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Design, synthesis and SAR studies of 4-allyoxyaniline amides as potent 15-lipoxygensae inhibitors

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ABSTRACT

A group of 4-allyloxyaniline amides **5a–o** were designed, synthesized and evaluated as potential inhibitors of soybean 15-lipoxygenase (SLO) on the basis of eugenol and esteragol structures. Compound **5e** showed the best IC_{50} in SLO inhibition (IC_{50} = 0.67 ± 0.06 μM). All compounds were docked in SLO active site retrieved from RCSB Protein Data Bank (PDB entry: 1IK3) and showed that allyloxy group of compounds is oriented towards the Fe^{3+}-OH moiety in the active site of enzyme and fixed by hydrogen bonding with two conserved His^{513} and Gln^{716}. It is resulted that molecular volume of the amide moiety would be a major factor in inhibitory potency variation of the synthetic amides, where the hydrogen bonding of the amide group could also involve in the activity of the inhibitors.

1. Introduction

It is well documented that mammalian lipoxygenases (LOs) are non-heme iron-containing enzymes responsible for the oxidation of polyunsaturated fatty acids and esters to hydroperoxy derivatives.[1] These are heterogeneous families of enzymes distributed widely throughout the plant and animal kingdoms,[2] and named according to the position at which a key substrate, arachidonic acid (AA), is oxidized. Among the mammalian lipoxygenases involved in the etiology of human disease, 5-lipoxygenase (5-LO) is now well established as a target for reducing the production of leukotrienes (important in particular asthma).[3,4] More recently, 15-lipoxygenase (15-LO) has emerged as an attractive target for therapeutic intervention.[5] 15-LO has been implicated in the progression of certain cancers[6,7] and chronic obstructive pulmonary disease (COPD).[6] Evidence for the inhibition of 15-LO in the treatment of vascular disease is, however, most compelling.[8] Both transgenic and knockout studies implicate a role for 15-LO in atherogenesis.[9,10] The enzyme is abundantly expressed in macrophages residing within the atherosclerotic lesion.[5] In addition, the immediate products of 15-LO oxidation of AA and linoleic acid (LA) have been shown to be pro-inflammatory[11] and pro-thrombotic.[12]

It is also found that 15-LO is linked to cardiovascular complications since it is known to participate in oxidative modification of low-density lipoproteins (LDL) leading to the development of atherosclerosis.[13]

Three different strategies have been developed to inhibit the LO's pathway.[12] They involve (i) redox inhibitors or antioxidants, which interfere with the redox cycle of 15-LO, (ii) iron-chelator agents and (iii) non-redox competitive inhibitors, which compete with AA to bind the enzyme active site.

There is reasonable homology between the SLO and the human one.[14] This homology becomes more identical (50%) within 8 Å in the active site pocket. Obviously soybean enzyme is much more accessible than the human one. Therefore, one can expect that the results can be extendable to the human LO.

Recently we reported the results of our studies on the soybean lipoxygenase (SLO) inhibitory activities of some eugenol esters and on the basis of the SAR (structure activity relationship) studies we suggested that the inhibitory activity of these molecules largely depends on the orientation of allyl group towards chelated Fe^{3+}-OH and the molecular volume of carboxylate moiety in active site pocket of the enzyme without hydroperoxidation of allylic carbons.[14] In this paper we wish to report the results of a comparative study on the 15-LO inhibitory activities of benzoate of eugenol, chavicol (4-allylphenol), 4-allyloxyphenol and a group of 4-allyloxyaniline amides.

2. Result and discussion

Considering our previous work on eugenol and esters[14] we tested the inhibitory property of eugenol, eugenyl benzoate, meth-

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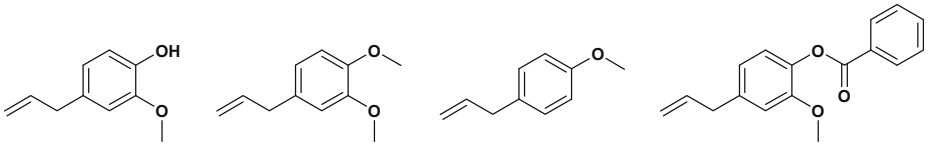


Figure 1. Molecular structure of some of allylbenzene analogs as lipoxygenase inhibitors.

Table 1
Enzyme inhibitory assessment, Van Der Waals molecular volum (VDW), HOMO energy and docking analysis data of consensus conformers of eugenol, methyleugenol, esteragol, eugenyl benzoate, compounds **1**, **2** and **5a–o**

Compound	IC_{50}	K_i	E_d (kcal/mol)	ΔG_b (kcal/mol)	RMSD (Å)	E_{HOMO} (eV)	VDW
Eugenol	38.2 ± 1.9	—	—	—	—	—	—
Methyleugenol	96.1 ± 3.3	—	—	—	—	—	—
Estragol	64.1 ± 1.5	—	—	—	—	—	—
Eugenyl benzoat	7.0 ± 0.4	2.09e−6	−9.49	−7.75	22.89	—	—
1	6.7 ± 0.2	2.74e−6	−8.55	−7.59	23.87	—	—
2	6.2 ± 0.7	4.03e−6	−8.78	−7.36	23.65	—	—
5a	6.1 ± 0.2	2.65e−5	−8.05	−6.24	23.79	−10.46	55.68
5b	2.2 ± 0.2	1.17e−5	−8.85	−6.73	23.11	−10.70	71.02
5c	4.1 ± 0.3	1.08e−6	−9.27	−8.14	23.43	−10.77	86.06
5d	6.4 ± 0.7	5.49e−7	−9.99	−8.54	24.07	−10.85	101.80
5e	0.67 ± 0.06	7.65e−9	−12.36	−11.07	23.12	−10.56	148.82
5f	3.3 ± 0.1	1.77e−6	−9.72	−7.58	23.45	−10.94	86.64
5g	3.8 ± 0.1	1.21e−6	−9.91	−8.07	23.47	−11.11	89.18
5h	5.6 ± 0.1	1.42e−6	−9.67	−7.98	23.60	−11.67	89.18
5i	10.1 ± 0.6	1.68e−6	−9.76	−7.88	23.02	−10.87	103.38
5j	6.7 ± 0.4	6.33e−7	−10.17	−8.64	23.42	−10.91	103.38
5k	8.8 ± 0.7	1.68e−6	−9.54	−7.99	23.15	−11.13	100.95
5l	8.1 ± 0.8	1.50e−6	−9.65	−7.94	23.39	−11.15	100.95
5m	13.8 ± 0.6	8.82e−7	−9.90	−8.26	22.91	−10.68	111.18
5n	17.0 ± 0.2	1.15e−6	−9.90	−8.10	23.37	−10.91	111.18
5o	12.9 ± 0.3	1.45e−5	−8.27	−6.60	23.72	−10.88	62.72

