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# Synthesis and pharmacochemical evaluation of novel aryl-acetic acid inhibitors of lipoxygenase, antioxidants, and anti-inflammatory agents

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**Abstract**—Lipoxygenase catalyzes the first two steps of the transformation of arachidonic acid into leukotrienes which are implicated in host defense reactions. It is well known that many acids possess potent anti-inflammatory activity. Taking into account that compounds bearing a thienyl, naphthyl, pyrollyl, and 2,4-di-*tert*-butyl-phenol moieties possess anti-inflammatory activity which is related to their capacity to transfer electrons and to scavenge reactive oxygen species, we synthesized some new aryl-acetic acids and we explored their ability to inhibit soybean lipoxygenase, to present antioxidant and anti-inflammatory activities, and to interact with glutathione. The compounds have shown important antioxidant activity, medium anti-inflammatory activity, and very good inhibition of soybean lipoxygenase. Compound 3-(3,5-di-*tert*-butyl-2-hydroxy-phenyl)-2-phenyl-acrylic acid (**1i**) showed significant in vitro LO inhibition ($IC_{50}$ 65 μM). The results are discussed in terms of structural and physicochemical characteristics of the compounds. The structures of the synthesized compounds were confirmed by spectral and elemental analysis. Their lipophilicity are experimentally determined by RPTLC method.


## 1. Introduction

Eicosanoids are a family of lipid mediators derived from the metabolism of arachidonic acid. Eicosanoids such as prostanoids and leukotrienes have a wide range of biological actions including potent effects on inflammation and immunity. Once liberated from the cell membrane, arachidonic acid may become substrate for various metabolic pathways that produce biological mediators.[1,2] The most important of these pathways are the cyclooxygenase and the lipoxygenase.

Lipoxygenases (LOs) constitute a family of dioxygenases, that catalyze the oxygenation of free and esterified polyunsaturated fatty acids containing a (1*Z*,4*Z*)-penta-1,4-diene system to produce the corresponding hydroperoxy derivatives.[3,4] They are monomeric proteins that contain a 'non-heme' iron per molecule in the active site as high-spin Fe(II) in the native state, and high-spin Fe(III) in the activated state, and they are categorized with respect to their positional specificity of arachidonic acid (AA) oxygenation.[5–7] Six LO families may be distinguished: 5-LOs, 8-LOs, 9-LOs, 11-LOs, 12-LOs, and 15-LOs.[8,4]

Lipoxygenases play an essential role in the biosynthesis of the leukotrienes (LTs). LTs are potent biological mediators in the pathophysiology of inflammatory diseases and host defense reactions.[9] These properties imply a significant role for $LTB_4$ in the pathogenesis of inflammatory diseases, such as asthma,[10] psoriasis,[11] atherosclerosis,[12] and cancer.[13,14]

It is well known that many natural products and synthetic compounds act by reducing the active site iron thereby uncoupling the catalytic cycle of the enzyme. Thus, phenols like nordihydroguaretic acid, caffeic acid, flavonoids, coumarins, or compounds like phenidone are efficient 5-LO inhibitors in vitro and in vivo.[15,16]

Recently we have reported a series of aryl-acetic and aryl-hydroxamic acids, having potent inhibitory activity against soybean lipoxygenase and possessing antioxidant and anti-inflammatory activities.[17–19] In continuation to

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our effort toward highly effective antioxidants, inhibitors of LOs, we synthesized some new aryl-acetic acids combining the appropriate physicochemical features in order to inhibit soybean lipoxygenase and to present antioxidant and anti-inflammatory activities. Further the synthesized compounds were screened for detailed free radical scavenging ability, in vitro lipoxygenase inhibition studies, and in vivo anti-inflammatory activity.

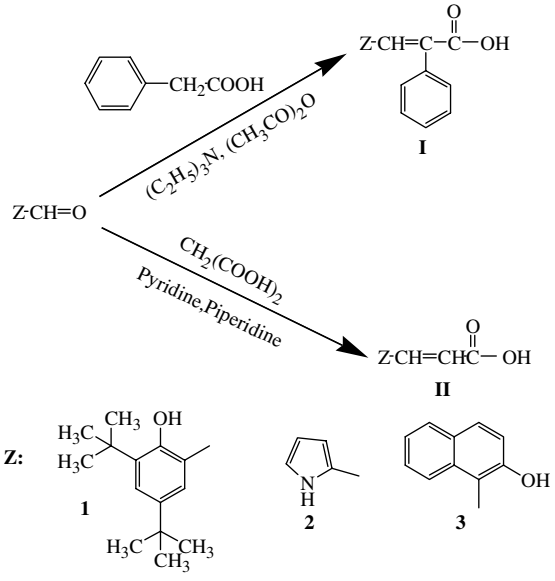
The suggested structural variations could affect both efficacy and their tolerability partly due to differences in their physicochemical properties, which determine their distribution in the body and their ability to pass through and to enter in the interior membranes.[20,21]

## 2. Results and discussion

### 2.1. Chemistry

The synthesis of the aryl-acetic acids was accomplished according to the Knoevenagel condensation as indicated in Scheme 1. The aryl-acetic acids of series I are obtained by the condensation of the suitable aldehyde with phenylacetic acid and acetic acid anhydride in the presence of triethylamine, while the aryl-acetic acids of series II are obtained by the condensation of the suitable aldehyde with malonic acid in the presence of pyridine and piperidine. Reactions were monitored by thin-layer chromatography.

