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Synthesis, Characterization, AMDET and DOCKING Studies of Novel Diclofenac Derivatives Containing Phenylalanine Moiety Acting as Selective Inhibitors Against Cyclooxygenase (COX-2)

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Abstract.

New diclofenac derivatives containing L-phenylanline moiety were synthesized, and fully characterized using different tools such IR, ^{1}H NMR, ^{13}C NMR spectroscopy. In order to gain the inhibition actions of the synthesized compounds against cyclooxygenases, its compounds were docked into the active sites of (COX-1 and COX-2) compared with reference drug. The Docking studies were predicted that, the lowest energies and high binding scores of docked compounds, which interacted with active site, perhaps making them possible physiologically active as selective inhibitors against (COX-2), and may considered them a suitable selective inhibitor against (COX-2).

Keywords: Phenylalanine, Diclofenac, COX, DOCKING, ADMET.

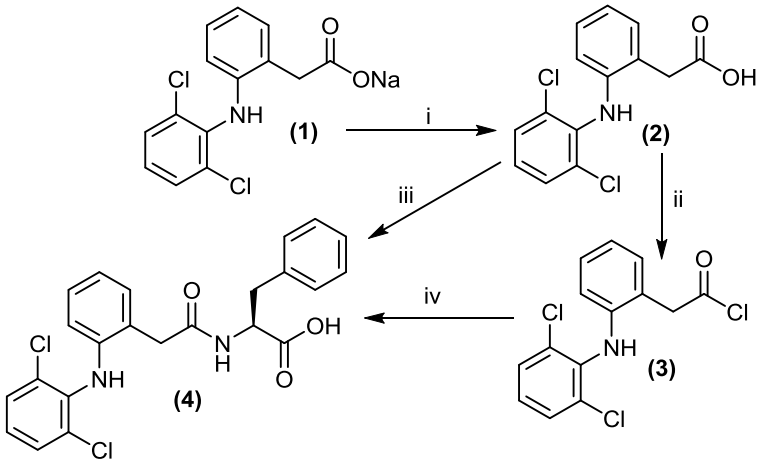
1. Introduction.

Non-steroidal anti-inflammatory drugs (NSAIDs) widely employed in musculoskeletal diseases, and anti-inflammatory properties [1]. The NSAIDs are effective in different clinical setting, and act as COX inhibitors (COX-1 and COX-2) through inhibiting the production of prostaglandins (PGs) [2-4]. Diclofenac is one of most famous available members of this drug's class under current clinical usage [5], and suffer from a common toxicity of gastrointestinal drawback, due to inhibition non-selectively of cyclooxygenases enzymes [6–8]. In addition, diclofenac have anti-microbial [9-11], ulcerogenic, analgesic, anti-inflammatory, lipid peroxidation [12,13], antitumor [14] and inhibitor formation of transthyretin amyloid fibril properties [15]. Fruthermore, the alaninyl derivatives especially containing amide and thioamide moieties possess diverse biological activities, as anti-inflammatory, anti-tumor and antimicrobial activities [16-18]. Hence, the present study aims to synthesis a novel series of diclofenac derivatives containing phenylalanine moiety acting as new NASIDs. The molecular docking was carried out, to predict the correct binding geometries for each ligands at the active sites, followed by molecular modeling to identify the structural features of these new series, which may support its postulation, the active compounds with high binding scores may act as COX inhibitors.

2. Results and discussion.

2.1. Chemistry.

The synthetic routes to obtain the target compounds **1-16** were depicted in Schemes **1-4**. The starting compound of 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetyl chloride **3** was carried out according to steps depicted in (Scheme 1).



Scheme **1**:regent and conditions; i- H_2O/ dil-HCl., ii-$SOCl_2$, iii-L-Phe/fusion, iv-L-Phe/THF/TEA.

(2-(2-((2,6-dichlorophenyl)amino)phenyl)acetyl)-L-phenylalanine **4** was synthesized with two strategies, first by coupling of **3** with L-Phenylalanine in THF/TEA/H_2O media, another strategy through fused diclofenac **2** with amino acid to afford compound **4.** The IR spectrum of compound **4** indicated that, the presence of a OH and NH function as a broad band (3266 cm^{-1}), and it's the ^{1}HNMR spectrum showed a singlet at (δH 12.68 ppm) due to OH protons of carboxylic. The compound **4**, which obtained from two methods of preparation, was examined via transmission electron microscope (TEM), and showed that, the average particle size obtained from fusion method with range of 10-15 nm diameters, but the particle produced from acid chloride method in the range 1 μm diameters (Fig.1). The simplicity preparation of L-free amino acid derivatives **4** and its high activity, may be suggest potential application of this synthetic model as anti COX agent.

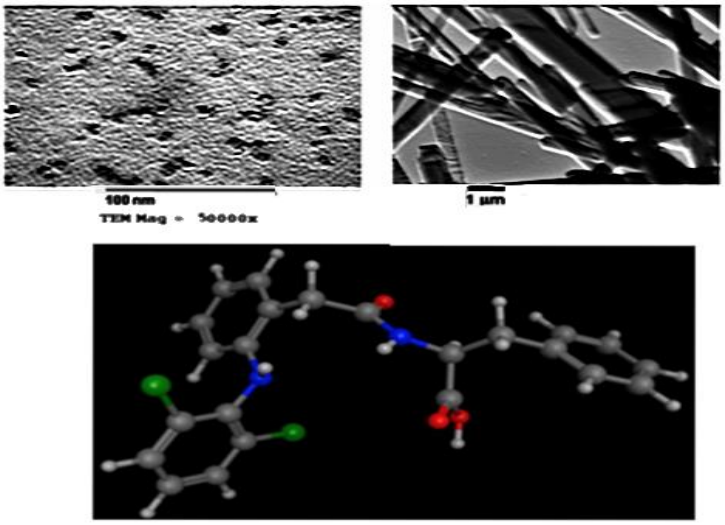
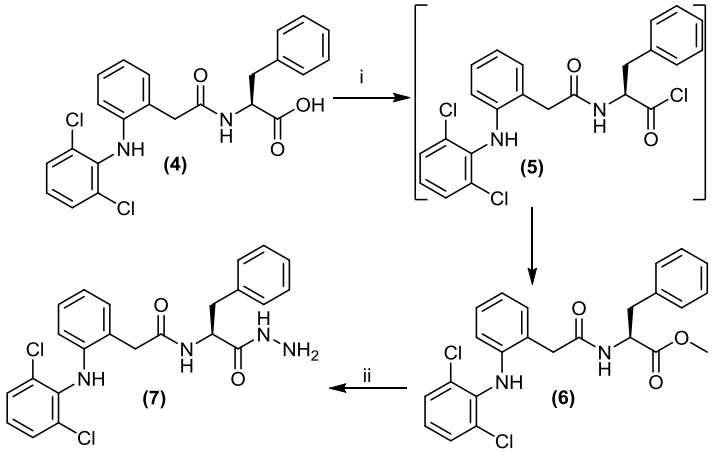


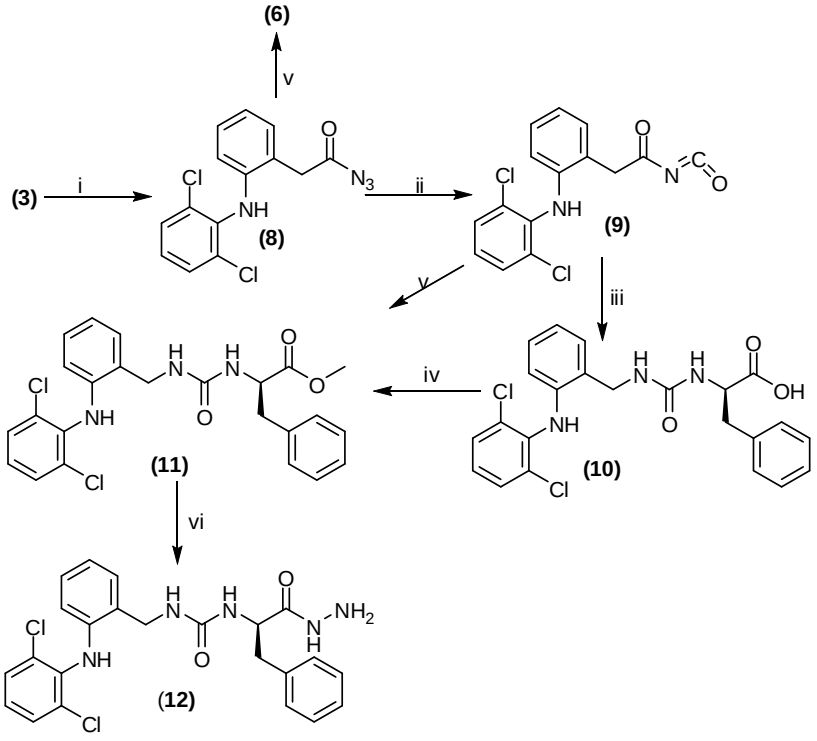
Fig. 1. TEM images of the **4** prepared with fission method(upper left), TEM images of the **4** prepared with acid chloride method (upper right), and representative ball and stick rendering for the most stable steroisomer form of compound **4** as calculated by PM3 semi-empirical molecular orbital calculations (lower).

