Design and Synthesis of Novel Quinolinone-3-aminoamides and Their α-Lipoic Acid Adducts as Antioxidant and Anti-inflammatory Agents

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A series of N-substituted-quinolinone-3-aminoamides and their hybrids containing the α-lipoic acid functionality were designed and synthesized as potential bifunctional agents combining antioxidant and anti-inflammatory activity. The new compounds were evaluated for their antioxidant activity and for their ability to inhibit in vitro lipoxygenase as well as for their anti-inflammatory activity in vivo. In general, the derivatives were found to be potent antioxidant or anti-inflammatory agents. The results are discussed in terms of structure−activity relationships and an attempt is made to define the structural features required for activity.

Introduction

The formation of reactive oxygen species (ROS) is characteristic of aerobic organisms that can normally defend themselves against these highly reactive species using enzymes, like superoxide dismutase and glutathione peroxidase, and naturally occurring antioxidants, such as α-tocopherol (vitamin E), ascorbic acid (vitamin C), and β-carotene. However, in many pathophysiological conditions, the excessive production of ROS overwhelms the natural antioxidant defense mechanisms. This imbalance is termed oxidative stress.

Oxidative stress has been associated with the inflammation process. ROS, like superoxide radical anion, hydrogen peroxide, and hydroxyl radical, are produced during the inflammation process by phagocytic leukocytes (e.g., neutrophils, monocytes, macrophages, eosinophils) that invade the tissue. Moreover, these reactive species are involved in the biosynthesis of prostaglandins and in the cycloxygenase- and lipoxygenase-mediated conversion of arachidonic acid into proinflammatory intermediates.[1,2] In addition, ROS may initiate inflammation via upregulating several different genes involved in this process by activating certain redox-sensitive transcription factors as the nuclear factor kB (NFkB) and activator protein-1 (AP-1).[3,4]

The rates of ROS production are increased in most pathophysiological conditions,[5] therefore, it is evident that the treatment of various diseases could benefit from the use of drugs that combine antioxidant and anti-inflammatory activity. This has already been proven for a number of commercially available nonsteroidal anti-inflammatory drugs (NSAIDs), which simultaneously possess radical scavenging properties.[6]

The quinolinone structure is characteristic of numerous natural products and synthetic analogues that exhibit a wide variety of biological activities. The antioxidant and anti-inflammatory properties of various compounds possessing the quinolinone moiety as a key structural feature have attracted the interest of several research groups.[7,8]

Linomide (*N*-phenylmethyl-1,2-dihydro-4-hydroxy-1-methyl-2-oxo-3-quinolinecarboxamide, Figure 1),[9] is a synthetic immunomodulator, which is effective against various types of cancers and autoimmune disorders, such as multiple sclerosis (MS), rheumatoid arthritis, systemic lupus erythematosis, and experimental autoimmune encephalomyelitis. The structure of linomide has served as the prototype for the synthesis of a variety of analogues in an effort to optimize this lead compound.[10−14]

Another synthetic quinolinone derivative, rebamipide[15] (2-(4-chlorobenzoylamino)-3-[2-(1*H*)-quinolinon-4-yl]propionic acid, Figure 1) is an effective antioxidant and antiulcer agent, mediating its pharmacological activity mainly by increasing endogenous prostaglandin synthesis and by ROS scavenging.[16] The structural characteristics of rebamipide that have been shown to be important for scavenging the hydroxyl radical are the 3,4-double bond and the 2-oxo functionality of the quinolinone moiety and the carbonyl part of the amido group in conjunction with a *p*-chlorobenzyl function.[17]

The research group of Ishiwara et al. has recently developed a novel quinolinone derivative, TA 270 (4-hydroxy-1-methyl-3-octyloxy-7-sinapinoylamino-2(1*H*)-quinolinone, Figure 1).[18] This compound was designed as an antioxidant, and further studies showed that it is as well a potent inhibitor of immediate- and late-airway responses, pulmonary inflammatory cell accumulation and, airway hyper-responsiveness, therefore, it may be of therapeutic use for the treatment of bronchial asthma. Studies of the action mechanisms of TA 270 in cellular level in vitro revealed that it specifically suppresses the antigen-induced leukotriene production, probably by inhibiting 5-lipoxygenase in inflammatory cells.[19]

A naturally occurring quinolinone alkaloid has been recently isolated from the aleurone layer of *Oryza sativa* cv *Hengjinmi*. This compound exhibited moderate antioxidant activity in a DPPH free radical scavenging assay.[20]

The potential use of quinolinone derivatives as therapeutic agents to treat a wide range of inflammation-mediated diseases

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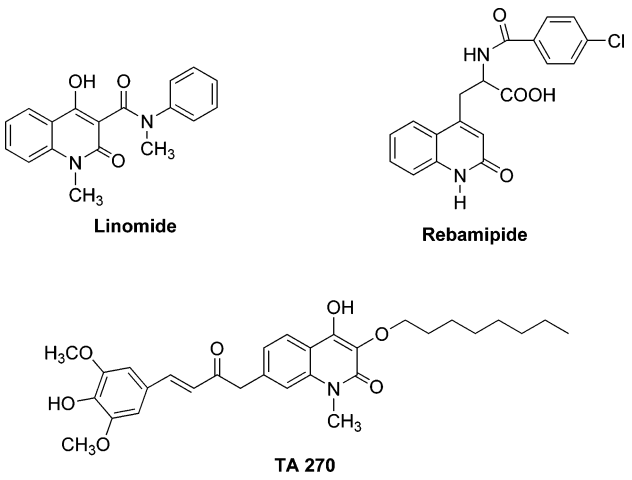


Figure 1.

is also depicted in several patents, which describe the synthesis and anti-inflammatory activity of a variety of quinolinone analogues.[21]

The aim of the present study was to synthesize novel quinolinone-3-aminoamides bearing a methyl or phenyl group on the nitrogen heteroatom and possessing a free amino functionality, which could allow further chemical modifications. The synthesis of the 4-hydroxy-2-quinolinone pharmacophoric moiety has already been reported by the research group of Igglessi-Markopoulou et al., as part of our long-standing interest in the development of novel methodologies for the synthesis of this molecular scaffold and analogous fused heterocyclic systems.[22,23] Moreover, because the combination of two pharmacophores on the same scaffold is a well established approach to the synthesis of more potent drugs with dual activity,[24−32] we decided to also incorporate the α-lipoic acid (LA) moiety and examine the influence of this modification on the activity of the aminoamides.

