Asian Journal of Chemistry; Vol. 33, No. 2 (2021), 423-438

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23036

Synthesis, Molecular Docking, Biological Potentials and Structure Activity Relationship of New Quinazoline and Quinazoline-4-one Derivatives

RITA M. BORIK[1] and MOHAMMED A. HUSSEIN[2,*]

[1]Chemistry Department, Faculty of Science (Female Section), Jazan University, Jazan 82621, Saudi Arabia
[2]Biochemistry Department, Faculty of Applied Medical Sciences, October 6 University, Sixth of October City, Egypt

*Corresponding author: Tel: +20 124832580; E-mail: prof.husseinma@o6u.edu.eg

Received: 21 October 2020; *Accepted*: 14 December 2020; *Published online*: 15 January 2021; AJC-20231

In this work, a new derivative of ethyl 5-chloro-2-(3-(4-hydroxyphenyl)propanamido)benzoate (**1**) was synthesized by reacting the amino group of 3-(4-hydroxyphenyl)propanoic acid (0.01 mol) and methyl 2-amino-5-chlorobenzoate in presence of PCl_3. Cyclcondensation of **1** with hydrazine hydrate afforded the corresponding 2-(4-hydroxyphenethyl)-3-amino-6-chloroquinazolin-4(3*H*)-one (**2**). Also, new Schiff base **3** was prepared *via* reaction of 2-(4-hydroxyphenethyl)-3-amino-6-chloroquinazolin-4(3*H*)-one (**2**) with 4-hydroxy-3-methoxybenzaldehyde. The synthesized compounds were characterized by elemental analysis, IR, ^{1}H NMR and mass spectral data. Also, the median lethal doses (LD_{50}s) of compounds **1-3** in rats were 1125, 835 and 1785 mg/kg b.w., respectively. IC_{50} values of compounds (**1**, **3**) as measured by DPPH$^{\bullet}$ method was 136.47 and 73.54 µg/mL, respectively. IC_{50} values of compounds (**1-3**) as measured by ABTS$^{\bullet+}$ radical method was 0.8, 0.92 and 0.08 mg/mL, respectively. Antiulcerogenic activity at dose 1/20 LD_{50} in albino rats was 47.94, 24.60 and 56.45%, respectively. However, the anti-inflammatory effect at dose 1/20 LD_{50} of compounds (**1-3**) induced edema model after 120 min were 74.19, 69.93 and 59.03%, respectively. The synthesized compounds also possess hepatocytes and gastric mucosa protective activity against ibuprofen induced ulceration and LPS-induced liver toxicity, respectively in rats *via* normalization of oxidative stress biomarkers and inflammatory mediators (Na^+/K^+-ATPase, ALT, AST, LDH, TNF-α, NO, TBARS, GPx, CAT and SOD). Also, TNF-α, NO, PGE2 and COX-2 were inhibited in peritoneal macrophage cells at a concentration of 100 µg/L. Molecular docking suggested that the most active compounds **1** and **2** can be positioned within the active sites of COX-2 at Arg121 & Tyr356 similarly to ibuprofen (Arg-120, Glu-524 and Tyr-355). The compound **3**–COX-2 complex generated by docking, revealed intricate interactions with a COX-2 channel, including hydrogen bonds with key residues Arg121 and phe519. These findings suggest that compounds **1-3** exhibited good antioxidant, antiulcer, anti-inflammatory activity and safe on liver enzymes in rats.

Keywords: Quinazoline, Quinazoline-4-one, Anti-inflammatory, Antioxidant, Antiulcer, COX-2.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs), are the active ingredient in several counter pain relievers [1]. Long-term use of NSAIDs has been associated with gastrointestinal (GI) ulceration, bleeding and nephrotoxicity [2]. Ibuprofen is one of the top-ten NSAIDs; has disadvantageous side effects such as dyspepsia, symptomatic and complicated gastric and duodenal ulcers [1]. COX-1 and COX-2 are the pharmacological targets of NSAIDs and COX-2 selective inhibitors (coxibs) [3]. It is intended to exploit biochemical differences between the two COX enzymes by conversion of carboxylate-containing NSAIDs into gastro-protective amide pro-drugs [4]. This can be achieved by masking the free carboxylic group and may shift its enzyme selectivity from COX-1 towards COX-2 led to reducing the NSAIDs topical irritant action [5,6].

Quinazolines are a common class of nitrogen-containing heterocyclic scaffolds exhibit a broad spectrum of pharmacological activities *viz*. anti-inflammatory, antioxidant [7], anti-tumor [8], anticancer [9], analgesic [10], antimicrobial [11] and antiviral [12,13] activities. Quinazolinone derived Schiff base derivatives are also considered good anti-inflammatory and antioxidants agents [14-16]. The structure activity relationship of these compounds plays an important role to illustrate their antioxidants and anti-inflammatory activities [14,15]. Also, Schiff base of quinazolinone derivatives with electron-

donating moiety (-OH, -OCH_3) were found to be excellent antioxidants and compounds with electron-withdrawing moiety (-Cl, -NO_2) were exhibit significant inhibition of gastric H^+/K^+-ATPase and found to be excellent antiulcer agents when compared to the reference drug omeprazole [17].

Phloretic acid (dihydrocoumaric acid) is biologically derived from *p*-coumaroyl-CoA [18,19] and then biotransformed to phloretin. Phloretin exhibit various bioactivities, including antioxidant [20], anti-inflammatory and antidiabetic [21] and antitumor [22]. As an extension of our studies interesting research program on the synthesis of some new biologically active heterocyclic compounds [7,14,15,23], now we wish to report the synthesis of combining form of these two structural features into one molecule might produce compounds with promising antioxidant, anti-inflammatory and anti-ulcer effects.

EXPERIMENTAL

Gallenkamp melting point apparatus was used to determine uncorrected melting points for all synthesized compounds. Shimadzu MR 470 infrared and ^{1}H Varian EM 360 (^{1}H NMR at 240 MHz) & (^{13}C NMR at 75 MHz) were used to record the spectral data of infrared (IR) using the KBr pellets, ^{1}H & ^{13}C NMR in DMSO-d_6 & CD_3OD, respectively. Also, HP Model MS-5988 was used to record the chemical shifts of mass spectra for all the synthesized compounds in part per million (δ ppm) downfield from internal tetramethylsilane (TMS). However. C, H and N microanalytical data were determined using CE440 Elemental Analyzer.

Ibuprofen, formalin and lipopolysaccharides (LPS) were obtained from Merck Ltd., Germany. 2,2′-Azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$), potassium persulfate, phosphate-buffered saline (pH 7.4), acetate buffer, pH 3.6 1, 2,4,6-tripyridyl-*s*-triazine, hydrochloric acid, ferric chloride, linoleic acid, butylated hydroxytoluene (BHT), β-carotene Type 1 (95%), $DPPH^{\bullet}$ (2,2-phenyl-1-picryl-hydroxyl) and thiobarbituric acid (TBA) were obtained from Sigma, USA, TPTZ (2,4,6-tripyridy-*s*-triazine) and ferric chloride from HmbG Chemicals, Germany, while ammonium thiocyanate was from AJAX Chemical, Australia and ferrous chloride and ferrous sulphate, acarbose (Bicon Ltd)., α-glucosidase (SRL), maltose (Loba cheme), Glucose assay reagent (Agappe Diagnostics), α-amylase (SRL) were from BDH, England.

Synthesis of ethyl 5-chloro-2-(3-(4-hydroxyphenyl)-propanamido)benzoate (1): Compound **1** was synthesized according to the method illustrated by Hussein [14] with some modifications. To a solution of 3-(4-hydroxyphenyl)propanoic acid (0.01 mol) and methyl 2-amino-5-chlorobenzoate (1.85 g, 0.01 mol) in xylene (50 mL), phosphorus trichloride (3 mL) was added. The reaction mixture was heated under reflux for 3-4 h. The crude product was recrystallized from ethanol to give compound **1**. Yield: 73%; m.p.: 132-134 ºC; elemental analysis of $C_{18}H_{18}NO_4Cl$ (*m.w.* 347) calcd. (found) %: C 62.2 (62.7); H 5.2 (5.2); N 2.03 (2.00) and Cl 10.08 (10.10). IR (KBr, ν_{max}, cm^{-1}): 3440 (OH), 3150 (NH), 2923 and 2854 (CH-arom.), 2648 (CH-aliph.), 1720 (C=O), 1512 (C=N), 756 (C-Cl, *str.*). MS (*m/z*) 3: 347 (M^+, 19.16 %), 226 (10.18 %), 183 (41.43 %), 110 (100 %), 77 (22.45 %). ^{1}H NMR (DMSO-d_6): 4.5 (s, 2H, CH_2), 6.8 (s, 1H, CH), 2.5 (s, 3H, $COCH_3$), 7.3–7.8 (m, 7H, Ar–H) 10.11 (s, 1H, OH), 11.6 (s, 1H, NH).

Synthesis of 2-(4-hydroxyphenethyl)-3-amino-6-chloroquinazolin-4(3*H*)-one (2): A mixture of compound **1** (0.01 mol) and hydrazine hydrate (95%) (0.05 mol) were dissolved in *n*-butanol (30 mL) and refluxed for 3-5 h [14]. The solvent was concentrated and the residue was recrystallized from ethanol to give compound **2**. Yield: 65%; m.p.: 188-190 ºC; elemental analysis of $C_{16}H_{14}N_3O_2Cl$ (*m.w.* 315) calcd. (found) %: C 60.9 (61.0); H 4.40 (4.44); N 13.30 (13.33) and Cl 11.11 (11.10). IR (KBr, ν_{max}, cm^{-1}): 3520 (OH), 3300, 3200 (NH_2), 3100 (NH), 3050 and 2970 (CH-arom), 2850 (CH-aliph.), 1710, 1685 (2C=O), 1593 (C=N) 743 (C-Cl, *str.*). ^{1}H NMR (DMSO-d_6): 4.4 (s, 2H, CH_2), 5.7 (s, 1H, CH), 7.31-8.3 (m, 7H, Ar-H), 5.8-6.3 (s, 1H, NH). Mass: *m/z* 315 (M^+; 36.58%) with a base peak at 156 (100%) and other significant peaks were observed at 195 (16.50%), 181 (9.23%), 113 (56.6%), 78 (58.2%).

Synthesis of (*E*)-3-(4-hydroxy-3-methoxybenzylideneamino)-2-(4-hydroxyphenethyl)-6-chloroquinazolin-4(3*H*)-one (3): A mixture of compound **2** (0.01 mol) and 4-hydroxy-3-methoxybenzaldehyde (0.01 mol) in *n*-butanol was refluxed for 4 h. The reaction mixture was poured onto ice water and the solid obtained was recrystallized from dioxane to give **3**. Yield: 84%; m.p.: 264-266 ºC; elemental analysis of $C_{24}H_{20}N_3O_4Cl$ (*m.w.* 449) calcd. (found) %: C 64.14 (64.20); H 4.44 (4.45); N 9.35 (9.40) and Cl 7.79 (8.10). IR (KBr, ν_{max}, cm^{-1}): 3409 (OH), 3240, 2923 (CH-arom.), 2723 (CH-aliph.), 1651 (C=O), 1573 (C=N), 771 (C-Cl, *str.*). ^{1}H NMR (DMSO-d_6): 1.24 (t, 3H, CH_3 ethyl), 2.4 (s, 3H, CH_3), 4.2 (q, 2H, CH_2 ethyl), 7.0-8.1 (m, 10H, Ar-H), 7.0-8.1 (m, 10H, Ar-H), 10.3 (s, 1H, OH). Mass: *m/z* 449 (M^+; 12.46%) with a base peak at 156 (100%) and other significant peaks were observed at 299 (49.30%), 252 (28.48%), 178 (36.50%), 77 (53.09%).

Biological testing

***in vitro* Antioxidant activity:** In the present study, 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$), 2,2′-azino*bis*(3-ethylbenzo-thiazoline-6-sulfonic acid) radical ($ABTS^{\bullet+}$), H_2O_2 and superoxide assays were applied to evaluate the *in vitro* antioxidant activity of compounds **1-3**:

$DPPH^{\bullet}$ assay: $DPPH^{\bullet}$ assay of compounds **1-3** was carried out according to the method of Hamauzu *et al.* [24]. A solution of $DPPH^{\bullet}$ (0.1 mM, 4 mL) in methanol was prepared then, added to different concentrations (50-300 µg/mL) of 0.2 mL compounds **1-3** as well as Trolox. The decrease in absorbance at 517 nm was measured at 60 min. A control test was carried out using 0.2 mL of distilled water instead of compounds **1-3**.

$ABTS^{\bullet+}$ radical assay: Re *et al.* [25] method was used to $ABTS^{\bullet+}$ decolourization assay. Briefly, 3 mL $ABTS^{\bullet+}$ (2.45 mM) was prepared and added to 1 mL potassium persulfate (2.45 mM) then mixed well and kept in the dark at room temperature for 16 h. The $ABTS^{\bullet+}$ solution was diluted with ethanol to obtain absorbance of 0.700 ± 0.020 at 734 nm. $ABTS^{\bullet+}$ (3 mL) solution was mixed with Trolox (standard antioxidant drug) as well as compounds **1-3** (0.1- 1.0 mg/mL), incubated at room temperature for 6 min and their absorbance was recorded at 734 nm. Blanks were run in each assay. The inhibition percentage calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{A_o - A_1}{A_1} \times 100$$

where A_0 = absorbance of the control and A_1 = absorbance of the sample.

Hydrogen peroxide assay: H_2O_2 scavenging activity assay was carried out using Guddadarangavvanahally *et al.* [26] method with some modifications. A 2 mL H_2O_2 (20 mM) solution prepared in PBS (pH 7.4) was added to 1 mL (100-250 µg/mL) compounds **1-3** and/or standard antioxidant drugs (BHT and α-tocopherol) then incubated for 10 min. The absorbance of the solution was measured at 230 nm against a blank solution containing compounds **1-3** without H_2O_2.