Compounds **5-7** were synthesized according to the methods were described in (Scheme 2). The free amino acid **4** was esterified to the corresponding L-phenylalanine methyl ester derivative **5**, characteristic ^{1}HNMR displayed peak at (δH 3.75 ppm) for (OMe) protons and disappeared of OH proton for carboxyl peak of free acid **4**. The L-phenylalanine hydrazide derivative **6** was carried out by refluxing compounds **5** with hydrazine hydrate in absolute ethanol, The IR spectrum was exhibited presence of NH_2 function at (3340 and 3139 cm^{-1}).



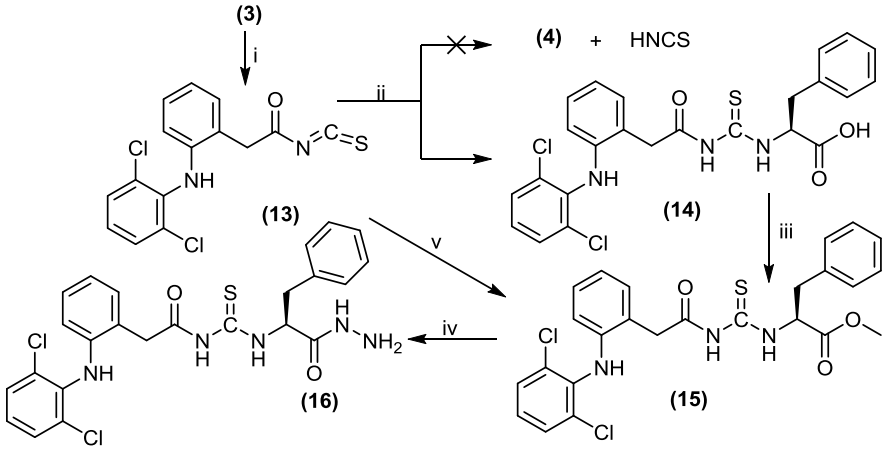
Scheme **2:**regents and conditions; i-$SOCl_2$.\ MeOH.,ii-alc.NH_2NH_2.

The synthetic pathway for preparing compounds **8-12** were outlined in Scheme 3. The acid chloride **3** was reacted with aqueous sodium azide to furnish the 2-(2-(2,6-dichlorophenylamino)phenyl)acetyl azide **8**, and converted to ((2-((2,6-dichlorophenyl)amino) benzyl)carbamoyl)-L-phenylalanine **10** via formation isocyanate **9**, the compound **8** was reacted with L-phenylalanine methyl ester to give compound **6**. The methyl ((2-((2,6-dichlorophenyl)amino)benzyl)carbamoyl)-L-phenylalaninate **11** was prepared by methylation of free amino acid derivative **10**, and\or by refluxing isocyanate **9** with L- phenylalanine methyl ester. The ((2-((2,6-dichlorophenyl)amino)benzyl)carbamoyl)-L-phenylalanine hydrazide **12** was synthesized by applying the hydrazinolysis of methyl ester **11** with hydrazine hydrate in ethanol.



Scheme **3:**regent and conditions; i- NaN_3, ii-THF/Reflux,iii-L-Phe\pyridine, iv-$SOCl_2$/ MeOH, v-L-PheOMe, vi-85% alc.NH_2NH_2.

The acid chloride **3**, was treated with ammonium thiocyanate in acetone to afford 2-(2-(2,6-dichlorophenylamino)phenyl)acetyl isothiocyanate **13**, which was not isolated from the mixture, and was converted into the corresponding ((2-(2-((2,6-dichlorophenyl)amino)phenyl)acetyl)carbamothioyl)-L-phenylalanine **14** by action of L-phenylalanine, and further modification with $SOCl_2$ in absolute methanol was achieved methyl ((2-(2-((2,6-dichlorophenyl)amino)phenyl)acetyl)carbamothioyl)-L-phenylalaninate **15**, which underwent hydrazonlysis to gave ((2-(2-((2,6-dichlorophenyl)amino)phenyl)acetyl)carbamothioyl)-L-phenylalanine hydrazide **16**, Scheme 4.



Scheme **4**:regent and conditions; i- NH_4NCS, ii-L-Phe./pyridine, iii-$SOCl_2$/MeOH, iv-85% alc.NH_2NH_2,v-L-Phe-OMe

In order to establish enantiomeric purity of isolated compounds, the specific rotation values were determined, which was remained unchanged after repeated crystallization for several times. Furthermore, the enantiomeric excess (ee) and distereoisomeric excess (de) values were determined for synthesized compounds, these values based on the stereo configuration of amino acid for amide part of the products **4, 10** and **14**, which were obtained through nucleophilic addition of free amino acids on diclofenac. The TLC analysis was showed, the optical purity of the resulting compounds were greater than 94 %. Thus, as expected, the stereo chemical configuration at α-carbon atom of the acid was practically unaffected, and this synthetic transformation from chiral α-amino acid could be applied at wide range without any significant loss of optical activity.

2.2. Molecular Modeling.

2.2.1. Docking studies:

In brief, two isoforms of COX protein are known: COX-1, is responsible for the physiological production of prostaglandins, which is expressed in most tissues, and COX-2, is responsible for the increasing production of prostaglandins during inflammation process, which is induced by endotoxins, cytokines and mitogens in inflammatory cells [19]. The recently analysis X-ray for the arachidonic acid with COX-2 co-crystal was showed, the carboxylate group was coordinated with Tyr-385and Ser-530[20], as well as the action of NSAIDs, through the interaction of carboxylate group with Tyr-385 and Ser-530, which was stabilized the negative charge of the tetrahedral intermediate [21], and demonstrated that, Tyr-385 and Ser-530 have a structural and functional evidence for the importance of them in the chelating of the ligands[22]. In order to obtained biological data on a structural basis, through rationalized ligand–protein interaction behavior, the molecular docking into the active site of COX was performed for the synthesized compounds using flexible method. All calculations for docking experiment were preformed with MVD 2008.4.00 using MOE 2008.10 [23,24]. The tested compounds were evaluated in silico (docking), using X-ray crystal structures of COX-1 (ID: 3N8Y; [25]) and COX-2 (ID: 1PXX; [20]) complexes with diclofenac. The tested compounds were docked into active sites of both enzymes COX-1 and COX-2. The active site of the enzyme was defined, to include residues within a 10.0 Å radius to any of the inhibitor atoms. The scoring functions were applied for the most stable

docking model to evaluate the binding affinities of the inhibitors, which complexes with (COX) active sites, table (1). The complexes were energy-minimized with an MMFF94 force field [26] until the gradient convergence 0.05 kcal/mol was reached. All isolated compounds were docked successfully into the COX active sites. Compared with reference inhibitor (diclofenac, **1**), the compounds 8 in COX-1 active site exhibited, higher binding scores of MOE and MVD (-121.78 and -113.28) Kcal/mol, respectively, (table 1, Fig.2). In addition, in COX-2 active site, the compounds **7, 12** and **16** were showed higher score than **1**, with MOE (136.94, -133.52 and 133.72) Kcal/mol and with MVD (-121.3, -102.16 and -106.27) Kcal/mol, respectively, table (1).

