Design, Synthesis, and Docking Studies of Novel Diarylpyrazoline and Diarylisoxazoline Derivatives of Expected Anti-inflammatory, and Analgesic Activities

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Abstract: Two series of novel non acidic 3, 5-diarylpyrazoline and 3, 5-diarylisoxazoline derivatives were designed to be synthesized and screened for anti-inflammatory and analgesic activities. In addition, molecular modelling and docking of the designed compounds into cyclooxygenase II (COX-II) using Molsoft ICM 3.4-8C program was performed in order to predict the affinity and orientation of the designed compounds at the active site compared with its binding inhibitor celecoxib. The ICM score values show good agreement with predicted binding affinities, where all the designed compounds exhibit ICM score values (range from -88.89 to -70.40) less than celecoxib (-60.71) revealing higher binding affinity with the enzyme. Accordingly, synthesis of the designed compounds *via* reaction of various propenone derivatives with hydrazine hydrate, phenyl hydrazine or hydroxylamine hydrochlorides were carried out. Evaluation of their activity as anti-inflammatory and analgesics using dextran-induced rat paw edema, formaldehyde arthritis test and paw pressure test, respectively and their ability to induce gastric toxicity was also estimated. All the synthesized compounds exhibited significant activity as anti-inflammatory and analgesic, where compounds **2** and **8** were the most active as anti-inflammatory in dextran-induced rat paw edema, while compounds **7** and **10** were the most active as anti-inflammatory in formaldehyde-induced arthritis rat paw edema test. All compounds showed analgesic activity with the most potent compounds were **3**, and **10** were the most active. No one of the tested compounds cause gastric toxicity. We can conclude that the synthesized compounds proved a successful hit and seem potentially attractive as anti-inflammatory and analgesic agents.
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1. Introduction:

Non steroidal anti-inflammatory drugs (NSAIDs) are the most important class of widely used therapeutics for the treatment of inflammation and pain. The principle pharmacological effects of NSAIDs arise from their inhibition of cyclooxygenases (COXs). Cyclooxygenases control the complex conversion of arachidonic acid to prostaglandins and thromboxanes, which trigger as autocrine and paracrine chemical messengers many physiological and pathophysiological responses [1-3]. The discovery of a second isoform of cyclooxygenase namely COX-II has opened a new line of research based on the assumption that pathological prostaglandins (PGs) are produced by the inducible isoform COX-II while physiological prostaglandins are produced by constitutive isoform COX-I [4]. These physiological protective PGs preserve the integrity of the stomach lining and maintain normal renal function in compromised kidney [5]. The separation of therapeutic effects from the side effects has been a major challenge in the design and synthesis of these drugs. A common structural feature of many selective COX-II inhibitors is the presence of two vicinal aryl rings attached to a central five member heterocyclic moieties [6]. Also, most of the side effects of (NSAIDs) are mainly due to inhibition of both isomers COX-I and COX-II, yet they may also relate to their acidic characters due to the presence of free carboxylic acid moiety [7]. Decreasing acidity or producing non acidic derivatives will solve a part of this problem. Moreover 2- pyrazoline derivatives have been reported to exhibit various pharmacological activities as antimicrobial [8-10] and anti-inflammatory [11-13]. Promoted with the above mentioned studies, it is intended in the present work to investigate the synthesis of novel non acidic 3,5-diarylpyrazoline or 3,5-diarylisoxazoline derivatives adopting facile synthetic approach and utilizing easily accessible starting materials. On the other hand computer docking technique plays an important role in the drug design as well as in the mechanistic study by placing a molecule into the binding site of the target macromolecule in a non-covalent fashion [14].

Molsoft [15] as flexible docking program enable us to predict favourable protein-ligand complex structures with reasonable accuracy and speed. The docking technique will undoubtedly continue to play an important role in drug discovery [16]. So, we docked the designed compounds into cycloxygenase II (COX-II) [17] active site in order to predict their binding modes, their binding affinities and orientation of the designed compounds at the active site of the cycloxygenase II enzyme.

2. Experimental protocols

2.1. General remarks:

All chemicals were obtained from Aldrich (Stenheim, Germany) or Merck chemical Co. (Darmstadt, Germany). Melting points were determined on electrothermal Griffin apparatus (London, UK) and are uncorrected. Microanalysis was carried out at the microanalytical centre, Cairo University, and is within ± 0.4% unless otherwise stated. IR spectra were determined using potassium bromide discs on Shimadzu IR-435 spectrometer (Kyoto, Japan). 1HNMR spectra were made on Joel NMR Varian Gemini 200 MHz spectrometer (Joel, Tokyo, Japan). Chemical shifts (δ) are given in parts per million (ppm) down field from TMS as the internal standard. Mass spectra were recorded on Hewlett Packard 5988 spectrometer at 70 eV (Hewlett-Packard, Palo Alto, CA, USA).