Superoxide assay: Superoxide radical assay was determined by the method of Elizabeth & Rao [27]. The reduction of nitroblue terazolium (NBT) by superoxide was measured in the presence and absence of compounds **1-3**. The assay mixture included 0.1 mL of NBT (0.1 mg) and 0.3 mL with concentration (100 mg/L) of compounds **1-3** and/or Trolox as standard antioxidant drug and 1 mL of alkaline DMSO in a final volume of 1.4 mL. Absorbance was measured at 560 nm.

in vivo Biological studies

LD_{50} estimation: Male albino rats weighing around 180 ± 15 g (300 rats; 180 for LD_{50} estimation, 54 for antiulcerogenic activity, 48 for anti-inflammatory, 48 for antinociceptive activities and 48 for hepatoprotective activity) were obtained from the animal house of Faculty of Veterinary Medicine, Cairo University, Giza, Egypt. They were housed in plastic cages with stainless steel covers at the National Cancer Institute Animal House. The animals were maintained at 22 ± 1 ºC and a humidity of 55-60% in a light-controlled room. The animals were kept for 1 week to acclimatize and then they were divided into groups according to the condition of each experiment. Animals were provided with a standard diet and water *ad libitum*. All approved procedures were following the guidelines of the AMS Directive 2019; 05/012/AMS for animal experiments and were approved by the Institutional Research Ethics Committee at the Faculty of Applied Medical Sciences, October 6 University, Egypt (No. 20191205).

Determination of LD_{50} of compounds 1-3: Preliminary experiments were carried out on 6 groups of 4 rats of compounds **1-3** were orally administrated in different doses to find out the range of doses that cause 0 and 100 % mortality of animals. A range dose was determined for each extract.

LD_{50} was determined by oral administration of compound **1** in different doses 400, 800, 1000, 1200, 1400 and 1500 mg/kg. In a group of 10 animals each, compound 2 was given orally in doses of 300, 600, 900, 1100, 1200 and 1300 mg/kg. In a group of 10 animals each, compound **3** was given orally in doses of 1200, 1400, 1600, 1900, 2100 and 2300 mg/k.g. b.w. After administration of the tested compounds **1-3** animals were observed individually every hour during the first day and every day for 21 days. Behaviour and clinical symptoms of animals were noted throughout the experiment. The LD_{50} was calculated by Finney [28] method by the application of the following formula:

$$LD_{50} = Dm - \sum(z.d)/n$$

Dm = The largest that kill all animals; Σ = The sum of (z x d); Z = Mean of dead animals between 2 successive groups; d = The constant factor between 2 successive doses; n = Number of animals in each group.

Antiulcerogenic activity of compounds 1-3 at doses of 1/20 LD_{50}: Induction of ulcer in rats was done on the last day of the experiment by oral administration of ibuprofen (50 mg/kg, p.o.) twice a day at an interval of 12 h [29]. The animals were deprived of food for 24 h prior to ulcer induction.

Experimental design: The rats were randomized and divided into six groups of six rats each. Food was withdrawn 24 h and water 2 h before the commencement of the experiment [30]. The animals were divided into the following groups:

Group I received 1 mL saline solution orally and was kept as a control group.

Group II was administered with 50 mg/kg of ibuprofen, p.o. twice a day at an interval of 12 h.

Groups III-V were pre-treated with 56.25, 41.75 and 89.25 mg/kg.b.w. of compounds **1**, **2** and **3**, respectively, in normal saline orally in a single daily for 20 days.

Groups VI-VIII were pre-treated with 56.25, 41.75 and 89.25 mg/kg.b.w. of compounds **1**, **2** and **3**, respectively, p.o. for 20 days) before induction of ulcer as described for group **II**.

Groups VIIII were pre-treated with omeprazole (20 mg/kg. b.w.) [31], p.o. for 20 days) before induction of ulcer as described for group **II**.

After 4 h, the animals were anesthesiated then the stomachs were removed and opened and its contents drained into a measuring cylinder. The pH of the contents was measured with a digital pH meter (PICO, Labindia Instruments Private Limited).

The macroscopic examination was carried out with a hand lens and scored for the presence of lesions [30]. Ulcer index (UI) and the preventive ratio of each of the groups pre-treated with extracts were calculated using the following formula:

$$UI = \frac{\text{Degree of ulceration} \times \text{Percentage of group ulcerated}}{100}$$

Preventive ratio (%):

$$PO = \frac{\text{Ulcerated group} - \text{Protected group}}{\text{Ulcerated group}} \times 100$$

$$\text{Degree of ulceration} = \frac{\text{Total ulcer score}}{\text{Number of ulcerated animals}}$$

Assay of acid concentration: The gastric contents were centrifuged at 1000 rpm for 10 min, 10 mL of gastric juice sample from the stomach of the animal was pipetted into a 250 mL conical flask 2-3 drops of phenolphthalein indicator was added and titrated against 0.1M NaOH till a faint pink color is obtained. The value obtained was used to measure the total acidity using the formula below [32] and expressed as in eqn. 1.

$$\text{Total acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1\text{m}} \times 100 \quad (1)$$

Also, the mucosal tissue was scraped from the stomach and the gastric mucosa was used to assay total protein contained and Na^+/K^+-dependent ATPase as described by Lowry *et al.* [33] and Bonting [34], respectively.

Anti-inflammatory and antinociceptive activities compounds 1-3 at doses of 1/40 and 1/20 LD_{50}: Domenjoz [35] method was used to evaluate the anti-inflammatory of compounds **1-3**. Rats (180 ± 10 g) were divided into 8 different groups, 6 animals in each (one positive control and 7 treatment groups). At the beginning, the thickness of the left paw was measured. They were treated orally with the compound **1** (28.15 and 56.25 mg/kg/b.w.), compound **2** (20.88 and 41.75 mg/kg/b.w.) and compound **3** (44.63 and 89.25 mg/kg/b.w.). ibuprofen 50 mg/kg as a reference standard [36].

The inflammation was induced by injection of 0.1 mL of 6% formalin solution S.C. in normal saline after 30 min of administration. The same volume of saline injected into the right hind paw. The thickness difference between the two paws gave the swelling induced by formalin. The efficacy of inflammation was estimated by comparing the swelling of the treated with the control.

The injected paw was measured using a transparent millimeter ruler for 2 h at 30 min. intervals after formalin solution injection. Increases in the linear diameter of the right hind paws were taken as indicators of paw edema. Edema was assessed in terms of the difference in the 'zero time' (C_o) linear diameter of the injected right hind paw and its linear diameter at 'time t' [(C_t) – that is, 30, 60, 90 and 120 min] following formalin solution administration.

Rats in the reference, comparative 'test' Group VIII received ibuprofen (30 mg/kg/b.w.); while rats in the 'control' Group I received distilled water (3 mL/kg/b.w.) only. Percentage inammation (edema) was calculated from the formula: $C_o/C_t \times 100$; while percentage inhibition of the edema was calculated from the formula:

$$\frac{C_o - C_t}{C_o} \times 100$$

where C_o is the average inammation (hind paw edema) of the 'control' Group I rats at a given time; and C_t is the average inammation of the (Group II-VII) compounds **1-3** or (Group VIII) ibuprofen-treated rats at the same time.

Antinociceptive activity of compounds 1-3: Koster *et al.* method [37] was used to investigate the antinociceptive effect of compounds **1-3**. Six groups of 8 rats (150 ± 10 g) each were used for the controls and treated rats. The compound **1** (28.15 and 56.25 mg/kg/b.w.), compound **2** (20.88 and 41.75 mg/kg/b.w.) and compound **3** (44.63 and 89.25 mg/kg/b.w.), aspirin (100 mg/kg) as standard analgesic and control vehicle were orally administrated 30 min before acetic acid (0.6%). Acetic acid was administrated intraperitoneally to all rats at the dose of 10 mL/kg and the number of abdominal constrictions with stretching of the hind limbs was counted for 30 min [38,39]. The percentage of inhibition was expressed as a percentage of reduction of the number of abdominal contractions in treated animals to the control.

Hepatoprotective effect of compound **1** (1/20 LD_{50}, 56.25 mg/kg.b.w.), compound **2** (1/20 LD_{50}, 41.75 mg/kg/b.w.) and compound **3** (1/20 LD_{50}, 89.25 mg/kg/b.w.) in LPS-induced liver toxicity in rats

Experimental design

Animals were randomly assigned to six groups, eight rats in each.

Group I: received 1 mL normal saline orally and kept as normal control.

Group II: received LPS at a single i.p. dose (30 mg/kg/b.w.) and kept as a positive control [38].

Group III-V: were treated with compound **1** (1/20 LD_{50}, 56.25 mg/kg.b.w.); compound **2** (1/20 LD_{50}, 41.75 mg/kg/b.w.) and compound **3** (1/20 LD_{50}, 59.25 mg/kg/b.w.) suspended in normal saline orally in a single daily dose for 10 days.

Group VI-VIII: were treated with compound **1** (1/20 LD_{50}, 56.25 mg/kg.b.w.), compound **2** (1/20 LD_{50}, 41.75 mg/kg/b.w.) and compound **3** (1/20 LD_{50}, 89.25 mg/kg/b.w.) suspended in normal saline orally in a single daily dose for 10 days followed by a single i.p. dose of LPS (30 mg/kg/b.w.).

On 11th day, blood was collected from the retro-orbital vein of each animal and each sample was collected into two heparinized tubes. The first part of heparinized blood samples was divided into two aliquots. The first aliquot was used for the determination of GPx and CAT activity according to the methods of Paglia & Valentine [39] and Sinha [40], respectively. The second aliquot was hemolyzed using bidistilled water and the hemolysate of each sample was divided into two portions.

The first portion was precipitated with chloroform/methanol (3:5 v/v) mixture and the resultant supernatant was used for the determination of SOD activity according to the methods of Marklund & Marklund [41]. The second portion was deproteinized with meta-phosphoric acid and the clear supernatant was used for the estimation of GSH level [42]. Hemoglobin and white blood cell count were determined in the heparinized blood samples [43] and used in the calculation of the enzyme activity (CAT and SOD) as well as the GSH level.

The second part of the heparinized blood samples was centrifuged at 1000 rpm for 20 min. The separated plasma was used for the estimation of serum activity of ALT, AST and LDH according to the methods of Reitman & Frankel [44] and King [45].

Rats were sacrificed by cervical dislocation after light anesthesia and placed on disposable pads abdomen up and the liver was excised immediately and washed off from blood with ice-cold physiological saline. Then, the tissue was blotted in between filter papers to absorb moisture. A 10% organ homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 3000 rpm for 15 min and the supernatant was used for the estimation of liver activity of GPx, CAT and SOD [39-42]. Also, levels of GSH, TNF-α, NO, TBARS and total protein in liver homogenate were estimated according to the methods previously described [43,46-48].

Histopathological examination: The liver and stomach were sliced and pieces were preserved in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5-6 μ in thickness were cut and stained with staining reagents. All the sections of the tissues

were examined under a microscope according to the method of Bancroft & Steven [49].

Isolation of peritoneal macrophages: Kolaczkowska *et al.* [51] method was used to isolated peritoneal macrophages of treated rats as follows:

Rats sacrificed and placed on disposable pads abdomen up, the skin was carefully cut by scissors above the peritoneum about midway the length of the abdomen. A 10 mL cold sterile PBS (invitrogen/gibco) was injected by a sterile syringe into the peritoneal cavity. The enriched fluid was collected from the peritoneal cavity by the same syringe into a 50 mL Falcon tube and centrifuged (10 min, 2000 rpm, 4 ºC). Cells were washed with complete medium and counted using a hemocytometer. The number of viable cells was calculated and kept constant at 1×10^6 cell/mL.

Assay of NO, PGE2 and TNF-α levels as well as COX-2 activity by compounds **1-3** in peritoneal macrophage suspensions. A 5×10^5 cells/mL of peritoneal macrophages was plated into 96-well plates as follows:

Well plate I (one well × 3): was seeded by macrophage suspensions (100 µL, at 6×10^6 cells/mL) kept as normal control well, incubated at 37 ºC for 18 h.

Well, plate II (one well × 3): was seeded by macrophage suspensions (100 µL, at 6×10^6 cells/mL) and added LPS (1 µg/mL) kept as positive control well, incubated at 37 ºC for 18 h.

Well, plates III-V (3 separate wells × 3): were seeded by macrophage suspensions (100 µL, at 6×10^6 cells/mL) and 100 µL of compounds **1-3** suspended in PBS were added to each well to obtain final concentrations of 100 µg/mL, incubated at 37 ºC for 18 h.

Group VI-VIIII (4 separate wells × 3): were seeded by macrophage suspensions (100 µL, at 6×10^6 cells/mL) and 100 µL of compounds **1-3**, as well as Ibuprofen, suspended in PBS were added to each well to obtain final concentrations of 100 µg/mL and later treated with LPS (1 µg/mL), incubated at 37 ºC for 18 h.

The NO and PGE2 levels were measured using diluted cell suspensions of peritoneal macrophages (1×10^6 cells/well) that were incubated with compounds **1-3** as well as ibuprofen at a concentration of (100 µg/mL) and later treated with LPS (1 µg/mL) for 18 h. The PGE2 level in the supernatants was estimated according to the method adapted by Chu *et al.* [51], Nakatsugi *et al.* [52] and Campelo *et al.* [53] respectively, using an enzyme immunoassay kit (Cayman Chemicals, USA). Also, COX-2 activity was assayed using a fluorescent activity assay kit (Cayman Chemicals, USA), according to the manufacturer's specifications [55]. Briefly, diluted cell suspensions of peritoneal macrophages (1×10^6 cells/well) were incubated with compounds **1-3** as well as Ibuprofen at a concentration of (100 µg/mL). Then wells were cultured with the vehicle, ibuprofen and test compounds **1-3** in the presence of 1 µg/mL of LPS for 18 h.

Molecular docking: A docking simulation study has been performed using molecular operating environment (MOE®) version 2014.09, Chemical Computing Group Inc., Montreal, Canada. The computational software operated under "Windows XP" installed on an Intel Pentium IV PC with a 1.6 GHz processor and 512 MB memory.

