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Design, Molecular docking, and Synthesis of New Derivatives of Diclofenac with expected anti-inflammatory and selectivity to COX-2 enzyme

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Abstract

A group of amine derivatives [5 - amino - 1,3,4 - thiadiazole - 2 - thiol and 5 - (methylsulfonyl) - 1, 3, 4 - thiadiazol - 2 - amine] binding to a well-known non-steroidal anti-inflammatory drug at α-carbon of diclofenac , where bulkiness will be increased and final target compounds (IX and X) have been expected to be anti-inflammatory and selectivity toward COX-2 enzyme. The synthesis of final target compounds (IX and X) was achieved successfully . The synthetic approach involved multi-steps procedures .The functional groups of these compounds were confirmed by some physicochemical properties, FTIR Spectroscopy , and elemental analysis CHN. Molecular docking was used to find the probability of binding position and binding strength of new derivatives of diclofenac with particular Prostaglandin G/H synthase 2 (PDB ID: 1CX2) .

Key word : Anti-inflammatory activity , Molecular docking **,** 5 - amino - 1,3,4 - thiadiazole - 2 - thiol , 5 - (methylsulfonyl) - 1, 3, 4 - thiadiazol - 2 - amine, Diclofenac .

INTRODUCTION

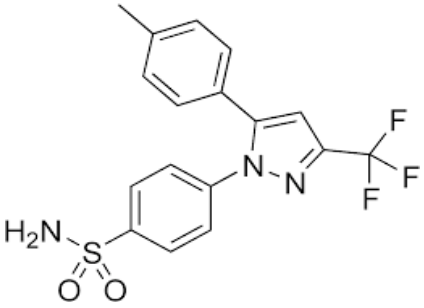
Prostaglandins and related products are yield in Extremely small amounts truthly in many tissue. It has biological activity on the tissues in which that will be present, and $t_{1/2}$ is very short where its metabolized to inactive compounds at the sites of action. Therefore, the prostaglandins do not circulate in the blood in significant concentration [1].

The prostaglandins are yield by the approach of cyclooxygenase. Where two form of the cyclooxygenase enzyme have been found .

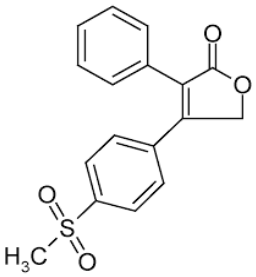
Prostanoids that produced normal physiological activity was yield by cyclooxygenase-1(COX-1) while Prostanoids that produced diseases and inflammation was yield by cyclooxygenase-2(COX-2) [2].

COX-1 will maintains the normal biological activity such as gastric cytoproticti on vascular homeostasis platelet aggregation and kidney function [3].In contras COX-2 will define in some tissue brain ;kidney ;and bone where expression at other sites is increased through out of inflammation [4,5]. Both isoform of cyclooxygenase enzyme are 60% identical in amino acid sequence. But the binding position & catalytic are somewhat are differ ,where COX-2has large side pocket & more flexible channel than COX-1 has & all of this at active site of enzyme this promote develop a selective COX-2 inhibitor [3].

Non-steroidal anti-inflammatory drugs (NSAIDs) are show many therapeutics uses as antipyretic , analgesic and anti-inflammatory , In spite of they have shown unwanted side effects [6,7]. In the beginning of nineteen century develop of new selective COX-II inhibiter create a new events where the selective COX-2 inhibitor act as 2nd generation & as group without side effect mainly gastric intestinal damage [8,9].In vivo study on an animal modal to evaluate biological activity of this theory has led produced to the market of both1,2-diarylheterocycles celecoxib (1) and rofecoxib (2) as selective COX-2 inhibitors (Figure 1) [10-15]. But still required for further research to produce new NSAIDs with more safety profile .



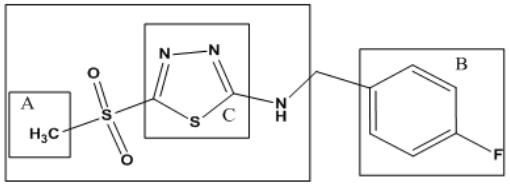
Celecoxib (1)



Rofecoxib (2)

Figure 1: The basic structures of selective COX-2 inhibitors

In recent time, design of new 1,3,4-thiadiazole derivative where structurally lined up by manipulation diuretic drug acetazolamide & a 1,3-bezodioxole ,which is a selective cyclooxygenase inhibiter .before The develop of the work recommended the matching of a cyclooxygenase enzyme pharmacophre which contained the 1,3,4-thiadiazole & existence of three demand (aryl hailed , thiadiazole ring & methyl sulfonyl substitution). The evolution of pharmacological activity of new products lead to the para-fluoro-substituted derivate (Figure 2) is a new prototype which is has higher activity than celecoxib at the same concentration with expected to be more selective cyclooxygenase inhibitor [16].



N-(4-fluorobenzyl)-5-(methylsulfonyl)-1,3,4-thiadiazol-2-amine

Figure 2: 1,3,4-thiadiazole derivative as COX-2 inhibitor

In consequence, the extension of this proposal was planned to produce a new arrangement with expected anti-inflammatory and selectivity toward COX-2 enzyme by the binding of an A group of amine derivatives [5 - amino - 1,3,4 - thiadiazole - 2 - thiol and 5 - (methylsulfonyl) - 1, 3, 4 - thiadiazol - 2 - amine] to a well-known non-steroidal anti-inflammatory drug at α-carbon of diclofenac to increase bulkiness .