The corresponding reactions proceeded smoothly and in good yields (46–73%), compound **2i** was the only exception (25%). All the compounds formed were recrystallized by using ethanol/water. The structures of the synthesized compounds are given in Table 1 and they are confirmed by UV, IR, $^{1}$H NMR, $^{13}$C NMR, and elemental analysis. All the acids present the characteristic absorption in the IR (Nujol) (cm$^{-1}$ 3200 (O–H), 2950, 1720 (C=O), 1625 (C=C)). $^{1}$H NMR spectroscopy revealed that coordination of the aromatic and CH= protons was given by integration. The results are consistent with the proposed structures and are in agreement with previous findings.[17–19]




**Scheme 1.** Synthesis of aryl-acetic acids.

### 2.2. Physicochemical studies

Since lipophilicity is a significant physicochemical property determining distribution, bioavailability, metabolic activity, and elimination, we tried to determine experimentally their lipophilicity from RPTLC method as $R_M$ values and to compare them with the corresponding theoretically calculated clog $P$ values in $n$-octanol-buffer.[21] This is considered to be a reliable, fast, and convenient method for expressing lipophilicity.[22] Apart from the important role of lipophilicity for the kinetics of biologically active compounds, antioxidants of hydrophilic or lipophilic character are both needed to act as radical scavengers in the aqueous phase or as chain-breaking antioxidants in biological membranes.

From our results (Table 1) it can be concluded that $R_M$ values could not be used as a successful relative measure of the overall lipophilic/hydrophilic balance of these molecules. We could attribute this to the different nature of the hydrophilic and lipophilic phases in the two systems.

### 2.3. Calculation methods

Some physicochemical properties like the energy values of the lowest and highest unoccupied molecular orbital ($E_{LUMO}$ and $E_{HOMO}$) of the aryl-acetic acids, the electrostatic potential, etc. were calculated by the program Spartan v. 5.1.3 (Wavefunction Inc.) on energy-minimized structures. These results were used in order to describe better the activity of the compounds in quantitative structure–activity relationships (QSARs).

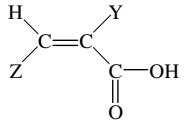
### 2.4. Biological assays

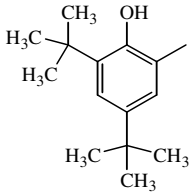
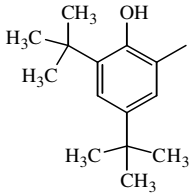
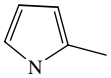
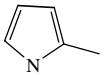
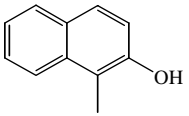
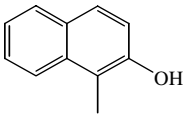
In this investigation, we synthesized some novel aryl-acetic acids that were expected to offer protection against inflammation and radical attack and inhibition of LO, by application of standard synthetic methods summarized in Scheme 1.

The reducing abilities of the examined compounds were determined by the use of the stable radical DPPH at 0.05 and 0.1 mM after 20 and 60 min (Table 2). This interaction indicates their radical scavenging ability in an iron-free system and expresses the reducing activity of compounds.[23] The reducing abilities ranged from 22% to 44%. Slight differences were observed within the compounds with the time and the concentration. The presence of the phenyl-ring does not offer to the reducing activity.

Regression analyses of the values of DPPH interaction (0.05 mM, 20 min and 0.1 mM, 20 min) revealed

**Table 1.** Synthesized aryl-acetic acids



| Compound | Z | Y | Formula* | $R_f$ | clog $P$** | $R_M$# (±SD) | Mp (°C) | Yield% |
|---|---|---|---|---|---|---|---|---|
| **1i** |  |  | $C_{23}H_{28}O_3$ | 0.86[a] | 6.47 | 0.87 (0.029)[c] | 135–137 | 73 |
| **1ii** |  | H | $C_{17}H_{24}O_3$ | 0.46[a] | 5.12 | −0.21 (0.011)[c] | 89–90 °C (760 mm Hg) | 46 |
| **2i** |  |  | $C_{13}H_{11}NO_2$ | 0.66[a] | 2.19 | 0.05 (0.003)[c] | 113–115 | 25 |
| **2ii** |  | H | $C_7H_7NO_2$ | 0.41[b] | 0.85 | 0.45 (0.026)[c] | 256–258 | 60 |
| **3i** |  |  | $C_{19}H_{14}O_3$ | 0.81[a] | 3.75 | 0.06 (0.004)[c] | 141–143 | 53 |
| **3ii** |  | H | $C_{13}H_{10}O_3$ | 0.66[b] | 2.36 | 0.85 (0.036)[c] | 126–128 | 72 |

