbance at 517 nm was determined spectrophotometrically and the percentage of activity was calculated. All tests were undertaken on three replicates and the results were averaged (Tables 1–3).

4.2.2.2. Soybean lipoxygenase inhibition study in vitro. The tested compounds dissolved in DMSO were incubated at room temperature with sodium linoleate (0.1 mL) and 0.2 mL of enzyme solution ($1/9 \times 10^{-4}$ w/v in saline). The conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was recorded and compared with the appropriate standard inhibitor.

4.2.2.3. Inhibition of linoleic acid lipid peroxidation. Production of conjugated diene hydroperoxide by oxidation of linoleic acid in an aqueous dispersion is monitored at 234 nm. AAPH is used as a free radical initiator. This assay can be used to follow oxidative changes and to understand the contribution of each tested compound.

Azo compounds generating free radicals through spontaneous thermal decomposition are useful for *in vitro* studies of free radical production. The water-soluble azo compound AAPH has been extensively used as a clean and controllable source of thermally produced alkylperoxyl free radicals. Ten microliters of the 16 mM linoleic acid dispersion was added to the UV cuvette containing 0.93 mL of 0.05 M phosphate buffer, pH 7.4 prethermostated at 37 °C. The oxidation reaction was initiated at 37 °C under air by the addition of 50 μL of 40 mM AAPH solution. Oxidation was carried out in the presence of aliquots (10 μL) in the assay without antioxidant, lipid oxidation was measured in the presence of the same level of DMSO. The rate of oxidation at 37 °C was monitored by recording the increase in absorption at 234 nm caused by conjugated diene hydroperoxides.

4.2.3. In vivo assays

4.2.3.1. Inhibition of the carrageenin-induced rat paw edema. Edema was induced in the right hind paw of Fisher 344 rats (150–200 g) by the intradermal injection of 0.1 mL 2% carrageenin in water. Both sexes were used. Females pregnant were excluded. Each group was composed of 6–15 animals. The animals, which have been bred in our laboratory, were housed under standard conditions and received a diet of commercial food pellets and water ad libitum during the maintenance but they were entirely fasted during the experiment period. Our studies were in accordance with recognized guidelines on animal experimentation. The tested complexes 0.01 mmol/kg body weight, were diluted in water and they were given intraperitoneally simultaneously with the carrageenin injection. The rats were euthanized 3.5 h after carrageenin injection. The difference between the weight of the injected and uninjected paws was calculated for each animal. The change in paw weight was compared with that in control animals (treated with water) and expressed as a percent inhibition of the oedema% ICPE values (Table 2 and Fig. 2). Indomethacin was tested as a reference compound in 0.01 mmol/kg (47%). Values% CPE are the mean from two different experiments with a standard error of the mean less than 10%.

4.2.3.2. Evaluation of the cytocompatibility of methoxypsoralen derivatives. The cytocompatibility of the compounds was evaluated by the viability of 1BR3 cells (ECACC, HPA, UK) cells in the presence of different concentrations of the compounds (1–100 μM) using the MTT test [31]. The cells were seeded in 24-well plates at a density of 20,000 cells per well in 500 μL EMEM medium (ATCC, USA) supplemented with 15% fetal bovine serum, 2 mM glutamine, 1% non essential aminoacids, 100 IU/mL penicillin, 100 μg/mL streptomycin and 2.5 μg/mL amphotericin B. Twenty-four hours after plating, different amounts of the compounds were added in the wells. After 24 h of incubation at 37 °C, 50 μL of MTT solution (5 mg/mL in phosphate buffered saline, pH 7.4) were added into each well and the plates were incubated at 37 °C for 2 h. The medium was withdrawn and 200 μL of DMSO was added in each well and agitated thoroughly to dissolve the formazan crystals. The solution was transferred to 96-well plates and immediately read on a microplate reader, at a wavelength of 540 nm. The experiments were performed in hexaplicate. Cell viability was calculated from the ratio between the absorbance provided by the cells treated with the different compounds and the absorbance provided by the non-treated cells (control).

4.3. Computational methods, docking simulations

All the molecules were constructed with ChemDraw program [32] and converted in 3D-Structures with the OpenBabel program [33], by using MMFF94 force field. Protein setup was performed using the UCSF Chimera software [34,35]. AnteChamber PYthon Parser interfacE (ACPYPE) tool [36] was employed to generate the topologies of the ligands. ACPYPE tool is written in python to use Antechamber [36,37] to generate topologies for chemical compounds was used for the parametrization of the ligands. Energy minimizations where carried out with the molecular simulation toolkit GROMACS [38] using the AMBER99SB-ILDN force field [39].

Docking calculations were performed with the software Autodock Vina [40,41] PyRx program [42] was employed to generate the docking input files and to analyze the docking results. The proteins were considered rigid. Performing a blind docking the protein 1RRH soybean lipoxygenase all the molecules were properly aligned in the active site. For all the ligands, the single bonds were considered as active torsional bonds. Docking was carried out with an exhaustiveness value of 64 and a maximum output of 100 binding modes. The results of ligands from protein 1RRH presented identical conformation in the binding site of 1RRH as they were crystallized in 1IK3.

The final output of the docking procedure is a set of solutions ranked according to the corresponding scoring function values, each defined by the 3D coordinates of its atoms and expressed as a PDB file.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.11.043. These data include MOL files and InChiKeys of the most important compounds described in this article.

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