MATERIAL AND METHODS

Material and Equipment's :

All reagent and anhydrous solvent were of analar type and usually obtain from the commercial supplier (Merk-Germany,Reidel-Dehean-Germany ,Sigma-England) Diclofenac sodium was obtain from Anyang Jiuzhou – china . Melting point were specify by capillary method on Thomas Hoover apparatus (England) and ascending thin layer chromatography (TLC) was run on DC-Kartan SI Alumin 0.2 mm to specify the purity and progress of reaction . The determination of products was achieved by using iodine vapor and the chromatograms were mobile phase is :

1-n-hexane : Ethyl acetate : Acetic acid (7: 2.5: 0.5) [17] .

2-THF :Ether : cyclohexane (4:4:2) [18].

IR spectra were recorded on model Pye-Unicam 1028, 110 FTIR

Spectrophotometer , Philips Company (England) as a KBr film . at the College of Science - University of Karbala. Elemental microanalysis was performed using CHN Euro EA Elemental analyzer (Italy), at the College of Pharmacy - University of Karbala.

Experimental Section:

A. Chemical synthesis:

The synthetic procedures that actually resulted in the production of the target compounds (IX and X) was prepared as described by Francis A. Carey [19,20] starting from Protection of carboxyl group of diclofenac as shown in the scheme 1. Their characterization and purity of these compounds (percent yields, melting points and Rf values) (table 1).

1.Synthesis of 2-[2-(2,6-diclorophenyl amine)phenyl]acetic acid compound (I) :

In the smallest volume of mixture of tetrahydrofuran and absolute ethanol (3:1) dissolved diclofenac sodium (4 gm,12.56 mmol). The prepared solution should be cool to 18 ° C with continues stirring for 10 min then HCl (2N,7.6ml,14.76 mmol) was added followed by excess of cold water was added, diclofenac acid will precipitate ,filter and dried to give a compound (I) which was used in next step without further purification [19] .The percent yield , physical data and Rf values were given in table 1. IR[3323 (N-H) stretching vibration of secondary amine , 3200-2500 (O-H) broad stretching vibration of carboxylic acid, 2891 (C-H) stretching vibration of alkane , 1710 (C=O) stretching vibration of carboxylic acid , 1587 and 1508 (C=C) stretching vibration of aromatic (skeletal vibration) , 1303 C-O stretching vibration] cm-1.

2.Synthesis of methyl 2-(2-(2,6-diclorophenyl amino) Phenyl) acetate compound (II): Suspension of compound I (2g, 6.74mmol) in 60ml of absolute methanol was cooling to -10°C followed by added (0.5ml, 6.74mmol) of thionyl chloride. The temperature should be kept blow (0 °C) for 10 minute . The prepared solution should be kept for 3hrs at 40°C ,then reflux for 3hrs & leave for 24 hrs. at room temperature followed by evaporate the solvent to dryness, then the excess of thionyle chloride removed by dissolved the dry powder in absolute methanol & evaporate repeat this process for several time , recrystallized the final product from mixture methanol & ether [20]. The percent yield, physical data and R_f values were given in tablet 1. IR[3352 (N-H) stretching vibration of secondary amine , 2952 (C-H) stretching vibration of alkane , 1745 (C=O) stretching vibration of ester , 1587 and 1506 (C=C) stretching vibration of aromatic] cm^{-1}.

3.Synthesis of methyl 2-bromo-2-(2-(2,6-diclorophynyl amino)phenyl)acetate compound (III) :

Compound (II) (1g, 3.37mmol) was dissolve in 15ml CCl_4 ,then step by step added NBS (0.57 gm, 3.37mmol) with mixing, leave the reaction for 3 hrs then filtrate is dried to given compound (III) [21,22]. The percent yield, physical data and R_f values were given in tablet (1). IR [3319 (N-H) stretching vibration of secondary amine, 3080 (C-H) stretching vibration of aromatic, 2987 (C-H) stretching vibration of CH3 , 1740 (C=O) stretching vibration of ester, 1579 and 1508 (C=C) stretching vibration of aromatic] cm^{-1}.

4. Synthesis of 2-amino -5-thiol -1, 3, 4-thiadiazol compound (IV):

Dissolved (2g , 0.021 mol) of thiosmicarbazide in absolute ethanol (15ml) in round flask (250ml), then sodium carbonate (1.16gm, 0.01mmol) and CS_2 (5ml, 0.062mmol) were added respectively with continues stirring. The reactant mixture was reflex for 3hours, and then evaporated to dryness. Dissolve the precipitate in cold distilled water (7ml) , then added slowly to concentration HCl(1ml) , white precipitate will formed filter the precipitate and wash with distilled water, recrystallized by used hot distilled water, faint yellow crystal will formed [23,24] . The percent yield, physical data and R_f values were given in table 1. IR [3408 and 3450 (N-H) stretching vibration of Primary amines, 1740 C=S stretching vibration gives evidence that compound (IV) can exist in two tautomeric form,thiol form and thion form., 1602 C=N stretching vibration of the thiadiazole ring moiety, 613 S-H stretching vibration.]cm^{-1} .

Table 1: The characterization and physical data of the intermediate compounds and final compounds.

