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Synthesis, in silico docking experiments of new 2-pyrrolidinone derivatives and study of their anti-inflammatory activity

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ABSTRACT

A new class of 2-pyrrolidinone derivatives was designed, synthesized, and tested for their antioxidant and anti-inflammatory activities. The compounds were evaluated for their inhibitory activity against LOX. The most potent among them, **14d** [IC_{50} 0.08 (±0.005) mM], and **14e** [IC_{50} 0.0705 (±0.003) mM], were also tested in vivo. The compound **14d** induced equipotent inhibition against rat paw edema, which is very close to the effect produced by the commonly used standard, namely indomethacin (47%). The LOX inhibitory activity of the compound **14e** proceeds in parallel to the % inhibitory value of lipid peroxidation meaning that this LOX inhibitory activity is supported by the lipid peroxidation inhibition. The molecular features that govern their bioactivity were explored through in silico docking experiments. The results showed that acidic moieties must be placed in certain distance and orientation in the active site of LOX enzyme in order to productively exhibit inhibitory activity. In addition, the 2-pyrrolidinone template significantly contributes in the inhibitory properties of the new compounds.

1. Introduction

Antioxidants are defined as substances that even at low concentration significantly delay or prevent oxidation of easily oxidizable substrates. There is an increased interest of using antioxidants for medical purposes in the recent years. It is known that free radicals play an important role in inflammatory process.[1] Compounds with antioxidant properties could be consequently expected to offer protection in rheumatoid arthritis and inflammation and to lead to potentially effective drugs.

Lipoxygenases oxidize certain fatty acids at specific positions to hydroperoxides, precursors of leukotrienes, which contain a conjugated triene structure, that is, soybean lipoxygenase converts linoleic to 13-hydroperoxylinoleic acid. Leukotrienes play an important role as mediators in a variety of inflammatory and allergic processes lipoxygenases (LOXs) play a role in membrane lipid peroxidation by forming hydroperoxides in the lipid bilayer.[2] Inhibitors of LOXs have attracted attention initially as potential agents for the treatment of inflammatory and allergic diseases but their therapeutic potential has been expanded to certain types of cancer and cardiovascular diseases.[3] Most of the LOXs inhibitors are antioxidants or free radical scavengers, since lipoxygenation occurs via a carbon-centered radical.[4]

A new class of nine 2-pyrrolidinone analogs was synthesized as potential leads against inflammation.[5] Three are the major structural features that characterize the new compounds: (a) they contain a 2-pyrrolidinone template; (b) they are substituted at five position with methylene imidazole ring unsubstituted or substituted by an acidic group; (c) they possess a benzyl group attached to the nitrogen of 2-pyrrolidinone template bearing an acidic group or metabolized to acidic one (Fig. 1).

As a reference molecule is considered the structurally simplest one (5*S*)-1-benzyl-5-(1*H*-imidazol-1-yl-methyl) pyrrolidin-2-one (**6a**)[6,7] which bears all the above three characteristics with no acidic groups. LOX was chosen as a target for the following reasons: (a) its 3D crystal structure alone or with anti-inflammatory agents has been published[8]; (b) pyrrolidinone analogs are well

Abbreviations: AAPH, 2,2′-azobis(2-amidinopropane)dihydrochloride; AcOH, acetic acid; AIBN, 2,2′-azobis(2-methylpropionitrile); DCC, dicyclohexylcarbodiimide; DCM, dichloromethane; DCU, dicyclohexylurea; DMAP, 4-dimethylaminopyridine; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; DPPH, 1,1-diphenyl-2-picryl-hydrazyl; EtOAc, ethyl acetate; 13-HPOD, 9*Z*,11*E*-13(*S*)-hydroperoxy-9, 11-octadecadienoic acid; LOX-3, soybean lipoxygenase-3; NBS, *N*-bromosuccinimide; NDGA, nordihydroguaiaretic acid; NP, normal phase; RP, reversed phase; TBAF, tetra-*n*-butylammonium fluoride; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin-layer chromatography; TMS, tetramethylsilane; $TMSN_3$, trimethylsilylazide; TESCl, triethylsilylchloride.

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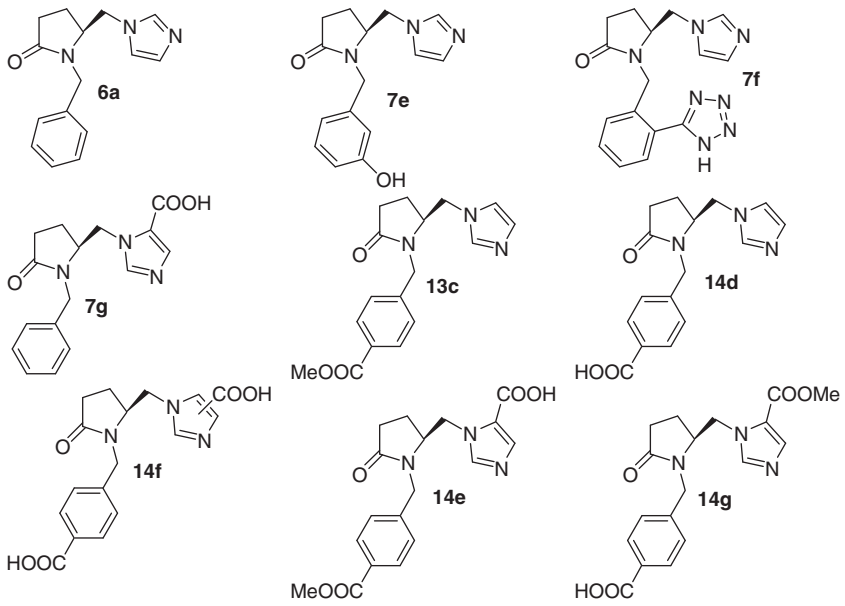


Figure 1. Structures of the synthetic compounds.

known to act as anti-inflammatory agents or to promote anti-inflammatory action[9,10]; (c) all the synthetic analogs in Figure 1 possess in their structure acidic group which is well known to be associated with anti-inflammatory activity; (d) biophysical studies results showed that the new analogs have similar thermal effects in lipid bilayers with those of NSAIDS.[11,12]

The experimental results justified in silico docking experiments and a correlation between the anti-inflammatory activity and binding affinity of the molecules under study was observed. Such a correlation would lead to understanding at a molecular level, the molecular features that govern the anti-inflammatory activity and more comprehensive rational design of new more potent compounds.

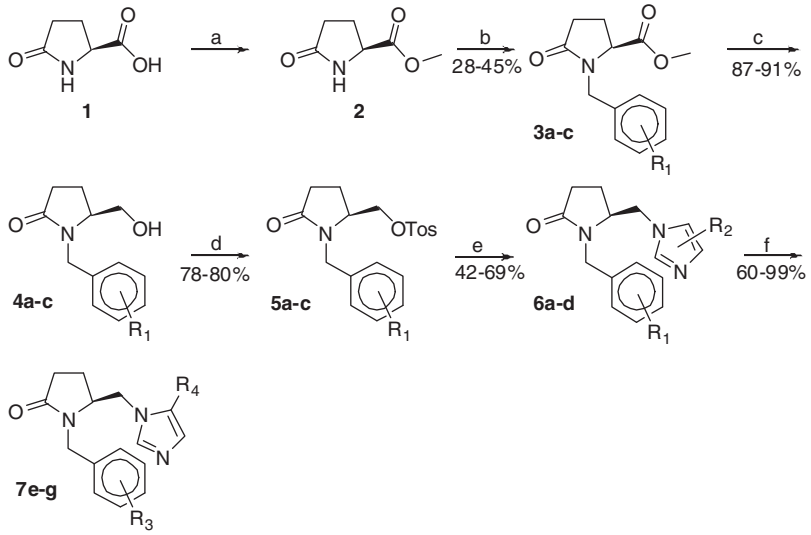
2. Results and discussion

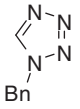
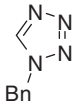
2.1. Chemistry

In this paper we present the synthesis of eight new, optically active compounds, derivatives of 2-pyrrolidinone (Fig. 1), starting from *S*-pyroglutamic acid. The pyrrolidinone ring possesses a benzyl type substituent at the N atom and an imidazole or substituted imidazole ring. All the substituents, namely carboxylic, its bio-isosteric tetrazole and phenolic groups have an acidic character. Our approach for the synthesis of these compounds is depicted in Schemes 1 and 2.

The synthetic route of Scheme 1 was followed for compounds not bearing a carboxylic group as substituent to the phenyl ring. Starting from *S*-pyroglutamic acid (**1**), it was converted to the methyl ester **2** using $SOCl_2$ and MeOH.[13] In the presence of the base NaH in dry THF, the N-substitution of the methyl ester with benzyl bromide (or bromides **16**, **20**, Schemes 3 and 4, respectively) was accomplished to afford compounds **3a–c**. After the reduction of the ester group using $LiBH_4$ in dry THF, the resulting alcohols **4a–c** were converted to the corresponding tosylesters **5a–c**. Nucleophilic attack on the former esters by freshly prepared lithium imidazolide or lithium salt of 1*H*-imidazole-4(5)-carboxylic acid benzylester (**22**, Scheme 5), in dry DMF at 50 °C under argon, afforded compounds **6a–d**. After catalytic hydrogenation in the presence of 10% Pd/C or $Pd(OH)_2$ as catalyst for the deprotection of tetrazole moiety of compound **6c**, the final products **7e–g** were obtained.

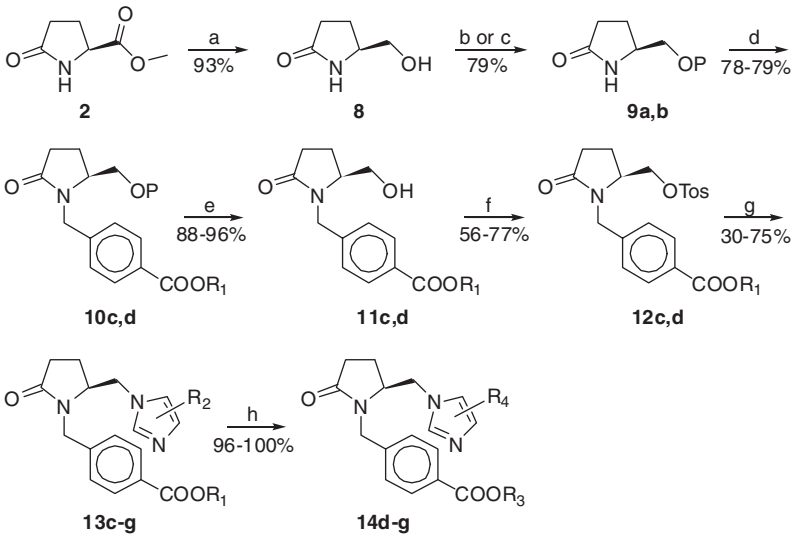
For compounds possessing a carboxylic group on the phenyl group, it was necessary first to reduce the carboxyl group of *S*-pyroglutamic acid to the corresponding *S*-pyroglutaminol, following the synthetic route of Scheme 2. The *S*-pyroglutaminol **8**, derived from the methyl *S*-pyroglutamate (**2**) by $NaBH_4$ in absolute EtOH,[14] was protected either as acetate by acetic anhydride in pyridine[15] or as TES ether using TESCl, Et_3N, and DMAP[16] to yield compounds **9a** and **9b**, respectively. N-Benzylation of compound **9a** with the bromide **25** (Scheme 6), using NaH in dry THF, afforded compound **10c**. When compound **9a** reacted with the bromide **26** (Scheme 6) under the same conditions, an almost equimolar mixture of the expected product **27** together with an undesired compound **28** (Scheme 7) was obtained. After separation using column chromatography, the product **28** was characterized by ^1H NMR, ^{13}C NMR, and MS. Probably, under the alkaline conditions of the reaction, the acetate group had been removed and the resulting alcohol had transesterified the benzyl ester of the used benzyl bromide **26**. Then, the TES group was used as protecting group of the alcohol **8** and the compound **10d** was finally obtained. After removal of the acetate group by K_2CO_3 in MeOH[17] and the TES group by TFA,[18] the resulting alcohols **11c** and **11d** were converted to their tosylesters **12c** and **12d**, respectively. Unexpectedly, when lithium salts of imidazole or 4(5)-substituted imidazole **22**, **23** (Scheme 5) reacted with the tosylesters **12c** and **12d**, the products 13c–**g** were provided in very low yield (8–10%). The desired products were obtained in much higher yields (30–75%) using the imidazole or the imidazole moiety **22**, **23** together with Cs_2CO_3 in dry DMF at 50 °C. It is known that the nucleophilic strength of nitrogen is enhanced via complexes with Cs^+, the so called 'cesium effect'.[19] After catalytic hydrogenation using 10% Pd/C as catalyst, the final products **14d–g** were obtained. As shown in Scheme 2, the compound **13f** was obtained as a mixture of two constitutional isomers, depending on which position (4 or 5) on the imidazole ring the ester group was located. The separation of the two isomers could not be achieved in a quantitatively acceptable yield neither in compound **13f** nor in its deprotected derivative, the final product **14f**. The last compound was the only one which was biologically tested as a mixture of the two isomers.



