Synthesis, lipoxygenase inhibition activity and molecular docking of oxamide derivative

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Abstract: In this study, a range of oxamide ligands were synthesized by the reaction of amines with oxalyl chloride in basic medium. Spectroscopic and analytical techniques such as IR, ^{1}H-NMR and ESI-MS techniques were used for characterization of the synthesized oxamides. The synthesized oxamides were screened for Lipoxygenase inhibition. Biological screening revealed that the oxamides possessed good lipoxygenase inhibition activities, whereas, the unsubstituted oxamide did not show any distinct lipoxygenase inhibition activity. Molecular docking studies of the oxamides were also carried out for lipoxygenase inhibition. The results obtained from molecular docking were well correlated with the empirical data.

Keywords: Oxamide, molecular docking, lipoxygenase inhibition.

INTRODUCTION

Enzyme inhibitors are very important class of compounds in the field of Pharmacology. It is because of their role in treatment of different pathological conditions. Most of the time the inhibitors bind themselves to the active site of target molecule thus making it unavailable for the enzymes.

Lipoxygenases are a group of compounds which are large protein monomers containing a single non-heme iron cofactor (Boyington *et al*, 1993). They are mainly involved in the hyper-oxidation of fatty acids such as linoleic and linolenic acids in plants and arachidonic acids in animals (Brash, 1999). Lipoxygenases also participate in different metabolic pathways that leads to pathological conditions such as cancer, heart problems etc. (Funk, 2006; Fürstenberger *et al*, 2006; Wu *et al*, 2012). It is reported that 5-LOX are often involved in the initial steps of metabolic pathways that leads to cancer and asthma (Rådmark & Samuelsson, 2010), whereas, involvement of Reticulocyte 15-LOX-1 have been observed in prostate cancer (Waller *et al*, 2008). Participation of lipoxygenase in different reactions is mainly dependent upon the oxidation state of iron present in the enzyme. The interconversion of Fe(II) to Fe(III) or vice versa is an essential step in the mechanism of the catalyzed reactions. It has been observed that the isolated lipoxygenases have iron in 2+ oxidation state but the active lipoxygenases which start peroxidation reactions have iron in 3+ oxidation state. In brief, catalytic cycles or peroxidation pathways start with the initial conversion of lipoxygenase-Fe(II) into active lipoxygenase-Fe(III) and the peroxidation reaction reverts back the oxidation state of iron from (III) to (II). Therefore, activity of lipoxygenase enzyme can be controlled by tuning the oxidation state of iron. Therefore, instead of binding to the active site of target molecule lipoxygenase inhibitors can perform their inhibitory function either by binding to or reducing the lipoxygenase-Fe (Ohri *et al*, 2005).

There are different types of LOXs such as 5-LOX, 8-Lox, 9-LOX, 12-LOX, and 15-Lox depending upon the position of oxygen insertion (Ivanov *et al*, 2010; Meruvu *et al*, 2005). Among all types of LOXs, 5-LOX are dominant due to their involvement in the synthesis of leukotrienes (LTs). These leukotrienes are involved in different pathways that leads to different inflammatory and allergic disorders like cancer, asthma etc. (Chu & Praticò, 2009; Funk, 2005; Rådmark *et al*, 2007; Reddanna *et al*, 2003). Leukotrienes also participate in damaging gastrointestinal track. Because of their involvement in different pathological conditions researchers are focusing on the synthesis of 5-LOX inhibitors. There are four kinds of 5-LOX inhibitors 1) redox inhibitors, 2) iron chelators, 3) competitive reversible inhibitors and 4) FLAP (5-Lipoxygenase-activating protein) inhibitors (Aparoy *et al*, 2012). Until yet only iron chelating inhibitors are successful (Young, 1999). The first 5-LOX inhibitor used for the treatment of asthma was Zileuton (Drazen *et al*, 1999). Flavonoids such as rutin, baicalein and quercetin have also been used as 5-LOX inhibitors (Yoshimoto *et al*, 1983). A major drawback with 5-LOX inhibitors is loss of effectiveness on clinical trial or they have associated toxic side effects on blood (Steele *et al*, 1999; Young, 1999).