Compounds and intermediates	Empirical formula	M Wt.	Description	% yield	Melting point ^{o}C	R_f value
I	C14H11Cl2NO2	296	White powder	98.36	160-162	A=0.85 B=0.65
II	C15H13Cl2NO2	310	White crystals	95.72	72-75	A=0.88 B=0.80
III	C15H12BrCl2NO2	389	Faint pink crystals	55	78-80	A=0.93 B=0.82
IV	C2H3N3S2	133	Faint Yellow crystals	60	220-222	A=0.63 B=0.51
V	C3H5N3S2	147	Green crystals	80	138-140d	A=0.65 B=0.6
VI	C3H5N3O2S2	179	Yellow crystals	75	295-297d	A=0.55 B=0.59
VII	C17H14Cl2N4O2S2	441	Red powder	66	64-66	A=0.89 B=0.82
VIII	C18H16Cl2N4O4S2	487	Black powder	75	182-184	A=0.91 B=0.82
IX	C16H12Cl2N4O2S2	427	White-Yellow crystals	30	105-107	A=0.79 B=0.7
X	C17H14Cl2N4O4S2	473	Gray powder	28	200-202	A=0.87 B=0.77

5.Synthesis of 5-(methylthio)-1, 3, 4-thiadiazol-2-amine compound (V):

In the mixture of smallest volum of DW & sufficient amount of KOH (85%) solution dissolve compound IV with continuous stirring at 25 °C, after 8 mints then put the solution in ice path and then added the CH_3I (0.623ml, 10 mmol) was added with vigorous stirring as 1drop every 2min, then continuous stirring for 1hour. Finally evaporate the solvent to get the product (V), which was used without further purification [25]. The percent yield, physical data and R_f values were given in table 1. IR [3296 – 3508 (N-H) stretching vibration of Primary amines, 2962 (C-H) stretching vibration of CH_3, 1599 C=N stretching vibration of the thiadiazole ring moiety , 1383 C-H bending vibration of CH_3.]cm^{-1} .

6.Synthesis of 5-(methylsulfonyl)-1, 3, 4-thiadiazol-2-amine compound (VI):

Compound (V) (0.147 g, 1mmol) was dissolved in ethanol (95%) (30ml), hydrogen peroxide (0.068 g ,2mmol) was added with continues stirring at room temperature for 2hrs,followed by evaporate the solution to get the product VI, Which was used without further purification [26]. The percent yield, physical data and R_f values were given in table 1. IR [3400 – 3522 (N-H) stretching vibration of Primary amines, 2932 (C-H) stretching vibration of CH_3, 1626 C=N stretching vibration of the thiadiazole ring moiety , 1359 C-H bending vibration of CH_3 , 1303 – 1238 S=O stretching vibration of sulfones]cm^{-1} .

7.Synthesis of methyl 2-(2-(2,6-dichorophenylamino)phenyl))-2-(5-mercapto-1,3,4-thiadiazol-2-ylamino)acetate compound (VII) :

in round flask dissolved Compound (III) (1g, 2.57 mmol) and compound (IV) (0.34 g, 2.57mmol) , then added mixture solvent (30 ml) which consist of (1:1) absolute ethanol & DMF , The reaction was proceed by reflux softly for 3.5 hrs evaporated the solvent then residue was dissolved in ethyl acetate , sodium hydroxide 5% are used to wash the solution ,repeat this process three time ,then anhydrous magnesium sulfate are used remove moisture followed by evaporated the solvent under vacuum to get the compound (VII) [27,28].The percent yield, physical data and R_f values were given in table 1 .IR [3321(N-H) stretching vibration of secondary amine, 1726 (C=O) stretching vibration of ester , 1614 (C=N) stretching vibration of the thiadiazole ring moiety, 1570 and 1496 (C=C) stretching vibration of aromatic, 787 (C-H) bending vibration of aromatic, 667 (S-H) stretching vibration] cm^{-1}.

8.Synthesis of methyl 2-(2-(2,6-dichorophenylamino)phenyl))-2-(5-(methylsulfonyl)-1,3,4-thiadiazol-2-ylamino)acetate compound (VIII):

in round flask dissolved Compound (III) (1g, 2.57 mmol) and (VI) (0.46g, 2.57mmol) , then added mixture solvent (30 ml) which consist of (1:1) absolute ethanol & DMF , The reaction was proceed by reflux softly for 3.5 hrs evaporated the solvent then residue was dissolved in ethyl acetate , sodium hydroxide 5% are used to wash the solution ,repeat this process three time ,then anhydrous

magnesium sulfate are used remove moisture followed by evaporated the solvent under vacuum to get the product (VIII) [27,28]. The percent yield, physical data and R_f values were given in table (1) .IR [3400 (N-H) stretching vibration of secondary amine, 3080 (C-H) stretching vibration of aromatic ,1739 (C=O) stretching vibration of ester ,1614 (C=N) stretching vibration of the thiadiazole ring moiety ,1568 and 1492 (C=C) bending vibration of aromatic, 1444 (C-H) bending vibration of CH3, 1311 and 1159 (S=O) stretching vibration of sulfones ,785 (C-H) bending vibration of aromatic] cm1.