	R_1	R_2	R_3	R_4
3a, 4a, 5a	H			
3b, 4b, 5b	(m) OBn			
3c, 4c, 5c	(o) 			
6a	H	H		
6b	(m) OBn	H		
6c	(o) 	H		
6d	H	COOBn		
7e			(m) OH	H
7f			(o) 	H
7g			H	COOH

Scheme 1. Reagents and conditions: (a) $SOCl_2$ in MeOH; (b) BnBr or **16** or **20**, NaH in THF; (c) $LiBH_4$ in THF; (d) TosCl, Et_3N in DCM; (e) lithium imidazolide or lithium salt of **22** in DMF, 50 °C; (f) H_2, Pd/C [$Pd(OH)_2$ in case of **6C**] in MeOH.

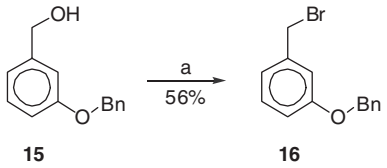
In case of the compounds **13e** and **13g** the separation of the other two isomers (**13e*** and **13g***, respectively) could be achieved using column chromatography whereas in case **6d** could not. After the deprotection of compound **6d** by catalytic hydrogenation, a mixture of the product **7g** and **7g*** was obtained (Scheme 8), which was subjected in column chromatography to afford an isomer with higher R_f in 87% yield. The other isomer had a lower R_f in the same solvent system and it was isolated only in traces. 2D NOESY NMR spectroscopy was used to distinguish whether the carboxyl group is attached on 4 or 5 carbon of the imidazole ring. The presence of the isomer **7g*** (carboxyl group attached at position 4) would be assumed if an NOE interaction between protons H5–H6 could be observed as it can be seen in the Figure 5 (right conformer), while the presence of the isomer **7g** (left conformer) would lack for such interaction, because H4 is oriented away from the methylene H6 protons. Indeed, such an interaction is lacking from the 2D NOESY spectrum proofing that the isolated isomer corresponds to **7g**. Differences in the ^1H NMR chemical shifts of imidazole ring



	P	R_1	R_2	R_3	R_4
9a	Ac				
9b	$SiEt_3$				
10c	Ac	Me			
10d	$SiEt_3$	Bn			
11c, 12c		Me			
11d, 12d		Bn			
13c		Me	H		
13d		Bn	H		
13e		Me	(5) COOBn		
13f		Bn	4(5) COOBn		
13g		Bn	(5) COOMe		
14d				H	H
14e				Me	(5) COOH
14f				H	4(5) COOH
14g				H	(5) COOMe

Scheme 2. Reagents and conditions: (a) $NaBH_4$ in EtOH; (Ac_2O) in Pyr; (c) TESCl, Et_3N, DMAP in DMF; (d) **25**, **26**; NaH in THF; (e) K_2CO_3 in MeOH for acetyl group or TFA for triethylsilyl group; (f) TosCl, Et_3N in DCM; (g) 1-*H*-imidazole or **22** or **23**, Cs_2CO_3 in DMF, 50 °C; (h) H_2, Pd/C in MeOH.

and diastereotopic H-12 protons were observed, providing further clarification for the identification of the two isomers in the cases of the compounds **13e**, **13e*** and **13g**, **13g***. More specifically, the H-4 of isomer with substitution at position 5 of the imidazole ring, resonates at lower field (ca. 7.8 ppm) in comparison to H-5 (<7.5 ppm) of isomer with substitution at position 4 of the imidazole ring. In addition, the diastereotopic proton of H-12 that points towards the carboxylate group in the isomer with substitution at position 5 resonates at lower field (ca. 4.6 ppm) in comparison to the corresponding diastereotopic proton of H-12 that is not deshielded by the carboxylate group in the isomer with substitution at position 4 and resonates at ca. 4.0 ppm. According to these observations, isomers **13e** and **13g** had the carboxylate group at the position 5 of the imidazole ring, whereas the compounds **13e*** and **13g*** were substituted at position 4. In order to have a family of structurally similar to compound **7g** isomers, for biological testing, only compounds **13e** and **13g** were subjected to catalytic hydrogenation affording the corresponding target products **14e** and **14g** both containing a carboxylic group at position 5 of the imidazole ring.



Scheme 3. Reagents: PBr_3 in Et_2O.

2.2. Biology

2.2.1. Antioxidant and anti-inflammatory activity

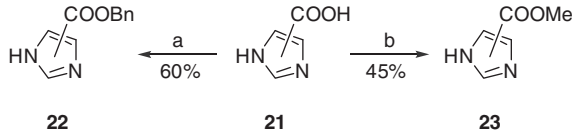
We evaluated in vitro and in vivo the new 2-pyrrolidinone derivatives that were expected to offer protection against inflammation and radical attack. Thus, we tested the new compounds with regard to their antioxidant ability and in comparison to well known antioxidant agents, for example, NDGA, caffeic acid (CA), and trolox.

For estimating the antioxidative potential of chemical components, different experimental approaches were used.[20] Most of them require a spectrophotometric measurement and a certain reaction time in order to obtain reproducible results[21] The use of DPPH for a radical scavenging measuring method was used. DPPH is a stable free radical in an ethanolic solution. In its oxidized form, the DPPH radical has an absorbance maximum centered at about 517 nm[22] The DPPH method is described as a simple, rapid and convenient method independent of sample polarity for screening of many samples for radical scavenging activity.[23] These advantages make the DPPH method interesting for testing our analogs.

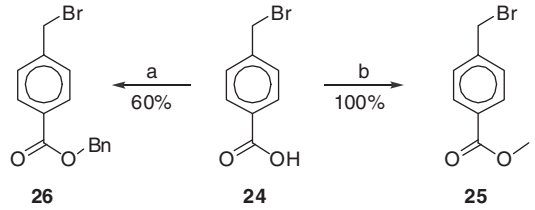
The difference in the reduction values of DPPH between the blank and the sample was used for determining the percent radical scavenging activity of the sample. The radical scavenging activity of the examined compounds against the stable free radical DPPH is shown in Table 1, in an iron-free system. All the compounds were tested at 0.05 mM without any effect (data not shown). With the exception of **7e** and **7f** the compounds did not interact with 0.1 mM DPPH. In addition, no changes were observed after 60 min. A study concerning the reaction kinetics with this free radical was not performed. The change of absorbance at 20 and 60 min was measured following our previously described experimental protocols[24] with which it was not possible to indicate the presence of slow reacting or fast reacting antioxidant compounds within our samples nor the time of maximum/minimum activity.

In the DPPH assay, the dominant chemical reaction involved is the reduction of the DPPH radical by a single electron transfer (ET) from the antioxidant. Particularly effective such antioxidants are the phenoxide anions from phenolic compounds. It is evident that the presence of the phenolic group and of the tetrazole group is correlated with higher values. The replacement of the phenolic group by H, or other groups leads to lower values or disappearance of the interaction. The results show that lipophilicity calculated theoretically *c log P* values and radical scavenging activity are not related.

The compounds were further evaluated for the inhibition of LOX by the UV absorbance based enzyme assay.[25] The compound **14e** is the most active presenting an IC_{50} value 70 μM. Perusal of % inhibition values at 100 μM, for the rest of the compounds shows that



Scheme 5. Reagents: (a) BnBr, Cs_2CO_3 in DMF; (b) CH_2N_2 in Et_2O.



Scheme 6. Reagents: (a) BnOH, DCC, DMAP; (b) CH_2N_2 in Et_2O.

compound **7g** is the most active (81%) and **7e** is inactive under the reported experimental conditions. Lipophilicity is referred[26,27] as an important physicochemical property for lipoxygenase inhibition. However in this case, lipophilicity does not influence positively the biological response.

In our studies the water soluble compound AAPH was used as a clean and controllable source of thermally produced alkylperoxyl free radicals. In our studies AAPH was used as a free radical initiator to follow oxidative changes of linoleic acid to conjugated diene hydroperoxide. Comparison between **14d** and **7f** ability to inhibit lipid hyperoxidation appears that tetrazole group is more effective than carboxylic group. No correlation between lipid peroxidation inhibitory activity and DPPH radical scavenging activity is observed. For example **14f** (39%) and **14e** (96%) have the highest ability to inhibit lipid peroxidation. However, they have low DPPH radical scavenging and opposite trend (20% and 3%, respectively). This is not surprising, since similar results have been observed for other classes of molecules.[28,29]

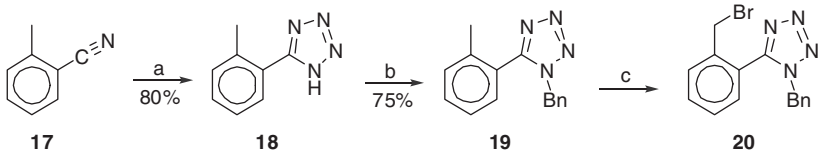
2.2.2. Anti-inflammatory activity in vivo

Compounds 14d and **14e** were selected to be examined in vivo by using the functional model of carrageenin-induced rat paw edema, on the basis that these two compounds highly inhibited LOX. Carrageenin-induced edema is a non-specific inflammation resulting from a complex of diverse mediators.[30] As shown in Table 1, the compound **14d** induced equipotent inhibition against rat paw edema, which is very close to the effect produced by the commonly used standard, namely indomethacin (47%).

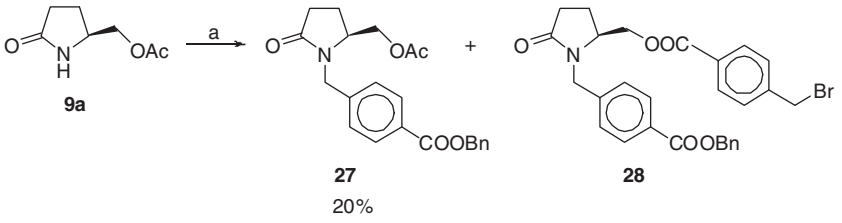
For compound **14e** the LOX IC_{50} value proceeds in parallel to the % inhibitory value of lipid peroxidation meaning that this LOX inhibitory activity is supported by the lipid peroxidation inhibition.

2.2.3. Docking studies of receptor–ligand interactions

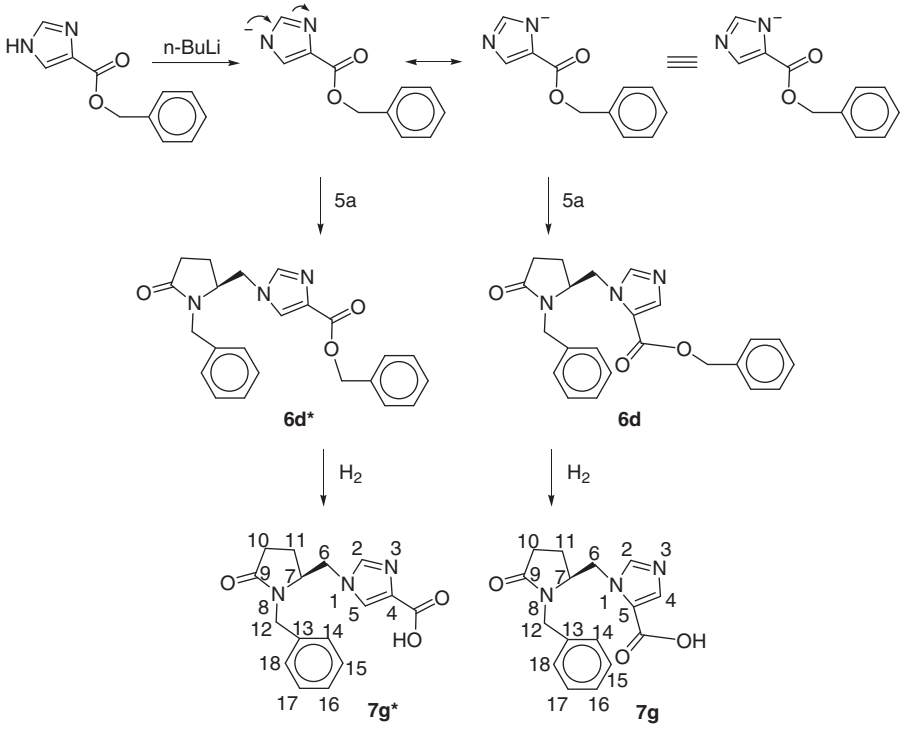
In order to explain the differences in bioactivity of the synthetic compounds towards soybean lipoxygenase, three representative ones, namely **6a**, **13c**, and **14d**, have been subjected to docking calculations using the Surflex-Dock program.



Scheme 4. Reagents and conditions: (a) TBAF·$3H_2O$, $TMSN_3$, 85 °C; (b) K_2CO_3, BnBr, 50 °C; (c) NBS, AIBN, 80 °C.



Scheme 7. Reagents: **26**, NaH in dry THF.



Scheme 8.

Table 1
Radical scavenging activity with DPPH (RSA%); in vitro inhibition of soybean lipoxygenase IC_{50}, or % inhibition at 0.1 mM; % inhibition of lipid peroxidation (AAPH%); % inhibition of carrageenin-induced rat paw edema (ICPE%)

Compds	*C* log *P*[a]	DPPH% (±SD)[b] 0.1 mM 20/60 min	IC_{50} mM or % inhibition (±SD)[b] at 0.1 mM	AAPH% (±SD)[b] 0.1 mM	ICPE% 0.01 mmol/kg
6a	1.80	9 (±0.5)	13% (±0.1)	11 (±0.4)	
7e	1.14	29 (±1.5)	na	13 (±0.08)	
7f	1.31	30 (±0.8)	5.2% (±0.5)	32 (±0.9)	
7g	1.43	na	81% (±4.5)	na	
13c	1.77	6 (±0.9)	na	13 (±0.7)	
14d	1.55	6 (±0.4)	0.08 (±0.005)	2 (±0.05)	41*
14f	1.18	20 (±1.2)	9% (±0.05)	39 (±2.1)	
14e	1.40	3 (±0.1)	0.0705 (±0.003)	96 (±3.7)	34**
14g	0.75	26 (±1.5)/13 (±0.2)	na	na	
NDGA		81 (±2.05)	84% (±3.3)		
Trolox				63 (±1.6)	
CA	0.82	5.5 (±0.1)	0.600 (±0.01)	17 (±0.4)	
Indomethacin					47**

The change in paw weight was compared with that in control animals and expressed as a percent inhibition of the edema% ICPE values. Each value represents the mean obtained from 6 to 15 animals in two independent experiments. In all cases, significant difference from control: $^{*}p < 0.1$ $^{**}p < 0.01$ (Student's *t* test).

