Egypt. J. Chem. **Vol. 65,** No. 8 pp. 163 - 176 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Synthesis, characterization, docking studies and anti-inflammatory activity of new safe NSAIDs agent based on Ibuprofen phenylalanine derivatives

Abdelhamid Selim[1*], Fatma A.A. El-Hag[2], Reem T. Attia[3], S.M. Shedid[1], MA El-Gazzar[1]

[1]*Chemistry Department, Faculty of Science, Al-Azhar University (Boys' Branch), Nasr City, Cairo, Egypt*

[2]*Department of Chemistry of Natural and Microbial Products, National Research Centre, Dokki, P.O. 12622, Giza, Egypt.*

[3] *Department of Pharmacology, Toxicology and Biochemistry, Future University in Egypt, Cairo, Egypt.*

Abstract

Ibuprofen phenylalanine derivatives **1-17** as new safe nonsteroidal anti-inflammatory drugs (NSAIDs) agents were synthesized and characterized depending on spectroscopic and analytical analyses. Starting from reaction between ibuprofen with PABA to have fussed derivative 1 that reacted with phenylalanine followed by hydrazine, ammonium thiocyanate, urea derivatives to afford the new compounds. For investigated drugs **1-17**, molecular docking was done at the cyclooxygenase-2 (COX-2) active site. For the purpose of discussing binding affinity, the position with the lowest root-mean square deviation (RMSD) has been chosen. The binding interaction was enhanced by adding a hydrazide fragment to the parent molecule, as shown in the docking technique. Compounds **5, 6, 12, 16** and **17** were investigated as anti-inflammatory and analgesic drugs. Using ~~a~~ carrageenan-induced mice of hind paw edoema, we investigated the synthesized compounds' potential anti-inflammatory activity in contrast to their parent molecule, ibuprofen. The antinociceptive and the ulcerogenic effect of the synthesized compounds have been measure. Compounds **5** and **6** are the best drug analogues and these compounds could be promising for anti-inflammatory agents. .

Ibuprofen; phenylalanine; Synthesis, docking, Analgesic, and anti-inflammatory

Introduction.

Ibuprofen is a derivative of propionic acid (2-arylpropionic acids) that was first offered as a superior alternative to aspirin in 1969[1]. Ibuprofen derivatives are widely used to alleviate acute arthritis, non-rheumatic inflammation, fever, pain, and primary dysmenorrehea; because of its good pharmacological properties and greater tolerability [2, 3]. In most situations, high dosages are required to produce good therapeutic benefits. Long-term oral treatment causes Gastrointestinal (GI) ulceration and hemorrhage, however these side effects are far less prevalent than aspirin [4, 5]. These adverse effects in the gastrointestinal tract (GIT) are directly attributed amalgamation of ibuprofen free carboxylic local irritation created that leads to inhibition of cyclooxygenase (COX) in GIT [4, 6]. Thus, the improvement of bioderivatives, such as prodrugs, is urgently needed to reduce the toxicity caused by nonsteroidal anti-inflammatory drugs NSAIDs.[7, 8] Prodrug is a substance that can undergoes conversion to active pharmacologically agent via physiochemical and metabolic transformation [4]. It is also have two active agents; one of them is a carrier and the other is a drug, and they may have additional pharmacological characteristics that are not included in parent drugs [9]. As a result, these pro-drugs provide significant therapeutic and alleviative efficacy while having less side effects than the parent drug [10]. Derivatives of Ibuprofen amide have an effective role of their importance as biodrug because of their action as gastro-protective via shifting selectivity of their enzymes from COX1 toward COX2[11]. NSAIDs' free carboxylic acid interacts with tyrosine, glutamine, and arginine remains in cyclooxygenase active site [12]. The principal source of decreased irritation

*Corresponding author e-mail aselemfue@gamil.com .; (AbdelHameed Selim).
Receive Date: 11 May 2022, Revise Date: 21 June 2022, Accept Date: 21 June 2022
DOI: 10.21608/EJCHEM.2022.137847.6069

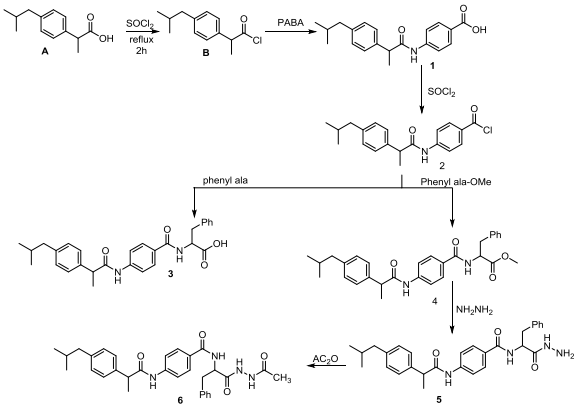
appears to be the masking effect of ibuprofen's free Carboxylic acid COOH [13]. Amidases responsible for amide bond hydrolysis are exclusively found in the intestine [14]. Clinically, amide derivatives of ibuprofen have pharmacological properties such as antifungal, anthelmintic, anti-HIV, antiulcer, cardiotonic, antihypertensive, antibacterial, and neuroleptic[15-17]. The aim of this study and others in our laboratory [18]was conduct the combination effect between p-aminobenzoic acid (p-ABA) with various phenyl alanines with evaluation of their pharmacological activity.

Results and Discussion

Chemistry

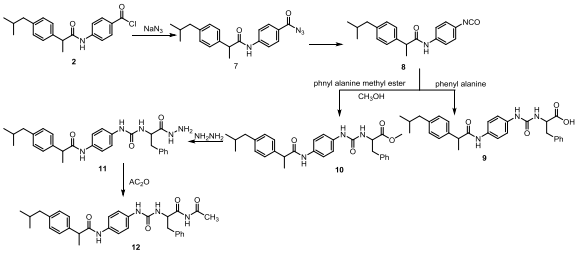
In continuation of synthesis of effective candidates in our laboratory as antimicrobial, antiviral, and anticancer agents [19-23]; we aim to conjugate Ibuprofen with phenyl alanine to be an effective agents as antiinflammatory and analgesic. Our strategy based on preparation of ibuprofen derivatives **1-17** starting from acid chloride **2** that may be expected to have biological activities (Scheme 1).

First, Ibuprofen **A** reacted with thionyl chloride (as a solvent and a reagent as with dual action) to afford its chloride derivative **B** that reacted with p-ABA to afford compound **1** in good yield. This respective compound is reacted with thionyl chloride under reflux condition to give compound **2** (Scheme 1). The staring material **2** reacted directly with phenyl alanine in to give amino acids derivative **3**, compound 2 also reacted with phenyl alanine methyl ester to give methyl derivative **4**. Compound **4** underwent hydrazinolysis to afford hydrazide **5** that reacted with acetic anhydride to get compound **6** (Scheme 1).



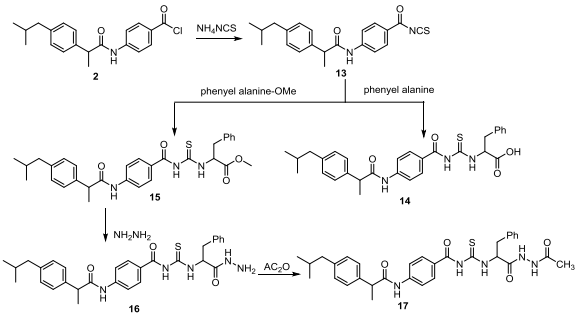
Scheme 1: Synthetic pathway of ibuprofen derivatives 1-6.