9.Synthesis of 2-(2-(2,6- dichorophenylamino)phenyl))-2-(5-mercapto-1,3,4-thiadiazol-2-ylamino)acetic acid compound (IX) :

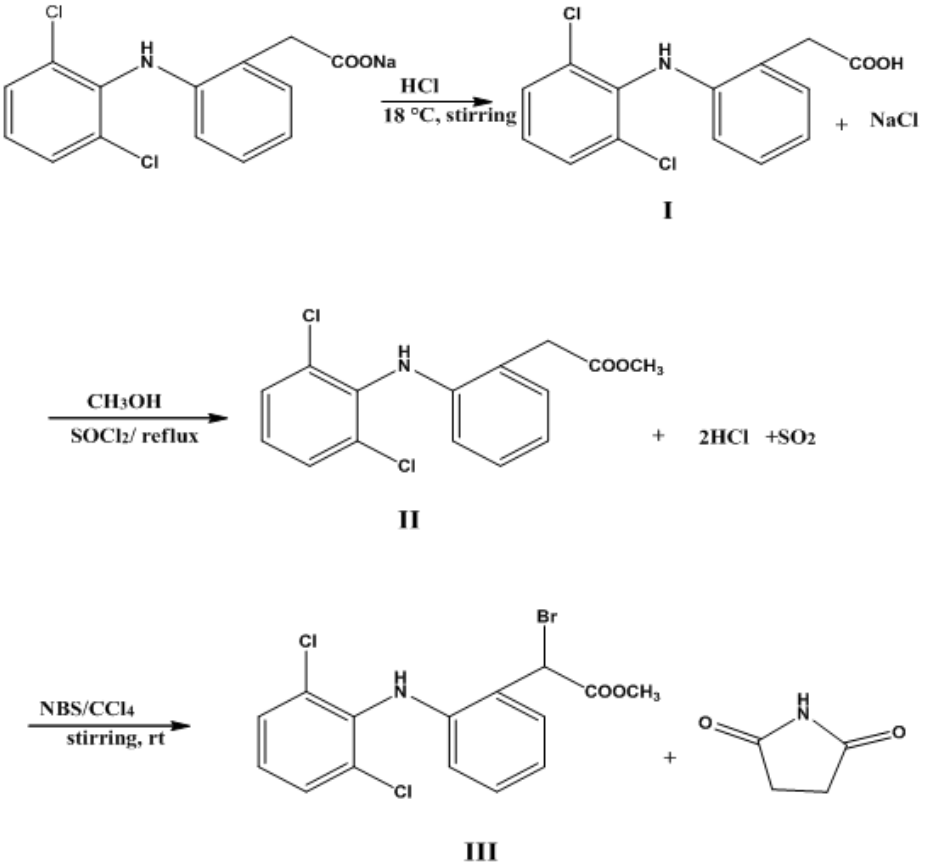
In the smallest volume of mixture solvent of (15:1) absolute ethanol & Tetrahydrofuran dissolved compound (VII) then put the solution in ice path &cool to 16 °C followed by added NaOH 2N(0.27ml, 0.68mmol) gradually & stirring continue for 3.5 hours , then added hydrochloric acid 2N (0.27ml, 0.68mmol), a lot off of cold water was added to precipitate acidic target product , filtered & dried to get compound

Compound (IX) [29]. The percent yield, physical data and R_f values were given in table (1).IR [3200 – 3600 Broad absorption band result from overlapping of (O-H) stretching vibration of carboxylic acid and (N-H) stretching vibration of secondary amine, 3172 (C-H) stretching vibration of aromatic ,1737 (C=O) stretching vibration of carboxylic acid, 1611 (C=N) stretching vibration of the thiadiazole ring moiety, 1445 and 1240 (O-H) bending and (C-O) stretching vibration of carboxylic acid respectively, 657 (S-H) stretching vibration] cm^{-1}. CHN analysis for C20H16N5NaO5S2, Calculate.: C, 44.97; H, 2.83; N, 13.11. Found: C, 45.001; H, 2.628; N, 13.003.