Table1: Docking energy scores (kcal/mol) derived from the MVD with MOE for isolated ligands:

Cpd.	dG.	Int.	RMS	Eele	Evdw	Esol	MVD	R. S.	Affinity	T.Int.	H.B.	LE1	LE2
							COX-1						
Dic.	-139.011	-78.583	1.576	2.275	-13.904	35.099	-105.918	-91.750	-41.401	-122.173	-2.492	-5.574	-4.828
4	-114.135	-70.099	1.357	5.276	-27.554	66.766	-73.2495	23.959	-13.946	-117.008	0	-2.441	0.798
6	-112.043	-75.852	1.668	0.812	-10.995	78.100	-64.598	129.773	-28.68	-97.552	-2.5	-2.083	4.186
7	-114.592	-77.949	1.099	30.48	-30.861	91.156	-89.348	42.462	-63.672	-128.488	-4.572	-2.882	1.369
8	-121.786	-47.346	1.409	-8.244	-43.300	118.335	-113.287	-100.01	-46.292	-136.203	22.916	-8.371	-5.394
10	-99.0466	-60.663	1.205	-25.855	-29.156	71.478	-22.656	222.54	-11.59	-78.191	-2.522	-0.666	6.545
11	-104.531	-43.461	1.895	-27.506	-37.526	125.942	-66.321	19.929	-21.55	-94.848	-1.922	-2.009	0.603
12	-105.772	-97.347	1.282	-4.533	-14.564	43.496	-94.219	86.838	-21.112	-123.217	-5.243	-2.771	2.554
14	-119.389	-80.023	1.503	-25.553	-16.973	116.436	-58.656	149.513	-47.575	-90.187	-3.687	-1.777	4.530
15	-119.05	-68.443	1.506	-21.150	-11.836	137.242	-70.273	86.945	-43.246	-107.896	-2.972	-2.066	2.557
16	-119.09	-50.250	1.502	6.645	-31.879	83.546	-96.309	-88.405	-19.728	-119.566	23.25	-1.314	-5.068
							COX-2						
Dic.	-87.5031	-60.580	1.554	4.56041	10689972	-12.516	-101.573	-1.838	-283.696	-145.109	-2.566	-3.211	-0.061
4	-118.077	-66.542	1.517	-2.03841	44424352	-18.150	-96.334	-87.851	-178.64	-118.524	-2.004	-5.345	-4.623
6	-115.515	-64.344	2.537	2.115024	53101028	-10.866	-70.607	113.98	-234.192	-140.187	-5.201	-2.076	3.352
7	-136.944	-86.591	1.760	-13.2268	64397116	-24.663	-121.317	-57.914	-40.2472	-144.777	-2.928	-3.913	-1.868
8	-109.616	-89.674	1.984	12.01661	64380680	-23.621	-98.946	0.605	-485.464	-145.237	-1.319	-2.910	0.017
10	-121.527	-38.7518	2.058	-22.4244	62614892	-11.642	-70.607	113.985	-229.488	-140.187	-5.201	-2.076	3.352
11	-132.68	-77.125	1.531	15.59195	60838292	-16.238	-93.15	60.298	-206.752	-143.456	-3.968	-2.739	1.773
12	-133.526	-113.74	1.908	-2.14498	51254160	-13.401	-102.16	25.160	-158.984	-159.507	-2.916	-3.095	0.7624
14	-132.401	-90.987	1.926	-9.29045	50268552	-13.521	-56.096	215.95	-214.2	-98.859	-5.774	-1.699	6.543
15	-121.684	-90.290	1.545	-11.5066	67707312	-20.524	-70.788	93.822	-45.416	-93.532	-0.313	-2.082	2.759
16	-133.727	-68.018	1.380	-26.026	70429696	-43.338	-106.271	-103.02	-25.48	-140.273	-10	42.06	-4.905

d.G.: *free binding energy of the ligand from a given conformer*, Int.: *binding energy of hydrogen bond interaction with receptor*, Eele: *the electrostatic interaction with the receptor*, Evdw*: van der Waals energies between the ligand and the receptor*, Esol.: *Solvation energy*., MVD: *Energy score used during docking*., R.S.:*The re-ranking score.* T.Int.:*The total interaction energy between the pose and the target molecule.* H.B.: *Hydrogen bonding energy between protein and ligand.* LE1:*MolDock Score divided by Heavy Atoms count.* LE2:*Rerank Score divided by Heavy Atoms count.*

2.2.2. Structures activity relationships:

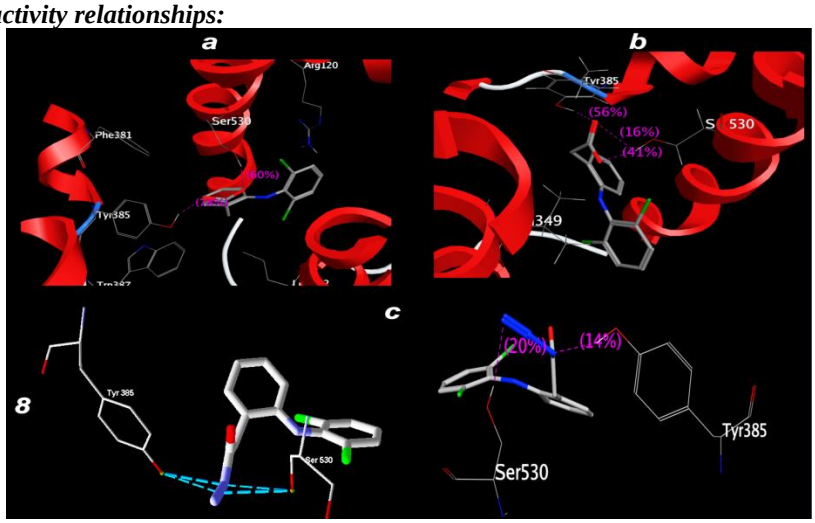


Fig. 2. The compounds were Docked into the active site; a: diclofenac into COX-1 using MOE tool. b: diclofenac into COX-2. using MOE tool; c: highest docking score compound **8** into COX-1 , H- bonds are in blue and pink, (left panel) using MVD and (right panel) using MOE .

In order to get a deeper insight into the nature and type of interactions of docked compounds, the complexes between each compound and COX receptor were visualized, and depicted in (Figs. 2 and 3). Since, the H bond interactions playing an important role in the structure and function of biological molecules, the current ligand-receptor interactions were analyzed on the basis of H bonding. In order to reduce the complexity, hydrophobic and π-cation interactions (>6Å) are not shown in (Figs. 2 and 3).

The highest binding score member is **8**, which complexes with COX-1, exhibited important interactions with Tyr-385 and Ser-530 through azido group; four hydrogen bonds in MVD, and two hydrogen bonds in MOE. The compound **8** was stabilized in this binding pocket by adjusting its dichlorophenyl and phenyl rings with phenyl ring of Tyr-385 by perpendicular and parallel, respectively. The compound 8 was stabilized with itself by arranged two phenyl rings in coplanar position, (Fig.2).

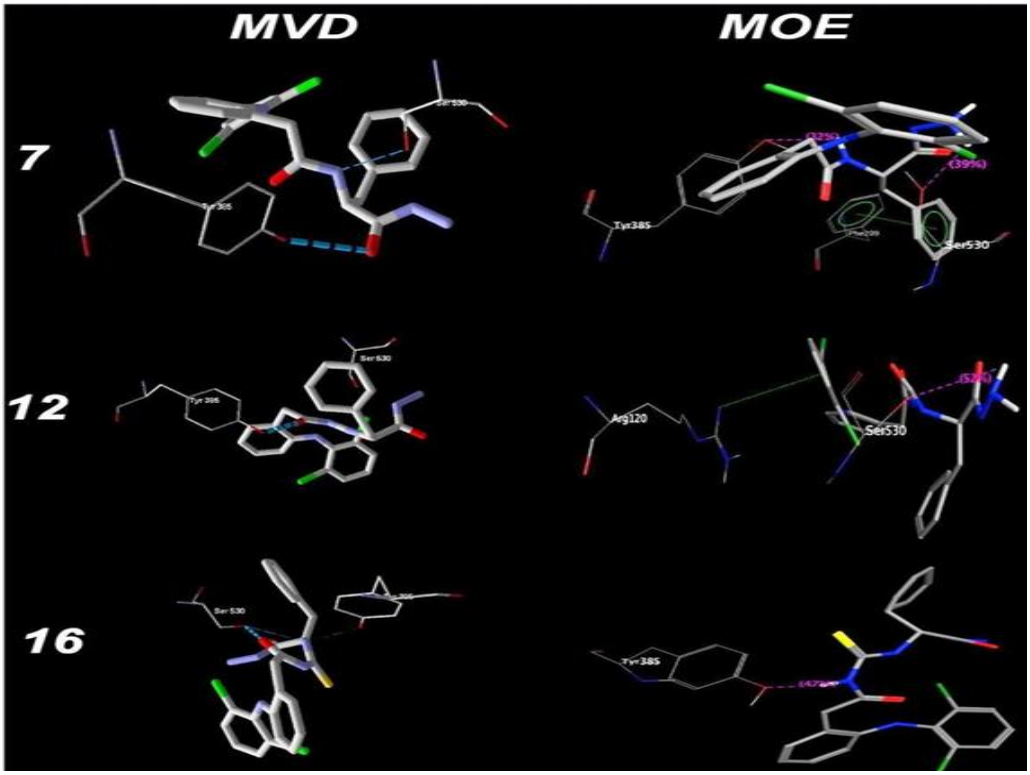


Fig. 3. The Compounds **7, 12** and **16** were docked into the active site of COX-2. H- bonds are in blue and pink.