Lipophilicity values: experimentally determined $R_M$ and theoretically calculated clog $P$ values.
[a] $CH_2Cl_2$.
[b] $CH_2Cl_2/CH_3COOC_2H_5$ (1:1).
[c] $CH_3OH/H_2O/CH_3COOH$, (77:23:0.1).
* Elemental analyses for molecular formula (±0.4%).
** Theoretically calculated clog $P$ values.
# $R_M$ values are the average of at least 10 measurements.

**Table 2.** Interaction % with DPPH (RA %); competition % with DMSO for hydroxyl radical (HO· %)

| Compound | RA % 0.05 mM | | RA % 0.1 mM | | HO· (%) | | |
|---|---|---|---|---|---|---|---|
| | 20 min | 60 min | 20 min | 60 min | 0.0 mM | 0.1 mM | 1 mM |
| **1i**[a] | 24 | 36 | 28 | 35 | 95 | 92 | * |
| **1ii** | 32 | 38 | 37 | 44 | 91 | 96 | 83 |
| **2i** | 22 | 29 | 23 | 35 | 86 | 96 | 88 |
| **2ii**[a] | 26 | 32 | 25 | 33 | no | 94 | * |
| **3i** | 29 | 37 | 26 | 32 | 97 | 94 | 86 |
| **3ii** | 32 | 38 | 34 | 42 | 90 | * | * |
| BHT | | | 31 | 60 | | | |
| NDGA | | | 81 | 83 | | | |
| Trolox | | | | | nt | 73.4 | 88.2 |

nt, not tested.
*Problems of dissolution under the experimental conditions.
[a] $k_{s\ (1i)}$, $0.908 \times 10^9\ M^{-1}\ s^{-1}$; $k_{s\ (2ii)}$, $2.911 \times 10^9\ M^{-1}\ s^{-1}$.

that $Elpot_{-min}$ (minimum electrostatic potential) is the main physicochemical parameter influencing their interaction:

$$\log\%(\mathrm{DPPH} - 20\ \mathrm{min} - 0.05\ \mathrm{mM}) = -0.015(\pm 0.011)Elpot_{-min} + 0.880(\pm 0.421) \quad (a)$$

$n = 5$, $r = 0.926$, $r^2 = 0.858$, $q^2 = 0.629$, $s = 0.032$, $F_{1,3} = 19$, $\alpha = 0.05$

$$\log\%(\mathrm{DPPH} - 20\mathrm{min} - 0.1\ \mathrm{mM}) = -0.013(\pm 0.012)Elpot_{-min} + 0.996(\pm 0.435) \quad (b)$$

$n = 5$, $r = 0.894$, $r^2 = 0.799$, $q^2 = 0.352$, $s = 0.043$, $F_{1,3} = 11$, $\alpha = 0.05$

It is consistent that rates of ROS production are increased in most diseases.[24,25] The cytotoxicity of $O_2^{-\cdot}$ and $H_2O_2$ in living organisms is mainly due to their transformation into ·OH, reactive radical metal complexes, and $^1O_2$. During the inflammatory process, phagocytes generate the superoxide anion radical at the inflamed site and this is connected to other oxidizing species as ·OH. Hydroxy radicals are among the most reactive oxygen species and are considered to be responsible for some of the tissue damage occurring in inflammation. It has been claimed that hydroxyl radical scavengers could serve as protectors, thus increasing prostaglandin synthesis.

The competition of aryl-acetic acids with DMSO for ·OH generated by the $Fe^{3+}$/ascorbic acid system expressed as percent inhibition of formaldehyde production was used for the evaluation of their hydroxyl radical scavenging activity. In this experiment compound **2ii** did not show any result at 0.01 mM concentration, all the other compounds showed high inhibition at 0.01 mM. All the compounds inhibited significantly the oxidation of DMSO (33 mM) at 0.1 mM except compound **3ii** which presents problems of dissolution under the experimental conditions. For compounds **1ii**, **2i**, and **2ii** the inhibition was found to be increased as the concentration of the tested compounds was increased. Lipophilicity is not correlated with the results. Antioxidants at hydrophilic or lipophilic character are both needed to act as radical scavengers in the aqueous phase or as chain-breaking antioxidants in biological membranes.

