Synthesis of Phenyl-substituted Amides with Antioxidant and Anti-inflammatory activity as Novel Lipoxygenase Inhibitors

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Abstract: Lipoxygenases (LO) have been implicated in several inflammatory diseases such as asthma, immune disorders, and cancers. Lipoxygenases play an essential role in the biosynthesis of the leukotrienes. Leukotrienes have been implicated as mediators in the pathophysiology of inflammatory diseases, host defense reactions and they were found to play important role in the propagation of the diseases states, exacerbating the local events and ultimately leading to tissue damage. As a consequence of these broad biological implications, there is a great interest in synthesising new compounds with this activity. The synthesis of new amides of aryl acetic acid is described. The structures of the synthesized compounds were confirmed by spectral and elemental analysis. Since lipophilicity is a significant physicochemical property determining distribution, bioavailability, metabolic activity and elimination, their lipophilicity is experimentally determined from RPTLC method. Several parameters were theoretically calculated and were used for a QSAR study. The compounds are tested *in vitro* on: a) soybean lipoxygenase inhibition, b) interaction with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical, c) the $HO^{\cdot}$ mediated oxidation of DMSO, d) inhibition of lipid peroxidation, e) scavenging of superoxide anion radicals f) interaction with glutathione and g) *in vivo* for the inhibition of carrageenin induced rat paw edema.

The compounds present significant antioxidant activities, medium anti-inflammatory activity and potent inhibition of soybean lipoxygenase as a result of their physicochemical features.

Key Words: Lipoxygenase inhibitors, antioxidant agents, anti-inflammatory agents, amides.

INTRODUCTION

It is well known that the epidermal layer is an active site of arachidonic acid metabolism, the main substrate of lipoxygenase. Arachidonic acid is esterified in the 2-position of the glycerol moiety of membrane phospholipids and its release is controlled by the activity of phospholipase A_2 mainly, or by the combined action of phospholipase C and diglyceride lipase [1]. Arachidonic acid liberated from the membranes, can be further metabolized by two major enzymatic pathways: cycloxygenase and lipoxygenase.

Lipoxygenases are a class of non heme iron-containing enzymes that contribute to the eicosanoid pathway [2] by the hydroperoxidation of arachidonic acid (AA) since they require a fatty acid substrate with two *cis* double bonds separated by a methylene group. These enzyme products are precursors for the inflammatory mediators, leukotrienes and lipoxins, but are also involved in a variety of human diseases such as asthma [3], psoriasis [4], atherosclerosis [5], and cancer [6,7] and thus are attractive pharmaceutical targets.

Today's anti-LT therapy applies two basic pharmacological strategies: suppression of the biosynthesis of LTs and the use of LT receptor antagonists. Suppression of LT synthesis can be achieved (I) by inhibition of phospholipases releasing the precursor AA, (II) direct inhibition of lipoxygenase and (III) inhibition of FLAP. Direct inhibitors can be classified in the following categories: a) Iron ligand inhibitors, containing hydroxamic acid or N-hydroxyurea groups that chelate the active site iron, represent potent LO inhibitors, b) non-redox competitive inhibitors, which compete with arachidonic acid to bind the enzyme active site and products analogues and c) a number of LO inhibitory compounds combine the properties of being both chelators and reductants. Many companies over the last decades have reported on compounds that inhibit both lipoxygenase and other enzymes involved in inflammation as approaches to treat a variety of inflammatory diseases.

It has been pointed out that inhibiting only one of these biosynthetic ways (cycloxygenase or lipoxygenase) could shunt the metabolism of arachidonic acid towards the other pathway, thus leading to potential side effects [8]. Pharmacologically active compounds that inhibit both enzymes at similar concentrations would have the potential to provide more complete relief for patients suffering from arthritis and inflammatory, hypersensitivity, dermatological or cardiovascular disorders [9].

The hydroxamic acid functionality was incorporated into a wide variety of molecules to produce potent inhibitors of LO. Recently we have reported a series of aryl-acetic and aryl-hydroxamic acids, having potent inhibitory activity against soybean lipoxygenase and possessing anti-oxidant and antiiflammatory activities [10, 11] (Scheme **1**).

As a further contribution to the understanding of the structure activity relationships of this class of compounds we described [12] a new series of amides combining the Z-

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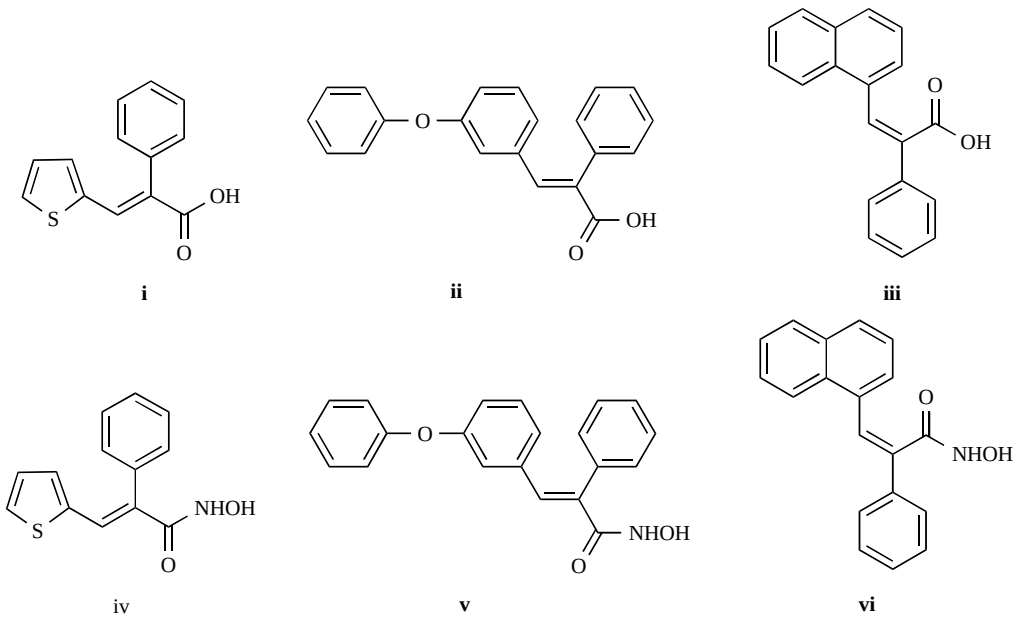
1573-4064/07 $50.00+.00



Scheme 1.