[a] Theoretically calculated values of lipophilicity according to Ref. 47.

[b] (±SD) standard deviation; na, no activity under the reported experimental conditions.

Docking studies of **14d** suggest that the compound is stabilized through van der Waals interactions with the amino acids His523, Ile557, Ala561, Trp519, Leu565, Ile572, His518, Glu514, Val769, Ile770, Leu773, and Ile857 (Fig. 2). The compound **14d** orients in such a way that its imidazole ring interacts electrostatically with Fe (III), (distance is indicated with green line and is calculated to be 2.35 Å). The orientation of the compound and the electrostatic interaction between the imidazole ring and Fe (III)) determine the entrance of substrate linolenic acid in the active site. The binding affinity score ($-\log K_d$) of the complex as calculated by Surflex-Dock is 4.15.

Docking studies of **6a** suggest that the compound is only stabilized through van der Waals interactions with the amino acids His518, His523, Leu773, Ile770, and Val769 (Fig. 3). The compound **6a** orients in the active site in such a position that the imidazole ring does not interact electrostatically with Fe (III) and the compound does not form hydrogen bonds insight the active site. The binding affinity score ($-\log K_d$) of the complex as calculated by Surflex-Dock is 2.45.

Docking studies of **13c** show stabilization of the compound with amino acids Phe576, Asp766, Glu716, Val372, His513, His523, His518, Ala561, and Glu514 through van der Waals interactions (Fig. 4). The imidazole ring of the compound is far by 3.97 Å (indicated as green line) from Fe (III) ion, showing not favoring electrostatic interaction. Furthermore, the compound does not form hydrogen bonds insight the active site. The binding affinity score ($-\log K_d$) of the complex as calculated by Surflex-Dock is 0.75.

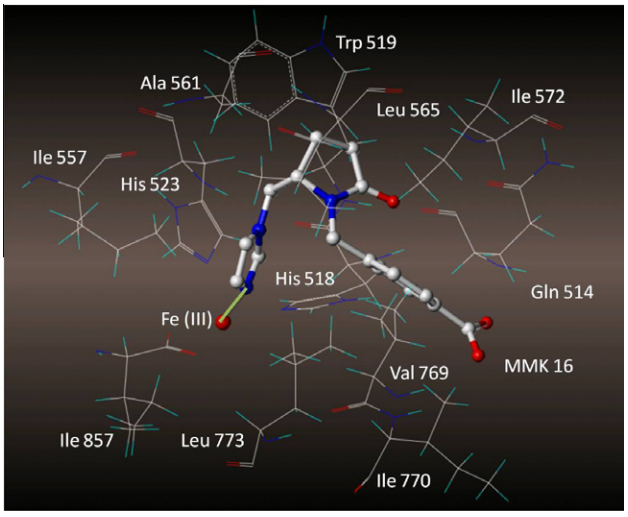


Figure 2. Docked orientation of **14d** using Surflex docking algorithm.

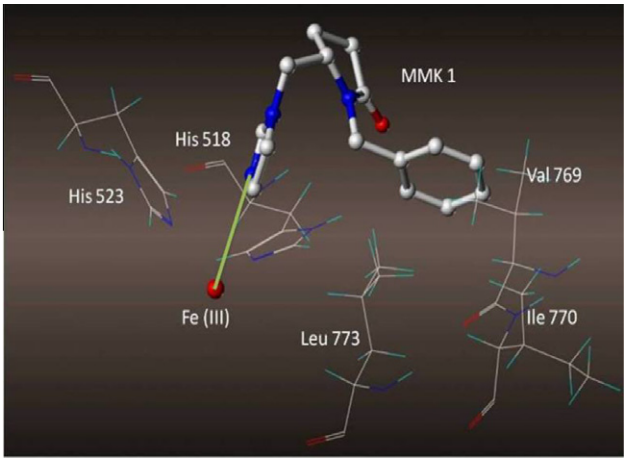


Figure 3. Docked orientation of **6a** using Surflex docking algorithm.

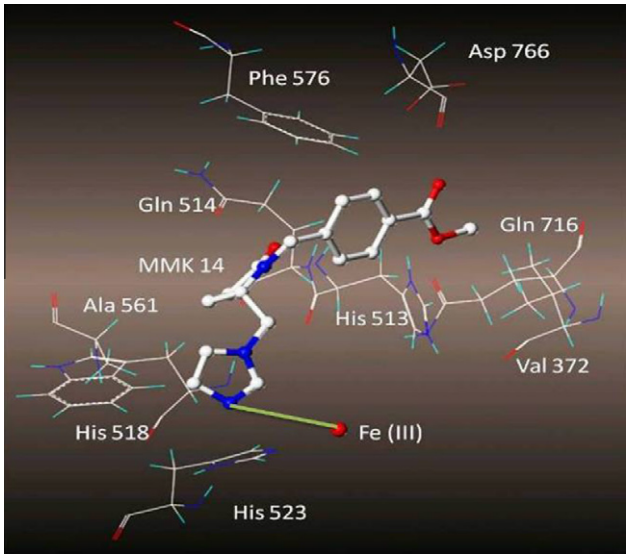


Figure 4. Docked orientation of **13c** using Surflex docking algorithm.

3. Conclusions

In this study the synthesis of a new class of compounds that possess 2-pyrrolidinone template and attached aromatic rings with acidic moieties is described. The anti-inflammatory and antioxidant activity of this class of molecules has been evaluated. It has been shown, as for other classes of molecules, that high DPPH radical scavenging activity or % inhibition of lipid peroxidation induced by AAPH are not necessarily accompanied by high LOX inhibitory activity. The interaction of the synthetic molecules with DPPH indicates their radical scavenging ability in an iron-free system. The results showed that compound **7e** possessing an acidic phenolic group and **7f** possessing a tetrazole group have the highest interactions. The compound **14f** bearing carboxylic groups and compound **14g** that bears carboxylic and ester groups present less inhibitory effect in comparison to **7e** and **7f** ones. The compounds **7g** and **13c** although appear with no or very low inhibition activity, possess acidic and ester groups. This indicates that these functional groups must appear to have the right position to exert the highest inhibitory effect.

Lipid peroxidation inhibition is increased in efficiency by the introduction of an ester group (compare higher activity of **14e** vs **14f**). The compound **7e** possesses only the acidic phenolic group and devoid of any inhibition. Compounds **14d** and **14e** show the highest in vitro inhibition to LOX. While the compound **14e** shows a slightly higher in vitro activity, it has lower in vivo one. This may signify the fact that two carboxylic groups or a combination of carboxylic with an ester group are not favored. The inferior in vivo results of **14e** led us to compare the binding properties of the most in vivo active compounds **14d** with the inactive compound **6a** and the compound **13c** possessing moderate in vitro activity.

Among all molecules synthesized **14d** showed the highest LOX inhibitory activity and the highest in vivo anti-inflammatory effect using the functional model of carrageenin-induced rat paw edema. Thus, this compound can serve as a lead for the design of more promising anti-inflammatory agents with higher LOX inhibitory activity. The promising effects of **14d** have been rationalized through in silico docking effects. Work under progress aims to optimize the results through simulated annealing Molecular

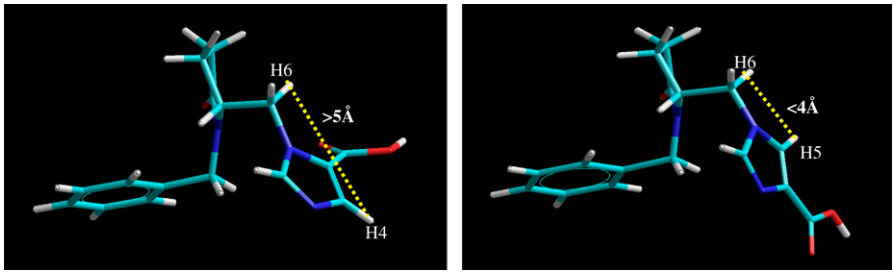


Figure 5. Low energy conformers of **7g** (left) and **7g*** (right).

Dynamics results. Such studies may lead to a possible application of a rational drug design to further optimize the anti-inflammatory activity.

4. Experimental

4.1. Instruments and materials

All chemicals and solvents were reagent grade and used without purification. Dry THF, was obtained by distillation of commercially available predried solvent from Na and stored over molecular sieves. Extra dry DMF (99.8%) over molecular sieves was purchased from Acros. DPPH, AAPH, NDGA, sodium linoleate, soybean lipoxygenase, caffeic acid, trolox were purchased from Aldrich–Sigma (St. Louis, MO, USA).

Melting points were determined on a Buchi 530 apparatus and are uncorrected. Specific rotations were measured at 25 °C on a Perkin–Elmer polarimeter using a 10 cm cell. Nuclear magnetic resonance spectra were obtained on a Varian Mercury spectrometer (^{1}H NMR recorded at 200 MHz and ^{13}C NMR recorded at 50 MHz and are referenced in ppm relative to TMS as an internal standard). The NOESY spectrum of compound **7g**, was obtained on a Varian 600 MHz spectrometer. TLC plates (Silica Gel 60 F_{254}) were purchased from Merck. Visualization of spots was effected with UV light and/or phosphomolybdic acid, in EtOH stain. Normal phase-HPLC analyses were carried out using a Thermo Scientific Hypersil Silica Column 250 × 4 mm, at a flow rate 1 mL/min, in solvent system A: Hex–*i*PrOH 25:75 B: Hex–*i*PrOH 45:55. Reversed phase-HPLC analyses were carried out using a Thermo Scientific C18 ODS Hypersil Column 250 × 4 mm, at a flow rate 1 mL/min, in solvent system C: 3% CH_3CN, 97% H_2O and solvent system D: 10% CH_3CN, 90% H_2O. The following gradient system was used: E t = 10 min (100% C, 0% D), t = 30 min (0% C, 100% D). Retention times are reported in minutes. All tested target compounds possessed ⩾95% purity as determined by combustion analysis. Compounds **6a** and lithium imidazolides were prepared by known methods.[7]

4.2. Compounds 3a–c, 10c, d: General procedure

Methyl (*S*)-pyrroglutamate (**2**) or O-protected-*S*-pyrroglutaminol (**9a**, **b**) (1 mmoL) was dissolved in dry THF (3 mL), followed by the addition of the appropriate benzyl bromide (1.1 mmoL). The reaction mixture was cooled at 0 °C and NaH (60% in paraffin oil, 60 mg) was added in portions. The stirring was continued for 30 min at 0 °C and 2–4 h at rt. The reaction was quenched by the addition of saturated solution of NH_4Cl, the organic solvent was evaporated and the residue was dissolved in ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The product was purified by column chromatography (Silica Gel 60) using the appropriate solvent systems as it will be defined in each case below.

4.2.1. (2*S*)-Pyrrolidinecarboxylic acid, 5-oxo-1-(phenylmethyl)-, methyl ester (3a)

See Ref. 7.

4.2.2. (2*S*)-Pyrrolidinecarboxylic acid, 5-oxo-1-[[3-(phenylmethoxy)phenyl]methyl]-, methyl ester (3b)

Prepared from ester **2** and the bromide **16**. Eluent EtOAc–petroleum ether (bp 40–60 °C), 9:1. Yield 65%; colorless oil; $[\alpha]_D$ +15.9 (c 1.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 7.40–7.18 (m, 6H, Ph), 6.81(m, 3H, Ph), 5.03(s, 2H, OCH_2Ph), 5.01(d, J = 14.8 Hz, 1H, C*H*HPh), 3.93(m, 1H, CH), 3.92(d, J = 14.8 Hz, 1H, CH*H*Ph) 3.66(s, 3H, CH_3), 2.59–2.02(m, 4H, 2 × CH_2).

^{13}C NMR δ: 174.8(CH_2CON), 172.0(*C*OOCH$_3$), 158.9, 137.2, 136.7, 129.6, 128.4, 127.8, 127.3, 120.8, 114.5, 114.1, 69.7(O*C*H$_2$Ph), 58.4(N*C*H), 52.2(*C*H$_3$), 45.3(*C*H$_2$Ph), 29.3(*C*H$_2$CO), 22.6 (*C*H$_2$CH$_2$CO). MS (ESI) m/z (%): 340[(M+H$^+$), 100].

Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.49; H, 6.47; N, 4.38.

4.2.3. 2-Pyrrolidinecarboxylic acid, 5-oxo-1-[[2-[1-(phenylmethyl)-1*H*-1,2,3,4-tetrazol-5-yl]phenyl]methyl]-, methyl ester (3c)

Prepared from the ester **2** and the bromide **20**.