In medicinal chemistry, amides have been center of attraction for a long time because of their great

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pharmacological history (Rajput & Sharma, 2018). Oxamides, an important group of amide family, are diamides and are derived from oxalic acid. They can form mononuclear metal complexes when acting as a bidentate ligand, however, bis bidentate property of these ligands yields polynuclear metal complexes (Soto *et al*, 1989; Steggerda *et al*, 1971).

5-LOX is a potential therapeutic target, so it is necessary to synthesize new molecules which possess distinct LOX inhibition activity with fewer side effects. Oxamides were selected for this study because of their ease of preparation and their reported biological importance. Therefore, in this study a series of oxamides were synthesized and screened for their lipoxygenase inhibition activity. The synthesized oxamides were then docked into the active site of human 5-lox and their structure-activity relationship was rationalized.

MATERIALS AND METHODS

General experimental

All the chemicals used in this study were reagent grade chemicals purchased from Sigma–Aldrich, Merck and BDH. Silica gel pre-coated aluminum plates were used for thin layer chromatography. Characterization of synthesized compounds was performed using Shimadzu 8900 FTIR spectrophotometer, Avane Bruker 300MHz and 400MHz ^{1}H NMR spectrometer, JEOL MS Route 600H EI Mass instrument.

General procedure for the synthesis of ligands

Oxalyl chloride (2.5mmole) was added dropwise to a cold solution of amine (5mmole) in tetrahydrofuran (40ml) containing triethyl amine (2-3drops). The solution was stirred at room temperature for 24 hours. Thin layer chromatography was used to observe completion of reaction. After reaction completion, desolvation was carried out using rotavapor, and solid product was treated with the solution of sodium bicarbonate in water and then dried to obtain desired product. The column chromatography was also performed for purification of some products using silica gel as stationary phase and n-hexane and ethyl acetate (7:3) as eluent.

Biological screening

Oxamides were screened for their lipoxygenase inhibition activities using lipoxygenase inhibition assay and their molecular docking.

Lipoxygenase inhibition assay

The modified Tappel spectrophotometric method was used for the analysis of lipoxygenase inhibitory activity (Safder *et al*, 2009). Lipoxygenase enzyme concentration in each sample was about 130U. 160µL of 100mM sodium phosphate buffer (pH 8.0) was added to each well labeled as enzyme blank, substrate blank, control and test. A 10 µL aliquot of each solution of oxamide sample series (5 – 500µM) prepared in DMSO was transferred to wells labeled as test. A 20µL of lipoxygenase (LOX) solution was added to each well except those labelled as substrate blank. After mixing the contents, wells were incubated for 10 min at 25°C. Autoxidation of substrate solution was avoided by flushing N_2 before addition of substrate to well. 10 µL substrate solution (linoleic acid, 0.5mM, 0.12 %w/v tween 20 in ratio of 1:2) was added to all wells except the one labeled enzyme blank. After completion of reaction absorbance was measured at 234 nm. Finally, the effect of sample concentration on the degree of inhibition was used to calculate IC_{50} values using EZ-Fit, Enzyme kinetics Program (Perrella Scientific In., Amherset, USA).

Molecular docking

The crystal structure of S663D mutant human lipooxygenase (5-LOX) in complex with arachidonic acid was fetched from the PDB using ID: 3V99 with 2.25 Å resolutions (Gilbert *et al*, 2012). Bound ligand and water molecules were deleted to setup the protein structure for docking. Polar hydrogen atoms and Kollman united atom charges were added using AutoDock Tools (Vina, 2010). ArgusLab (Thompson, 2004) program modeled the 3D structures of synthesized oxamides with semi-empirical (AM1) parameterization (Dewar *et al*, 1985). Restricted Hartree-Fock (RHF) closed shell method was used to carry out energy minimization. The Maximum iterations and geometry search gradient conversion was set to 200 and 10^{-1} Kcal/mol/Å, respectively. The ligand structures were prepared for docking using AutoDock Tools. The tools were used for addition of Gasteiger partial charges, merging polar hydrogen atoms, and defining rotatable bonds. Lamarckian Genetic Algorithm was used for docking calculations performed using AutoDock Vina (Morris *et al*, 1998). The AutoGrid generated a 25 x 25 x 25 a point's grid box and the grid spacing was set to 1 Å. The binding site residues of arachidonic acid were reported as center of grid and the x,y,z coordinates for grid center were 16.317, -78.717, -32.292, respectively. The van der Waals terms were calculated using AutoDock parameter set and the electrostatic terms were calculated using distance-dependent dielectric functions. The docked conformations of oxamide derivatives were derived from 100 independent docking runs. The termination procedure was set to 2.5×10^6 energy evaluations with mutation rate of 0.02 and crossover rate of 0.8.