CH=C(C_6H_5)-C=O group with a tertiary or secondary amidic nitrogen which presented interesting antioxidant/anti-inflammatory properties. From the literature it is well known several amides containing the hydroxyurea moiety such as zileuton-A-64077 [13], available in the USA for the treatment of asthma, ABT-761 [14], LDP-977 (CMI-977) [15] and BW-B70C [16] (dual 5- and 15-lipoxygenase inhibitor), or simple amides derivatives of 4-[5-fluoro-3-[4-(2-methyl-1H-imidazol-1-yl)benzyloxy]-phenyl]-4-methoxy-3,4,5,6-tetrahydro-2H-pyran [17] and amides containing the furan moiety like ABT-761, inhibitor of 5-LO and antagonist of histamine H1 [18]. N-substituted indole-2 and 3-carboxamides were also evaluated as antioxidants with reactive oxygen species (ROS) [19, 20] Fig. (**1**).

Compounds bearing a thiazolyl, benzothiazolyl, hydrazinyl moieties posses a wide spectrum of biological activities which is related to their capacity to transfer electrons and to scavenge reactive oxygen species (ROS) [21]. In continuation to our effort toward highly effective antioxidants, inhibi-

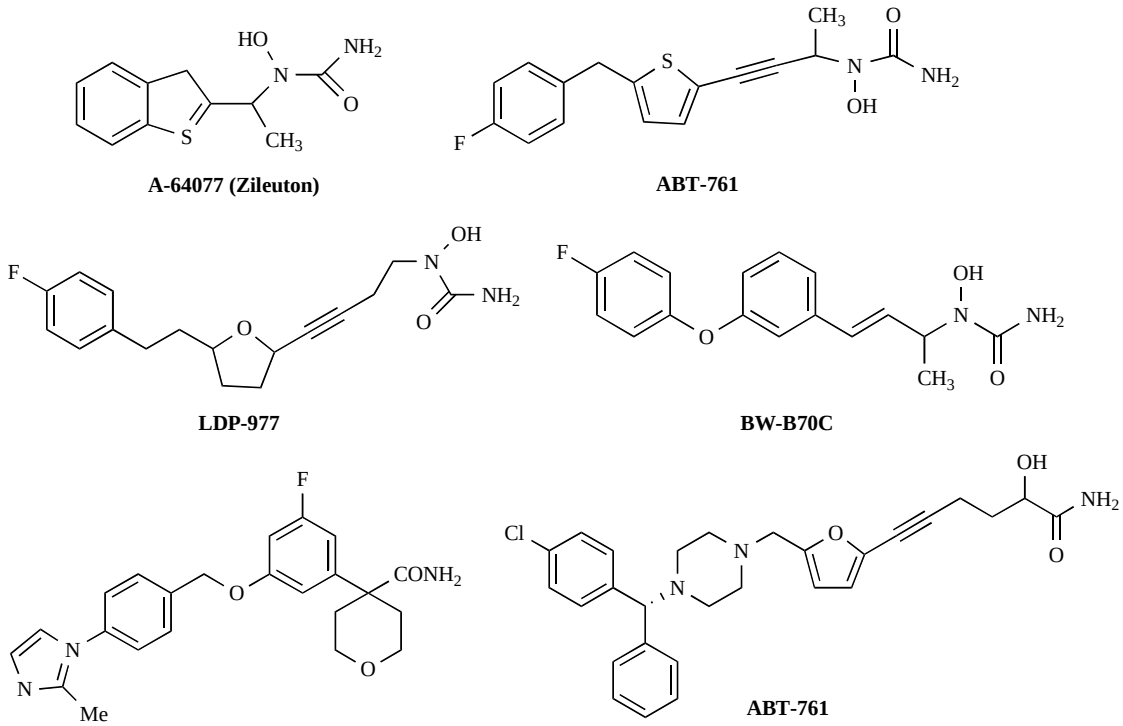


Fig. (1). Lipoxygenase inhibitors.

tors of LOs, we synthesized five series of amides hybrids containing the aryl-acetic acid moiety and a thiazolyl, benzothiazolyl, hydrazinyl (methyl) and benzyl-amine groups and we explored their ability to inhibit soybean lipoxygenase and to present antioxidant and anti-inflammatory activities.

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 [22, 23].

Today there is an increase interest in the development of effective lipoxygenase inhibitors (Fig. **1**) that are non acidic anti-inflammatory agents, since the used acidic NSAIDs cause development untoward side effects in a significant fraction of people [24, 25].

CHEMISTRY

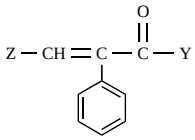
The synthesis of the amides (Table **1**) is indicated in Scheme **2**. The synthesis of the aryl-acetic acids was accomplished according to the Knoevenangel condensation as indicated to (Scheme 2) by the condensation of the suitable aldehyde with phenylacetic acid and acetic acid anhydride in the presence of triethylamine [10, 12]. The formed acids [10, 18] are converted to the corresponding chlorides by $SOCl_2$. Finally the appropriate amine dissolved in dry acetone was added to a solution of the corresponding acid chloride in the same solvent. Reactions were monitored by thin layer chromatography.

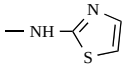
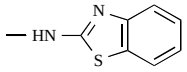
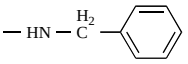
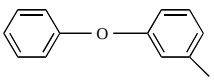
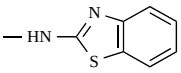
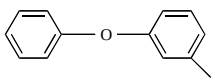
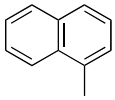
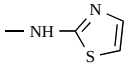
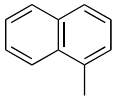
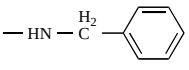
The corresponding reactions proceeded smoothly and in good yields (30.6-72.5%) with the exception of compounds 5 (17.4%) and 7 (22.2%). The structures of the synthesized compounds are given (Scheme **3**) and they are confirmed by UV, IR, ^{1}H-NMR, ^{13}C-NMR, MS and elemental analysis. All the amides present the characteristic absorption in the IR (nujol) (cm^{-1} 3100 (N-H), 1720 (C=O), 1625 (C=C)). ^{1}H-NMR spectroscopy revealed that the olefinic bond in these compounds adopted the *E*-configuration (trans-derivatives).