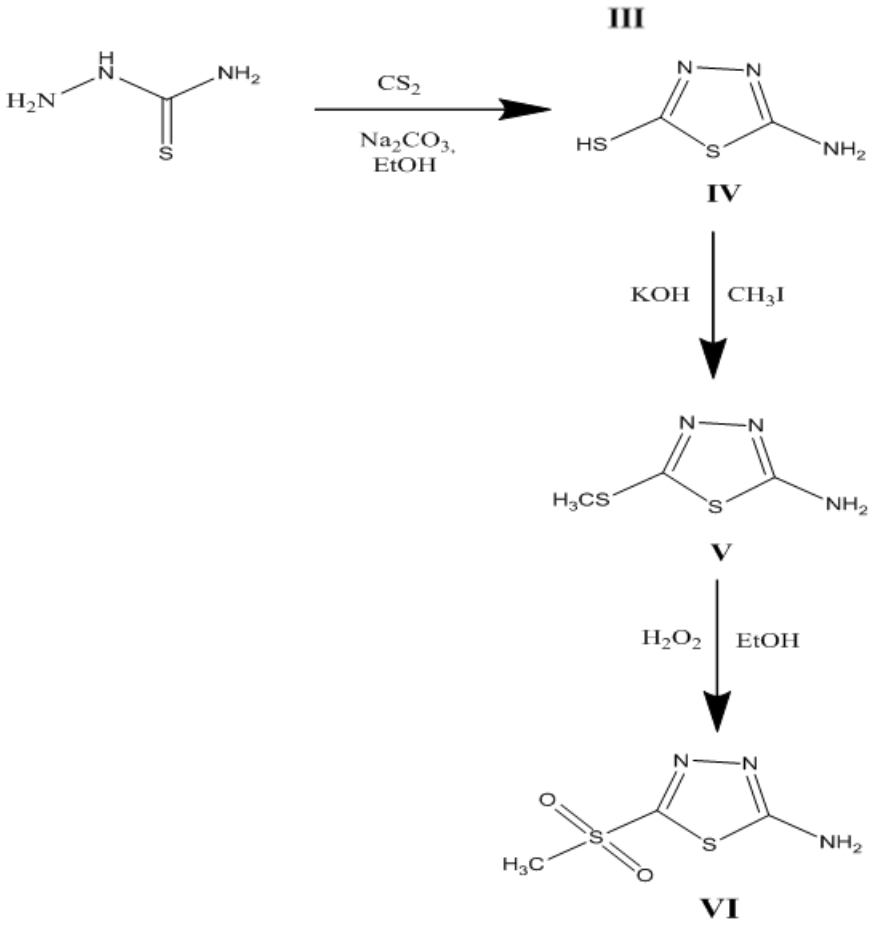
10.Synthesis of 2-(2-(2,6- dichorophenylamino)phenyl))-2-(5-(methylsulfonyl)-1,3,4-thiadiazol-2-ylamino)acetic acid compound (X):

In the smallest volume of mixture solvent of (20:1) absolute ethanol & Tetrahydrofuran dissolved compound (VIII) then put the solution in ice path &cool to 16 °C followed by added NaOH 2N(0.27ml, 0.68mmol) gradually & stirring continue for 3.5 hours , then added hydrochloric acid 2N (0.27ml, 0.68mmol), a lot off of cold water was added to precipitate acidic target product , filtered & dried to get compound

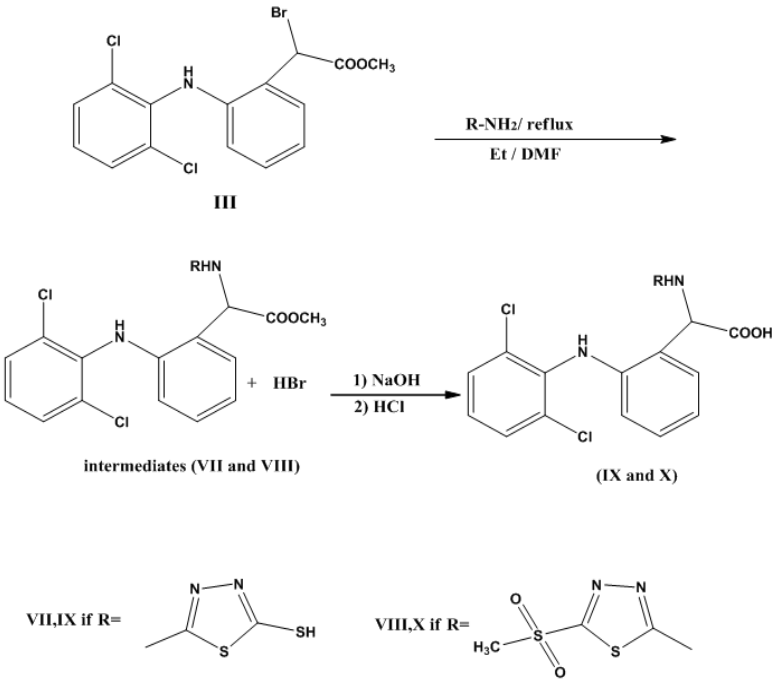
Compound (X) [29]. The percent yield, physical data and R_f values were given in table (1) .IR [3100 -3650 Broad absorption band result from overlapping of(O-H) stretching vibration of carboxylic acid and (N-H) stretching vibration of secondary amine ,3090 (C-H) stretching vibration of aromatic, 1742 (C=O) stretching vibration of carboxylic acid, 1606 (C=N) stretching vibration of the thiadiazole ring moiety, 1572 and 1484 (C=C) bending vibration of aromatic, 779 (C-H) bending vibration of aromatic] cm^{-1}. CHN analysis for C20H16N5NaO5S2, Calculate.: C, 43.14; H, 2.98; N, 11.84. Found: C, 43.686; H, 2.85; N, 11.089.



Scheme 1: The synthesis of intermediates (I, II and III).



Scheme 2: The synthesis of intermediates (IV, V and VI).



Scheme 3: The synthesis of intermediates (VII and VIII) and target compounds (IX and X).

B. In Silico Study of the anti-inflammatory activity of the synthesized compounds IX & X :

In silico technician are used to evaluate the anti-inflammatory effect of target compound ,where the orientation & affinity of binding the ligand (synthesized compound) to receptor (COX-2) by using 1-click Docking (www. mcule.com) is the simplest way on line .this method of evolution give good impression how drugs & receptor will bind at active site .docking usually composed of two steps: (i) pose generation, (ii) affinity prediction (scoring).the docking scores with highest negative charge will be used ,where highest score value refer to the best affinity. Binding poses can be displayed ("Visualize pose" button) and downloaded ("Download pose" button) (figure 3&4) [30].