The remarkable antioxidant properties of LA[33−35] have been shown to be coupled with interesting anti-inflammatory activity: LA possesses skin anti-inflammatory activity when administered orally[36] and can effectively suppress allergic inflammation and airway hyper-responsiveness in a mouse model of asthma, therefore, it is a possible candidate for adjuvant antiasthmatic therapy.[37] Moreover, thiazolidinedione-LA adducts can be potentially useful as oral and topical agents for treating inflammatory skin conditions.[38] LA has been widely used for the synthesis of various conjugates, possessing multifunctional activity.[25,27,31,32,39−45]

Chemistry

The synthesis of the starting 1-methyl and 1-phenyl-4-hydroxy-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid methyl esters **3** and **4** (Scheme 1) was accomplished using our previously developed methodology.[22] The appropriate commercially available N-substituted anthranilic acids **1** and **2** were treated with equimolar amounts of N-hydroxybenzotriazole and dicyclohexylcarbodiimide (DCC) in anhydrous THF, and the nonisolated active ester was used as an acylating agent. The C-acylation of dimethyl malonate was performed using NaH in THF to generate the malonate anion, which attacked the carbonyl carbon of the active ester to produce the intermediate C-acylation products **3a** and **4a**. The latter spontaneously cyclized in the basic reaction conditions to provide compounds **3** and **4** in good yields.

The preparation of the quinolinone-3-aminoamides **5**−**12** was succeeded via two methods, A and B, as depicted in Scheme 1. According to Method A, quinolinone **3** or **4** was dissolved in 1,2-ethylenediamine or 1,6-hexamethylenediamine, and the mixture was heated at 100−110 °C for 5−24 h[38,46] to afford aminoamides **5**−**8** in good yields (62−65%). In the case of aromatic diamines, anhydrous toluene was used as a solvent, and the mixture of quinolinone (1 equiv) and diamine (2 equiv) was heated overnight at 100 °C (Method B). The aminoamides formed were insoluble in toluene and precipitated upon formation, therefore, the isolation procedure involved only filtration and washing with diethylether. Compounds **9**−**12** were obtained in high yields (85−99%), and we were gratified to find out that no further purification was needed, because they produced excellent elemental analyses data without recrystallization (see Experimental Section). Because Method B was more advantageous than Method A in isolation, yields, and purity of the products, we have employed it also for the synthesis of the aliphatic aminoamides and succeeded in improving the yields considerably (75−80%) and obtaining analytically pure compounds in a less tedious way. The use of a solvent in which the resulting amide has low solubility has been previously reported to increase the yields and purities of quinolinecarboxamides.[13,47]

The ^{1}H NMR spectra of aminoamides **5**−**9**, containing aliphatic amines, were obtained in a mixture of $CDCl_3/CD_3OD$ or in $CDCl_3$ containing drops of CF_3COOD and are characterized by the presence of a broad signal at 10−10.45 ppm, owed to the proton of the amide group (−CONH−). Compounds **9** and **10**, produced from 1,2-phenylenediamine, were soluble in $CDCl_3$, and their spectra revealed the presence of a strongly hydrogen-bonded proton at 16.6−16.8 ppm, the hydroxyl proton on position 4 of the heterocyclic ring. This signal, appears at a lower field than in the corresponding 1-methyl 4-hydroxy-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid methyl ester **(3)**, indicating that the electron density of the 3-carbonyl group has been intensified. In the case of the 1,4-phenylenediamine-containing aminocarboxamides **11** and **12**, CF_3COOD had to be added in $CDCl_3$ to transform the insoluble compounds to their corresponding soluble TFA salts.

To synthesize the hybrids of quinolinone-3-aminoamides with LA (Scheme 2), racemic α-LA was converted to its *N*-hydroxysuccinimidyl-ester **13**.[48] This activation was chosen instead of, for example, transformation to the acyl-chloride, because the ester is easily prepared in high yields, can be stored for a long time, and the *N*-hydroxysuccinimide formed as a byproduct is a water-soluble compound and can be efficiently removed from the reaction mixture.

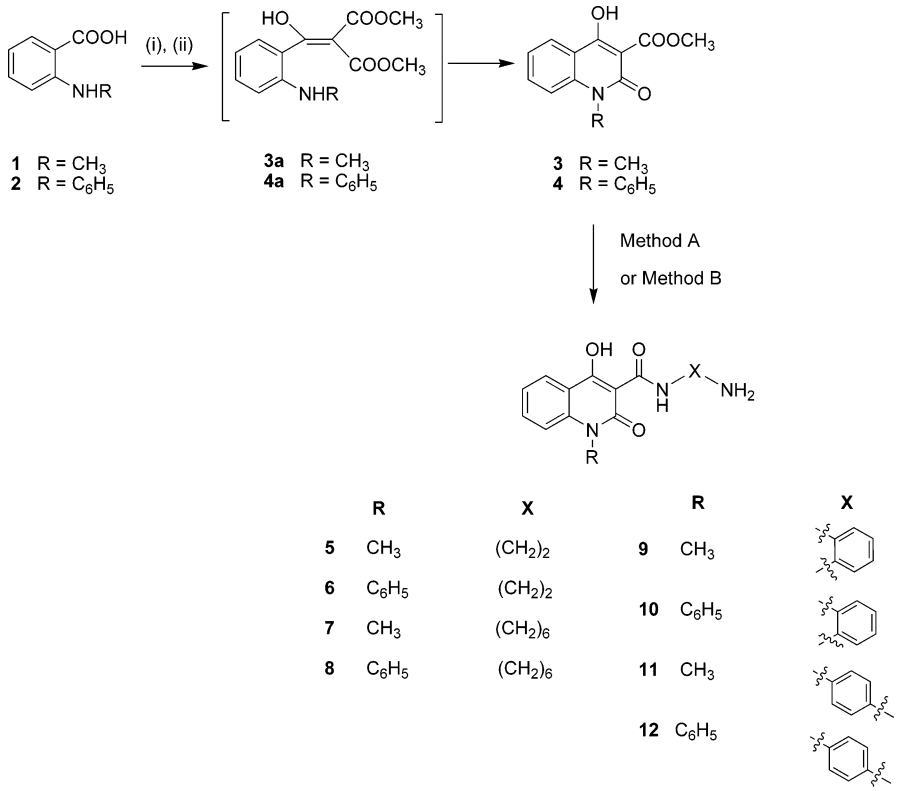
The synthesis of conjugates **14**−**18** was effected by dissolving equimolar amounts of compounds **5**−**8** or **10** with the active ester **13** in dichloromethane containing a few drops of dimethylformamide to facilitate dissolution. Their ^{1}H NMR spectra were characterized by the signal of the intramolecular hydrogen-bonded hydroxyl proton at low field (16.7−17.5 ppm) and the signals of the amide protons at 10−11.9 ppm for the 3-CONH− group and at 5.6−6.2 ppm for the −CONH− connected to α-LA.