Physicochemical Studies

Since lipophilicity is a significant physicochemical property determining distribution, bioavailability, metabolic ac-

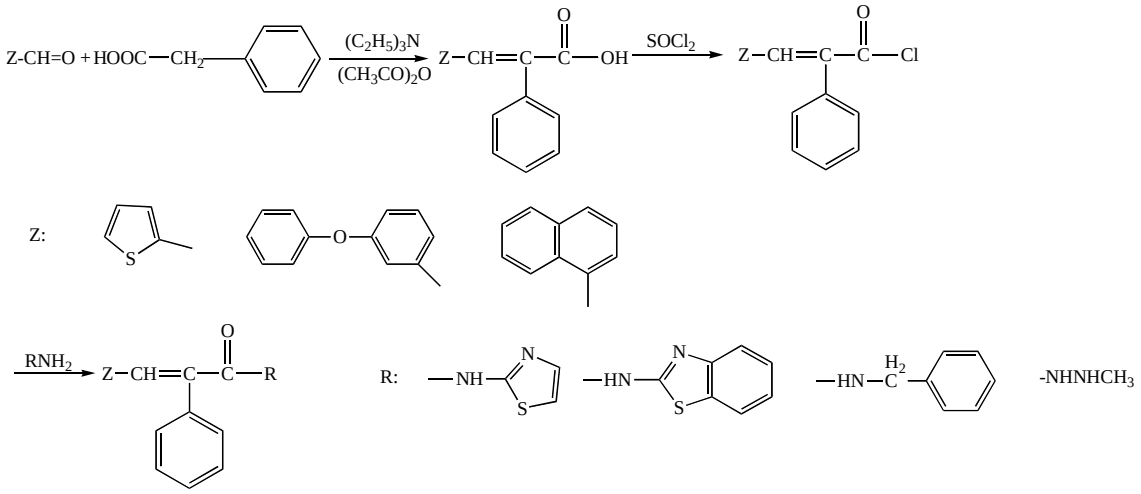
Table 1. Chemical Data of Aryl-acetic Acids and the Corresponding Amides



No	Z	Y	Molecular Formula*	Rf	clog *P***	R_M# (±SD)	mp ^{0}C	yield%
1			$C_{16}H_{12}N_2OS_2$	0.86[a]	3.89	0.26 (± 0.017)	138-139	66.7
2			$C_{20}H_{14}N_2OS_2$	0.90[a]	5.49	0.56 (± 0.023)	175-177	55.2
3			$C_{20}H_{17}NOS$	0.35[a]	4.57	0.12 (± 0.011)	82-84	43.2
4			$C_{28}H_{20}N_2O_2S$	0.90[a]	7.94	1.33 (± 0.041)	165-167	72.5
5		-NHNHCH$_3$	$C_{22}H_{20}N_2O_2$	0.93[b]	4.07	-0.38 (±0.023)	129-130	17.4
6			$C_{22}H_{16}N_2OS$	0.83[a]	5.42	1.02 (± 0.043)	165-167	30.6
7			$C_{26}H_{21}NO$	0.64[a]	6.10	-0.38 (± 0.019)	136-138	22.2

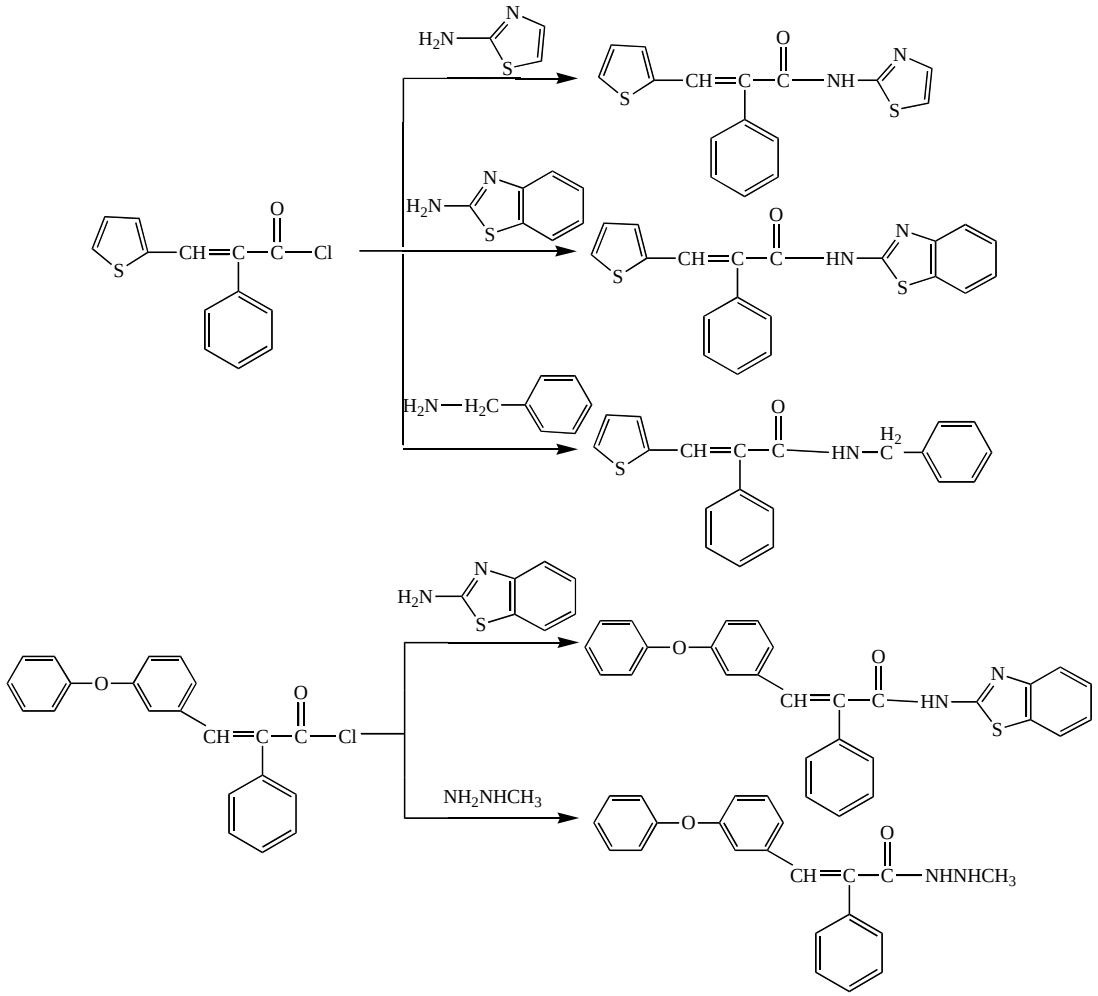
[a]C_6H_5 : C_2H_5OH, 4:1, [b]$CHCl_3$: C_2H_5OH, 1:1.

