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Original article

Syntheses and evaluation of the antioxidant activity of acitretin analogs with amide bond(s) in the polyene spacer

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ABSTRACT

Ester analogs of the antipsoriatic drug acitretin were synthesized by coupling either anilines with N-protected indole-3-carboxylic acid, followed by deprotection and coupling with O-monoprotected dicarboxylic acids or Wittig reaction of indole-3-carboxaldehyde, 3-acetyl-1-tosylpyrrole and 4-amino-9-fluorenone with $Ph_3P{=}CHCO_2tBu$, followed by N-deprotection, where necessary, and finally coupling with cinnamoyl fluorides. Corresponding free acids were obtained through TFA-mediated carboxyl group deprotection. Although these analogs and acitretin showed very low reducing abilities, analogs **5**, **6**, **8** and **12** strongly inhibited LOX with IC_{50} values ranging from 35–65 μM. Acitretin and its analogs **5**–**7**, **10**, **11** and **15** inhibited lipid peroxidation more strongly than trolox whereas acitretin and analog **4** were in vivo more potent anti-inflammatory agents on rat paw oedema induced by Carrageenan than indomethacin.

1. Introduction

Acitretin (ACI) is a second generation retinoid which is currently used orally as a drug against severe psoriasis and other skin disorders [1a]. Its mechanism of action is not completely understood, and there are few studies focusing on histological and immunohistochemical differences before and after treatment of psoriasis with ACI [1b]. Acitretin is known to re-establish a more normal pattern of cell growth and, in a lesser degree, to have some effect on immune cell processes [1b]. Plewig and Wagner noted in vivo significant anti-inflammatory effects with retinoids [2a]. Acitretin was found to inhibit superoxide anion formation [2b]. Its use is however associated by a number of unwanted side-effects [2c]. The molecule of ACI incorporates an aromatic lipophilic part which is connected to a carboxylic group (the hydrophilic part of the retinoid) through a conjugated tetraene chain (Fig. 1). In an effort to improve the therapeutic index of this compound and its selectivity towards several biological targets, a variety of ACI analogs have been synthesized [3].

We have recently described the synthesis of easily assembled ACI analogs in which two double bonds of the tetraene chain have been replaced by the isosteric amide bond and a third one has been restricted within the heterocyclic ring of either indole (see e.g. compounds **1**, **2** and **3**, Fig. 1) or pyrrole. In addition, the remaining fourth E double bond has been either retained (**1**) or isomerized (**2**) or reduced (**3**) whereas the aromatic part of ACI was varied at will by choosing the appropriate anilines [4]. For the purpose of the present study, four new analogs with changes in the aromatic part, namely compounds **4**–**7**, were synthesized. We reasoned that similar changes may be introduced in the tetraene chain by using cinnamic acids in the place of anilines as exemplified with ACI analogs **8** and **9** and their corresponding *tert*-butyl esters **10** and **11** (Fig. 1). As it will be described below, attempted synthesis of **9** eventually led to the isomeric acid **12**. Furthermore, the indole residue may be substituted by either the pyrrole (analog **13**) or the 4-amino-9-fluorenone (analogs **14** and **15**) moieties, whereas structural characteristics of the two types of ACI analogs may be combined as exemplified with analog **16**. Earlier studies have shown that inclusion of aromatic heterocyclic rings in retinoids is associated with reduced toxicity [5]. The involvement of these particular heterocyclic rings in the design of our analogs stemmed from the anticipation that they could be readily incorporated into the spacer by using the commercially available indole-3-carboxylic acid and -carboxaldehyde or the readily synthesized

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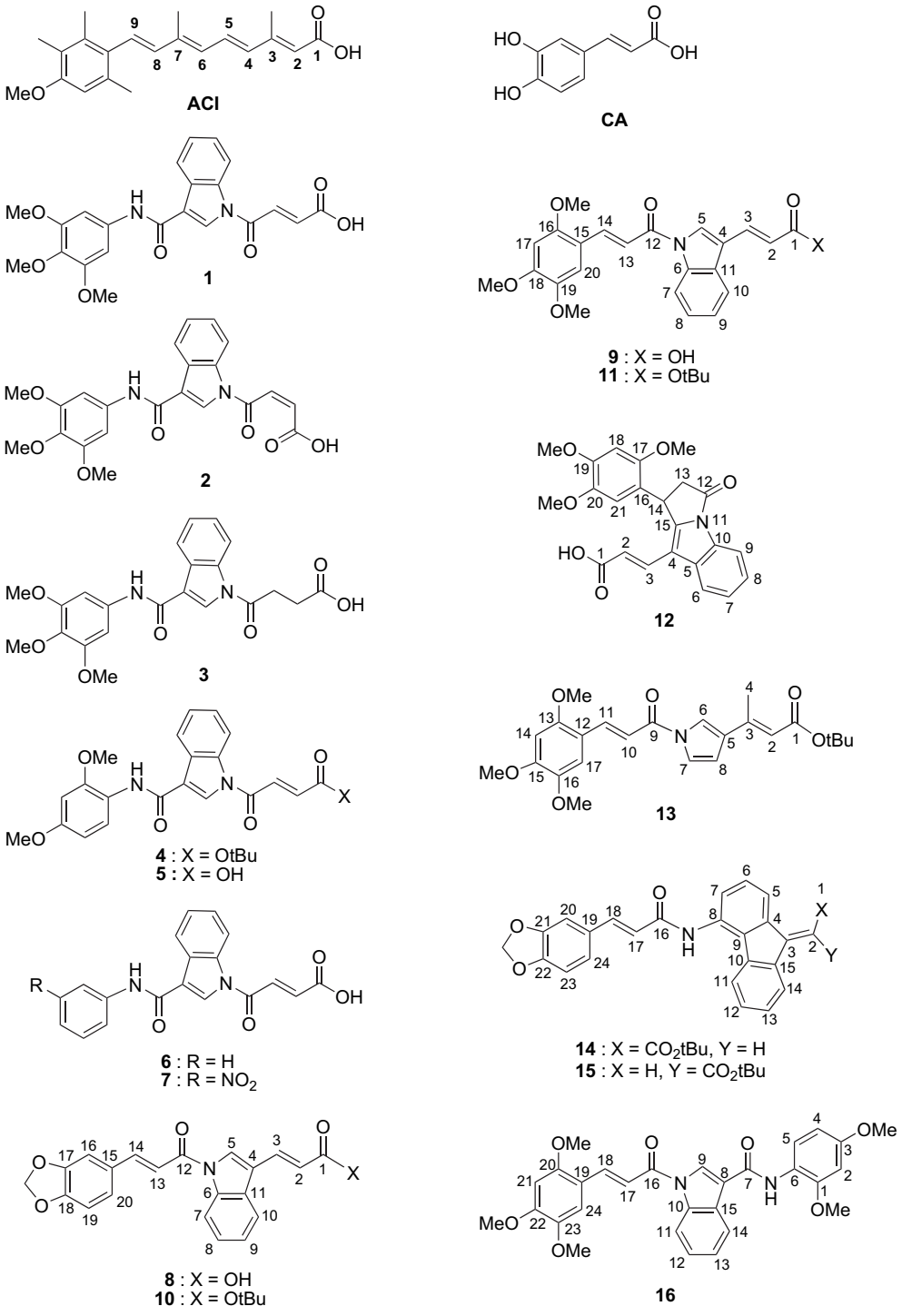


Fig. 1. Structures of compounds encountered in the present work.

3-acetylpyrrole, which include the hydrolytically stable N–C═C–CO structural motif. The latter, would allow for the replacement of either three or two conjugated double bonds of the spacer by the isosteric structural units NH–CO–C(R)═CH–N(R)–CO (analogs **1**–**7**) and CO–N(R)–CH═C(R) (analogs **8**–**11**, **13**), respectively. Finally, the implication of *tert*-butyl esters in the present study was thought of interest because we had observed, from biological studies of other types of α,β-unsaturated acids, that their corresponding *tert*-butyl esters were more potent inhibitors of lipoxygenase (LOX) than the free acids [6].

Taking into consideration that ACI and the present ACI analogs bear structural characteristics of cinnamic acids with established antioxidant activities, we decided to examine the possibility of ACI and the currently described analogs to act as inhibitors of LOX activity and of the peroxidation of biological membranes as well as to present anti-inflammatory activity. Through these compounds, we could hopefully determine the effect of the following structural elements on the biological activity: (a) the variation of the electron density and/or lipophilicity of the aromatic part, (b) the presence of one or two amide bonds in various positions of the tetraene chain

(spacer), (c) the nature of the heterocyclic ring in the spacer (d) the presence of an E double bond next to the hydrophilic part of the molecule and (e) the presence of a free carboxyl group or its corresponding lipophilic *tert*-butyl ester group at the hydrophilic part of the molecule.