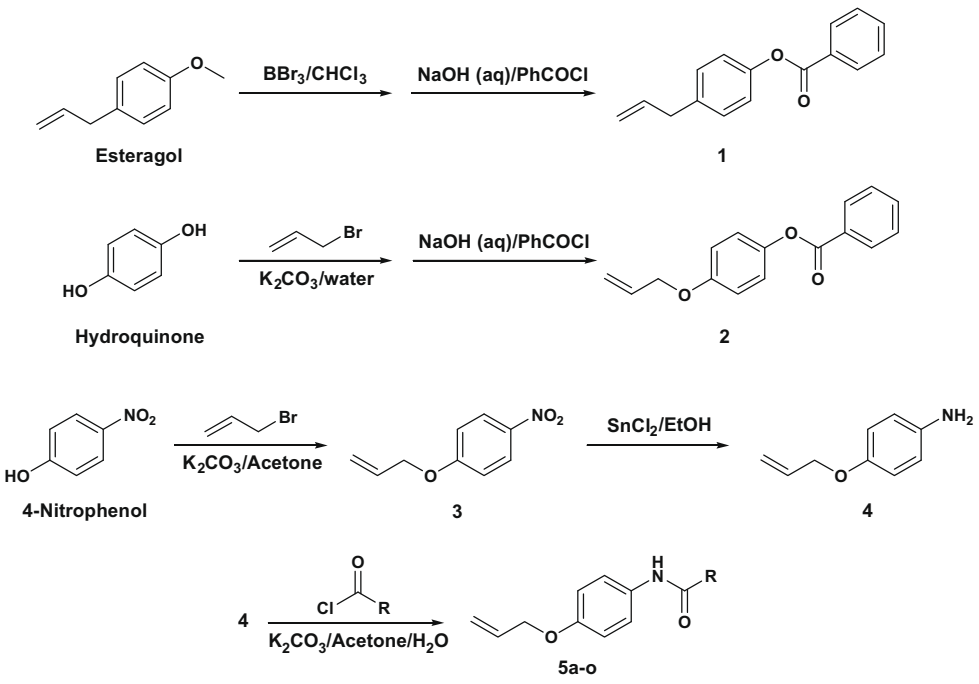
ΔG_b: Estimated free energy of bonding, E_d: final docking energy, K_i: estimated inhibition constant and RMSD: root mean square deviation from reference structure. The IC_{50} values are given as mean ± SD.

yleugenol and esteragol (Fig. 1) on the SLO (substrate: linoleic acid). The results showed IC_{50} = 38.2 ± 1.9, 7.0 ± 0.4, 96.1 ± 3.3 and 64.1 ± 1.5 μM for the mentioned compounds, respectively (Table 1). It is notable that no other products such as hydroperoxy are isolated from action of the LO enzyme on eugenyl benzoate, methyleugenol and esteragol as substrate (assuming hydroproxy is supposed to be obtained if the redox pathway is blocked and the inhibitor acts through its allylic group in reaction with the enzyme active site similar to the oxidation of natural unsaturated fatty acids).[†] Considering the IC_{50} results of the above compounds, we decided to demethylate esteragol and synthesize the benzoate ester **1** (Scheme 1) and study its inhibitory potency. Based on the IC_{50} of compound **1** (Table 1) comparing to eugenyl benzoate, it is suggested that the methoxy group of eugenyl benzoate have no effective role in inhibitory potency. Then the allyl group of compound **1** was replaced with allyloxy (Scheme 1) to study the effect of HOMO energy of allyl group on inhibitory potency. Interestingly, the IC_{50} of allyloxy analog **2** was comparable with compound **1** (Table 1). The experimental results matched with theoretical K_i of docking study for those models in which allylic double bound oriented towards iron atom similar to orientation of linoleic acid (LA) in the active site of SLO (Fig. 2A). We generated 100 docked conformers of desired compounds corresponding in AutoDockTools software.[15] One conformer from each esters cluster which had more similarity with optimum conformer (lowest K_i) of eugenyl benzoate was adopted as the 'consensus' structure and used for further analysis.[14,16] The results of docking analysis showed that the consensus structure of eugenyl benzoate, compounds **1** and **2** have the similar estimated inhibitory constant (K_i) in a rang of 2–4 × 10^{-6} (Table 1).

It seems that the allyl and allyloxy benzene portion of the compounds has hydrophobic interaction with Ile^{557}, Leu^{565}, Leu^{773} and Ile^{572}, respectively in such an orientation (Fig. 2A). The most critical residues that is Ile^{557}, Leu^{565}, Leu^{773} and Ile^{572} are close to the active site. X-ray presentation of LA into SLO[17] indicates that Ile^{557}, Leu^{565} and Leu^{773} lay within 4–6 Å of Fe^{3+}-OH and both Leucines are the proximity of the reactive C-11–C-13 of LA (C-11: hydrogen abstraction site, C-13: oxygenation site). Although Ile^{572} is far from Fe^{3+}-OH, (at 9 Å) but still forms part of the substrate-bonding cavity. Each of these residues provides a large surface to interact with natural substrate, particularly Leu^{565} and Leu^{773}. Mutating large residues such as Ile or Leu to an Ala opens up space within the bonding pocket of SLO, leading to altered H· transfer kinetics.[18] The Ile^{557}→Ala and Ile^{572}→Phe mutants decreased k_{cat} by twofold from WT (wild type), While Leu^{565}→Ala and Leu^{773}→Ala decreased k_{cat} by 60- and 1000-fold, respectively, indicating that these hydrophobic residues (specially Leu^{565} and Leu^{773}) contribute significantly to catalysis.[18] According to the result of multiple alignment, three amino acids Ile^{557}, Leu^{565} and Leu^{773} are found to be conserved over all species (Fig. 3).

As shown in Figure 2A, the molecules in presented orientation has hydrophilic and hydrophobic interaction with conserved His^{513}, Gln^{514} and Gln^{716}. The conserved amino acids Gln^{514} and Gln^{716} play a key role in oxidation potential of Fe^{3+} via hydrogen

[†] Substrate (100 μM) was reacted with soybean LO enzyme (167 U/mL) in 3 mL borate buffer solution (0.1 M, pH 9) at 20 °C for 15 min. The mixture was then analyzed by UV at 230–270 nm and no absorption of vinyl benzene formation was appeared over the blank solution.