2.2. Chemistry

2.2.1. General procedure for preparation of 5-aryl- 3-(4-bromophenyl)-4,5-dihydro-*1H*-pyrazole **2** and **3**.

A mixture of appropriate propenone **1a-d** (10.0 mmol) and hydrazine monohydrate (99%) (1.0 g, 1.0 mL, 20.0 mmol) was heated at reflux for 6 hr in absolute ethanol (50 mL). The solution was left to cool at room temperature and the solid formed was filtered off, washed with water, dried and crystallized from absolute ethanol.

2.2.1.1. 3-(4-Bromophenyl)-4,5-dihydro-5-(4-methoxyphenyl)-*1H*-pyrazole **2**.

Yield: 67%; m.p: 256-257°C. IR (cm^{-1}): 3220 (NH). ^{1}H-NMR (DMSO-d_6, D_2O) δ ppm: 3.57 (s,3H,OCH_3) , 4.75 (t,1H,CH-NH), 4.98 (d,2H,CH_2), 8.50 (s,1H,NH exch.), 7.21-7.87 (m,8H,Ar). MS: m/z 330 [M^+], 332 [M^++2]. Anal.Calcd. for $C_{16}H_{15}BrN_2O$ (331.21): C, 58.02; H, 4.56; N, 8.46. Found: C, 57.79; H, 4.49; N, 8.81.

2.2.1.2. 3-(4-Bromophenyl)-4,5-dihydro-5-(4-methylphenyl)-*1H*-pyrazole **3**.

Yield: 60%; m.p: 274-276°C. IR (cm^{-1}): 3300 (NH). ^{1}H-NMR (DMSO-d_6, D_2O) δ ppm: 2.35(s,3H,CH_3) , 4.50 (t,1H,CH-NH), 4.90 (d,2H,CH_2), 5.37(s,1H,NH exch.), 7.17-7.51 (m,8H,Ar). MS: m/z 314 [M^+], 316 [M^++2]. Anal.Calcd. for $C_{16}H_{15}BrN_2$ (315.21): C, 60.97; H, 4.76; N, 8.88. Found: C, 60.76; H, 4.60; N, 8.61.

2.2.2. General procedure for preparation of 5-aryl-3-(4-bromophenyl)-1-phenyl-4,5-dihydro-1-phenyl-*1H*-pyrazole **4-6**.

A mixture of appropriate propenons **1a-d** (10.0 mmol) and phenyl hydrazine (1.08 g, 1.0 mL, 10.0 mmol) was heated at reflux for 8 hr in acetic acid. The solution was then cooled and ice was added .the solid formed was filtered off, washed with water and crystallized from ethanol.

2.2.2.1. 3-(4-Bromophenyl)-4,5-dihydro-5-(4-methoxyphenyl)-1-phenyl-*1H*-pyrazole **4**.

Yield: 57%; m.p: 250-252°C. ^{1}H-NMR (DMSO-d_6) δ ppm: 3.45 (s,3H,OCH_3), 3.85 (t,1H,CH-N), 4.75 (d,2H,CH_2), 6.40-7.50 (m,13H,Ar). Anal.Calcd. for $C_{22}H_{19}BrN_2O$ (407.30): C, 64.87; H, 4.70; N, 6.88. Found: C, 64.70; H, 4.52; N, 6.60.

2.2.2.2. 3-(4-Bromophenyl)-4,5-dihydro-5-(2-hydroxyphenyl)-1-phenyl-*1H*-pyrazole **5**.

Yield: 68%; m.p: 130-132°C. IR (cm^{-1}): 3450 (OH). ^{1}H-NMR (DMSO-d_6, D_2O) δ ppm: 4.55 (t,1H,CH-N), 4.65 (d,2H,CH_2), 6.67-7.76 (m,13H,Ar), 8.80 (s,1H,OH exch.). Anal.Calcd. for $C_{21}H_{17}BrN_2O$ (392.8): C, 64.13; H, 4.36; N, 7.12. Found: C, 64.00; H, 4.20; N, 7.01.

2.2.2.3. 3-(4-Bromophenyl)-4,5-dihydro-5-(4-methylphenyl)-1-phenyl-*1H*-pyrazole **6**.

Yield: 75%; m.p: 260-261°C. ^{1}H-NMR (DMSO-d_6) δ ppm: 2.49 (s,3H,CH_3), 3.88 (t,1H,CH-N), 4.85 (d,2H,CH_2), 6.67-7.53 (m,13H,Ar). Anal.Calcd. for $C_{22}H_{19}BrN_2$ (391.3): C, 67.53; H, 4.89; N, 7.16. Found: C, 67.60; H, 4.70; N, 6.92.

2.2.3. General procedure for preparation of 5-aryl- 3-(4-bromophenyl)-4,5-dihydroisoxazole **7-10**.

A mixture of appropriate propenone **1a-d** (10 mmol), hydroxylamine hydrochloride (0.7 g, 10 mmol) and sodium hydroxide (1.0 g) was refluxed in ethanol (50 mL) for 5 hr. The mixture then cooled and solution of diluted ammonium hydroxide was then added drop wise till complete precipitation. The precipitate is filtered and crystallized from ethanol.