In COX-2 binding site (Fig. 3); i- Compound **7** was arranged in binding pocket by adjusting dichlorophenyl ring perpendicular with phenyl ring of Tyr-385, the highest score may be due to formation hydrogen bonding interaction between two important residues Tyr-385 and Ser-530; ii- Compound **12**, was interacted with different modes in two programs, which was interacted by formation of one hydrogen bond with Tyr-385 in MVD tool, its compound **12** was stabilized in MOE by formation H-bond with Ser-530 and electrostatic bond with Arg-120; iii- Compound 16 formed H-bond interaction with different manners, by formation of H-bond with Ser-530 in MVD tool, and H-bond with Tyr-385 with MOE, and was stabilized in binding pocket by arrangement of dicloro- phenyl ring parallel with Tyr-385 residue.

The results obtained clearly revealed that, the amino acid residues close to the reference molecules diclofenac (**1**) are mostly the same as observed in the currently compounds under investigation, which complexes with proteins (Figs. 2 and 3). Compared with the original inhibitor 1, the higher binding energies and binding process interaction were observed in case of compounds **7, 12** and **16** in COX-2, and the lower binding energies of tested compounds in COX-1, these results indicated that, the compounds **7, 12** and **16** act as selective inhibitors against COX-2, this could probably due to the presence of hydrazido phenylanline Fragment in the synthesized compounds.

2.2.2. Conformational analysis.

It is obvious that, possibility existence of the synthesized amino acid derivatives **4-7, 10-12** and **14-16** in L- and D- optical isomer forms. In trying to achieve better insight into the molecular structure of the most preferentially stereoisomer forms for its compounds, the conformational analysis of the target compounds has been performed using the MMFF94 force-field [26] (calculations in vacuo, bond dipole option for electrostatics, PolakeRibiere algorithm, RMS gradient of 0.01 kcal/ mol) implemented in MOE [23]. The most

stable conformer for **4-7, 10-12** and **14-16** were fully geometrical optimized with molecular orbital function PM3 semi-empirical *Hamiltonian* molecular orbital calculation MOPAC 7 package [27]. The computed molecular parameters, total energy, electronic energy, heat of formation, the highest occupied molecular orbital (HOMO) energies, the lowest unoccupied molecular orbital (LUMO) energies and the dipole moment for studied compounds were calculated , (table 2).

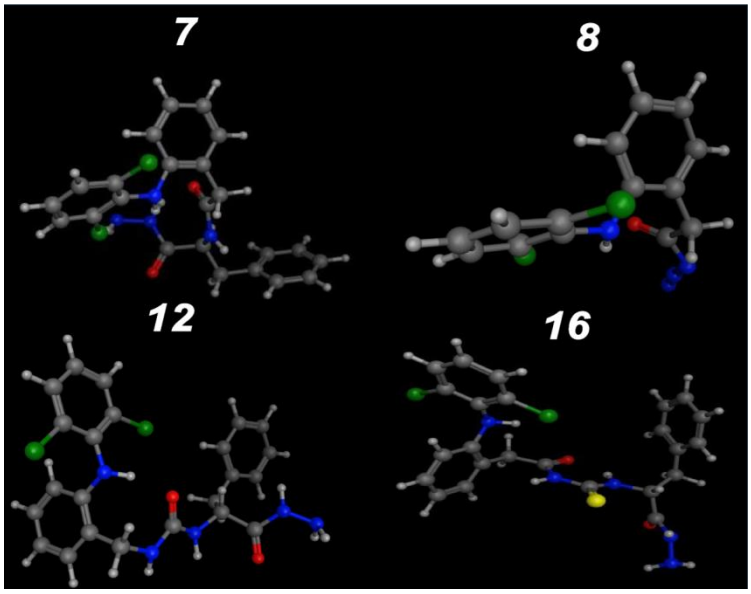


Fig. 4. The Lowest energy conformers of the most active compounds **7,8,12** and **16** as representative examples in rendering ball and stick.

The calculated molecular parameters (table 2) showed, the most stable stereoisomer is the *(L)* form, this may be explained by slightly reduces its calculated energy, and this leads to predominance (L) form structures over (D) forms. The lowest minimization energy for the isolated structures **4-7, 10-12** and **14-16** was exhibited common arrangement of three phenyl rings in coplanar position, as shown in represented example of most active compounds **7, 8, 12** and **16** against (COX) enzymes (Fig. 4), the higher HOMO energy values show the molecule is a good electron donor, on other hand, the lower HOMO energy values indicate that, a weaker ability of the molecules for donating electron. LUMO energy presents the ability of a molecule for receiving electron [28].

2.2.3. ADMET factors profiling.

Oral bioavailability was considered playing an important role for the development of bioactive molecules as therapeutic agents. Many potential therapeutic agents fail to reach the clinic because their ADMET (absorption, distribution, metabolism, elimination and toxic) Factors. Therefore, a computational study for prediction of ADMET properties of the molecules was performed for tested compounds **1-16**, by determination of topological polar surface area (TPSA), a calculated percent absorption (%ABS) which was estimated by Zhao et al. equation[29], and ‘‘rule of five’’ formulated by Lipinski[30] and established that, the chemical compound could be an orally active drug in humans, if no more than one violation of the following rule: i) ClogP (partition coefficient between water and octanol < 5, ii) number of hydrogen bond donors sites ≤ 5, iii) number of hydrogen bond acceptors sites ≤ 10, iv), molecular weight <500 and molar refractivity should be between 40-130. In addition, the total polar surface area (TPSA) is another key property linked to drug bioavailability, the passively absorbed molecules with (TPSA>140) have low oral bioavailability [31].

Table 2: The Optimized Calculations Energies at PM3 molecular orbital for **1-16**.

	L							D						
Cpd	**E**	**Eele**	**HF**	**IP**	**HOMO**	**LUMO**	**μ**	**E**	**Eele**	**HF**	**IP**	**HOMO**	**LUMO**	**μ**
1	**-67008**	**-420062**	**-68.750**	**6.839**	**-6.839**	**-0.108**	**9.911**	**-67008**	**-420062**	**-68.750**	**6.839**	**-6.839**	**-0.108**	**9.911**
2	**-74052.4**	**-475725**	**-52.761**	**8.634**	**-8.634**	**-0.266**	**0.753**	**-74052.4**	**-475725**	**-52.761**	**8.634**	**-8.634**	**-0.266**	**0.753**
3	**-74215.8**	**-470924**	**-4.8395**	**8.783**	**-8.783**	**-0.410**	**2.500**	**-74215.8**	**-470924**	**-4.8395**	**8.783**	**-8.783**	**-0.410**	**2.500**
4	**-112351**	**-930612**	**-66.277**	**8.815**	**-8.815**	**-0.399**	**2.508**	**-112341**	**-917698**	**-66.443**	**8.711**	**-8.711**	**-0.265**	**1.732**
5	**-112512**	**-923769**	**-15.808**	**8.659**	**-8.659**	**-0.338**	**2.893**	**-112508**	**-915558**	**-16.846**	**8.710**	**-8.710**	**-0.279**	**2.985**
6	**-115785**	**-989671**	**-57.266**	**8.627**	**-8.627**	**-0.305**	**1.787**	**-115780**	**-975750**	**-58.395**	**8.667**	**-8.667**	**-0.236**	**1.503**
7	**-113744**	**-997388**	**11.8597**	**8.693**	**-8.693**	**-0.358**	**3.183**	**-113734**	**-984784**	**10.1223**	**8.666**	**-8.666**	**-0.243**	**2.360**
8	**-78084.5**	**-526686**	**71.8880**	**8.681**	**-8.681**	**-0.336**	**1.454**	**-78084.5**	**-526686**	**71.8880**	**8.681**	**-8.681**	**-0.336**	**1.454**
9	**-80142.6**	**-526778**	**-13.710**	**8.635**	**-8.635**	**-0.319**	**1.355**	**-80142.6**	**-526778**	**-13.710**	**8.635**	**-8.635**	**-0.319**	**1.355**
10	**-129386**	**-1117041**	**-88.388**	**8.499**	**-8.499**	**-0.244**	**3.413**	**-129349**	**-1112926**	**-92.690**	**8.815**	**-8.815**	**-0.335**	**3.316**
11	**-125953**	**-1045955**	**-98.99**	**8.599**	**-8.599**	**-0.272**	**3.057**	**-125932**	**-1054926**	**-100.44**	**8.809**	**-8.809**	**-0.335**	**3.335**
12	**-127346**	**-1117378**	**-19.687**	**8.584**	**-8.584**	**-0.325**	**5.074**	**-127324**	**-1109640**	**5.3761**	**8.816**	**-8.816**	**-0.391**	**3.912**
13	**-77650.2**	**-520068**	**43.686**	**8.799**	**-8.799**	**-0.936**	**2.967**	**-77650.2**	**-520068**	**43.686**	**8.799**	**-8.799**	**-0.936**	**2.967**
14	**-123454**	**-1065171**	**-10.104**	**8.989**	**-8.989**	**-1.042**	**2.024**	**-123432**	**-1056425**	**8.5913**	**1.271**	**-8.591**	**-0.9765**	**4.381**
15	**-126890**	**-1124079**	**-27.749**	**8.970**	**-8.970**	**-1.002**	**1.831**	**-126885**	**-1116060**	**8.9239**	**-23.965**	**-8.923**	**-0.958**	**2.080**
16	**-124850**	**-1140585**	**8.9780**	**39.24**	**-8.978**	**-0.928**	**4.176**	**-124838**	**-1123632**	**8.7754**	**52.897**	**-8.775**	**-0.974**	**3.253**