1. Preparation of small molecule

A group of amine derivatives [5 - amino - 1,3,4 - thiadiazole - 2 - thiol and 5 - (methylsulfonyl) - 1, 3, 4 - thiadiazol - 2 - amine] binding to a well-known non-steroidal anti-inflammatory drug at α-carbon of diclofenac , where bulkiness will be increased and final target compounds (IX and X) have been expected to be anti-inflammatory and selectivity toward COX-2 enzyme was compiled by us earlier; Chem Draw 3D structures were constructed using Chem 3D ultra 12.0 software [Molecular Modelling and Analysis; Cambridge Soft Corporation, USA (2004).

2 .Determination of Enzyme Structures

From the protein Data Bank the crystal structure of Prostaglandin G/H synthase 2 (PDB ID: 1CX2) [31] removed all bound waters ,ligands & cofactors .specify the active site by using data from Garavito RM, Malkowski MG and DeWitt DL [31].

3. Molecular Visualization

Analysis molecular structure ,visualized the interactivity & related data , which consist of conformation structure of ligand & target , sequence of amino acid , result of docking & and so on ,can be doing by using UCSF Chimera is a highly extensible program . Chimera includes complete documentation and several tutorials, and can be downloaded free of charge for academic, government, non-profit, and personal use [32].

4. Evaluation of toxicity

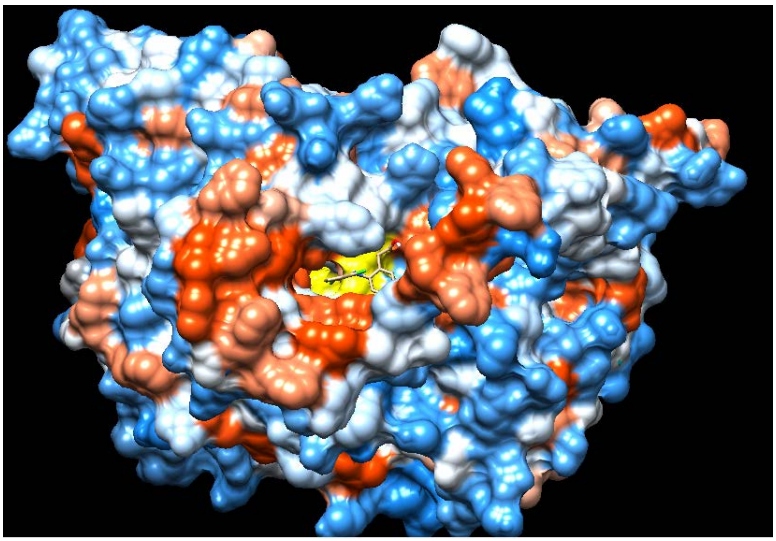
Evaluation of toxicity to the target compound by using Toxicity Checker (Mcule) which based on more than 100 SMARTS toxic matching rules. Reject problematic compounds to avoid toxicity, selectivity and pharmacokinetic issues in further development [30] .

5. Evaluate the physicochemical properties of target compounds

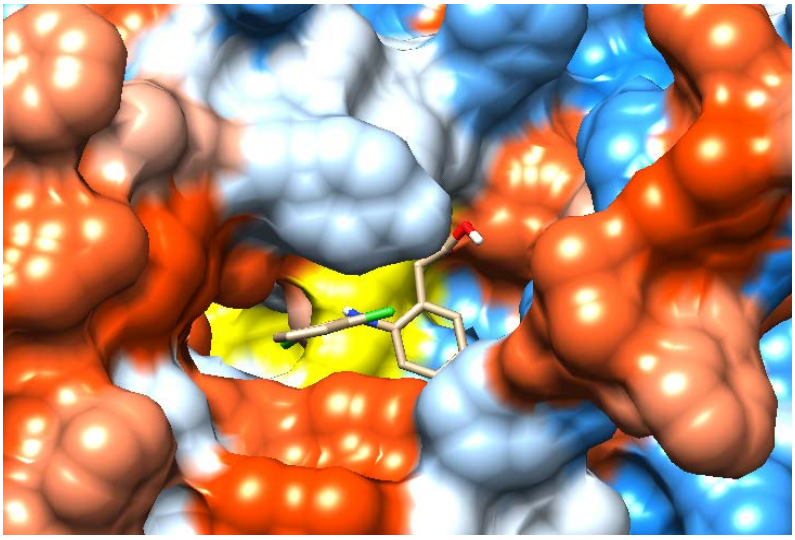
Newly design compounds to produce certain pharmacological effect should be survived through different barriers to reach site of action , where Christopher A. Lipinski design rule of five to evaluate the physicochemical properties of chemical compounds which expected have biological activity [33,34]**.** This rule was study the properties of molecules which have a direct effect on the pharmacokinetic of drug (absorption, distribution, metabolism, and excretion) without predicate their biological activity [35]. Application of this rule has greaten benefit to decrease the time of retardation under clinical trials & increase the probability to be used as medication [33,36]. Factors that assumed by Lipinski can be enumerate easily as hydrogen bond donor & acceptors should be not more than 5& 10 consequently , molecular weight not more than 500 daltons , rotatable bonds fewer than 10 and finally the partition coefficient (Log P) about 5 or less [37].

RESULTS

According to the aim of current study, and bioinformatics calculations of COX-2 and ligand's (design compounds) properties. The following figures and tables summarized the results of current study.