2.2.3.1. 3-(4-Bromophenyl)-5-(4-chlorophenyl)-4,5-dihydroisoxazole **7**.

Yield: 74%; m.p: 295-297°C. ^{1}H-NMR (DMSO-d_6) δ ppm: 4.62 (t,1H,CH-O), 4.88 (d,2H,CH_2), 7.16-7.52 (m,8H,Ar). Anal.Calcd. for $C_{15}H_{11}BrClNO$ (336.61): C, 53.52; H, 3.29; N, 4.16. Found: C, 53.70; H, 3.24; N, 4.30.

2.2.3.2. 3-(4-Bromophenyl)-4,5-dihydro-5-(4-methoxyphenyl) isoxazole **8**.

Yield: 81%; m.p: 293-295°C. ^{1}H-NMR (DMSO-d_6) δ ppm: 3.45 (s,3H,OCH_3), 3.85 (t,1H,CH-O), 4.75 (d,2H,CH_2), 7.23-7.80 (m,8H,Ar). MS: m/z 332 [M^+], 333 [M^++2]. Anal.Calcd. for $C_{16}H_{14}BrNO_2$ (332.19):

C, 57.85; H, 4.25; N, 4.22. Found: C, 58.19; H, 4.38; N, 4.00.

2.2.3.3. 3-(4-Bromophenyl)-4,5-dihydro-5-(4-methylphenyl)- isoxazole **9**.

Yield: 64%; m.p: 218-220°C. ^{1}H-NMR (DMSO-d_6) δ ppm: 2.50 (s,3H,CH_3), 4.40 (t,1H,CH-O), 4.60 (d,2H,CH_2), 7.10-7.46 (m,8H,Ar). MS: m/z 316 [M^+], 318[M^++2]. Anal.Calcd. for $C_{16}H_{14}BrNO$ (316.19): C, 60.78; H, 4.46; N, 4.43. Found: C, 61.14; H, 4.46; N, 4.70.

2.2.3.4. 3-(4-Bromophenyl)-4,5-dihydro-5-(2-hydroxyphenyl)- isoxazole **10**.

Yield: 82%; m.p: 320°C. IR (cm^{-1}): 3420 (OH). ^{1}H-NMR (DMSO-d_6, D_2O) δ ppm: 3.80 (d,2H,CH_2), 3.83 (t,1H,CH-O), 7.09-7.81 (m,8H,Ar), 8.49 (s,1H,OH exch.). Anal.Calcd. for $C_{15}H_{12}BrNO_2$ (318.17): C, 56.62; H, 3.80; N, 4.40. Found: C, 56.21; H, 3.80; N, 4.72.

2.3. Pharmacological testing

2.3.1. Anti-inflammatory activity.

All animals use procedures complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

2.3.1.1. Dextran-induced rat paw edema:

Edema was induced by injecting 0.1 mL dextran (4% w/v) into the subplanter region of the left hind paw one hour after the oral administration of the tested compound in a dose 100 mg/kg [19]. Paw volume was measured immediately before, 1, 3, and 5 hours after dextran injection using digital plethysmograph (table 1).

2.3.1.2. Formaldehyde arthritis test.

Each test compound was administrated orally at dose of 25, 50 and 100 mg/kg one hour prior to formaldehyde injection and continued for the duration of the experiment [20]. Arthritis was induced by injecting 0.1 mL formaldehyde solution (2% w/v) into the subplanter region of the left hind paw of the rats. The mean change in the paw volume of each treated group was measured at day 3, 5, 7 and 9 by using digital plethysmograph. At the end of the experiment, the animals were sacrificed and the stomach was excised and inspected for haemorrhage and erosions (table 2).

2.3.2. Analgesic activity.

Nociceptive Test (Paw Pressure Test), during all the experiments, the evaluation of antinociceptive effects was carried out using the paw pressure test (Randall and Selitto method) [21]. Increasing pressures measured with an analgesimeter (tip diameter of the probe, 1 mm; weight, 30 g; Apelex; Ugo Basile, Comerio, Italy) were applied to the left hind paw of rats, with a cut-off at 300 g. Vocalization thresholds, considered nociceptive thresholds, were expressed in grams (baseline predrug vocalization thresholds range from 284 ± 28 g to 316 ± 25 g). . An analgesimeter, with a cone-shaped paw pressure and a round tip which applies a pressure of increasing intensity to punctiform area on hind paw of the rat was used. The weight in grams required to elicit nociceptive responses such as paw flexion (reflux withdrawal) of the paw was taken to be the nociceptive threshold. Rats were divided into fifteen groups (n= 6). All tested compounds were given orally at dose of 50 mg/kg. Indomethacin and celecoxib were used as standards. Indomethacin treated group receive a dose 10mg/kg; while celecoxib treated group received 40 mg/kg (table 3).