Eluent EtOAc–petroleum ether (bp 40–60 °C), 9:1. Yield 48%; colorless oil; $[\alpha]_D$ +8.0 (c 1.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 7.91–7.78(m, 1H, Ph), 7.39–7.26(m, 8H, Ph), 5.74(s, 2H, NCH_2Ph), 5.18(d, J = 15.2 Hz, 1H, C*H*HPh), 4.52(d, J = 15.2 Hz, 1H, CH*H*Ph), 3.92(m, 1H, CH), 3.49(s, 3H, CH_3), 2.47–2.04(m, 4H, 2 × CH_2).

^{13}C NMR δ: 174.9(N*C*O), 172.1(*C*OO), 164.5(N*C*N), 135.3, 133.4, 131.1, 130.7, 130.5, 130.3, 130.2, 129.3, 128.7, 126.8, 58.8(NNCH_2Ph), 56.6(*C*H), 52.0(*C*H$_3$), 43.1(*C*H$_2$Ph), 29.1(*C*H$_2$CO), 22.8(*C*H$_2$CH$_2$CO). MS (ESI) m/z (%): 392[(M+H$^+$), 100].

Anal. Calcd for $C_{21}H_{21}N_5O_3$: C, 64.44; H, 5.41; N, 17.89. Found: C, 64.71; H, 5.64; N, 17.62.

4.2.4. Benzoic acid, 4-[[2-[(acetyloxy)methyl]-5-oxo-1-pyrrolidinyl]methyl]-, methyl ester (10c)

Prepared from the compound **9a** and the bromide **25**. Eluent EtOAc. Yield 28%; yellow oil; $[\alpha]_D$ +46.3 (c 0.95, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 7.90(d, J = 8.3 Hz, 2H, Ph), 7.22(d, J = 8.2 Hz, 2H, Ph), 4.85(d, J = 15.5 Hz, 1H, C*H*HPh), 4.13(d, J = 15.3 Hz, 1H, CH*H*Ph), 4.11(d, J = 3.8 Hz, 1H, CH*H*OCO), 3.90(d, J = 4.2 Hz, 1H, CH*H*OCO), 3.82(s, 3H, COOC*H*$_3$), 3.60(m, 1H, C*H*CH$_2$OCO), 1.92(s, 3H, OCOC*H*$_3$), 2.49–1.79(m, 4H, 2 × CH_2).

^{13}C NMR δ: 175.2(N*C*O), 170.2(CH$_3$*C*O), 166.4(CH$_3$O*C*O), 141.7, 129.8, 129.3, 127.5 64.0(*C*H$_2$OCOCH$_3$), 55.8(*C*HCH$_2$OCO), 51.9(*C*H$_3$OCO), 44.3(*C*H$_2$Ph), 29.7(*C*H$_2$CO), 21.4(*C*H$_2$CH$_2$CO), 20.5(*C*H$_3$COO). MS (ESI) m/z (%): 328 [(M+Na$^+$), 100].

Anal. Calcd for $C_{16}H_{19}NO_5$: C, 62.94; H, 6.27; N, 4.59. Found: C, 63.15; H, 6.18; N, 4.48.

4.2.5. Benzoic acid, 4-[[2-oxo-5-[[(triethylsilyl)oxy]methyl]-1-pyrrolidinyl]methyl]-, phenylmethyl ester (10d)

Prepared from the compound **9b** and the bromide **26**.

Eluent EtOAc–petroleum ether (bp 40–60 °C), 7:3. Yield 45%; colorless oil; $[\alpha]_D$ +39.93 (*c* 1.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 8.02(d, J = 8.2 Hz, 2H, Ph), 7.46–7.29(m, 7H, Ph), 5.36(s, 2H, OCH_2-Ph), 5.00(d, J = 15.3 Hz, 1H, C*H*HPh), 4.18(d, J = 15.3 Hz, 1H, CH*H*Ph), 3.68–3.47(m, 3H, C*H*, CHCH_2O), 2.63–1.77(m, 4H, 2 × CH_2), 0.92(t, J_1 = 7.8 Hz, J_2 = 15.4 Hz, 9H, 3 × CH_3), 0.55(q, J = 7.9, 6H, 3 × (CH_2CH$_3$).

^{13}C NMR δ: 175.7(NCO), 166.1(CH_3CO), 166.2(*C*OOPh), 142.5, 136.0, 130.0, 129.2, 128.6, 128.2, 128.1, 127.9, 66.6(CHCH_2O), 63.7(COOCH_2Ph), 58.6(*C*H), 44.5(NCH_2Ph), 30.2(CH_2CO), 21.5(CH_2CH_2CO), 6.7(SiCH_2CH$_3$), 4.2(SiCH_2CH_3).

MS (ESI) *m/z* (%): 454 [(M+H$^+$), 100].

Anal. Calcd for $C_{26}H_{35}NO_4Si$: C, 68.84; H, 7.78; N, 3.09. Found: C, 68.63; H, 7.59; N, 3.22.

4.3. Compounds 4a–c: General procedure

In a two necked flask, $LiBH_4$ (1 mmoL) was suspended in dry THF (0.6 mL) under argon at rt. A solution of the N-substituted-(2*S*)-methyl pyrroglutamate (**3a–c)** (1 mmoL) in dry THF (0.5 mL) was added dropwise. The reaction was completed within 15–30 min and it was quenched by the addition of a 20% solution of AcOH at 0 °C, until the production of the gas was ceased. The excess of acetic acid was neutralized by the addition of a small quantity of Na_2CO_3. The organic solvent was evaporated under reduced pressure and the residue was dissolved in EtOAc. The organic phase was then washed with brine and dried over Na_2SO_4. After evaporation of the solvent the product was purified by column chromatography (Silica Gel 60) using $CHCl_3$–MeOH, 9:1 as eluent.

4.3.1. (5*S*)-(Hydroxymethyl)-1-(phenylmethyl)-2-pyrrolidinone (4a)

See Ref. 31.

4.3.2. (5*S*)-(Hydroxymethyl)-1-[[3-(phenylmethoxy)phenyl]-methyl]-2-pyrrolidinone (4b)

Prepared from the compound **3b**. Yield 87%; colorless oil; $[\alpha]_D$ +27.0 (*c* 1.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 7.38–7.17(m, 6H, Ph), 6.88–6.80(m, 3H, Ph), 5.02(s, 2H, OCH_2Ph), 4.84(d, J = 15.1 Hz, 1H, C*H*HPh), 4.11(d, J = 15.1 Hz, 1H, CH*H*Ph), 3.72(m, 1H, CH), 3.43(m, 2H, CH_2OH), 2.78(s, 1H, OH), 2.60–1.93(m, 4H, 2 × CH_2).

^{13}C NMR δ: 176.1(CH_2*C*ON), 159.0, 138.3, 136.7, 129.8, 128.5, 127.9, 127.4, 120.3, 114.2, 113.9, 69.8(OCH_2Ph), 62.1(CH_2OH), 58.8(*C*CH_2OH), 44.4(CH_2Ph), 30.4(CH_2CO), 20.8(CH_2CH_2CO). MS (ESI) *m/z* (%): 312 [(M+H$^+$), 100].

Anal. Calcd for $C_{19}H_{25}NO_3$: C, 73.29; H, 6.80; N, 4.50. Found: C, 73.11; H, 6.59; N, 4.62.

4.3.3. (5*S*)-(Hydroxymethyl)-1-[[2-[1-(phenylmethyl)-1*H*-1,2,3,4-tetrazol-5-yl]phenyl]methyl]-2-pyrrolidinone (4c)

Prepared from the compound **3c**. Yield 91%; colorless oil; $[\alpha]_D$ +18.5 (*c* 1.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 7.90(m, 1H, Ph), 7.42–7.31(m, 8H, Ph), 5.73(s, 2H, NCH_2Ph), 5.11(d, J = 15.8 Hz, 1H, C*H*HPh), 4.71(d, J = 15.8 Hz, 1H, CH*H*Ph), 3.54(dd, J_1 = 3.4 Hz, J_2 = 11.4 Hz, 1H, CH*H*OH), 3.46(m, 1H, CH), 3.32(dd, J_1 = 3.0 Hz, J_2 = 11.4 Hz, 1H, C*H*HOH), 2.88(br s, 1H, OH), 2.86–1.92(m, 4H, 2 × CH_2).

^{13}C NMR δ: 176.3(*C*ON), 164.7(NCN), 135.9, 133.1, 130.5, 130.0, 129.0, 128.7, 128.5, 127.6, 126.0, 62.5(CH_2OH), 59.0(*C*H), 56.8(NNCH_2Ph), 42.2(CH_2Ph), 30.3(CH_2CO), 21.3(CH_2CH_2CO). MS (ESI) *m/z* (%): 364 [(M+H$^+$), 100].

Anal. Calcd for $C_{20}H_{21}N_5O_2$: C, 66.10; H, 5.82; N, 19.27. Found: C, 66.32; H, 5.66; N, 19.13.

4.4. (5*S*)-(Hydroxymethyl)-2-pyrrolidinone (8)

See Ref. 14.

4.5. Acetic acid, (5-oxo-2-pyrrolidinyl)methyl ester (9a)

See Ref. 15.

4.6. 5-[[(Triethylsilyl)oxy]methyl]-2-pyrrolidinone (9b)

In a solution of the alcohol 9a (0.57 g, 5 mmol) in DMF (5 mL), Et_3N (0.84 mL, 6 mmol, 1.2 equiv) and DMAP (0.12 g, 1 mmol) were added, followed by the addition of TESCl (0.9 g, 6 mmol, 1.2 equiv) in small portions. After being stirred for 2.5 h at room temperature the reaction mixture was quenched with MeOH (1 mL) followed by the addition of some drops of saturated solution of NH_4Cl. The solvent was evaporated in high vacuo and the residue dissolved in Et_2O was washed with a saturated solution of NH_4Cl. The aqueous phase was extracted once again with Et_2O and the two combined organic phases were washed with brine and dried over Na_2SO_4. After evaporation of the solvent under reduced pressure, purification was achieved by column chromatography (Silica Gel 60), eluting with $CHCl_3$–MeOH, 9.5:0.5. The product was obtained as a colorless oil in 78% yield (0.9 g). $[\alpha]_D$ +31.04 (*c* 1.0, EtOH).

^{1}H NMR ($CDCl_3$) δ: 6.12(s, 1H, N*H*), 3.73(m, 1H, C*H*), 3.59(dd, J_1 = 4.1 Hz, J_2 = 9.9 Hz, 1H, CHC*H*HO), 3.42(dd, J_1 = 7.6 Hz, J_2 = 9.9 Hz, 1H, CHCH*H*O), 2.36–1.63(m, 4H, 2 × CH_2), 0.93(t, J_1 = 8.3 Hz, J_2 = 15.8 Hz, 9H, 3 × CH_3), 0.57(q, J = 7.9, 6H, 3 × (CH_2CH$_3$).

^{13}C NMR δ: 178.3(*C*ON), 65.9(CH_2OSi), 55.6(*C*H), 29.6(CH_2CO), 22.6(CH_2CH_2CO), 6.36(SiCH_2CH$_3$), 3.95(SiCH_2CH_3). MS (ESI) *m/z* (%): 230 [(M+H$^+$), 100].

Anal. Calcd for $C_{11}H_{23}NO_2Si$: C, 57.59; H, 10.11; N, 6.11. Found: C, 57.71; H, 10.34; N, 6.05.

4.7. Benzoic acid, 4-[[2-(hydroxymethyl)-5-oxo-1-pyrrolidinyl]methyl]-, methyl ester (11c)

K_2CO_3 (142 mg, 1.03 mmoL) was added to a solution of the compound **10c** (315 mg, 1.03 mmoL) in MeOH (25 mL) and. After stirring for 30 min at room temperature the reaction mixture was acidified with an aqueous solution of 1 N HCl and evaporated in vacuo at 25 °C. The residue was dissolved in EtOAc and the organic phase was washed with brine, dried over Na_2SO_4 and evaporated. The product was isolated as a pure white solid in 88% yield (241 mg); mp. 95–98 °C; $[\alpha]_D$ +65.3 (*c* 0.97, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 7.99(d, J = 8.2 Hz, 2H, Ph), 7.33(d, J = 8.2 Hz, 2H, Ph), 4.86(d, J = 15.3 Hz, 1H, C*H*HPh), 4.33(d, J = 15.3 Hz, 1H, CH*H*Ph), 3.91(s, 3H, OCH_3), 3.71(m, 1H, CHC*H*HOH), 3.56(m, 1H, CHCH*H*OH), 3.50(m, 1H, C*H*CH$_2$OH), 2.58–1.93(m, 4H, 2 × CH_2), 1.67(br s, 1H, O*H*).

^{13}C NMR δ: 176.2(CH_2*C*ON), 166.7(*C*OCH$_3$), 142.1, 130.0, 129.4, 127.8, 62.5(CH_2OH), 61.0(CHCH_2OH), 52.1(CH_3), 44.4(CH_2Ph), 30.3(CH_2CO), 21.0(CH_2CH_2CO). MS (ESI) *m/z* (%): 286 [(M+Na$^+$), 87].

Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32. Found: C, 63.95; H, 6.37; N, 5.48.