Compd.	R	Compd.	R	Compd.	R
5a		5f		5k	
5b		5g		5l	
5c		5h		5m	
5d		5i		5n	
5e		5j		5o	

Scheme 1. General procedures for the synthesis of compounds **1**, **2** and **5a–o**.

bonding with Asn^{713} and His^{518}.[19] This hydrogen bond network is exist in both SLO and 15-RLO (rabbit 15-LO) structures and also play a steric role in orienting the substrate and inhibitor bound to LO.[19] The C-3–C-8 hydrocarbon tail of LA is flanked by the hydrophobic portion of the Gln^{514} and Gln^{716} (Fig. 2A). Disrupting this bonding pocket by changing the position of Gln^{514} and Glu^{716} may affect the proper positioning of the substrate for C-H bond cleavage so that abstraction becomes more rate-limiting (as was observed in the Gln^{514}→Ala, Gln^{716}→Asn and Gln^{716}→Glu mutants by 4-, 3- and 6-fold decrease in k_{cat} from WT SLO, respectively[19]). Proposed inhibitory model of docked molecules have hydrogen bond with Gln^{716} via C=O portion except compound **2**. The aromatic part of benzoate moiety in eugenyl benzoate, compound **1** and **2** is flanked by the hydrophobic portion of the Gln^{716} side chain like LA (Fig. 2A). As seen in Figure 2A, the benzoate moiety of compound **2** is in the far distance of the pocket then the same moiety of compound **1**, causes the hydrogen bonding of C=O portion with His^{513} instead of Gln^{716}. Considering the orientation of ester moiety of compound **2** towards His^{513} and Gln^{716}, we decided to replace the esteric bond by amide to achieve two hydrogen bonds between the mentioned amino acids and inhibitor (this can obviously hold the inhibitor more tightly in the active site pocket). Upon this suggestion, 4-allyloxyphenylbenzamide (**5f**) was synthesized (Scheme 1) and its inhibitory potency was determined. The IC_{50} value of **5f** (3.3 ± 0.1 μM) was nearly two fold less than **2**, which was in accordance with our prediction.

In the next step, other benzoate and cycloalkylate analogs **5a–e** and **5g–n** were designed, synthesized and docked into the active site to obtain the best inhibitor. The synthetic amids **5a–n** showed a broad range of inhibition activity on the enzyme (IC_{50} = 0.67–17 μM; Table 1). Compound **5e** having an adamantanecarboxylate substituent was the most potent inhibitor at 0.67 μM while the 3- and 4-methoxybenzamid analogues (**5n** and **5m**) presented less activity (IC_{50} = 17.0 and 13.8 μM, respectively).

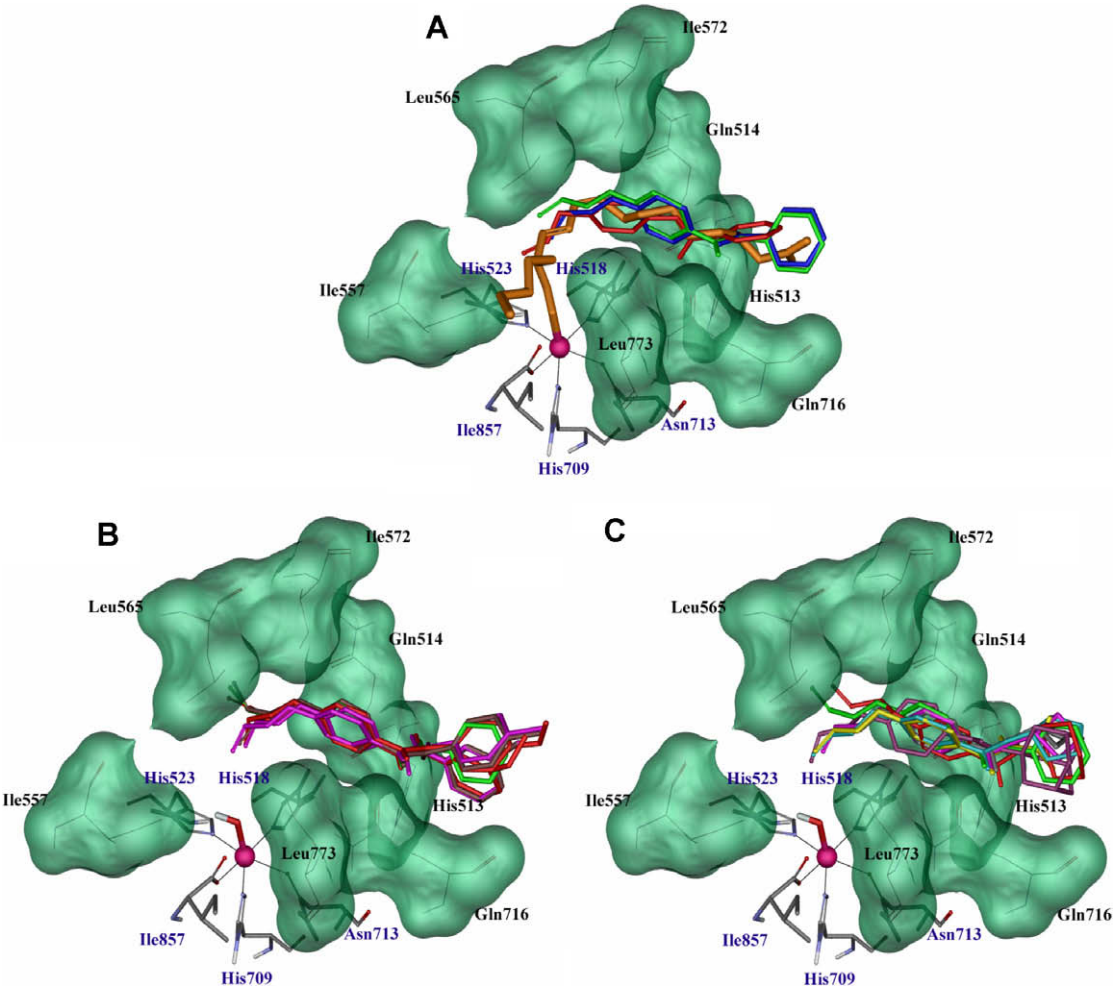


Figure 2. Consensus bonding conformations (stick view) of compounds **1** (red), **2** (blue) and **5f** (green) with linoleic peroxide (orange) bonded to Fe in the SLO active site (A). Consensus bonding conformations (stick view) of compounds **5f**–**n** (B) and **5a**–**e** (C) in the SLO active site. The amino acids bonded to Fe are shown in stick.

The K_i of consensus structure of amides **5a–n**, did not show rational relation with IC_{50} results. This may comes from tendency of the amide moiety for making hydrogen bond with His^{513} and Gln^{716} side chains (Fig. 4). This result can be explained by considering HOMO energy and Van Der Waals volume (VDW) of amide moiety of compounds **5a–n** (Table 1). A non-linear relation between IC_{50} values and VDW of the amides was observed except for **5e** (Fig. 5). The best IC_{50} belongs to the compounds with molecular volume of 70–80 $Å^3$ (Fig. 5). The proposed relation in Figure 5 between IC_{50} and molecular volume is only reserved for expansion of the amide moiety in two dimensions while the adamantanamide group (compound **5e**) which also expand in the third dimension dose not follow the series. Considering the free space which is formed by Val^{372}, Ser^{510}, Phe^{576}, Gln^{716}, Gly^{720}, Ile^{723}, Arg^{726}, Thr^{728}, Asp^{766} and Ile^{770} (amide pocket—Fig. 7), we expected to see direct relation between molecular volume and inhibitory potency for compounds **5a–e** but it did not. As the HOMO energy is closely related to the electronic density of C=O group and therefore support the hydrogen bonding potency,[20] it is assumed that HOMO energy of the amide moiety could be responsible for this observation.