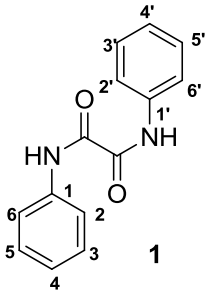
RESULTS

Chemistry

N1,N2-diphenyloxalamide (1)

White solid, 38% Yield, melting point= 252C.

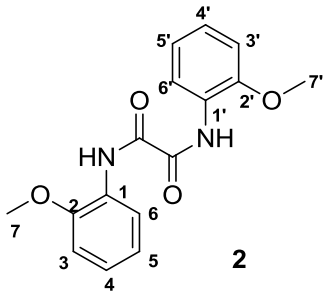
IR (KBr) cm^{-1}: υ (C-N) 1311.5, (CH bending aromatic) 1436.9, (C=N, C=C) 1596.9, 1523.7, (C=O) 1668.3, (sp2-CH) 3055.0, (NH) 3303.8.



^{1}H-NMR (DMSO, 300 MHz) *δ(ppm)* = 7.15 (4H, t, *J*(Hz) = 7.2 , H-4, H-4'), 7.37 (4H, t, *J*(Hz) = 7.5, H-3, H-3', H-5, H-5'), 7.87 (4H, d, *J*(Hz) = 8.4 , H-2, H-2', H-6' H-6'), 10.83 (2H, s, N-H). EI-MS : [M$^+$]240.0, (100%)120.93.

N1,N2-bis(2-methoxyphenyl)oxalamide (2)

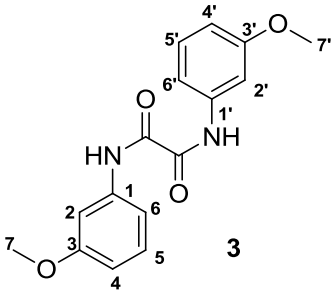
White solid, 79.4% Yield, melting point= 250-252C.
IR (KBr) cm^{-1}: υ (C-N) 1294.1, (CH bending aromatic) 1400.2, (C=C) 1525.6, (C=N) 1583.4, (C=O) 1691.5, (sp2-CH) 3055.0, (NH) 3340.5.



^{1}H-NMR (DMSO, 300 MHz) *δ(ppm)* = 3.70 (6H, s, 2× CH_3)7.03 (2H, t, *J*(Hz) = 6, H-5, H-5'), 7.35 (4H, m, H-4, H-4',H-6, H-6'), 8.15 (2H, d, *J*(Hz) = 5.4, H-3, H-3'), 9.87 (s, 2H, N-H). EI-MS : [M$^+$]300.1, (100%)123.

N1,N2-bis(3-methoxyphenyl)oxalamide (3)

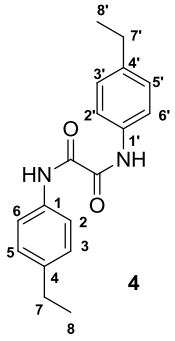
White solid, 61.8% Yield, melting point= 187-189C.
IR (KBr) cm^{-1}: υ (C-N) 1211.6, (CH bending aromatic) 1425.3, (C=N) 1598.9, (C=O) 1672.2, (sp2-CH) 3020.3.0, (NH) 3284.5.



^{1}H-NMR (DMSO, 300 MHz) *δ(ppm)* = 3.74 (6H, s, 2× CH_3), 6.75 (d, 2H, H-4, H-4'), 7.27(2H, t, *J*(Hz) = 8.1, H-5, H-5'), 7.47 (d, 2H, *J*(Hz) = 8.1, H-6, H-6'), 7.55 (2H, s, H-2, H-2'), 11.79 (s, 2H, N-H). EI-MS: [M$^+$] 300.1, (100%) 123.