IR spectra of new compounds showed carboxylic OH absorption band and NH group at 3363 and 3460 cm^{-1}, with appearance of carbonyl group at 1720 cm^{-1}. Also, 1H-NMR of compound **1** revealed signal of COOH of new coupled amino acids at δ =12.21 ppm that attributed hydroxyl group. NH for phenylalanine were appeared in 1H-NMR spectra at rang 3.44-8.05 and for compounds **3-6**.



Scheme 2: Synthetic pathway of ibuprofen derivatives 7-12

Besides, Azidation as nucleophilic substitution occurred between ibuprofen derivative 2 and sodium azide to give 4-(2-(4-isobutylphenyl) propanamido)benzoyl azide (**7**) , which converted to 2-(4-isobutylphenyl)-N-(4-isocyanatophenyl) propenamide (**8**) by heating. Compound **8** reacted with phenyl alanine to give ((4-(2-(4-isobutylphenyl) propanamido)phenyl)carbamoyl) phenyl alanine (**9**), Compound **10** reacted with hydrazide to give hydrazide derivative **11**, which acylated by acetic anhydride to give N-(3-(3-(1-(2-acetylhydrazinyl)-1-oxo-3-phenylpropan-2-yl)ureido)phenyl)-2-(4-isobutylphenyl)propanamide **12** (Scheme 2)



Scheme 3: Synthetic pathway of ibuprofen derivatives 13-17

Structures of new compounds were deduced on their elemental analyses with spectroscopic techniques, taking compound **12** as an example, it revealed in its FTIR peaks at 3463, 3436, 3401, 3355

cm^{-1} of NH groups with characteristic peak at 1724 cm^{-1} of C=O. 1H NMR of derivative 12 afford signals at δ 8.14 as singlet of NH that exchangeable with D2O and at δ 7.57 (d, J = 8.2, 2H, CH_{arom}), δ 7.24 (d, J = 8.2, 5.7 Hz, 2H, CH_{arom}), δ 7.14 (d, 2H, CH_{arom}), δ 7.06 (m, 5H, CH_{arom}), δ 6.50 (d, J = 8.3, 5.8 Hz, 2H, CH_{arom}), and signals at δ 6.20-5.47 as singlets of NH that exchangeable with D2O. Also, signals at δ 3.81 (s, 3H, Me), δ 3.69 (t, J = 5.2 Hz, 1H, CH-Me), δ 2.47 (d, J = 6.4 Hz, 2H, CH_2), δ 2.34 (s, 2H, CH_2), δ 1.81 (t, J = 6.1 Hz, 1H, CH), δ 1.68 (t, J = 6.1 Hz, 1H, CH_3), and at δ 0.81 (t, J = 6.3 Hz, 6H, CH_3).

Compound **2** were reacted with ammonium isothiocyanate to give isothiocyanate derivative **13**, that reacted with phenyl alanine in THF and few drops of pyridine for 5 h at room temperature to give ((3-(2-(4-isobutylphenyl)propanamido)benzoyl) carbamothioyl) phenylalanine **14** (Scheme 3). While compound **13** reacted with phenyl alanine methylester afforded compound **15** directly as methyester derivative , which reacted with hydrazine to give N-((1-hydrazinyl-1-oxo-3-phenylpropan-2-yl)carbamothioyl)-3-(2-(4-isobutylphenyl) propanamido)benzamide (**16**). Compound **16** was acylated to N-((1-(2-acetylhydrazinyl)-1-oxo-3-phenylpropan-2-yl)carbamothioyl)-3-(2-(4-isobutylphenyl)propanamido)benzamide (**17**) (Scheme 3).

Molecular Docking studies.

Antiinflammatory action for compounds 3-17 was identified using the molecular docking experiment against COX-2. The COX-2 (ID: 1PXX; [24]) crystal structure of X-ray bonded with original inhibitor was utlized in this experiment [25]. The Tyrosin-385 (Tyr-385) and Serine-530 Ser-530 have a crucial function for chelating of the ligand. They amino acids chelated with COOH for arachidonicacid which was coordinated with Tyr-385&Ser-530 and make the tetrahedral intermediate geometry [26, 27].

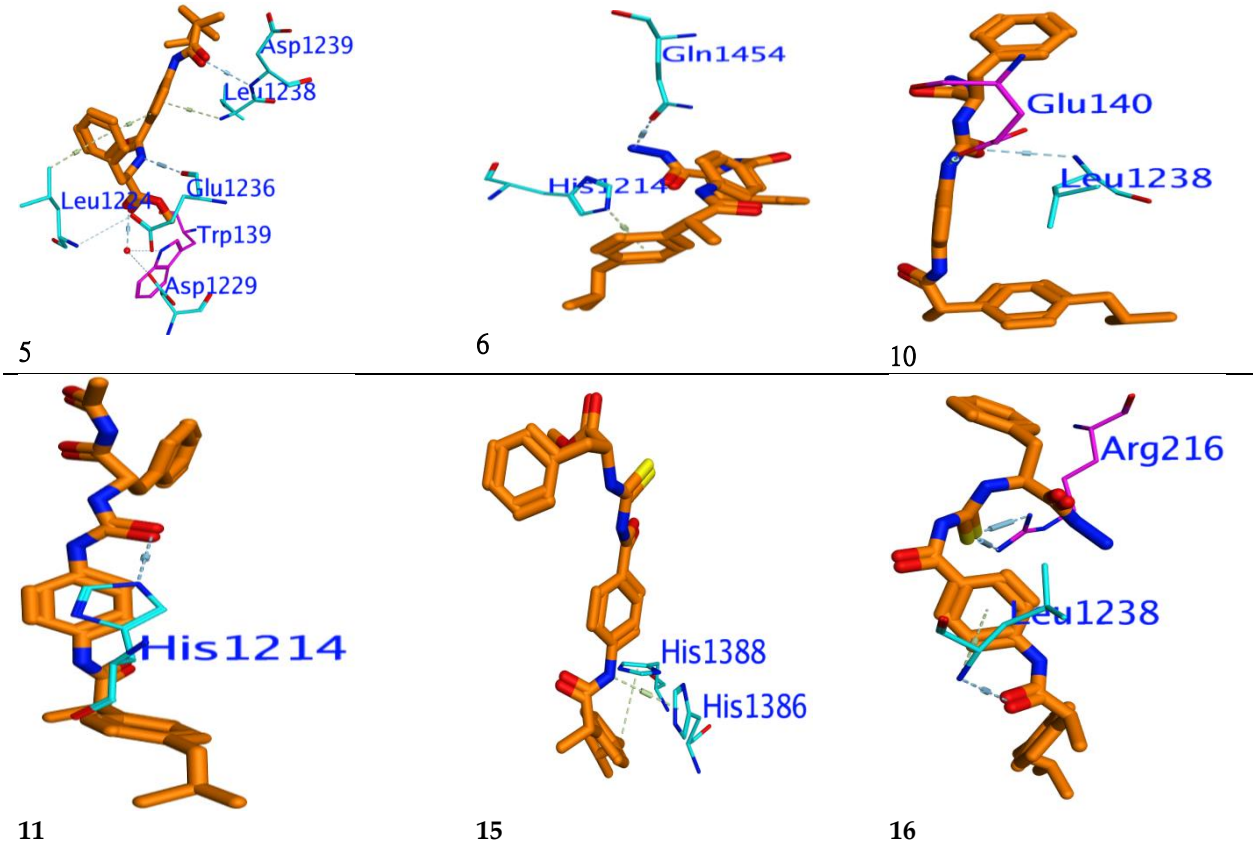


Fig.1 : The interaction approach of most active (5, 6,10,11,14 and 15) inside the COX-2active site, H-bond represented as blue dashed color.