Mixing heme proteins with $H_2O_2$ generates powerfully oxidizing activated heme species and radicals on amino acid side chains that can cause lipid peroxidation. As a model of such reactions we used the peroxidation of arachidonic acid by a mixture of heme and $H_2O_2$. The tested compounds inhibit the lipid peroxidation almost equally (69–74%) with the exception of compound **2i** (60%). Lipophilicity does not affect inhibition.

Non-enzymatic superoxide anion radicals were generated. The majority of the compounds present high scavenging activity. Lipophilicity does not seem to increase the scavenging activity. Compound **1i** (61.5%) did not present high biological response. It seems that as the concentration increases the inhibitory activity decreases.

We tried to linearly correlate the superoxide anion scavenging activity and it seems that the energy of the lowest unoccupied molecular orbital ($E_{LUMO}$) is important for this activity. We can explain that by the ability of the compounds to gain an electron at an unoccupied molecular orbital.

Compounds were further evaluated for the inhibition of soybean lipoxygenase LO by the UV absorbance based enzyme assay.[26] While one may not extrapolate the quantitative results of this assay to the inhibition of mammalian 5-LO, it has been shown that inhibition of plant LO activity by NSAIDs is qualitatively similar to their inhibition of the rat mast cell LO and may be used as a simple qualitative screen for such activity.

Perusal of $IC_{50}$ values shows that compound **1i** is the most active, within the set followed by compound **2i** (Table 3). In this investigation all compounds were studied in order to gain insight into their LO-inhibition. Most of the LO inhibitors are antioxidants or free radical scavengers,[27] since lipoxygenation occurs via a carbon centerd radical. Some studies suggest a relationship between LO inhibition and the ability of the inhibitors to reduce the $Fe^{3+}$ at the active site to the catalytically inactive $Fe^{2+}$.[28,29] LOs 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 in the activated state $Fe^{3+}$. Several LO inhibitors are excellent ligands for $Fe^{3+}$. It has been demonstrated that their

**Table 3.** Inhibition % of carrageenin induced rat paw edema (CPE %); in vitro inhibition of soybean lipoxygenase (LO) ($IC_{50}$), % inhibition of heme dependent lipid peroxidation (LP %); % superoxide radical scavenging activity $O_2^{-\cdot}$

| Compound | CPE (%)[a] 0.01 mmol/kg body weight | LO $IC_{50}$ (μM) | A % LP-1 mM | $O_2^{-\cdot}$ (%) 0.1 mM |
|---|---|---|---|---|
| **1i** | 43.4** | 65 | 71 | 61 |
| **1ii** | 45.7** | 425 | 71 | 97 |
| **2i** | 49.8* | 70 | 60 | 90 |
| **2ii** | 29.5* | 170 | 73 | 85 |
| **3i** | 25.2* | 0.01 mM (66.3%)<br>0.1 mM (70.4%) | 74 | 96 |
| **3ii** | 44.1* | 415 | 69 | 93 |
| Caffeic acid | | 600 | 22 | 48 |
| Indomethacin | 47* | | | |

[a] Statistical studies were done with Student's *T*-test.
* $p < 0.01$.
** $p < 0.05$.

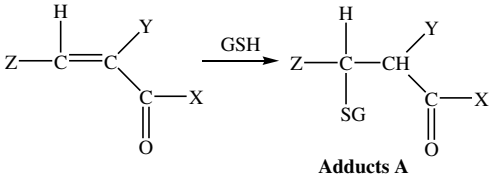
mechanism of action is presumably related to its coordination with a catalytically crucial $Fe^{3+}$. Lipophilicity is referred[30–33] to as an important physicochemical property for lipoxygenase inhibition. However in this data set lipophilicity does not seem to affect absolutely the LO inhibition. The presence of the phenyl ring seems to improve the inhibitory activity. For compound **3i** it was not possible to determine the $IC_{50}$. This derivative seems to inhibit constantly soybean LO (approximately 68.3%). This effect does not depend on the concentration used.

In acute toxicity experiments, the in vivo examined compounds did not present toxic effects in doses up to 0.5 mmol/kg body weight. Ulcerogenicity was not found. Acute inflammation is due to the release of chemical mediators, which cause edema as a result of extravasations of fluid and proteins from the local microvasculature and accumulation of polymorphonuclear leukocytes at the inflammatory site. The in vivo anti-inflammatory effects of the tested acids were assessed by using the carrageenin-induced rat paw edema (CPE) model and are presented in Table 3, percentage of weight increase at the right hind paw. The induced edema is a non-specific inflammation highly sensitive to non-steroidal anti-inflammatory agents (NSAIDs). Thus it has been accepted as a useful tool for studying new anti-inflammatory agents.[34] It reliably predicts the anti-inflammatory potency of the NSAIDs and detects during the second phase that are anti-inflammatory agents as a result of inhibition of prostaglandin amplification.[35] All the tested acids induced protection (ranged from 25.2% to 49.8%) against carrageenin induced paw edema while the reference drug indomethacin induced 47% protection at an equivalent dose. Compound **2i** was the most potent (49.8%). Compounds **1i** and **1ii** had almost equipotent effect. Thus, the presence of the phenyl-group in compound **1i** does not seem to affect the biological response. Between compounds **2i** and **2ii** the phenyl substituted derivative (**2**i) was found to be more potent than the 2ii. On the contrary, compound **3ii**, in which no phenyl group is present, inhibits stronger the carrageenin induced paw edema. Regression analysis was performed to discover whether any correlation existed between anti-inflammatory activity and several physicochemical parameters (lipophilicity, steric and electronic variables). Unfortunately the confidence limits were found to be poor.