N1,N2-bis(4-ethylphenyl)oxalamide (4)

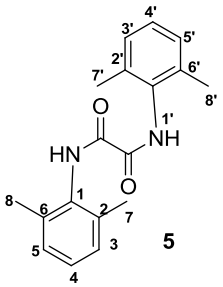
White solid, 85% Yield, melting point= 248C.
IR (KBr) cm^{-1}: υ (C-N) 1235.6, (CH bending aromatic) 1407.9, (C=C) 1517.9, (C=N) 1591.2, (C=O) 1658.7, (sp3-CH) 2964.4, (sp2-CH) 3039.6, (NH) 3294.2.



^{1}H-NMR (DMSO, 300 MHz) *δ(ppm)* = 1.16 (6H, s, 2× CH_3), 2.57(4H, q, *J*(Hz) = 6.0, 2× CH_2), 7.21 (4H, d, *J*(Hz) = 6.0, H-2, H-2', H-6, H-6'), 7.75(d, 4H, *J*(Hz) = 6.0, H-3, H-3', H-5, H-5'), 10.70 (2H, s, N-H). EI-MS : [M$^+$]296, (100%)121.

N1,N2-bis(2,6-dimethylphenyl)oxalamide (5)

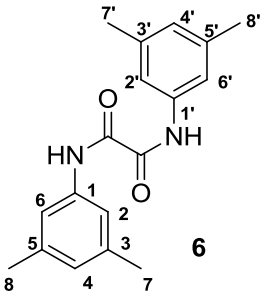
White solid, 41% Yield, melting point= 253C. IR (KBr) cm^{-1}: υ (C-N) 1218.9, (CH bending aromatic) 1494.7, (C=C) 1480.7, (C=N) 1593.1, (C=O) 1664.5, (sp3-CH) 2962.5, (sp2-CH) 3018.4, (NH) 3294.2.



^{1}H-NMR (DMSO, 300 MHz) *δ(ppm)* = 2.17 (s, 12H, 4× CH_3), 7.15 (s, 6H, H-3, H-3', H-4, H-4', H-5, H-5'), 10.27 (s, 2H, N-H). EI-MS : [M$^+$]296, (100%)121.

N1,N2-bis(3, 5-dimethylphenyl)oxalamide (6)

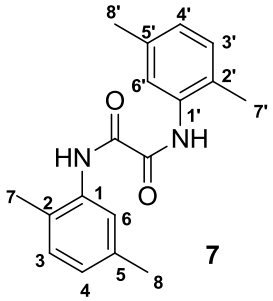
White solid, 69.5% Yield, melting point= 204C. IR (KBr) cm^{-1}: υ (C-N) 1207.4, (CH bending aromatic) 1433.0, (C=C) 1521.7, (C=N) 1608.5, (C=O) 1666.4, (sp3-CH) 2914.22, (sp2-CH) 3010.7, (NH) 3265.3.



^{1}H-NMR (DMSO, 300 MHz) *δ(ppm)* = 2.26 (s, 12H, 4× CH_3), 6.80 (s, 2H, H-4, H-4'), 7.45(s, 4H, H-2, H-2', H-6, H-6'), 10.54 (s, 2H, N-H). EI-MS: [M^+]296, (100%)121.

N1,N2-bis(2,5-dimethylphenyl)oxalamide(7)

White solid, 39% Yield, melting point= 228C. IR (KBr) cm^{-1}: υ (C-N) 1217.4, (CH bending aromatic) 1471.6, (C=C) 1521.7, (C=N) 1577.2, (C=O) 1670.2, (sp3-CH) 2920.0, (sp2-CH) 3050.6, (NH) 3255.6.



7

^{1}H-NMR (DMSO, 300 MHz) *δ(ppm)* = 2.28 (6H, s, 2× CH_3), 2.28 (6H, s, 2× CH_2), 7.00 (d, 2H, *J*(Hz) = 7.8, H-3, H-3'), 7.16 (2H, d, *J*(Hz) = 7.8, H-4, H-4'), 7.33 (s, 2H, H-6, H-6') 10.16 (2H, s, N-H).
EI-MS : [M^+]296.1 (100%).

Biological screening

Lipoxygenase inhibition

Lipoxygenase inhibition activities of oxamides (1-7) using Baicalein as a standard reference inhibitor are reported in table 1.

Binding of Oxamides to the human 5-LOX

Binding of all the seven synthesized oxamides with human 5- LOX is shown in fig. 2-4.