Table 1: Docking scores of the synthesized compounds (3-17)

Cpd	ΔE	rmsd	H.B	Int.	E_ele
3	-8.33	1.01	-62.47	-19.51	-4.99
4	-7.79	1.51	-14.97	-30.03	-5.80
5	-6.92	1.36	16.61	-1.69	-4.31
6	-6.75	1.48	45.05	-6.15	-4.96
7	-7.58	1.38	-103.26	0.29	-6.22
9	-6.02	1.49	-99.38	-8.41	-4.83
10	-7.76	1.81	-72.80	-19.32	-6.34
11	-8.88	1.01	-47.21	-35.59	-4.72
12	-7.60	1.57	-166.29	-18.05	-7.34
14	-8.41	1.18	-76.82	-19.18	-5.16
15	-7.49	1.40	-48.60	-15.19	-5.67
16	-8.36	1.27	-18.59	-28.75	-4.92
17	-9.01	1.10	-61.45	-15.77	-4.45

DG: Free binding energy of the ligand, Int.: Affinity binding energy of hydrogen bond interaction with receptor, H.B.: Hydrogen bonding energy between protein and ligand. Eele: Electrostatic interaction with the receptor.

NSAIDs inhibit COX2 as arachidonic acid manner [24]. The tested compounds (**3-17**) re-docked into COX-2 active site after eliminated reference inhibitor. The docking protocol that has scoring function with the lowest root-mean square deviation (RMSD) was carefully selected to analysis of the interaction-affinity. MMFF94 force field was used to minimize the energy of the resulting complexes. The MOE docking has a lowest (RMSD) utilized in (table 1). From (table 1) we noticed that, all of compounds showed an accurate binding energies which has (RMSD<1°A).

Generally, the binding interaction has increased by introducing methoxy and hydrazide fragments to the parent compound. Compounds 5,6, 10, 11,15 and 16 forms important H-bond interactions with active binding pocket. They trapped the amino acid backbone of the binding pocket, and modifying the phenyl rings in perpendicular mode with Hisitdin through adjusting phenyl rings in perpendicular mode with Hisitdine (Fig. 1). Compounds 5,6, 10, 11,15 and 16 were anchored themselves to caped binding pocket and arranging naphthalene-rings in orthogonal alignments with amino residues (Fig.1). The results revealed that the amino acid residues located near the reference molecule are very similar to those found in the structures analyzed. The binding score with the promising values for compounds 5,6, 10, 11,15 and 16 indicated its compounds may be effective against COX-2 inhibitors.

Pharmacological activities

Paw edema experiment:

The carrageenan induced paw edema method used for evaluated the anti-inflammatory activity for 5 , 6 , 12 , 16 and 17 [21]. The compounds were examined at a 35 mg kg–1 body mass oral dosage and compared to the conventional medication ibuprofen at the same dose. The studied 12, 16, and 17 molecules were fatal at this therapeutic level, and more toxicological research is being conducted to understand the compounds' toxicity at this dose. Compounds 5 and 6 showed significant anti-inflammatory activity ranging from 45 to 54% (Table 2), while Ibuprofen showed 47% inhibition after 4 hours. Compound 6 showed the greatest inhibitory percentage after 4 hours (54%). When eating the chemical number, the lowest percent inhibition (45%) was observed.

Tail flick test:

The antinociceptive impact of the synthesized substances was evaluated using latency time and the Maximum Possible Effect (MPE%). The latency time is the amount of time it takes for a radiant heat stimulus to be administered 5–6 cm from the tip of the rattail. MPE% was calculated by "difference percentage between the measured response and the baseline response, divided by the difference between the cutoff-time and the baseline response". To avoid tissue damage the irradiation was automatically stop after 15 s (cut-off = 15 s). Before every trial, the baseline was measured three times, and animals having outranged latency periods were eliminated from the study[22].

Figure 2 depicts the antinociceptive impact of following injection in the tail-flick test. In comparison

to ibuprofen, the latency time-response bar chart of distinct groups (Fig. 3a) revealed that variance was considerably different among groups. And the temporal response curve differed depending on the medication administered; the synthetic compounds 26 had the longest latency period, with an 81 percent MPE (Fig 2b), compared to just 22 percent in rats given the positive control (ibuprofen).

Table 2: Percent inhibition of inflammatory response post-carrageenan injection.

Drug/time	% inhibition after 1 hr	% inhibition after 2 hrs	% inhibition after 3 hrs	% inhibition after 4hrs
BRU	19.4±2	33.2±4	40.3±2	47.1±1.5
5	66.49±3.5	44.31±0.6	48.12±1	45.41±1
6	57.77±1.7	43.80±0.5	46.66±0.6	54.36±0.8

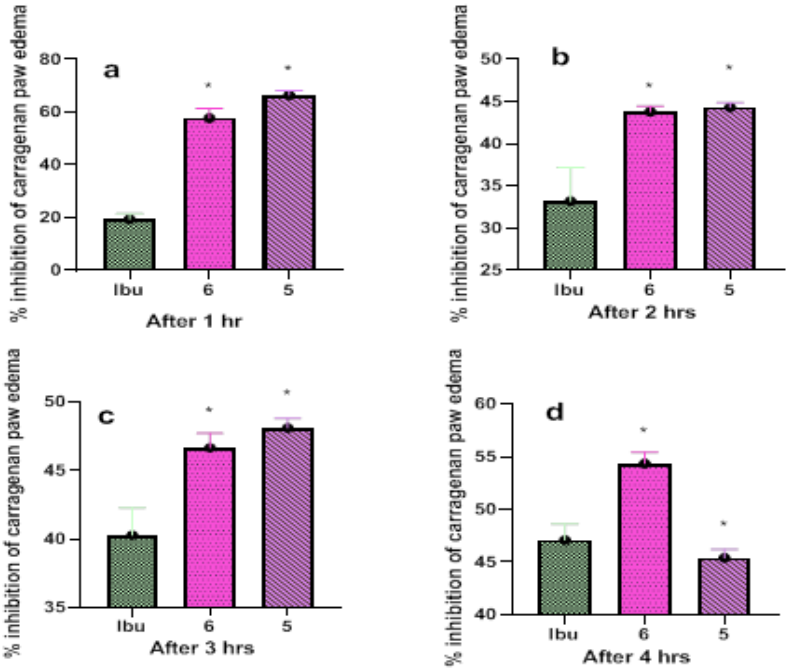


Fig.2: Percent inhibition of inflammatory response post-carrageenan injection. Synthesized products were administered orally at the dose of 35 mg/kg after the administration of 1% carrageenan solution. * means significant difference at P <0.05 compared to brufen. Data are presented as means ± SD, n=6

Acute ulcerogenesis test was done according to Cioli et al. The animals were separated into six groups of six, with group I serving as the control group and receiving just DMSO, while the other groups got an oral dosage of 35 mg/kg dissolved in DMSO. Food, not water, was withdrawn before the 24 hour administration of the investigated products; the rats were then provided a regular diet for 17 hours before being slaughtered after the treatment of drug. The stomach was evacuated and opened along its wider curvature, then gently scrubbed with saline and washed with distilled water. A magnifying glass was used to assess the mucosal injury. The tested compounds didn't show any ulceration or redness. This means that the therapeutic dose of either brufen or the synthesized drugs 5 and 6 didn't show any acute ulcerogenic potential.