A number of α,β-unsaturated ketones have demonstrated preferential reactivity toward thiols in contrast to amino and hydroxyl groups,[36] and hence these compounds may be free from the problems of mutagenicity and carcinogenicity which are associated with a number of alkylating agents used in cancer chemotherapy.[37] Alkylation with a cellular thiol such as glutathione GSH may occur with the α,β-unsaturated acids, leading to the adducts A and we have studied this possibility.

Glutathione conjugation is an important pathway by which reactive electrophilic compounds are detoxified. It protects vital cellular constituents against chemical reactive species by virtue of its nucleophilic sulfhydryl group and constitutes an in vivo antioxidant protective mechanism. The nucleophilic addition of GSH to electron-deficient carbon double bonds occurs mainly in compounds with α,β-unsaturated double bonds. In most instances the double bond is rendered electron deficient by resonance or conjugation with a carbonyl group. It should be emphasized that all α,β-unsaturated compounds are conjugated with GSH (**1i**, **1ii**, **2i**, **2ii**, **3i**, **3ii**).

For these compounds an alkylation may occur leading to the adducts A:




**Adducts A**

Only for compound **2i** the alkylation proceeds, higher when the concentration of GSH is high (10GSH). All compounds present high alkylation rates, whereas **3ii** presents slight alkylation rate. It seems that the stereochemistry of substituent Z affects the alkylation.

We tried to linearly correlate the expressions of anti-inflammatory, antioxidant, free radical scavenging activity, and LO inhibition activity for all tested compounds. None of these correlations were satisfactory enough ($r < 0.6$). Presumably these activities proceed via at least partially different mechanisms. Attempts to correlate these expressions of activity with $R_M$ values in a linear or non-linear regression analysis gave statistically non-significant equations.

## 3. Conclusions

Synthesis of aryl-acetic acids was achieved. Compound **1i** possesses good LO inhibitory activity. Almost all the compounds showed good rate toward various free radicals $HO^{\bullet}$, $O_2^{-\bullet}$. They were also found to be potent inhibitors of lipid peroxidation and to significantly be conjugated to GSH. All these warrant further structural optimization of **1i** optimum anti-inflammatory/LO activity.

## 4. Experimental

### 4.1. General

All the chemicals used were of analytical grade and commercially available from Merck, 1,1-diphenyl-2-picrylhydrazyl (DPPH), nordihydroguairetic acid (NDGA) are purchased from the Aldrich Chemical Co. Milwaukee, WI, USA. Soybean Lipoxygenase, linoleic acid sodium salt, arachidonic acid (AA), NADH, nitrotetrazolium blue (NBT), porcine heme, 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. *N*-Methylphenazonium-methyl sulfate was purchased from Fluka.

All starting materials were obtained from commercial sources and used without further purification. Melting Points (uncorrected) were determined on a MEL-Temp II (Lab. Devices, Holliston, MA, USA). UV–vis spectra were obtained on a Perkin-Elmer 554 beam spectrophotometer and on a Hitachi U-2001 spectrophotometer. Infrared spectra (film as Nujol mulls) were recorded with a Shimadzu FTIR-8101 M. The $^1$H nuclear magnetic resonance (NMR) spectra were recorded at 300 MHz on a Bruker AM 300 spectrometer (Bruker Analytische Messtechnik GmbH, Rheinstetten, Germany) in $CDCl_3$ or DMSO using tetramethylsilane as an internal standard unless otherwise stated. $^{13}$C NMR spectra were obtained at 75.5 MHz on a Bruker AM 300 spectrometer in $CDCl_3$ or DMSO solutions with tetramethylsilane as internal reference unless otherwise stated. Elemental analyses were obtained in an acceptable range (±0.4%) in a Perkin-Elmer 240B CHN analyzer (The Perkin-Elmer Corporation Ltd). Reactions were monitored by thin-layer chromatography (TLC) by Fluka, on aluminum cards precoated with 0.2 mm of silica gel and fluorescent indicator.