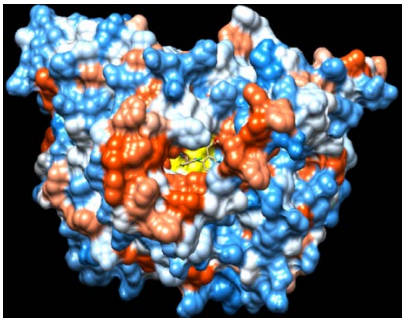
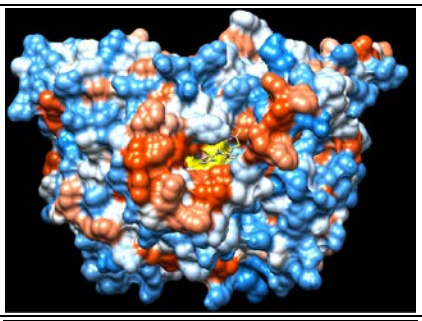
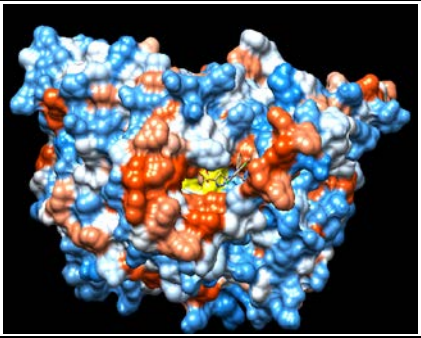


Figure(3): active site of COX-2(PDB ID: 1CX2) with position x=-18.697 , y=10.782 , z=28.377 bound with diclofenac , In the active site of cyclooxygenase the membrane binding domains are spirals around the enzyme (COX) channel to create the mouth through which all arachidonate fatty acid substrate & ligand (NSAIDs) are entered to the active site . Arg-120 (yellow) ,which is part of channel aperture, where their active center is present in the med of this channel between Arg-120 & Tyr-385 (yellow) [31].



Figure(4): Enlarged picture shows active site of COX-2(PDB ID: 1CX2) with position x=-18.697 , y=10.782 , z=28.377 bound with diclofenac , In the active site of cyclooxygenase the membrane binding domains are spirals around the enzyme (COX) channel to create the mouth through which all arachidonate fatty acid substrate & ligand (NSAIDs) are entered to the active site . Arg-120 (yellow) ,which is part of channel aperture, where their active center is present in the med of this channel between Arg-120 & Tyr-385 (yellow) [31].

Table (3-2): docking score and active site binding position of several compounds to COX-2 .

No.	Ligand	Docking score (Kcal/mol)	Binding position
1	Diclofenac	**-6.6**	
2	2-(2-(2,6-dichorophenylamino)phenyl))-2-(5-mercapto-1,3,4-thiadiazol-2-ylamino)acetic acid compound (IX)	**-7.3**	
3	2-(2-(2,6-dichorophenylamino)phenyl))-2-(5-(methylsulfonyl)-1,3,4-thiadiazol-2-ylamino)acetic acid compound (X)	**-7.8**	

Table(3-2): COX-2 (PDB ID: 1CX2) docking score against studied compounds, in addition to their predicted Lipinski rule of five .

No.	Ligand	Docking score (Kcal/mol)	M. Wt	H- Bond Acceptors	H-Bond donors	Rotable Bonds	Log P	Score of priority
1	Diclofenac	-6.6	296	3	2	4	4.43	5
2	2-(2-(2,6-dichorophenylamino)phenyl))-2-(5-mercapto-1,3,4-thiadiazol-2-ylamino)acetic acid compound (IX)	-7.3	427	6	3	6	5.26	5
3	2-(2-(2,6-dichorophenylamino)phenyl))-2-(5-(methylsulfonyl)-1,3,4-thiadiazol-2-ylamino)acetic acid compound (X)	-7.8	473	8	3	7	5.45	5

DISCUSSION:

The purpose of this study to design & synthesis of new derivative of a well-known NSAIDs (diclofenac) through binding of an bulk heterocyclic ring [namely 5-amino-1,3,4-thiadiazole-2-thiol and 5-(methylsulfonyl)-1, 3, 4-thiadiazol-2-amine] by amination to α-carbon of diclofenac to increase the bulkiness with expected to increase its activity & selectivity to COX-2 enzyme .The synthesis of target compounds (XI& X) are succeed by using easily method lead to obtain pure & good yields products .

The synthesis of heterocyclic ring was achieved by condensing of thiosmicarbazide

With carbon disulfide under alkaline media to get (compound IV) followed by S-methylation to obtain (compound V) then finally which oxidized by hydrogen peroxide to obtain (compound VI) . Both compound (IV & VI) are used as a bulk heterocyclic ring that will binding α-carbon of diclofenac to produce target compound (IX & X) , where the structure of final products proofed & characterization by physicochemical parameter presented in Table 1 , FTIR & elemental microanalysis (CHN) .

Molecular docking:

The biological effect of target compounds (IX&X) on particular Prostaglandin G/H synthase 2 (PDB ID: 1CX2) have been evaluated in comparison with a reference drug (Diclofenac) , The target compounds (IX&X) showed lower docking scores (more negative scores) on COX-2 enzyme , which indicate that these may have better activities. The most potent compounds based on the lowest docking scores on the particular Prostaglandin G/H synthase 2 (PDB ID: 1CX2).