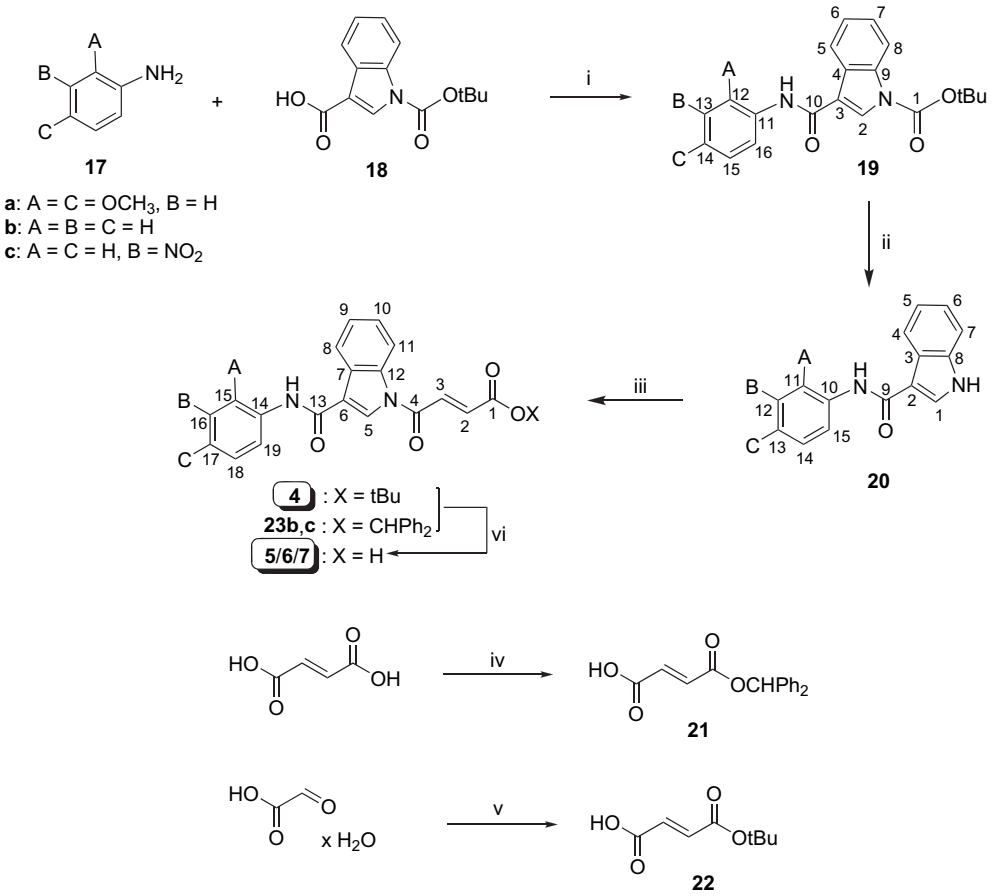
2. Results and discussion

2.1. Chemistry

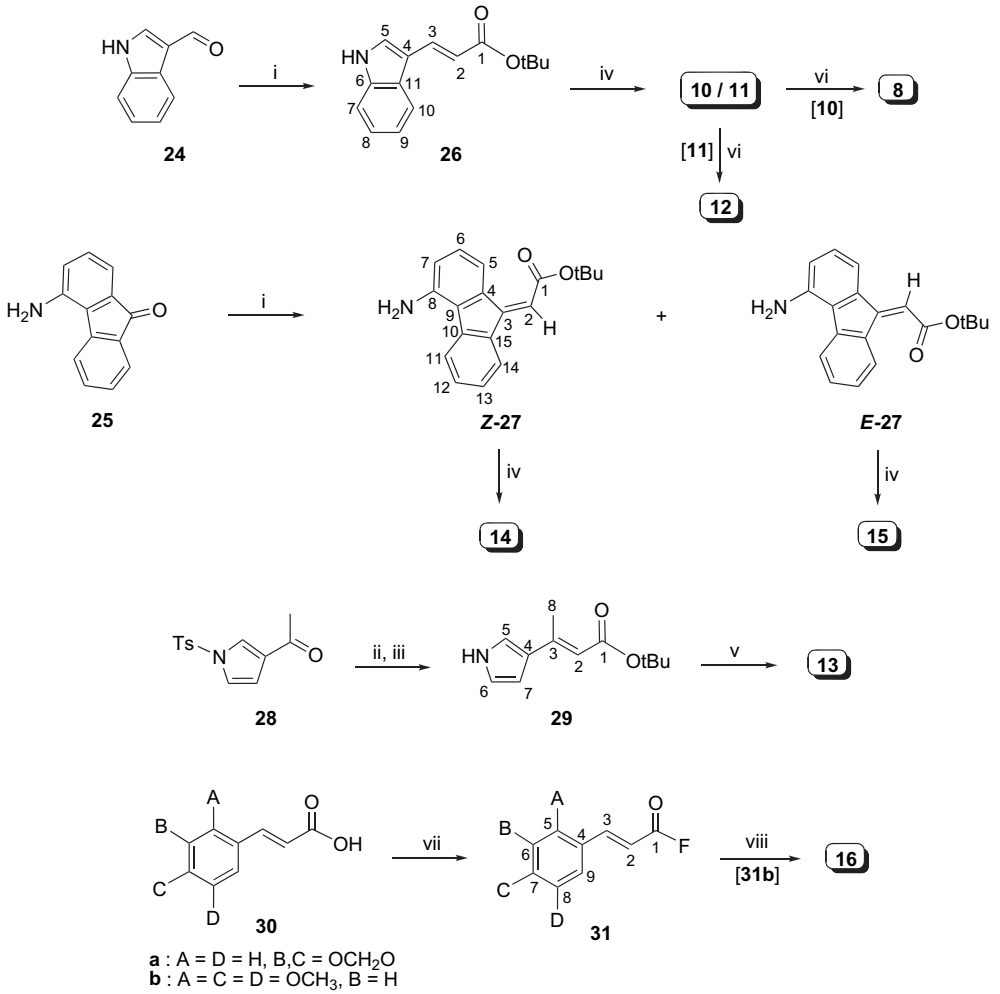
Syntheses of ACI analogs **1**, **2** and **3** have been previously reported [4]. ACI analogs **4**–**7** were synthesized by coupling initially the commercially available anilines **17a**–**c** with 1-(*tert*-butoxycarbonyl)indole-3-carboxylic acid (**18**) [4] using the powerful coupling agent bromotripyrrolidinophosphonium hexafluorophosphate (PyBrOP) and diisopropylethylamine (DIPEA) in $CHCl_3$ at 40 °C for 4 h. That way, the corresponding N-protected anilides **19** were obtained in 74–91% yields (Scheme 1). Removal of the N-protecting group with a 50% solution of trifluoroacetic acid (TFA) in dichloromethane (DCM) at room temperature (RT) for 1 h and successive treatment of the thus obtained trifluoroacetate salt with a 5% $NaHCO_3$ aq. solution, gave the free anilides **20** in 63–70% overall yield.

Attachment of the fumaryl unit was realized by coupling anilides **20** with the suitably *O*-monoprotected fumarates **21** or **22** which were prepared through two different approaches. Thus, *O*-diphenylmethyl fumarate (**21**) was synthesized directly through the reaction of fumaric acid and diphenylmethyl bromide in DMF in the presence of DIPEA at RT. This ester was easily isolated through routine flash column chromatography (FCC) in 34% yield. On the other hand, *O*-*tert*-butyl fumarate (**22**) was synthesized through a Wittig reaction of the commercially available glyoxylic acid monohydrate with the stabilized phosphorane $Ph_3P{=}CHCO_2tBu$ in DMF at RT [7], followed by aqueous work-up. The thus obtained ester **22** had the E configuration, as shown by ^1H NMR. Coupling of anilide **20a** with acid **22** and of anilides **20b**,**c** with acid **21** using PyBrOP in the presence of DIPEA and a catalytic amount of 4-dimethylaminopyridine (DMAP) in DCM, at RT for overnight, afforded the anticipated esters **4** and **23b**,**c** respectively, in 81–86% yields. Routine deprotection of the afore mentioned esters using a 25% or 50% solution of TFA in DCM for 1 h gave the projected ACI analogs **5**–**7** in 79–90% yields.

On the other hand, Wittig reaction of $Ph_3P{=}CHCO_2tBu$ with the commercially available indole-3-carboxaldehyde (**24**) or 4-amino-9-fluorenone (**25**) provided adducts **26** and **27**, respectively, in 93–95% yields (Scheme 2). It is worth mentioning that compound **27** was obtained as a mixture of isomers (ratio $E/Z = 55{:}45$ by ^1H NMR). Crystallization of said mixture from PhMe, gave in low yield the *Z* isomer (*Z* – **27**), as identified by single crystal X-ray crystallography [8]. Similarly, Wittig reaction of $Ph_3P{=}CHCO_2tBu$ with 3-acetyl-1-tosylpyrrole (**28**), readily synthesized from pyrrole [9], provided the anticipated adduct as a mixture of geometric isomers (ratio $E/Z = 65{:}35$, as shown by ^1H NMR). Crystallization from ethyl acetate/hexane, afforded the desirable pure *E* isomer. The latter was



Scheme 1. Synthesis of acitretin analogs **4**–**7**. Reagents and conditions: (i) PyBrOP, DIPEA, $CHCl_3$, 40 °C, 4 h, 74–91%; (ii) 50% TFA/DCM, RT, 1 h, then 5% aq. $NaHCO_3$, 63–70%; (iii) **22** (for **20a**) or **21** (for **20b**,**c**), PyBrOP, DIPEA, DMAP (cat.), DCM, RT, 12 h, 81–86%; (iv) Ph_2CHBr, DIPEA, DMF, RT, 12 h, 34%; (v) $Ph_3P{=}CHCO_2tBu$, DMF, RT, 12 h, 56%; (vi) 50% TFA/DCM (for **4**) or 25% TFA/DCM (for **23b**,**c**), 1 h, RT, 79–90%.



Scheme 2. Synthesis of acitretin analogs **8**–**16**. Reagents and conditions: (i) $Ph_3P{=}CHCO_2tBu$/DCM, RT, 16 h, 93% (for **26**) or $Ph_3P{=}CHCO_2tBu$/DMF, 100 °C, 16 h, 95% (for *E*/*Z* – **27**); (ii) $Ph_3P{=}CHCO_2tBu$/MeCN, 80 °C, 16 h, 64%; (iii) 10 N NaOH/DMSO, RT, 2 h, 98%; (iv) **31a** or **31b**/DIPEA/$CHCl_3$, 40 °C, 2–3 h, 50–76%; (v) **31a**, NaH, THF, RT, 16 h, 65%; (vi) 50% TFA/DCM, 0 °C, 1 h, 80–90%; (vii) cyanuric fluoride/Py/DCM, −10 °C, 45 min, 30–50%; (viii) **20a**, DIPEA, $CHCl_3$/DMF (85:15), 40 °C, 12 h, 43%.

treated with a 10 N NaOH solution in DMSO in order to remove the tosyl protecting group. Compound **29** was thus obtained in 63% overall yield.

The amines **26**, *E*/*Z* – **27**, pure *Z* – **27** and **29**, were then condensed with the cinnamoyl fluorides **31a** or **31b**, to give the *tert*-butyl esters **10**, **11**, **13**, **14**/**15** and pure **14** in 50–76% yields. These reactions generally took place in the presence of DIPEA as the base in $CHCl_3$ at 40 °C for 1.5–3 h, with the exception of the reactants **29** and **31b** which required NaH (as the base) in tetrahydrofuran (THF) at RT for overnight for their efficient condensation. The required acyl fluorides **31** were readily obtained from the corresponding commercially available cinnamic acids (**30**) upon cyanuric fluoride treatment [10]. Finally, TFA-mediated deprotection of ester **10** provided the corresponding acid **8** in 90% yield. Unexpectedly, under identical reaction conditions, attempted deprotection of ester **11**, in order to obtain acid **9**, led to the rearranged acid **12** in 84% yield. The structure of this compound was deduced by spectroscopic data and in particular ^1H NMR data. Indeed, two vinylic protons and the aromatic proton of the indole ring next to N had been replaced by three protons in the aliphatic region of the spectrum. A possible mechanism for the formation of this unexpected product, through an acid-mediated Michael-type nucleophilic attack, is put forward in Fig. 2. The formation of the resonance form **II** should be facilitated by the two MeO groups at positions o- and p- of the aromatic ring and this may be the reason why the corresponding ester **10**, with only one alkoxy group at position p- of the aromatic ring, does not give this rearrangement.

Finally, analog **16**, which is a hybrid of compounds **4** and **11**, was readily obtained by coupling acyl fluoride **31b** with anilide **20a** in the presence of DIPEA in $CHCl_3$/DMF (85:15) overnight at 40 °C. Routine FCC purification of the crude product afforded pure analog **16** in 43% yield (Scheme 2).

2.2. Antioxidant and anti-inflammatory activity

2.2.1. In vitro antioxidant activity studies

In the present investigation, the ACI analogs **1**–**8** and **10**–**16** as well as the parent molecule ACI were studied with regard to their antioxidant ability as well as their ability to inhibit soybean LOX. Antioxidants are defined as substances that even at low concentration significantly delay or prevent oxidation of easily oxidizable substrates. There is an increased interest of using antioxidants for medical purposes in the recent years. It is well known that free radicals play an important role in the inflammatory process [11]. Many non-steroidal anti-inflammatory drugs have been reported to act either as inhibitors of free radical production or as radical scavengers [12]. Consequently, compounds with antioxidant properties could be expected to offer protection in rheumatoid

arthritis and inflammation and to lead to potentially effective drugs. Thus, we tested the present ACI analogs with regard to their antioxidant ability and in comparison to well known antioxidant agents, such as caffeic acid (CA), nordihydroguairetic acid (NDGA) and trolox.

Several assays should be used in order to assess in vitro antioxidant activity [13a]. In this way, factors such as solubility or steric hindrance, which may be of overriding importance in one environment but not in another, can be varied and the antioxidant ability of a compound in a variety of milieus may be evaluated. For these reasons, we have used two different types of assays to measure *in vitro* antioxidant activity of ACI analogs: a) interaction with the stable free radical 1,l-diphenyl-2-picrylhydrazyl radical (DPPH), b) interaction with the water soluble azo compound 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH). Both require a spectrophotometric measurement and a certain reaction time in order to obtain reproducible results [13b].

DPPH has been used in a radical scavenging measuring method. DPPH is a stable free radical in an ethanolic solution. Because of the unpaired electron of DPPH, it gives a strong absorption maximum at 517 nm by visible spectroscopy (purple color). In addition, the unpaired electron of the radical becomes paired in the presence of a hydrogen donor (a free radical scavenging antioxidant), decreasing the absorption. Therefore, DPPH loses color in proportion to the number of electrons captured [13c]. As a result, this reaction has been widely used as a simple, rapid and convenient method independent of sample polarity for screening many samples for radical scavenging activity [13d] as free radical scavengers or hydrogen donors [13e]. These advantages made the DPPH method interesting for testing our analogs.

The use of the AAPH peroxyl radical is recommended as more appropriate for measuring radical scavenging activity in vitro, because the activity of the AAPH peroxyl radical shows a greater similarity to cellular activities such as lipid peroxidation [14]. The water soluble azo compound AAPH has been extensively used as a clean and controllable source of thermally produced alkylperoxyl free radicals [15].

The interaction of the examined compounds with the stable free radical DPPH is shown in Table 1. This interaction indicates their radical scavenging ability and electron donating activity in an iron-free system. Comparing the efficiencies of all the ACI analogs with those of ACI, only compounds **1** and **8** (both presenting a free carboxyl function), displayed medium interaction values whereas compounds **2**, **6** and **7** (all with a free carboxyl group) were inactive. Taking into consideration the mechanism of action of DPPH, analog **1** has both, the electron-rich ring of trimethoxyaniline to offer an electron to DPPH radical and a free carboxyl group as an efficient proton source. On the other hand, the comparable interaction value of the next most potent analog **8** in the series, might be mainly attributed to the presence of the (*E*)-3-(1*H*-indol-3-yl)acrylic acid substructure.