In this regard we found an interesting exception in cyclopropyl analog. We expected higher IC_{50} for this analog than the others of the series (**5b–e**) regarding its smaller volume but the lower IC_{50} is being explained considering higher HOMO (E_{HOMO} = −10.46 eV) and more strong hydrogen bound of the C=O group. To prove this observation, isopropylcarboxamide analog (**5o**) with comparable molecular volume to cyclopropyl (62.72 vs 55.68 $Å^3$) but having lower HOMO energy of amide moiety (E_{HOMO} = −10.88 eV) was synthesized and its inhibitory potency determined. The IC_{50} result of **5o** (12.9 ± 0.3 μM) was a good evidence for proving the mentioned hypothesis. With the exception of **5a**, we see a good non-linear relationship between IC_{50} value and E_{HOMO} for compounds **5b–e** and **5o** (Fig. 6).

It is clear that the inhibitory potency of the aromatic analogs decrease while their size increases (Fig. 5). We can not see such a relationship between IC_{50} and E_{HOMO} for the aromatic analogs. It might be due to two dimensional expansion of *meta* and *para* substituent of phenyl portion (it is more than the expansions of cycloalkane analog) which cause steric hindrance in filling the amide pocket. The steric hindrance can disturb the planarity between phenyl portion and C=O and therefore decreasing in mesomeric effects.[20] The HOMO energy level of amide moiety of **5f–n** can rationalize the IC_{50} value deference between *meta* and *para* isomer. For example compound **5g** with less IC_{50} value in comparison with its isomer **5h** (3.8 ± 0.1 vs 5.6 ± 0.1 μM), has higher HOMO energy level and it is the same for compounds **5k** and **5l** (IC_{50} = 8.8 ± 0.7 and 8.1 ± 0.8 μM, respectively).

In summary we have carried out a SAR comparative studies on allyl and allyloxy benzene derivatives as 15-lipoxygenase inhibi-

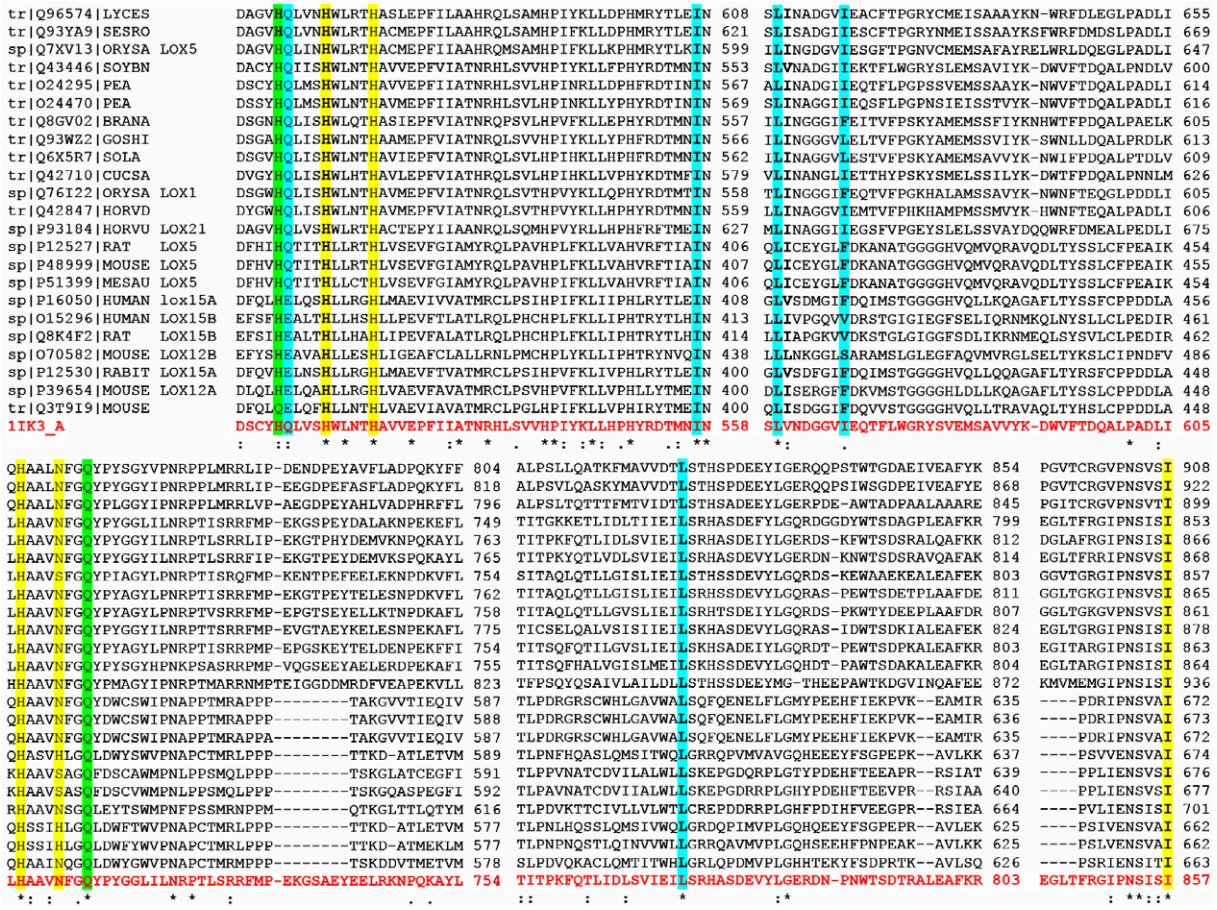


Figure 3. Multiple alignment of SLO (1ik3_A). The conserved residues which have hydrogen bond and lipophilic interactions with docked ligands are highlighted in green and blue, respectively. The iron-bond residues are highlighted in yellow.

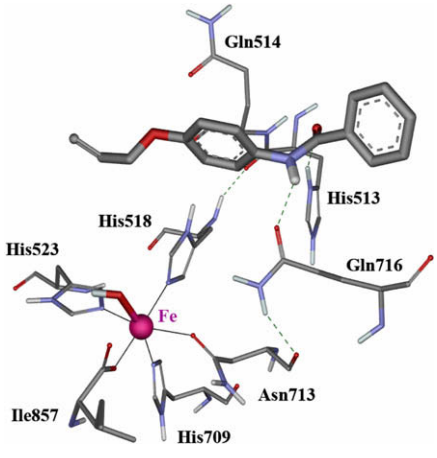


Figure 4. Stick view of compound **5f** interacting with His^{513} and Gln^{716} via hydrogen bonding of amid bond. Hydrogen bonds are revealed by dashed green lines.