2.3.3 Acute ulcerogenicity studies

Acute ulceroginicity screening was done according to method reported by Cioli *et al* [22]. The mucosal damage was examined by means of an electron microscope. For each stomach specimen, the mucosal damage was reported.

2.4. Drug modelling studies

All docking studies were performed using "Internal Coordinate Mechanics (Molsoft ICM 3.4-8C)". ICM docking is probably the most accurate predictive tool of binding geometry today [14-17].

Preparation of small molecule

A set of diaryl pyrazoline, and diaryl isoxazoline analogues designed to inhibit cyclooxygenase II was compiled by us earlier; Chem Draw 3D structures were constructed using Chem 3D ultra 8.0 software [Molecular Modelling and Analysis; Cambridge Soft Corporation, USA (2004)], and then they were energetically minimized by using MOPAC (semi-empirical quantum mechanics), Jop Type with 100 iterations and minimum RMS gradient of 0.01, and saved as MDL Mol File (*.mol).

Generation of Ligand and Enzyme Structures

The crystal structure of target protein cyclooxygenase (1CX2) is a COX-II [17] was retrieved from the Protein Data Bank (http://www.rcsb.org/pdb/welcome.do). All bound waters ligands and cofactors were removed from the protein. The amino acids of the binding site where defined using data in pdbsum (http://www.ebi.ac.uk/thorontonsrv/databases/pdbsum

Docking using Molsoft ICM 3.4-8C program

Conversion of our PDB file into an ICM object involves addition of hydrogen bonds, assignment of

atoms types, and charges from the residue templates, then perform ICM small molecule docking through setup the receptor, review and adjust binding site make receptor maps, then start docking simulation, followed by displaying the results. ICM stochastic global optimization algorithm attempts to find the global minimum of the energy function that include five grid potentials describing interaction of the flexible ligand with the receptor and internal conformational energy of the ligand, during this process a stack of alternative low energy conformations is saved. All inhibitors were compared according to the best binding free energy (minimum) obtained among all the run.

3. Results and discussion

3.1. Chemistry

The synthetic pathways utilized to prepare the target compounds are illustrated in scheme 1. Propenone derivatives **1a-d** were synthesized by a base catalyzed Claisen-Schmidt condensation reaction of *p*-bromoacetophenone and substituted aromatic aldehydes in presence of 10% sodium hydroxide in ethanol [18]. Refluxing propenones **1a-d** with hydrazine monohydrate (99%) in absolute ethanol or with phenyl hydrazine in acetic acid afforded the corresponding pyrazolines **2, 3** or phenyl pyrazolines **4-6** respectively. While, reaction of propenones **1a-d** with hydroxylamine hydrochloride yielded isoxazoline derivatives **7-10**. Spectral data (IR, ^{1}H-NMR and MS) of all the newly synthesized compounds were in full agreement with the proposed structures.

3.2. Anti-inflammatory activity

All the newly synthesized compounds **2-10** were evaluated for their anti-inflammatory activity using dextran-induced rat paw edema method [19]. The tested compounds and reference drugs were administered orally at a dose of 100 mg/kg one hr before dextran injection into the subplanter region of the left hind paw. Paw volume was measured immediately before and 1, 3, 5 hr after dextran injection by using digital plethysmograph. Results are listed in table 1 and illustrated in figure 1-A. Where, compounds **2** and **8** were the most active. The anti-inflammatory activity was also measured using formaldehyde arthritis test [20]. Arthritis was induced by formaldehyde injection into subplanter region of the left hind paw of the rats; the mean change in the paw volume of each treated group was measured at day 3, 5, 7 and 9 by using digital plethysmograph. The results are listed in table 2 and illustrated in figure 1-B. It was found that all the synthesized compounds possess significant anti-inflammatory activity, where compounds **7** and **10** were the most active.

3.3. Analgesic activity

Paw pressure test was carried out according to the Randall and Selitto test [21] which is based on determination of the animal threshold response to pain induced in the paw by the application of a uniformly increasing pressure. The results are listed in table 3 and illustrated in figure 1-C. It was found that all the synthesized compounds show good analgesic activity and compounds **3** and **10** were the most active.

3.4. Acute ulcerogenicity studies

All the synthesized compounds were subjected to ulcerogenicity potential test [22] at 12 times the therapeutic dose of diclofenac with additional physical (cold) stress for 2 hr at -20 ^{o}c. Ulcerogenic effect of the synthesized compounds in animal efficacy model was evaluated for gastric ulcerogenic potential in rat stress model. When compared with diclofenac, all the compounds did not cause any gastric ulceration at the above mention doses. Hence gastric tolerance to these compounds was better than that of diclofenac.