All other compounds presented very low, if any, interaction to DPPH at 100 μM. The low interaction values of ACI and its analogs, compared to NDGA, should be mainly attributed to the absence of easily oxidized functionalities like the ones present in NDGA (two catechol subunits). The absence of hydrogen atoms (phenolic hydroxyl groups) which could be donated to stabilize the DPPH radical is obvious within the given results

Comparing the interaction values between the couples of compounds **4** and **5**, **10** and **8**, and **11** and **12**, it is evident that the presence of the free carboxyl function is correlated with higher interaction values. The introduction of an alkyl ester group in the conjugated side chain did not increase the oxidation potentials, when compared to the lead compound. The data obtained strongly suggest that alkyl ester group have no effect on the electron density. The replacement of the *trans* with the *cis* double bond resulted in very high decrease of the inhibitory value (compound **2**, 2%),

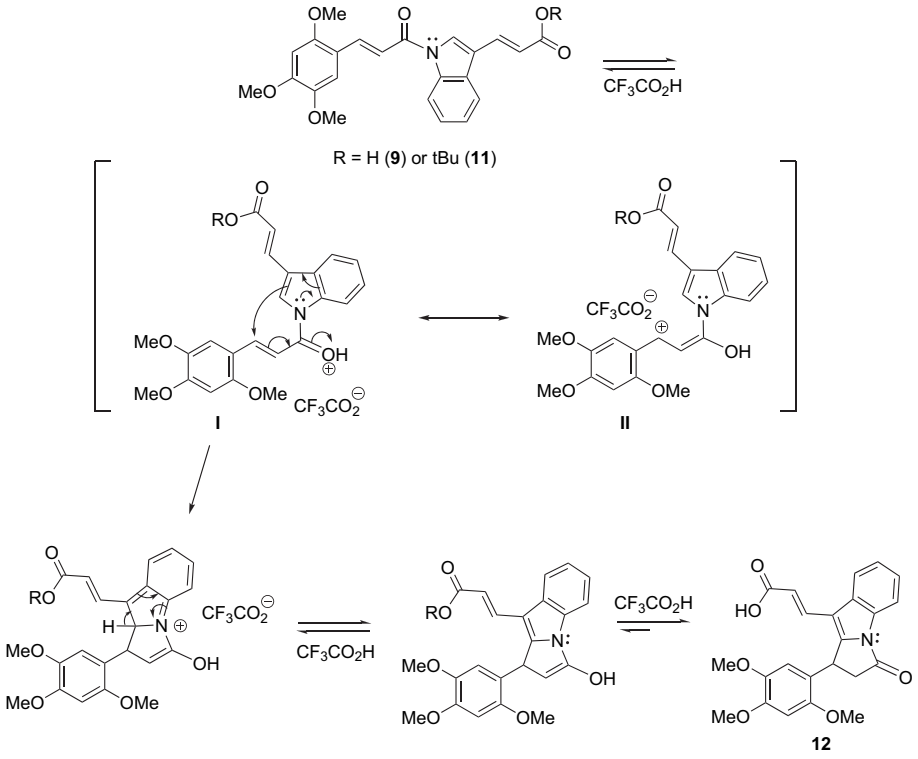


Fig. 2. Proposed mechanism for the formation of the unexpected product **12**.

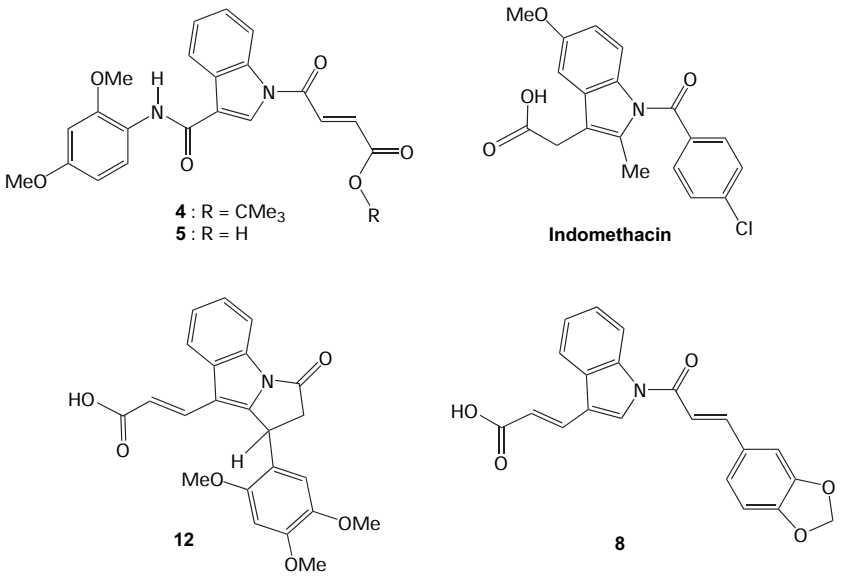


Fig. 3. Structures of indomethacin and ACI analogs (redrawn in a similar way to indomethacin) tested for anti-inflammatory activity in the context of the present work.

probably due to unfavourable interaction with the oxidant. Hybridization of structures **4** and **11** (analog **16**) resulted to a very low interaction value. The interaction remains highly constant after 60 min. Lipophilicity, as $\sum\pi$ values (the sum of the π values, assigned for lipophilic contribution of the substituents on the phenyl ring) at the anilide type analogs, influences the reducing activity e.g **1** > **7** (for compound **1** $\sum\pi = -0.06$ whereas for compound **7** $\sum\pi = -0.28$). The role of molar lipophilicity is contradictory since the two most active analogs **1** and **8** present big differences in terms of calculated values of lipophilicity e.g. clog *P* values **1** = 1.60 and **8** = 4.63. It is worth to mention that the parent compound ACI presented zero interaction. The same result has been found for ACI by Packer [16], who earlier studied the antioxidant efficiency of several retinoids using the same assay. He did not find any interaction with DPPH for ACI as well as for *all-trans*-retinoic acid. From his studies he concluded that the retinoids were not efficient antioxidants for hydrophobic radicals. Packer found that acitretin was quite effective in a different antioxidant protocol (the phycoerythrin acid/2,2′-azobis(2,4-dimethylvaleronitrile -AMVN assay) [16].

After treatment with etretinate (the ethyl ester of ACI), a significant increase in erythrocyte membrane fluidity and in antioxidant activity as well as a decrease in lipid peroxidation were observed in erythrocytes from psoriatic patients. The results confirmed the improvement of the antioxidant defence system and the cell protection against lipid peroxidation [17]. Taking this under

Table 1
Interaction % with DPPH (DPPH %); % Inhibition of Lipid Peroxidation (AAPH %); *In vitro* inhibition of soybean lipoxygenase (LOX) (IC_{50} or %); % Inhibition of Carrageenan-induced rat paw oedema (ICPE %)

Compds	Clog *P* [31]	DPPH % @ 100 μM 20/60 min (±SD)[a]	AAPH % @ 100 μM (±SD)[a]	IC_{50} μM or % LOX Inh. @ 100 μM (±SD)[a]	ICPE % 0.01 mmol/Kg (±SD)[a]
CA	0.82	5.5 ± 0.3	17.5 ± 0.8	$IC_{50} = 600 \pm 15$ μM	
ACI	6.07	na	85 ± 1.6	$1\% \pm 10^{-3}$	$63^{**} \pm 3.4$
1	1.60	26 ± 1.3	64 ± 1.2	57% ± 3.2	
2	1.60	2 ± 10^{-3}	8 ± 0.5	42% ± 0.8	
3	1.54	12.5 ± 0.02	34 ± 1.2	$10\% \pm 10^{-3}$	
4	3.92	5 ± 0.04	68 ± 3.2	$IC_{50} = 100 \pm 2.8$ μM	$58.6^{**} \pm 4.2$
5	2.16	10 ± 0.8	77 ± 4.1	$IC_{50} = 35 \pm 1.4$ μM	$46^{**} \pm 1.8$
6	2.66	1 ± 10^{-3}	85 ± 1.9	$IC_{50} = 52.4 \pm 2.5$ μM	
7	2.95	na	94 ± 2.2	26% ± 1.3	
8	4.63	21 ± 1.5	22 ± 0.7	$IC_{50} = 65 \pm 1.8$ μM	$34^{**} \pm 1.3$
10	6.25	13 ± 0.035	95 ± 2.1	$IC_{50} = 82.5 \pm 2.7$ μM	
11	5.98	8.3 ± 0.06	80 ± 1.6	85% ± 5.6 @ 100 μM 85% ± 3.8 @ 50 μM 0% @ 10 μM	
12	3.90	12 ± 0.06	57 ± 1.4	$IC_{50} = 45.2 \pm 4.4$ μM	$9^{*} \pm 0.7$
13	5.24	8 ± 0.3	46 ± 3.2	39% ± 2.9	
14 + **15**		na	100 ± 2.9	63% ± 3.3	
14	6.15	na 6 ± 0.02 (60 min)	49 ± 1.7	43% ± 2.5	
16	4.78	5 ± 0.02	42 ± 0.7	69% ± 3.1	
NDGA		81 ± 1.3/83 ± 2.4			
Trolox			63 ± 1.2		
Indomethacin					$47^{**} \pm 3.1$

The effect on oedema is expressed as percent of weight increase of hind paw (and as percent of inhibition of oedema) 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: $^{*}p < 0.1$ $^{**}p < 0.01$ (Student's *t* test); na, no activity under the reported experimental conditions. SD standard deviation.

[a] Values are means ± SD of three or four different determinations. Means within each column differ significantly ($p < 0.05$).

consideration, all the ACI analogs were studied in order to identify their possible inhibitory activity on lipid peroxidation.

In our studies, AAPH was used as a free radical initiator to follow oxidative changes of linoleic acid to conjugated diene hydroperoxide. Compounds **5**, **6**, **7**, **10** and **11** showed substantially higher inhibition of lipid peroxidation than the reference compound Trolox (63%). Comparing the anti-lipid peroxidation activities of all ACI analogs with that of ACI, only compounds **7** and **10** showed higher inhibition (94 and 95%) followed by **11** (80%) and **5** (77%). Also, the most potent anti-lipid peroxidation sample seemed to be the mixture of the geometric isomers **14** and **15** containing them in comparable amounts. The unsubstituted analog **6** presented activity equipotent to ACI. Within the benzodioxolyl-couple, ester **10** (95%) was by far more potent than the corresponding acid **8** (22%). It is interesting to note that the presence of the indole ring (analog **11**) is more favourable than the pyrrole ring (analog **13**). Although high inhibition values (>80%) are observed for compounds (ACI, **10**, **11**, **15**) with the highest lipophilicities (clog *P* 5.98–6.15), there are some exceptions, such as compounds **6** and **7** presenting clog *P* 2.66 and 2.95 and **14** presenting clog *P* 6.15 (49%). A clear trend is observed in the series of carboxylic acids **1**, **5**–**7**, wherein increased inhibition of lipid peroxidation is well-correlated to the increase of lipophilicity of said compounds Within the series of compounds **1**–**7**, compounds bearing a *trans* double bond (**1**, **4**–**7**) are more active than the one bearing the ethylene unit (reduced double bond, compound **3**, 34%) whereas replacement of the *trans* with the *cis* double bond resulted in very high decrease of the inhibitory value (compound **2**, 8%).

The standard inhibitor trolox obviously exerts its inhibitory effect on lipid peroxidation mainly through the ability of its 6-hydroxy-5,7,8-trimethylchromane moiety to break the radical chain. Although no phenol moieties are present in ACI and its analogs, both ACI and the anilides **1**, **4**–**7** and **15** could break the radical chain through the initial abstraction of a hydrogen from either a methyl group of the spacer (ACI) or the amide function (anilides). The thus created new radicals are efficiently stabilized through resonance. A nitro group in position meta to the N radical would provide additional stabilization through its electron-withdrawing effect and therefore analog **7** should be expected to be, and actual is, the most effective inhibitor among the analogs of the anilide type. On the other hand, the *tert*-butyl esters **10** and **11** will obviously exert their inhibitory activity on lipid peroxidation through other mechanism(s), e.g. through interception of the alkylperoxyl radicals mainly by the indole moiety and possible formation oxidized derivatives. Of course, ACI might exert its inhibitory effect, alternatively or in addition to hydrogen abstraction, through interception of the alkylperoxyl radicals by its conjugated tetraene chain.