E: Total energy (kcal/mol)., ***E-ele***:Electronic energy (kcal/mol),***HF***: Heat of formation (kcal/mol), ***IP***: Ionization potential energy(kcal/mol), ***HOMO***: Highest Occupied Molecular Orbital(eV), ***LUMO***: Lowest Occupied Molecular Orbital(eV), **μ**: *Dipole moment*(*Deby)*.

All calculated descriptors were performed using MOE Package [23], and their results were disclosed in (table 3). Our results revealed that, the CLogP (factor of the lipophilicity [32] was less than 5.0, excluding 3 and 5, hydrogen bond acceptors between (2-6), hydrogen bond donors between (1-4) and molar refractivity values in rang (~73-139), these data show these compounds fulfill Lipinski's rule. Also, the absorption percent between (~ 60-99%).

The HOMO and LUMO of a molecule play important roles in intermolecular interactions[33], through the interaction between the HOMO of the drug with the LUMO of the receptor and vice versa. The interactions were stabilized inversely with energy gap between the interacting orbital's. Increasing HOMO energy and decreasing LUMO energy in the drug molecule lead to enhanced stabilizing interactions, and hence, binding with the receptor. Furthermore, the global and local chemical reactivity descriptors for molecules have been defined (table 3), like softness (measures stability of molecules and chemical reactivity [34], hardness (reciprocal of softness), chemical potential, electronegativity (strength atom for attracting electrons to itself), electrophilicity index (measuring lowering energy due to maximal flowing electron between donor and acceptor) [34-39]. From (tables 2 and 3), and from compared with reference drug 1, these results were concluded, the higher potency compounds **7, 8, 12** and **16** have, higher energy gap, higher hardness, lower softness, lower electronegativity, higher chemical potential and higher electrophilicty, lower dipolemoment, and this may explain the less toxicity and high affinity of its compounds against COX.

2.3. Pharmacology

2.3.1. Determination of acute toxicity (LD_{50})

The LD_{50} for diclofenac sodium and isolated compounds were screened, all rats were alive over period 24 h of observation during treated with dose up to 500 mg\ kg for different compounds, and did not show a visible signs of acute toxicity. LD_{50} (750 mg\kg) of compounds **4, 7, 10, 12, 15** and 16, LD_{50} of compounds **6, 8, 11** and **14** was 550 mg\kg, and for diclofenac sodium was 530 mg\kg. The tested compounds are considered non-toxic. Therapeutic index was calculated as (LD_{50} \ ED_{50}), the compounds **4,7,10,12** and **15** with value 37.5, compounds **6,8,11** and **14** were displayed value 27.5, and 26.5 for diclofenac sodium (table 4). So, the isolated compounds **4, 6, 7, 8, 10-12** and **14-17** have higher therapeutic index compared with diclofenac sodium, and may be appear to be relatively less toxic as anti-COX agents.

Table 3: Pharmacokinetic parameters important for good oral bioavailability of compounds **1-16**:

CPD	HBD	HBA	CLogP	V	Vol.	TPSA	%ABS	Log S	mr	ΔE	η	S	χ	σ	ω
1	1	2	4.202	0	278.75	29.1	98.9605	-4.91458	73.18	6.73142	3.36571	0.297114	-3.47382	3.47382	1.792701
2	2	3	4.231	0	255.75	49.33	91.98115	-5.6614	75.46	8.36734	4.18367	0.239025	-4.45039	4.45039	2.367057
3	3	5	5.131	1	264.5	29.1	98.9605	-5.00443	79.32	8.37253	4.186265	0.238876	-4.59725	4.597255	2.524297
4	2	4	5.355	1	403.75	78.43	81.94165	-5.41676	117.38	8.4156	4.2078	0.237654	-4.60777	4.60777	2.522879
5	2	5	6.255	1	410.5	58.2	88.921	-5.26708	79.32	8.3218	4.1609	0.240333	-4.49906	4.49906	2.432351
6	4	6	5.619	0	420.75	67.43	85.73665	-5.177	122.15	8.32181	4.160905	0.240332	-4.46657	4.466575	2.39735
7	1	5	4.207	1	419.625	96.25	75.79375	-5.58933	135.65	8.33471	4.167355	0.23996	-4.52585	4.525855	2.457598
8	1	3	2.915	0	273.375	53.82	90.4321	-5.43965	80.63	8.34486	4.17243	0.239668	-4.50865	4.50865	2.435982
9	4	6	4.031	1	279.125	58.53	88.80715	-6.16118	105.77	8.31628	4.15814	0.240492	-4.47746	4.47746	2.410651
10	3	6	5.240	2	451	96.53	75.69715	-5.06224	125.62	8.25563	4.127815	0.242259	-4.37186	4.371855	2.315161
11	1	2	4.976	0	430.875	107.53	71.90215	-4.79691	130.39	8.32732	4.16366	0.240173	-4.43613	4.43613	2.363215
12	2	3	3.828	0	445.75	125.35	65.75425	-5.23296	131.93	8.25889	4.129445	0.242163	-4.45494	4.454935	2.40304
13	3	5	4.685	1	289	73.55	83.62525	-5.64529	108.99	7.86316	3.93158	0.254351	-4.8682	4.8682	3.013975
14	2	4	5.165	1	443.5	122.55	66.72025	-5.1684	133.61	7.94654	3.97327	0.251682	-5.01624	5.01624	3.166493
15	2	5	5.429	1	463.25	111.55	70.51525	-6.05019	138.38	7.9685	3.98425	0.250988	-4.98641	4.98641	3.120322
16	4	6	4.017	0	458.5	140.37	60.57235	-6.46792	139.92	8.0495	4.02475	0.248463	-4.95326	4.95326	3.047989

TPSA**: Polar surface area* **(A^2)**, %**ABS**: *Absorption percentage,* ***Vol**: Volume (A^3),* ***HBA**: Number of hydrogen bond acceptor,* ***HBD**: Number of hydrogen bond donor,* ***V**: Number of violation from Lipinski's rule of five.,* ***Log P**: Calculated lipophilicity.,* ***Log S**: Solubility parameter,* ***mr**: Molar Refractivity,* **Δ*E: *Energy Gaps*(ev), ***η**: Hardness*(ev)**, S:** Softness(ev)**, χ :** Electronegativity (ev), **σ:** chemical potential (ev), **ω**:Electrophilicity (ev).

2.3.2. Acute ulcer genesis.

The compounds were screened for gastric irritation activity. The ulcerogenic effect of indomethacin and newly synthesized compounds were studied at 20 mg /kg in rats. All tested compounds exhibited significant reduction in ulcerogenic activity compared with the indomethacin (standard drug), which were showed high severity index of 4.50 ± 0.316. This data confirmed that (table 4), these compounds were showed negligible ulcerogenic effect, and may be considered as safer drugs for treating inflammation conditions.

Table 4: Ulcer gastric effect of rats for tested compounds.