### 4.2. Synthesis

**4.2.1. Synthesis of phenyl-substituted aryl-acetic acids.**[17,18] The reaction was performed according to the literature.[17,18] The compounds reported here were generally prepared as illustrated in Scheme 1. The aryl-acetic acids were synthesized by a Knoevenagel condensation of the suitable aldehyde (0.015 mol) with phenylacetic acid (0.015 mol) and acetic acid anhydride (10 mL) in the presence of triethylamine (5 mL). The mixture was refluxed for 5 h. The solution was poured into 2 N HCl, then on ice and a precipitate was formed which was collected by filtration and recrystallized from 50% aqueous ethanol. In case that no precipitate was formed after the ice dropping an extraction with 3× 100 mL $CHCl_3$ was made and the organic phase was collected and dried over $MgSO_4$.

**4.2.1.1. 3-(3,5-Di-*tert*-butyl-2-hydroxy-phenyl)-2-phenyl-acrylic acid (1i).** UV (ethanol absolute) $\lambda_{max}$: 220, 310, $\varepsilon_{max}$: 15870, 12730. IR (Nujol) ($cm^{-1}$): 2970, 2950, 1720, 1625, 1470, 1375. $^1$H NMR (DMSO-$d_6$, $CDCl_3$): $\delta$ (ppm) 1.26–1.54 (s, 9H, C(C*H*$_3$)$_3$), 1.62–1.76 (s, 9H, C(C*H*$_3$)$_3$), 7.26 (d, 3H), 7.30–7.58 (m, 5H), 7.72–7.74 (m, 1H, O*H*), 7.81 (s, 1H, COO*H*). $^{13}$C NMR (DMSO-$d_6$, $CDCl_3$): 31.2, 31.3, 31.4, 34.4, 34.5, 34.5, 34.6, 35.1, 35.6, 113.0, 122.5, 124.0, 126.4, 126.6, 128.4, 128.5, 128.6, 131.0, 135.6, 137.1, 141.1, 142.3, 152.0, 164.0. Anal. C, H, N. Expected %: ($C_{23}H_{28}O_3$) C, 78.38; H, 8.01. Calculated %: C, 78.35; H, 7.74.

**4.2.1.2. 2-Phenyl-3-(1*H*-pyrrol-2-yl)-acrylic acid (2i).** UV (ethanol absolute) $\lambda_{max}$: 220, 320 $\varepsilon_{max}$: 10700, 3330. IR (Nujol) ($cm^{-1}$): 3000, 2950, 1750, 1625. $^1$H NMR (DMSO-$d_6$, $CDCl_3$): $\delta$ (ppm) 5.95–6.51 (br, 3H), 6.92–7.36 (m, 7H), 8.2 (s, 1H, COO*H*). $^{13}$C NMR (DMSO-$d_6$, $CDCl_3$): 105.0, 110.9, 118.0, 127.0, 127.5, 127.9, 128.3, 128.5, 128.8, 133.0, 135.0, 141.9, 165.0. Anal. C, H, N. Expected %: ($C_{13}H_{11}NO_2$) C, 73.2; H, 6.56; N, 5.19. Calculated %: C, 73.14; H, 6.35; N, 4.92.

**4.2.1.3. 3-(2-Hydroxy-naphthalen-1-yl)-2-phenyl-acrylic acid (3i).** UV (ethanol absolute) $\lambda_{max}$: 230, 360 $\varepsilon_{max}$: 17760, 11420. IR (Nujol) ($cm^{-1}$): 3050, 2950, 1770, 1650, 1470, 1375. $^1$H NMR (DMSO-$d_6$, $CDCl_3$): $\delta$ (ppm) 7.42–7.61 (m, 6H), 7.67–7.73 (m, 2H), 7.80–7.83 (m, 1H, O*H*), 7.90–8.33 (m, 4H), 8.6 (s, 1H, COO*H*). $^{13}$C NMR (DMSO-$d_6$, $CDCl_3$): 116.7, 117.9, 123.5, 126.0, 126.4, 127.1, 128.0, 128.2, 128.6, 128.7, 128.9, 130.4, 132.0, 132.7, 135.7, 155.8, 168.0. Anal. C, H, N. Expected %: ($C_{19}H_{14}O_3$) C, 78.61; H, 4.86. Calculated %: C, 78.46; H, 4.81.

**4.2.2. General procedure for the synthesis of aryl-acetic acids II.**[18] The compounds reported here were generally prepared as illustrated in Scheme 1. The aryl-acetic acids were synthesized by a Knoevenagel condensation of the suitable aldehyde (0.01 mol). The malonic acid (0.01 mol) was dissolved in pyridine (1.12 mol). Aldehyde and piperidine (0.01 mol) were added. The mixture was refluxed under water until the emission of $CO_2$ stops. Then the solution was poured into 2 N HCl, on ice and a precipitate was formed which was collected

by filtration and recrystallized from water or from 3:1 water/ethanol. For compounds where no precipitate was formed after the ice dropping, an extraction with 3× 100 mL $CHCl_3$ or $CH_2Cl_2$ was made and the organic phase was collected and dried over $MgSO_4$.