Leukotrienes play an important role as mediators of a variety of inflammatory and allergic reactions and are derived from the biotransformation of arachidonic acid catalyzed by Lipoxygenase (LOX). LOXs play a role in membrane lipid peroxidation by forming hydroperoxides in the lipid bilayer [18,19]. Inhibitors of LOX have attracted attention initially as potential agents for the treatment of inflammatory and allergic diseases but their therapeutic potential has now been expanded to certain types of cancer and cardiovascular diseases [20,21].

In this context, we decided to further evaluate the synthesized ACI analogs for their ability to inhibit soybean LOX by the UV absorbance based enzyme assay [22]. 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. Most of the LOX inhibitors are antioxidants or free radical scavengers [23]. Other 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+}. Several LOX inhibitors are excellent ligands for Fe^{3+}. This inhibition is related to their ability to reduce the iron species in the active site to the catalytically inactive ferrous form [23].

Perusal of the IC_{50}'s or % inhibition values (Table 1) shows that the most potent inhibitor, namely compound **5** (IC_{50} 35 μM), is found within the subset of the anilide type compounds (**1**–**7**), followed by compound **12** (IC_{50} 45.2 μM) belonging to the other subset of compounds (**8**–**15**), namely of the cinnamic acid-type. In these two subsets of compounds, two other potent inhibitors may be also identified, namely compounds **6** (IC_{50} 52.4 μM) and **8** (IC_{50} 65 μM) respectively. Notably, the parent compound ACI showed essentially negligible interaction with LOX (1%) under our experimental conditions at 100 μM.

A comparison of the inhibition values in the couples of compounds **4** and **5** and **8** and **10** indicates that the free acid seems to be stronger inhibitor than the corresponding *tert*-butyl ester, whereas the comparison between *tert*-butyl esters **11** and **13** indicates that the indole ring is probably more important than the pyrrole ring for the biological activity. Also, isomer **15** seems to be more potent inhibitor than isomer **14**. On the other hand, comparison of compounds **1**, **2** and **3** shows that the double bond next to the carboxyl group is correlated with higher inhibitory activity in particular when it has the *trans* configuration. Furthermore, comparison of acids **1** and **5**–**7** reveals that the presence of an electron-withdrawing group, such as the nitro group, in the aniline ring is correlated with the lowest inhibitory activity, whereas the highest activity is secured by the presence of two methoxy groups in the ring. Finally, hybridization of structures **4** and **11** (analog **16**) did not show to offer any advantage over the parent compounds. Although lipophilicity is referred as an important physicochemical property for LOX inhibitors [24], herein the most potent compound **5** had an IC_{50} value of 35 μM and clog $P = 2.16$ and does not follow this concept. On the contrary, analogs **8** and **10** had IC_{50} values of 65 μM and 82.5 μM and clog $P = 4.63$ and 6.25, respectively.

Compared to the standard inhibitor CA and taking into consideration the mode of action of LOX, the most active analogs of the present set of compounds do not contain a catechol moiety, which efficiently scavenges free radicals and might also chelate Fe^{3+} and reduce it to the catalytically inactive Fe^{2+}. Goldreich et al [25] examined the effect of retinol, *all-trans*-retinoic acid and 13-*cis*-retinoic acid on the activity of lipoxygenase-1 and lipoxygenase-2 towards linoleic acid. *All-trans*-retinoic acid and 13-*cis*-retinoic acid inhibited lipoxygenase-1 activity competitively, whereas *all-trans*-retinol inhibited lipoxygenase-1 activity in a mixed manner. These findings suggest that retinoids may bind to the active site of the enzyme or simultaneously act as antioxidants. Thus, it is tempting to propose that our ACI analogs exert their inhibitory effect mainly through binding to the active site of the enzyme with different efficiencies and to a lesser degree to their electron donating abilities. Compounds **5** and **6,** the most potent compounds (with lower lipophilicity values), probably present different anchorage to the lipophilic region of the enzyme due to different orientation/position of the molecules. Compared to the almost inactive ACI, the most active ACI analogs are characterized by the presence of the indole ring which imposes a conformational restriction in either the C4 to C6 spacer segment (analogs **8** and **12**) or better the C5 to C7 spacer segment (analogs **5** and **6**). Worth noting is the fact that analog **12** is more potent than analog **8**, and this might be attributed to the additional restriction imposed on the C5 to C9 spacer segment by the pyrrolidinone ring. It also evident that the *trans* configuration of the terminal –CH=CH–CO_2H moiety is preferred (it is also present in the standard inhibitor CA) over the *cis* and even more over the flexible –CH_2CH_2–CO_2H moiety (reduced double bond) and that the first moiety in that region of

the molecule can be better accommodated than the corresponding bulkier –CH=CH–CO_2tBu moiety.

2.2.2. Anti-inflammatory activity in vivo

We had observed, from biological studies of other types of α,β-unsaturated acids, that their corresponding *tert*-butyl esters were more potent inhibitors of lipoxygenase (LOX) than the free acids [6]. Several esters of a non-steroidal anti-inflammatory drug have been synthesized and showed long acting anti-inflammatory activity [26]. Indomethacin amides and esters are orally active, non-ulcerogenic, anti-inflammatory agents in an in vivo model of acute inflammation [27].

The most interesting compounds in this series of ACI analogs, namely compounds **4**, **5**, **8** and **12** as well as ACI were selected to be examined in vivo by using the functional model of Carrageenan-induced rat paw oedema. Carrageenan-induced oedema is a non-specific inflammation resulting from a complex of diverse mediators [28]. As shown in Table 1, ACI and the *tert*-butyl ester **4** (clog *P* 3.92) showed superior anti-inflammatory properties than the commonly used standard indomethacin, whereas free acid **5** (clog *P* 2.16) induced equipotent inhibition to indomethacin. Lipophilicity seems therefore to be the main cause for the higher activity of ester **4** compared to acid **5**. On the other hand, compounds **8** and **12** were less potent with the compound **12** presenting the lowest inhibition (9%). This behaviour might be possibly attributed to the conformational restriction imposed by the additional five-membered ring in structure **12** compared to the structure of compound **8** (Fig. 3). Furthermore, it seems that the decoration of the indole nucleus by the types of substructures present in compound **12** (and **8**) are not as good as the ones in compounds **4** and **5** for improved or equipotent biological activity to indomethacin.

3. Conclusions

ACI analogs, bearing a variety of substituents in the aromatic part of the molecule, incorporating one or two amide bonds and an indole or a pyrrole ring in the spacer, and the hydrophilic carboxyl group or the lipophilic *tert*-butoxycarbonyl group at the polar end of the molecule, were readily assembled by combining either anilines, indole-3-carboxylic acid and 1,4-dicarboxylic acids or cinnamic acids, indole-3-carboxaldehyde or 3-acetylpyrrole and the phosphorane Ph_3P=$CHCO_2$tBu.

These analogs and ACI exhibited in general low reducing abilities. The *tert*-butyl ester **10** and the carboxylic acid **7** presented higher inhibition of lipid peroxidation than ACI. Analog **5** showed the highest inhibitory activity against soybean LOX, *in vitro*, followed by analogs **12**, **6** and **8**. Finally, ACI and analog **4** showed higher anti-inflammatory activity, *in vivo*, than indomethacin.

Our study indicates that LOX or lipid peroxidation inhibitory activity is not always accompanied by DPPH radical scavenging activity. Thus, although compounds such as **5**, **6**, **7**, **10** and **11** inhibit lipid peroxidation potently they present low, if any, DPPH scavenging activity. On the other hand, the compounds **5**, **6**, **8**, **10**, **11**, **12** and **16** are potent LOX inhibitors possessing low to moderate DPPH radical scavenging activity. This is in accordance with the finding of Curini *et al.* [29] who have studied the antioxidant and LOX inhibitory activity of five natural prenyloxycarboxylic acids and showed that the most efficient LOX inhibitor (boropinic acid) is not the most active DPPH radical scavenger. However, a better correlation exists between LOX and lipid peroxidation inhibitory activity.

In general, the comparison of the antioxidant and anti-inflammatory effects of the parent molecule ACI and its analogs **1**–**8**, and **10**–**16** examined in the present work, leads to the conclusion that the most important contribution to the significant increase of the inhibitory activity of LOX comes from the change of the spacer and in particular by incorporating the indole ring and by replacing one or two double bonds with the isosteric amide bond. On the other hand, improved inhibitory activities on lipid peroxidation are observed by retaining the afore mentioned changes in spacer and by replacing either the carboxyl polar group of ACI with the highly lipophilic *tert*-butoxycarbonyl group or the electron-rich aromatic ring of ACI with an electron-deficient phenyl ring, respectively. Analog **4**, presenting in vivo equipotent anti-inflammatory activity to ACI could be used as a lead compound for the design of long acting anti-inflammatory agents with LOX inhibitory activity.

4. Experimental section

4.1. General synthetic

Melting points were determined with a Buchi SMP-20 apparatus and are uncorrected. IR spectra were recorded for KBr pellets on a Perkin Elmer 16PC FT-IR spectrophotometer. ^{1}H NMR spectra were obtained at 400.13 MHz and ^{13}C NMR spectra at 100.62 MHz on a Bruker DPX spectrometer; TMS was used as reference. ESI-MS were recorded at 30 V, on a Micromass-Platform LC spectrometer 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 μm; 0.25 μm nominal) having as stationary phase HP-5MS 5% Phenyl Methyl Siloxane. Carrier gas flow-rate: 26.4 mL min^{-1} He; injection port temperature 300 °C; program: 70–300 °C at 14.45 min. Electron Impact mass spectra were recorded at 20 eV on an Agilent Technologies 5975B instrument, tandem to the above mentioned GC spectrometer. Microanalyses were performed on a Carlo Erba EA 1108 CHNS elemental analyzer in the Laboratory of Instrumental Analysis of the University of Patras. Flash column chromatography was performed on Merck silica gel 60 (230–400 mesh) and TLC on 60 Merck 60F_{254} films (0.2 mm) precoated on aluminium foil. Spots were visualized with UV light at 254 nm and charring agents. The eluent systems used were: (**A**) PhMe/Hex (1:1), (**B**) PhMe/AcOEt (95:5), (**C**) PhMe/AcOEt (9:1), (**D**) PhMe/AcOEt (8:2), (**E**) PhMe/AcOEt (7:3), (**F**) PhMe/AcOEt (1:1), (**G**) AcOEt, (**H**) $CHCl_3$/MeOH (95:5), (**I**) $CHCl_3$/MeOH (7:3).

All the chemicals used were of analytical grade and commercially available from Merck. ACI was purchased from CHEMOS GmbH. ACI analogs **1**–**3** were synthesized, for the purpose of the present study, according to literature protocols [4].

*4.2. General procedure for the preparation of anilides **19***

To a solution of the commercially available anilines **17a**–**c** (10 mmol) and acid **18** (2.61 g, 10 mmol) [4] in dry $CHCl_3$ (15 mL) anhydrous DIPEA (6.97 mL, 40 mmol) and PyBrOP (6.99 g, 15 mmol) were added sequentially. The thus obtained solution was set at 40 °C for 4 h. Upon completion, the reaction mixture was diluted with $CHCl_3$ and washed sequentially once with a 5% aqueous $NaHCO_3$ solution, twice with H_2O and brine. Drying over Na_2SO_4 and evaporation to dryness left a residue, from which pure amides **19a**–**c** were obtained through FCC.