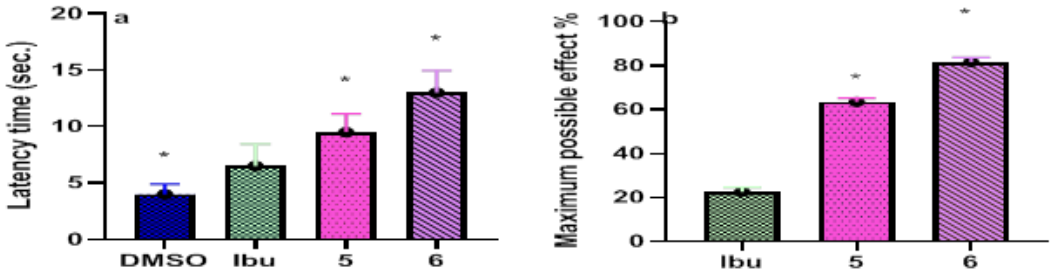


Fig 3: The antinociceptive effect of brufen and synthetic compounds **5** and **6** in tail flick test. Animals received DMSO (vehicle), synthetic drugs in the dose of 35 mg/kg. (a) The time course of latency times of drugs. (b) Maximum Possible Effect percent (%MPE) of groups. Data were compared using one way ANOVA. * means significant difference at P <0.05 compared to brufen. Data are presented as means ± SD, n=6

Table3: latency time and MPE% of tail flick test in response to the administration of DMSO (vehicle), Brufen (Bru) and synthetic compounds **5** and **6**.

Drug	**Latency time (sec.)**	**MPE%**
DMSO	4±0.89	___
Bru	6.5±1.96	22.7±1.96
(5)	11±1.64	63.6±1.64
(6)	13±1.96	81.8±1.96

Acute ulcerogenesis:

Table 4: Severity index of ibuprofen and synthetic compounds **5 and 6**

Drug	**Severity index**
Bru	0
(5)	0
(6)	0

Materials and Methods

Animals (See supplementary material)

Experimental

Chemistry (See supplementary material)

Synthesis of 4-(2-(4-isobutylphenyl)propanamido)benzoic acid (1)

Ibuprofen **A** refluxed with pure thionyl chloride in Dioxane for 5 h to give product **B**. Then stirring product (**B**) with para amino benzoic acid in dioxin (20 ml) for 3hrs. The solvent was removed under vacuum to get product **1**.

Product **1** was separated as colorless crystals, yield 95 %. mp 177-178 oC. IR (KBr, cm-1): 3460 (NH), 3363 (OH), 3043 (CH aromatic), 2954 (CH aliphatic), 1720 (C=O), 1670 (C=O), 1624 (C=C), 1508 (C-N), 1323 (CH3). 1H NMR (500 MHz, DMSO-D6) δ 12.21 (s, 1H, OH), 7.71 (d, J = 9.2 Hz, 2H, CHarom), 7.24 (d, J = 8.5 Hz, 2H, CHarom), 6.64 (d, J = 9.0 Hz, 2H, CHarom), 5.97 (d, 2H, CHarom), 3.73 (t, J = 7.4 Hz, 1H, CH-Me), 1.91 (d, J = 7.0 Hz, 2H, CH2), 1.44 (m, J = 7.9 Hz, 1H, CH), 0.95 (2d, J = 7.1 Hz, 6H, CH3). MS (m/z): M+ 325 (15%). Analysis for C20H23NO3 (325.17). Calced: % C, 73.82; H, 7.12; N, 4.30. Found: % C, 73.79; H, 7.20; N, 4.15.

(4-(2-(4-isobutylphenyl) propanamido) benzoyl) phenylalanine (3)

The titled compound was synthesized by stirring of phenyl alanine with compound 2 in THF (50 ml) and few drops of pyridine for 5 h. at room temperature. The solvent was removed under vacuum to get product 3

Product 3 was separated as colorless crystals (ethanol), yield 85 %. mp 177-179 oC.

IR (KBr, cm-1): 3463 (OH), 3363 (NH), 2954 (CH aliphatic), 1716, 1666, 1623 (3 C=O), 1596 (C=C), 1504 (C-N), 1307 (CH3). 1H NMR (500 MHz, DMSO-D6) δ 9.66 (s, 1H, OH), 7.58 (d, J = 8.4Hz, 2H, CHarom), 7.57 (d, J = 11.5 Hz, 2H, CHarom), 7.55 (d, J = 8.4 Hz, 2H, CHarom), 7.12 (m, 5H, CHarom), 6.51 (d, J = 8.4 Hz, 2H, CHarom), 3.70 (t, 1H, CH-Me), 3.59 (br. s, 2H, NH), 3.28 (dd, 1H, CH2), 2.37 (d, 1H, CH2), 1.77 (dd, J = 12.1 Hz, 2H, CH2), 1.38 (m, J = 7.9 Hz, 1H, CH), 0.94 – 0.63 (2d, J = 7.1 Hz, 6H, CH3). MS (m/z): M+ 472 (40%). Analysis for C29H32N2O4 (472.59). Calced.: % C, 73.73; H, 6.78; N, 5.93. Found: % C, C, 73.78; H, 6.65; N, 5.75.

Methyl (4-(2-(4-isobutyl phenyl) propanamido) benzoyl) phenyl alaninate (4)

The titled compound was synthesized by stirring of phenyl alanine methyl ester hydrochlorides with compound (**2**) in THF (50 ml) and few drops of tri ethyl amine (TEA) for 3hrs. The solvent was removed under vacuum to get product **4**.

Product **4** was separated as colorless crystals (ethanol), yield 75 %. mp 145-147. oC.

IR (KBr, cm-1): 3463 (NH), 3355 (NH), 3089 (CH aromatic), 2950 (CH aliphatic), 1720 (C=O), 1666 (C=O), 1558 (C=C), 1438 (C-N), 1319 (CH3). 1H NMR (500 MHz, DMSO-D6) δ 8.30 (s, 1H, NH), 7.58 (d, J = 8.4Hz, 2H, CHarom), 7.12 (m, 5H, CHarom), 7.15 (d, J = 11.5 Hz, 2H, CHarom), 7.05 (d, J = 8.4 Hz, 2H, CHarom), 6.49 (d, J = 8.4 Hz, 2H, CHarom), 5.86 (s, 1H, NH), 3.72 (t, 1H, CH-Me), 3.58 (br. s, 2H, NH), 3.29 (dd, 1H, CH2), 2.35 (d, 1H, CH2), 1.77 (dd, J = 12.1 Hz, 2H, CH2), 1.29 (m, J = 7.9 Hz, 1H, CH), 0.81–0.80 (2d, J = 7.1 Hz, 6H, CH3). MS (m/z): M+ 486 (20%). Analysis for C30H34N2O4 (486.61). Calced.: % C, 74.07; H, 7.00; N, 5.76. Found: % C, 74.25; H, 7.15; N, 5.55.

N-(1-hydrazinyl -1-oxo-3-phenyl propan-2-yl)-4-(2-(4-isobutylphenyl)propanamido) benzamide (5)

The titled compound was synthesized by heating compound 4 with ethanolic solution of hydrazine hydrate solution (40 ml) for 1/2hr. The solvent was removed under vacuum to get product **5**.