Optimization of target compounds: The target compounds were constructed into a 3D model using the builder interface of the MOE program. After checking their structures and the formal charges on atoms by 2D depiction, the following steps were carried out: (i) the target compounds were subjected to a conformational search; (ii) all conformers were subjected to energy minimization, all the minimizations were performed with MOE until an RMSD gradient of 0.05 kcal mol^{-1} $Å^{-1}$ with MMFF94x force field and the partial charges were automatically calculated; and (iii) the obtained database was then saved as a Molecular DataBase (MDB) file to be used in the docking calculations.

Optimization of enzyme active site: The X-ray crystallographic structure of COX-2 co-crystallized with ibuprofen as an inhibitor (PDB ID: 4PH9) [55,56] was downloaded from the protein data bank (http://www.rcsb.org/). The enzyme was prepared for docking study by (i) removal of chain A of its dimer, water molecules and ligands that are not involved in the binding; (ii) protonate 3D protocol in MOE with default options; and (iii) the co-crystalized ligand was then used to define the active site for docking.

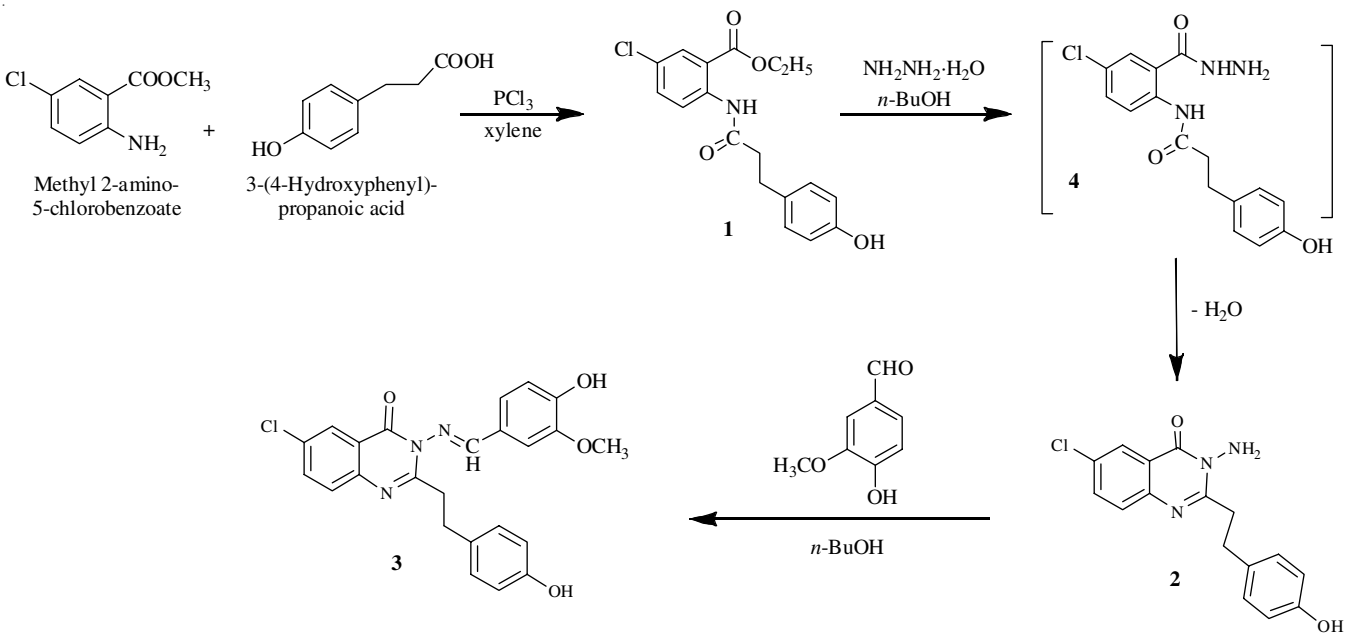
Docking of the target molecules to COX-2 active site: Docking of the conformation database of the target compounds was done using MOE software. The following methodology was generally applied (i) the enzyme active site file was loaded and the Dock tool was initiated. The program specifications were adjusted to the ligand atoms as the docking site, triangle matcher as the placement methodology to be used and London dG as scoring methodology to be used and was adjusted to its default values; (ii) The MDB file of the ligand to be docked was loaded and dock calculations were run automatically. The obtained poses were studied and the poses showed the best ligand-enzyme interactions were selected and stored for energy calculations.

The mentioned docking set up was first validated by re-docking of the co-crystallized ligand (ibuprofen) in the active site of the enzyme before predicting the binding mode for the target compounds **1-3**.

Statistical analysis: The results are expressed as means ± SD. Comparisons between groups were performed using a one-way analysis of variance (ANOVA). Differences between individual treatment groups were compared using Dunnett's test. Statistical significance was set at $p < 0.05$ and $p < 0.01$ and the statistical analyses were performed using SPSS software, version 15.0 (SPSS, Inc., USA).

RESULTS AND DISCUSSION

The starting compound **1**, ethyl 5-chloro-2-(3-(4-hydroxyphenyl)propanamido)benzoate was synthesized as described by Hussein [14] with some modifications by reaction of 3-(4-hydroxyphenyl)propanoic acid and methyl 2-amino-5-chlorobenzoate. The synthesis of target compounds **2**, **3** was achieved by the route depicted in **Scheme-I**. The structure of compounds **1-3** were supported by elemental analyses, IR, ^{1}H NMR and mass spectral data.



Scheme-I: Synthesis of target compounds **1-3**

Thus, condensation of N-amino derivative **3** with aromatic aldehydes in refluxing *n*-butanol afforded the corresponding Schiff base (**Scheme-I**). The structure of the newly prepared compounds was examined by elemental analysis and spectral data. Cyclcondensation of compound **2** with hydrazine hydrate afforded the corresponding (*E*)-3-(4-hydroxy-3-methoxybenzylidene-amino)-2-(4-hydroxyphenethyl)-6-chloroquinazolin-4(3*H*)-one. The reaction proceeds *via* the intermediate **4**, which undergoes a nucleophilic addition to the carbonyl of the side chain followed by the loss of 1 mol of water. IR spectra of compound **2** showed the distinct bands for amino group (NH_2) and one carbonyl group (C=O). Their ^1H NMR spectra revealed the presence of primary amine protons at δ = 6.3-5.8 ppm that were D_2O exchangeable, the methylene protons in the side chain were found at around δ = 4.6-4.2 ppm.

The mass spectra of compound **3** showed the molecular ion peak and prominent M^+ peak indicating the early loss of free amino function group at position N-3. Further fragmentation pattern of the molecule was found to be satisfactory.

***in vitro* Antioxidant activity:** Scavenging activity of compounds **1-3** and Trolox as a reference standard at different concentrations against DPPH$^•$ is illustrated in Fig. 1. The maximum activity for compound **1** is 40% at 150 µg/mL with IC_{50} =136.4 µg/mL. Compounds **2** and **3** had the highest radical scavenging activity 25% and 50% (IC_{50} = 73.54 µg/mL) at 100 µg/mL, respectively. Also, IC_{50} of Trolox = 107.28 µg/mL.

Fig. 2 showed the ABTS$^{•+}$ radical scavenging activity of compounds **1-3** at different concentrations. The maximum ABTS$^{•+}$ free radical scavenging activity of compounds **1-3** and Trolox at 1.0 mg/mL 65, 40 and 85% with IC_{50} = 0.8, 0.92, 0.08 and 0.17 mg/mL, respectively (Table-1). Compound **3** had the highest radical scavenging activity when compared with compounds **1**, **2** and Trolox. Radical scavenging activity of compounds **1-3** and Trolox is arranged in the following order: compound **3** > Trolox > compound **1** > compound **2**.

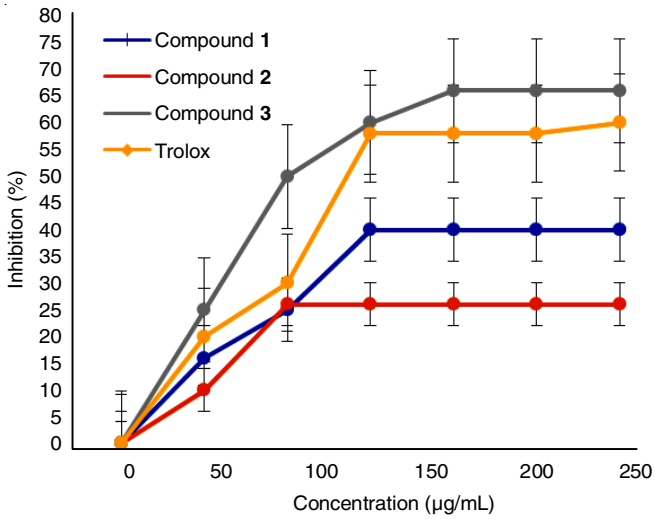


Fig. 1. Scavenging activities of different concentrations of compounds **1-3** and Trolox against DPPH$^•$ radical

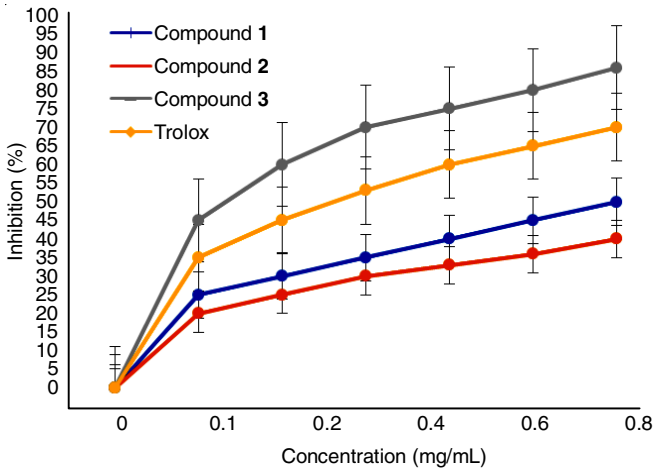


Fig. 2. Scavenging activities of different concentrations of compounds **1-3** and Trolox against ABTS$^{•+}$ radical

TABLE-1
in vitro FREE RADICAL SCAVENGING ACTIVITY
OF THE SYNTHESIZED COMPOUNDS **1-3**

Compound	DPPH$^{\bullet}$ radical IC_{50} (μg/mL)	$ABTS^{\bullet+}$ radical IC_{50} (mg/mL)
1	136.47 ± 6.25[a]	0.80 ± 6.250[a]
2	–	0.92 ± 0.013[b]
3	73.54 ± 3.14[c]	0.08 ± 0.006[d]
Trolox	107.08 ± 5.73[b]	0.17 ± 0.009[c]

Values are mean of three determinations. Data shown are mean ± standard deviation. Data followed by the same capital letters are not significantly different at $p \leq 0.05$

The scavenging activity of the compounds **1-3** on H_2O_2 was presented in Fig. 3. The results are compared with BHT and α-tocopherol as standards. At 100 μg/mL, compo-unds **1-3**, BHT and α-tocopherol 57, 30, 78, 67 and 40%, inhibition, respectively. Also, the superoxide radical scavenging activity of compounds **1-3**, ascorbic acid and Trolox at 100 mg/L as illustrated in Fig. 4. Compound **3** showed a higher superoxide radical scavenging activity than ascorbic acid, Trolox and compounds **1** and **2**. In short, Compound **3** is the best one and followed by compound **2**.

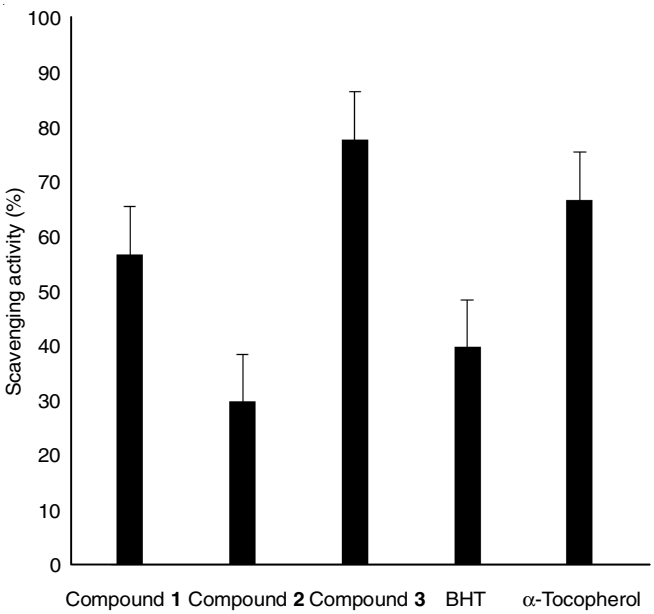


Fig. 3. Hydrogen peroxide scavenging activity of compounds **1-3**, α-tocopherol and BHT at 100 μg/mL concentration

The antioxidant activity of compounds **1** and **3** depends on their structural features. The presence of chlorine, a good electron-accepting group as well as ethyl ester and amide groups induce compound **1** to give 2 resonating structures (**Scheme-II**). The antioxidant activity of compound **3** (four resonating structures) more pronounced than compound **1** (two resonating structures) due to the presence of conjugated double bond (HC=N), which makes the electrons more delocalized from C ring to the D ring (**Scheme-II**) [14].