The predominance of the enol tautomeric form in the 3-aminoamides and the LA conjugates guarantees the existence of a 3,4-double bond of the 2-quinolinone moiety.

Results and Discussion

In Vivo Anti-Inflammatory Activity. In this work, we designed and synthesized a series of novel quinolinone derivatives that were expected to offer protection against inflammation and radical attack by application of standard synthetic methods summarized in Schemes 1 and 2.

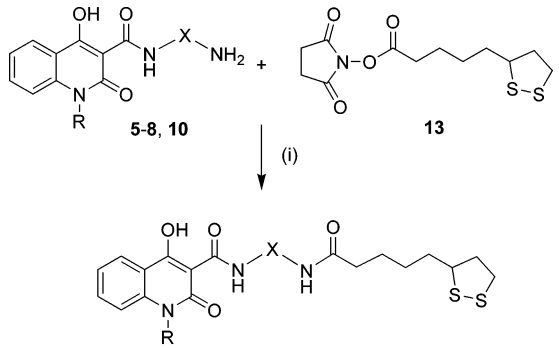
Scheme 1[a]



	R	X		R	X
5	CH_3	$(CH_2)_2$	**9**	CH_3	
6	C_6H_5	$(CH_2)_2$	**10**	C_6H_5	
7	CH_3	$(CH_2)_6$	**11**	CH_3	
8	C_6H_5	$(CH_2)_6$	**12**	C_6H_5	

[a] Reagents and conditions: (i) HOBt, DCC, THF; (ii) NaH, dimethyl malonate, THF; Method A (for 1,2-ethylenediamine and 1,6-hexamethylenediamine): **3** or **4**, diamine, 100−110 °C; Method B: **3** or **4**, diamine, toluene, 100 °C.

Scheme 2[a]



	R	X
14	CH_3	$(CH_2)_2$
15	C_6H_5	$(CH_2)_2$
16	CH_3	$(CH_2)_6$
17	C_6H_5	$(CH_2)_6$
18	C_6H_5	

[a] Reagents and conditions: (i) CH_2Cl_2, DMF, rt.

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-inflammatory effects of the tested quinolinone-3-aminocarboxamides and their adducts with α-LA were assessed by using the functional model of carrageenin-induced rat paw oedema and are presented in Table 1 as percent inhibition of induced rat paw oedema. Carrageenin-induced oedema is a nonspecific inflammation resulting from a complex of diverse mediators.[49] Because oedemas of this type are highly sensitive to NSAIDs, carrageenin has been accepted as a useful agent for studying new anti-inflammatory drugs.[50] This model reliably predicts the anti-inflammatory efficacy of the NSAIDs, and during the second phase, it detects compounds that are anti-inflammatory agents as a result of inhibition of prostaglandin amplification.[51] As shown in Table 1, all the investigated compounds inhibited carrageenin−induced paw oedema. The inhibition ranged from 37.1−85.3%, while the reference drug, indomethacin, induced 47% inhibition at an equivalent concentration. Compound **9** was the most potent (85.3%), followed

Table 1. Inhibition % LOX of Soybean Lipoxygenase; Inhibition % of Induced Carrageenin Rat Paw Edema (CPE %) at 0.01 mmol/Kg Body Weight; and Calculated Lipophilicity Clog P[62]

cmpd	ClogP	LOX %[a] (0.1 mM)	CPE %[b]
3	1.74	72.4	58.8**
4	3.74	100	37.1**
5	0.61	99.5	67*
6	2.60	82.3	68.1**
7	1.86	92.1	68.5**
8	3.82	17.6	68.8*
9	1.66	71.3	85.3*
10	3.65	no[d]	48.5*
11	1.66	50.8	69.5*
12	3.65	58.9	56.4*
14	3.08	66.5	48.2*
15	5.07	no[d]	45.5**
16	4.02	79.1	53*
17	5.18	64.0	50*
18	5.42	100	63*
lipoic acid	2.39	29.1	29.6**
indomethacin		nt[e]	47*
NDGA[c]		83.7	nt[e]

[a] SD < 10%. [b] Statistical studies were done with student's T-test, ** $p < 0.01$, * $p < 0.05$. [c] Nordihydroguaiaretic acid. [d] no = no action under the reported experimental conditions. [e] nt = not tested.

by compounds **8**, **6**, **11**, **5**, **7**, **3**, and **16**, whereas compound **4** had the lowest effect (37.1%).

For the starting quinolinones **3** and **4**, as well as for the aminoamides **9** and **10**, **11** and **12**, which contain an aromatic diamine moiety, the nature of the substituent on the nitrogen of the heterocyclic ring seems to have a distinct influence on the inhibition values. Apparently, the presence of the small methyl group (lower lipophilic contribution, minor steric effects, and electron-donating nature) instead of the large phenyl group is correlated with higher inhibition values. However, for compounds **5** and **6**, **7** and **8**, which contain an aliphatic diamine, the nature of the substituent on the nitrogen of the ring did not affect the activity significantly. For the same compounds, the number of methylene fragments did not alter the activity either.

The presence of the amine group in the *ortho*- or *para*-position of the 3-aminoamide phenyl ring (compounds **9**−**12**) influences biological response. Thus, compound **9**, a derivative of 1,2-phenylenediamine, exhibits 85.3% inhibition, whereas the corresponding compound **11**, containing 1,4-phenylenediamine, shows lower inhibition value (69.5%). The opposite phenomenon was observed for the *N*-phenyl-substituted compounds **10** and **12**, in which the 1,2-phenylenediamine derivative **10** is less active than the 1,4-phenylediamine analogue **12**.