Compounds	LD_{50}	Therapeutic index	Ulcerogenic index	
			Ulcer severity	Ulcer Number
Control	ND	ND	0.0	0.0
Indomethacin	ND	ND	22.4 ±1.652	11.8 ±0 .985
Diclofenac	530	26.5	0.0	0.0
4	750	37.5	0.0	0.0
6	550	27.5	0.0	0.0
7	750	37.5	0.0	0.0
8	550	27.5	0.0	0.0
10	750	37.5	0.0	0.0
11	550	27.5	0.0	0.0
12	750	37.5	0.0	0.0
14	550	27.5	0.0	0.0
15	750	37.5	0.0	0.0
16	750	37.5	0.0	0.0

* *Dose= 20 mg/kg., ND: Not detected, Statistically significant at the corresponding time ($p < 0.05$).*

3. Conclusion.

A series of Diclofenac derivatives containing L-phenylanline moiety **4-16** were synthesized. The comparative docking experiments were carried out on compounds **4-8**, **10-12** and **12-16**, and reference molecules **1**. These compounds were stabilized in the binding pocket of COX with a similar binding mode of diclofenac, some compounds **7, 12** and **16** with high binding score were showed suitable selective inhibitors against COX-2. The compounds under investigation were subjected to ADMET profile theoretically reveled that, these compounds were considered well passive oral absorption. The ulcerogenic studies were screened for isolated compounds **4-8**, **10-12** and **14-16**, and showed negligible ulcerogenic effect with higher safety and better therapeutic index than diclofenac. The molecular docking studies were supported with ulcerogenic effect and through understanding the various interactions of ligands and active sites of enzymes, help design novel potent selective COX-2 inhibitors.

4. EXPERMINTAL:

4.1. Instrumentation and materials:

Melting points were taken on a Griffin melting point apparatus and are uncorrected. Thin layer chromatography (R_f) for analytical purposes was carried out on silica gel and developed. Benzidine, ninhydrin, and hydroxamate tests used for detection reactions. The IR spectra of the compounds were recorded on a Perkin–Elmer spectrophotometer model 1430 as potassium bromide pellets and frequencies are reported in cm^{-1}. The NMR spectra were observed on a Varian Genini-300 MHz spectrometer and chemical shifts (δ) are in ppm. The mass spectra were recorded on a mass spectrometer HP model MS–QPL000EX (Shimadzu) at 70 eV. Elemental analyses (C, H, N) were carried out at the Microanalytical Centre of Cairo University, Giza, Egypt. Transmission Electron Microscope (TEM) micrographs were measured using JEOL JEM-1010 Transmission Electron Microscope, at an accelerating voltage of 60 kV. Suspensions of the samples were put on carbon foil with a micro grid. TEM images were observed with minimum electron irradiation to prevent damage to the sample structure.

4.2. Synthesis:

4.2.1. 2-[(2,6-Dichlorophenyl)amino]phenyl acetyl chloride (3)

Prepared by reported method and directly used in the next step (40, 41).

4.2.2. (2-(2-((2,6-dichlorophenyl)amino)phenyl)acetyl)-L-phenylalanine (4):

4.2.2.1. Path 1:

A mixture of L-phenylalanine (0.01 mol) and 2-(2-(2,6-dichlorophenyl-amino)phenyl)acetic acid (**2**; 0.01 mol) was fused at 280 ℃ in an oil bath for 15 min. The fused mass was dissolved in ethanol and poured onto cold water, the solid obtained was recrystallized from ethanol to give compound (**4**).

4.2.2.1. Path 2:

The L-phenylalnine (0.01 mol) was dissolved in a mixture of water (25ml), THF (15ml) and triethylamine (2 ml) were added, followed by portionwise addition of acid chloride (3; 0.01 mmol) during 30 mins, temperature of the reaction mixture was kept at 10 ℃ during the addition. Stirring was continued for 3 h at 10 ℃. THF was removed by concentration of the reaction mixture under reduced pressure; water (30 ml) was added and acidified with 1 N HCL to pH =5. The crude products were filtered and recrystallized from ethanol. The product 4 was chromatographically homogeneous by iodine and benzidine development. Brown crystal : yields=81%; R_f=0.55 ($CHCl_3$/EtOH=3/1); mp: 150-52 ℃; =+49.1 ° (Et$[\alpha]_D^{20}$) ; IR (KBr cm^{-1}) ν; 3266 broad band (OH,NH), 3055 ($CH_{arom.}$), 2954 ($CH_{ali.}$), 1694(CO),1596(CONH) cm^1; ^{1}H NMR (300 MHz, Chloroform) δ= 12.68 (s, 1H-O<u>H</u>), 8. 55 (d, H-N<u>H</u>-$_{Dic.}$), 7.72 – 7.05 (m, 12H-aromatic proton), 6.36(d, H-N<u>H</u>-$_{Phe}$), 3.85(s, 2H, C<u>H$_2$</u>-$_{Dic.}$), 2.31-2.04 (*t*, 1H-C<u>H</u>-$_{Phe}$), 1.27 (d, 2H-C<u>H$_{2\text{-}Phe}$</u>); Anal./Calcd. for $C_{23}H_{20}Cl_2N_2O_3$ (442): C (62.44%), H (4.52%), N (6.33%). Found: C (62.31); H (4.55); N, (6.32).

4.2.2. General procedures for synthesis L- amino acid methyl ester derivatives (6, 11 and 15).

The free amino acid derivatives (**4**, 10 and 14; 0.01 mol) in absolute methanol (50 ml) was cooled to 0-5 ℃, and pure thionylchlorid (0.015 mmol) was added dropwise during one hour. The reaction mixture was stirred for an additional 3 h at room temperature, and kept overnight. The solvent was removed by vacuum distillation, the residual solid material was recrystallized from ethanol. The products 6, 11 and 15 were chromatographically homogeneous by iodine and benzidine development.

4.2.2.1. methyl (2-(2-((2,6-dichlorophenyl)amino)phenyl)acetyl)-L-phenylalaninate (6).

Yellowish brown crystal : yields=57%; R_f=0.86 ($CHCl_3$/EtOH=3/1); mp: 131-33 ℃ $[\alpha]_D^{20}$ = +52.38 ° (*c*=0.1, EtOH) ; IR (KBr cm^{-1}) ν; 3262 (NH), 3036($CH_{arom.}$), 2957 (CH_{ali}), 1713(CO), 1659 (CONH) cm^1; ^{1}H NMR (300 MHz, Chloroform) δ= 8. 55 (d, H-N<u>H</u>-$_{Dic.}$), 7.72 – 7.05 (*m*, 12H- aromatic proton), 6.36(d, H-N<u>H</u>-$_{phe}$), 3.85(*s*, 2H, C<u>H$_2$</u>-$_{Dic.}$), 3.75(*s*, 3H, OC<u>H$_3$</u>), 2.31-2.29 (*t*, 1H-C<u>H</u>-$_{Phe}$), 1.77 (*d*, 2H-C<u>H$_{2\text{-}Phe}$</u>); Anal./Calcd. for $C_{24}H_{22}Cl_2N_2O_3$ (456): C (63.15 %), H (4.82%), N (6.14%). Found: C (63.03); H (4.85); N, (6.13).

4.2.2.2. methyl ((2-((2,6-dichlorophenyl)amino)benzyl)carbamoyl)-L-phenylalaninate (11).

Yellowish brown crystal : yields=57%; R_f=0.71 ($CHCl_3$/EtOH=3/1); mp: 144-46 ℃; $[\alpha]_D^{20}$ = +51.84 ° (EtOH) ; IR (KBr cm^{-1}) ν; 3396,3277 (NH), 3109($CH_{arom.}$), 2988 (CH_{ali}), 1696(CO), 1607 (CONH) cm^1; ^{1}H NMR (300 MHz, Chloroform) δ= 8. 55 (d, H-NH-$_{Dic.}$), 7.52 – 6.81 (*m*, 12H- Ar-H), 6.34 (*d*, H-NHCONH), 6.21(d, H-NHPh-$_{phe}$), 3.80(*s*, 2H, CH_2-$_{Dic.}$), 3.77(*s*, 3H, OCH_3), 3.35-3.32 (*t*, 1H-CH-$_{Phe}$), 1.19 (*d*, 2H-$CH_{2\text{-}Phe}$); Anal./Calcd. for C24H23Cl2N3O3 (471): C(61.14 %), H (4.88%), N (8.91%). Found: C (61.01); H (4.63); N, (8.40).

4.2.2.3. methyl ((2-(2-((2,6-dichlorophenyl)amino)phenyl)acetyl)carbamothioyl)-L-phenylalaninate (15).