Many attempts for the explaining the structure-activity relationships of 4-quinazolines have been reported and showed the ability of such compounds to neutralize of free radicals

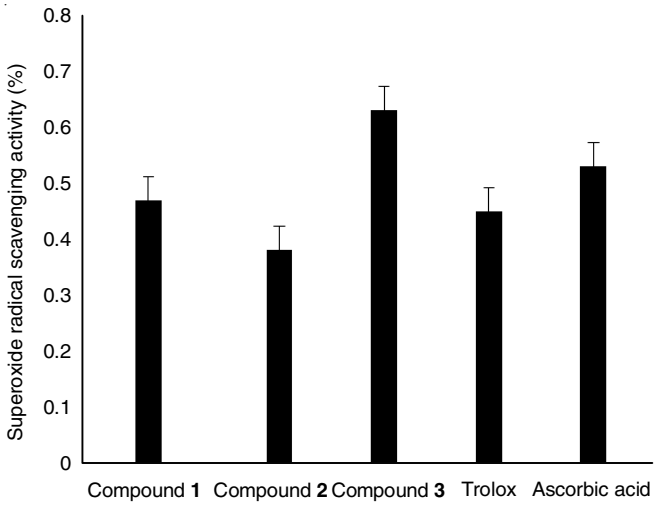
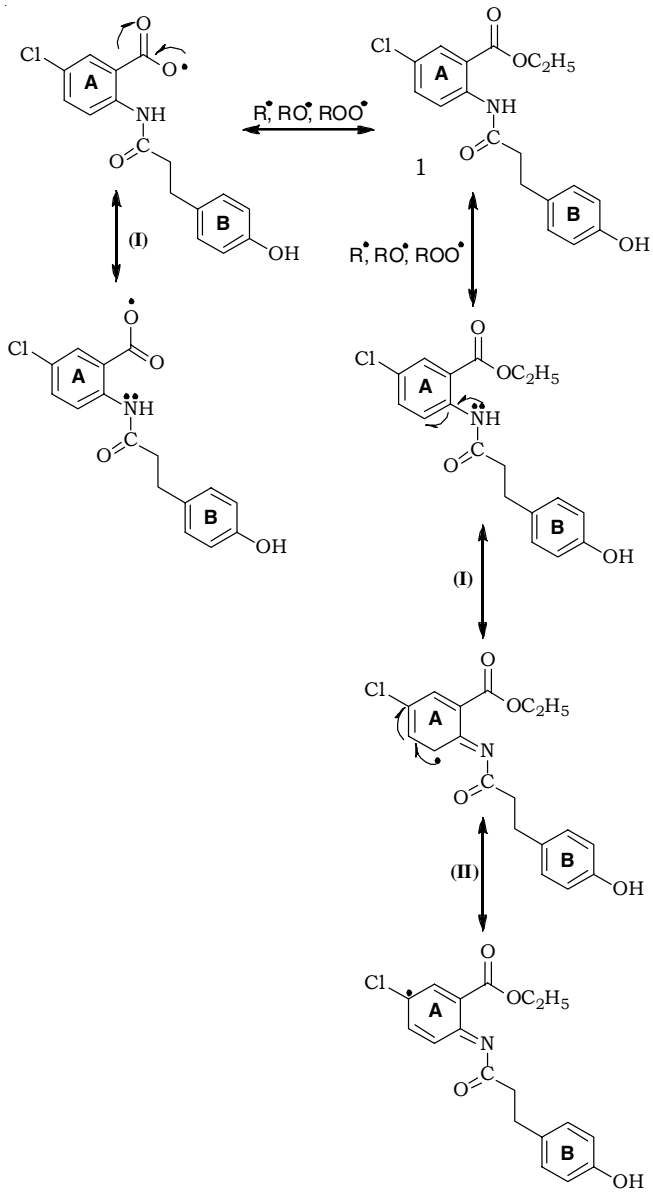


Fig. 4. Superoxide radical scavenging activity of compounds **1-3**, trolox and ascorbic acid at 100 mg/L concentration



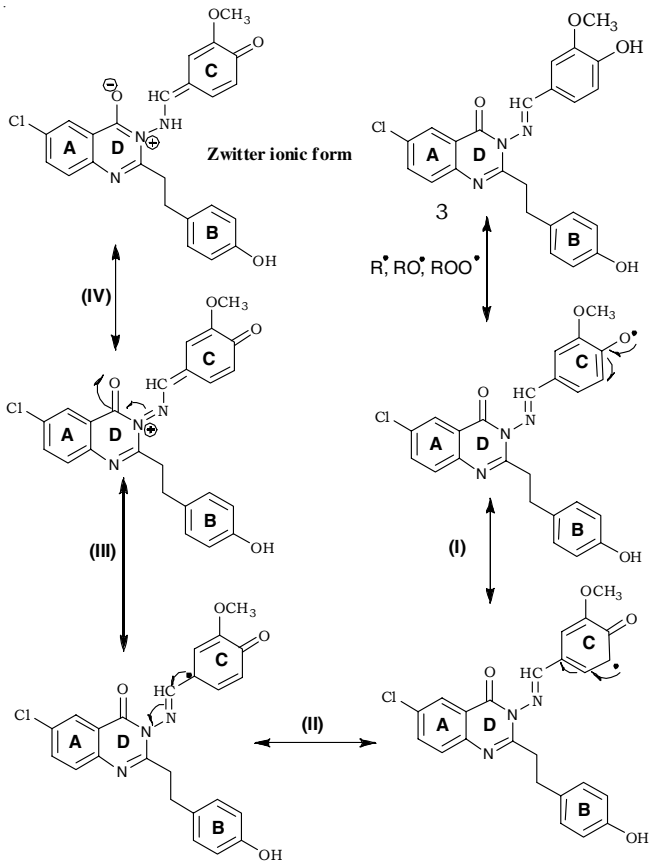
Scheme-II: Proposed antioxidant activity mechanism of compound **1**

initiating oxidation processes or from the termination of radical chain reactions, due to their hydrogen donating ability [14,57]. Also, Hussein [14] and Hussein & Samir [57] showed that the antioxidant activity of compounds is closely associated with their structures, such as substitutions on the aromatic ring and side chain structure. Free radical scavenging activity of phenolic compounds is believed to be influenced by the number and position of phenolic hydrogen in their molecules [7].

It is also proposed that the higher antioxidant activity of compounds **1** and **3** is related to the presence of hydroxyl and ethoxide groups [7]. The structural requirement considered essential for effective radical scavenging by compounds **1-3** is the presence of *p*-hydroxyl group in C ring and conjugated double bond (HC=N). The presence of a double bond between C ring and D ring in compound **3** makes the electrons more delocalized to form quinone structure which possesses electron donating properties and is a radical target [57] (**Scheme-III**).

Assay of LD_{50} of compounds 1-3 in adult rats: The LD_{50} of compounds **1-3** were found to be 1125, 835 and 1185 mg/kg/b.w., respectively. Increase the heart rate and rapid respiration as well as change the colour of eyes and skin to brownish and bluish color, respectively. Also, hypothermia was observed with the toes and tail.

The results are given in Table-2 shows that the oral administration of compound **1** in doses of 400, 800, 1000, 1200, 1400 and 1500 mg/kg b.w. resulted in mortalities of 0, 1, 2, 6, 9 and 10, respectively. The dose of compound **1** that killed half of the rats (LD_{50}) was 1125 mg/kg b.w. The oral administration of compound **2** in doses of 300, 600, 900, 1100, 1200 and 1300 mg/kg b.w. resulted in mortalities of 0, 2, 4, 7, 9 and 10, respectively (Table-3). The dose of compound **2** that killed half of the rats (LD_{50}) was 835 mg/kg.b.w. For the oral administration of compound **3** in doses of 1200, 1400, 1600, 1900, 2100 and 2300 mg/kg.b.w. resulted in mortalities of 0, 1, 3, 6, 8 and 10, respectively (Table-4). The dose of compound **3** that killed half of the rats (LD_{50}) was 1785 mg/kg.b.w.



Scheme-III: Proposed antioxidant activity mechanism of compound **3**

The high LD_{50} values of compounds **1-3** indicated their safety. There were no changes in animal behaviour, but the bodyweight gains were significantly different in the treated rats. In the acute toxicity in male rats of quinazoline orally at dose 500-2500 mg/kg/b.w. [58]. Since the changes in animal behaviour have been used as an indicator of the adverse effects of drugs and chemicals [59].

TABLE-2
DETERMINATION OF LD_{50} OF COMPOUND **1** GIVEN ORALLY IN ADULT RATS

Group number	Dose (mg/kg)	Number of animals/group	Number of dead animals	(Z)	(d)	(Z.d)
1	400	10	0	0.5	400	200
2	800	10	1	1.5	200	300
3	1000	10	2	4.0	200	800
4	1200	10	6	7.5	200	1500
5	1400	10	9	9.5	100	950
6	1500	10	10	0	00	00

TABLE-3
DETERMINATION OF LD_{50} OF COMPOUND **2** GIVEN ORALLY IN ADULT RATS

Group number	Dose (mg/kg)	Number of animals/group	Number of dead animals	(Z)	(d)	(Z.d)
1	300	10	0	1	300	300
2	600	10	2	3	300	900
3	900	10	4	5.5	300	1650
4	1100	10	7	8.5	100	850
5	1200	10	9	9.5	100	950
6	1300	10	10	0	00	00

TABLE-4
DETERMINATION OF LD_{50} OF COMPOUND **3** GIVEN ORALLY IN ADULT RATS

Group number	Dose (mg/kg)	Number of animals/group	Number of dead animals	(Z)	(d)	(Z.d)
1	1000	10	0	0.5	400	200
2	1400	10	1	2.0	200	400
3	1600	10	3	4.5	300	1350
4	1900	10	6	7.0	200	1400
5	2100	10	8	9.0	200	1800
6	2300	10	10	0.0	00	00

Administration of ibuprofen (50 mg/kg.b.w) induced gastric lesions in rats with mean gastric ulcer index which is significantly higher and lower of Na^+/K^+-ATPase and total protein levels than for the control (Table-5). Treatment rats with compounds **1-3** as well as omeprazole at 56.25, 41.75, 89.25 and 20 mg/kg/b.w, respectively, lowered the gastric ulcer index as well as increased Na^+/K^+-ATPase and total protein levels significantly ($p < 0.05$) when compared with ibuprofen-treated groups. Also, the administration of ibuprofen resulted in elevated significantly of acid concentration in gastric contents when compared with the control group. Compounds **1-3** as well as omeprazole at 56.25, 41.75, 89.25 and 20 mg/kg.b.w, respectively, lowered significantly ($p < 0.05$) the level of acid in gastric content when compared with ibuprofen-treated groups. Also, administration of ibuprofen resulted in depletion of gastric pH significantly ($p < 0.05$) than for the control group. Compounds **1-3** as well as omeprazole at 56.25, 41.75, 89.25 and 20 mg/kg/b.w, respectively elevated gastric pH significantly when compared with ibuprofen treated groups ($p < 0.05$).

On the other hand, ibuprofen-induced lesion formation may be multifactorial, with stasis of gastric flow contributing significantly to the hemorrhagic as well as the necrotic aspects of the tissue injury [60]. Ibuprofen induced gastric lesion by chondrocytes inhibition of proteoglycans synthesis and interfere with *trans*-membrane ion fluxes as well as a cell to cell binding [30]. The present study indicated that gastric acidity parameters and ulcer index were elevated as well as lowered of gastric mucosa contained from Na^+/K^+-ATPase and protein in rats treated with ibuprofen oral administration.

In present study, quinazoline and quinazolinone derived Schiff base derivative especially, hydroxy and methoxy derivatives were the most potent compounds, contributing positively to gastric Na^+/K^+-ATPase inhibition. The preliminary structure activity relationship revealed that the compounds **1-3** with electron-donating moiety (OH, OCH_3, OC_2H_5 and methoxy benzene) were found to be excellent activity and compounds **1-3** with electron-withdrawing moiety (Cl) were found to be least antiulcer agents.

The present results also showed that the anti-ulceration activity of compounds **1-3** was against ibuprofen induced ulceration in rats *via* normalization of gastric acidity parameters, ulcer index, Na^+/K^+-ATPase and protein levels. The maximum effect was showed in compounds **1** & **3** and followed by compound **2**. The presence of pharmacophores (5-Cl and 2-ethyl, 4-OH, 3-amino, 3-N substitutions) are both essential for the activity. Present results are in agreement with the results of Rakesh *et al.* [17], who reported that quinazolinone derivatives containing -OH, -OCH_3 and -Cl moieties classified as potent antiulcer agents.

Microscopic pictures of H&E stained sections from glandular stomach showing normal mucosa, submucosa and muscular coat (MC) in control negative (I) and compounds **1-3** treated groups (III-V) (Fig. 5a,c-e). Histopathological sections showed marked histopathological changes including mucosal ulceration and congestion in ibuprofen treated group II (Fig.

TABLE-5
EFFECT OF COMPOUNDS **1-3** ON GASTRIC ACIDITY PARAMEETERS AND ULCER INDEX AS WELL AS GASTRIC MUCOSA LEVELS OF Na^+/K^+-ATPase AND PROTEIN ON IBUPROFEN-INDUCED ULCERATION IN RATS

Groups	Treatment (mg/kg)	Ulcer index (UI)	Protection (%)	Concentration of acid in gastric contents (meq/L)	pH of gastric contents	Na^+/K^+-TPase	Total protein (mg/g tissues)
I	Negative control (2 mL saline)	0.54 ± 0.17^{a}	–	0.22 ± 0.01^{a}	3.20 ± 0.35^{a}	5.25 ± 0.38^{a}	34.7 ± 2.87^{a}
II	Ibuprofen (50 mg/kg, p.o.)	15.66 ± 4.50^{e}	–	0.42 ± 0.04^{e}	2.25 ± 0.17^{e}	3.75 ± 0.44^{c}	12.87 ± 1.50^{b}
III	Compound 1 (56.25 mg/kg. b.w.)	0.57 ± 0.33a	–	0.23 ± 0.01^{a}	3.19 ± 0.43^{a}	5.11 ± 0.50^{a}	33.40 ± 3.98^{a}
IV	Compound 2 (41.75 mg/kg.b.w.)	0.54 ± 1.0^{a}	–	0.24 ± 0.02^{a}	3.10 ± 0.20^{d}	5.33 ± 0.37^{a}	35.00 ± 4.10^{a}
V	Compound 3 (89.25 mg/kg. b.w.)	0.52 ± 0.34^{a}	–	0.21 ± 0.01^{a}	3.24 ± 0.40^{a}	5.20 ± 0.54^{a}	34.76 ± 0.40^{a}
VI	Compound 1 (56.25 mg/kg. b.w.) + Ibuprofen (50 mg/kg. b.w.)	8.15 ± 0.86^{c}	47.95	0.29 ± 0.04^{c}	3.24 ± 0.43^{bc}	4.85 ± 0.36^{a}	32.21 ± 3.25^{a}
VII	Compound 2 (41.75 mg/kg. b.w.) + Ibuprofen (50 mg/kg. b.w.)	11.80 ± 1.86^{d}	24.6	0.35 ± 0.03^{d}	2.96 ± 0.20^{d}	4.60 ± 0.55^{b}	28.90 ± 2.90^{b}
VIII	Compound 3 (89.25 mg/kg. b.w.) + Ibuprofen (50 mg/kg. b.w.)	6.66 ± 0.27^{b}	56.45	0.25 ± 0.03^{ab}	3.22 ± 0.40^{ab}	5.10 ± 0.60^{a}	30.58 ± 4.65^{a}
VIIII	Omeprazole (20 mg/kg. b.w.) + Ibuprofen (50 mg/kg. b.w.)	10.75 ± 0.52^{c}	31.35	0.29 ± 0.01^{b}	3.24 ± 0.34^{b}	5.35 ± 0.54^{a}	32.17 ± 3.27^{a}

#Omeprazole is used as a reference. Data followed by the same capital letters are not significantly different at $p \leq 0.05$. Data shown are mean ± standard deviation of number of observations within each treatment, n = 6. Gastric mucosa contained from Na^+/K^+-ATPase was calculated as mmol Pi liberated per mg protein per h.