LA-quinolinone hybrids showed higher anti-inflammatory activity than LA alone (29.6% inhibition in the carragennin-induced rat paw edema). However, these hybrids did not show enhanced anti-inflammatory activity compared to that of the quinolinone aminoamides, with the exception of compound **18**.

The results show that, in the case of the synthesized quinolinone derivatives, overall lipophilicity does not increase in parallel to inhibition (Table 1). On the contrary, compounds with significantly high in vivo anti-inflammatory activity present lower clogP values, for example, **9**, **11**, **5**, **7**, and **3**.

In Vitro Antioxidant Activity and LOX Inhibition. It is well-known that free radicals play an important role in the inflammatory process.[52] Many nonsteroidal anti-inflammatory drugs have been reported to act either as inhibitors of free radical production or as radical scavengers.[6,53] 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 novel quinolinone derivatives with regard to their antioxidant ability and in comparison to well-known antioxidant agents, for example, nordihydroguaiaretic acid and trolox (Tables 1 and 2).

The interaction of the examined compounds with the stable free radical DPPH is shown in Table 2. This interaction indicates their radical scavenging ability in an iron-free system. It is evident that the presence of an amine group in a position para on the 3-carboxamide phenyl ring is correlated with higher values, for example, compounds **11** and **12**, which exhibited the highest interactions (90.2 and 89%). The main reason for this might be that the free amine group is at the 4-position far from the carbonyl group and no intermolecular interactions could happen.

All the other analogues were found to have very low activity at 0.1 mM. LA exhibits a mild interaction. For the majority of the compounds, the interaction was time and concentration dependent.

The number of CH_2 groups, the nature of the substituent on the nitrogen of the heterocyclic ring, and the ester functionality do not correlate with higher interaction values. Moreover, introduction of the LA moiety did not improve scavenging activity. Lipophilicity does not seem to play a significant role under the reported experimental conditions.

Table 2. Interaction % with DPPH (RA %); Competition % with DMSO for Hydroxyl Radical (•OH %)

cmpd	0.1 mM[a] RA% 20 min	0.1 mM[a] RA% 60 min	0.5 mM[a] RA% 20 min	0.1 mM[a] RA% 60 min	0.1 mM[a] •OH%
3	8.3	7.4	17.5	18.4	45
4	9.1	9.1	16.8	19.3	95
5	5.6	11.5	20.9	37.1	40.1
6	7.2	10.0	1.4	7.9	78.6
7	3.1	8.3	13.7	21.9	59.3
8	11.6	17.5	19.3	28.1	27.1
9	6.0	13.5	28.8	41.4	60.5
10	9.4	16.3	21.3	34.7	98
11	90.2	88.1	85.1	83.7	20.7
12	89.0	88.3	94.4	89.2	10
14	10.9	3.4	28.8	40.1	67.9
15	6.4	7.8	no[c]	14.1	0
16	9.1	13.0	28.4	38.8	28.6
17	6.2	10.2	23.9	29.5	no[c]
18	9.7	16.1	11.0	28.5	no[c]
lipoic acid	20.3	27.1	5.2	6.8	38.6
NDGA	81	82.6	96.5	98	nt[b]
trolox	nt[b]	nt[b]	nt[b]	nt[b]	88.2

[a] SD < 10%. [b] nt = not tested. [c] no = no action under the reported experimental conditions.

Hydroxyl 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 the quinolinone analogues with DMSO for HO•, 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 these experiments (Table 2), compounds **15**, **17**, and **18** did not show any inhibition at 0.1 mM. *N*-phenyl-quinolinones **4**, **10**, and **6** were the most active under these experimental conditions. LA presents 38.6% competition at 0.1 mM. Among the quinolinone-LA hybrids, compound **14** showed the highest inhibitory activity (67.9%), whereas compound **16** was slightly active. The overall low lipophilicity is correlated with higher competition values (compounds **7** and **16**, compounds **6** and **15**, compounds **18** and **10**, and compounds **17** and **8**), with the exception of compounds **5** (clogP = 0.61) and **14** (clogP = 3.08).

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 5-lipoxygenase (5-LOX). Inhibitors of 5-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.[54] Several quinoline derivatives have been synthesized as inhibitors of human 5-LOX.[55−57]

In this context, we decided to further evaluate the synthesized compounds for inhibition of soybean lipoxygenase LOX by the UV absorbance-based enzyme assay (Table 1).[58] While one may not extrapolate the quantitative results of this assay to the inhibition of mammalian 5-LOX, it has been shown that inhibition of plant LOX activity by NSAIDs is qualitatively similar to their inhibition of the rat mast cell LOX and may be used as a simple qualitative screen for such activity. Most of the LOX inhibitors are antioxidants or free radical scavengers,[59] because lipoxygenation occurs via a carbon-centered radical. Some studies suggest a relationship between LOX inhibition

and the ability of the inhibitors to reduce Fe^{3+} at the active site to the catalytically inactive Fe^{2+}. 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. Several LOX inhibitors are excellent ligands for Fe^{3+}. It is possible that our compounds follow a mechanism of inhibition acting as chelators for iron at the active site of LOX.

Perusal of % inhibition values shows that compounds **4**, **5**, **6**, **7**, and **18** are active, while compounds **10** and **15** do not inhibit LOX. Compounds **3**, **9, 14**, **16**, and **17** also show inhibitory activity, followed by derivatives **11** and **12**, whereas analogue **8** slightly inhibits LOX. No inhibition was observed by lowering the concentration to 0.01 mM. Thus, inhibition vanishes in 0.01 mM, with the exception of quinolinone **4**, which presents 7.5% inhibition at 0.01 mM. LA presents moderate inhibitory activity (29.1%). In the case of 3-aminoamide **10**, which shows no inhibitory activity, incorporation of the LA moiety resulted to an impressive increase of activity (compound **18**, inhibition 100%). The presence of an amine group in a position para to the 3-carboxamide phenyl ring (compound **12**) also enhances inhibitory activity. A preliminary QSAR study reveals that higher molar volume values are correlated with a decrease in biological activities. More research is in progress to delineate the role of structural characteristics.