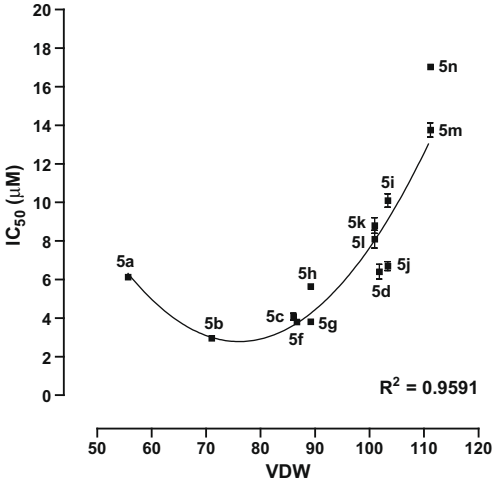


Figure 5. Diagram of IC_{50} value versus Van Der Waals molecular volume (VDW) for compounds **5a–d** and **5f–n**.

tors. It could be suggested that the application of single point properties like HOMO energy might be applicable to predict the inhibitory potency the synthetic amides. This study has also shown the important role of molecular volume in the inhibitory activity of 4-allyloxyaniline amides. The importance of these compounds could be more highlighted when we rank their easy synthesis pathway and their high yield.

3. Materials and methods

3.1. Chemistry

Compound **1** was synthesized via demethylation of esteragol using boron tribromide[21] and then the followed with benzoyl chlo-

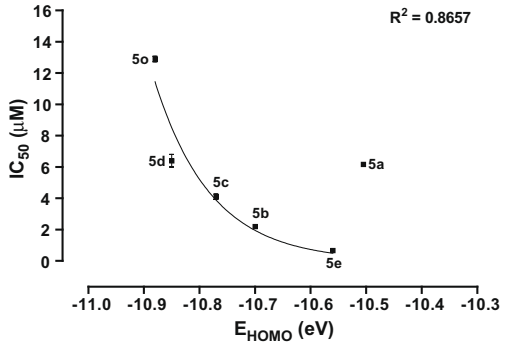


Figure 6. Diagram of IC_{50} value versus HOMO energy (E_{HOMO}) of the amide moiety for compounds **5a–e** and **5o**.

ride in aqueous solution of sodium hydroxide.[14] Compound **2** was synthesized by allylation of hydroquinone in the presence of saturated potassium carbonate in water[22] and immediate reaction with benzoyl chloride in aqueous solution of sodium hydroxide. The amides **5a–o** were prepared by reaction of desired acid chlorides with 4-allyloxyaniline (**4**) in the presence of potassium carbonate in acetone–water.[23] 4-Allyloxyaniline was synthesized via reduction of 4-allyloxynitrobenzene (**3**)[24] using stannous chloride.[25]

3.2. Molecular modeling, docking and SAR study

3.2.1. Multiple alignment

Conserved amino acids were identified through multiple alignment in clustalX 1.81.[26] Sequences of lipoxygenase (LO) family were selected from blasted sequences via ExPASY proteomics server.[27] Multiple alignment process was then carried out on the selected sequences (protein weight matrix: BLOSUM series, opening gap penalty = 10).

3.2.2. Calculations

Structures of desired compounds were simulated in chem3D professional; Cambridge software; using MM2 method (RMS gradient = 0.05 kcal/mol).[28] Output files were minimized under semi-empirical AM1 method in the second optimization (Convergence limit = 1e−5; Iteration limit = 100; RMS gradient = 0.05 kcal/mol; Fletcher-Reeves optimizer algorithm) in HyperChem7.5.[29,30] Single point properties of molecules such as energy of HOMO and LUMO were calculated using ab initio RHF/6-311G* methods (Convergence limit = 1e−5; Iteration limit = 100) in HyperChem7.5. In this study Van Der Waals molecular volume (VDW) was measured by QSAR properties tool in HyperChem7.5.

Crystal structure of soybean lipoxygenase-3 (arachidonate 15-lipoxygenase) complex with 13(*S*)-hydroproxy-9(*Z*)-2,11(*E*)-octadecadienoic acid was retrieved from RCSB Protein Data Bank (PDB entry: 1IK3).

3.2.3. Molecular docking

Automated docking simulation was implemented to dock eugenyl benzoate, **1**, **2** and **5a–o** into the active site of SLO with AutoDock-Tools (ADT) version 1.4[15] using Lamarckian genetic algorithm.[31] This method has been previously shown to produce bonding models similar to the experimentally observed models.[14,31,32] The torsion angles of the ligands were identified, hydrogens were added to the macromolecule, bond distances were edited and solvent parameters were added to the enzyme 3D structure. Partial atomic charges were then assigned to the macromolecule as well as ligands (Gasteiger for the ligands and Kollman for the protein).

The regions of interest of the enzyme were defined by considering Cartesian chart 19, 2 and 19 as the central of a grid size of 50, 50 and 50 points in *X*, Y and *Z axes*. The docking parameter files were generated using Genetic Algorithm and Local Search Parameters (GALS) while number of generations was set to 100. The mentioned compounds were each docked into the active site of SLO enzyme and the simulations were composed of 100 docking runs, each of 50 cycles containing a maximum of 10,000 accepted and rejected steps. The simulated annealing procedure was started at high temperature (RT = 616 kcal/mol, where R is the gas constant and T is the steady state temperature) and was decreased by a fraction of 0.95 on each cycle[33] The 100 docked complexes were clustered with a root-mean-square deviation tolerance of 0.2 Å. Autodock generated 100 docked conformers of **1**, **2** and **5a–o**-corresponding to the lowest-energy structures. After docking procedure in ADT4, docking results were submitted to Weblab Viewerlite 4.0[34] and Swiss-PdbViewer 3.7 (spdbv)[35] for further evaluations. The results of docking processing (ΔG_b: estimated free energy of bonding, E_d: final docked energy and K_i: estimated inhibition constant) are outlined in Table 1.

3.3. 15-LO inhibitory assessment

Lipoxygenase activity was measured in borate buffer solutions (0.1 M, pH 9) using the method described in literature,[36,37] by measuring the absorbance at 234 nm for 60 s after addition of the enzyme (soybean 15-lipoxygenase), and linoleic acid (final

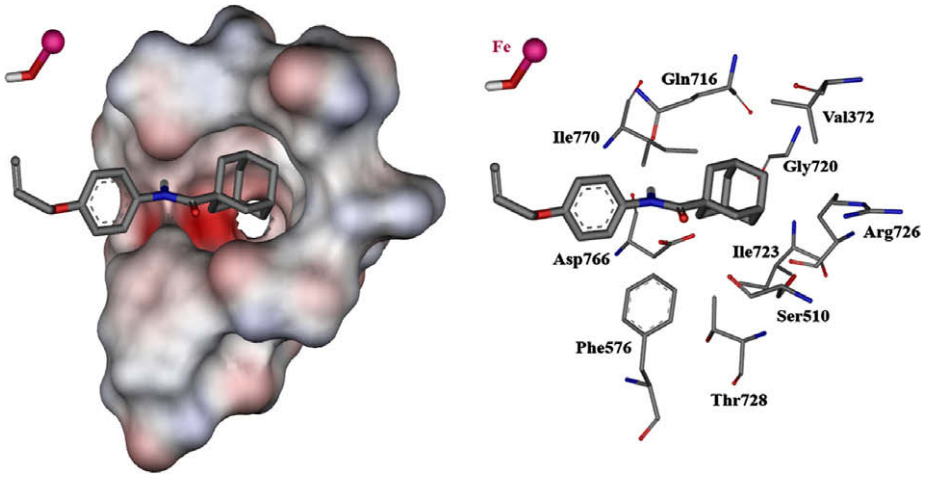


Figure 7. The cavity for amide moiety of consensus structure of compounds **5a–o** is shown in stick (right) and solvent surface (left) view.