**4.2.2.1. 3-(3,5-Di-*tert*-butyl-2-hydroxy-phenyl)-acrylic acid (1ii).** UV (ethanol absolute) $\lambda_{max}$: 220, 260, 340 $\varepsilon_{max}$: 14630, 8590, 4010. IR (Nujol) ($cm^{-1}$): 3000, 2970, 1770, 1650, 1440, 1390. $^1H$ NMR (DMSO-$d_6$, $CDCl_3$): $\delta$ (ppm) 1.23–1.67 (m, 18H, 6× C*H*$_3$), 6.38 (d, 1H, $J = 12$), 6.84–7.13 (m, 2H), 7.67–7.71 (d, 1H, $J = 12$), 9.89 (s, 1H, O*H*), 11.65 (s, 1H, COO*H*). $^{13}C$ NMR (DMSO-$d_6$, $CDCl_3$): 31.3, 31.4, 31.5, 31.6, 31.7, 32.3, 40.4, 115.2, 115.5, 120.9, 123.4, 137.4, 141.6, 147.9, 159.0, 171.0. Anal. C, H, N. Expected %: ($C_{17}H_{24}O_3$) C, 73.88; H, 8.75. Calculated %: C, 73.53; H, 8.81.

**4.2.2.2. 3-(1*H*-Pyrrol-2-yl)-acrylic acid (2ii).** UV (ethanol absolute) $\lambda_{max}$: 220, 370 $\varepsilon_{max}$: 678, 14,990. IR (Nujol) ($cm^{-1}$): 3070, 1750, 1670. $^1H$ NMR (DMSO-$d_6$, $CDCl_3$): $\delta$ (ppm) 6.43–6.46 (m, 2H), 6.96–6.98 (m, 1H), 7.37 (s, 1H), 7.58 (s, 1H), 8.24 (br, 1H, $J = 15$, C*H*═CH), 13.02 (s, 1H, COO*H*). $^{13}C$ NMR (DMSO-$d_6$, $CDCl_3$): 109.1, 112.8, 121.6, 127.6, 129.7, 137.6, 174.1. Anal. C, H, N. Expected %: ($C_7H_7O_2N$) C, 61.31; H, 5.14; N, 10.21. Calculated %: C, 61.59; H, 5.49; N, 10.58.

**4.2.2.3. 3-(2-Hydroxy-naphthalen-1-yl)-acrylic acid (3ii).** UV (ethanol absolute) $\lambda_{max}$: 230, 280 $\varepsilon_{max}$: 17,210, 4190. IR (Nujol) ($cm^{-1}$): 3010, 1750, 1650. $^1H$ NMR (DMSO-$d_6$, $CDCl_3$): $\delta$ (ppm) 7.19–7.21 (m, 1H), 7.28–7.56 (m, 5H), 7.61 (d, 1H, $J = 15$), 7.83 (d, 1H, $J = 15$), 8.10 (s, 1H, O*H*), 13.02 (s, 1H, COO*H*). $^{13}C$ NMR (DMSO-$d_6$, $CDCl_3$): 116.3, 117.4, 118.7, 122.3, 124.4, 126.8, 128.8, 129.2, 130.3, 131.0, 149.0, 160.9, 170.8. Anal. C, H, N. Expected %: ($C_{13}H_{10}O_3$) C, 72.89; H, 4.71. Calculated %: C, 73.14; H, 4.61.

### 4.3. Physicochemical studies

**4.3.1. Determination of lipophilicity as $R_M$ values.** Reversed phase TLC (RPTLC) was performed on silica gel plates impregnated with 55% (v/v) liquid paraffin in light petroleum ether. The mobile phase was a methanol/water mixture (77:23, v/v) containing 0.1 acetic acid. The plates were developed in closed chromatography tanks saturated with the mobile phase at 24 °C. Spots were detected under UV light or by iodine vapors. $R_M$ values were determined from the corresponding $R_f$ values (from 10 individual measurements) using the equation $R_M = \log [(1/R_f) - 1]$.[38]

**4.3.2. Determination of lipophilicity as clog *P*.** Lipophilicity was theoretically calculated as clog *P* values in *n*-octanol-buffer by CLOGP Programe of Biobyte Corp.[39]

### 4.4. Biological experiments

**4.4.1. Experiments in vitro.** In the in vitro assays each experiment was performed at least in triplicate and the standard deviation of absorbance was less than 10% of the mean.