Product 5 was separated as colorless crystals, yield 65 %. mp 213-215oC. IR (KBr, cm-1): 3297 (NH2, NH), 3124 (CH aromatic), 2958 (CH aliphatic), 1720, 1627 (amide C=O), 1562 (C=C), 1500 (C-N), 1311 (CH3). 1H NMR (500 MHz, DMSO-D6) δ 8.48 (d, J = 7.7 Hz, 2H, CHarom), 8.39 (d, 2H, CHarom), 8.27 (s, 1H, NH), 7.59 (s, 1H, NH), 7.16 (d, J = 6.6 Hz, 2H, CHarom), 6.52 (s, 1H, NH), 6.23 (m, 5H, CHarom), 5.87 (br s, 3H, NH, NH2), 3.59 (t, J = 5.2 Hz, 1H, CH-Me), 2.86 (s, 2H, CH2), 2.47 (s, 2H, CH2), 1.76 (d, J = 5.9 Hz, 2H, CH), 1.30 (d, J = 4.9 Hz, 3H, CH3), 0.81 (t, J = 5.8 Hz, 6H, CH3). MS (m/z): M+1 485(25%). Analysis for C29H34N4O3 (486.62). Calced.: % C, 71.60; H, 7.00; N, 11.52. Found: % C, 71.71; H, 7.25; N, 11.40.

N-(1-(2-acetylhydrazinyl)-1-oxo-3-phenylpropan-2-yl)-4-(2-(4-isobutyl phenyl) propanamido) benzamide (6)

The titled compound was synthesized by refluxing of compound 5 in acetic anhydride (50 ml) for 12 hrs. The solvent was removed under vacuum to get product **6**

Product **6** was separated as colorless crystals (ethanol), yield 40 %. mp 198-200oC. IR (KBr, cm-1): 3536, 3293(NH), 3131 (CH aromatic), 2989 (CH aliphatic), 1700 (C=O), 1664 (C=O), 1535 (C=C), 1400 (C-N), 1319 (CH3). 1H NMR (500 MHz, DMSO-D6) δ 10.25 (s, 1H, NH), 8.60 (d, J = 8.2, 2H, CHarom), 8.34 (d, J = 8.2, 5.7 Hz, 2H, CHarom), 7.90 (s, 1H, NH), 7.70 (s, 1H, NH),7.24 (m, 5H, CHarom), 7.18 (d, 2H, CHarom), 6.20 (s, 1H, NH), 6.51 (d, J = 8.3, 5.8 Hz, 2H, CHarom), 3.57 (t, J = 5.2 Hz, 1H, CH-Me), 3.25 (s, 3H, Me), 2.46 (s, 2H, CH2), 2.37 (s, 2H, CH2), 2.04 (s, 3H, COCH3), 1.87 (d, J = 5.9 Hz, 2H, CH), 1.30 (d, J = 4.9 Hz, 3H, CH3), 0.80 (t, J = 5.8 Hz, 6H, CH3). MS (m/z): M+1 527 (35%). Analysis for C31H36N4O4 (528.65). Calced: % C, 70.45; H, 6.82; N, 10.61. Found: % C, 70.62; H, 6.65; N, 10.70.

4-(2-(4-isobutyl phenyl) propanamido) benzoyl azide (7)

The titled compound was prepared by the reaction of compound **2** with NaN_3 in acetone (20 ml) by stirring for 5 h. The solvent was removed under vacuum to get product 7.

Product **7** was separated as colorless crystals, yield 78 %. mp 181-182 ºC. IR (KBr, cm-1): 3363 (NH), 3045 (CH aromatic), 2993 (CH aliphatic), 2137 (N3), 1705 (C=O), 1650 (C=O), 1608 (C=C), 1508 (C-N), 1400 (CH3). 1H NMR (500 MHz, DMSO-D6) δ 7.96 (d, J = 8.2, 2H, CHarom), 7.72 (d, J = 8.2, 5.7 Hz, 2H, CHarom), 7.45 (d, 2H, CHarom), 6.24 (d, J = 8.3, 5.8 Hz, 2H, CHarom), 6.61 (s, 1H, NH), 3.55 (q, J = 5.2 Hz, 1H, CH-Me), 2.14 (m, 2H, CH2), 1.97 (t, J = 6.1 Hz, 1H, CH), 1.61 (d, 3H, CH3), 1.06 (d, J = 6.3 Hz, 6H, 3 CH3). MS (m/z): M+ 350 (15%). Analysis

for C20H22N4O2 (350.42). Calced.: % C, 68.55; H, 6.33; N, 15.99. Found: % C, 68.85; H, 6.46; N, 15.63.

2-(4-isobutyl phenyl) -N-(4-isocyanatophenyl) propanamide (8)

The titled compound was prepared by refluxing compound 7 in dioxane for 5 hrs . The solvent was removed under vacuum to get product 8.

Product 8 was separated as colorless crystals (ethanol), yield 74 %. mp 155-156 oC. IR (KBr, cm-1): 3360 (NH), 3093 (CH aromatic), 2954 (CH aliphatic), 2048 (NCO), 1660 (C=O), 1608 (C=C), 1504 (C-N), 1323 (CH3). 1H NMR (500 MHz, DMSO-D6) δ 8.20 (d, J = 8.2, 2H, CHarom), 7.76 (d, J = 8.2, 5.7 Hz, 2H, CHarom), 7.32 (d, 2H, CHarom), 7.11 (d, J = 8.3, 5.8 Hz, 2H, CHarom), 6.67 (s, 1H, NH), 3.54 (q, J = 5.2 Hz, 1H, CH-Me), 2.40 (m, 2H, CH2), 2.13 (t, J = 6.1 Hz, 1H, CH), 1.69 (d, 3H, CH3), 1.14 (d, J = 6.3 Hz, 6H, 3 CH3). MS (m/z): M+ 321 (20%). Analysis for C20H22N2O2 (322.41). Calced.: % C, 74.51; H, 6.88; N, 8.69.Found: % C, 74.25; H, 6.63; N, 8.84.

((3-(2-(4-isobutylphenyl) propanamido) phenyl) carbamoyl) phenylalanine (9)

The titled compound was synthesized stirring by Phenylalanine (10 mmole) with product 8 (10 mmole) in THF (35 ml) and few drops of pyridine for 5h at room temperature (TLC). Pour the reaction into water with acidification by 1N HCl to get a crude material that recrystallized with ethanol to afford 9 as colorless crystals, yield 69 %. mp 231-233 oC. IR (KBr, cm-1): 3455 (OH), 3355 (NH), 3031 (CHarom), 2958 (CHaliph), 1716, 1666 (C=O), 1623 (C=C), 1562, 1496 (C-N), 1307 (CH3). MS (m/z): M+ 487 (30%). Analysis for C29H33N3O4 (487.60). Calced: % C, 71.46; H, 6.78; N, 8.62. Found: %, C 71.52; H, 6.78; N, 8.43.

N-(3-(3-(1-hydrazinyl-1-oxo-3-phenylpropan-2-yl)ureido)phenyl)-2-(4-isobutylphenyl)propanamide (11)

The titled compounds were synthesized by heating compound **10** with ethanolic hydrazine hydrate solution for 1/2 hr. The progress of the reaction was monitored by TLC. The desired materials were filtered and recrystallized from ethanol to afford product **11**.