concentration: 134 μM) as substrate at 20 ± 1 °C. The final enzyme concentration was 167 U/mL. Synthesized substances were added in DMSO solutions (final DMSO concentration 1%); whereas DMSO was added in control experiments with no inhibitor. The mixture of each inhibitors and linoleic acid was set as blank sample in testing step. At least six control test tubes and three tubes for each inhibitor solution were measured. To ensure constant enzyme activity throughout the experiment, the enzyme solution was kept in ice, and controls were measured at regular intervals. Calculation of enzyme activity was carried out as previously described[37] and IC_{50} values were determined by linear interpolation between the points around 50% activity.

4. Experimental

4.1. Instruments

Melting points were recorded on an Electrothermal type 9100 melting point apparatus. The ^{1}H NMR (500 MHz) spectra were recorded on a Bruker Avance DRX-500 spectrometer. Elemental analysis was obtained on a Thermo Finnigan Flash EA microanalyzer. The IR spectra were obtained on a 4300 Shimadzu Spectrometer. All measurements of lipoxygenase activities were carried out using an Agilent 8453 spectrophotometer. The soybean 15-lipoxygenase and other chemicals were purchased from Sigma, Aldrich and Merck Co., respectively.

4.2. 4-Allylphenyl benzoate (1)

A solution of 0.30 g (2 mmol) of esteragol in 5 mL of $CHC1_3$ was added during 2 min to a well-stirred solution of 3.0 g (12 mmol) of BBr_3 in 35 mL of $CHC1_3$ maintained in the range 23–26 °C. Stirring was continued for 15 min at 23–26 °C. The reaction mixture was then poured into a well-stirred mixture of 30 g of ice-water. After 15 min the organic phase was separated, washed with water (2 × 20 mL) and then extracted by NaOH 5% (2 × 10 mL). The extract was washed with $CHCl_3$ (2 × 20 mL) and then acidified by HCl 10% to appearance of milky emulsion of 4-allylphenol. The product was extracted by ether and after removal of the solvent, dissolved in stirred NaOH 5% (5 mL) and then benzoyl chloride (0.3 mL) was added. After 30 min stirring, the precipitate of compound **1** was separated and recrystallized from methanol 90% (0.32 g, 67% yield).

White solid; mp: 61–62 °C; ^{1}H NMR ($CDCl_3$): δ 3.42 (d, J = 6.6 Hz, 2H, –CH_2–), 5.79–6.23 (m, 2H, H_2C=), 5.94–6.33 (m, 1H, HC=), 7.00–7.36 (m, 5H, H-2, H-4, H-3′, H-4′, H-5′), 7.56 (d, J = 9.5 Hz, 2H, H-3, H-5), 8.24 (d, J = 7.9 Hz, 2H, H-2′, H-6′); IR cm^{-1}: 1735 (OC=O). $C_{16}H_{14}O_2$ requires: C, 80.65; H, 5.92. Found: C, 80.39; H, 5.83.

4.3. 4-(Allyloxy)phenyl benzoate (2)

A mixture of 5.5 g (50 mmol) of hydroquinone, 6.6 g (55 mmol) of allyl bromide and 7.0 g of anhydrous potassium carbonate (50 mmol) in water (12 mL) was refluxed for 5 h and cooled. The mixture was extracted with ether (2 × 20 mL) and washed with water (3 × 20 mL). After removal of the solvent, the residual oil was dissolved in stirred NaOH 10% (50 mL) and then benzoyl chloride (7.0 mL) was added. After 30 min stirring, the precipitate of compound **2** was separated and recrystallized from ethanol (5.5 g, 43% yield).

White solid; mp: 102–103 °C; ^{1}H NMR ($CDCl_3$): δ 3.40 (d, J = 6.6 Hz, 2H, –CH_2–), 4.94–5.19 (m, 2H, H_2C=), 5.74–6.19 (m, 1H, HC=), 7.56 (d, J = 9.5 Hz, 2H, H-3, H-5), 7.20–7.38 (m, 5H, H-2, H-4, H-3′, H-4′, H-5′), 8.22 (d, J = 7.9 Hz, 2H, H-2′, H-6′); IR cm^{-1}: 1730 (OC=O). $C_{16}H_{14}O_3$ requires: C, 75.57; H, 5.55. Found: C, 75.37; H, 5.53.

4.4. General procedure for preparation of compounds 5a–o

A mixture of 69.5 g (0.50 mol) of 4-nitrophenol, 66.0 g (0.55 mmol) of allyl bromide and 70.0 g of anhydrous potassium carbonate (0.50 mol) in dry acetone (150 mL) was refluxed for 8 h and cooled. The mixture was diluted with water (250 mL) and then extracted with ether (2 × 150 mL). The combined extracts were washed with NaOH 10% (2 × 100 mL) and dried with anhydrous sodium carbonate. After removal of the solvent the residual oil of **3** was distilled under reduced pressure (74.4 g, 83% yield).

A mixture of 64.4 g (0.36 mol) of **3** and 406.0 g (1.80 mol) of $SnCl_2{\cdot}2H_2O$ in 600 mL of absolute ethanol was refluxed under nitrogen for 20 min. After cooling; the mixture was poured into cold water (1500 mL). The pH was made basic by adding sodium bicarbonate. The 4-(allyloxy)aniline (**4**) was extracted by ethyl acetate (3 × 300 mL). The combined organic phase dried with anhydrous sodium sulfate. After removal of the solvent the residual oil of **4** was distilled under reduced pressure (40.3 g, 75% yield).

To a stirred mixture of **4** (1.1 g, 7.3 mmol), potassium carbonate (1.5 g, 10.8 mmol), water (10 mL), and acetone (5 mL), was added desired acid chlorides (9.0 mmol) at 0 °C dropwise. The mixture was stirred at room temperature for 30 min and then diluted with water (20 mL). The resulting precipitate of **5a–o** was separated and recrystallized from ethanol.

4.4.1. 1-(Allyloxy)-4-nitrobenzene (3)

Colourless oil; pb (3 mm): 126–129 °C; ^{1}H NMR ($CDCl_3$); δ 3.47 (s, 2H, –NH_2), 4.49 (d, J = 5.20 Hz, 2H, –CH_2–), 5.18–5.53 (m, 2H, H_2C=), 5.89–6.29 (m, 1H, HC=), 6.88 (d, J = 8.90 Hz, 2H, H-3, H-5), 7.48 (d, J = 8.90 Hz, 2H, H-2, H-6).