3.5. Docking studies

To understand the pharmacological data on structural basis, we evaluate the designed compounds (three different classes of diarylpyrazoline, triarylpyrazoline and diarylisoxazoline) through docking techniques using Molsoft ICM 3.4-8C program. We docked our designed compounds on one of the crystal structures of cyclooxygenase II available through the RCSB Protein Data Bank (PDB entry 1CX2) [17]. The scoring functions of the compounds were calculated from minimized ligand protein complexes. In order to compare the binding affinity of the newly synthesized diarylpyrazoline and diarylisoxazoline analogues, we docked compounds **2 -10** into the empty binding site of cyclooxygenase II with its bound inhibitor celecoxib (1CX2), figures 2a-c show the docking solutions with the highest predicted binding affinity for cyclooxygenase II. Figure 2a shows orientation of celecoxib, while figures 2b and 2c show orientations of compounds **2** and **4** respectively. As shown from the (tables 1-4, figures 2a-c) the following results can be drawn: The ICM score values show good agreement with predicted binding affinities obtained by molecular docking studies as verified by pharmacological screening. Where the designed compounds shows ICM score values (range from -88.89 to -70.40) less than celecoxib (-60.71) revealing higher binding affinity with the enzyme table 4.

Celecoxib (the original ligand) reveals ICM score of -60.71 and form seven hydrogen bonds with Tyr355, His90, and Arg513 (table 4, figure 2a).

Diarylpyrazoline derivatives **2** and **3** show relatively high binding affinity, where compound **2** has ICM score of –77.98 and form one hydrogen bond with Ser530 (table 4, figure 2b), while compound **3** exhibit ICM score of -75.77 without hydrogen bond but one of its conformers has a score of -68.16 and form three hydrogen bonds with His90, Arg513 (table 4). Triarylpyrazoline derivatives **4, 5** and **6** exhibit lesser score values relatively to diaryl derivatives revealing higher binding affinity, compounds **4** possess ICM scores of -87.02 and form four hydrogen bonds with Arg120, Tyr355 (table 4, figure 2c), while compound **5** has ICM score of -88.67 and form one hydrogen bond with Val523, and compound **6** exhibit ICM score of -88.89 without hydrogen bond but one of its conformers has a score of -79.67 and form one bond with Tyr355. Diarylisoxazoline compounds **7, 8, 9**, and **10** possess ICM scores ranges from –81.71 to -70.40, where compound **7** has ICM score of -79.76 and form two hydrogen bonds with Arg120, and compound **8** possesses ICM score of -79.41 and forms three bonds with Ala378, and Asp125 and compound **9** has ICM score of -70.40 with no hydrogen bond while another conformer has ICM score of -69.44 and form six bonds with His90, Arg513. Finally, compound **10** which has the most anti-inflammatory effect possesses ICM score of -81.71 and form one hydrogen bond with Asn375, where another conformer possesses ICM score of -78.39 and form eight hydrogen bonds with His90, Ser530, and Arg513 (table 4).

Table 1: Effect of the test compounds on dextran-induced rat paw edema compared with indomethacin and celecoxib.

Compound	Edema volume (mL)		
	1 hr	3 hr	5 hr
Control	0.86±0.10	1.90±0.32	1.17±0.21
Indomethacin	0.67±0.11	0.71±0.12*	0.51±0.13*
Celecoxib	0.71±0.11	1.44±0.51*	0.74±0.22*
2	0.74±0.09	1.29±0.31	0.80±0.22
3	0.81±0.15	1.44±0.17	0.90±0.31
4	0.78±0.14	1.39±0.61	0.88±0.19
5	0.81±0.15	1.55±0.51*	0.97±0.12*
6	0.76±0.13	1.28±0.45	0.82±0.09
7	0.79±0.07	1.41±0.12	0.87±0.31
8	0.73±0.18	1.2±0.35	0.77±0.08
9	0.83±0.11	1.73±0.38*	1.08±0.22*
10	0.84±0.08	1.77±0.41*	1.09±0.21*

* Significant from control at $p < 0.01$ compared to control using ANOVA followed by Tukey- Kramer as post ANOVA test.

Table 2: Effect of the test compounds on formaldehyde-induced arthritis rat paw edema compared with indomethacin and celecoxib.

Compound	Edema volume (mm)			
	3 days	5 days	7 days	9 days
Control	2.30 ±0.11	3.5 ±0.61	3.20±1.01	3.06±1.11
Indomethacin	1.05±0.17*	1.41±0.08*	1.31±0.07*	1.10±0.60*
Celecoxib	1.17±0.08*	1.66±0.09*	1.60±0.33*	1.24±0.22*
2	2.18±0.41	3.10±0.32	2.97±0.09	2.30±0.71
3	1.53±0.21*	2.27±0.41*	2.14±0.54*	1.70±0.22*
4	1.73±0.18	2.62±0.09*	2.50±0.19	1.80±0.04*
5	1.64±0.71*	2.43±0.62*	2.19±0.13*	1.90±0.32*
6	2.25±0.18	2.45±0.09*	2.40±0.13*	2.30±0.61
7	1.45±0.08*	2.17±0.19*	2.08±0.17*	1.50±0.33*
8	1.58±0.19*	2.38±0.41*	2.30±0.15*	1.61±0.16*
9	2.27±0.28	3.45±0.21	3.09±0.15	2.80±0.01
10	1.42±0.32*	2.00±0.28	1.89±0.41*	1.40±0.11*

* Significant from control at $p < 0.01$ compared to control using ANOVA followed by Tukey- Kramer as post ANOVA test.