DISCUSSION

Chemistry

The preparation of oxamide ligands is summarized in fig. 1. The synthesis was carried out by mixing the stoichiometric amount of oxalyl chloride (1eq) and the corresponding amine (2eq). The solvent used was THF and triethyl amine was used as base. Characterization of synthesized oxamides was done using ^{1}H-NMR, EI-MS and IR spectroscopy EI-MS showed molecular ion peak for the oxamides. IR spectra of all the compounds showed clear peaks for C=O and NH in the range of 1650-1700and 3250-3300 cm^{-1} respectively. ^{1}H-NMR data of compounds further supported the synthesis of oxamide by showing distinct peaks for all the aliphatic, aromatic and amide protons. Hence all the spectroscopic data confirmed the synthesis of oxamides. All the synthesized compounds (1-7) were white, amorphous, non-hygroscopic, stable and showed solubility in DMSO and dissolve on heating in ethanol and ethyl acetate.

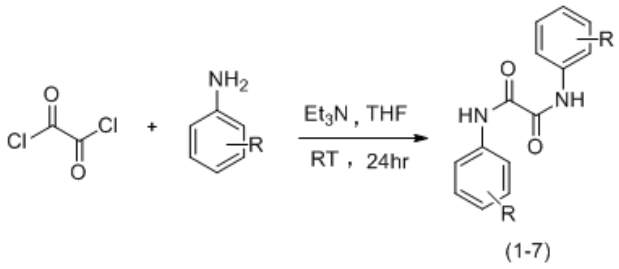
Biological screening

Lipoxygenase inhibition

Lipoxygenase inhibition activities of oxamides (1-7) were evaluated using Baicalein as a standard reference inhibitor (IC50 = 22.6 ± 0.08μM). The bioassay results revealed that some of the compounds had good Lipoxygenase inhibition properties which were comparable with the inhibitory potential of Baicalein. It was also observed that the nature and position of substituent on oxamide can moderate the Lipoxygenase inhibition. The oxamides with ortho substitution exhibited better lipoxygenase inhibitory property as compared to the substitutions at other positions, whereas, unsubstituted oxamide showed very less intermediate activity (IC_{50} >200μM).

Binding of Oxamides to the human 5-LOX

Seven synthesized oxamide derivatives were docked into the active site of human 5-LOX using the binding site of arachidonic acid. Structures, lipoxygenase inhibition potential and the binding energy calculated from Autodock for all oxamides are included in figs. 2-4 and table 1 respectively. According to the experimental and in-silico binding affinities of the synthesized oxamides, they have been grouped into strongly active, weakly active and inactive compounds.



(1-7)

1. R - H
2. R = 2-OCH_3
3. R = 3-OCH_3
4. R = 4-CH_2-CH_3
5. R = 2,6-dimethyl
6. R = 3,5-dimethyl
7. R = 2,5-dimethyl

Fig. 1: Schematic of oxamide ligand synthesis

Strongly active oxamides

Fig. 2 (A, B, C) displayed the binding of strongly active oxamides (Compounds # 2, 5 and 7) which were able to replace the third iron-bound water molecule in order to coordinate with the iron metal. Compounds 2, 5 and 7 established the coordinating distances of 2.7 Å, 2.9 Å, and 2.9 Å, respectively with the Fe (II) by using the oxygen atoms of the 2-methoxy substituent and the carbonyl groups of their phenyl ring 1. The nitrogen atom (N_1) of the compound 2 was involved in the hydrogen bonding at the distance of 3.0 Å with the iron-bound water molecule HOH922 while the nitrogen atom (N_2) of compounds 5 and 7 formed similar hydrogen bonds at the distance of