Product **11** was separated as colorless crystals (ethanol), yield 65 %. mp 192-194 ºC. IR (KBr, cm^{-1}): 3451, 3340, 3208 (NH), 3043 (CH aromatic), 2958 (CH aliphatic), 1716 (C=O), 1666 (C=O), 1573 (C=C), 1457 (C-N), 1311 (CH3). 1H NMR (500 MHz, DMSO-D6) δ 9.25 (s, 1H, NH), 7.83 (s, 1H, NH), 7.58 (d, J = 7.7 Hz, 2H, CHarom), 7.32 (d, 2H, CHarom), 7.12 (m, 5H, CHarom), 7.05 (d, J = 6.6 Hz, 2H, CHarom), 6.66 (d, 2H, J = 7.7 Hz, CHarom), 6.17 (br s, 3H, NH, NH2), 3.55 (t, J = 5.2 Hz, 1H, CH-Me), 2.33 (s, 2H, CH2), 1.74 (d, J = 5.9 Hz, 2H, CH2), 1.29 (t, J = 6.1 Hz, 1H, CH), 0.79 (t, J = 5.8 Hz, 6H, CH3). MS (m/z): M+ 501 (40%). Analysis for C29H35N5O3 (501.63). Calced.: % C, 69.46; H, 6.99; N, 13.97. Found: % C, 69.39; H, 6.99; N, 13.92.

N-(3-(3-(1-(2-acetyl hydrazinyl)-1-oxo-3-phenyl propan-2-yl) ureido) phenyl)-2-(4-isobutyl phenyl) propanamide (12)

The titled compound was synthesized by refluxing of compound 11 in acetic anhydride (50 ml) for 8 hrs. The solvent was removed under vacuum to get product **12**

Product **12** was separated as colorless crystals (ethanol), yield 58 %. mp 278-280 oC. IR (KBr, cm-1): 3463, 3436, 3401, 3355 (NH), 2950 (CH aliphatic), 1724 (C=O) 1619 (C=C), 1442 (C-N), 1392 (CH3). 1H NMR (500 MHz, DMSO-D6) δ 8.14 (s, 1H, NH), 7.57 (d, J = 8.2, 2H, CHarom), 7.24 (d, J = 8.2, 5.7 Hz, 2H, CHarom), 7.14 (d, 2H, CHarom), 7.06 (m, 5H, CHarom), 6.50 (d, J = 8.3, 5.8 Hz, 2H, CHarom), 6.20 (s, 1H, NH), 5.61 (s, 1H, NH), 5.47 (s, 1H, NH), 3.81 (s, 3H, Me), 3.69 (t, J = 5.2 Hz, 1H, CH-Me), 2.47 (d, J = 6.4 Hz, 2H, CH2), 2.34 (s, 2H, CH2), 1.81 (t, J = 6.1 Hz, 1H, CH), 1.68 (t, J = 6.1 Hz, 1H, CH3), 0.81 (t, J = 6.3 Hz, 6H, CH3). Analysis for C31H37N5O4 (543.67). Calced.: % C, 68.51; H, 6.81 ; N, 12.89. Found: % C, 68.55; H, 6.81; N, 12.75.

4-(2-(4-isobutylphenyl)propanamido)benzoyl isothiocyanate (13)

The titled compound was prepared by the reaction of 2-(4-isobutylphenyl)propanoyl chloride (**2**) with NH4NCS (10 mmole) in acetone (50 mL) by refluxing for 10 hr, The progress of the reaction was monitored by TLC After removing NH4Cl residue, the solvent was removed in vacuo to get product **13**

Product **13** was separated as colorless crystals (ethanol), yield 74 %. mp 191-192 ºC. IR (KBr, cm-1): 3452 (NH), 3093 (CH aromatic), 2954 (CH aliphatic), 1716 (C=O), 1681 (C=O), 1604 (C=C), 1539 (C-N), 1323 (CH_3). 1H NMR (500 MHz, DMSO-D_6) δ 7.56 (d, J = 10Hz, 2H, CHa_{rom}), 7.12 (d, J = 10 Hz, 2H, CHa_{rom}), 7.04 (d, 2H, CHa_{rom}), 6.50 (d, J = 10 Hz, 2H, CHa_{rom}), 5.81 (s, 1H, NH), 3.55 (q, J = 5.2 Hz, 1H, CH-Me), 2.35 (m, 2H, CH_2), 2.22 (t, J = 6.1 Hz, 1H, CH), 1.27 (d, 3H, CH_3), 0.79 (d, J = 6.3

Hz, 6H, 3 CH3). MS (m/z): M+ 365 (20%). Analysis for C21H22N2O2S (366.48). Calced.: % C, 68.83; H, 6.05; N, 7.64; S, 8.75.Found: % 68.55; H, 6.35; N, 7.49.

((3-(2-(4-isobutyl phenyl) propanamido) benzoyl) carbamothioyl) phenylalanine (14)

The titled compound was synthesized by stirring phenyl alanine amino acid with the compound 13 in THF (35 ml) and few drops of pyridine for 5 hrs at room temperature. The solvent was removed under vacuum to get product 14.

Product 14 was separated as colorless crystals, yield 60 %. mp 265-267 oC. IR (KBr, cm-1): 3463 (OH), 3359 (NH), 3023 (CH aromatic), 2958 (CH aliphatic), 1716 (C=O), 1689 (C=S), 1562 (C=C), 1496 (C-N), 1311 (CH3). 1H NMR (500 MHz, DMSO-D6) δ 12.04 (s, 1H, OH), 7.56 (d, J = 10 Hz, 2H, CHarom), 7.13 (d, J = 10 Hz, 2H, CHarom), 7.05 (d, J = 10 Hz, 2H, CHarom), 6.50 (d, J = 10 Hz, 2H, CHarom), 5.78 (s, 1H, OH), 5.00 (s, 1H, NH), 3.57 (q, J = 5.2 Hz, 1H, CH-Me), 3.37 (br. s, 2H, 2 NH), 3.05 (d, J = 12.1 Hz, 2H, CH2), 2.35 (m, J = 7.9 Hz, 1H, CH), 1.72 (d, J = 10 Hz, 2H, CH2), 0.79 (d, J = 10 Hz, 6H, 2 CH3). MS (m/z): M+ 531 (10%). Analysis for C30H33N3O4S (531). Calced: % C, 67.80; H, 6.21; N, 7.91; S, 6.03. Found: % C, 67.85; H, 6.35; N, 7.75; S, 6.26

N-((1-hydrazinyl-1-oxo-3-phenyl propan-2-yl) carbamothioyl)-3-(2-(4-isobutyl phenyl) propanamido) benzamide (16)

The titled compound was synthesized by heating compound (15) with ethanolic hydrazine hydrate solution (40 ml) for 1/2hr. The product 16 was obtained via solvent removal under vacuum as colorless crystals after crystallization with ethanol, yield 50 %. mp >300 oC. IR (KBr, cm-1): 3602 (NH), 3498 (NH), 3193 (CH aromatic), 2958 (CH aliphatic), 1716 (C=O), 1666 (C=S), 1616 (C=C), 1457 (C-N), 1315 (CH3). 1H NMR (500 MHz, DMSO-D6) δ 7.59 (d, J = 7.7 Hz, 2H, CHarom), 7.23 (d, 2H, CHarom), 7.21 (m, 5H, CHarom), 7.15 (d, J = 6.6 Hz, 2H, CHarom), 6.50 (d, 2H, J = 7.7 Hz, CHarom), 5.70 (s, 1H, NH), 5.49 (t, J = 5.2 Hz, 1H, CH-Me),5.49 (br.s, 1H, NH), 4.17 (br s, 1H, NH), 3.36 3.34 (br s, 2H, NH2), 3.02 (s, 1H, NH), 2.46 (s, 1H, NH), 2.37 (d, 2H, CH2), 1.77 (d, J = 5.9 Hz, 2H, CH2), 1.75 (m, 1H, CH), 1.30 (d, J = 6.1 Hz, 1H, CH3), 0.80 (t, J = 5.8 Hz, 6H, 2 CH3). MS (m/z): M+1 544 (15%). Analysis for C30H35N5O3S (545.70). Calced: %C, 66.06; H, 6.472; N, 12.83; S, 5.87. Found: %. C, 66.12; H, 6.39; N, 12.72; S, 5.69