*4.2.1. tert-Butyl 3-(2,4-dimethoxyphenylcarbamoyl)-1H-indole-1-carboxylate (**19a**)*

Yield: 3.01 g (76%), light red solid, m.p. 132–133 °C, R_f (B): 0.27, ESI-MS (*m*/*z*): 815.16 (2*M* + Na), 793.25 (2*M* + H), 419.15 (*M* + Na), 397.19 (*M* + H). IR (KBr, cm^{-1}): 3435, 2971, 1746, 1658. ^{1}H NMR ($CDCl_3$): δ 8.40 (1H, d, *J* 8.0 Hz, H-16), 8.22 (1H, dd, *J* 7.2 and 1.6 Hz, H-8), 8.21 (1H, d, *J* 2.8 Hz, H-13), 8.20 (1H, s, H-2), 8.12 (1H, dd, *J* 6.0 and 1.6 Hz, H-5), 7.40 (1H, td, *J* 8.0 and 2.8 Hz, H-7), 7.37 (1H, td, *J* 7.2 and 1.6 Hz, H-6), 6.56 (1H, dd, *J* 8.0 and 2.8 Hz, H-15), 6.54 (1H, s,

NH), 3.93 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 1.71 (9H, s, C(CH_3)$_3$) ppm. ^{13}C NMR ($CDCl_3$): δ 161.6, 156.5 (two C), 149.2, 135.9, 129.4, 127.5, 127.2, 125.2, 123.9, 120.9, 120.6, 117.1, 115.5, 103.9, 98.7, 85.0, 55.9, 55.6, 28.1 (three C) ppm. Anal. Calcd. for $C_{22}H_{24}N_2O_5$: C, 66.65; H, 6.10; N, 7.07. Found: C, 66.40; H, 6.22; N, 7.38.

4.2.2. tert-Butyl 3-(phenylcarbamoyl)-1H-indole-1-carboxylate (**19b**)

Yield: 2.49 g (74%) white foam; R_f(B): 0.32; ESI-MS (*m*/*z*): 695.16 (2*M* + Na), 673.19 (2*M* + H), 375.45 (*M* + K), 359.49 (*M* + Na), 337.51 (*M* + H); IR (neat, cm^{-1}): 3299, 2981, 1741, 1649; ^{1}H NMR ($CDCl_3$): δ 8.21 (1H, d, *J* 8.0 Hz, H-8), 8.19 (1H, s, H-2), 8.13 (1H, d, *J* 7.6 Hz, H-5), 7.76 (1H, br.s, N*H*), 7.67 (2H, d, *J* 8.0 Hz, H-12/16), 7.42 (1H, td, *J* 7.2 and 1.6 Hz, H-7), 7.40 (2H, t, *J* 8.0 Hz, H-13/15), 7.38 (1H, td, *J* 7.2 and 1.6 Hz, H-6), 7.17 (1H, t, *J* 8.0 Hz, H-14), 1.18 (9H, s, C(CH_3)$_3$) ppm; ^{13}C NMR ($CDCl_3$): δ 162.1, 149.1, 137.8, 135.6, 129.1 (two C), 127.8, 127.3, 125.4, 124.4, 123.9, 120.9, 120.1 (two C), 116.4, 115.4, 85.2, 28.1 (three C) ppm. Anal. Calcd. for $C_{20}H_{20}N_2O_3$: C, 71.41; H, 5.99; N, 8.33. Found: C, 71.58; H, 5.80; N, 8.62.

4.2.3. tert-Butyl 3-(3-nitrophenylcarbamoyl)-1H-indole-1-carboxylate (**19c**)

Yield: 3.47 g (91%), pale yellow oil, R_f (C): 0.37, ESI-MS (*m*/*z*): 420.38 (*M* + K), 404.43 (*M* + Na), 382.12 (*M* + H). IR (neat, cm^{-1}): 3254, 2980, 1746, 1657, 1372, 856. ^{1}H NMR ($CDCl_3$): δ 8.49 (1H, t, *J* 2.0 Hz, H-12), 8.20 (1H, s, H-2), 8.16 (1H, d, *J* 8.0 Hz, H-8), 8.12 (1H, s, N*H*), 8.09 (2H, d, *J* 6.4 Hz, H-14/16), 7.97 (1H, dd, *J* 7.2 and 1.2 Hz, H-5), 7.52 (1H, t, *J* 8.4 Hz, H-15), 7.40 (1H, td, *J* 7.2 and 1.2 Hz, H-7), 7.36 (1H, td, *J* 7.2 and 1.2 Hz, H-6), 1.70 (9H, s, C(CH_3)$_3$) ppm. ^{13}C NMR ($CDCl_3$): δ 162.4, 148.9, 148.6, 139.1, 135.6, 129.9, 128.2, 127.1, 125.8, 125.6, 124.1, 120.9, 118.8, 115.5, 115.4, 114.8, 85.5, 28.1 (three C) ppm. Anal. Calcd. for $C_{20}H_{19}N_3O_5$: C, 62.99; H, 5.02; N, 11.02. Found: C, 63.12; H, 4.88; N, 10.74.

4.3. General procedure for the preparation of indole derivatives **20**

Anilides **19** (5 mmol) were treated with a 50% solution TFA in DCM (15 mL) at RT for 1 h and then evaporated to dryness. The oily residues were triturated with Et_2O and refrigerated overnight to give, upon filtration, anilides **20** as the coresponding trifluoroacetate salts. These, were then treated with an excess of a 5% aqueous $NaHCO_3$ solution and extracted twice with EtOAc. The organic layers were combined and washed twice with H_2O, dried (Na_2SO_4) and evaporated to leave a residue. Addition of Et_2O and overnight refrigeration gave pure free indole derivatives **20**.

4.3.1. N-(2,4-Dimethoxyphenyl)-1H-indole-3-carboxamide (**20a**)

Yield: 1.04 g (70%), purple solid, m.p. 145–146 °C, R_f (F): 0.29, ESI-MS (*m*/*z*): 615.29 (2*M* + Na), 319.27 (*M* + Na), 297.23 (*M* + H). IR (KBr, cm^{-1}): 3448, 3167, 1635. ^{1}H NMR (DMSO-d_6): δ 11.70 (1H, s, N*H*), 8.79 (1H, s, N*H*CO), 8.24 (1H, d, *J* 2.8 Hz, H-1), 8.13 (1H, d, *J* 7.6 Hz, H-7), 7.68 (1H, d, *J* 8.6 Hz, H-15), 7.47 (1H, d, *J* 7.6 Hz, H-4), 7.18 and 7.14 (2H, two t, *J* 7.6 Hz, H-5/6), 6.66 (1H, d, *J* 2.6 Hz, H-12), 6.54 (1H, dd, *J* 8.6 and 2.6 Hz, H-14), 3.84 (3H, s, OCH_3), 3.78 (3H, s, OCH_3) ppm. ^{13}C NMR (DMSO-d_6): δ 162.8, 156.8, 152.1, 136.1, 128.5, 125.8, 124.8, 121.9, 120.5 (two C), 120.4, 111.9, 110.5, 104.7, 98.7, 55.6, 55.2 ppm. Anal. Calcd. for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45. Found: C, 69.21; H, 5.13; N, 9.26.

4.3.2. N-Phenyl-1H-indole-3-carboxamide (**20b**)

Yield: 0.80 g (68%) pale yellow solid; m.p. 173–175 °C; R_f (F): 0.31; ESI-MS (*m*/*z*): 259.40 (*M* + Na), 237.11 (*M* + H); IR (KBr, cm^{-1}): 3376, 3247, 1643, 1602; ^{1}H NMR (DMSO-d_6): δ 11.73 (1H, s, N*H*), 9.71 (1H, s, N*H*CO), 8.29 (1H, s, H-1), 8.19 (1H, d, *J* 7.6 Hz, H-7), 7.77 (2H, d, *J* 7.6 Hz, H-11/15), 7.47 (1H, d, *J* 7.6 Hz, H-4), 7.30 (2H, t, *J* 7.6 Hz, H-12/14), 7.16 and 7.11 (2H, two t, *J* 7.6 Hz, H-5/6), 7.03 (1H, t, *J* 7.6 Hz, H-13) ppm. ^{13}C NMR ($CDCl_3$): δ 166.5, 140.3, 136.7, 129.1, 129.0 (two C), 126.9, 123.1, 122.6, 121.6, 121.1, 120.2 (two C), 112.4, 111.0 ppm. Anal. Calcd. for $C_{15}H_{12}N_2O$: C, 76.25; H, 5.12; N, 11.86. Found: C, 76.02; H, 5.40; N, 12.11.

4.3.3. N-(3-Nitrophenyl)-1H-indole-3-carboxamide (**20c**)

Yield: 0.89 g (63%), yellow solid, m.p. 240–242 °C, R_f (F): 0.36, ESI-MS (*m*/*z*): 320.25 (*M* + K), 304.23 (*M* + Na), 282.32 (*M* + H). IR (KBr, cm^{-1}): 3373, 3272, 1638, 1372, 856. ^{1}H NMR (DMSO-d_6): δ 11.85 (1H, s, N*H*), 10.19 (1H, s, N*H*CO), 8.81 (1H, s, H-11), 8.36 (1H, s, H-1), 8.21 and 8.19 (2H, two d, *J* 8.0 Hz, H-13/15), 7.90 (1H, d, *J* 7.6 Hz, H-7), 7.63 (1H, t, *J* 8.0 Hz, H-14), 7.50 (1H, d, *J* 7.6 Hz, H-4), 7.22 and 7.18 (2H, two t, *J* 7.6 Hz, H-5/6) ppm. ^{13}C NMR ($CDCl_3$): δ 164.1, 148.5, 141.6, 136.8, 130.4, 129.8, 126.8, 125.9, 122.9, 121.5, 121.4, 117.4, 114.0, 112.6, 110.4 ppm. Anal. Calcd. for $C_{15}H_{11}N_3O_3$: C, 64.05; H, 3.94; N, 14.94. Found: C, 63.88; H, 4.30; N, 15.24.

4.4. Synthesis of O-monoprotected fumarates **21** and **22**

4.4.1. (E)-4-(Benzhydryloxy)-4-oxobut-2-enoic acid (**21**)

To a solution of fumaric acid (90 mmol, 10.44 g) and DIPEA (180 mmol, 31.4 mL) in DMF (60 mL), was added dropwise a solution of bromodiphenylmethane (18 mmol, 4.44 g) in DMF (30 mL) over a period of 2 h. The reaction mixture left to stir at RT overnight, quenched with ice, and acidified to pH 6 with careful addition of gl. AcOH. The mixture was extracted twice with AcOEt and the combined organic layers washed with water and brine, dried over Na_2SO_4 and evaporated to dryness. The crude product was subjected to FCC to afford pure ester **21**. Yield: 1.73 g (34%), m.p. 141–142 °C, R_f(G): 0.2, ESI-MS (*m*/*z*): 305.17 (*M* + Na), 283.65 (*M* + H). IR (KBr, cm^{-1}): 3200-2400, 1718, 1692. ^{1}H NMR (DMSO-d_6): δ 7.46 (4H, d, *J* 7.2 Hz, *o*-Ph*H*), 7.36 (4H, t, *J* 7.2 Hz, *m*-Ph*H*), 7.29 (2H, t, *J* 7.2 Hz, *p*-Ph*H*), 6.90 (1H, s, PhC*H*), 6.84 (2H, s, C*H*=C*H*) ppm. ^{13}C NMR (DMSO-d_6): 165.9, 163.8, 140.3 (two C), 135.6, 132.4, 128.8 (four C), 128.1 (two C), 126.7 (four C), 77.5 ppm. Anal. Calcd. for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00. Found: C, 72.56; H, 4.79.

4.4.2. (E)-4-(tert-Butyloxy)-4-oxobut-2-enoic acid (**22**)

To a solution of glyoxylic acid monohydrate (10 mmol, 0.92 g) in DMF (15 mL) phosphonate Ph_3P=$CHCO_2$tBu (15 mmol, 5.65 g) was added. The resulting mixture left to stir at RT overnight, diluted with EtOAc and washed several times with water, dried over Na_2SO_4 and evaporated to dryness. The oily residue was triturated with Et_2O and refrigerated. The precipitated Ph_3P=O was filtered off and the filtrate diluted with EtOAc and washed twice with an aq. solution 0.5 N NaOH. The aqueous phase was carefully acidified to pH 5 with an aq. solution 5% citric acid and was extracted twice with EtOAc. The combined organic layers washed twice with water, dried over Na_2SO_4 and evaporated to dryness to afford pure ester **22** (0.96 g, 56%) which had identical spectrometric data to those reported in the literature [7].

4.5. General procedure for coupling indole derivatives **20** with fumarates **21** and **22**

To a suspension of **20a** (2 mmol, 0.59 g) and **22** (2.2 mmol, 0.38 g) or **20b**,**c** (2 mmol) and **21** (2.2 mmol, 0.62 g) in DCM (1.5 mL), were added sequentially DIPEA (6 mmol, 1.05 mL), a catalytic quantity of DMAP and finally PyBrOP (3 mmol, 1.4 g). The resulting solution was left at RT overnight, diluted with DCM and washed once with 5% aq. $NaHCO_3$, twice with water, dried over Na_2SO_4 and evaporated to dryness. The oily residues were subjected to FCC to afford pure esters **4** and **23b**,**c**.