4.8. Benzoic acid, 4-[[2-(hydroxymethyl)-5-oxo-1-pyrrolidinyl]methyl]-, phenylmethyl ester (11d)

In a stirred solution of the compound **10d** (250 mg, 0.55 mmol) in DCM (25 mL), TFA was added (1.3 mL, 17 mmol) at room temperature. Once the reaction was finished (10 min), the solvent

was evaporated to dryness. The residue was dissolved in toluene (2 mL) and the solvent was evaporated (twice) for the removal of the acid. Finally the residue was dissolved in ethyl acetate and the organic phase was washed with brine to neutral pH. After drying over Na_2SO_4 and evaporation of the solvent, the product was obtained as a pure colorless oil. Yield 0.180 g (96%); $[\alpha]_D$ +43.6 (*c* 1.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 7.99(d, *J* = 6.1 Hz, 2H, Ph), 7.39–7.27(m, 7H, Ph), 5.32(s, 2H, C*H*$_2$Ph), 4.92(d, *J* = 15.3 Hz, 1H, C*H*HPh), 4.19(d, *J* = 15.4 Hz, 1H, CH*H*Ph), 3.72(m, 1H, CHCH*H*O), 3.47(m, 1H, CHC*H*HO), 3.47(m, 1H, C*H*CH$_2$O), 3.10(br s, 1H, O*H*), 2.54–1.94(m, 4H, 2 × CH_2).

^{13}C NMR δ: 176.2(N*C*O), 166.0(*C*OO), 142.2, 136.2, 130.3, 129.7, 128.8, 128.4, 128.3, 128.0, 66.9(O*C*H$_2$Ph), 62.4(*C*H$_2$OH), 58.8(*C*HCH$_2$OH), 44.4(*C*H$_2$Ph), 30.3(*C*H$_2$CO), 21.0(CH$_2$*C*H$_2$CO). MS (ESI) *m/z* (%): 362 [(M+Na$^+$), 100].

Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.95; H, 6.37; N, 4.41.

4.9. Compounds 5a–c and 12c, d: General procedure

In an ice cooled stirred solution of the alcohols **4a–c** and **11c**, **d** (1 mmoL) in DCM (5 mL), *p*-toluenesulfonylchloride (1.5 mmoL) was added, followed by the addition of Et_3N (1.1 mmoL). The reaction mixture was stirred at 0 °C for 10 min and overnight at room temperature. The organic layer was subsequently washed with a 5% solution of H_2SO_4, brine, 5% solution of $NaHCO_3$ and brine. After drying over Na_2SO_4 and evaporation in vacuo, the residual product was purified by column chromatography (Silica Gel 60) using the appropriate solvent systems as it will be defined in each case below.

4.9.1. Benzenesulfonic acid, 4-methyl-, [5-oxo-1-(phenylmethyl)-(2*S*)-pyrrolidinyl]methyl ester (5a)

See Ref. 32.

4.9.2. Benzenesulfonic acid, 4-methyl-, [5-oxo-1-[[3-(phenylmethoxy)phenyl]methyl]-2-pyrrolidinyl]methyl ester (5b)

Prepared from the compound **4b**. Eluent, EtOAc. Yield 78%; colorless oil; $[\alpha]_D$ +19.9 (*c* 1.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 7.70(m, 2H, Ph), 7.38–7.14(m, 8H, Ph), 6.85 (m, 1H, Ph), 6.69(m, 2H, Ph), 5.02(s, 2H, OC*H*$_2$Ph), 4.85(d, *J* = 15.1 Hz, 1H, C*H*HPh), 3.91(m, 2H, CH_2OS), 3.72(d, *J* = 15.1 Hz, 1H, CH*H*Ph), 3.58(m, 1H, CH), 2.42(s, 3H, CH_3), 2.58–1.82(m, 4H, 2 × CH_2).

^{13}C NMR δ: 175.0(CH$_2$*C*ON), 159.0, 145.3, 137.5, 136.8, 132.2, 129.9, 129.8, 128.5, 127.9, 127.8, 127.4, 120.4, 114.2, 114.2, 69.6, 68.8, 55.3(*C*CH$_2$OS), 44.3(*C*H$_2$Ph), 29.6(*C*H$_2$CO), 21.6(*C*H$_2$CH$_2$CO), 21.0(*C*H$_3$). MS (ESI) *m/z* (%): 466 [(M+H$^+$), 100].

Anal. Calcd for $C_{26}H_{27}NO_5S$: C, 67.08; H, 5.85; N, 3.01. Found: C, 67.34; H, 5.98; N, 3.27.

4.9.3. Benzenesulfonic acid, 4-methyl-, [5-oxo-1-[[2-[1-(phenylmethyl)-1*H*-1,2,3,4-tetrazol-5-yl]phenyl]methyl]-2-pyrrolidinyl]methyl ester (5c)

Prepared from the compound **4c**. Eluent EtOAc–petroleum ether (bp 40–60 °C). Yield 80%; colorless oil; $[\alpha]_D$ +12.1 (*c* 1.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 7.90(m, 1H, Ph), 7.62(d, *J* = 8.3 Hz, 2H, Ph), 7.46–7.28(m, 8H, Ph), 7.24(d, *J* = 8.3 Hz, 2H, Ph), 5.81(s, 2H, NNCH$_2$Ph), 4.99(d, *J* = 15.8 Hz, 1H, C*H*HPh), 4.48(d, *J* = 15.8 Hz, 1H, CH*H*Ph), 4.07(dd, J_1 = 4.0 Hz, J_2 = 10.6 Hz, 1H, CH*H*OS), 3.84(dd, J_1 = 3.2 Hz, J_2 = 10.6 Hz, 1H, CH*H*OS), 3.57(m, 1H, CH), 2.38(s, 3H, CH_3), 2.33–1.78(m, 4H, 2 × CH_2).

^{13}C NMR δ: 175.1(*C*ON), 164.5(N*C*N), 145.0, 135.3, 133.1, 132.2, 130.4, 129.8, 129.2, 128.9, 128.4, 127.7, 127.6, 126.0, 68.8(*C*H$_2$OS), 56.8(NNCH$_2$Ph), 55.6(*C*H), 41.8(*C*H$_2$Ph), 29.5(*C*H$_2$CO), 21.5 (*C*H$_2$CH$_2$CO), 21.2 (*C*H$_3$).

MS (ESI) *m/z* (%): 518 [(M+H$^+$), 100].

Anal. Calcd for $C_{27}H_{27}N_5O_4S$: C, 62.65; H, 5.26; N, 13.53. Found: C, 62.48; H, 5.37; N, 13.72.

4.9.4. Benzoic acid, 4-[[2-[[[(4-methylphenyl)sulfonyl]oxy]-methyl]-5-oxo-1-pyrrolidinyl]methyl]-, methyl ester (12c)

Prepared from the compound **11c**. Eluent EtOAc. Yield 77%; colorless oil; $[\alpha]_D$ +11.8 (*c* 1.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 7.93(d, *J* = 7.4 Hz, 2H, Ph), 7.69(d, *J* = 7.4 Hz, 2H, Ph), 7.33(d, *J* = 7.9 Hz, 2H, Ph), 7.19(d, *J* = 7.9 Hz, 2H, Ph), 4.86(d, *J* = 15.4 Hz, 1H, C*H*HPh), 3.96(d, *J* = 15.4 Hz, 1H, CH*H*Ph), 4.00–3.93(m, 2H, C*H*$_2$OSO$_2$), 3.90(s, 3H, OC*H*$_3$), 3.60(m, 1H, C*H*CH$_2$N), 2.45(s, 3H, SO$_2$PhC*H*$_3$), 2.39–1.77(m, 4H, 2 × CH_2).

^{13}C NMR δ: 175.1(N*C*O), 166.6(*C*OOCH$_3$), 145.3, 141.4, 132.2, 130.0, 129.5, 128.2, 128.1, 127.8, 69.0(*C*H$_2$OSO$_2$), 55.7(*C*HCH$_2$OS), 52.1(COO*C*H$_3$), 44.4(*C*H$_2$Ph), 29.5(*C*H$_2$CO), 21.6(OSO$_2$PhC*H*$_3$), 21.1(*C*H$_2$CH$_2$CO). MS (ESI): *m/z* (%): 440 [(M+Na$^+$), 100].

Anal. Calcd for $C_{21}H_{23}NO_6S$: C, 60.42; H, 5.55; N, 3.36. Found: C, 60.28; H, 5.47; N, 3.42.

4.9.5. Benzoic acid, 4-[[2-[[[(4-methylphenyl)sulfonyl]oxy]methyl]-5-oxo-1-pyrrolidinyl]methyl]-, phenylmethyl ester (12d)

Prepared from the compound **11d**. Eluent EtOAc. Yield 56%; colorless oil; $[\alpha]_D$ +15.3 (*c* 0.8, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 7.96(d, *J* = 8.2 Hz, 2H, Ph), 7.66(d, *J* = 8.3 Hz, 2H, Ph), 7.41–7.17(m, 9H, Ph), 5.34(s, 2H, C*H*$_2$Ph), 4.86(d, *J* = 15.5 Hz, 1H, CH*H*PhCOO), 3.98–3.91(m, 3H, CH*H*PhCOO, C*H*$_2$O-SO$_2$), 3.58(m, 1H, C*H*CH$_2$OS), 2.40(s, 3H, SO$_2$PhC*H*$_3$), 2.58–1.77(m, 4H, 2 × CH_2).

^{13}C NMR δ: 175.1(N*C*O), 165.9(*C*OOCH$_2$Ph), 145.4, 141.5, 135.8, 132.1, 130.1, 130.0, 129.5, 128.6, 128.3, 128.1, 127.8, 69.0(*C*H$_2$O-SO$_2$), 66.7(*C*H$_2$Ph), 55.7(*C*HCH$_2$OS), 44.3(*C*H$_2$Ph), 29.5(*C*H$_2$CO), 21.6(SO$_2$PhC*H*$_3$), 21.1(*C*H$_2$CH$_2$CO).

MS (ESI): *m/z* (%): 494 [(M+H$^+$), 100].

Anal. Calcd for $C_{27}H_{27}NO_6S$: C, 65.70; H, 5.51; N, 2.84. Found: C, 65.58; H, 5.57; N, 3.01.

4.10. Compounds 6a–d and 13c–g: General procedure

Method A: In a solution of the tosylesters **5a–c** (1 mmol) in dry DMF (5 mL), the freshly prepared lithium imidazolide or 4(5)-substituted-imidazolide (**22**, 2 mmol) was added. The reaction mixture was stirred at 50 °C overnight under argon. After evaporation of DMF in high vacuo, the residue was dissolved in EtOAc and the organic phase was washed with brine to neutral pH, dried over Na_2SO_4 and evaporated under reduced pressure. The residual product was purified by column chromatography (silica gel) using the appropriate solvent systems as it will be defined in each case below.

Method B: Compounds **22** or **23** (1.2 mmol) and cesium carbonate (390 mg, 1.2 mmol) were dissolved in dry DMF (3 mL) under argon. After stirring of the mixture for 30 min at 50 °C, a solution of the tosyl ester **12c** or **12d** (1 mmol) dissolved in dry DMF (3 mL) was added and the stirring was continued overnight at 50 °C under argon. After evaporation of DMF in high vacuo, the residue was dissolved in EtOAc and the organic phase was washed with brine to neutral pH, dried over Na_2SO_4 and evaporated under reduced pressure. The residual product was purified by column chromatography (silica gel) using the appropriate solvent systems as it will be defined, in each case, below.

4.10.1. (5*S*)-(1*H*-Imidazol-1-ylmethyl)-1-(phenylmethyl)-2-pyrrolidinone (6a)

See Ref. 7.

4.10.2. (5*S*)-(1*H*-Imidazol-1-ylmethyl)-1-[[3-(phenylmethoxy)phenyl]methyl]-2-pyrrolidinone (6b)

Prepared from the compound **5b** and lithium imidazolide by method A. Eluent EtOAc–MeOH, 1:1. Yield 42%; colorless oil; $[\alpha]_D$ +17.0 (*c* 1.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 7.38–6.72(m, 12H, Ph), 5.05(s, 2H, OC*H*$_2$Ph), 4.93(d, *J* = 15.4 Hz, 1H, C*H*HPh), 3.90(m, 2H, CH_2N), 3.84(d, *J* = 15.4 Hz, 1H, CH*H*Ph), 3.64(m, 1H, CH), 2.28–1.58(m, 4H, 2 × CH_2).

^{13}C NMR δ: 175.1(CH_2*C*ON), 159.1, 137.5, 137.3, 136.7, 130.1, 130.0, 128.5, 127.9, 127.3, 120.5, 119.2, 114.5, 114.3, 69.8(O*C*H$_2$Ph), 56.9, 48.6, 44.8, 29.1(*C*H$_2$CO), 22.3(*C*H$_2$CH$_2$CO). MS (ESI): *m*/*z* (%): 362 [(M+H$^+$), 100].

Anal. Calcd for $C_{22}H_{23}N_3O_2$: C, 73.11; H, 6.41; N, 11.63. Found: C, 73.32; H, 6.57; N, 11.48.

4.10.3. (5*S*)-(1*H*-Imidazol-1-ylmethyl)-1-[[2-[1-(phenylmethyl)-1*H*-1,2,3,4-tetrazol-5-yl]phenyl]methyl]-2-pyrrolidinone (6c)

Prepared from the compound **5c** and freshly prepared lithium imidazolide by method A. Eluent EtOAc–MeOH, 1:1. Yield 48%; colorless oil; $[\alpha]_D$ +11.6 (*c* 1.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 7.95(m, 1H, NC*H*N), 7.48–7.32(m, 8H, Ph), 7.08(s, 1H, Ph), 6.99(s, 1H, Ph), 6.69(m, 1H, Ph), 5.82(s, 2H, NNCH_2Ph), 5.18(d, *J* = 15.3 Hz, 1H, CH*H*Ph), 4.72(d, *J* = 15.3 Hz, 1H, C*H*HPh), 3.85(m, 2H, CH_2N), 3.70(m, 1H, CH), 2.24–1.67(m, 4H, 2 × CH_2).