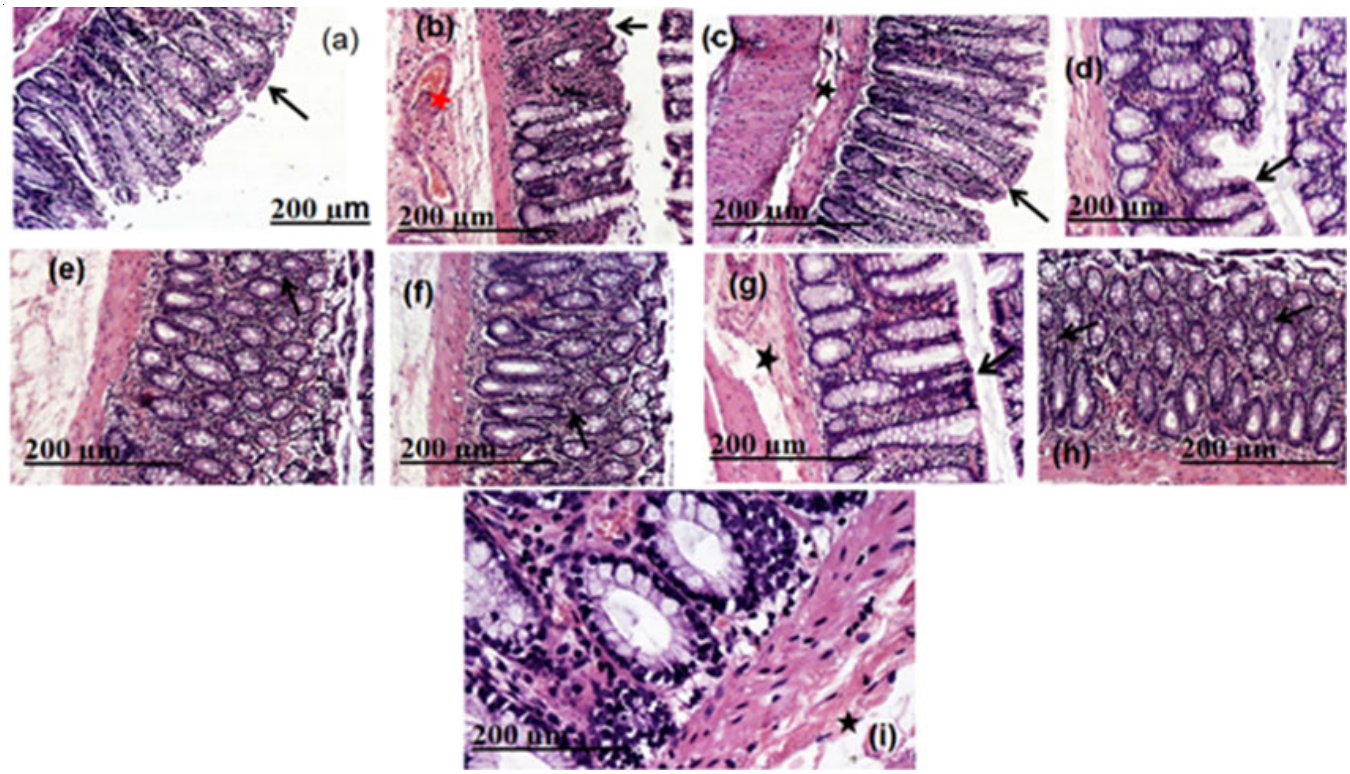


Fig. 5. Sections stained with hematoxylin and eosin (H&E; 200 X) displaying the regenerated glandular epithelium width in stomachs of rats treated with ibuprofen. Also, antiulcerogenic activity of compounds **1-3** on ibuprofen -Induced Ulceration in Rats. a, Negative control (2 mL saline); b, ibuprofen (50 mg/kg.b.w.); c, compound **1** (56.25 mg/kg. b.w.); d, compound **2** (41.75 mg/kg.b.w.); e, compound **3** (89.25 mg/kg. b.w.); f, compound **1** (56.25 mg/kg. b.w.) + ibuprofen (50 mg/kg.b.w.); g, compound **2** (41.75 mg/kg.b.w.) + ibuprofen (50 mg/kg.b.w.); h, compound **3** (89.25 mg/kg. b.w.) + ibuprofen (50 mg/kg.b.w.); i, Omeprazole (20 mg/kg. b.w.) + ibuprofen (50 mg/kg.b.w.)

5b). Also, sections of the ulcerated area revealed that there was a significant increase in regenerated glandular The glandular stomach retained its normal histological picture and no significant difference in capillary density in scar tissue was observe after treatment with compounds **1-3** as well as omeprazole at 56.25, 41.75, 89.25 and 20 mg/kg.b.w, respectively (Fig. 5f-i).

Table-6 showed the anti-inflammatory activity of compounds **1-3** at two different doses 1/40 and 1/20 LD_{50} as well as ibuprofen (30 mg/kg.b.w.) reference drug. The present data showed that 6% formalin solution injection induces edema within 5-8 min. However, the maximal swelling occurred approximately at 120 min. Compounds **1-3** at two different doses 1/40

TABLE-6
ANTI-INFLAMMATORY ACTIVITY OF THE SYNTHESIZED COMPOUNDS **1-3**

Groups	Treatment (mg/kg)	Formalin induced rat paw oedema thickness (mm)/min			
		30	60	90	120
I	Control positive	15.65 ± 0.15^{aA}	14.80 ± 0.39^{aA}	15.00 ± 0.29^{aA}	15.50 ± 0.25^{aA}
II	Compound **1** (28.15 mg/kg. b.w.)	9.80 ± 0.11 (37.38 %)^{bcA}	8.32 ± 0.12 (43.73%)^{cA}	6.10 ± 0.08 (59.33%)^{bBC}	4.50 ± 0.07 (70.96%)^{cC}
III	Compound **1** (56.25 mg/kg. b.w.)	8.54 ± 0.32 (45.43%)^{bcA}	7.51 ± 0.43 (49.25%)^{cA}	5.77 ± 0.22 (61.53%)^{bBC}	4.00 ± 0.21 (74.19%)^{cC}
IV	Compound **2** (20.88 mg/kg .b.w.)	10.74 ± 0.09 (31.37 %)^{bA}	7.10 ± 0.07 (52.02%)^{cB}	6.25 ± 0.12 (58.33%)^{bB}	5.11 ± 0.08 (67.03%)^{bcB}
V	Compound **2** (41.74 mg/kg. b.w.)	10.13 ± 0.27 (39.15 %)^{bcA}	6.30 ± 0.38 (57.43%)^{cA}	5.34 ± 0.25 (64.4%)^{bBC}	4.66 ± 0.36 (69.93%)^{cC}
VI	Compound **3** (44.63 mg/kg. b.w.)	13.50 ± 0.09 (13.73%)^{aA}	11.25 ± 0.17 (23.98%)^{bA}	8.90 ± 0.14 (40.66%)^{bB}	7.66 ± 0.09 (50.58%)^{bB}
VII	Compound **3** (89.25 mg/kg. b.w.)	10.56 ± 0.28 (32.52%)^{bA}	9.65 ± 0.43 (34.79%)^{bA}	7.32 ± 0.38 (51.2%)^{bB}	6.35 ± 0.32 (59.03%)^{bB}
VIII	Ibuprofen (30 mg/kg.b.w.)	7.25 ± 0.09 (53.67%)^{cA}	6.80 ± 0.069 (54.05%)^{cA}	6.56 ± 0.071 (56.26%)^{bA}	5.80 ± 0.0488 (62.58%)^{bcA}

#Ibuprofen is used as a reference. Data shown are mean ± standard deviation of number of observations within each treatment, n = 6. Data followed by the same small letters are not significantly different at $p \leq 0.05$ in the same column. Data followed by the same capital letters are not significantly different at $p \leq 0.05$ in the same row.

and 1/20 LD_{50} as well as ibuprofen produced a significant reduction ($p < 0.05$) the volume of edema and acute inammation of the rat hind paw (Table-4). The anti-inflammatory activity of compounds **1** and **2** are more pronounced than the activity of compound **3** and ibuprofen.

Histological examination of gastric mucosa sections of compounds **1-3** groups showed a decrease in gastric injuries and inflammation than that of ibuprofen-treated group. This effect may be due to the reason mentioned in the present study (normalization of gastric acidity parameters, ulcer index, Na^+/K^+-ATPase and protein levels and anti-inflammatory activity). All compounds preserved the structural integrity of the gastric mucosa membrane, with no signs of hemorrhage or apoptosis.

In the present study, the anti-inflammatory potency of compounds **1-3** were assayed in formalin treated rat model. The maximum effect was recorded for compounds **1** & **2** and followed by compound **3**. The presence of 5-Cl and 2-ethyl and 4-OH substitutions in compound **1** as well as 6-Cl, 3-NH_2 and 4-OH substitutions in compound 2 nucleus results in more anti-inflammatory activity than compound **3** [61]. Also, compounds **1** and **2** showed significant anti-inflammatory activity comparable to ibuprofen. Current result was confirmed with the results of Saxena *et al*. [62] who reported that some quinazolinones showed superior activity compared to standard ibuprofen.

Table-7 showed the antinociceptive activity of compounds **1-3** at two different doses 1/40 and 1/20 LD_{50} as well as aspirin (100 mg/kg.b.w.) reference drug. The compounds **1-3** at both doses as well as aspirin-induced a significant reduction in the number of contortions provoked by an i.p. injection of acetic acid in rats. The maximum reduction of the writhing response of compounds **1-3** was 68.21, 63.00 and 61.09 % at doses of 56.25, 41.75 and 89.25 mg/kg, respectively. Also, the positive control drug, aspirin (100 mg/kg) also provoked a significant protective effect (60.91%) against acetic acid-induced pain.

The present study suggested that good antinociceptive activity was shown by compounds **1** and **2** with ethyl, hydroxyl and Cl substitutions, due to an increase in lipophilicity [63]. The increase in activity was observed in compound **3** with N-3 substitution. The activity was retained when the heteroaryl group was placed at N-3 position [64]. Thus, these compounds may contribute to the antinociceptive and anti-inammatory activities reported here and the combination of these two properties could help to support the usefulness of the synthesized compounds in the treatment of arthritis.

The activity of ALT, AST and LDH enzymes as well as TNF-α, NO, TBARS levels in plasma and liver homogenates was increased significantly in LPS-treated rats (group **II**) ($p < 0.05$) when compared with a control group **I** (Tables 8 and 9). Treatment of normal animals in groups **III**-**V** with compounds **1-3** at doses of 56.25, 41.50 and 59.25 mg/kg/b.w.) was a non-significant change of these enzymes as well as TNF-α, NO, TBARS when compared with the control group **I**. However, Treatment of LPS-treated rats in groups **VI**-**VIII** with compounds **1-3** at doses of 56.25, 41.50 and 89.25 mg/kg.b.w.) was reduced significantly of these enzymes as well as TNF-α, NO, TBARS when compared with LPS-treated group **II**.

On the other hand, LPS (30 mg/kg), injection subcutaneously markedly decreased GPx, CAT and SOD activity as well as GSH levels ($p < 0.05$) indicating acute hepatotoxicity compared with saline-treated "normal" rats. The compounds **1-3** were shown to possess significant hepatoprotective activity in the LPS-induced liver toxicity in rats *via* normalization of the levels of normalizing these oxidative stress biomarkers. Also, oral administration of rats in groups **III**-**V** with compounds **1-3** was a non-significant change of GPx, CAT and SOD activity as well as GSH when compared with the control group **I**. Oral treatment with compounds **1-3** significantly increases in the activities of these liver oxidative stress biomarker enzymes ($p < 0.05$) compared with LPS-treated rats.

The hepatoprotective activity of prepared compounds is closely associated with their structures. The present results were confirmed with the results of $DPPH^{\bullet}$, $ABTS^{\bullet+}$, hydrogen peroxide and superoxide radicals scavenging activity of compounds **1-3**. However, compounds **1-3** may protect the hepatocytes against LPS- induced liver toxicity *via* scavenging free radicals produced by LPS administration. Also, the anti-inflammatory activity results of compounds **1-3** in the formalin treated rat model support the normalization of TNF-α and NO levels in LPS treated rats. The results indicated that compounds **1-3** could induce apoptosis *via* a mechanism that involves either extrinsic or intrinsic pathways [65].