*4.5.1. tert-Butyl (E)-4-(3-(2,4-dimethoxyphenylcarbamoyl)-1H-indol-1-yl)-4-oxobut-2-enoate (**4**)*

Yield: 0.73 g (81%), yellow solid, m.p. 118–120 °C, R_f (B): 0.15, ESI-MS (*m*/*z*): 923.55 (2*M* + Na), 489.63 (*M* + K), 473.62 (*M* + Na), 451.66 (*M* + H). IR (KBr, cm^{-1}): 3444, 2924, 1714, 1690, 1672. ^1H NMR ($CDCl_3$): δ 8.56–8.52 (1H, m, H-11), 8.38 (1H, d, *J* 9.6 Hz, H-19), 8.27 (1H, s, N*H*CO), 8.16 (1H, s, H-5), 8.08-8.03 (1H, m, H-8), 7.64 (1H, d, *J* 15.2 Hz, H-3), 7.46 and 7.45 (2H, two t, *J* 7.6 Hz, H-9/10), 7.06 (1H, d, *J* 15.2 Hz, H-2), 6.57–6.51 (2H, m, H-16/18), 3.93 (3H, s, OC*H*$_3$), 3.82 (3H, s, OC*H*$_3$), 1.56 (9H, s, C(C*H*$_3$)$_3$) ppm. ^{13}C NMR ($CDCl_3$): δ 163.8, 162.8, 160.9, 156.7, 149.4, 137.9 (two C), 136.3, 131.0, 127.3, 127.1, 126.2, 125.4, 121.0, 120.3, 119.6, 117.3, 103.9, 98.7, 82.6, 55.9, 55.6, 28.0 (three C) ppm. Anal. Calcd. for $C_{25}H_{26}N_2O_6$: C, 66.65; H, 5.82; N, 6.22. Found: C, 66.40; H, 6.01; N, 6.55.

*4.5.2. Benzhydryl (E)-4-oxo-4-(3-(phenylcarbamoyl)-1H-indol-1-yl)but-2-enoate (**23b**)*

Yield: 0.81 g (81%), white solid, m.p. 198–199 °C, R_f(C): 0.39, ESI-MS (*m*/*z*): 523.13 (*M* + Na), 501.07 (*M* + H). IR (KBr, cm^{-1}): 3326, 1720, 1698, 1650, 1598. ^1H NMR (DMSO-d_6): δ 10.13 (1H, s, N*H*CO), 8.94 (1H, s, H-5), 8.44 (1H, d, *J* 7.6 Hz, H-11), 8.27 (1H, d, *J* 7.6 Hz, H-8), 7.99 (1H, d, *J* 15.2 Hz, H-3), 7.75 (2H, d, *J* 8.0 Hz, H-15/19), 7.52 (4H, d, *J* 7.6 Hz, *o*-Ph*H*), 7.48-7.29 (11H, m, Ph*H*), 7.25 (1H, d, *J* 15.2 Hz, H-2), 7.01 (1H, s, C*H*Ph$_2$) ppm. ^{13}C NMR (DMSO-d_6): δ 164.0, 163.2, 161.8, 140.2, 139.1, 135.5, 134.2, 134.1, 128.9 (three C), 128.8 (four C), 128.2 (three C), 126.9 (four C), 125.9, 125.2, 123.8, 122.0, 120.4 (three C), 116.8, 116.4, 77.9 ppm. Anal. Calcd. for $C_{32}H_{24}N_2O_4$: C, 76.78; H, 4.83; N, 5.60. Found: C, 76.97; H, 4.73; N, 5.39.

*4.5.3. Benzhydryl (E)-4-(3-(3-nitrophenylcarbamoyl)-1H-indol-1-yl)-4-oxo-but-2-enoate (**23c**)*

Yield: 0.94 g (86%), white solid, m.p. 206–207 °C, R_f(B): 0.22, ESI-MS (*m*/*z*): 568.15 (*M* + Na), 546.45 (*M* + H). IR (KBr, cm^{-1}): 3330, 1726, 1694, 1656. ^1H NMR (DMSO-d_6): δ 10.58 (1H, s, N*H*CO), 8.97 (1H, s, H-5), 8.74 (1H, t, *J* 1.5 Hz, H-15), 8.43 (1H, dd, *J* 8.0 and 1.5 Hz, H-17), 8.28 (2H, dd, *J* 8.0 and 1.5 Hz, H-19), 8.17 (1H, unresolved dd, *J* 8.4 Hz, H-8), 7.99 (1H, d, *J* 15.2 Hz, H-3), 7.96 (1H, unresolved d, *J* 8.4 Hz, H-8), 7.68 (1H, t, *J* 8.0 Hz, H-18), 7.53 (4H, d, *J* 7.8 Hz, *o*-Ph*H*), 7.47 and 7.46 (2H, two t, *J* 8.4 Hz, H-9/10), 7.40 (4H, t, *J* 7.8 Hz, *m*-Ph*H*), 7.32 (2H, t, 7.6 Hz, *p*-Ph*H*), 7.26 (1H, d, *J* 15.2 Hz, H-2), 7.02 (1H, s, C*H*Ph$_2$) ppm. ^{13}C NMR (DMSO-d_6): δ 163.9, 163.2, 161.8, 148.2, 140.3, 140.2 (two C), 135.5, 134.2 (two C), 130.4, 129.6, 128.8 (four C), 128.5, 128.2 (two C), 126.9 (four C), 126.0, 125.9, 125.3, 121.9, 118.1, 116.4, 116.1, 114.2, 77.9 ppm. Anal. Calcd. for $C_{32}H_{23}N_3O_6$: C, 70.45; H, 4.25; N, 7.70. Found: C, 70.15; H, 4.58; N, 7.91.

*4.6. General procedure for TFA-mediated deprotection of esters **4** and **23b,c***

An ice-cold solution of 50% TFA in DCM (15 mL) was added to ester **4** (1.5 mmol, 0.68 g) or 25% TFA in DCM (12 mL) to ester **23b** or **23c** (1 mmol). The resulting solution left at 0 °C for 30 min and then at RT for additional 30 min. Solvents were removed under reduced pressure and the residual solids were treated with Et_2O and refrigerated overnight. The precipitates were filtered to afford pure acids **5**–**7**.

*4.6.1. (E)-4-(3-(2,4-Dimethoxyphenylcarbamoyl)-1H-indol-1-yl)-4-oxobut-2-enoic acid (**5**)*

Yield: 0.53 g (90%), yellow solid, m.p. 273–274 °C, R_f (H): 0.13, ESI-MS (*m*/*z*): 417.39 (*M* + Na), 395.45 (*M* + H). IR (KBr, cm^{-1}): 3428, 3200-2400, 1702, 1625, 1543. ^1H NMR (DMSO-d_6): δ 9.41 (1H, s, N*H*CO), 9.03 (1H, s, H-5), 8.44 (1H, d, *J* 7.6 Hz, H-11), 8.24 (1H, d, *J* 7.6 Hz, H-8), 7.86 (1H, d, *J* 15.2 Hz, H-3), 7.48 (1H, d, *J* 8.4 Hz, H-19), 7.44 and 7.40 (2H, two t, *J* 7.6 Hz, H-9/10), 6.98 (1H, d, *J* 15.2 Hz, H-2), 6.67 (1H, d, *J* 2.4 Hz, H-16), 6.56 (1H, dd, *J* 7.6 and 2.4 Hz, H-18), 3.83 (3H, s, OC*H*$_3$), 3.79 (3H, s, OC*H*$_3$) ppm. ^{13}C NMR (DMSO-d_6): δ 165.6, 163.1, 161.5, 157.6, 153.2, 135.5, 135.1, 132.2, 128.5, 128.2, 126.5, 125.3, 124.6, 121.6, 119.0, 116.3, 115.9, 104.1, 98.8, 55.5, 55.1 ppm. Anal. Calcd. for $C_{21}H_{18}N_2O_6$: C, 63.96; H, 4.60; N, 7.10. Found: C, 64.28; H, 4.41; N, 6.95.

*4.6.2. (E)-4-oxo-4-(3-(Phenylcarbamoyl)-1H-indol-1-yl) but-2-enoic acid (**6**)*

Yield: 0.28 g (85%), yellow solid, m.p. 143–145 °C, R_f (I): 0.32, ESI-MS (*m*/*z*): 691.20 (2*M* + Na), 357.30 (*M* + Na), 335.11 (*M* + H). IR (KBr, cm^{-1}): 3338, 3138, 1718, 1698, 1644. ^1H NMR (DMSO-d_6): δ 10.14 (1H, s, N*H*CO), 8.97 (1H, s, H-5), 8.44 (1H, d, *J* 7.0 Hz, H-11), 8.29 (1H, d, *J* 7.0 Hz, H-8), 7.86 (1H, d, *J* 15.2 Hz, H-3), 7.76 (2H, d, *J* 7.6 Hz, H-15/19), 7.52–7.28 (4H, m, H-9/10/16/18), 7.12 (1H, t, *J* 7.2 Hz, H-17) 7.01 (1H, d, *J* 15.2 Hz, H-1) ppm. ^{13}C NMR (DMSO-d_6): δ 166.3, 163.7, 162.1, 139.3, 136.2, 135.8, 132.9, 129.2 (two C), 129.1, 129.0, 126.1, 125.4, 124.0, 122.3, 120.7 (two C), 116.9, 116.6 ppm. Anal. Calcd. for $C_{19}H_{14}N_2O_4$: C, 68.26; H, 4.22; N, 8.38. Found: C, 68.01; H, 4.44; N, 8.68.

*4.6.3. (E)-4-(3-(3-Nitrophenylcarbamoyl)-1H-indol-1-yl)-4-oxobut-2-enoic acid (**7**)*

Yield: 0.30 g (79%) yellow solid; m.p. 278–280 °C; R_f(I): 0.26; ESI-MS (*m*/*z*): 418.12 (*M* + K), 402.09 (*M* + Na), 380.17 (*M* + H); IR (KBr, cm^{-1}): 3420, 3200-2500, 1728, 1702, 1654; ^1H NMR (DMSO-d_6): δ 10.51 (1H, s, N*H*CO), 8.95 (1H, s, H-5), 8.73 (1H, s, H-15), 8.40 (1H, d, *J* 8.0 Hz, H-17), 8.27 (1H, d, *J* 8.0 Hz, H-11), 8.14 (1H, d, *J* 8.0 Hz, H-19), 7.94 (1H, d, *J* 8.0 Hz, H-8), 7.82 (1H, d, *J* 15.2 Hz, H-3), 7.65 (1H, t, *J* 8.0 Hz, H-18), 7.43 and 7.40 (2H, two t, *J* 8.0 Hz, H-9/10), 6.99 (1H, d, *J* 15.2 Hz, H-2) ppm; ^{13}C NMR (DMSO-d_6): δ 166.3, 163.6, 162.5, 148.4, 140.6, 136.3, 135.7, 132.7, 130.6, 129.6, 128.8, 126.2 (two C), 125.5, 122.2, 118.3, 116.6, 116.2, 114.4 ppm. Anal. Calcd. for $C_{19}H_{13}N_3O_6$: C, 60.16; H, 3.45; N, 11.08. Found: C, 59.84; H, 3.68; N, 11.38.

*4.7. Procedures for the Wittig reaction between the carbonyl compounds **24**, **25** and **28** and the phosphorane $Ph_3P{=}CHCO_2tBu$*

*4.7.1. For adduct **26***

To a solution of $Ph_3P{=}CHCO_2tBu$ (2.2 mmol, 0.83 g) in DCM (2 mL) was added indole-3-carboxaldehyde (**24**) (2 mmol, 0.29 g). The resulting solution left for 16 h at RT and then evaporated to dryness. The oily residue was subjected to FCC to afford ester **26**.

*4.7.2. For adduct **27***

The procedure has been previously described [9].