^{13}C NMR δ: 174.9(*C*ON), 164.8(NC*N*), 137.4, 135.5, 132.9, 130.8, 130.1, 129.9, 129.7, 129.1, 129.0, 128.5, 128.2, 126.1, 119.3, 56.9, 56.7, 47.8(*C*H), 41.5(NC*P*h), 28.7(*C*H$_2$CO), 22.0(*C*H$_2$CH$_2$CO). MS (ESI): *m*/*z* (%): 414 [(M+H$^+$), 100].

Anal. Calcd for $C_{23}H_{23}N_7O$: C, 66.81; H, 5.61; N, 23.73. Found: C, 66.95; H, 5.48; N, 23.57.

4.10.4. 1*H*-Imidazole-4(5)-carboxylic acid, 1-[[5-oxo-1-(phenylmethyl)-(2*S*)-pyrrolidinyl]methyl]-, phenylmethyl ester (6d)

Prepared from the compound **5a** and the lithium salt of **22** by method A. The product was obtained as a mixture of the two substitutional isomers, possessing the carboxylate group at position 4 or 5 on the imidazole ring. The separation of the two isomers could not be achieved in a quantitatively acceptable yield. Yield 69% (mixture of both isomers).

MS (ESI) *m*/*z* (%): 390 [(M+H$^+$), 100].

4.10.5. Benzoic acid, 4-[[(2*S*)-(1*H*-imidazol-1-ylmethyl)-5-oxo-1-pyrrolidinyl]methyl]-, methyl ester (13c)

Prepared from **12c** and imidazole by method B. Eluent EtOAc–MeOH, 7:3. Yield 65%; colorless oil; $[\alpha]_D$ +14.6 (*c* 0.9, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 8.00(d, *J* = 8.1 Hz, 2H, Ph), 7.65(s, 1H, NC*H*N), 7.37(d, *J* = 8.1 Hz, 2H, Ph), 7.06(d, 2H, *J* = 18.0 Hz NC*H*C*H*N),4.98(d, *J* = 15.6 Hz, 1H, CH*H*Ph), 4.25(m, 2H, C*H*$_2$N), 4.15(d, *J* = 15.6 Hz, 1H, CH*H*Ph), 3.90(s, 3H, OC*H*$_3$), 3.30(m, 1H, C*H*CH$_2$N), 2.21–1.71(m, 4H, 2 × CH_2).

^{13}C NMR δ: 178.1(NCO), 167.7(*C*OO), 143.2, 139.2, 131.1, 131.0, 129.4, 129.0, 121.6, 66.0(*C*H$_2$N), 59.0(*C*HCH$_2$N), 52.7(*C*H$_3$), 45.2(*C*H$_2$Ph), 30.0(*C*H$_2$CO), 20.3(*C*H$_2$CH$_2$CO). MS (ESI): *m*/*z* (%): 336 [(M+Na$^+$), 100].

Anal. Calcd for $C_{17}H_{19}N_3O_3$: C, 65.16; H, 6.11; N, 13.41. Found: C, 64.89; H, 5.94; N, 13.29.

4.10.6. Benzoic acid, 4-[[(2*S*)-(1*H*-imidazol-1-ylmethyl)-5-oxo-1-pyrrolidinyl]methyl]-, phenylmethyl ester (13d)

Prepared from **12d** and imidazole by method B. Eluent EtOAc–MeOH, 7:3. Yield 75%; colorless oil; $[\alpha]_D$ +10.7 (*c* 1.1, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 8.00(d, *J* = 8.3 Hz, 2H, Ph), 7.43–7.31(m, 6H, Ph), 7.22 (d, *J* = 8.3 Hz, 2H, Ph), 7.04(s, 1H, CH_2NC*H*CHN), 6.75(s, 1H, CH_2NCHC*H*N), 5.32(s, 2H, C*H*$_2$Ph), 5.01(d, *J* = 15.4 Hz, 1H, C*H*HPhCOO), 3.97(d, *J* = 5.0 Hz, 2H, CHC*H*$_2$), 3.85(d, *J* = 15.4 Hz, 3.85(d, *J* = 15.4 Hz, 1H, CH*H*PhCOO), 3.74–3.63(m, 1H, C*H*CH$_2$O), 2.36–1.65(m, 4H, 2 × CH_2).

^{13}C NMR δ: 175.1(N*C*O), 165.8(*C*OOCH$_2$Ph), 141.2, 137.3, 135.7, 130.2, 130.1, 129.7, 128.5, 128.2, 128.0, 127.8, 119.1, 66.7(OCH$_2$Ph), 56.8(CHCH$_2$), 48.8(*C*HCH$_2$), 44.4(NCH$_2$Ph), 28.9(*C*H$_2$CO), 22.3(*C*H$_2$CH$_2$CO). MS (ESI) *m*/z (%): 390[(M+H$^+$), 100].

Anal. Calcd for $C_{23}H_{23}N_3O_3$: C, 70.93; H, 5.95; N, 10.79. Found: C, 70.77; H, 5.84; N, 10.83.

4.10.7. 1*H*-Imidazole-5-carboxylic acid, 1-[[1-[[4-(methoxycarbonyl)phenyl]methyl]-5-oxo-(2*S*)-pyrrolidinyl]methyl]-, phenylmethyl ester (13e)

Prepared from **12c** and **22** by method B. Eluent EtOAc–MeOH, 9:1. Yield 50%; yellow oil; $[\alpha]_D$ +12.4 (*c* 1.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 7.95(d, *J* = 7.9 Hz, 2H, Ph), 7.78(s, 1H, NC*H*N), 7.60(s, 1H, NC*H*CCOO), 7.36(s, 5H, Ph), 7.29(d, *J* = 7.9 Hz, 2H, Ph), 5.24(s, 2H, COOC*H*$_2$Ph), 4.86(d, *J* = 15.3 Hz, 1H, NC*H*HPh), 4.58(dd, J_1 = 4.6 Hz, J_2 = 13.3 Hz, 1H, CHC*H*HN), 4.01(dd, J_1 = 7.3 Hz, J_2 = 13.3 Hz, 1H, CHCH*H*N), 4.00(d, *J* = 15.3 Hz, 1H, NCH*H*Ph), 3.88(s, 3H, C*H*$_3$), 3.82(m, 1H, NC*H*CH$_2$), 2.44–1.74(m, 4H, 2 × CH_2).

^{13}C NMR δ: 175.1(CH_2*C*ON), 166.6(*C*OOCH$_2$), 159.9(*C*OOCH$_3$) 142.2, 142.0, 138.1, 135.3, 129.9, 129.5, 128.6, 128.5, 128.2, 128.0, 122.2, 66.4(COOCH$_2$Ph), 57.2(CHCH$_2$N), 52.1(OCH$_3$), 48.9(CHCH$_2$N), 44.9(*C*H$_2$Ph), 28.9(*C*H$_2$CO), 22.5(*C*H$_2$CH$_2$CO). MS (ESI) *m*/*z* (%): 470 [(M+Na$^+$), 100].

Anal. Calcd for $C_{25}H_{25}N_3O_5$: C, 67.10; H, 5.63; N, 9.39. Found: C, 66.95; H, 5.78; N, 9.25.

4.10.8. 1*H*-Imidazole-4-carboxylic acid, 1-[[1-[[4-(methoxycarbonyl)phenyl]methyl]-5-oxo-(2*S*)-pyrrolidinyl]methyl]-, phenylmethyl ester (13e*)

Yield 10%; colorless oil.

^{1}H NMR ($CDCl_3$) δ: 7.98(d, *J* = 8.1 Hz, 2H, Ph), 7.47–7.23(m, 9H, NC*H*N, NC*H*CCOO, Ph), 5.31(s, 2H, COOC*H*$_2$Ph), 4.95(d, *J* = 15.4 Hz, 1H, NC*H*HPh), 4.04–3.97(m, 3H, CHC*H*$_2$N, NCH*H*Ph), 3.89(s, 3H, C*H*$_3$), 3.75(m, 1H, NC*H*CH$_2$), 2.36–1.66(m, 4H, 2 × CH_2).

^{13}C NMR δ: 175.0(CH_2*C*ON), 166.4(*C*OOCH$_2$), 161.9(*C*OOCH$_3$) 141.0, 138.3, 135.8, 134.1, 130.3, 130.0, 128.5, 128.4, 128.2, 127.8, 125.3, 66.4(COOCH$_2$Ph), 57.0(CHCH$_2$N), 52.2(OCH$_3$), 49.5(CHCH$_2$N), 44.9(*C*H$_2$Ph), 28.9(*C*H$_2$CO), 22.4(*C*H$_2$CH$_2$CO). MS (ESI) *m*/*z* (%): 470 [(M+Na$^+$), 100].

4.10.9. 1*H*-Imidazole-4(5)-carboxylic acid, 1-[[5-oxo-1-[[4-[(phenylmethoxy)carbonyl]phenyl]methyl]-(2*S*)-pyrrolidinyl]methyl]-, phenylmethyl ester (13f)

Prepared from **12d** and **22** by method B.

The product was obtained as a mixture of the two substitutional isomers, possessing the carboxylate group at position 4 or 5 on the imidazole ring. The separation of the two isomers could not be achieved in a quantitatively acceptable yield. Yield 75% (mixture of both isomers).

4.10.10. Methyl 1-[((2*S*)-1-4-[(benzyloxy)carbonyl]benzyl-5-oxotetrahydro-1*H*-pyrrol-2-yl)methyl]-1*H*-imidazole-5-carboxylate (13g)

Prepared from **12d** and **23** by method B. Eluent EtOAc–MeOH, 9:1. Yield 38%; colorless oil; $[\alpha]_D$ +9.4 (*c* 0.8, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 8.01(d, J = 8.2 Hz, 2H, Ph), 7.77–7.71(m, 2H, NC*H*NC*H*), 7.41–7.30(m, 7H, Ph), 5.35(s, 2H, CH_2Ph), 4.88(d, J = 15.2 Hz, 1H, NC*H*HPh), 4.60(dd, J_1 = 4.5 Hz, J_2 = 13.3 Hz, 1H, CHCH*H*N), 4.04(m, 2H, CHCH*H*N, NCH*H*Ph), 3.89(m, 1H, NC*H*CH_2), 3.81(s, 3H, CH_3), 2.60–1.78(m, 4H, 2 × CH_2).

^{13}C NMR δ: 175.2(CH_2CON), 166.0(*C*OOPh), 160.5(*C*OO CH_3), 142.2, 137.6, 135.9, 130.1, 129.5, 128.6, 128.2, 128.1, 128.0, 66.7(*C*H_2Ph), 57.3(CH*C*H_2N), 51.8(CH_2*C*H), 49.0(*C*H_3), 44.9(*C*H_2Ph), 28.9(*C*H_2CO), 22.5(*C*H_2CH_2CO).

MS (ESI) m/z (%): 358 [(M+H$^+$), 100].

Anal. Calcd for $C_{18}H_{19}N_3O_5$: C, 60.50; H, 5.36; N, 11.76. Found: C, 60.72; H, 5.47; N, 11.69.

4.10.11. Methyl 1-[((2*S*)-1-4-[(benzyloxy)carbonyl]benzyl-5-oxotetrahydro-1*H*-pyrrol-2-yl)methyl]-1*H*-imidazole-4-carboxylate (13g*)

Yield 26%; colorless oil.

^{1}H NMR ($CDCl_3$) δ: 8.04(d, J = 8.2 Hz, 2H, Ph), 7.44–7.24(m, 9H, Ph, NC*H*NC*H*), 5.35(s, 2H, CH_2Ph), 4.99(d, J = 15.3 Hz, 1H, NC*H*HPh), 4.12–3.91(m, 3H, NCHCH_2, NCH*H*Ph), 3.86(s, 3H, CH_3), 3.75(m, 1H, NC*H*CH_2), 2.30–1.67(m, 4H, 2 × CH_2).

^{13}C NMR δ: 175.0(CH_2CON), 166.0(*C*OOPh), 162.6(*C*OOCH_3), 141.1, 138.1, 135.8, 134.3, 130.4, 130.0, 128.6, 128.3, 128.2, 127.8, 125.1, 66.8(*C*H_2Ph), 56.9(CH*C*H_2N), 51.8(CH_2*C*H), 49.4(*C*H_3), 44.9(*C*H_2Ph), 28.9(*C*H_2CO), 22.3(*C*H_2CH_2CO).

MS (ESI) m/z (%): 358 [(M+H$^+$), 100].

4.11. Compounds 7e–g and 14d–g: General procedure

A mixture of a compound possessing a benzyl ether (**6b**), benzyl ester (**6d**, **13d**, **13e**, **13f**, and **13g**) and benzyl protected tetrazole group (**6c**) (1 mmol) in MeOH (10 mL) and 10% palladium on activated carbon (0.02 g) or Pd(OH)$_2$ (0.02 g) for compound **6c**, was hydrogenated for 1.5–3 h under atmospheric conditions. After filtration through a pad of Celite, the solvent was removed in vacuo to give the deprotected final compound in almost quantitative yield and high purity.

4.11.1. 1-[(3-Hydroxyphenyl)methyl]-(5*S*)-(1*H*-imidazol-1-ylmethyl)-2-pyrrolidinone (7e)

Prepared from the compound **6b**. Yield 93%; colorless oil; $[\alpha]_D$ −1.1 (*c* 1.5, CH_3OH).