Table 3: Study of the analgesic effect of the test compounds using the paw pressure test.

Compound	Weight by gm			% Change in weight		
	1 hr	3 hr	6 hr	1 hr	3 hr	6 hr
Control	201±0.12	204±0.19	204±0.18	-	-	-
Indomethacin	426±0.12*	442±0.29*	298±0.27*	111.94	116.67	46.08
Celecoxib	410±0.13*	448±0.10*	286±0.13*	103.98	119.61	40.20
2	374±0.11*	397.5±0.36*	228±0.16*	86.07	94.85	11.76
3	398±0.21*	432.5±0.25*	231±0.06*	98.01	112.01	13.24
4	321±0.21*	350±0.19*	249±0.27*	59.7	71.57	22.06
5	341±0.17*	374±0.29*	245±0.33*	69.65	83.33	20.10
6	271±0.10*	305±0.61*	242±0.18*	34.83	49.51	18.63
7	211±0.31*	240±0.19*	206±0.29*	4.98	17.65	98.00
8	244±0.21*	275±0.40*	219±0.26*	21.39	34.80	7.35
9	297±0.23*	322.5±0.20*	258±0.35*	47.76	58.09	26.47
10	379±0.13*	402±0.36*	240±0.31*	88.56	97.06	17.65

All compounds are significant from control at 1, 3 and 6 hours at $p < 0.05$ compared to control using ANOVA followed by Tukey- Kramer as post ANOVA test.

Table 4: ICM Scores of celecoxib, the compounds, and hydrogen bonds formed with amino acid residues

Compounds	ICM scores	No. of Hydrogen bonds	Involved amino acid
Celecoxib	-60.71	7	Tyr355, Tyr355, His90, Arg513, Arg513, Arg513, Arg513
2	-77.98	1	Ser530
3	-75.77 or -68.16	0 3	…….. His90, His90, Arg513
4	-87.02	4	Arg120, Arg120, Arg120, Tyr355
5	-88.67	1	Val523
6	-88.89 or -79.67	0 1	…..... Tyr355
7	-79.76	2	Arg120, Arg120
8	-79.41	3	Ala378, Ala378, Asp125
9	-70.40 or -69.44	0 6	…….. His90, His90, Arg513, Arg513, Arg513, Arg513
10	-81.71 -78.39	1 8	Asn375 Ser530, His90, His90, His90, Arg513, Arg513, Arg513, G354

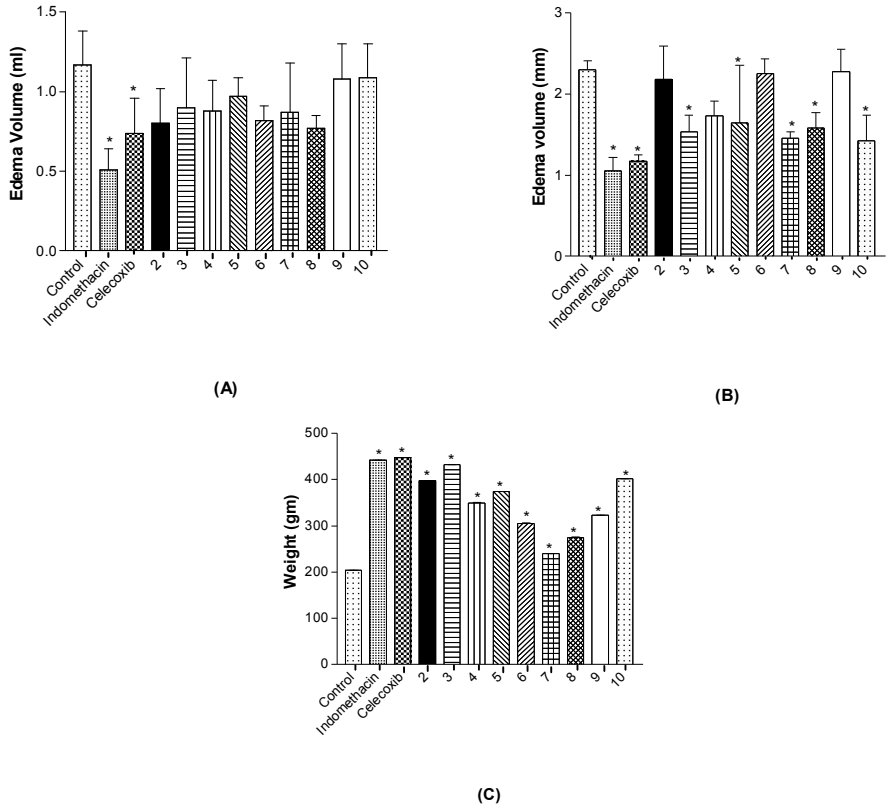


Figure 1: Anti-inflammatory and analgesic effects of diarylpyrazolin and diarylisoxazolin derivatives on (A) dextran-induced rat paw edema after 5 hr; (B) formaldehyde arthritis test after 3 days; (C) paw pressure test after 3 hr. Values are mean of 6 data points ± S.D. *P< 0.01 compared to control group using ANOVA followed by Tukey-Kramer as post ANOVA test.