N-((1-(2-acetylhydrazinyl)-1-oxo-3-phenyl propan-2-yl) carbamothioyl)-3-(2-(4-isobutylphenyl) propanamido)benzamide (17)

The titled compound was synthesized by refluxing of compound 16 in acetic anhydride (50 ml) for 15 h. product 17 was obtained after vacuum solvent removal and recrystallized with ethanol as colorless crystals, yield 45 %. mp >300. oC. IR (KBr, cm-1): 3471, 3432, 3351 (NH), 2950 (CH aliphatic), 1720 (C=O), 1604 (C=C), 1442 (C-N), 1388 (CH3). 1H NMR (500 MHz, DMSO-D6) δ 8.16 (s, 1H, NH), 7.57 (d, J = 7.7 Hz, 2H, CHarom), 7.23 (d, 2H, CHarom), 7.06 (m, 5H, CHarom), 7.05 (d, J = 6.6 Hz, 2H, CHarom), 6.51 (d, 2H, J = 7.7 Hz, CHarom), 5.51 (s, 1H, NH), 5.50 (t, J = 5.2 Hz, 1H, CH-Me),5.49 (br.s, 1H, NH), 4.17 (br s, 1H, NH), 3.69 3.68 (br s, 2H, NH2), 3.57 (s, 1H, NH), 2.47 (s, 1H, NH), 2.37 (d, 2H, CH2), 1.84 (s, 3H, CH3), 1.81 (d, J = 5.9 Hz, 2H, CH2), 1.77 (m, 1H, CH), 1.30 (d, J = 6.1 Hz, 1H, CH3), 0.80 (t, J = 5.8 Hz, 6H, 2 CH3). MS (m/z): M+1 587 (25%). Analysis for C32H37N5O4S (587.74) Calced.: % C, 65.42; H, 6.30; N, 11.93; S, 5.45. Found: % C, 65.49; H, 6.30; N, 11.97; S, 5.51

Computational Model:

Docking study :

Docking study was recorded for the investigated compounds into EGFR using MOE 2015 [28]. The crystal structures of the (COX-2) complexes with diclofenac (ID: 1PXX[26]) was prepared using MOE 2015. All the remaining procedures were peformed as reported [29].

Pharmacological activity

The new compounds pharmacological properties were evaluated in (130-150 g) rats. In 36 rats in six groups, animals got oral dose (35 mg/kg) in DMSO for the new compounds and ibuprofen in a preliminary test to determine the dose for biological testing. The animals were monitored for indicators of toxicity and the number of deaths for 24 hours. There were no deaths reported, and no evidence of distress, seizures, impaired movement, dyspnea, or any other abnormal clinical signs were seen.

Anti-inflammatory activity

The activity was tested according to winter et al method, in which edema was induced by injecting a freshly synthesized suspension of carrageenan (1.0% m/v, 0.1 mL) in the right hind paw in the plantar region

of each rat. Six groups of six rats each were formed. The first group served as a control group, receiving the same amount of solvent as the other groups (1% DMSO orally).

Both other groups were given the drugs orally at a dose of 35 mg/kg body weight (dissolved in 1% DMSO) one hour before receiving the injection of carrageenan. The percentage inhibition was used to express the results. The following formulas were used to calculate the inhibition and edema rates for each group:

(E %) Percentage of Edema rate change = ((Vt-Vo) \Vo)*100

(I %) Inhibition rate = ((Ec-Et)\Et) *100

Where:

Vo: volume before carrageenan injection (ml). Vt : volume at t hour after carrageenan injection (ml).Ec : edema rate of control group. Et : edema rate of treated group.

Analgesic activity

By using Tail-flick test method, the analgesic test of target compounds was investigated [30]. Also anti-nociceptive activities were examined by using the same test. The rat was gently handled while the tail was placed on the apparatus to measure the latency of the tail-flick reaction. The tail flick reflex was obtained by putting radiant heat on the mouse's dorsal surface. The period in seconds between the first activation of the heat source and the removal of the tail was recorded. The tail withdrawal latency was calculated using the average of two measurements for each experimental animal. The nociceptive pain was measured by using test based on latency time, which was defined as the time it took to avoid a thermal stimulus provided by radiant heat 5–6 cm from the rat tail tip. Before any experiment, the baseline was obtained three times. . Latency times (LT) were recorded after 60 min of drug administration to determine the time of a drug's effect. Maximum Possible effect (MPE%) was used to measure the Antinociceptive effect in tail-flick. MPE% in 60th min was evaluated based on the following formula. The MPE% = [(LT min60 - T0)/(Tc - T0)] _ 100. LT min 60 was the latencies time 60 min after drug administration, T0 was the base line and Tc was cut-off time (15 s).

Acute ulcerogenesis

The test of acute ulcerogenesis was determined as previous method [30]. The animals were 36 rats in 6 groups. First group as control that was injected with DMSO, while the other groups got an oral dosage of 35mg/kg dissolved in DMSO and their food-not water-was not provided before the 24 hour administration. of the investigated synthetic derivatives; the normal diet has been fed to rats after the drug treatment for 17 hours before being slaughtered. We got the stomach to open it along its greater curvature, then distilled water cleansing and gently saline cleaning. A magnifying glass was used to examine the mucosal injury. The mucosal injury in each stomach was measured using a macroscopic examination. The stomach was evacuated and opened along its larger curvature, then cleaned with saline and evaluated with a magnifier lens (x10) to check for lesions and ulcer formation. The number of ulcers was recorded, and the severity of the ulcers was evaluated by assigning a score between 0 and 5 on a scale of 1 to 5. (5: perforation, 4: large ulcers, 3: Small; ulcers, 2: hemorrhagic streaks, 1: erythema, 0: no lesions).

Statistical analysis

Values are presented as mean ± SD of 6 animals. Significant difference between groups was carried out using ANOVA (analysis of variance) then test of Tukey's post hoc. The statistical significance level was taken ($P < 0.05$). Software of Graph pad prism® package, version8 (GraphPad Software, CA, USA).

Conclusion

From the previous results, it was concluded that phenyl alanine derivatives were synthesized to enhance the anti-inflammatory activity with decreasing the ulcer genic activity. The synthesized compound were characterized by different spectral data. The molecular docking study of the synthesized compounds 3-17 has performed in COX-2 active site. The pharmacological studies has evaluated to the compounds 5, 6, 12, 16 and 17. The compounds 12, 16, and 17 molecules were fatal at the therapeutic level. Compounds 5 and 6 showed higher anti-inflammatory and analgesic activity compared with the parent compound "Ibuprofen" with negligible ulcerogenic effect.

References

[1] K.J. NICHOL, The Medicinal Chemistry ofIbuprofen, Ibuprofen: a critical bibliographic review (1999) 23.

[2] P. Chander Sharma, S. Yadav, R. Pahwa, A. Sharma, S. Jain, Naproxen: an update on physicochemical, analytical and pharmacological

aspects, Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents) 10(5) (2011) 339-350.