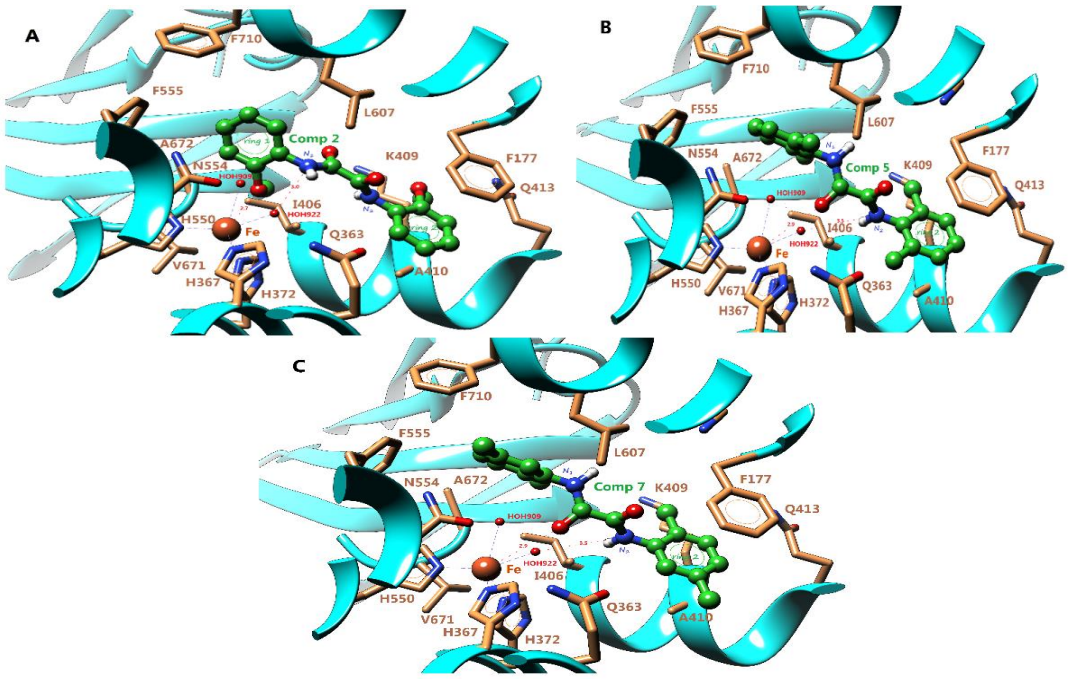


Fig. 2: Docked poses of compounds (A) 2 (B) 5 and (C) 7 at the active site of human 5-Lipoxygenase.

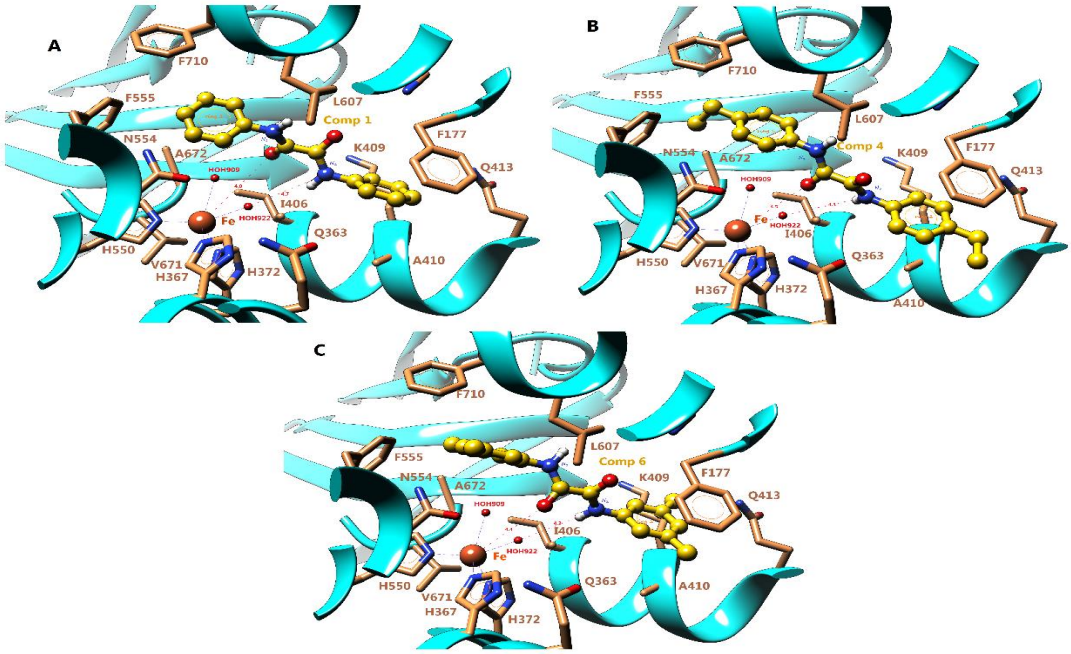


Fig. 3: Docked poses of compounds (A) 1 (B) 4 and (C) 6 at the active site of human 5-Lipoxygenase.