Yellowish brown crystal : yields=60%; R_f=0.73 ($CHCl_3$/EtOH=3/1); mp: 148-150 ℃; $[\alpha]_D^{20}$ =3.77 ° (EtOH) ; IR (KBr cm^{-1}) ν; 3362 (NH), 3150($CH_{arom.}$), 2950 (CH_{ali}), 1693(CO), 1586 (CONH) cm^1; ^{1}H NMR (300 MHz, Chloroform) δ= 8. 55 (*d*, H-NH $_{Dic.}$), 7.72 – 7.15 (*m*, 12H- Ar-H), 7.05 (*d*, H-NHCSNH), 6.36(*d*, H-NHPh-$_{phe}$), 3.85(*s*, 2H, CH_2-$_{Dic.}$), 3.71(*s*, 3H, OCH_3), 2.04 (*t*, 1H-CH-$_{Phe}$), 1.32 (*d*, 2H-$CH_{2\text{-}Phe}$); Anal./Calcd. for $C_{25}H_{23}Cl_2N_3O_3S$ (515): C(58.25 %), H (4.46%), N (8.15%). Found: C (58.14); H (4.49); N, (8.14).

4.2.3. General procedures for synthesis L- amino acid hydrazide (7, 12 and 16).

L-amino acid methyl ester derivatives (6, 11 and 15; 0.01 mol) were dissolved in a solution containing ethanol (100 ml) and 85 % hydrazine hydrate (6.3 ml), the mixture was refluxed for 1/2h., then left overnight at 25 ℃. The product was separated, and collected by suction filtration, washed with methanol and light petroleum ether, and recrystallized from ethanol to give desired hydrazid compound derivatives (7, 12 and 16).

4.2.3.1(2-(2-((2,6-dichlorophenyl)amino)phenyl)acetyl)-L-phenylalanine hydrazide (7).

white crystal; yields=84%; R_f=0.80 ($CHCl_3$/MeOH=3/1); mp: 178-181 ℃; $[\alpha]_D^{20}$ =+52.81 ° (EtOH); IR (KBr cm^{-1}) ν; 3340, 3139 (NH_2 ,NH), 3014($CH_{arom.}$), 2911,2851(CH_{ali}), 1687 (CO), 1617,1582 (CONH) cm^{-1}; ^{1}H NMR (300 MHz, Chloroform) δ = 8.01(*d*, 1H-NH-$_{Dic.}$) 7.66– 7.12 (*m*,12H-Ar-H), 6.89(*d*,1H, NH- $_{phe.}$), 4.90(s, 1H-NHNH$_2$), 4.25(s, 2H-NHNH$_2$), 3.84 (s, 2H- $CH_{2\text{-}DIC.}$), 1.90(*t*, 1H-CH-$_{Phe}$), 1.14 (*d*, 2H-$CH_{2\text{-}Phe}$); ^{13}CNMR (CD3OD); δ= 161.74,160.11 (CONH and CONHNH$_2$), 114.77, 116.26, 118.41, 120.25, 123.95, 127.91, 129.84, 132.81, 134.079, 145.64, 153.40, 155.20, 157.71 (ArC, ArCH), 40.33 (CH-$_{Phe}$), 39.77 (CH_2-$_{Phe}$), 38.66(CH2-$_{Dic.}$); Anal./Calcd. for $C_{23}H_{22}Cl_2N_4O_2$ (456): C (60.52%), H (4.82%), N (12.28%). Found: C (60.40); H (4.85); N, (12.25).

4.2.3.2. ((2-((2,6-dichlorophenyl)amino)benzyl)carbamoyl)-L-phenylalanine hydrazide (12).

white crystal; yields= 50%; Rf=0.69 (CHCl3/MeOH=3/1); mp:210-12 ℃; $[\alpha]_D^{20}$ =+53.01 ° (EtOH); IR (KBr cm-1) ν; 3331, 3248 (NH2 ,NH), 3018(CH arom.), 2959 (CHali), 1694 (CO), 1582 (CONH) cm-1; 1H NMR (300 MHz, Chloroform) δ =8.11(d, 1H-NH-Dic.), 7.66– 7.12 (m,13H,[12H-ArH+1H-NHCONH), 6.89(s,1H, NH- phe.), 4.90 (s, 1H-NHNH2), 4.55(s, 2H-NHNH2), 3.84 (s, 2H- CH2-DIC.), 3.33 (t, 1H-CH-Phe), 1.14 (d, 2H-CH2-Phe); 13CNMR (CD3OD); δ= 163.56, 162.93 (CONHCO and CONHNH2), 153.93(CONHCO), 115.66, 116.53, 120.11, 120.67, 120.72 (ArC, ArCH), 40.66 (CH-Phe), 39.22 (CH2-Phe), 37.08(CH2-Dic.); Anal./Calcd. for $C_{23}H_{23}Cl_2N_5O_2$ (471): C (58.59%), H (4.88%), N (14.86%). Found: C (57.61); H (4.63); N, (14.00).

4.2.3.3. ((2-(2-((2,6-dichlorophenyl)amino)phenyl)acetyl)carbamothioyl)-L-phenylalanine hydrazide (16).

white crystal; yields=63%;R_f=0.80 ($CHCl_3$/MeOH=3/1); mp:183-85 ℃ ; $[\alpha]_D^{20}$ =+50.32 ° (EtOH); IR (KBr cm^{-1}) ν; 3252, 3248 (NH_2 ,NH), 3050(CH $_{arom.}$), 2992 (CH_{ali}), 1710 (CO), 1588 (CONH) cm^{-1}; ^{1}H NMR (300 MHz, Chloroform) δ =8.21(*d*, 1H-NH-$_{Dic.}$), 7.66– 7.01 (*m*,12H-ArH), 6.34(d,1H-NHCSNH), 6.22(*d*,1H, NH- $_{phe.}$), 5.21 (s, 1H-NHNH$_2$), 4.72(s, 2H-NHNH$_2$), 3.90 (s, 2H- $CH_{2\text{-}DIC.}$), 3.67 (*t*, 1H-CH-$_{Phe}$), 2.84 (*d*, 2H-$CH_{2\text{-}Phe}$); ^{13}CNMR (CD3OD); δ= 185.03(CSNHCO), 160.88 (CONHNH$_2$), 150.72(CONHCS), 120.71, 120.73, 128.67, 129.81, 129.85, 134.95(ArC, ArCH), 47.96 (CH-$_{Phe}$), 39.77 (CH_2-$_{Phe}$), 32.35(CH2-$_{Dic.}$); Anal./Calcd. for $C_{24}H_{23}Cl_2N_5O_2S$ (515): C (55.92%), H (4.46%), N (13.59%). Found: C (55.82); H (4.49); N, (13.56).

4.2.4. 2-(2-(2,6-dichlorophenylamino)phenyl)acetyl azide (8).

The compounds 3 (0.01 mol) in THF (20 ml), was added to 35% aqueous sodium azide (2 ml) in acetone(15 ml) at 0 ℃. The resulting mixture was stirred at 0–5 ℃ for 5hs. The progress of the reaction was monitored by TLC using 40% EtOAc in petroleum ether as a mobile phase. The solvent was evaporated on a rotatory evaporator under reduced pressure, and residue obtained was triturated in diethyl, the solid material was

filtered under suction and dried to afford the title compound 8 as yellow solid : yields=75%; R_f=0.57 ($CHCl_3$/MeOH=3/1); mp 102-104 ℃; IR (KBr cm^{-1}) ν; 3248 (NH), 3025 ($CH_{arom.}$), 2978 (CH_{ali}), 1694 (CO) cm^1; ^{1}H NMR (300 MHz, Chloroform) δ = 8.20(*s*,1H, NH), 7.13 – 8.01 (*m*,7H-Ar-H), 3.88 (s, 2H- CH_2); Anal./Calcd. for $C_{14}H_{10}Cl_2N_4O$ (320): C (52.50%), H (3.12%), N (17.50%). Found: C (52.36); H (3.14); N, (17.45).

4.2.5. Action of L-Phenylalnine methyl ester on azide(8).

Azide (8; 0.01 mole) in THF (10 ml.) was treated with L-phenylalnine methyl ester (0.015 mole) at room temperature. The reaction mixture was refluxed for 10 h. The excess solvent was removed and the solid residue was washed with water, and then crystallized from EtOH to give L- amino acid methyl ester derivatives (6).

4.2.6. 2-(2-(2,6-dichlorophenylamino)phenyl)acetyl isocyanate (9).