Histopathology examination of rat liver tissue in different experimental groups showed normal organized hepatic cords, central veins and portal areas in control negative group **I** as well as compounds **1-3** treated groups **III-V** (Fig. 6a, c-e). Rats injected with LPS (30 mg/kg/b.w.) revealed histopathological changes including congestion of central vein with disorganized hepatic cords, apoptotic hepatocytes, moderate portal

TABLE-7
EFFECT OF THE SYNTHESIZED COMPOUNDS **1-3** ON ACETIC ACID INDUCED ABDOMINAL CONSTRICTION IN RATS

Groups	Treatment (mg/kg)		Number of writhings for 30 min	Inhibition (%)
I	Control		96.40 ± 4.77 [a]	–
II	Compound **1**	(28.15 mg/kg. b.w.)	37.25 ± 5.09[b]	(61.35%)
III		(56.25 mg/kg. b.w.)	30.65 ± 2.75[d]	(68.21%)
IV	Compound **2**	(20.88 mg/kg.b.w.)	35.47 ± 3.21[b]	(63.20%)
V		(41.75 mg/kg.b.w.)	32.77 ± 2.70[d]	(66.00%)
VI	Compound **3**	(44.63 mg/kg. b.w.)	42.80 ± 4.00[b]	(55.60%)
VII		(89.25 mg/kg. b.w.)	37.50 ± 3.15[c]	(61.09%)
VIII	Aspirin (100 mg/kg.b.w.)		38.00 ± 2.40[c]	(60.91%)

[#]Aspirin is used as a reference. Data shown are mean ± standard deviation of number of observations within each treatment, n = 6. Data followed by the same letter are not significantly different at $p \leq 0.05$.

TABLE-8
EFFECT OF THE SYNTHESIZED COMPOUNDS **1-3** ON OXIDATIVE STRESS BIOMARKERS IN PLASMA AND BLOOD

No.	Groups	ALT (U/L)	AST (U/L)	LDH (U/L)	GPx (U/mL)	CAT (U/g Hb)	SOD (U/g Hb)	GSH (mg/g Hb)	TBARS (nmol/mL)
I	Normal 1 mL normal saline orally	14.54 ± 1.35[a]	17.68 ± 2.34[a]	135.87 ± 9.43[a]	40.54 ± 3.65[a]	84.60 ± 6.09[ab]	175.00 ± 12.07[a]	27.60 ± 3.10[a]	3.05 ± 0.12[a]
II	Control (LPS 30 mg/kg.b.w.)	42.11 ± 3.65[d]	63.90 ± 4.25[d]	280.34 ± 9.76[e]	13.64 ± 1.44[d]	30.70 ± 2.25[f]	86.98 ± 4.70[e]	10.32 ± 1.40[c]	7.54 ± 0.66[d]
III	Compound **1** (56.25 mg/k.g.b.w.)	11.90 ± 0.55[a]	18.56 ± 1.57[a]	135.78 ± 12.80[a]	37.60 ± 2.90[a]	85.43 ± 3.25[a]	175.50 ± 8.70[a]	24.30 ± 1.77[a]	2.75 ± 0.30[a]
IV	Compound **2** (41.75 mg/k.g.b.w.)	13.00 ± 0.87[ac]	18.70 ± 2.11[a]	134.06 ± 11.20[a]	40.90 ± 4.06[a]	84.00 ± 3.98[a]	174.9 ± 7.64[a]	24.04 ± 1.65[a]	2.66 ± 0.11[a]
V	Compound **3** (89.25 mg/k.g.b.w.)	11.05 ± 1.76[a]	17.45 ± 2.90[a]	134.00 ± 9.50[a]	39.55 ± 2.90[a]	90.54 ± 4.98[b]	177.43 ± 10.54[a]	23.20 ± 1.05[a]	2.77 ± 0.32[a]
VI	Compound **1** (56.25 mg/k.g.b.w.) + LPS (10 mg/kg.b.w.)	15.87 ± 1.65[c]	23.76 ± 2.40[b]	162.80 ± 8.70[c]	31.60 ± 2.76[b]	68.25 ± 3.77[e]	159.83 ± 8.74[c]	22.61 ± 2.09[a]	3.42 ± 0.11[b]
VII	Compound **2** (41.75 mg/k.g.b.w.) + LPS (10 mg/kg.b.w.)	19.70 ± 2.05[b]	36.87 ± 3.87[c]	183.60 ± 13.65[d]	26.09 ± 3.80[c]	59.64 ± 4.05[d]	143.80 ± 12.36[d]	18.42 ± 1.55[b]	4.53 ± 0.32[c]
VIII	Compound **3** (89.25 mg/k.g.b.w.) + LPS (10 mg/kg.b.w.)	13.27 ± 1.53[ac]	20.87 ± 2.67[a]	152.76 ± 10.60[b]	35.65 ± 3.08[a]	77.40 ± 6.52[c]	164.90 ± 11.87[b]	23.44[a] ± 2.00	3.17 ± 0.24[a]

LPS was given i.p as a single dose of 30 mg/kg.b.w. to 18 h fasted animals. It was given to all groups except the normal one. Compounds **1-3** were orally given daily for 10 days. Blood samples were collected 24 h after the last dose administration. Values are given as mean ± SD for groups of eight animals each. Data followed by the same letter are not significantly different at $p \leq 0.05$.

TABLE-9
ACTIVITY OF GLUTATHIONE PEROXIDASE (GPx), CATALSE (CAT), SUPEROXIDE DISMUTASE (SOD) AS WELL AS LEVEL OF REDUCED GLUTATHIONE (GSH), TUMOR NECROSIS FACTOR-α (TNF-α), NITRIC OXIDE (NO) AND THIBARBATURIC ACID REACTIVE SUBSTANCES (TBARs) IN LIVER OF NORMAL AND EXPERIMENTAL GROUPS OF RATS

No.	Groups	GPx	CAT	SOD	GSH (mg/g tissue)	TNF-α (pg/g protein)	NO (Umol/g protein)	TBARS (nmol/g protein)
I	Normal 1 mL normal saline orally	8.75±0.37	40.54±3.78	15.47±1.08[@]	7.54±0.98	9.10±0.43	14.53±0.44	1.54±0.07
II	Control (LPS 10 mg/kg.b.w.)	2.63±0.22	14.65±1.43	4.32±0.08	2.33±0.25	27.65±2.80	39.43±4.25	3.54±0.42
III	Compound **1** (56.25 mg/k.g.b.w.)	6.56±0.25	42.80±3.10	11.00±0.54	5.22±0.61	5.74 ±0.43	9.00±1.08	1.71±0.14
IV	Compound **2** (41.75 mg/k.g.b.w.)	7.15 ±0.63	45.00±3.66	11.06±0.24	5.71±0.53	6.84±0.54	9.25±1.14	1.70±0.11
V	Compound **3** (89.25 mg/k.g.b.w.)	7.95±0.50	43.87±3.20	10.76±0.98	5.32±0.33	6.75±0.43	9.00±1.08	1.68±0.16
VI	Compound **1** (56.25 mg/k.g.b.w.) + LPS (10 mg/kg.b.w.)	5.74 ±0.47	38.68±4.00	9.70±0.79	5.00±0.48	11.35±0.76	11.76±1.08	1.73±0.08
VII	Compound **2** (41.75 mg/k.g.b.w.) + LPS (10 mg/kg.b.w.)	4.69±0.82	32.76±3.16	6.54±0.86	4.34±0.65	15.70±1.45	22.90±3.00	1.90±0.05
VIII	Compound **3** (89.25 mg/k.g.b.w.) + LPS (10 mg/kg.b.w.)	7.10±0.60	42.90±3.98	10.25±0.83	5.43±0.40	8.09±0.62	12.65±1.54	1.70±0.07

LPS was given i.p as a single dose of 10 mg/kg.b.w. to 18 h fasted animals. It was given to all groups except the normal one. Compounds (1-3) were orally given daily for 10 days. Liver samples were collected 24 h after the last dose administration. Data shown are mean ± standard deviation of number of observations within each treatment, n = 8. Data followed by the same letter are not significantly different at $p \leq 0.05$. SOD: one unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in 1min/mg protein; GPx: μg of GSH consumed/min mg protein; CAT: μmol of H_2O_2 utilized/min mg protein.

lymphocytic inflammation (red arrow) are seen in Fig. 6b. Liver sections of LPS-treated rats administrated with compounds **1-3** at doses of 57.50, 41.75 and 89.25 mg/kg.b.w.) showed apparent normal histological structures of hepatic tissue (Fig. 6f-h).

Histological examination of liver sections of compounds **1-3** groups showed a decrease in hepatocyte necrosis and inflammation than that of LPS-treated group. This effect may be due to the reason mentioned in the present study (free radical scavenging properties and anti-inflammatory activity). All compounds preserved the structural integrity of the hepatocellular membrane, with no signs of hemorrhage or apoptosis.

Table-10 showed that the levels of peritoneal macrophage cells (PMCs) and white blood cells (WBCs) per 1 mL of isolated peritoneal fluid. Also, the results exhibited the levels of tumor necrosis factor-α (TNF-α), nitric oxide (NO), prostaglandin E2 (PGE2) and cyclooxygenase (COX-2) enzyme in 1×10^6 peritoneal macrophage cells. The results showed that significant increase in PMCs, TNF-α, NO, PGE2 and COX-2 enzyme as well as a significant decrease in WBCs levels, in isolated peritoneal fluid treated with LPS (1 μg/mL) well plats (**II**) ($p < 0.05$) when compared to cells of the normal control well plats (**I**). The treatment of PMCs injected with LPS with both compounds **1-3** as well as ibuprofen (100 μg/mL) reduced the levels of PMCs, TNF-α, PGE2 and COX-2 as well as elevated WBCs level significantly ($p < 0.05$) compared with LPS-treated cells group **II**. The level of WBCs in peritoneal macrophage cells was decreased significantly in peritoneal macrophage cells (group **II**) injected with LPS (1 μg/mL) ($p < 0.01$) compared with the normal control group **I**. The activity of compounds **1**

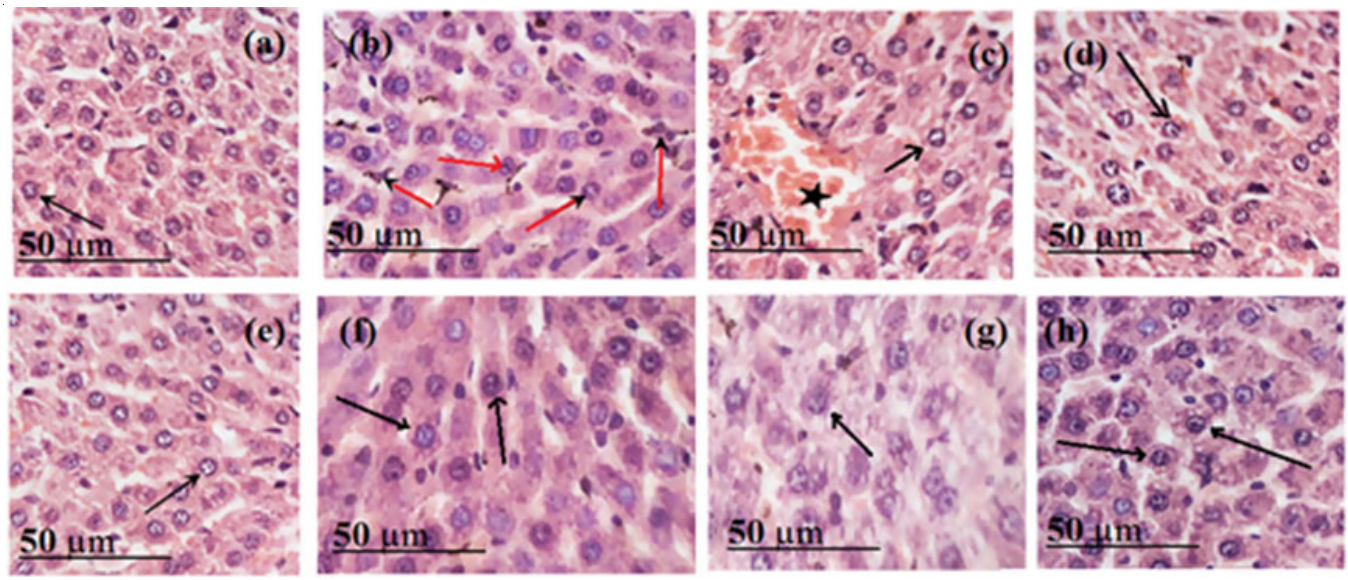


Fig. 6. Sections stained with hematoxylin and eosin (H&E; 50 X) histological examination of rats hepatocytes of different groups compared to control group (a); b, LPS (10 mg/kg.b.w.); c, compound **1** (57.50 mg/kg. b.w.)**;** d**,** compound **2** (41.75 mg/kg.b.w.); e, compound **3** (89.25 mg/kg. b.w.); f, compound **1** (57.50 mg/kg. b.w.) **+** LPS (10 mg/kg.b.w.)**;** g**,** compound **2** (41.75 mg/kg.b.w.) + LPS (10 mg/kg.b.w.) ; h, compound **3** (89.25 mg/kg. b.w.) + LPS (10 mg/kg.b.w.)