^{1}H NMR (CD_3OD) δ: 8.89(s, 1H, NCHN), 7.60(s, 2H, NCHCHN), 7.17(t, J_1 = 8.1 Hz, J_2 = 7.4 Hz, 1H, Ph), 6.76–6.69(m, 3H, Ph), 4.79(d, J = 15.4 Hz, 1H, C*H*HPh), 4.46(m, 2H, CH_2N), 4.19(d, J = 15.4 Hz, 1H, CH*H*Ph), 4.06(m, 1H, CH), 2.30–1.88(m, 4H, 2 × CH_2).

^{13}C NMR δ: 177.9(CH_2*C*ON), 159.2, 139.0, 137.3, 131.1, 123.8, 121.9, 119.8, 115.9, 115.5, 58.6(*C*H_2N), 51.6(*C*H_2Ph), 45.9(N*C*H), 30.1(*C*H_2CO), 22.8(*C*H_2CH_2CO).

MS (ESI) m/z (%): 272 [(M+H$^+$), 100].

NP Analytical HPLC in solvent A: *t*R = 13.8 min. HPLC purity >96%.

Anal. Calcd for $C_{15}H_{17}N_3O_2$: C, 66.40; H, 6.32; N, 15.49. Found: C, 66.22; H, 6.17; N, 15.33.

4.11.2. 2-Pyrrolidinone, (5*S*)-(1*H*-imidazol-1-ylmethyl)-1-[[2-(1*H*-1,2,3,4-tetrazol-5-yl)phenyl]methyl] (7f)

Prepared from the compound **6c**. Yield 90%; colorless oil; $[\alpha]_D$ +18.6 (*c* 1.0, MeOH).

^{1}H NMR (CD_3OD) δ: 8.77(s, 1H, NC*H*N), 7.72(d, J = 8.0 Hz, 2H, NC*H*C*H*N), 7.56–7.46(m, 4H, Ph), 5.07(d, J = 15.6 Hz, 1H, C*H*HPh), 4.63(d, J = 15.6 Hz, 1H, CH*H*Ph), 4.48(dd, J_1 = 4.1 Hz, J_2 = 14.0 Hz, 1H, CHC*H*HN), 4.34(dd, J_1 = 6.3 Hz, J_2 = 14.0 Hz, 1H, CHCH*H*N), 4.01(m, 1H, C*H*CH$_2$N), 2.25–1.87(m, 4H, 2 × CH_2).

^{13}C NMR δ: 177.8(*C*ON), 160.6(NCN), 138.0, 136.7, 131.3, 131.2, 130.7, 129.2, 129.1, 124.7, 123.0, 58.7(CHCH_2N), 50.5(*C*HCH_2N), 43.3(*C*H_2Ph), 29.8(*C*H_2CO), 22.8(*C*H_2CH_2CO). MS (ESI) m/z (%): 324 [(M+H$^+$), 100].

NP Analytical HPLC in solvent A: *t*R = 18.5 min. HPLC purity >99%.

Anal. Calcd for $C_{16}H_{17}N_7O$: C, 59.43; H, 5.30; N, 30.32. Found: C, 59.57; H, 5.39; N, 30.41.

4.11.3. 1*H*-Imidazole-5-carboxylic acid, 1-[[5-oxo-1-(phenylmethyl)-(2*S*)-pyrrolidinyl]methyl] (7g)

Compound **6d** was hydrogenated according to the general procedure. The product was obtained as a mixture of the two constitutional isomers. It was purified using silica gel column chromatography and EtOAc–MeOH, 1:1 as eluent. The pure isomer with the carboxylic group at position 5 on the imidazole ring was obtained as a colorless oil. Yield 65%; $[\alpha]_D$ +22.5 (*c* 0.93, MeOH).

^{1}H NMR (CD_3OD) δ: 7.63(s, 1H, NC*H*N), 7.52(s, 1H, NC*H*CCOO), 7.32–7.23(m, 5H, Ph), 4.85(dd, J_1 = 5.1 Hz, J_2 = 13.5 Hz, 1H, CHC*H*HN), 4.82(d, J = 15.0 Hz, 1H, CH*H*Ph), 4.35(dd, J_1 = 6.2 Hz, J_2 = 13.5 Hz, 1H, C*H*CHHN), 4.00(m, 1H, C*H*CH$_2$N), 3.94(d, J = 15.0 Hz, 1H, CH*H*Ph), 2.34–1.95(m, 4H, 2 × CH_2).

^{13}C NMR δ: 178.1(*C*ON), 167.1(*C*OOH), 141.6, 138.2, 134.7, 130.6, 129.8, 129.1, 128.7, 59.6(CH*C*H_2N), 47.7(*C*HCH_2N), 45.9(*C*H_2Ph), 30.3(*C*H_2CO), 23.2(*C*H_2CH_2CO).

MS (ESI) m/z (%): 300 [(M+H$^+$),100].

Anal. Calcd for $C_{16}H_{17}N_3O_3$: C, 64.20; H, 5.72; N, 14.04. Found: C, 64.48; H, 5.87; N, 14.12.

4.11.4. 4-[(2*S*)-2-(1*H*-Imidazol-1-ylmethyl)-5-oxotetrahydro-1*H*-pyrrol-1-yl]methylbenzenecarboxylic acid (14d)

Prepared from the compound **13d**. Yield 96%; colorless oil; $[\alpha]_D$ +19.8 (*c* 1.00, MeOH).

^{1}H NMR (CD_3OD) δ: 8.80(s, 1H, NCHN), 7.87(d, J = 8.0 Hz, 2H, Ph), 7.48(d, J = 10 Hz, 2H, Ph), 7.24 (d, J = 8.0 Hz, 2H, Ph), 4.7(d, 1H, J = 15.9 Hz, CH_2Ph), 4.37–4.32(m, 2H, CHCH_2), 4.23(d, J = 15.9 Hz, 2H, CH_2Ph), 3.85(d, J = 15.4 Hz, 1H, CH*H*PhCOO), 3.99–3.92(m, 1H, C*H*CH$_2$N), 2.28–1.73(m, 4H, 2 × CH_2).

^{13}C NMR δ: 178.1(NCO), 170.2(*C*OOH), 142.5, 138.9, 132.5, 131.3, 128.9, 128.4, 121.9(CH$_2$NC*H*CHN), 58.9(CH*C*H_2N), 49.6(CH$_2$*C*HCH$_2$), 45.3(NC*H*$_2$Ph), 30.0(*C*H_2CO), 23.1(*C*H_2CH_2CO). MS (ESI) m/z (%): 300 [(M+H$^+$), 100].

NP Analytical HPLC in solvent B: *t*R = 5.9 min. HPLC purity >98%.

Anal. Calcd for $C_{16}H_{17}N_3O_3$: C, 64.20; H, 5.72; N, 14.04. Found: C, 64.39; H, 5.85; N, 14.12.

4.11.5. 1-((2*S*)-1-[4-(Methoxycarbonyl)benzyl]-5-oxotetrahydro-1*H*-pyrrol-2-ylmethyl)-1*H*-imidazole-5-carboxylic acid (14e)

Prepared from the compound **13e**. Yield 99%; colorless oil; $[\alpha]_D$ +43.7 (*c* 1.00, MeOH).

^{1}H NMR (CD_3OD) δ: 8.09(s, 1H, NC*H*N), 7.96(d, J = 8.1 Hz, 2H, Ph), 7.74(s, 1H, NC*H*CCOO), 7.37(d, J = 8.1 Hz, 2H, Ph), 4.87(d, J = 15.6 Hz, 1H, NC*H*HPh), 4.78(dd, J_1 = 4.4 Hz, J_2 = 13.6 Hz, 1H, CHC*H*HN), 4.31(dd, J_1 = 7.3 Hz, J_2 = 13.6 Hz, 1H, C*H*CHHN), 4.15(d, J = 15.6 Hz, 1H, NCH*H*Ph), 4.00(m, 1H, NC*H*CH$_2$), 3.88(s, 3H, CH_3), 2.45–1.74(m, 4H, 2 × CH_2).

^{13}C NMR δ: 178.2(CH_2*C*ON), 168.2(*C*OOH), 162.6(*C*OOCH_3) 143.7, 143.4, 136.1, 130.9, 129.0, 125.5, 62.2(CH*C*H_2N), 59.2(*C*HCH_2N), 52.7(*C*OCH_3), 45.7(*C*H_2Ph), 29.9(*C*H_2CO), 23.1(*C*H_2CH_2CO). MS (ESI) m/z (%): 356 [(M−H$^+$), 100].

RP Analytical HPLC in solvent E: *t*R = 14.4 min. HPLC purity >95%.

Anal. Calcd for $C_{18}H_{19}N_3O_5$: C, 60.50; H, 5.36; N, 11.76. Found: C, 60.38; H, 5.22; N, 11.80.

4.11.6. 1-[(2*S*)-1-(4-Carboxybenzyl)-5-oxotetrahydro-1*H*-pyrrol-2-yl]methyl-1*H*-imidazole-4(5)-carboxylic acid (14f)

Prepared from the compound **13f**. Quantitative yield; yellow oil; MS (ESI) *m/z* (%): 342[(M−H$^+$), 100].

4.11.7. 4-[((2*S*)-2-[5-(Methoxycarbonyl)-1*H*-imidazol-1-yl]methyl-5-oxotetrahydro-1*H*-pyrrol-1-yl)methyl]benzenecarboxylic acid (14g)

Prepared from the compound 13 g. Yield 85%; colouless oil; $[\alpha]_D$ +10.2 (*c* 0.8, MeOH).

^{1}H NMR (CD_3OD) δ: 8.23(s, 1H, NC*H*N), 7.99(d, *J* = 8.2 Hz, 2H, Ph), 7.84(s, 1H, NC*H*CCOO), 7.37(d, *J* = 8.2 Hz, 2H, Ph), 4.88(d, *J* = 15.7 Hz, 1H, NCH*H*Ph), 4.77(dd, J_1 = 4.7 Hz, J_2 = 13.6 Hz, 1H, C*H*CHHN), 4.31(dd, J_1 = 7.6 Hz, J_2 = 13.6 Hz, 1H, CHCH*H*N), 4.19(d, *J* = 15.7 Hz, 1H, NCH*H*Ph), 4.00(m, 1H, NC*H*CH_2), 3.84(s, 3H, C*H*$_3$), 2.47–1.88(m, 4H, 2 × CH_2).

^{13}C NMR δ: 178.2(CH_2CON), 169.4(*C*OOH), 161.4(*C*OOCH_3) 143.7, 143.6, 143.4, 136.0, 131.4, 131.2, 128.9, 59.1(*C*HCH_2N), 52.6(*C*HCH_2N), 49.7(COCH_3), 45.8(*C*H$_2$Ph), 29.9(*C*H$_2$CO), 23.1(*C*H$_2$CH$_2$CO). MS (ESI) *m/z* (%): 356 [(M−H$^+$), 100].

Anal. Calcd for $C_{18}H_{19}N_3O_5$: C, 60.50; H, 5.36; N, 11.76. Found: C, 60.26; H, 5.19; N, 11.65.

4.12. 1-(Bromomethyl)-3-(phenylmethoxy)-benzene (16)

In an ice cooled solution of 3-benzyloxy-phenylmethanol (2.14 g, 10 mmol) in Et_2O (25 mL), PBr_3[33] (1.41 mL, 15 mmol) was added dropwise. The mixture was stirred at room temperature for 3 h and the reaction mixture was quenched by the addition of H_2O (15 mL) in small portions at 0 °C. The aqueous phase was removed and the organic layer was washed with H_2O, dried over Na_2SO_4 and evaporated in vacuo. The product was purified by column chromatography (silica gel) using petroleum ether (bp 40–60 °C)–EtOAc, 7:3 as eluent. Yield 1.5 g (56%); yellow oil.

^{1}H NMR ($CDCl_3$) δ: 7.42–6.82(m, 9H, Ph), 5.06(s, 2H, O*C*H$_2$Ph), 4.46(s, 2H, Br*C*H$_2$Ph).

^{13}C NMR δ: 159.0, 139.2, 136.7, 129.8, 128.6, 128.0, 127.5, 121.5, 115.4, 114.9 70.0(O*C*H$_2$Ph), 33.4(Br*C*H$_2$Ph).

Anal. Calcd for $C_{14}H_{13}BrO$: C, 60.67; H, 4.73. Found: C, 60.46; H, 4.69.

4.13. 5-(2-Methylphenyl)-1*H*-1,2,3,4-tetrazole (18)

2-Methyl-benzonitrile (0.59 mL, 5 mmol), TBAF.3H_2O (0.79 g, 2.5 mmol) and $TMSN_3$[34] (0.99 mL, 7.5 mmol) were mixed in a wheat on microreactor. After stirring of the reaction mixture at 85 °C for 72 h, it was diluted with EtOAc and the organic phase was successively washed with aqueous solution of 1 N HCl, H_2O and twice with aqueous solution of 5% $NaHCO_3$. The alkaline aqueous extracts were combined, acidified with 1 N HCl and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O, dried over Na_2SO_4 and evaporated under reduced pressure. The product was obtained as a pure white solid. Yield 0.64 g (80%); mp 142–144 °C.

^{1}H NMR (CD_3OD) δ: 7.64–7.36(m, 4H, Ph), 2.49(s, 3H, C*H*$_3$)

^{13}C NMR δ: 157.2(N*C*N), 138.9, 132.5, 132.2, 130.5, 127.5, 124.9, 20.7(*C*H$_3$).