3.5 Å. The π-π interactions were observed between the phenyl ring 1 of compounds (2, 5, 7) and the aromatic side chains of F555 and F710 while the residues I406, L607 and A672 of 5-LOX formed hydrophobic contacts.

Another π-π stacking was found between the phenyl ring 2 of compounds (2, 5, 7) and the aromatic side chain of F177 whereas the hydrophobic contact was developed by the residue A410 of 5-LOX.

Weakly active oxamides

Fig. 3 (A, B, C) presented the binding of weakly active oxamides (Compounds # 1, 4 and 6) which might be able to coordinate with the iron metal. Compounds 1, 4 and 6 showed weak bonding interactions at the distances of 4.0 Å, 4.5 Å, and 4.4 Å, respectively with the Fe (II) by means of the carbonyl oxygen atoms of their phenyl ring 1. Consequently, the nitrogen atom (N_2) of compounds 1, 4, and 6 were trying to establish very weak hydrogen

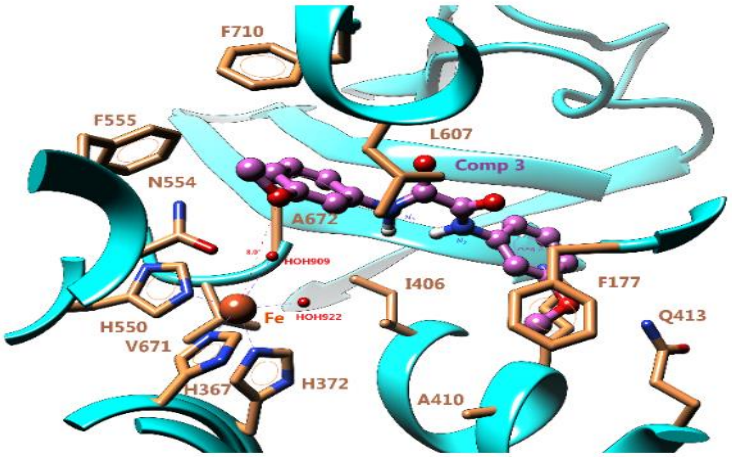


Fig. 4: Docked pose of the compound 3 at the active site of human 5-Lipoxygenase.

Table 1: Lipoxygenase inhibition activities for oxamides (1-7) and the respective binding energy from molecular docking

Sample ID	R	Lipoxygenase inhibition IC_{50} (μM± SEM)	Binding Energy (Kcal/mol)
1	H	> 200	-6.3
2	2-methoxy	42.4± 0.14	-8.3
3	3-methoxy	N.A.	-
4	4-ethyl	>200	-6.5
5	2,6-dimethyl	50.6 ± 0.08	-7.9
6	3,5-dimethyl	>200	-6.4
7	2,5-dimethyl	51.2 ± 0.34	-7.8
Baicalein (standard)	-	22.6 ± 0.08	-

bonds at the distance of 4.7 Å, 4.3 Å and 4.3 Å, respectively with the iron-bound water molecule HOH922. The π-π interactions were observed between the phenyl ring 1 of compounds (1, 4, 6) and the aromatic side chains of F555 and F710 while the residues I406, L607, and A672 of 5-LOX formed hydrophobic contacts. Another π-π stacking was found between the phenyl ring 2 of compounds (1, 4, 6) and the aromatic side chain of F177 whereas the hydrophobic contact was developed by the residue A410 of 5-LOX.

Fig. 4 revealed the docked conformation of inactive compound 3 which illustrated well the reason of the non-binding behavior of this oxamide derivative. As shown in the figure the compound 3 was unable to approach iron and was also unable to show any of the above-mentioned hydrogen bonds and π-π interactions.

CONCLUSION

In this study, N,N-substituted oxamide derivatives were synthesized by reacting oxalyl chloride with a variety of amines in the presence of base. After purification oxamides were characterized using different spectroscopic techniques such as IR, ^{1}H-NMR and ESI-MS. All the spectroscopic techniques confirm the synthesis of oxamide. Oxamide derivatives were then screened for lipoxygenase inhibition activity. Compounds showed good to moderate lipoxygenase inhibition. All the results are in good coordination with the molecular docking studies.

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