4.4.2. 4-(Allyloxy)aniline (4)

Colourless oil; bp (3 mm): 108–110 °C; ^{1}H NMR ($CDCl_3$); δ 3.47 (s, 2H, –NH_2), 4.49 (d, J = 5.20 Hz, 2H, –CH_2–), 5.18–5.53 (m, 2H, H_2C=), 5.89–6.29 (m, 1H, HC=), 6.7 (AB, qarter, 4H, H-2, H-3, H-5, H-6).

4.4.3. *N*1-(4-(Allyloxy) phenyl)-1-cyclopropanecarboxamide (5a)

White solid; mp: 147–148 °C; ^{1}H NMR ($CDCl_3$): δ 0.20–1.20 (m, 4H, –CH_2– (cyclopropyl)), 1.24–1.73 (m, 1H, –CH– (cycloropyl)), 4.51 (d, J = 5.1 Hz, 2H, –CH_2–), 5.17–5.54 (m, 2H, H_2C=), 5.85–6.27 (m, 1H, HC=), 6.83 (d, J = 8.94 Hz, 2H, H-3, H-5), 7.42 (d, J = 8.94 Hz, 2H, H-2, H-6), 7.53 (s, 2H, –NH–); IR cm^{-1}: 1653 (NC=O). $C_{13}H_{15}NO_2$ requires: C, 71.87; H, 6.96; N, 6.45. Found: C, 72.02; H, 6.93; N, 6.41.

4.4.4. *N*1-(4-(Allyloxy) phenyl)-1-cyclobutanecarboxamide (5b)

White solid; mp: 129–130 °C; ^{1}H NMR ($CDCl_3$): δ 1.21–2.12 (m, 8H, –CH_2– (cyclobutyl)), 2.96–3.32 (m, 1H, –CH– (cyclobutyl)), 4.53 (d, J = 5.1 Hz, 2H, –CH_2–), 5.20–5.52 (m, 2H, H_2C=), 5.88–6.29 (m, 1H, HC=), 6.84 (d, J = 9 Hz, 2H, H-3, H-5), 7.12 (s, 2H, –NH–), 7.42 (d, J = 9 Hz, 2H, H-2, H-6); IR cm^{-1}: 1652 (NC=O). $C_{14}H_{17}NO_2$ requires: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.89; H, 7.33; N, 6.05.

4.4.5. *N*1-(4-(Allyloxy) phenyl)-1-cyclopantanecarboxamide (5c)

White solid; mp: 138–140 °C; ^{1}H NMR ($CDCl_3$): δ 1.64–1.91 (m, 8H, –CH_2– (cyclopentyl)), 2.50–2.85 (m, 1H, –CH– (cyclopentyl)), 4.52 (d, J = 5.1 Hz, 2H, –CH_2–), 5.20–5.54 (m, 2H, H_2C=), 5.85–6.26 (m, 1H, HC=), 6.85 (d, J = 8.94 Hz, 2H, H-3, H-5), 7.23 (s, 1H, –NH–), 7.42 (d, J = 8.94 Hz, 2H, H-2, H-6); IR cm^{-1}: 1652 (NC=O).

$C_{15}H_{19}NO_2$ requires: C, 73.44; H, 7.81; N, 5.71. Found: C, 73.59; H, 7.83; N, 5.75.

4.4.6. *N*1-(4-(Allyloxy) phenyl)-1-cyclohexanecarboxamide (5d)

White solid; mp: 148–149 °C; ^{1}H NMR ($CDCl_3$): δ 1.10–2.36 (m, 6H, cyclohexyl), 4.50 (d, J = 5.1 Hz, 2H, –CH_2–), 5.19–5.53 (m, 2H, H_2C=), 5.84–6.26 (m, 1H, HC=), 6.85 (d, J = 8.9 Hz, 2H, H-3, H-5), 7.12 (s, 1H, –NH–), 7.40 (d, J = 8.9 Hz, 2H, H-2, H-6); IR cm^{-1}: 1645 (NC=O). $C_{16}H_{21}NO_2$ requires: C, 74.10; H, 8.16; N, 5.40. Found: C, 74.21; H, 8.13; N, 5.48.

4.4.7. *N*1-(4-(Allyloxy) phenyl)-1-admantancarboxamide (5e)

White solid; mp: 180–181 °C; ^{1}H NMR ($CDCl_3$): δ 1.76 (m, 6H, –CH_2– (adamantylyl)), 1.91–2.22 (m, 9H, –CH–, –CH_2– (adamantyl)), 4.52 (d, J = 5 Hz, 2H, –CH_2–), 5.19–5.52 (m, 2H, H_2C=), 5.88–6.28 (m, 1H, HC=), 6.88 (d, J = 8.90 Hz, 2H, H-3, H-5), 7.21 (s, 1H, –NH–), 7.48 (d, J = 8.90 Hz, 2H, H-2, H-6); IR cm^{-1}: 1650 (NC=O). $C_{20}H_{25}NO_2$ requires: C, 77.14; H, 8.09; N, 4.50. Found: C, 76.99; H, 8.15; N, 4.46.

4.4.8. *N*1-(4-(Allyloxy) phenyl)benzamide (5f)

White solid; mp: 150–151 °C; ^{1}H NMR ($CDCl_3$): δ 4.55 (d, 2H, –CH_2–), 5.22–5.58 (m, 2H, H_2C=), 5.91–6.31 (m, 1H, HC=), 6.92 (d, J = 9.5 Hz, 2H, H-3, H-5), 7.29–7.65 (m, 5H, H-2, H-4, H-3′, H-4′, H-5′), 7.86 (d, J = 7.9 Hz, 2H, H-2′, H-6′), 8.02 (s, 1H, –NH–); IR cm^{-1}: 1648 (NC=O). $C_{16}H_{15}NO_2$ requires: C, 75.87; H, 5.97; N, 5.53. Found: C, 75.79; H, 6.03; N, 5.59.

4.4.9. *N*1-(4-(Allyloxy) phenyl)-4-fluorobenzamide (5g)

White solid; mp: 169–170 °C; ^{1}H NMR ($CDCl_3$): δ 4.55 (d, J = 5 Hz, 2H, –CH_2–), 5.20–5.57 (m, 2H, H_2C=), 5.86–6.28 (m, 1H, HC=), 6.95 (m, 2H, H-3, H-5), 7.18 (d, J = 9.50 Hz, 2H, H-3′, H-5′), 7.50 (m, 2H, H-2, H-6), 7.71 (s, 1H, –NH–), 7.88 (d, J = 9.50 Hz, 2H, H-2′, H-6′); IR cm^{-1}: 1649 (NC=O). $C_{16}H_{14}FNO_2$ requires: C, 70.84; H, 5.20; N, 5.16. Found: C, 70.92; H, 5.14; N, 5.11.