Anal. Calcd for $C_8H_8N_4$: C, 59.99; H, 5.03; N, 34.98. Found: C, 59.78; H, 5.26; N, 34.76.

4.14. 5-(2-Methylphenyl)-1-(phenylmethyl)-1*H*-1,2,3,4-tetrazole (19)

In a solution of compound **18** (120 mg, 0.75 mmol) in acetone (3 mL) potassium carbonate (125 mg, 0.9 mmol) and benzylbromide (0.1 mL, 0.9 mmol) were added and the reaction mixture was warmed at 50 °C for 1 h under argon. After evaporation of the solvent, the residue was dissolved in ethyl acetate and the organic phase was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The residual oil was purified by column chromatography on silica gel, eluting with petroleum ether (bp 40–60 °C)–EtOAc, 9:1. Yield 140 mg (75%); yellow oil.

^{1}H NMR ($CDCl_3$) δ: 7.97(m, 1H, Ph), 7.42–7.24(m, 8H, Ph), 5.80(s, 2H, C*H*$_2$Ph) 2.59(s, 3H, C*H*$_3$).

^{13}C NMR δ: 165.8(N*C*N), 137.4, 133.4, 131.3, 129.8, 129.4, 129.1, 129.0, 128.9, 128.4, 125.9, 56.7(*C*H$_2$Ph), 21.6(*C*H$_3$).

Anal. Calcd for $C_{15}H_{14}N_4$: C, 71.98; H, 5.64; N, 22.38. Found: C, 72.04; H, 5.72; N, 22.15.

4.15. 5-[2-(Bromomethyl)phenyl]-1-(phenylmethyl)-1*H*-1,2,3,4-tetrazole (20)

In a solution of compound **19** (0.360 g, 1.44 mmol) in CCl_4 (6 mL), NBS (0.436 g, 2.44 mmol) and AIBN (3.11 mg, 0.019 mmol) were dissolved and the mixture was warmed at 80 °C for 5 h. The solvent was evaporated under reduced pressure and the residual product (0.500 g) was used without further purification.

4.16. 1*H*-Imidazole-4(5)-carboxylic acid, phenylmethyl ester (22)

In a solution of 1H-imidazole-4(5)-carboxylic acid (0.760 mg, 6.78 mmol) in DMF (10 mL) Cs_2CO_3 (1.11 g, 3.39 mmol) was added with some drops of H_2O. The solvent was evaporated under reduced pressure and the residue dissolved in DMF (10 mL), was stirred for 5 h at room temperature followed by the addition of benzyl bromide (0.93 mL, 7.8 mmol). After stirring overnight at room temperature and evaporation of DMF under reduced pressure, the residue was dissolved in EtOAc and the organic phase was washed with H_2O, 5% aqueous solution of $NaHCO_3$ and H_2O. The organic layer was dried over Na_2SO_4, evaporated in vacuo and the residue was purified by column chromatography on silica gel, eluting with EtOAc–AcOH, 9.5:0.5. The product was isolated as a white solid (0.822 g, 60%); mp 152–153 °C (mp $_{lit}$ = 159–163 °C).[35]

^{1}H NMR (CD_3OD) δ: 7.78(s, 2H, NC*H*NHC*H*), 7.42–7.34(m, 5H, Ph), 5.32(s, 2H, Ph).

^{13}C NMR δ: 163.4(*C*OO), 138.9, 138.7, 137.7, 129.8, 129.6, 129.4, 129.3, 67.1(*C*H$_2$Ph).

MS (ESI) *m/z* (%): 225 [(M+Na$^+$), 96].

Anal. Calcd for $C_{11}H_{10}N_2O_2$: C, 65.34; H, 4.98; N, 13.85. Found: C, 65.48; H, 5.03; N, 13.62.

4.17. 1*H*-Imidazole-4(5)-carboxylic acid, methyl ester (23)

1*H*-Imidazole-4(5)-carboxylic acid (0.560 g, 5 mmol) was dissolved in DMF (20 mL) and a small amount of insoluble solid was filtered off. A cooled (0 °C) solution of diazomethane in ether[36] was added until no more bubbles were formed. After evaporation of the solvent under reduced pressure the residue was dissolved in ether and washed with water to neutral pH. Then the ethereal solution was dried and evaporated. The product was obtained as a white solid homogeneous by TLC in 40% yield (0.252 g); mp 144–147 °C (mp.lit[37] = 153–154 °C).

^{1}H NMR (CD_3OD) δ: 7.77(d, *J* = 4.5 Hz, 2H, NC*H*NHC*H*), 3.85(s, 3H, CH_3).

^{13}C NMR δ: 164.2(*C*OO), 138.6, 126.6, 123.4, 52.0(*C*H$_3$).

Anal. Calcd for $C_5H_6N_2O_2$: C, 47.62; H, 4.80; N, 22.21. Found: C, 47.58; H, 4.96; N, 22.12.

4.18. Benzoic acid, 4-(bromomethyl)-, methyl ester (25)

To a solution of 4-bromomethyl benzoic acid (0.5 g, 2.33 mmol) in THF (15 mL), a cooled (0 °C) solution of diazomethane in ether was added until no more bubbles were formed. Some drops of glacial AcOH were added (to destroy any excess of diazomethane) and the ether solution was washed with water, 5% aqueous potassium hydrogen carbonate, and water (to neutral pH), then dried and evaporated. The product was obtained as a white solid homogeneous by TLC in quantitative yield (0.535 g); mp: 52–54 °C.

^{1}H NMR ($CDCl_3$) δ: 8.00(d, J = 8.3 Hz, 2H, Ph), 7.45(d, J = 8.3 Hz, 2H, Ph), 4.49(s, 2H, CH_2Ph), 3.91(s, 3H, CH_3).

^{13}C NMR δ: 166.4(*C*OO), 142.5(*C*CH$_2$Br), 130.0, 128.9, 52.1(*C*H$_3$), 32.1(*C*H$_2$Br).

Anal. Calcd for $C_9H_9BrO_2$: C, 47.19; H, 3.96. Found: C, 47.48; H, 3.75.

4.19. Benzoic acid, 4-(bromomethyl)-, phenylmethyl ester (26)

To a stirred solution of 4-bromomethyl benzoic acid (1.00 g, 4.88 mmol) and benzyl alcohol (0.5 mL, 4.88 mmol) in THF (30 mL), DMAP (0.06 g, 4.88 mmol) and subsequently DCC (1.01 g, 4.88 mmol) were added at 0 °C. After stirring of the reaction mixture for 1 h at rt, the DCU formed was filtered off and the solvent was evaporated under reduced pressure. Purification of the product was achieved by column chromatography using hexane–EtOAc 8:2 as eluent. Yield 0.9 g (60%); colorless oil.

^{1}H NMR ($CDCl_3$) δ 7.99(d, J = 8.3 Hz, 2H, Ph), 7.41–7.33(m, 7H, Ph), 5.30(s, 2H, CH_2Ph), 4.42(s, 2H, CH_2Br).

^{13}C NMR δ: 166.5(*C*OO), 142.5, 135.7, 130.2, 129.0, 128.8, 128.4, 128.1, 127.9, 66.6(*C*H$_2$Ph), 32.0(*C*H$_2$Br).

Anal. Calcd for $C_{15}H_{13}BrO_2$: C, 59.04; H, 4.29. Found: C, 59.32; H, 4.34.

4.20. Benzoic acid, 4-(bromomethyl)-, [5-oxo-1-[[4-[(phenylmethoxy)carbonyl]phenyl]methyl]-2*S*-pyrrolidinyl]methyl ester (28)

Sticky oil.

^{1}H NMR ($CDCl_3$) δ: 7.95(d, J = 8.1 Hz, 2H, Ph), 7.86(d, J = 8.2 Hz, 2H, Ph), 7.43–7.26(m, 9H, Ph), 5.32(s, 2H, COOCH_2Ph), 4.93(d, J = 15.5 Hz, 1H, NC*H*HPh), 4.44(s, 2H, CH_2Br), 4.37–4.18(m, 3H, NCH*H*Ph, CH_2OCOPh), 3.78(m, 1H, NC*H*), 2.60–1.93(m, 4H, 2 × CH$_2$).

^{13}C NMR δ: 175.2(N*C*O), 165.8(*C*OO), 165.4(*C*OO), 143.1, 141.7, 135.8, 130.0, 129.9, 129.3, 129.0, 128.5, 128.1, 128.0, 127.6, 66.5(N*C*H$_2$Ph), 64.4(*C*H$_2$OCOPh), 56.1(N*C*H), 44.5(*C*H$_2$PhCOO), 31.9(*C*H$_2$Br), 29.8(*C*H$_2$CO), 21.6(*C*H$_2$CH$_2$CO).

MS (ESI) m/z (%): 536 [(M+H$^+$), 80 for ^{79}Br] and 538 [(M+H$^+$), 100 for ^{81}Br].

4.21. Biological assays in vitro

Each experiment in vitro was performed at least in triplicate and the standard deviation of absorbance was less than 10% of the mean.

4.21.1. Determination of the radical scavenging activity (RSA) against DPPH[24,38]

An equal volume of the compounds (final concentration 0.05 and 0.1 mM dissolved in DMSO) was added to an ethanolic solution of DPPH (0.05 mM in absolute ethanol). The mixture was vigorously shaken and allowed to stand for 20 or 60 min; absorbance at 517 nm was determined spectrophotometrically and the percentage of activity was calculated. The radical scavenging activity (RSA) was assessed as a percentage of DPPH discolouration, using the equation: % RSA = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the DPPH solution with the tested compound-sample and A_{DPPH} is the absorbance of the DPPH solution.[39] All tests were undertaken on three replicates and the values (results presented in Table 1) were averaged and compared with the appropriate standard NDGA. Ethanol was used as a control.

4.21.2. Soybean lipoxygenase inhibition

DMSO solution of the tested compound was incubated with sodium linoleate (0.1 mM) and LOX (0.2 mL, 1:9 × 10^{-4} m/v in saline) at room temperature. The conversion of sodium linoleate to 13-hydroperoxylinoleic acid was recorded at 234 nm and compared with the standard inhibitor caffeic acid (Table 1), according to the procedure previously reported.[24,38]

4.21.3. Inhibition of linoleic acid lipid peroxidation[40]

Oxidation of linoleic acid to conjugated diene hydroperoxide in an aqueous dispersion was monitored at 234 nm. AAPH was used as a free radical initiator. Linoleic acid dispersion (10 μL, 16 mM) was added to the UV cuvette containing phosphate buffer pH 7.4 (0.93 mL, 0.05 M) at room temperature. The oxidation reaction was initiated under air by the addition of AAPH solution (50 μL, 40 mM). Oxidation was carried out in the presence of compounds (10 μL, 0.1 mM). In the assay with no antioxidant, lipid oxidation was measured in the presence of the same level of DMSO. The rate of oxidation was monitored at 37 °C by recording the increase of absorption at 234 nm caused by conjugated diene hydroperoxides. The results were compared to the standard inhibitor trolox.

4.22. Biological assays in vivo

4.22.1. Inhibition of the carrageenin-induced edema[24,38]

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. Female pregnants 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 compound (0.01 mmol/kg body weight), were suspended in water, with few drops of Tween 80 and ground in a mortar before use and then they were given intraperitoneally simultaneously with the carrageenin injection. The rats were euthanized 3.5 h after carrageenin injection. The difference between the weight of the injected and uninjected paws was calculated for each animal. The change in paw weight was compared with that in control animals (treated with water) and expressed as a percent inhibition of the edema% ICPE values (Table 1). Indomethacin was tested as a reference compound in 0.01 mmol/kg (47%).

Values% ICPE are the mean from two different experiments with a standard error of the mean less than 10%.

4.23. Computational methods

The structures of compounds, **6a**, **13c**, and **14d** were constructed using the 2D sketcher module of SYBYL 8.0 molecular modeling interface 41. The structures were minimized using Tripos Force Field, Steepest Descent Conjugated Gradient and Powell algorithms (termination: Gradient 0.01 kcal/mol, max iterations: 5000). The conformational space of the compounds was explored using the Simulated Annealing method.[41,42] The compounds were

heated at 2000 K for 2000 fs and annealed at 0 K for 10,000 fs for 100 cycles. This method resulted to 100 low energy conformations for each compound that were used for docking calculations.

The X-ray crystal structure LOX (pdb entry: 1IK3) was obtained from RCSB Protein Data Bank in order to get the detailed sights of the interactions between the enzyme and compounds. Prior to docking studies a validation test was performed in order to reproduce the conformation of the co-crystallized ligand 13-HPOD, derived from linolenic acid, at the active site of LOX-3. The validation test using Surflex-Dock[43–45] predicted the binding mode of the ligand at the active site of the enzyme with RMSD 0.65.

The parameters used for validation test were used for all docking calculations followed. The crystal structure (1IK3) was prepared using the Protein Preparation Wizard utility provided by Schrödinger.[46] According to this utility bond orders were assigned, water molecules of crystallization were removed, hydrogen atoms were added and het states were generated for pH 7.0 for amino acids Histidine, Glutamic acid and Aspartic acid. The 3D structure of the protein was further refined using restraint minimization method for hydrogens only and constraint was set to 0.3 Å (Force field OPLS 2005). Docking calculations using Surflex-Dock referred to 1IK3 were performed through protomol generation by ligand. The parameters used were threshold 0.3 and bloat 0. At the Surflex-Dock panel was chosen to perform pre- and post-dock minimization and to provide five possible solutions for each annealed conformation.

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