TABLE-10
LEVELS OF PERITONEAL MACROPHAGE CELLS (PMCs), WHITE BLOOD CELLS (WBCs), TUMOR NECROSIS FACTOR-α (TNF-α), NITRIC OXIDE (NO) AND PROSTAGLANDIN E2 (PGE2) AS WELL AS CYCLOOXYGENASE-2 (COX-2) ENZYME ACTIVITY OF NEGATIVE CONTROL AND TREATED WELL PLATES

No.	Well plates	Number of PMCs × 10^6	WBCs ×10^3	TNF-α (pg/mL PM)	NO (Umol/mL PM)	PGE2 (pg/mL PM) × 10^3	COX-2 (U/mL PM)
I	Negative control	1.70±0.14[a]	4.50±0.63[a]	6.10 ±0.48[a]	3.50±0.17[a]	0.60±0.32[a]	22.50±2.50[a]
II	Control (LPS 1 µg/mL)	4.20 ±0.50[c]	2.10 ±0.22[b]	18.90 ±2.08[c]	6.10 ±0.40[c]	2.50 ±0.35[c]	85.00±5.42[e]
III	Compound **1** (100 µg/L)	1.66 ±0.10[a]	4.40±0.60[a]	5.90 ±0.50[a]	3.70±0.19[a]	0.63±0.38[a]	20.86±1.58[a]
IV	Compound **2** (100 µg/mL)	1.73 ±0.15[a]	4.60±0.50[a]	6.00 ±0.35[a]	4.00±0.30[a]	0.55±0.28[a]	21.90±3.05[a]
V	Compound **3** (100 µg/L)	1.68±0.20[a]	4.45±0.60[a]	6.25 ±0.55[a]	3.80±0.22[a]	0.58±0.40[a]	20.65±1.87[a]
VI	Compound **1** (100 µg/L) + LPS (1 µg/mL)	2.10±0.25[b]	4.10 ±0.76	8.26 ±0.65[b]	3.20 ±0.40[a]	0.68 ±0.09[a]	32.54±3.25[b]
VII	Compound **2** (100 µg/mL) + LPS (1 µg/mL)	2.40±0.10	4.30 ±0.36[a]	8.10±0.40[b]	3.70±0.21[a]	0.71 ±0.08[a]	38.32 ±2.96[c]
VIII	Compound **3** (100 µg/L) + LPS (1 µg/mL)	1.95±0.35[a]	4.20 ±0.17[a]	10.80±0.96[b]	5.70±0.60[b]	1.20 ±0.16[b]	48.80±5.10[d]
VIIII	Ibuprofen (100 µg/mL) + LPS (1 µg/mL)	2.50 ±0.20[b]	4.00 ±0.35[a]	5.80±0.43[a]	3.80±0.28[a]	0.75 ±0.09[a]	29.80±3.66[b]

[#]Ibuprofen is used as a reference. LPS was added as a single dose of 1 µg/mL. It was added to all groups except the normal and III-IV groups. Data followed by the same letter are not significantly different at $p \leq 0.05$. Data shown are mean ± standard deviation of number of observations within each treatment, n = 3.

and **2** are more pronounced than the activity of compound **3** and ibuprofen. Hydrophobic (Cl), hydrogen bonding (OH, OCH_3) substituents (compound **1**); Cl, OH and free NH_2 (compound **2**) and (Cl, OH and ethoxy benzene) (compound **3**) showed a higher preference towards COX-2. The effect was a selective inhibition of COX-2.

Present results agreed with Wahby *et al.* [66] who reported that LPS caused elevation of the levels of inflammatory mediators in peritoneal macrophages. The present data showed that the treatment of peritoneal macrophages cells with compounds **1-3** led to normalizing the levels of PMCs, WBCs, TNF-α, NO and PGE2 and COX-2.

Molecular docking: A molecular docking study was carried out to predict the possible binding mode of the newly synthesized compounds **1-3** with COX-2 enzyme and to study their interaction with the enzyme hot spots (key amino acids) to explain their good enzyme inhibitory activity and analgesic and anti-inflammatory activities.

The docking protocol was initiated by a validation step involving the redocking of the co-crystallized ligand into the binding site. Fig. 7 revealed binding of compounds **1** through its carbonyl group of ethoxy moiety by hydrogen bonding interactions with both Tyr356 and Arg121 amino acids, with bond length 2.60 and 2.61 Å, respectively, as well as docking score (S) = -13.68 kcal/mol, in addition to the same interaction pattern of the docked pose to that of the co-crystal-lized ligand within the binding site. Also, compound **2** binds with COX-2 enzyme through its carbonyl group of amino-quinazolin-4-one moiety by hydrogen bonding interactions with both Tyr356 and Arg121 amino acids, with bond length 3.40 and 2.48 Å, respectively, as well as docking score (S) = -13.06 kcal/mol (Fig. 8).

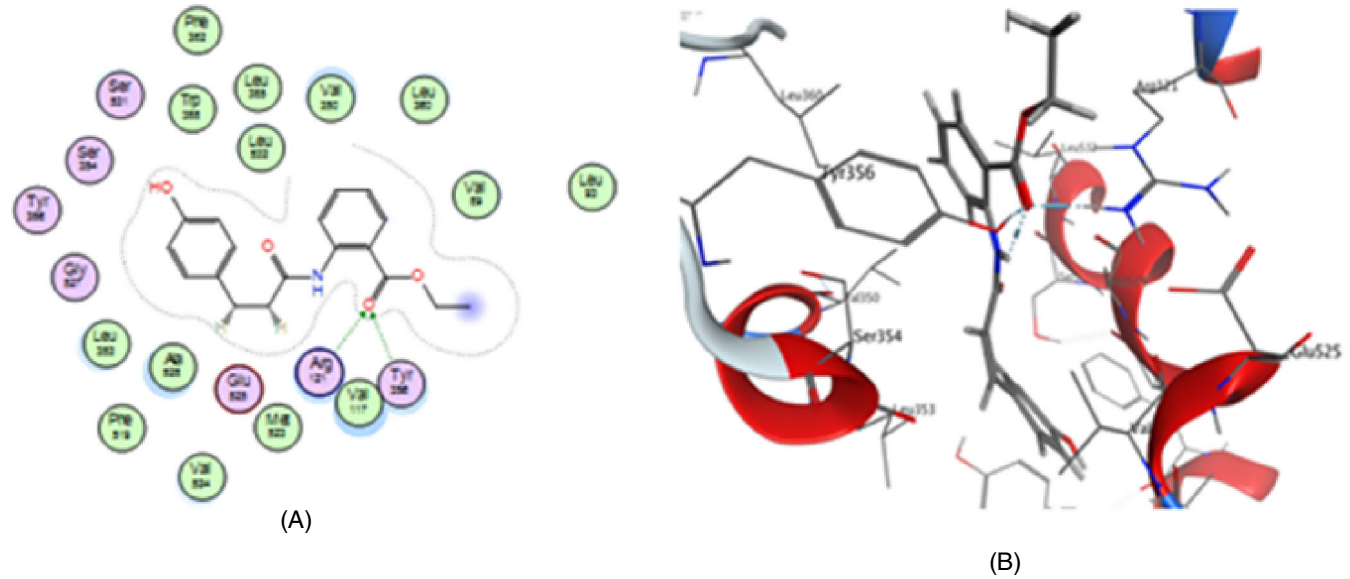


Fig. 7. 2D diagram (A) and 3D representation in (B) of compound **1** in the COX-2 binding site

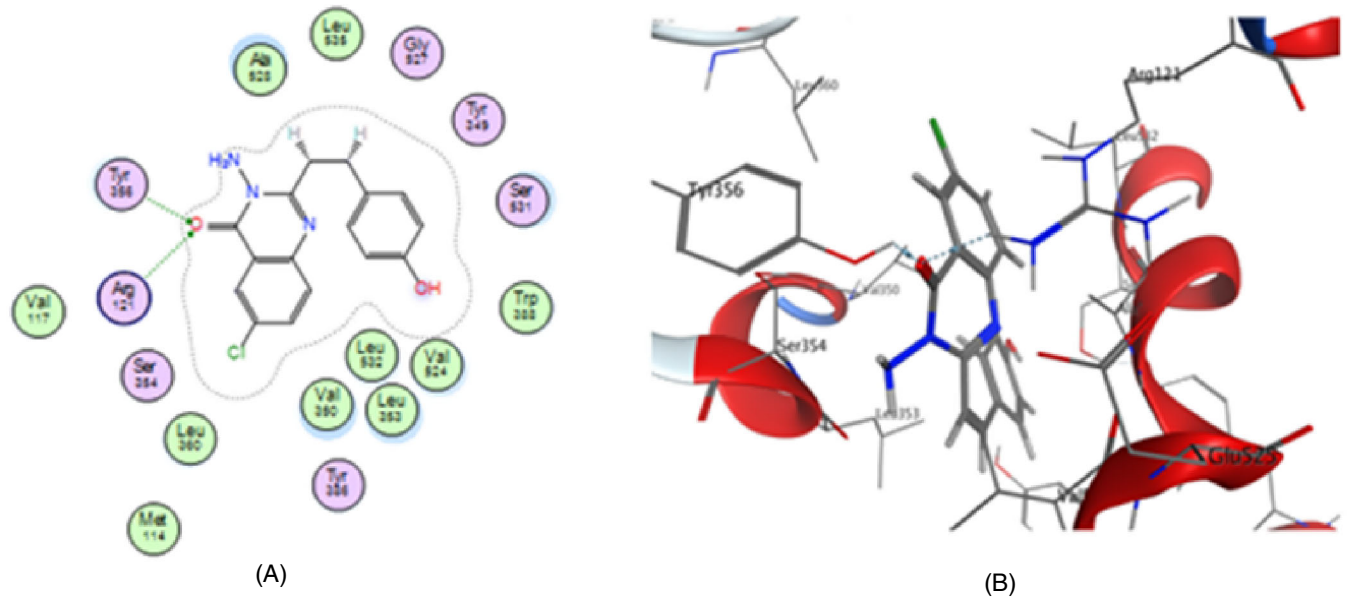


Fig. 8. 2D diagram (A) and 3D representation (B) of compound **2** in the COX-2 binding site

However, compounds **3** binds with COX-2 enzyme through its carbonyl group of aminoquinazolin-4-one and hydroxyl of phenoxy moieties by hydrogen bonding interactions with Arg121 and phe519 amino acids, with bond length 3.32 and 4.09 Å, respectively, as well as docking score (S) = -14.54 kcal/mol (Fig. 9).

Present data (Table-11) suggested that the presence of free (C=O) and (OH) increases in polarity, H-bonding and basicity of compounds structure and promote compound **1** to bind to COX-2 at the hydrophilic groups of Arg121 & Tyr356; compound **2** (Arg121 & Tyr356); compound **3** (Arg121 & phe519) at the entrance of the cyclooxygenase channel.

TABLE-11
DOCKING ENERGY SCORES (S) IN kcal/mol FOR THE REFERENCE AND SYNTHESIZED COMPOUNDS **1-3**

Compounds	Docking score (S) (kcal/mol)	Interaction	Distance (Å)	E (kcal/mol)
Compound **1**	-13.68	H-accept. Arg 121 – C=O (ethoxy).	2.60	-2.8
		H-accept. Tyr356 – C=O (ethoxy).	2.61	-1.0
Compound **2**	-13.06	H-accept. Arg 121 – C=O (aminoquinazolin-4-one)	3.40	-1.1
		H-accept. Tyr356 – C=O (aminoquinazolin-4-one)	2.48	-1.2
Compound 3**1**	-14.54	H-accept. Arg 121 – C=O (aminoquinazolin-4-one)	3.32	-1.2
		H-pi. phe 519 – OH. (phenoxy)	4.09	-1.3

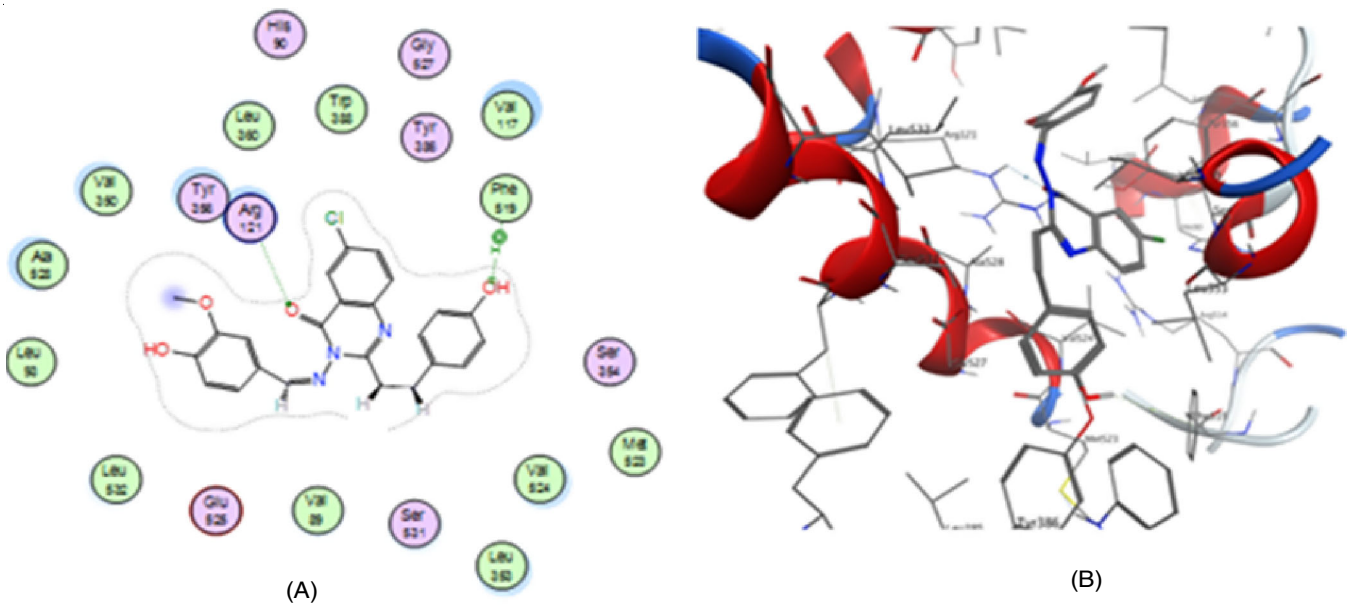


Fig. 9. 2D diagram (A) and 3D representation (B) of compound **3** in COX-2 binding site

The free carboxylic acid group found in ibuprofen forms critical interactions with residues Arg-120, Glu-524 and Tyr-355 within the cyclooxygenase active site [67]. The masking of the ibuprofen-free carboxylic group seems to be principally the basis of this reduced topical irritant action [68]. According to the molecular docking data mentioned in the present study, we suggest that anti-COX-2 properties of compounds **1-3** are close with ibuprofen.