The chemical stability of selected aminoamides and quinolinone-LA adducts has been tested to investigate the integrity of the molecules under pH and temperature conditions that mimic those of the biological experiments. Thus, we have tested the stability of compounds **5**, **6**, **9**, **10**, **15**, and **18** in pH 9 (borax buffer) at rt for 1 h and of compounds **5**, **6**, **9**, **14**, and **15** at pH 7.4 (phosphate buffer) at 37 °C for 4 h: a 0.1 M solution of the tested compound in DMSO was diluted with the appropriate aqueous buffer solution to 0.01 M, and the slurry was stirred vigorously. TLC of the compounds showed that no degradation had taken place. To verify this finding, after extraction with CH_2Cl_2, drying, and evaporation of the solvent, the ^1H NMR spectrum of each compound was obtained. The spectra were identical with the spectra of the untreated compounds.

Conclusion

In the present study, we have synthesized and biologically evaluated novel quinolinone 3-aminoamides and their α-LA hybrids. The synthesis is straightforward and efficient and provides the desired compounds in satisfactory yields, without demanding tedious workup and purifying procedures. The compounds are stable under basic and neutral conditions. We have shown that the synthesized compounds possess significant anti-inflammatory activity in vivo, whereas most of them are potent $^{\bullet}OH$ scavengers and inhibit in vitro soybean lipoxygenase.

Our studies confirm that the presence of a free hydroxyl group at position 4, a 3,4-double bond, and an aminoamide functionality at position 3 are important structural features for the antioxidant/anti-inflammatory activity, as it is already known for NSAIDs containing a β-ketocarboxamide functionality ("acidic amide")[51] and has been recently shown for the quinolinone antioxidant rebamipide.[17]

An overview of the results of the in vivo experiment reveals that all the synthesized quinolinone-LA hybrids (compounds **14**−**18**) exhibit significantly higher activity than LA. The chemical stability of the compounds shows that they constitute new, promising, anti-inflammatory agents as intact molecules and are not LA prodrugs. This provides an impetus for designing new anti-inflammatory agents using the quinolinone-LA scaffold as the starting point.

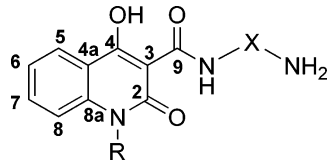
However, incorporation of the LA moiety to the quinolinone aminoamide scaffold did not provide compounds with enhanced dual antioxidant and anti-inflammatory activity. More specifically, in this series of compounds, the presence of LA increased the antioxidant activity of compound **5** (compare **5** with the LA adduct **14**) and the anti-inflammatory activity of compound **10** (compare **10** with **18**). It seems that, even though LA possesses all the characteristics required for enhancing antioxidant and anti-inflammatory activity, its hybrids do not always possess improved activity compared to that of the parent compounds (the quinolinone aminoamides in this case). This observation is not unique because other LA adducts have been synthesized and reported to exhibit lower activity than the parent compounds.[27,31]

The results of this study show that quinolinone aminoamides **5**−**12** exhibit satisfactory anti-inflammatory activity in vivo. It is noteworthy that the most satisfactory combined antioxidant−anti-inflammatory activity was exhibited by compounds **6** and **9**, which show the most appealing pharmacological profile. It is, therefore, evident that the design of this type of dual-acting molecules should be further explored based on the structural features of these compounds.

Experimental Section

Melting points were determined on a Gallenkamp MFB-595 melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet Magna IR 560. ^1H NMR spectra (300 MHz) and ^{13}C NMR spectra (75 MHz) were recorded on a Varian Gemini 2000 300 MHz spectrometer. The numbering of carbon atoms is shown on the structure in Chart 1. Elemental analyses were obtained on a Euro EA3000 Series Euro Vector CHNS Elemental Analyzer.

Chart 1



Materials. All the chemicals used were of analytical grade and commercially available by Merck, 1,1-diphenyl-2-picrylhydrazyl (DPPH), nordihydroguairetic acid (NDGA) were purchased from the Aldrich Chemical Co., Milwaukee, WI (U.S.A.). Soybean lipoxygenase, linoleic acid sodium salt, and indomethacin were obtained from Sigma Chemical Co. (St. Louis, MO), and carrageenin, type K, was commercially available. For the in vivo experiments, male and female Fischer-344 rats (180−240 g) were used.

General Procedure for the Synthesis of Quinolinone-3-aminoamides 5−12. Method A: A mixture of 1-methyl- or 1-phenyl-4-hydroxy-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid methyl ester (1.9 mmol) and the appropriate diamine (5.7mmol) was heated at 100−110 °C for 24 h. The reaction mixture was diluted with $CHCl_3$ and washed with saturated aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4) and evaporated in vacuo. The residue was triturated with diethylether, and the solid product was filtered off.

Method B: 1-methyl- or 1-phenyl-4-hydroxy-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid methyl ester (1.4 mmol) and the appropriate diamine (2.7mmol) were added to 7 mL of dry toluene, and the mixture was heated at 100 °C for 4 h. The product precipitated in the reaction mixture and, after cooling at ambient temperature, the solid was filtered off and washed with diethylether.

***N*-Ethylamino-1,2-dihydro-4-hydroxy-2-oxo-1-phenyl-3-quinolinecarboxamide (6).** Compound **6** was prepared from quinolinone **4** and 1,2-ethylenediamine: yield 62% (Method A) and 89% (Method B), yellowish solid, mp 163−165 °C. ^1H NMR ($CDCl_3$/

CF_3COOD): δ 10.47 (br s, 0.4H, CONH), 8.30 (dd, 1H, $J_{5,6}$ = 8.1 Hz, $J_{5,7}$ = 1.2 Hz, 5-H), 7.68−7.61 (m, 3H), 7.60−7.56 (m, 1H), 7.41 (pseudotriplet, 1H), 7.28−7.23 (m, 2H), 6.72 (d, 1H, *J* = 8.7 Hz), 3.79 (br s, 2H, NH−CH_2−), 3.39 (br s, 2H, −C$H_2$$NH_2$). ^{13}C NMR ($CDCl_3$/$CF_3COOD$): δ 173.4 (9-C), 172.6 (4-C), 163.3 (2-C), 140.6 (8a-C), 136.0 (1′-C), 134.8 (7-C), 130.9, 130.2, 128.7, 125.6, 124.3, 116.8 (4a-C), 113.0 (8-C), 109.2 (3-C), 41.6 (10-C), 37.5 (11-C). Anal. ($C_{18}H_{17}N_3O_3$) C, H, N.