*Elemental analyses for molecular formula (± 0.4%), **Theoretically calculated clog *P* values; #R_M values are the average of at least 10 measurements

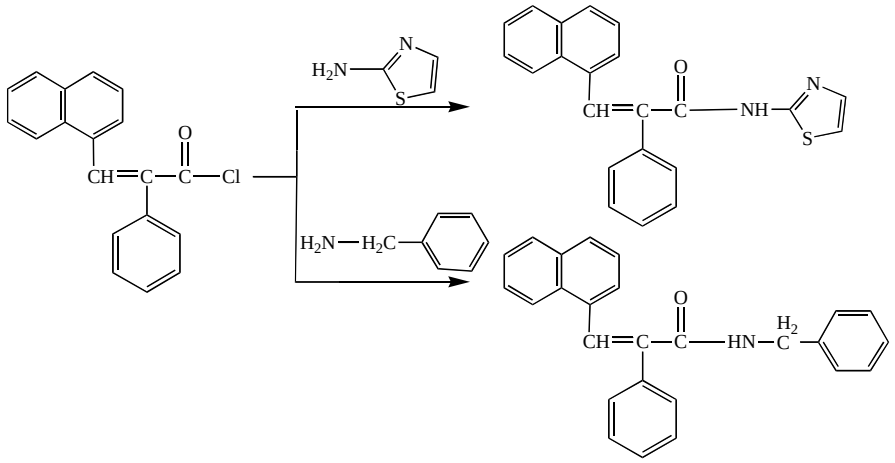


Scheme 2.

tivity 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 [23]. This is considered to be a reliable, fast and convenient method for expressing lipophilicity [24]. 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.



(Scheme 3. Contd…..)



Scheme 3.

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 and to the presence of the amide group, which could disturb the absorption/desorption process.

Calculation Methods

The energy values of the lowest and highest unoccupied molecular orbital (E_{LUMO} and E_{HOMO}) of the amides were calculated by the program Spartan v. 5.1.3 (Wavefunction Inc.) on energy minimized structures (semi-empirical AM1). A conformation analysis MMFFS4 (conformer search Monte Carlo) was done followed by an ab initio RHF/6-31G* calculation.

RESULTS AND DISCUSSION

Discussion

In this investigation, we synthesized some novel amides that was expected to offer protection against inflammation and radical attack and inhibition of LO, by application of standard synthetic methods summarized in Scheme **2**.

In acute toxicity experiments, the *in vivo* examined compounds did not present toxic effects in doses up to 0.5 mmol/kg body weight.

The *in vivo* anti-inflammarory effects of the tested amides were assessed by using the carrageenin-induced rat paw edema (CPE) model and are presented in Table **2**, 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 antiiflammatory agents [26]. It reliably predicts the anti-inflammatory potency of the NSAIDs and detects during the second phase that are anti-inflammmatory agents as a result of inhibition of prostaglandin amplification [27]. All the tested amides induced protection (ranged from 6.5-67.6%) against to carragenin induced paw edema while the reference drug indomethacin induced 47% protection at an equivalent concentration. Compound **3** was the most potent (67.6%) whereas compounds **5**, **6** and **7** had very low effect (6.5-9.6%). Com-

Table 2. Energy Values of the Lowest/Highest Unoccupied Molecular Orbital (E_{LUMO}/E_{HOMO}) by the Spartan. Physicochemical Parameters used for the Derivation of eqs. 1-4

α/α	E_{LUMO} (eV)	$E_{LUMO(\alpha/\alpha)}$-$E_{HOMO(GSH)}$ (eV)	MR_Z [a]	MgVol [a]
1	2.167	9.548	2.40	312.44
2	2.468	9.849	2.40	362.50
3	2.361	9.742	2.40	319.45
4	2.361	9.742	5.25	448.57
5	2.691	10.072	5.25	344.44
6	2.290	9.671	4.27	356.47
7	2.171	9.552	4.27	363.48

$E_{HOMO(GSH)}$ = -7.381eV

[a][25]

pound **1** and **2** had almost equipotent effect. The nature of R group does not seem to affect these two compounds. Both present a thienyl group at Z. Compared to the parent acid **i** (Scheme **1**) (65.5%) [10, 11] these two amides seem to be weaker. Comparison of compounds **6** and **7** with their parent acid iii (80.1%) [12] showed a high decrease in inhibition values. The nature of Z seems to influence the inhibition in compounds **1** and **6**. The presence of a thienyl group is correlated with higher values.

Lipophilicity influences the results. Higher clog *P* value 5.42 (compound **6**) is correlated with lower *in vivo* results compared to compound **1** (3.89). Benzylamino group is correlated with significant anti-inflammatory activity (compound **3**, 67.6%). Lipophilicity seems to be important for the biological effect of compound **4** (7.94, 30%), compared to compound **5** ((4.07, 9.3%). Stereochemistry of Z and Y should be an important parameter for the *in vivo* behaviour as it is shown by the following equation:

log % CPE = - 0.308 (± 0.193) MR_Z + 2.376 (± 0.711) **(1)**

n = 6, r = 0.912, r^2 = 0.831, s = 0.195, $F_{1,6}$ = 19.73, α = 0.01

MR_Z describes the molecular refractivity of substituent Z. Since MR is primarily a measure of bulk and of polarizability of the substituent, the negative coefficient suggests steric hindrance. Clog *P* cannot replace MR. Compound **4** which presents the higher MR_Z value (5.25) was omitted.

Higher lipophilicity does not seem to affect the inhibitory activity, e.g. compound **4** with clog *P* 7.94 inhibits the carrageenin induced edema at a range of 30%.