**4.4.1.1. Determination of the reducing activity of the stable radical 1,1-diphenyl-picrylhydrazyl (DPPH).**[17–19,40,41] To a solution of DPPH in absolute ethanol an equal volume of the compounds dissolved in ethanol was added. As control solution ethanol was used. The concentrations of the solutions of the compounds were 0.1 and 0.05 mM. After 20 and 60 min at room temperature the absorbance was recorded at 517 nm (Table 2) and compared with the appropriate standards NDGA and BHT.

**4.4.1.2. Competition of the tested compounds with DMSO for hydroxyl radicals.**[17–19,42,43] The hydroxyl radicals generated by the $Fe^{3+}$/ascorbic acid system were detected according to Nash, by the determination of formaldehyde produced from the oxidation of DMSO. The reaction mixture contained EDTA (0.1 mM), $Fe^{3+}$ (167 μM), DMSO (33 mM) in phosphate buffer (50 mM, pH 7.4), the tested compounds (concentration 0.01 and 0.1 mM), and ascorbic acid (10 mM). After 30 min of incubation (37 °C) the reaction was stopped with $CCl_3COOH$ (17%, w/v) (Table 2). Trolox was used as an appropriate standard.

**4.4.1.3. Heme protein-dependent lipid degradation.**[18,19,44,45] Fifty micromolar of heme, arachidonic acid (0.4 mM), the compounds at various concentrations tested, and $H_2O_2$ (0.5 mM) were incubated together for 10 min at 37 °C in $KH_2PO_4$–KOH buffer (50 mM, pH 7.4). The product of peroxidation was detected using the TBA test.[45,46] The compounds were added in DMSO solution, which has no effect on the assay (Table 3). Caffeic acid was used as an appropriate standard.

**4.4.1.4. Non-enzymatic assay of superoxide radicals—measurement of superoxide radical scavenging activity.**[18,19,46] The superoxide producing system was set up by mixing PMS, NADH, and air–oxygen. The production of superoxide was estimated by the nitroblue tetrazolium method. The reaction mixture containing compounds, 3 μM PMS, 78 μM NADH, and 25 μM NBT in 19 μM phosphate buffer, pH 7.4, was incubated for 2 min at room temperature and the absorption measured at 560 nm against a blank containing PMS. The tested compounds were preincubated for 2 min before adding NADH (Table 3). Caffeic acid was used as an appropriate standard.

**4.4.1.5. Soybean lipoxygenase inhibition study in vitro.**[17–19,47] In vitro study was evaluated as reported previously. The tested compounds dissolved in ethanol were incubated at room temperature with sodium linoleate (0.1 mM) 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 (Table 3). Caffeic acid was used as an appropriate standard.

**4.4.2. Experiments in vivo**

**4.4.2.1. Inhibition of the carrageenin-induced edema.**[17–19,48] Edema was induced in the right hind paw of Fisher-344 rats (150–200 g) by the intradermal injection of 0.1 mL

of 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 compounds 0.01 mmol/kg body weight were suspended in water, with few drops of Tween 80 and ground in a mortar before use and 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 edema, CPE % values Table 3. Indomethacin at 0.01 mmol/kg (47%), was used for comparison reasons as a reference compound. CPE % values are means from two different experiments with a standard error of the mean less than 10% (Table 3).

**4.4.3. Stability studies and incubation with glutathione (GSH).**[18,19,36] Solutions of the compounds were prepared in water using phosphate buffer solution (PBS), pH 7.4, and in order to achieve dissolution the solvent contained approximately 10%, v/v alcohol. The concentrations of the solutions were chosen so that the absorption maxima were between 0.5 and 1. The test compounds are incubated for 24 h at 37 °C and their UV spectra were recorded. All determinations were carried out in duplicate. The error limits of the $\varepsilon$ values were approximately 2%.

The experiment was repeated in the presence of GSH using thiol-test compound, 2/1 and 10/1, and incubation at 37 °C for 24 h and their UV spectra were recorded. The results are given in Table 4.

**Table 4.** Stability studies and incubation with glutathione (GSH)

| α/α | λ | $\varepsilon_{max}$ |
|---|---|---|
| **1i** | 250 | 729 |
| **1i** + 2GSH | 250 | 210 |
| **1i** + 10GSH | 250 | 356 |
| **1ii** | 340 | 892 |
| **1ii** + 2GSH | 340 | 228 |
| **1ii** + 10GSH | 340 | 404 |
| **2i** | 280 | 307 |
| **2i** + 2GSH | 280 | 123 |
| **2i** + 10GSH | 280 | 110 |
| **2ii** | 280 | 287 |
| **2ii** + 2GSH | 280 | 109 |
| **2ii** + 10GSH | 280 | 146 |
| **3i** | 340 | 892 |
| **3i** + 2GSH | 340 | 228 |
| **3i** + 10GSH | 340 | 404 |
| **3ii** | 280 | 412 |
| **3ii** + 2GSH | 280 | 242 |
| **3ii** + 10GSH | 280 | 386 |

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