*4.7.3. For adduct **29***

To a solution of $Ph_3P{=}CHCO_2tBu$ (2.2 mmol, 0.83 g) in MeCN (2 mL) was added 1-tosyl-3-acetylpyrrole (**28**)(2 mmol, 0.53 g). The resulting solution was set at 80 °C for overnight and then evaporated to dryness. The anticipated ester was obtained, as a mixture of geometric isomers, through FCC using as an eluant the solvent system C. Pure *E*-ester was isolated as a white solid in 64% yield after crystallization from AcOEt/hexane. The thus obtained ester (1.2 mmol, 0.43 g) was treated with a 10 N NaOH solution (0.5 mL) in DMSO (5 mL) at RT for 2 h. The reaction mixture was diluted with EtOAc and washed twice with water. The organic layer was dried over Na_2SO_4 and evaporated to dryness to afford ester **29** in 98% yield.

*4.7.3.1. tert-Butyl (E)-3-(1H-indol-3-yl)acrylate (**26**).* Yield: 0.45 g (93%), white solid, m.p. 124–125 °C, R_f (D): 0.33, GC MS (EI): t_R = 11.241 min, *m*/*z* (rel.int.), 243 (16, *M*), 187 (100, *M* − $CH_2{=}CMe_2$), 170 (35, *M* − OtBu). IR (KBr, cm^{-1}): 3276, 2982,

2926, 1678, 1612. ^{1}H NMR ($CDCl_3$): δ 8.77 (1H, br.s, N*H*), 7.92 (1H, d, *J* 7.2 Hz, H-7), 7.84 (1H, d, *J* 16 Hz, H-3), 7.42 (1H, d, *J* 2.4 Hz, H-5), 7.41 (1H, d, *J* 7.2 Hz, H-10), 7.27 and 7.24 (2H, two t, *J* 7.2 Hz, H-8/9), 6.42 (1H, d, *J* 16 Hz, H-2), 1.57 (9H, s, C(CH_3)$_3$) ppm. ^{13}C NMR ($CDCl_3$): δ 167.8, 137.2, 129.1, 128.5, 128.3, 123.2, 121.3, 120.5, 115.4, 113.6, 111.7, 79.9, 28.4 (three C) ppm. Anal. Calcd. for $C_{15}H_{17}NO_2$: C, 74.05; H, 7.04; N, 5.76. Found: C, 73.78; H, 7.34; N, 6.03.

*4.7.3.2. tert-Butyl (Z)-2-(4-amino-9H-fluoren-9-ylidene)acetate (Z – **27**).* Reddish solid, m.p. 113–114 °C, R_f (B): 0.30, GC-MS (EI): t_R = 13.040, *m*/*z* (rel.int.), 293 (9, *M*), 237 (100, *M* – CH_2=CMe_2), 220 (11, *237* – OH), 193 (13, *237* – CO_2). IR (KBr, cm^{-1}): 3404, 3338, 1700, 1628. ^{1}H NMR ($CDCl_3$): δ 8.32 (1H, d, *J* 7.6 Hz, H-14), 7.62 (1H, d, *J* 7.6 Hz, H-11), 7.53 (1H, d, *J* 7.6 Hz, H-5), 7.32 (1H, t, *J* 6.8 Hz, H-13), 7.15 (1H, t, *J* 6.8 Hz, H-12), 7.09 (1H, t, *J* 7.6 Hz, H-6), 6.71 (1H, d, *J* 7.6 Hz, H-7), 6.63 (1H, s, H-2), 3.96 (2H, s, NH_2), 1.54 (9H, s, (CH_3)$_3$) ppm. ^{13}C NMR ($CDCl_3$): δ 165.9, 146.6, 141.6, 140.4, 138.7, 136.6, 130.3, 128.7, 125.9, 121.5, 121.0, 120.3, 119.9, 115.7, 112.4, 81.0, 28.3 (three C) ppm. Anal. Calcd. for $C_{19}H_{19}NO_2$: C, 77.79; H, 6.53; N, 4.77. Found: C, 78.00; H, 6.28; N, 4.50.

*4.7.3.3. tert-Butyl (E)-3-(1H-pyrrol-3-yl)but-2-enoate (**29**).* Yield: 0.26 g (63% overall yield), white solid, m.p. 93–95 °C, R_f (C): 0.30, GC-MS (EI): t_R = 9.181 min, *m*/*z* (rel.int.), 207 (12, *M*), 151 (93, *M* – CH_2=CMe_2), 133 (100, *151* – H_2O), 105 (35, *133* – CO). IR (KBr, cm^{-1}): 3356, 2974, 1676, 1606. ^{1}H NMR ($CDCl_3$): δ 8.35 (1H, br.s, N*H*), 7.04 (1H, q, *J* 2.6 Hz, H-5), 6.77 (1H, dd, *J* 4.6 and 2.6 Hz), 6.46 (1H, dd, *J* 4.6 and 2.6 Hz, H-7), 6.05 (1H, q, *J* 1.2 Hz, H-2), 2.48 (3H, d, *J* 1.2 Hz, H-8), 1.51 (9H, s, C(CH_3)$_3$) ppm. ^{13}C NMR ($CDCl_3$): δ 165.5, 154.6, 128.1, 119.4, 118.2, 112.8, 106.2, 81.4, 28.2 (three C), 14.3 ppm. Anal. Calcd. for $C_{12}H_{17}NO_2$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.86; H, 8.01; N, 6.43.

*4.8. General procedure for the preparation of the cinnamoyl fluorides **31***

To an ice-cold solution (−10 °C) of cinnamic acids **30** (6 mmol) and pyridine (42 mmol, 3.38 mL) in DCM (8 mL) was added dropwise a solution of cyanuric fluoride (12 mmol, 1.03 mL) in DCM (4 mL). The resulting solution was left at −10 °C for 1 h and at RT for additional 30 min. The reaction mixture was diluted with $CHCl_3$ and washed twice with water. Drying over Na_2SO_4 and evaporation to dryness left a residue, from which pure fluorides **31** were obtained through FCC.

*4.8.1. (E)-3-(Benzo[d][1,3]dioxol-5-yl)acryloyl fluoride (**31a**)*

Yield: 0.58 g (50%), white solid, m.p. 103–104 °C, R_f (A): 0.3, GS-MS (EI): t_R = 8.183, *m*/*z* (rel.int.), 194 (100, *M*), 166 (23, *M* – CO), 146 (17, *166* – HF), 136 (6, *166* – CH_2O). IR (KBr, cm^{-1}): 1819, 1797, 1773, 1628. ^{1}H NMR ($CDCl_3$): δ 7.73 (1H, d, *J* 16 Hz, H-3), 7.06 (1H, d, *J* 8.4 Hz, H-9), 7.05 (1H, s, H-5), 6.85 (1H, d, *J* 8.4 Hz, H-8), 6.16 (1H, dd, *J* 16 and 7.2 Hz, H-2), 6.05 (2H, s, OCH_2O) ppm. ^{13}C NMR ($CDCl_3$): δ 157.6 (d, *J* 335 Hz), 151.3, 151.2 (d, *J* 11 Hz), 149.0, 128.0, 126.2, 110.1 (d, *J* 68.2 Hz), 109.1, 107.0, 102.2 ppm. Anal. Calcd. for $C_{10}H_7FO_3$: C, 61.86; H, 3.63. Found: C, 61.60; H, 3.90.

*4.8.2. (E)-3-(2,4,5-Trimethoxyphenyl)acryloyl fluoride (**31b**)*

Yield: 0.43 g (30%), yellow solid, m.p. 93–95 °C, R_f (C): 0.28, GS-MS: (EI): t_R = 9.609, *m*/*z* (rel.int.), 240 (100, *M*), 225 (49, *M* – Me), 209 (3, *M* – OMe), 197 (20, *225* – CO), 178 (8, *197* – F), 163 (5, *178* – Me). IR (KBr, cm^{-1}): 2944, 2838, 1770, 1604. ^{1}H NMR ($CDCl_3$): δ 8.10 (1H, d, *J* 16 Hz, H-3), 6.98 (1H, s, H-9), 6.50 (1H, s, H-6), 6.28 (1H, dd, *J* 16 and 7.5 Hz, H-2), 3.95 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.87 (3H, s, OCH_3) ppm. ^{13}C NMR ($CDCl_3$): δ 158.3 (d, *J* 333.7 Hz), 155.0, 153.7, 146.4 (d, *J* 6.6 Hz), 143.4, 113.7, 111.3, 108.8 (d, *J* 65.8 Hz), 96.4, 56.5, 56.2, 56.1 ppm. Anal. Calcd. for $C_{12}H_{13}FO_4$: C, 60.00; H, 5.45. Found: C, 60.25; H, 5.17.

*4.9. Procedures for coupling the cinnamoyl fluorides **31** with the amines **26**, **27** and **29***

*4.9.1. For compounds **10**, **11**, **14**/**15** and pure **14***

To an ice-cold (0 °C) solution of cinnamoyl fluoride **25a** or **25b** (1 mmol) and amine **26** or **27** or *Z* – **27** (1.05 mmol) in dry $CHCl_3$ (1 mL) DIPEA (2 mmol, 0.35 mL) was added. The thus obtained solution was set at 40 °C for 2–3 h, diluted with $CHCl_3$ and then washed twice with water. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The resulting residues were subjected to FCC to afford *tert*-butyl esters **10**, **11**, **14** and **14**/**15** (only the data for pure **14** are provided below).

*4.9.2. For compound **13***

To an ice-cold suspension of NaH (0.6 mmol, 14.5 mg) in THF (0.65 mL) was added portionwise amine **29** (0.55 mmol, 0.11 g). After 30 min, fluoride **31b** (0.5 mmol, 0.12 g) was added and the resulting solution was left at RT for overnight. Evaporation to dryness and routine FCC purification afforded *tert*-butyl ester **13**.

*4.9.2.1. tert-Butyl (E)-3-(1-((E)-3-benzo[d][1,3]dioxol-5-yl)acryloyl)-1H-indol-3-yl)acrylate (**10**).* Yield: 0.30 g (71%), yellow solid, m.p. 141–143 °C, R_f (B): 0.37, ESI-MS (*m*/*z*): 857.39 (2*M* + Na), 835.35 (2*M* + H), 440.21 (*M* + Na), 418.18 (*M* + H). IR (KBr, cm^{-1}): 2978, 2904, 1686, 1628. ^{1}H NMR ($CDCl_3$): δ 8.53 (1H, d, *J* 8.2 Hz, H-20), 7.91 (1H, d, *J* 15.2 Hz, H-3), 7.87 (1H, d, *J* 7.2 Hz, H-7), 7.86 (1H, s, H-5), 7.75 (1H, d, *J* 16 Hz, H-14), 7.43 (1H, t, *J* 7.2 Hz, H-8), 7.38 (1H, t, *J* 7.2 Hz, H-9), 7.16 (1H, s, H-16), 7.14 (1H, d, *J* 8.2 Hz, H-19), 7.03 (1H, d, *J* 15.2 Hz, H-2), 6.86 (1H, d, *J* 7.2 Hz, H-10), 6.53 (1H, d, *J* 16 Hz, H-13), 6.04 (2H, s, OCH_2O), 1.57 (9H, s, C(CH_3)$_3$) ppm. ^{13}C NMR ($CDCl_3$): δ 166.6, 164.2, 150.4, 148.6, 147.3, 144.3, 137.0, 134.9, 128.6, 128.1, 126.7, 125.8, 125.5, 124.4, 120.2, 118.3, 117.1, 114.3, 108.8, 106.7, 101.8, 80.5, 28.3 (three C) ppm. Anal. Calcd. for $C_{23}H_{25}NO_5$: C, 71.93; H, 5.55; N, 3.36. Found: C, 72.28; H, 5.27; N, 3.12.