Compounds were further evaluated for inhibition of soybean lipoxygenase by the UV absorbance based enzyme assay [28]. Perusal of IC_{50}s values show that compound **6** is the most active within the set followed by compounds **5**, **7** and **4**. In general all the amides are found to be stronger inhibitors than the corresponding acids. The most of the lipoxygenase inhibitors are antioxidants or free radical scavengers [29] since lipoxygenation occurs *via* a carbon centred radical. Some studies suggest a relationship between lipoxygenase inhibition and the ability of the inhibitors to reduce Fe^{+3} at the active site to the catalycally inactive site Fe^{+2}. Lipophilicity is referred [30] as an important physicochemical property for lipoxygenase inhibition. However the QSAR analysis of our data did not verify this:

log $1/IC_{50}$ (LO) = 0.066 (± 0.045) MR_Z + 4.034 (± 0.185) **(2)**

n = 6, r = 0.898, r^2 = 0.806, s = 0.047, $F_{1,6}$ = 16, α = 0.01

Compound **1** was omitted.

Eq. (2) seems strange because it contains no π or clog *P* term. MR_Z refers to the molar refractivity of substituent and the positive sign indicates that the larger the molar refractivity the more it promotes the LO inhibition.

Carrageenin model after 3.5 hrs, detects compounds that act as anti-inflammatory agents, as a result especially of cycloxygenase inhibition [31]. An attempt was made to correlate the CPE *in vivo* activity with the IC_{50} values of LO. Eq. (3) was derived:

log % (CPE) = -3.575 (± 3.402) log $1/IC_{50}$ (LO) + 16.632 (± 14.612) **(3)**

n = 6, r = 0.825, r^2 = 0.680, s = 0.259, $F_{1,6}$ =8.52, α = 0.05

Compound **1** was omitted. It has the lowest IC_{50} value. Compounds **5**, **6** and **7** with high IC_{50}'s values on lipoxygenase, present low CPE% values.

We determined the inhibition of Fe^{2+} stimulated oxidation of linoleic acid by compounds **3**, **4**, **5**, **6** and **7** (they present the higher LO inhibition) in order to find out if the tested compounds act as antioxidants in a non biological system (Table **5**). Only compounds-substrates **3**, **6** and **7** inhibited this type of lipid peroxidation. Compounds **3** and **7** at 0.1mM presented 63.8 and 33.9% inhibition respectively. Compound **5** shown the highest inhibition (IC_{50} 10μM) whereas compounds **4** and **6** did not present any inhibition under the reported experimental conditions. In continuation the amides were tested with regard to their antioxidant activity. The interaction of the examined amides with the stable free radical DPPH was studied, indicating their scavenging/reducing ability in an iron-free system.

Compounds **1**, **2**, **4** were found to have low activity. Compounds **3**, **6** and 7 showed higher interactions. For the most of the tested amides the interaction was time and concentration dependent. However, it was found to be lower than the interaction presented by the corresponding acids. Preliminary QSAR studies on the values of DPPH interaction (0.1mM, 20 min) have shown that the steric effects as MgVol (refers to the molecular volume of the molecules) are significant. The negative sign indicates that the larger the molecular volume, the less it promotes the DPPH interaction. Naphthalinyl amides were most potent in interacting with DPPH.

log % DPPH (20min-0.1mM) = -0.007 (± 0.005) MgVol + 4.005 (± 1.758) **(4)**

n = 6, r = 0.892, r^2 = 0.796, s = 0.168, $F_{1,6}$ = 15.68, α = 0.01

Compound **1** is omitted (MgVol = 312.44).

Eqs. **2**, **3**, **4** are not sharp in terms of r^2. However they illustrate in terms of QSAR the most significant structural characteristics for anti-inflammatory/antioxidants activities.

It is consistent that rates of ROS production are increased in most diseases [32, 33]. The cytotoxicity of $O_2^{\cdot-}$ and H_2O_2 in living organisms is mainly due to their transformation into $^{\cdot}OH$, reactive radical metal complexes and $^1O_2$. During the inflammatory process, phagocytes generate the superoxide anion radical at the inflammed site and this is connected to other oxidizing species as $^{\cdot}OH$. 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 amides with DMSO for $^{\cdot}OH$ 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 **5** did not show any result. Com-

pounds **3 and** 7 showed high inhibition at 0.001mM and 0.01mM whereas compounds **1**, **2**, **4** and **6** inhibited significant the oxidation of DMSO (33mM) at 0.1mM. Lipophilicity is not correlated with the results.

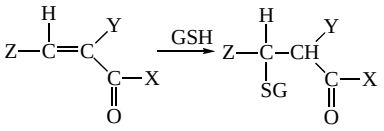
Mixing heme proteins with H_2O_2 generates powerfully oxidizing activated heme species and radicals on amino-acids side chains that can cause lipid peroxidation. As a model of such reactions we used the peroxidation of arachidonic acid by a mixture of heme and H_2O_2.

The tested compounds did not highly inhibit the lipid peroxidation (10.3-24.3%). Compounds **5** and **7** did not present any inhibition. 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 **3** did not present biological response at 0.1mM. On the contrary it is very potent at 1mM (100%). Compounds **5** and **6** seem to be equipotent. Naphthalinyl amides seem to be more potent compared to the phenoxy-phenyl-derivatives. We tried to linearly correlate the superoxide anion scavenging activity without any success.

A number of α,β-unsaturated ketones have demonstrated preferential reactivity towards thiols in contrast to amino and hydroxyl groups [34] 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 [35]. 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 sulphydryl 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 not all α,β-unsaturated compounds are conjugated with GSH.

For compounds **1**, **2**, **3**, **4**, **6** and **7** an alkylation may occur leading to the adducts A Fig. (**2**).



Adducts A

Fig. (2).

Compound **4** presents the higher alkylation. Compounds **3** and **6** present higher alkylation when the concentration of GSH is low (2GSH). For compounds **1**, **2**, **4** and **7** the alkylation proceed, higher when the concentration of GSH is high (10GSH). It seems that alkylation proceeds parallel to the electronic affinity of the compound, expressed as E_{LUMO} (Table **4**). E_{LUMO} is an electrophilicity parameter, directly proportional to the electronic affinity. E_{LUMO} values show the tendency of chemicals to undergo orbital-controlled reactions. The lower the E_{LUMO} values the stronger the electrophilicity [36].

We tried to linearly correlate the *in vivo/ in vitro* expressions of anti-inflammatory, antioxidant activity for the 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 in terms of confidence limits.