***N*-Hexylamino-1,2-dihydro-4-hydroxy-1-methyl-2-oxo-3-quinolinecarboxamide (7).** Compound **7** was prepared from quinolinone **3** and 1,6-hexamethylenediamine: yield 46% (Method A) and 69% (Method B), white solid, mp 156−158 °C. ^{1}H NMR ($CDCl_3$/CD_3OD): δ 10.27 (br s, 1H, CONH), 8.12 (dd, 1H, $J_{5,6}$ = 8.1, $J_{5,7}$ = 1.2 Hz, 5-H), 7.62 (pseudotriplet, 1H, 7-H), 7.32−7.21 (m, 2H, 6-H, 8-H), 3.59 (s, 3H, N−CH_3), 3.37−3.30 (m, 2H, NH−CH_2−), 2.56 (pseudotriplet, 2H, −C$H_2$$NH_2$), 1.58−1.29 (m, 8H, −$(CH_2)_4$−). Anal. ($C_{17}H_{23}N_3O_3$) C, H, N.

***N*-*o*-Phenylamino-1,2-dihydro-4-hydroxy-1-methyl-2-oxo-3-quinolinecarboxamide (9).** Compound **9** was prepared from quinolinone **3** and 1,2-phenylenediamine, according to Method B: yield 65%, light green solid, mp 230−232 °C (lit.[60] mp 230−232 °C). ^{1}H NMR ($CDCl_3$): δ 16.61 (s, 1H, OH), 12.17 (s, 1H, CONH), 8.26 (dd, 1H, $J_{5,6}$ = 8.1, $J_{5,7}$ = 1.5 Hz, 5-H), 7.73 (dtd, 1H, $J_{7,8}$ = 8.7 Hz, $J_{7,6}$ = 6.9 Hz, $J_{7,5}$ = 1.5 Hz, 7-H), 7.55 (dd, 1H, *J* = 8.4, 1.8 Hz), 7.09 (ddd, 1H, *J* = 9.3, 7.8, 1.5 Hz), 6.85 (pseudotriplet, 2H), 7.41 (d, $J_{8,7}$ = 9.0 Hz, 1H, 8-H), 7.35 (ddd, 1H, $J_{6,5}$ = 8.1 Hz, $J_{6,7}$ = 7.2 Hz, $J_{6,8}$ = 1.2 Hz, 6-H), 3.93 (br s, 2H, −NH_2), 3.75 (s, 3H, N−CH_3). ^{13}C NMR ($CDCl_3$): δ 169.7 (4-C), 159.9 (CONH), 155.0 (2-C), 140.3, 140.2 (8a-C), 134.2 (7-C), 127.1, 125.7, 125.2 (5-C), 122.7, 119.3, 117.3 (4a-C), 114.4 (8-C), 100.0 (3-C), 29.1 (N−CH_3). Anal. ($C_{17}H_{15}N_3O_3$) C, H, N.

***N*-*p*-Phenylamino-1,2-dihydro-4-hydroxy-1-methyl-2-oxo-3-quinolinecarboxamide (11).** Compound **11** was prepared from quinolinone **3** and 1,4-phenylenediamine, according to Method B: yield 97%, green solid, mp 228 °C (dec). ^{1}H NMR ($CDCl_3$/CF_3COOD): δ 8.34 (dd, 1H, $J_{5,6}$ = 7.8, $J_{5,7}$ = 1.5 Hz, 5-H), 7.90−7.77 (m, 3H), 7.63−7.43 (m, 5H), 3.82 (s, 3H, N−CH_3). ^{13}C NMR ($CDCl_3$/CF_3COOD): δ 172.7 (9-C), 169.8 (4-C), 164.0 (2-C), 139.7, 138.2 (8a-C), 135.8, 130.6 (7-C), 126.3 (5-C), 123.9 (6-C), 120.2, 116.5, 115.4 (4a-C), 112.7 (8-C), 108.9 (3-C), 30.8 (N−CH_3). Anal. ($C_{17}H_{15}N_3O_3$) C, H, N.

***N*-*p*-Phenylamino-1,2-dihydro-4-hydroxy-2-oxo-1-phenyl-3-quinolinecarboxamide (12).** Compound **12** was prepared from quinolinone **4** and 1,4-phenylenediamine, according to Method B: yield 99%, light green solid, mp > 280 °C (dec). ^{1}H NMR ($CDCl_3$/CF_3COOD): δ 8.37 (dd, 1H, $J_{5,6}$ = 8.1, $J_{5,7}$ = 1.5 Hz, 5-H), 7.75−7.62 (m, 6H), 7.45 (pseudotriplet, 3H), 7.34−7.31 (m, 2H), 6.78 (d, 1H, *J* = 8.7 Hz). ^{13}C NMR ($CDCl_3$/CF_3COOD): δ 173.8 (9-C), 169.8 (4-C), 163.9 (2-C), 140.8, 138.2 (8a-C), 135.9, 135.5, 131.3 (7-C), 130.8, 128.9, 125.8, 125.7 (5-C), 124.5, 124.0 (6-C), 120.3, 117.3 (4a-C), 116.5, 112.8 (8-C), 109.0 (3-C). Anal. ($C_{22}H_{17}N_3O_3$) C, H, N.

General Procedure for the Synthesis of Compounds 14−18. Quinolinone 3-aminocarboxamides **5**−**8** or **10** (0.46 mmol) were mixed with *N*-(lipoyloxy)succinimide (**13**; 0.14 g, 0.46 mmol) in dichloromethane (3 mL), and drops of DMF were added to facilitate dissolution of the reactants. The mixture was stirred and light-protected overnight. H_2O (10 mL) was added to the mixture and afterward it was extracted with dichloromethane (3 × 10 mL) and washed repeatedly with H_2O. The organic extracts were combined, dried (Na_2SO_4), and concentrated in vacuo to afford the final compounds, which were triturated with diethylether and filtered off.