*4.9.2.2. tert-Butyl (E)-3-(1-((E)-3-(2,4,5-trimethoxyphenyl)acryloyl)-1H-indol-3-yl)acrylate (**11**).* Yield: 0.35 g (76%), yellow solid, m.p. 158–159 °C, R_f (B): 0.10, ESI-MS (*m*/*z*): 949.67 (2*M* + Na), 486.44 (*M* + Na), 464.45 (*M* + H), 390.26 ([*M* + H] − tBuOH). IR (KBr, cm^{-1}): 2970, 2944, 1702, 1676, 1636. ^{1}H NMR ($CDCl_3$): δ 8.56 (1H, d, *J* 7.7 Hz, H-7), 8.21 (1H, d, *J* 16 Hz, H-3), 7.89 (1H, s, H-5), 7.87 (1H, d, *J* 7.7 Hz, H-10), 7.77 (1H, d, *J* 16 Hz, H-14), 7.45 (1H, t, *J* 7.7 Hz, H-8), 7.37 (1H, t, *J* 7.7 Hz, H-9), 7.23 (1H, d, *J* 16 Hz, H-2), 7.08 (1H, s, H-20), 6.54 (1H, s, H-17), 6.52 (1H, d, *J* 16 Hz, H-13), 3.96 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 1.56 (9H, s, C(CH_3)$_3$) ppm. ^{13}C NMR ($CDCl_3$): δ 166.8, 165.1, 155.1, 153.2, 143.6, 143.2, 137.2, 135.3, 128.2, 127.2, 125.7, 124.3, 120.3, 120.0, 118.0, 117.1, 115.2, 114.8, 113.0, 97.2, 80.5, 56.9, 56.5, 56.3, 28.4 (three C) ppm. Anal. Calcd. for $C_{27}H_{29}NO_6$: C, 69.96; H, 6.31; N, 3.02. Found: C, 69.70; H, 6.52; N, 3.38.

*4.9.2.3. tert-Butyl (E)-3-(1-((E)-3-(2,4,5-trimethoxyphenyl)acryloyl)-1H-pyrrol-3-yl)but-2-enoate (**13**).* Yield: 0.14 g (65%), yellow foam, R_f (C): 0.20, ESI-MS (*m*/*z*): 450.30 (*M* + Na), 428.31 (*M* + H), 372.27 ([*M* + H] − CH_2=CMe_2), 221.36 ([*M* + H] − [pyrrole − C(Me) = $CHCO_2$tBu]). IR (KBr, cm^{-1}): 2976, 2946, 1704, 1694, 1638. ^{1}H NMR ($CDCl_3$): δ 8.21 (1H, d, *J* 15.4 Hz, H-11), 7.68 (1H, br.s, H-6), 7.42 (1H, unresolved dd, H-7), 7.10 (1H, d, *J* 15.4 Hz, H-10), 7.06 (1H, s, H-17), 6.55 (unresolved dd, H-8), 6.52 (1H, s, H-14), 6.08 (1H, s, H-2), 3.95 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 2.48 (3H, s, H-4), 1.51 (9H, s, C(CH_3)$_3$) ppm. ^{13}C NMR ($CDCl_3$): δ 166.8, 163.5, 155.0, 153.0, 146.7, 143.7, 143.3, 130.2, 120.4, 118.4, 115.6, 114.8, 112.7, 112.3,

110.4, 96.6, 79.8, 56.7, 56.3, 56.1, 28.3 (three C), 16.2 ppm. Anal. Calcd. for $C_{24}H_{29}NO_6$: C, 67.43; H, 6.84; N, 3.28. Found: C, 67.63; H, 6.57; N, 3.04.

*4.9.2.4. tert-Butyl (Z)-2-(4-((E)-3-benzo[d][1,3]dioxol-5-yl)acrylamido)-9H-fluoren-9-ylidene)acetate (**14**).* Yield: 0.23 g (50%), yellow solid, m.p. 208–209 °C, R_f (B): 0.15, ESI-MS (*m*/*z*): 957.06 (2*M* + Na), 935.13 (2*M* + H), 490.09 (*M* + Na), 468.07 (*M* + H), 411.96 ([*M* + H] − tBu). IR (KBr, cm^{-1}): 2960, 2922, 1710, 1656, 1630. ^{1}H NMR ($CDCl_3$, 45 °C): δ 8.72 (1H, br.s, NHCO), 7.71 (1H, d, *J* 15.6 Hz, H-18), 7.70–7.60 (2H, m, H-11/14), 7.51 (1H, m, H-13), 7.40–7.20 (3H, m, H-5/23/24), 7.30 (1H, s, H-20), 7.10–6.94 (2H, m, H-6/12), 6.81 (1H, m, H-7), 6.69 (1H, s, H-2), 6.47 (1H, d, *J* 15.6 Hz, H-17), 5.99 (2H, s, OCH_2O), 1.62 (9H, s, $C(CH_3)_3$) ppm. ^{13}C NMR ($CDCl_3$): δ 165.7, 165.6, 149.4 (two C), 148.2, 145.7, 140.8, 139.1 (two C), 135.8, 132.0, 130.8, 129.0, 128.9, 128.2, 127.6, 127.2, 124.3, 121.1, 118.6, 116.4, 108.5, 106.6 (two C), 101.5, 81.3, 28.2 (three C) ppm.

4.10. General procedure for carboxyl group deprotection

tert-Butyl ester **10** or **11** (0.20 mmol) was treated with an ice-cold solution of 50% TFA in DCM (2 mL) for 1 h and then evaporated to dryness. Addition of Et_2O and overnight refrigeration gave a precipitate, which upon filtration afforded the acid **8** or **12**, respectively.

*4.10.1. (E)-3-(1-((E)-3-benzo[d][1,3]dioxol-5-yl)acryloyl)-1H-indol-3-yl)acrylic acid (**8**)*

Yield: 0.065 g (90%), white solid, m.p. 240–242 °C, R_f (H): 0.17, ESI-MS (*m*/*z*): 400.03 (*M* + K), 384.03 (*M* + Na), 362.09 (*M* + H). IR (KBr, cm^{-1}): 3412, 2978, 1698, 1654, 1634. ^{1}H NMR (DMSO-d_6): δ 8.89 (1H, s, H-5), 8.52 (1H, d, *J* 8.0 Hz, H-7), 7.95 (1H, d, *J* 7.2 Hz, H-20), 7.88 (1H, d, *J* 15.2 Hz, H-3), 7.79 (1H, d, *J* 16 Hz, H-14), 7.66 (1H, s, H-16), 7.60 (1H, d, *J* 15.2 Hz, H-2), 7.43 and 7.39 (2H, two t, *J* 8.0 Hz, H-8/9), 7.36 (1H, d, *J* 7.2 Hz, H-19), 7.02 (1H, d, *J* 8.0 Hz, H-10), 6.63 (1H, d, *J* 16 Hz, H-13), 6.13 (2H, s, OCH_2O) ppm. ^{13}C NMR (DMSO-d_6): δ 168.3, 164.7, 150.4, 148.7, 147.2, 136.8, 135.9, 129.8, 129.3, 128.3, 126.7, 125.9, 124.9, 120.5, 118.8, 117.3, 117.1, 115.5, 109.0, 107.3, 102.3 ppm. Anal. Calcd. for $C_{21}H_{15}NO_5$: C, 69.80; H, 4.18; N, 3.88. Found: C, 69.53; H, 4.38; N, 4.05.

*4.10.2. (E)-3-(3-oxo-1-(2,4,5-trimethoxyphenyl)-2,3-dihydro-1H-pyrrolo[1,2-a]indol-9-yl)acrylic acid (**12**)*

Yield: 0.068 g (84%), white solid, m.p. 235–236 °C, R_f (H): 0.22, ESI-MS (*m*/*z*): 430.57 (*M* + Na), 408.60 (*M* + H), 390.63 ([*M* + H] − H_2O), 240.53 ([*M* + H] − $(MeO)_3Ph$]), 214.59 (240 − C_2H_2), 158 (214 − 2CO), 141.6 (158 − OH). IR (KBr, cm^{-1}): 3446, 3200–2400, 1750, 1684, 1618. ^{1}H NMR (DMSO-d_6): δ 12.1 (1H, br.s, CO_2H), 8.08–8.02 (1H, m, H-9), 7.88–7.84 (1H, m, H-6), 7.44 (1H, d, *J* 16 Hz, H-3), 7.42–7.35 (2H, m, H-7/8), 7.05 (1H, s, H-21), 6.69 (1H, s, H-18), 6.20 (1H, d, *J* 16 Hz, H-2), 4.98 (1H, dd, *J* 9.2 and 3.2 Hz, H-14), 3.77 (3H, s, OCH_3), 3.68 (3H, s, OCH_3), 3.62 (1H, dd, *J* 18 and 9.2 Hz, H-13a), 3.58 (3H, s, OCH_3), 2.98 (1H, dd, *J* 18 and 3.2 Hz, H-13b) ppm. ^{13}C NMR (DMSO-d_6): δ 172.2, 168.2, 151.7 (two C), 149.5, 143.0, 134.9, 132.4, 130.5, 125.2, 124.6, 121.0, 119.4, 117.4, 114.8, 113.6, 108.9, 99.4, 56.9, 56.8, 56.2, 43.2, 34.9 ppm. Anal. Calcd. for $C_{23}H_{21}NO_6$: C, 67.80; H, 5.20; N, 3.44. Found: C, 68.10; H, 4.98; N, 3.21.

4.11. Preparation of (E)-N-(2,4-dimethoxyphenyl)-1-(3-(2,4,5-trimethoxyphenyl)acryloyl)-1H-indole-3-carboxamide (**16**)

To a solution of anilide **20a** (1.05 mmol, 0.31 g) and DIPEA (2 mmol, 0.35 mL) in $CHCl_3$/DMF (85:15, 1.2 mL) was added fluoride **31b** (1 mmol, 0.24 g). The reaction mixture was stirred at 40 °C for overnight, diluted with DCM and washed once with 5% aq. $NaHCO_3$ and twice with water. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Pure analog **16** was obtained through FCC purification.

Yield: 0.22 g (43%), yellow solid, m.p. 275–276 °C, R_f (E): 0.22, ESI-MS (*m*/*z*): 1055.53 (2*M* + Na), 1033.57 (2*M* + H), 539.71 (*M* + Na), 517.68 (*M* + H). IR (KBr, cm^{-1}): 2932, 2834, 1684, 1662. ^{1}H NMR ($CDCl_3$, 40 °C): δ 8.61 (1H, dd, *J* 6.8 and 1.6 Hz, H-11), 8.41 (1H, d, *J* 9.2 Hz, H-4), 8.38 (1H, s, NHCO), 8.36 (1H, d, *J* 9.2 Hz, H-5), 8.28 (1H, d, *J* 15.2 Hz, H-18), 8.04 (1H, dd, *J* 6.8 and 1.6 Hz, H-14), 7.50–7.38 (2H, m, H-12/13), 7.28 (1H, d, *J* 15.2 Hz, H-17), 7.11 (1H, s, H-9), 6.56 (1H, s, H-24), 6.54 (2H, s, H-2/21), 3.96 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 3.83 (3H, s, OCH_3) ppm. ^{13}C NMR ($CDCl_3$, 40 °C): δ 165.3, 161.6, 156.6, 155.0, 153.1, 149.4, 143.4, 136.6, 128.7, 126.6, 125.5, 124.7, 124.6, 121.3, 120.9, 119.6, 117.8, 117.3, 114.8, 113.8, 111.8, 104.0, 98.7, 96.7, 56.6, 56.4, 56.1, 56.0, 55.6 ppm. Anal. Calcd. for $C_{29}H_{28}N_2O_7$: C, 67.43; H, 5.46; N, 5.42. Found: C, 67.18; H, 5.67; N, 5.75.

4.12. General biological assays

DPPH, NDGA and CA 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). Carrageenan 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.

4.13. In vitro assays

4.13.1. Determination of the reducing activity of the stable radical DPPH [30]

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. The 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 (Table 1).

4.13.2. Inhibition of linoleic acid lipid peroxidation [15]

Ten microliters of the 16 mM sodium linoleate solution 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.

4.13.3. Soybean LOX inhibition study in vitro [30]

The tested compounds dissolved in DMSO were incubated at RT with sodium linoleate (0.1 ml) and 0.2 ml of enzyme solution (1/9×10^{-4} w/v in saline). The conversion of sodium linoleate to 13-hydroperoxylinoleate was recorded at 234 nm and compared with the appropriate standard inhibitor.

4.13.4. In vivo assays: inhibition of the Carrageenan-induced oedema [30]

Oedema was induced in the right hind paw of Fisher 344 rats (150–200 g) by the intradermal injection of 0.1 ml 2% Carrageenan in water.

Both sexes were used. Pregnant females 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 recognised guidelines on animal experimentation. The tested compounds (0.01 mmol/kg body weight), were diluted in water with few drops of Tween 80 and ground in a mortar before use and they were given intraperitoneally simultaneously with the Carrageenan injection. The rats were euthanized 3.5 h after Carrageenan administration. 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 1). Indomethacin was tested (0.01 mmol//kg body weight) as a reference compound (47%). Values % ICPE are the mean from two different experiments with a standard error of the mean less than 10%.

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