CONCLUSION

In conclusion the broad spectrum of the observed antioxidant as well as the LO inhibitory activity of the majority of the examined amides allows us to propose them as potential LO inhibitors. The importance of the above properties may also be significant in treating human diseases that involves ROS.

EXPERIMENTAL SECTION

Materials

All the chemicals used were of analytical grade and commercially available by Merck, 1,1-diphenyl-2-picrylhydrazyl (DPPH), nordihydroguairetic acid (NDGA), NADH are purchased from the Aldrich Chemical Co. Milwaukee, WI, (USA). Soybean Lipoxygenase, linoleic acid sodium salt Arachidonic Acid (AA), NADH, Blue Nitroblue tetrazolium (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 PMS was purchased by Fluka.

Synthesis

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 (Lamda 20) beam spectrophotometer and on a Hitachi U-2001 spectrophotometer. Infrared spectra (film as Nujol mulls) were recorded with Perkin-Elmer 597 spectrophotometer (The Perkin-Elmer Corporation Ltd., Lane Beaconsfield, Bucks, England) and a Shimadzu FTIR-8101M. The ^1H Nucleic Magnetic Resonance (NMR) spectra were recorded at 300MHz 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. Mass spectra were determined on a VG-250 spectrometer (VG-Labs., Tritech, England) with ionization energy maintained at 70 eV. Elemental analyses were obtained on 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. R_M determinations were performed on Silica Gel 60 F_{254} 5715 Merck.

I. Synthesis of Phenyl-substituted aryl-acetic Acids I [10, 11, 37, 38]

The reaction was performed according to the literature [10, 11, 37, 38] after modification. The compounds reported here were generally prepared as illustrated in Scheme **2**. The aryl-acetic acids were synthesized by a Knoevenangel 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 hours. 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 3x100 mL $CHCl_3$ was made and the organic phase was collected and dried over Mg_2SO_4.

II. General Procedure for the Synthesis of aryl-acetic Chlorides [12]

Thionyl chloride ($SOCl_2$-16mmol) was added dropwise to the corresponding acid (equimolar amount) in a round bottom flask with strirring. The mixture was refluxed (TLC monitoring). The volatile materials were then removed under reduced pressure and the resulting solid was used without further purification in the subsequent step.

III. General Procedure for the Synthesis of Amides [12]

The appropriate amine dissolved in dry acetone (30ml) was added dropwise to a solution of the corresponding acid chloride (equimolar amount) in the same solvent (30ml). The mixture was stirred at room temperature for 1 hour (TLC monitoring) and then was mild refluxed for 10 hours. After acetone removal under vacuum evaporation, the residue was purified by recrystallization from ethanol 95°. The precipitate was filtered and dried. The compounds reported here were generally prepared as illustrated in Scheme **2**.

2-Phenyl-N-thiazol-2-yl-3-thiophen-2-yl-acrylamide (1)

From the reaction of thiophen-carbaldehyde with phenylacetic acid 2-Phenyl-3-thiophen-2-yl-acrylic acid was formed which is converted to the corresponding chloride. 2-Phenyl-3-thiophen-2-yl-acryloyl chloride reacts with thiazol-2-ylamine according to the general procedure. The product was collected and recrystallized from ethanol. (66.7 %); mp 138-139°C; UV (ethanol absolute) λ_{max}: 320, 260, ε_{max}: 7020, 8020; IR (Nujol) (cm^{-1}): 3200 (N-H), 1720-1700 (C=O), 1650-1620 (C=C); ^{1}H-NMR ($CDCl_3$): δ; ppm 6.94-6.96 (m, 1H), 7.00-7.15 (b, 1H), 7.18-7.20 (d, 1H), 7.22-7.25 (d, 1H), 7.32-7.37 (m, 3H), 7.47-7.50 (d, 1H) *J = 9*, 7.56-7.58 (m, 3H), 8.26 (s, 1H); ^{13}C-NMR ($CDCl_3$): 113.9, 126.4, 126.8, 127.4, 128.4, 128.5, 128.6, 128.9, 130.4, 130.5, 131.3, 137.3, 137.5, 138.4, 158.1, 164.0; MS: 312 [M^+] (75), 99.2 (24), 84.2 (18.4), 83.18 (52.25), 77.14 (56.15), 43.12 (82.98); Anal.: ($C_{16}H_{12}N_2OS_2$) Expected %: C:61.52, H:3.87, N:8.97 Calculated %: C:61.76, H:4.02, N:8.65

N-Benzothiazol-2-yl-2-phenyl-3-thiophen-2-yl-acrylamide (2)

According to the general procedure, 2-Phenyl-3-thiophen-2-yl-acryloyl chloride reacts with benzothiazol-2-ylamine and the product was collected and recrystallized from ethanol. (55.2 %); mp 175-177°C; UV (ethanol absolute) λ_{max}: 350, 330, 270, ε_{max}: 11190, 13530, 11600; IR (Nujol) (cm^{-1}): 3200 (N-H), 1730-1700 (C=O), 1650-1600 (C=C); ^{1}H-NMR ($CDCl_3$): δ ppm 6.98 (m, 1H), 7.30-7.38 (m, 5H), 7.43-7.46 (d, 1H) *J = 9*, 7.48-7.73 (m, 4H), 7.83-7.85 (d, 2H), 8.32 (s, 1H); ^{13}C-NMR ($CDCl_3$):119.4, 121.7, 124.7, 125.6, 126.2, 126.3, 127.0, 128.8, 128.6, 129.2, 130.0, 130.2, 130.5, 130.7, 132.0, 135.0, 135.3, 138.3, 172.9, 173.0; MS: 361 [M^+] (46.9), 213.1 (95.2), 185.1 (95.9), 150.1 (100), 134.1 (20.8); Anal.: ($C_{20}H_{14}N_2OS_2$) Expected %: C: 66.27, H:3.89, N:7.73 Calculated %: C:66.01, H:4.21, N:7.62

N-Benzyl-2-phenyl-3-thiophen-2-yl-acrylamide (3)