***N*-(1,2-Dihydro-4-hydroxy-1-methyl-2-oxo-3-quinolinecarbonyl)-*N*′-(1,2-dithiolane-3-pentanoyl)-ethylenediamine (14).** Compound **14** was obtained by reaction of **5** with **13**, as a yellowish solid, after purification with flash column chromatography (CH_2Cl_2/MeOH, 97:3): yield 69%, mp 146−148 °C. ^{1}H NMR ($CDCl_3$): δ 16.71 (s, 1H, OH), 10.57 (br, 1H, CON*H*−$(CH_2)_2$−), 8.22 (d, 1H, $J_{5,6}$ = 8.1 Hz, 5-H), 7.71 (pseudotriplet, 1H, 7-H), 7.39−7.26 (m, 2H, 6-H, 8-H), 6.19 (br, 1H, −$(CH_2)_2$−N*H*CO), 3.68 (s, 3H, N−CH_3), 3.65−3.59 (m, 2H, −CONHC$H_2$$CH_2$-NHCO−), 3.51−3.44 (m, 3H, −CONHC$H_2$$CH_2$NHCO− and 17-H), 3.14−3.04 (m, 2H), 2.41 (sextet, 1H, *J* = 6.3 Hz), 2.20 (m, 2H, −NHCOC$H_2$$CH_2$$CH_2$$CH_2$−), 1.86 (sextet, 1H, *J* = 6.4 Hz), 1.65−1.61 (m, 4H, −NHCOCH_2C$H_2$$CH_2CH_2$−), 1.46−1.43 (m, 2H, −NHCOC$H_2$$CH_2CH_2$$CH_2$−). ^{13}C NMR ($CDCl_3$): δ 173.3 (9-C), 172.3 (4-C), 171.9, 162.8 (2-C), 140.1 (8a-C), 134.1 (7-C), 125.6 (5-C), 122.6 (6-C), 116.2 (8-C), 114.4 (4a-C), 96.8 (3-C), 56.3, 40.3, 40.1, 38.5, 38.3, 36.4, 34.5, 29.1 (N−CH_3), 28.7, 25.2. Anal. ($C_{21}H_{27}N_3O_4S_2$) C, H, N, S.

***N*-(1,2-Dihydro-4-hydroxy-2-oxo-1-phenyl-3-quinolinecarbonyl)-*N*′-(1,2-dithiolane-3-pentanoyl)-ethylenediamine (15).** Compound **15** was obtained by reaction of **6** with **13**, as a white solid, after purification with flash column chromatography (CH_2Cl_2/MeOH, 97:3) and recrystallization from $CHCl_3$/petroleum ether: yield 70%, mp 170−172 °C. ^{1}H NMR ($CDCl_3$): δ 16.92 (s, 1H, OH), 10.38 (br, 1H, CON*H*−$(CH_2)_2$−), 8.24 (dd, 1H, $J_{5,6}$ = 8.1 Hz, $J_{5,7}$ = 1.5 Hz, 5-H), 7.66−7.53 (m, 3H), 7.48 (dtd, 1H, *J* = 1.8, 8.7, 7.2 Hz), 7.32−7.26 (m, 3H), 6.66 (d, 1H, *J* = 8.4 Hz), 6.14 (br, 1H, −$(CH_2)_2$−N*H*CO), 3.60−3.42 (m, 5H), 3.15−3.02 (m, 2H), 2.42 (sextet, 1H, *J* = 6.3 Hz), 2.17 (m, 2H, −NHCOCH_2-$CH_2$$CH_2$$CH_2$−), 1.88 (sextet, 1H, *J* = 6.4 Hz), 1.70−1.61 (m, 4H, −NHCOCH_2C$H_2$$CH_2CH_2$−), 1.48−1.40 (m, 2H, −NHCOCH_2-CH_2C$H_2$$CH_2$−). ^{13}C NMR ($CDCl_3$): δ 173.2 (9-C), 172.9 (4-C), 172.2, 163.1 (2-C), 141.0 (8a-C), 137.1, 133.7 (7-C), 130.5, 129.4, 129.0, 125.3 (5-C), 122.9 (6-C), 116.2 (8-C), 116.0 (4a-C), 96.7 (3-C), 56.3, 40.2, 40.1, 38.6, 38.3, 36.3, 34.5, 28.7, 25.2. Anal. ($C_{26}H_{29}N_3O_4S_2$) C, H, N, S.

***N*-(1,2-Dihydro-4-hydroxy-1-methyl-2-oxo-3-quinolinecarbonyl)-*N*′-(1,2-dithiolane-3-pentanoyl)-hexamethylenediamine (16).** Compound **16** was obtained by reaction of **7** with **13**, as a yellowish solid, after purification with flash column chromatography (CH_2Cl_2/MeOH, 97:3): yield 60%, mp 93−95 °C. ^{1}H NMR ($CDCl_3$): δ 17.19 (s, 1H, OH), 10.30 (br, 1H, CON*H*−$(CH_2)_6$−), 8.18 (dd, 1H, $J_{5,6}$ = 8.1 Hz, $J_{5,7}$ = 1.5 Hz, 5-H), 7.71 (ddd, 1H, $J_{7,8}$ = 8.4 Hz, $J_{7,6}$ = 7.5 Hz, $J_{7,5}$ = 1.8 Hz, 7-H), 7.35−7.25 (m, 2H, 6-H, 8-H), 5.68 (br, 1H, −$(CH_2)_6$−N*H*CO), 3.65 (s, 3H, N−CH_3), 3.54 (quintet, 1H, *J* = 6.6 Hz), 3.42 (q, 2H, *J* = 6.6 Hz, CONH−C$H_2$$(CH_2)_4$$CH_2$−NHCO−), 3.22 (q, 2H, *J* = 6.9 Hz, CONH−CH_2-$(CH_2)_4$CH_2−NHCO−), 3.17−3.30 (m, 2H), 2.42 (sextet, 1H, *J* = 6.6 Hz), 2.15 (m, 2H, −NHCOC$H_2$$CH_2$$CH_2$$CH_2$−), 1.87 (sextet, 1H, *J* = 6.0 Hz), 1.70−1.30 (m, 14H). ^{13}C NMR ($CDCl_3$): δ 172.8 (9-C), 172.0 (4-C), 171.1, 162.8 (2-C), 139.9 (8a-C), 133.7 (7-C), 125.5 (5-C), 122.4 (6-C), 116.3 (8-C), 114.2 (4a-C), 96.7 (3-C), 56.3, 40.1, 39.2, 38.7, 38.3, 36.4, 34.5, 29.3 (N−CH_3), 29.0, 28.8, 26.4, 26.3, 25.3. Anal. ($C_{25}H_{35}N_3O_4S_2$) C, H, N, S.