[3] F. Seraj, K.M. Khan, A. Khan, M. Ali, R. Khalil, Z. Ul-Haq, S. Hameed, M. Taha, U. Salar, S. Perveen, Biology-oriented drug synthesis (BIODS), in vitro urease inhibitory activity, and in silico studies on ibuprofen derivatives, Molecular diversity 25(1) (2021) 143-157.

[4] I. Alkabodi, S. Almekhlafi, A.I. Doa'a, Synthesis and anti-inflammatory activity of novel aspirin and ibuprofen amide derivatives, Journal of Chemical and Pharmaceutical Research 8(3) (2016) 307-313.

[5] K.K. Angajala, S. Vianala, R. Macha, M. Raghavender, M.K. Thupurani, P. Pathi, Synthesis, anti-inflammatory, bactericidal activities and docking studies of novel 1, 2, 3-triazoles derived from ibuprofen using click chemistry, SpringerPlus 5(1) (2016) 1-15.

[6] S. Chadha, A. Kumar, S.A. Srivastava, T. Behl, R. Ranjan, Inulin as a delivery vehicle for targeting colon-specific cancer, Current Drug Delivery 17(8) (2020) 651-674.

[7] J.L. Wallace, How do NSAIDs cause ulcer disease?, Best Practice & Research Clinical Gastroenterology 14(1) (2000) 147-159.

[8] P.N. Kourounakis, K. Tsiakitzis, A.P. Kourounakis, D. Galanakis, Reduction of gastrointestinal toxicity of NSAIDs via molecular modifications leading to antioxidant anti-inflammatory drugs, Toxicology 144(1-3) (2000) 205-210.

[9] K.M. Huttunen, H. Raunio, J. Rautio, Prodrugs—from serendipity to rational design, Pharmacological reviews 63(3) (2011) 750-771.

[10] J.B. Zawilska, J. Wojcieszak, A.B. Olejniczak, Prodrugs: a challenge for the drug development, Pharmacological reports 65(1) (2013) 1-14.

[11] R.M. Borik, M.A. Hussein, A Novel Quinazoline-4-one Derivatives as a Promising Cytokine Inhibitors: Synthesis, Molecular Docking, and Structure-activity Relationship, Current Pharmaceutical Biotechnology 23(9) (2022) 1179-1203.

[12] B.J. Orlando, M.J. Lucido, M.G. Malkowski, The structure of ibuprofen bound to cyclooxygenase-2, Journal of structural biology 189(1) (2015) 62-66.

[13] J. Wang, D. Dai, Q. Qiu, X. Deng, H. Lin, H. Qian, W. Huang, Evaluation of Anti-inflammatory and Analgesic Effects of Synthesized Derivatives of Ibuprofen, Chemical Biology & Drug Design 85(5) (2015) 623-632.

[14] D. Piomelli, L. Scalvini, Y. Fotio, A. Lodola, G. Spadoni, G. Tarzia, M. Mor, N-acylethanolamine acid amidase (NAAA): structure, function, and inhibition, Journal of Medicinal Chemistry 63(14) (2020) 7475-7490.

[15] A. Deplano, J. Karlsson, M. Svensson, F. Moraca, B. Catalanotti, C.J. Fowler, V. Onnis, Exploring the fatty acid amide hydrolase and cyclooxygenase inhibitory properties of novel amide derivatives of ibuprofen, Journal of Enzyme Inhibition and Medicinal Chemistry 35(1) (2020) 815-823.

[16] H. Rafea, M.S. Farhan, A.A. Fadhil, Synthesis of New Ibuprofen Derivatives Containing (Oxothiazolidin-3-yl) Amino Moiety with Expected Biological Activity, Systematic Reviews in Pharmacy 11(12) (2020) 1851-1856.

[17] V.O. Novellino, C.J. Fowler, B. Catalanotti, Design and pharmacological evaluation of Ibuprofen amides derivatives as dual FAAH/COX inhibitors. Name: Federica Moraca1, Carmine Marco Morgillo2, Alessandro Deplano3, Ettore.

[18] A.A. Elhenawy, L. Al-Harbi, G.O. Moustafa, M. El-Gazzar, R.F. Abdel-Rahman, Synthesis, comparative docking, and pharmacological activity of naproxen amino acid derivatives as possible anti-inflammatory and analgesic agents, Drug design, development and therapy 13 (2019) 1773.

[19] M. Abo-Ghalia, A. Amr, Synthesis and investigation of a new cyclo (N α-dipicolinoyl) pentapeptide of a breast and CNS cytotoxic activity and an ionophoric specificity, Amino Acids 26(3) (2004) 283-289.

[20] E.F. Ewies, M. El-Hussieny, N.F. El-Sayed, M.A. Fouad, Design, synthesis and biological evaluation of novel α-aminophosphonate oxadiazoles via optimized iron triflate catalyzed reaction as apoptotic inducers, European Journal of Medicinal Chemistry 180 (2019) 310-320.

[21] E.F. Ewies, F.A. El-Hag, Synthesis, reactions, and antimicrobial evaluations of new benzo [e][1, 3] thiazine derivatives, Journal of Heterocyclic Chemistry 57(1) (2020) 163-172.

[22] N.F. El-Sayed, M. El-Hussieny, E.F. Ewies, M.F. El Shehry, H.M. Awad, M.A. Fouad, Design, synthesis, biological evaluation, and molecular docking of new benzofuran and indole derivatives as tubulin polymerization inhibitors, Drug Development Research (2021).
[23] E. Ewies, M. El-Hussieny, N.F. El-Sayed, M. Abdelaziz, Synthesis and Antimicrobial Evaluation of New 5-Amino-2, 3-dihydrophthalazine-1, 4-dione Derivatives, Egyptian Journal of Chemistry 64(12) (2021) 2-3.
[24] G.P. Hochgesang, S.W. Rowlinson, L.J. Marnett, Tyrosine-385 Is Critical for Acetylation of Cyclooxygenase-2 by Aspirin, Journal of the American Chemical Society 122(27) (2000) 6514-6515.
[25] J.R. Kiefer, J.L. Pawlitz, K.T. Moreland, R.K. Stegeman, Structural insights into the stereochemistry of the cyclooxygenase reaction, Nature 405(6782) (2000) 97.
[26] S.W. Rowlinson, J.R. Kiefer, J.J. Prusakiewicz, J.L. Pawlitz, K.R. Kozak, A.S. Kalgutkar, W.C. Stallings, R.G. Kurumbail, L.J. Marnett, A novel mechanism of cyclooxygenase-2 inhibition involving interactions with Ser-530 and Tyr-385, J Biol Chem 278(46) (2003) 45763-9.
[27] J. Kieffer, É. Brémond, P. Lienard, G. Boccardi, In silico assessment of drug substances chemical stability, Journal of Molecular Structure: THEOCHEM 954(1) (2010) 75-79.
[28] O. Korb, T. Stutzle, T.E. Exner, Empirical scoring functions for advanced protein− ligand docking with PLANTS, Journal of chemical information and modeling 49(1) (2009) 84-96.
[29] D.S. BIOVIA, Discovery Studio Modeling Environment, Release 2017, San Diego: Dassault Systèmes, 2017.
[30] H.J. Witchel, J.C. Hancox, Familial And Acquired Long QT Syndrome And The Cardiac Rapid Delayed Rectifier Potassium Current, Clinical and Experimental Pharmacology and Physiology 27(10) (2000) 753-766.