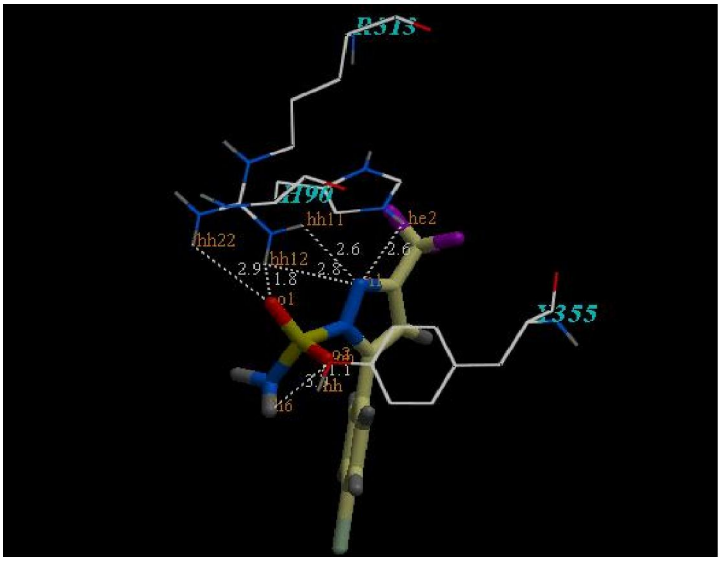


Figure 2a: Binding mode of the original ligand celecoxib into its binding site of cycloxygenase II, it has ICM score -60.71, and form 7 hydrogen bonds shown as white dotted lines (table 4), showing one hydrogen bond between NH of His90 with N1 of pyrazoline moiety distance 2.01 A^o, and two hydrogen bonds between H of OH of Tyr-355 and O of SO_2NH_2, and between O of OH of Tyr355 and H of SO_2NH_2 distances 1.13 A^o and 2.70 A^o respectively, and another four bonds between 4H of 2 NH_2 of Arg513 with N1 of pyrazoline moiety, and 2O of SO_2NH_2 distances 2.41 A^o, 2.54 A^o, 1.74 A^o, and 2.69 A^o respectively.

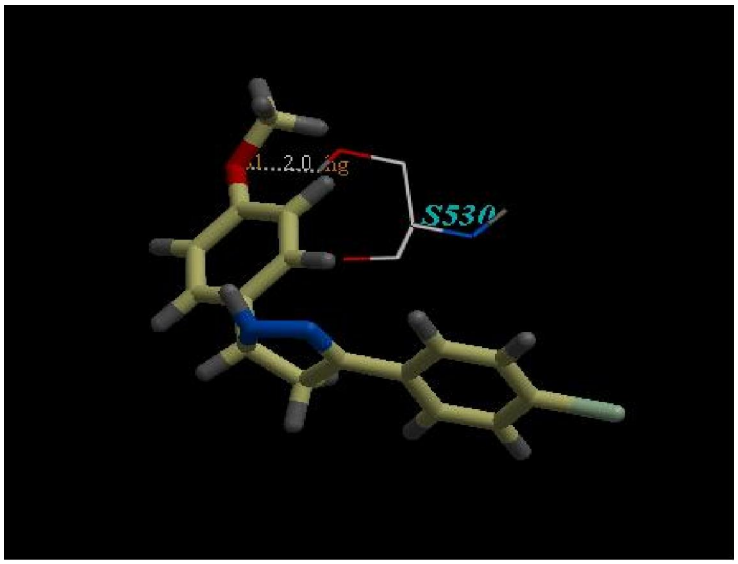


Figure 2b: Binding mode compound **2** into its binding site of cycloxygenase II, it has ICM score -77.98, and form one hydrogen bond shown as white dotted lines (table 4), showing one hydrogen bond between O (OCH_3), with OH of Ser530 distance 1.77 A^o.