The azide (8; 0.01mmol) in dry THF (17 ml.) was refluxed at 150–180 ℃ for a period of 5 h. The progress of the reaction mixture was monitored by TLC using 45% EtOH in petroleum ether as a mobile phase. The solvent was concentrated, and used immediately in the other reactions without further purification.

4.2.7. General procedures for synthesis of L- amino acid ureido(10) and thioureido (14) derivatives.

The isocyanate (9; 0.01mol) and\ or isothiocyanate (13; 0.01mol) in THF, the L-Phenylalanine and few drops of pyridine (0.5 ml.) was added at 0–5 ℃, and stirred at same temperature for over a period of 21–26 h. The solvent was concentrated, and the mixture was treated with cooled ice-water and 0.1N HCl to afford crude product. The obtained residue was washed with water, and recrystallized from ethanol to provide the products 10 and 14.

4.2.6.1. ((2-((2,6-dichlorophenyl)amino)benzyl)carbamoyl)-L-phenylalanine (10) .

brown crystal: yields=60%; R_f=0.60 (petroleum ether /EtOH = 3/1); mp: 205-207 ℃; $[\alpha]_D^{20}$ = +51.42 ° (EtOH); IR (KBr cm^{-1}) ν; 3210 broad band (OH, NH), 3055 ($CH_{arom.}$), 2958 (CH_{ali}), 1694 (CO), 1602 (CONH) cm^1; ^{1}H NMR (300 MHz, Chloroform) δ =11.04(*s*, 1H,OH), 8.44 (*d*, H-N<u>H</u>-$_{Dic.}$), 7.72 – 7.05 (*m*, 12H- Ar-H), 6.57 (*d*, H-N<u>H</u>CONH), 6.36(*d*, H-N<u>H</u>Ph-$_{Phe.}$), 3.85(*s*, 2H, C<u>H</u>$_2$-$_{Dic.}$), 2.09 (*t*, 1H-C<u>H</u>-$_{Phe}$), 1.87 (*d*, 2H-C<u>H</u>$_{2\text{-}Phe}$); Anal./Calcd. for **$C_{23}H_{21}Cl_2N_3O_3$** (457): C(60.03 %), H (4.59%), N (9.19%). Found: C (59.27); H (4.35); N, (8.64).

4.2.6.2. ((2-(2-((2,6-dichlorophenyl)amino)phenyl)acetyl)carbamothioyl)-L-phenylalanine (14).

browen crystal: yields=78%; R_f=0.65 (petroleum ether /EtOH=3/1); mp: 196-98 ℃; $[\alpha]_D^{20}$ = +53.82 ° (EtOH) ; IR (KBr cm^{-1}) ν; 3324, 3128 broad band (OH overlapping NH), 3064 ($CH_{arom.}$), 2978 (CH_{ali}), 1656(CO), 1586 (CONH) cm^1; ^{1}H NMR (300 MHz, Chloroform) δ = 9.93(*s*, 1H,OH), 8.02 (*d*, H-N<u>H</u>$_{phe}$), 7.61 – 7.36 (*m*, 12H- aromatic proton), 6.82 (*s*, H-N<u>H</u>CSNH), 6.58(*d*, H-N<u>H</u>Ph-$_{Dic}$), 3.85(*s*, 2H, C<u>H</u>$_2$-$_{Dic.}$), 1.74 (*t*, 1H-C<u>H</u>-$_{Phe}$), 1.22 (*d*, 2H-C<u>H</u>$_{2\text{-}Phe}$); Anal./Calcd. for $C_{24}H_{21}Cl_2N_3O_3S$ (501): C(57.48 %), H (4.19%), N (8.38%). Found: C (57.37); H (4.21); N, (8.36).

4.2.7. 2-(2-(2,6-dichlorophenylamino)phenyl)acetyl isothiocyanate (13).

The ammonium isothiocyanate (0.01 mol.) in dry acetone (15 ml) was stirred with acid chloride (3; 0.01 mmol) in dry THF (10 ml.) at 0–5 ℃ over a period of 5 h. The progress of the reaction mixture was monitored by TLC using 30% EtOH in petroleum ether as a mobile phase. The solvent was concentrated, and was used immediately in the other reactions without further purification.

4.2.7.1. Action of L-Phenylalnine methyl ester on isocynate (9) and isothiocyanate (13).

The L-Phenylalanine methyl ester (0.01mol) was added to isocyanate (9) isothiocyanate (13) in THF at 0–5 ℃, and stirred at for a period of 5 hrs. The solvents were evaporated on a rotator evaporator under reduced pressure to afford a crude product. The solids were filtered under suction and dried to afford the compounds 11 and 15.

4.3. Molecular Modeling Study:

4.3.1. ***Generation of Ligand and Enzyme Structures.***

4.3.1.1. Selection of COX structures.

Docking study was carried out for the target compounds into (COX-1 (ID: 3N8Y) and COX-2 (ID: 1PXX) using MVD,4.0 and MOE,10. The crystal structure of the (COX) complexes with (1), which is a selective inhibitor of COX-2 in co-crystallized form in the active site of the receptor. From X-ray crystal structure studies

of the COX enzyme, the mouse enzyme is expected to be very similar to the human [19], and can be used as model for human COX enzyme.

4.3.2. Preparation of Small Molecule:

Molecular modeling of the target compounds were built using MOE, and were minimized their energy with PM3 through MOPAC. Our compounds were introduced into the (COX) binding sites according to the published crystal structures of (1) bound to kinase.

4.3.3. Stepwise Docking Method:

4.3.3.1. MOE Stepwise

The crystal structures of the (COX) with a diclofenac (1) as an reference inhibitor molecule was used, Water and inhibitor molecule was removed, and hydrogen atoms were added. The parameters and charges were assigned with MMFF94x force field. After alpha-site spheres were generated using the site finder module of MOE. The optimized 3D structures of molecules were subjected to generate different poses of ligands using triangular matcher placement method, which generating poses by aligning ligand triplets of atoms on triplets of alpha spheres represented in the receptor site points, a random triplet of alpha sphere centers is used to determine the pose during each iteration. The pose generated was rescored using London dG scoring function. The poses generated were refined with MMFF94x forcefield, also, the solvation effects were treated. The Born solvation model (GB/VI) was used to calculate the final energy, and the finally assigned poses were assigned a score based on the free energy in kJ/mol

4.3.3.2. *MVD Scorings Stepwise*

Molecular docking was carried out using Molegro Virtual Docker (MVD). MVD is based on a differential evolution algorithm; the solution of the algorithm takes into account the sum of the intermolecular interaction energy between the ligand and the protein, and the intramolecular interaction energy of the ligand. The docking energy scoring function is based on a modified piecewise linear potential (PLP) with new hydrogen bonding and electrostatic terms included. Full description of the algorithm and its reliability compared to other common docking algorithm can be found in reference [42, 43]. The small molecules and the PDB crystal structure atomic coordinates determined by x-ray crystallography of 1G54 were imported, potential binding sites were predicted. The binding cavities were set at X: -6.51, Y: 2.79, Z: 17.44, grid resolution was set to 0.3 Å, while the binding site radius was set to 10 Å. The RMSD threshold for multiple cluster poses was set at < 1.00Å. The docking algorithm for molecular docking is based on a differential evolution algorithm; the solution of the algorithm takes into account the sum of the intermolecular interaction energy between the ligand and the protein, and the intramolecular interaction energy of the ligand.

4.4. Pharmacology.

4.4.1. Determination of acute toxicity

The acute toxicity and lethality (LD_{50}) for the new isolated compounds were estimated in albino mice (25–30 g). In a preliminary test, animals in groups of three, received one of 300, 500, 600 or 700 mg\ kg for the tested compounds and diclofenac. Animals were observed for 24 hrs for signs of toxicity and number of deaths. The LD_{50} calculated as the percentage mortality in each group was determined 24 h after administration[44].

4.4.2. Acute ulcer genesis.

The studies were carried out on healthy Albino rats at a dose 20 mg\ kg. The animals were divided into different groups of six each, group I served as control and received vehicle only, groups II received pure indomethacin 20 mg\kg, the other groups were administered test compounds in dose molecularly equivalent to 20 mg\ kg of indomethacin. Before 24 hrs administration of the tested compounds, Food not water was removed; the rats were fed normal diet for 17 hrs and then sacrificed after the drug treatment. The stomach was removed and opened along the greater curvature, washed with distilled water and cleaned gently by dipping in saline. The mucosal damage was examined by means of a magnifying glass. For each stomach, the mucosal damage was assessed according to the following scoring system[45].

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