***N*-(1,2-Dihydro-4-hydroxy-2-oxo-1-phenyl-3-quinolinecarbonyl)-*N*′-(1,2-dithiolane-3-pentanoyl)-hexamethylenediamine (17).** Compound **17** was obtained by reaction of **8** with **13**, as a yellowish solid, after purification with flash column chromatography (CH_2Cl_2/MeOH, 97:3): yield 72%, mp 110−112 °C. ^{1}H NMR ($CDCl_3$): δ 17.47 (s, 1H, OH), 10.11 (br, 1H, CON*H*−$(CH_2)_6$−), 8.22 (dd, 1H, $J_{5,6}$ = 8.1 Hz, $J_{5,7}$ = 1.5 Hz, 5-H), 7.64−7.54 (m, 3H), 7.44 (dtd, 1H, *J* = 1.5, 8.4, 7.5 Hz), 7.28−7.24 (m, 3H), 6.62 (d, 1H, *J* = 8.4 Hz), 5.63 (br, 1H, −$(CH_2)_6$−N*H*CO), 3.54 (quintet, 1H, *J* = 6.6 Hz), 3.39 (q, 2H, *J* = 6.9 Hz, CONH−C$H_2$$(CH_2)_4$-$CH_2$−NHCO−), 3.19 (q, 2H, *J* = 6.6 Hz, CONH−$CH_2$$(CH_2)_4CH_2$−NHCO−), 3.16−3.04 (m, 2H), 2.43 (sextet, 1H, *J* = 6.6 Hz), 2.12 (m, 2H, −NHCOC$H_2$$CH_2$$CH_2$$CH_2$−), 1.88 (sextet, 1H, *J* = 6.9 Hz), 1.71−1.31 (m, 14H). ^{13}C NMR ($CDCl_3$): δ 173.2 (9-C), 172.9 (4-C), 171.1, 163.3 (2-C), 141.0 (8a-C), 137.3, 133.4 (7-C), 130.5, 129.3, 129.1, 125.3 (5-C), 122.7 (6-C), 116.1 (8-C), 100.0 (4a-C), 96.7 (3-C), 56.4, 40.2, 39.2, 38.8, 38.4, 36.4, 34.5, 32.5, 29.3, 28.8, 26.3, 25.3. Anal. ($C_{30}H_{37}N_3O_4S_2$) C, H, N, S.

***N*-(1,2-Dihydro-4-hydroxy-2-oxo-1-phenyl-3-quinolinecarbonyl)-*N*′-(1,2-dithiolane-3-pentanoyl)-1,2-phenylenediamine (18).** Compound **18** was obtained by reaction of **10** with **13**, as a green solid, after purification with flash column chromatography (CH_2Cl_2/MeOH, 99:1): yield 68%, mp 196−198 °C. ^{1}H NMR ($CDCl_3$): δ 16.81 (s, 1H, OH), 11.88 (s, 1H, CON*H*C_6H_4−), 8.27 (dd, 1H, $J_{5,6}$ = 7.9 Hz, $J_{5,7}$ = 1.2 Hz, 5-H), 7.66−7.57 (m, 3H),

7.52−7.47 (m, 1H), 7.39 (d, 1H, J = 8.1 Hz), 7.33−7.26 (m, 3H), 7.07 (pseudotriplet, 1H), 6.82−6.78 (m, 2H), 6.67 (d, 1H, J = 8.4 Hz), 3.58 (quintet, 1H, J = 7.2 Hz), 3.20−3.09 (m, 2H), 2.47 (sextet, 1H, J = 6.7 Hz), 2.62 (t, 2H, J = 6.9 Hz, −NHCOCH_2-$CH_2CH_2CH_2$−), 1.93 (sextet, 1H, J = 6.7 Hz), 1.84−1.52 (m, 6H). ^{13}C NMR ($CDCl_3$): δ 173.1 (9-C), 169.7 (4-C), 169.3, 163.3 (2-C), 141.0 (8a-C), 140.7, 137.1, 133.7, 130.5, 129.4, 129.1, 127.4, 125.7, 125.4, 123.0, 122.8, 119.1, 117.3, 116.2, 116.0 (4a-C), 97.2 (3-C), 56.0, 40.1, 38.4, 34.3, 30.9, 28.2, 25.2. Anal. ($C_{30}H_{29}N_3O_4S_2$) C, H, N, S.

Biological Experiments. Experiments In Vivo. Toxicity of the Examined Compounds. Toxicity experiments were carried out using both male and female Fischer 344 rats. Pregnant females were excluded. The tested compounds were dissolved in water (salts) or suspended with a few drops of Tween 80, and ground in water, and administered by intraperitoneal injection at various concentrations. Mortality was recorded after 24 h.

Inhibition of the Carrageenin-Induced Edema.[61] 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. 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 recognized guidelines on animal experimentation.

The tested compounds, 0.01 mmol/kg body weight, were suspended in water with a few drops of Tween 80 and ground in a mortar before use and were given intraperitoneally simultaneously. 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 1, 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%.

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).[61] To a solution of DPPH in absolute ethanol an equal volume of the compounds (0.1−0.5 mM) dissolved in ethanol was added. As control solution, ethanol was used. The concentrations of the solutions of the compounds were 0.1 and 0.5 mM. After 20 and 60 min at room temperature, the absorbance was recorded at 517 nm (Table 2).

Effect of the Tested Compounds on the $^{\bullet}$OH Radical-Mediated Oxidation of DMSO.[61] 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 (0.1 mM), and ascorbic acid (10 mM). After 30 min of incubation (37 °C), the reaction was stopped with CCl_3-COOH (17% w/v), and the absorbance was recorded at λ 425 nm. The results are shown in Table 1.

Soybean Lipoxygenase Inhibition Study In Vitro.[61] 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 (nordihydroguaiaretic acid 0.1 mM 83.7%).

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Supporting Information Available: Full experimental details, analytical and spectroscopic data for compounds **5**, **8**, and **10**, additional ^{1}H NMR and IR spectroscopic data for all compounds, and combustion analysis data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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