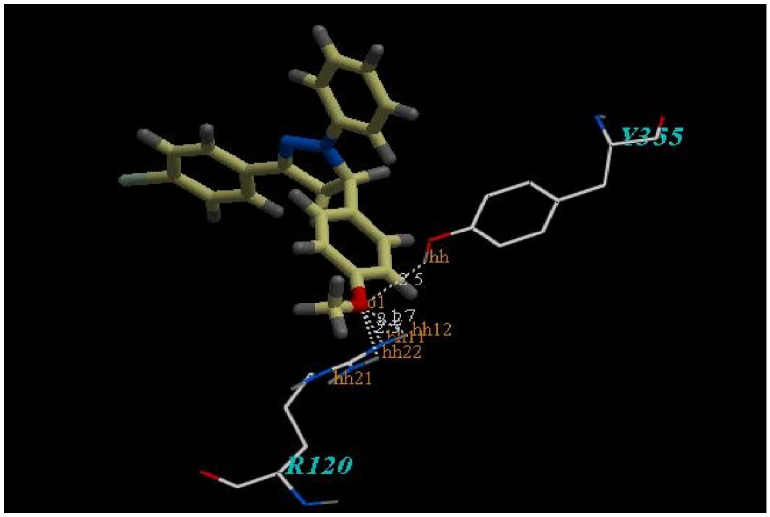
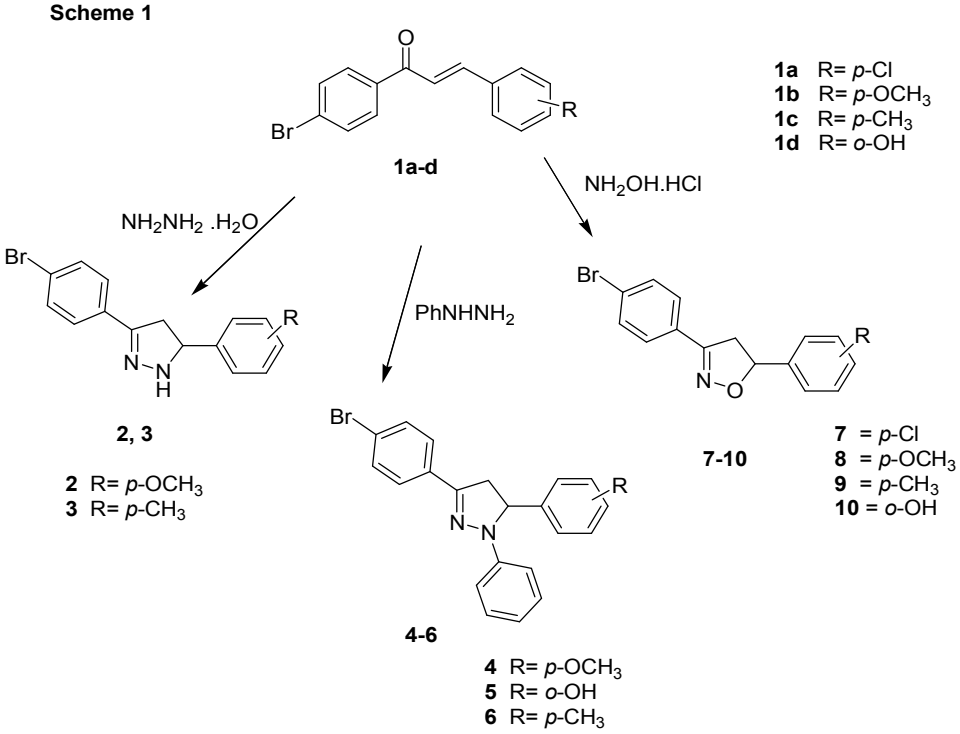


Figure 2c: Binding mode of compound **4** into the binding site of cycloxygenase II, it has ICM score -87.02, and form four hydrogen bonds shown as white dotted lines (table 4), showing three hydrogen bonds between O of (OCH_3), with NH_2 of Arg120 distance 2.74 A^o and 2.09 A^o, and 2.44 A^o respectively, and one bond between O of (OCH_3) and OH of Tyr355 distance 2.03 A^o.

Scheme 1



4. Conclusion

From the previous data we can conclude that acidic group sulfamido which is found in most COX-II inhibitor is not essential for the anti-inflammatory and analgesic activity but it enhances it, where diarylpyrazoline and diarylisoxazoline moieties may be responsible for activity. Compound **8** is the most active one as anti-inflammatory in dextran-induced rat paw edema, while compound **10** is the most active one in formaldehyde arthritis test. Other compounds showed activity ranging from mild to moderate that compared to celecoxib. For the analgesic activity, all the test compounds showed promising activity, where the most active compounds were **3** (diaryl pyrazoline moiety) and **10** showing activity parallel to celecoxib. It is clear that presence of *p*-methoxy group in phenyl ring increased the anti-inflammatory activity of compounds **2, 4** and **8**. To less extent the presence of *p*-methyl group may also increase the activity as shown by compounds **6** and **9**. Furthermore, the presence of *o*-hydroxyl group increases the analgesic and anti-inflammatory activity as shown by compounds **5** and **10**. Also, the smaller the ICM score value and/or formation of hydrogen bonds with Arg120, Arg513 , Ser530, Ser353, Try355, and His90 amino acids lining the pocket of COX-II enzyme, increasing the binding affinity, and hence the anti-inflammatory effect. No one of the tested compounds show ulcerogenic activity.

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