4.4.10. *N*1-(4-(Allyloxy) phenyl)-3-fluorobenzamide (5h)

White solid; mp: 155–156 °C; ^{1}H NMR ($CDCl_3$): δ 4.71 (d, J = 5.1 Hz, 2H, –CH_2–), 5.35–5.70 (m, 2H, H_2C=), 6.02–6.44 (m, 1H, HC=), 7.09 (d, J = 9.8 Hz, 2H, H-3, H-5), 7.29–7.85 (m, 4H, H-2′, H-3′, H-5′, H-6′), 7.62 (d, J = 9.8 Hz, 2H, H-2, H-4), 7.94 (s, 1H, –NH–); IR cm^{-1}: 1650 (NC=O). $C_{16}H_{14}FNO_2$ requires: C, 70.84; H, 5.20; N, 5.16. Found: C, 70.69; H, 5.18; N, 5.20.

4.4.11. *N*1-(4-(Allyloxy) phenyl)-4-methylbenzamide (5i)

White solid; mp: 149–150 °C; ^{1}H NMR ($CDCl_3$): δ 2.39 (s, 3H, –CH_3), 4.50 (d, J = 5 Hz, 2H, –CH_2–), 5.20–5.48 (m, 2H, H_2C=), 5.87–6.27 (m, 1H, HC=), 6.89 (d, J = 9.8 Hz, 2H, H-3, H-5), 7.27 (d, J = 9.50 Hz, 2H, H-3′, H-5′), 7.50 (d, J = 9.8 Hz, 2H, H- 2, H-6), 7.72 (s, 1H, –NH–),7.83 (d, J = 9.50 Hz, 2H, H-2′, H-6′); IR cm^{-1}: 1632 (NC=O). $C_{17}H_{17}NO_2$ requires: C, 76.38; H, 6.41; N, 5.24. Found: C, 70.44; H, 6.33; N, 5.25.

4.4.12. *N*1-(4-(Allyloxy) phenyl)-3-methylbenzamide (5j)

White solid; mp: 108–109 °C; ^{1}H NMR ($CDCl_3$): δ 2.45 (s, 3H, –CH_3), 4.52 (d, J = 5 Hz, 2H, –CH_2–), 5.20–5.50 (m, 2H, H_2C=), 5.88–6.27 (m, 1H, HC=), 6.92 (d, J = 9.8 Hz, 2H, H-3, H-5), 7.28–7.82 (m, 5H, H-2′,H-3′,H-5′,H-6′,–NH–) 7.55 (d, J = 9.8 Hz, 2H, H-2, H-6); IR cm^{-1}: 1655 (NC=O). $C_{17}H_{17}NO_2$ requires: C, 76.38; H, 6.41; N, 5.24. Found: C, 76.29; H, 6.37; N, 5.20.

4.4.13. *N*1-(4-(Allyloxy) phenyl)-4-chlorobenzamide (5k)

White solid; mp: 179–180 °C; ^{1}H NMR ($CDCl_3$): δ 4.53 (d, J = 5 Hz, 2H, –CH_2–), 5.30–5.58 (m, 2H, H_2C=), 5.86–6.20 (m, 1H, HC=), 6.90 (d, J = 9.5 Hz, 2H, H-3, H-5), 7.12–8.20 (m, 5H, H-2′, H-3′, H-5′, H-6′, –NH–), 7.55 (d, J = 9.5 Hz, 2H, H-2, H-6); IR cm^{-1}: 1650 (NC=O). $C_{16}H_{14}ClNO_2$ requires: C, 66.79; H, 4.90; N, 4.87. Found: C, 66.86; H, 4.83; N, 4.92.

4.4.14. *N*1-(4-(Allyloxy) phenyl)-3-chlorobenzamide (5l)

White solid; mp: 157–158 °C; ^{1}H NMR ($CDCl_3$): δ 4.55 (d, J = 5.1 Hz, 2H, –CH_2–), 5.22–5.54 (m, 2H, H_2C=), 5.88–6.44 (m, 1H, HC=), 6.82 (d, J = 9.8 Hz, 2H, H-3, H-5), 7.16–7.88 (m, 5H, H-2′, H-3′, H-5′, H-6′, –NH–), 7.55 (d, J = 9.8 Hz, 2H, H-2, H-4); IR cm^{-1}: 1648 (NC=O). $C_{16}H_{14}ClNO_2$ requires: C, 66.79; H, 4.90; N, 4.87. Found: C, 66.71; H, 4.92; N, 4.79.

4.4.15. *N*1-(4-(Allyloxy) phenyl)-4-methoxybenzamide (5m)

White solid; mp: 162–163 °C; ^{1}H NMR ($CDCl_3$): δ 3.83 (s, 3H, –OCH_3), 4.52 (d, J = 5.5 Hz, 2H, –CH_2–), 5.23–5.54 (m, 2H, H_2C=), 5.86–6.32 (m, 1H, HC=), 6.88 (d, J = 10 Hz, 2H, H-3, H-5), 6.88 (d, J = 10 Hz, 2H, H-3′, H-5′),7.50 (d, J = 10 Hz, 2H, H-2, H-6), 7.80 (d, J = 10 Hz, 2H, H-2′, H-4′), 8.02 (s, 1H, –NH–); IR cm^{-1}: 1645 (NC=O). $C_{17}H_{17}NO_3$ requires: C, 72.07; H, 6.05; N, 4.94. Found: C, 72.19; H, 6.13; N, 5.01.

4.4.16. *N*1-(4-(Allyloxy) phenyl)-3-methoxybenzamide (5n)

White solid; mp: 109–110 °C; ^{1}H NMR ($CDCl_3$): δ 3.88 (s, 3H, –OCH_3), 4.52 (d, J = 5.5 Hz, 2H, –CH_2–), 5.19–5.52 (m, 2H, H_2C=), 5.88–6.28 (m, 1H, HC=), 6.92 (d, J = 9.8 Hz, 2H, H-3, H-5), 6.88 (d, J = 10 Hz, 2H, H-2′, H-6′), 7.50 (d, J = 9.8 Hz, 2H, H- 2, H-6), 7.55 (d, J = 10 Hz, 2H, H-3′, H-5′), 7.74 (s, 1H, –NH–); IR cm^{-1}: 1648 (NC=O). $C_{17}H_{17}NO_3$ requires: C, 72.07; H, 6.05; N, 4.94. Found: C, 71.94; H, 6.00; N, 4.88.

4.4.17. *N*1-(4-(Allyloxy) phenyl)-2-methylpropanamide (5o)

White solid; mp: 121–122 °C; ^{1}H NMR ($CDCl_3$): δ 1.22 (d, J = 7 Hz, 6H, –CH_3 (propan)), 2.26–2.64 (m, 1H, –CH– (propan)) 4.52 (d, J = 5.1 Hz, 2H, –CH_2–), 5.18–5.52 (m, 2H, H_2C=), 5.85–6.28 (m, 1H, HC=), 6.85 (d, J = 9.8 Hz, 2H, H-3, H-5), 7.14 (s, 1H, –NH–), 7.42 (d, J = 9.8 Hz, 2H, H-2, H-4); IR cm^{-1}: 1648 (NC=O). $C_{13}H_{17}NO_2$ requires: C, 71.21; H, 7.81; N, 6.39. Found: C, 71.37; H, 7.83; N, 6.45.

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