Conclusion

In this study, the synthesis and characterization of the new quinazoline and quinazoline-3-one derivatives (**1-3**) are reported. The new chemical structure of the synthesized compounds was proved by elemental analysis and spectral data. The new compounds exhibited high LD_{50} value, promising antioxidant, anti-inflammatory and protective activity on the liver as well as gastric mucosa tissues *via* inhibition of the production of pro-inflammatory mediators, including TNF-α, NO and PGE2, COX-2 in rats and peritoneal macrophage cells. The molecular docking data suggested that the most active compounds **1** and **2** can be positioned within the active sites of COX-2 at Arg121 and Tyr356 similarly to ibuprofen (Arg-120, Glu-524 and Tyr-355). Compound **3**–COX-2 complex generated by docking, revealed intricate interactions with a COX-2 channel, including hydrogen bonds with key residues Arg121 and phe519. More studies are needed to prove their medicinal importance, which may pave the way for possible therapeutic applications.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. D. Tripathi, Non-steroidal Anti-inflammatory Drugs and Anti-pyretic Analgesics. In: Essentials of Medical Pharmacology, Jaypee Brothers, New Delhi, edn 5, p. 176 (2003).
2. B.J. Orlando, D.R. McDougle, M.J. Lucido, E.T. Eng, L.A. Graham, C. Schneider, D.L. Stokes, A. Das and M.G. Malkowski, *Arch. Biochem. Biophys.*, **546**, 33 (2014); https://doi.org/10.1016/j.abb.2014.01.026
3. A.L. Blobaum and L.J. Marnett, *J. Med. Chem.*, **50**, 1425 (2007); https://doi.org/10.1021/jm0613166
4. J.J. Prusakiewicz, K.C. Duggan, C.A. Rouzer and L.J. Marnett, *Biochemistry*, **48**, 7353 (2009); https://doi.org/10.1021/bi900999z
5. W.L. Smith, Y. Urade and P.J. Jakobsson, *Chem. Rev.*, **111**, 5821 (2011); https://doi.org/10.1021/cr2002992
6. K.C. Duggan, D.J. Hermanson, J. Musee, J.J. Prusakiewicz, J.L. Scheib, B.D. Carter, S. Banerjee, J.A. Oates and L.J. Marnett, *Nat. Chem. Biol.*, **7**, 803 (2011); https://doi.org/10.1038/nchembio.663
7. M.A. Hussein, *Med. Chem. Res.*, **22**, 4641 (2013); https://doi.org/10.1007/s00044-013-0468-9
8. M.N. Noolvi, H.M. Patel, V. Bhardwaj and A. Chauhan, *Eur. J. Med. Chem.*, **46**, 2327 (2011); https://doi.org/10.1016/j.ejmech.2011.03.015
9. W. Dohle, F.L. Jourdan, G. Menchon, A.E. Prota, P.A. Foster, P. Mannion, E. Hamel, M.P. Thomas, P.G. Kasprzyk, E. Ferrandis, M.O. Steinmetz, M.P. Leese and B.V.L. Potter, *J. Med. Chem.*, **61**, 1031 (2018); https://doi.org/10.1021/acs.jmedchem.7b01474
10. A.A.-M. Abdel-Aziz, L.A. Abou-Zeid, K.E.H. ElTahir, R.R. Ayyad, M.A.-A. El-Sayed and A.S. El-Azab, *Eur. J. Med. Chem.*, **121**, 410 (2016); https://doi.org/10.1016/j.ejmech.2016.05.066
11. L. Yang, S. Ge, J. Huang and X. Bao, *Mol. Divers.*, **22**, 71 (2018); https://doi.org/10.1007/s11030-017-9792-1
12. R. Al-Salahi, H.A. Abuelizz, H.A. Ghabbour, R. El-Dib and M. Marzouk, *Chem. Cent. J.*, **10**, 21 (2016); https://doi.org/10.1186/s13065-016-0168-x
13. V. Alagarsamy, K. Chitra, G. Saravanan, V.R. Solomon, M.T. Sulthana and B. Narendha, *Eur. J. Med. Chem.*, **151**, 628 (2018); https://doi.org/10.1016/j.ejmech.2018.03.076
14. M.A. Hussein, *Med. Chem. Res.*, **21**, 1876 (2012); https://doi.org/10.1007/s00044-011-9707-0
15. M.A. Hussein, R.F. Omar and H.S. Farghaly, *Int. J. Acad. Res.*, **3**, 454 (2011).
16. K.P. Rakesh, C.S. Shantharam and H.M. Manukumar, *Bioorg. Chem.*, **68**, 1 (2016); https://doi.org/10.1016/j.bioorg.2016.07.001

17. K.P. Rakesh, H.M. Manukumar and D.C. Gowda, *Bioorg. Med. Chem.*, **25**, 1072 (2015); https://doi.org/10.1016/j.bmcl.2015.01.010
18. F. Koopman, J. Beekwilder, B. Crimi, A. van Houwelingen, R.D. Hall, D. Bosch, A.J.A. van Maris, J.T. Pronk and J.-M. Daran, *Microb. Cell Fact.*, **11**, 155 (2012); https://doi.org/10.1186/1475-2859-11-155
19. N. Tamilarasan and U. Rajangam, *J. Biosci. Med.*, **4**, 85 (2016); https://doi.org/10.4236/jbm.2016.41010
20. B.G. Auner, C. Valenta and J. Hadgraft, *J. Control. Release*, **89**, 321 (2003); https://doi.org/10.1016/S0168-3659(03)00124-X
21. C.H. Wu, Y.S. Ho, C.Y. Tsai, J. Wang, H. Tseng, L. Wei, C.H. Lee, R.S. Liu and S.Y. Lin, *Int. J. Cancer*, **124**, 2210 (2009); https://doi.org/10.1002/ijc.24189
22. X. He and R.H. Liu, *J. Agric. Food Chem.*, **55**, 4366 (2007); https://doi.org/10.1021/jf063563o
23. M.A. Hussein, *Int. J. Org. Bioorg. Chem.*, **1**, 12 (2011).
24. Y. Hamauzu, H. Yasui, T. Inno, C. Kume and M. Omanyuda, *J. Agric. Food Chem.*, **53**, 928 (2005); https://doi.org/10.1021/jf0494635
25. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, *Free Radic. Biol. Med.*, **26**, 1231 (1999); https://doi.org/10.1016/S0891-5849(98)00315-3
26. G.K. Jayaprakasha, L. Jaganmohan Rao and K.K. Sakariah, *Bioorg. Med. Chem.*, **12**, 5141 (2004); https://doi.org/10.1016/j.bmc.2004.07.028
27. E. Kunchandy and M.N.A. Rao, *Int. J. Pharm.*, **58**, 237 (1990); https://doi.org/10.1016/0378-5173(90)90201-E
28. D.J. Finney, Statistical Methods in Biological Assay, Charles Griffen & Company Ltd., London (1964).
29. S. Santhosh, R. Anandan, T.K. Sini and P.T. Mathew, *J. Gastroenterol. Hepatol.*, **22**, 949 (2007); https://doi.org/10.1111/j.1440-1746.2007.04840.x
30. P.A. Nwafor, F.K. Okwuasaba and L.G. Binda, *J. Ethnopharmacol.*, **72**, 421 (2000); https://doi.org/10.1016/S0378-8741(00)00261-0
31. F.A.S. Alasmary, A.S. Awaad, A.M. Alafeefy, R.M. El-Meligy and S.I. Alqasoumi, *Saudi Pharm. J.*, **26**, 183 (2018); https://doi.org/10.1016/j.jsps.2017.09.011
32. A.P. Kulkarni, R.S. Policegoudra and S.M. Aradhya, *J. Food Biochem.*, **31**, 399 (2007); https://doi.org/10.1111/j.1745-4514.2007.00122.x
33. O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951); https://doi.org/10.1016/S0021-9258(19)52451-6
34. S.L. Bonting, eds.: E.E. Bittar, Sodium Potassium Activated Adenosine Triphosphatase and Carbon Transport, In: Membrane and Iron Transport, Wiley-Interscience: London, vol. 1, pp. 257-363 (1970).
35. R. Domenjoz, *Schweiz. Med. Wochenschr.*, **82**, 1023 (1952).
36. C. Sudhakaran, *J. Clin. Diagn. Res.*, **12**, FC05 (2018).
37. R. Koster, N. Anderson and E.J. Debber, *Fed. Proc.*, **18**, 412 (1959).
38. W. Reanmongkol, S. Subhadhirasakul, J. Kongsang, M. Tanchong and J. Kitti, *Pharm. Biol.*, **38**, 68 (2000); https://doi.org/10.1076/1388-0209(200001)3811-BFT068
39. D. Paglia and W. Valentine, *J. Lab. Clin. Med.*, **70**, 158 (1967).
40. A.K. Sinha, *Ann. Biochem.*, **47**, 389 (1972); https://doi.org/10.1016/0003-2697(72)90132-7
41. S. Marklund and D. Marklund, *Eur. J. Biochem.*, **47**, 469 (1974); https://doi.org/10.1111/j.1432-1033.1974.tb03714.x
42. J. Sedlak and R.H. Lindsay, *Anal. Biochem.*, **25**, 192 (1968); https://doi.org/10.1016/0003-2697(68)90092-4
43. E. Van Kampen and W. Zijlstra, *Clin. Chim. Acta*, **6**, 538 (1961); https://doi.org/10.1016/0009-8981(61)90145-0
44. S. Reitman and S.A. Frankel, *Am. J. Clin. Pathol.*, **28**, 56 (1957); https://doi.org/10.1093/ajcp/28.1.56
45. J. King, The Dehydrogenase or Oxidoreductatse, Lactate Dehydrogenase, In: Practical Clinical Enzymology, Nostrand Co.: London, pp. 106-115 (1965).
46. R. Beyaert and W. Fiers, eds.: R. Mire-Sluis and R. Thorpe, Tumor Necrosis Factor and Lymphotoxin, In: Cytokines, Academic Press: San Diego, pp. 335-360 (1998).
47. K.M. Miranda, M.G. Espey and D.A. Wink, *Nitric Oxide*, **5**, 62 (2001); https://doi.org/10.1006/niox.2000.0319
48. W.G. Niehaus and B. Samuelsson, *Eur. J. Biochem.*, **6**, 126 (1968); https://doi.org/10.1111/j.1432-1033.1968.tb00428.x
49. G.D. Bancroft and A. Steven, Theory and Practice of Histological Technique, Churchill Livingstone: New York, edn 4, pp. 99-112 (1983).
50. E. Kolaczkowska, B. Arnold and G. Opdenakker, *Immunobiology*, **213**, 109 (2008); https://doi.org/10.1016/j.imbio.2007.07.005
51. H. Chu, Q. Tang, H. Huang, W. Hao and X. Wei, *Environ. Toxicol. Pharmacol.*, **41**, 159 (2016); https://doi.org/10.1016/j.etap.2015.11.018
52. S. Nakatsugi, N. Sugimoto and M. Furukawa, *Prostaglandins Leukot. Essent. Fatty Acids*, **55**, 451 (1996); https://doi.org/10.1016/S0952-3278(96)90130-1
53. S.R. Campelo, M.B. da Silva, J.L.F. Vieira, J.P. da Silva and C.G. Salgado, *BMC Res. Notes*, **4**, 24 (2011); https://doi.org/10.1186/1756-0500-4-24
54. M.-Z. Zhang, B. Yao, Y. Wang, S. Yang, S. Wang, X. Fan and R.C. Harris, *J. Clin. Invest.*, **125**, 4281 (2015); https://doi.org/10.1172/JCI81550
55. http://www.rcsb.org/.
56. B.J. Orlando, M.J. Lucido and M.G. Malkowski, *J. Struct. Biol.*, **189**, 62 (2015); https://doi.org/10.1016/j.jsb.2014.11.005
57. M.A. Hussein and M.O. Samir, *IJPI's J. Med. Chem.*, **2**, 7 (2010).
58. V. Alagarsamy, K. Chitra, G. Saravanan, V.R. Solomon, M.T. Sulthana and B. Narendhar, *Eur. J. Med. Chem.*, **151**, 628 (2018); https://doi.org/10.1016/j.ejmech.2018.03.076
59. J.E. Hilaly, Z.H. Israili and B. Lyoussi, *J. Ethnopharmacol.*, **91**, 43 (2004); https://doi.org/10.1016/j.jep.2003.11.009
60. F.L. Lanza, D. Fakouhi and A. Rubin, *Am. J. Gastroenterol.*, **84**, 633 (1989).
61. K. Bothara, S. Kadam and V. Shivram, *Indian Drugs*, **35**, 372 (1998).
62. S. Saxena, M. Verma and A.K. Saxena, *Indian J. Chem.*, **30B**, 453 (1991).
63. V. Alagarsamy, G. Murugananthan and R. Venkateshperumal, *Biol. Pharm. Bull.*, **26**, 1711 (2003); https://doi.org/10.1248/bpb.26.1711
64. V. Alagarsamy, S. Revathi and R. Kalaiselvi, S. Phuvaneshwari, R. Revathi, S. Amuthalakshmi, S. Vijayakumar, S.M. Sivakumar, T. Angayarkanni, M. Sarathadevi, S. Saravanakumar, A. Thangathiruppathy, R. Venkatnarayanan and R. Venkatesaperumal, *Indian J. Pharm. Sci.*, **65**, 534 (2003).
65. M. Zahedifard, F. Lafta Faraj, M. Paydar, C. Yeng Looi, M. Hajrezaei, M. Hasanpourghadi, B. Kamalidehghan, N. Abdul Majid, H. Mohd Ali and M. Ameen Abdulla, *Sci. Rep.*, **5**, 11544 (2015); https://doi.org/10.1038/srep11544
66. M.W. Wahby, G. Yacout, K. Kamal and D. Awad, *Beni-Suef J. Basic Appl. Sci.*, **4**, 291 (2015); https://doi.org/10.1016/j.bjbas.2015.11.004
67. V. Limongelli, M. Bonomi, L. Marinelli, F.L. Gervasio, A. Cavalli, E. Novellino and M. Parrinello, *Proc. Natl. Acad. Sci. USA*, **107**, 5411 (2010); https://doi.org/10.1073/pnas.0913377107
68. O. Unsal-Tan, K. Ozadali, K. Piskin and A. Balkan, *Eur. J. Med. Chem.*, **57**, 59 (2012); https://doi.org/10.1016/j.ejmech.2012.08.046