According to the general procedure, 2-Phenyl-3-thiophen-2-yl-acryloyl chloride reacts with benzylamine and the product was collected and recrystallized from ethanol. (43.2%); mp 82-84°C; UV (ethanol absolute) λ_{max}: 320, 260, ε_{max}: 10310, 11320; IR (Nujol) (cm^{-1}): 3100-3200 (N-H), 1750-1720 (C=O), 1650-1600 (C=C); ^{1}H-NMR ($CDCl_3$): δ ppm 4.51-4.52 (d, 2H), 6.93-7.13 (m, 3H), 7.20-7.62 (m, 11H), 8.14 (s,1H); ^{13}C-NMR ($CDCl_3$): 43.9, 126.4, 126.5, 126.6, 127.2, 128.0, 128.6, 129.0, 129.2, 129.5, 129.6, 130.0, 130.2, 131.0, 132.6, 135.2, 139.0, 143.0, 172.0; MS: 319 [M^+] (54.6), 187.3 (27.1), 106.1 (26.2), 77.1 (23.1), 43.3 (13.65); Anal.: ($C_{20}H_{17}NOS$) Expected %: C:75.20, H:5.361, N:4.38 Calculated %: C:75.02, H:5.61, N:4.01

N-Benzothiazol-2-yl-3-(3-phenoxy-phenyl)-2-phenyl-acrylamide (4)

According to the general procedure, 3-(3-Phenoxy-phenyl)-2-phenyl-acryloyl chloride reacts with thiazol-2-ylamine and the product was collected and recrystallized from ethanol. (72.5 %); mp 165-167°C; UV (ethanol absolute) λ_{max}: 290, 250, ε_{max}: 19490, 19160; IR (Nujol) (cm^{-1}): 3100-3200 (N-H), 1750-1700 (C=O), 1650-1550 (C=C); ^{1}H-NMR ($CDCl_3$): δ ppm 6.69-6.95 (m, 5H), 7.15-7.30 (m, 9H), 7.49-7.53 (d, 1H), 7.56-7.68 (m, 2H), 8.1-8.4 (m, 2H), 9.14 (s,1H); ^{13}C-NMR ($CDCl_3$): 116.7, 116.9, 117.0, 117.9, 119.2, 119.8, 120.3, 120.8, 124.3, 124.5, 126.4, 126.6, 127.9, 128.2, 128.4, 128.5, 128.7, 129.2, 129.5, 132.1, 134.8, 141.6, 149.0, 155.2, 157.3, 171.4, 173.5; MS: 315 (37.4), 270.6 (27.2), 178.9 (69.3), 177.8 (50.7), 102.2 (15.4), 93.2 (12.2), 77.2 (100); Anal.: ($C_{28}H_{20}N_2O_2S$) Expected %: C:74.98, H:4.49, N:6.25, Calculated %: C:74.73, H:4.87, N:5.84

3-(3-Phenoxy-phenyl)-2-phenyl-acrylic acid N'-methyl-hydrazide (5)

According to the general procedure, the product was collected and recrystallized from ethanol. (17.4%); mp 129-130 °C; UV (ethanol absolute) λ_{max}: 280, 250, ε_{max}: 11950, 12590, ^{1}H-NMR ($CDCl_3$): δ 2.46 (s, 3H), 6.71-6.92 (b, 4H), 7.15-7.17 (m, 3H), 7.28-7.34 (m, 5H), 7.54-7.68 (m, 4H), 9.13 (s, 1H); ^{13}C-NMR ($CDCl_3$): 29.7, 112.0, 119.1, 119.5, 119.7, 121.3, 125.3, 126.6, 127.3, 127.7, 127.9, 128.0, 128.7, 129.1, 129.9, 131.7, 133.7, 139.8, 156.6, 157.2, 166.7; MS: 343 [M^+] (22.9), 299.3 (12.6), 180.7 (22.1), 166.7 (10.7),

92.9 (13.9), 84.8 (78), 82.9 (96.2), 76.9 (47.2), 75.9 (7.6), 44.9 (15.4), 43 (90.0); Anal.: ($C_{22}H_{20}N_2O_2$) Expected %: C:76.72, H:5.85, N:8.13, Calculated %: C:76.76, H:5.74, N:7.84

3-Naphthalen-1-yl-2-phenyl-N-thiazol-2-yl-acrylamide (6)

According to the general procedure, the product was collected and recrystallized from ethanol. (30.6%); mp 165-167 ^oC**;** UV (ethanol absolute) λ_{max}: 290, 250, ε_{max}: 14510, 22740, ^{1}H-NMR ($CDCl_3$): δ 7.02-7.05 (d, 2H), 7.20-7.43 (m, 5H), 7.47-7.48 (m, 2H), 7.6-7.64 (m, 3H), 7.70-7.72 (m, 2H), 7.84-7.92 (m, 2H); ^{13}C-NMR ($CDCl_3$): 101.0, 124.3, 124.4, 125.8, 126.0, 126.3, 126.4, 126.8, 127.8, 128.3, 128.5, 128.8, 130.0, 132.0, 133.0, 134.0, 137.0, 138.5, 140.0, 166.0, 172.6; MS: 355 [M^+] (14), 257.7 (86), 228.6 (83.4), 227.5 (100), 126.8 (9.9), 76.9 (29.9), 69.9 (12.7), 42.9 (12.3); Anal.: ($C_{22}H_{16}N_2OS$) Expected %: C:74.13, H:4.52, N:7.86, Calculated %: C:74.36, H:4.46, N:7.65

N-Benzyl-3-naphthalen-1-yl-2-phenyl-acrylamide (7)

According to the general procedure, the product was collected and recrystallized from ethanol. (22.2%); mp 136-138 ^oC; UV (ethanol absolute) λ_{max}: 250, 240, ε_{max}: 16350, 15610, ^{1}H-NMR ($CDCl_3$): δ 4.22 (s, 1H), 6.71-7.73 (b, 18H), 7.85-7.92 (m, 2H); MS: 257.9 (17.8), 228.7 (20.2), 151.8 (9.7), 105.9 (10.4), 91.0 (100), 76.9 (18.4), 44.0 (51.0); Anal.: ($C_{26}H_{21}NO$) C, H, N. Expected %: C:85.92, H:5.82, N:3.85 Calculated %: C:85.67, H:5.93, N: 3.58

Physicochemical Studies

a) 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 (70/30, v/v) for the amides 1, 2, 3 and 6 and (75/25, v/v) containing 2ml NH_4OH for the amides 4, 5 and 7. The plates were developed in closed chromatography tanks saturated with the mobile phase at 24^{o}C. Spots were detected under UV light or by iodine vapours. R_M values were determined from the corresponding R_f values (from ten individual measurements) using the equation $R_M = \log [(1/R_f) -1]$ [39].

***b) Determination of Lipophilicity as Clog* P**

Lipophilicity was theoretically calculated as Clog *P* values in n-octanol-buffer by CLOGP Programme of Biobyte Corp [25].

Biological Experiments

Experiments In Vivo

Inhibition of the Carrageenin-induced Edema [10, 11, 40, 41]

Edema was induced in the right hind paw of Fisher 344 rats (150-200 g) by the intradermal injection of 0.1 ml 2% carrageenin in water. Both sexes were used. Females pregnant were excluded. Each group was composed of 6-15 animals. The animals, which have been bred in our laboratory, were housed under standard conditions and received a diet of commercial food pellets and water ad libitum during the maintenance but they were entirely fasted during the experiment period. Our studies were in accordance with recognised 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 **2**. Indomethacin in 0.01 mmol/kg (47%). Values CPE % are the mean from two different experiments with a standard error of the mean less than 10 % [40, 41] (Table **2**).

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.

Determination of the Reducing Activity of the Stable Radical 1,1-diphenyl-picrylhydrazyl (DPPH) [10, 11, 18, 42]

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 0.05mM. After 20 and 60 min at room temperature the absorbance was recorded at 517nm (Table **3**).

Competition of the Tested Compounds with DMSO for Hydroxyl Radicals [10, 11, 18, 43, 44]

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 mM and 0.1mM) and ascorbic acid (10 mM). After 30 min of incubation (37^o C) the reaction was stopped with CCl_3COOH (17 % w/v) (Table **3**).

Soybean Lipoxygenase Inhibition Study In Vitro [10, 11, 45]

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 x 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 **2**).

Heme Protein-dependent Lipid Degradation [11, 46, 47]

50μM heme, arachidonic acid (0.4mM) the compounds at the various concentrations tested, H_2O_2 (0.5mM) were incubated together for 10 min at 37^{o}C in KH_2PO_4-KOH buffer (50mM, pH 7.4). The product of peroxidation was detected using the TBA test [34, 35]. The compounds were added in DMSO solution, which has no effect on the assay (Table **2**).

Table 3. Inhibition % of Induced Carrageenin Rat Paw Edema (CPE %); *In Vitro* Inhibition of Soybean Lipoxygenase (LO) (IC_{50}), % Inhibition of Heme Dependent Lipid Peroxidation (LP %); % Superoxide Radical Scavenging Activity ($O_2^{\cdot-}$)

compd	CPE (%)[a] 0.01 mM/ kg body weight	LO IC_{50} (μM)	A % LP-1mM	$O_2^{\cdot-}$ (%)0.1mM
1	39.5*	119	21.8	82.2
2	38.9*	71	24.4	41.9
3	67.6**	63	14.7	100 %, 1mM
4	30**	46	14.7	27.8
5	9.3*	44	no	76.7
6	6.5*	43	10.3	78.5
7	9.6*	44	no	99.3
Caffeic acid		600	21.9	5.5
Indomethacin	47*			
NDGA			26.1	no

[a] statistical studies were done with student's T-test, * $p<0.01$, ** $p<0.05$; no: no action under the reported experimental conditions

Non Enzymatic Assay of Superoxide Radicals-Measurement of Superoxide Radical Scavenging Activity [11, 48]

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 **2**).

Effects of the Test Compounds on the Fe^{+3} –ion-stimulated Peroxidation of Linoleic Acid [11, 49]

Linoleic acid sodium salt (90mg) was dissolved and diluted to 50mL with 0.2M phosphate buffer pH 7.4 and oxygen (100%) bubbled through the solution. 2mL aliquots of the linoleic acid sodium solution were incubated for 2h at 37°C with 2.5mM Fe^{+3} ($FeSO_4$) and 1mM of the test compounds. The amount of peroxidation which occurred during this time was measured by the 2-thiobarbituric acid (TBA) method. The amount of TBA-reactive material in each sample was determined by measuring the absorbance of the

Table 4. Interaction % with DPPH (RA %); Competition % with DMSO for Hydroxyl Radical ($HO^{\cdot}$ %)

compd.	RA % 0.05mM		RA % 0.1mM		$HO^{\cdot}$ (%)		
	20min	60min	20min	60min	0.001mM	0.01mM	0.1mM
1	28.1	25.9	27.7	33.3	no	no	96.5
2	28.5	19.1	43.4	70	no	no	98
3	46.5	53.1	77.2	70.8	97.7	94.3	91.6
4	7.9	24.1	26.5	42.3	no	no	90.5
5	50.4	45.1	52.3	62.5	no	no	no
6	39.9	74.7	71.9	72.2	no	no	48.5
7	63	65.9	92.9	84.7	96.4	95.3	95.5
BHT			31.3	60			
NDGA			81	82.6			
Caffeic acid			9.3	9.02			
Trolox					nt	73.4	88.2

no: no action under the experimental conditions; nt: not tested.

aqueous layer at 535 nm. The inhibition of Fe^{+2}-stimulated oxidation of linoleic acid caused by each compound is the mean value of three or five experiments (Table **5**).

Table 5. Effects of the Tested Compounds on the Fe^{3+}-ion Stimulated Peroxidation of Linoleic Acid

α/α	% Inhb. 0.1mM
3	63.8
4	no
5	10μM, IC_{50}
6	no
7	33.9
NDGA	83.7

no: no results under the reported experimental conditions, nt: not tested

Stability Studies and Incubation with Glutathione (GSH) [34]

Solutions of the compounds were prepared in water using phosphates 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 24h at 37°C and their UV spectra were recorded. All determinations were carried out in duplicate. The error limits of the ε 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 24h and their UV spectra were recorded. The results are given in Table **6**.

Table 6. Stability Studies and incubation with glutathione (GSH)

α/α	λ_{max}	ε_{max}
1	230	1791
1 + 2GSH	230	1705
1 + 10GSH	230	1361
2	230	1717
2 + 2GSH	230	1684
2 + 10GSH	230	1374
3	310	223
3 + 2GSH	310	174
3 + 10GSH	310	190
4	290	955
4 + 2GSH	300	91
4 + 10GSH	300	17
5	290	274
5 + 2GSH	290	304
5 + 10GSH	290	545
6	300	101
6 + 2GSH	300	82
6 + 10GSH	300	141
7	220	1979
7 + 2GSH	230	1859
7 + 10GSH	230	1615

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