Analytical comparison:

The analytical comparison between the final target compounds & diclofenac (reference drug) as following:

a) The finally designed compounds (IX&X) showed lower docking scores on COX-2 enzyme , which indicate that these may have good activity as NSAIDs.

b) Compound IX expressed a comparable docking scores to that of Diclofenac on COX-2 enzyme.

c) Compound X showed higher docking scores than Diclofenac on COX-2 enzyme **.**

d) Comparison between final target compounds & reference drug (diclofenac) depended on results scores of docking & rule of five (Lipinski's rule of five) show all of them have equal score of priority five with advantage to the new design compounds expected to be more affinity (activity) & less side effect (selective to COX-2 enzyme) .

CONCLUSION:

1. successfully achieved the synthesis of the finally designed compounds (IX &X).
2. Increasing the time of reflux in the preparation of (5-amino-1, 3, 4- thiadiazole-2-thiol) ring led to increase the percent yield of the product.
3. The using of CCl4 solvent instead of CH2Cl2 in synthesis of intermediate (III) led to give higher percent yield of the product.
4. the molecular docking screening is suggested as a very useful new program that could be used prior the chemical synthesis to predict the more effective NSAIDs by measuring the docking scores.

RECOMMENDATIONS:

1.Pharmacological evaluation of tested compounds as anti-inflammatory agents.

2.Incorporation of another amino groups as C6H5-NH2 and X-C6H4-NH2 into α-carbon of diclofenac and tested their anti-inflammatory activity.

3. Study the sub-acute anti-inflammatory effect and chronic anti-inflammatory effect of the synthesis finally designed compounds (IX &X).

4.Spesify the selectivity to COX-2 enzyme of the finally designed compounds (IX &X) by evaluation COX-2:COX1 inhibitory ratio using human whole blood assay.

REFERENCES:

1- Harvey, R.A. and Champe, P.C. (Eds.). Lippincott's illustrated reviews pharmacology , 3rd ed., 2006;p.495-500.

2-Marnett,L.J.;Rowlinson, S.W.;Goodwin,D.C.;Kalgutkar,A.S. and Lanzo, C.A, J.Biol.Chem.1999,274,22903-22906.

3- Vane, J. , Garavito, M.,Picot, D., Loll, P.J., Nature. 1994, 367,243–249.

4-Hardman,J.G.(Eds.). Goodman and Gilman,s The pharmacological Basis of Therapeutics ,10th ed., McGraw-Hill,New York, 2001, pp.689.

5-Lipsky,P.E., Abramson, S.B., Breedveld, F.C., et al., Rheunatol. 2000;27(1338-1340).

6-Lombardino,G.(Eds.). Non-steroidal Anti-inflammatory Drugs, 1st ed., John Wiley and Sons, New York, 1985, p.442.

7-Garcia, R. , Jick, H., Lancet .1994,343,769-72.

8-Talley , J. J., Brown , D. L., Carter ,J.S., Graneto, M.J., Med Chem . 2000,43,775.

9- Penning T.D., Talley J.J., Bertenshaw, S.R., J. Med. Chem. 1997,401347.

10- Simon, L.S., Lanza, F.L., Lipsky, P.E., Hubbard, R.C., Talwalker, S., Schwartz , B.D., Isakson, P.C., Arthritis Rheumatism, 1998, 41,1591-1602.

11- Ehrich, E.W., Dallob, A., De Lepeleire , I., VanHecken, A., Riendeau, D., Yuan, W., Clin. Pharmacol. Ther., 1999, 65, 336-347.

12- Kalgutkar, A.S., Exp. Opin. Ther. Patents, 1999, 9, 831-849.

13- Talley, J.J., Exp. Opin. Ther. Patents, 1997, 7, 55-62.

14- Carter, J.S., Exp. Opin. Ther. Patents, 1997, 8,21-29.

15- Prasit, P., Riendeau, D., Annual Reports in Medicinal Chemistry, Hagmann, W. K., Ed.; Academic Press Inc., New York, 1997, 32, 211-220.

16- Varandas, L.S., Fraga, C.A.M., Miranda1, A.L.P., Barreiro, E.J., Letters in Drug Design & Discovery, 2005, 2, 62-67.

17- Ali, M., Sudhanshu, S., Showkat A. B., Indian Journal of chemical Technology ,2009, 16, 344-350.

18- AL-Mikhlafi S., Ph.D. Thesis, College of Pharmacy, Baghdad University, Baghdad, 2004.

19- Francis A. Carey (Eds.). Organic Chemistry , 3rd ed. McGraw–Hill, New York,1996, pp. 1092 –1124.

20-Francis A. Carey & Richard J.S. (Eds.). Advance Organic chem., 4th ed. Plenum publishers, New York,2006.

21- Hori, T., Sharpless, K.B. J., Org. Chem. 1979, 44, 4204.

22- E. Hogarth, J,Chem.Soc. , 1952, p4811.

23-K. Fujii M. Yoskikawa and M. Yuasa , J.pharm. Soc.,japan ,1955, 1056-8.

24- Petrow, V., Stephenson, O., Thomas, A., Wild,J.Chem.Soc.,1958,1506-8.

25- M. B. Smith, and J. March (Eds.). March Advanced Organic Chemistry ,6th ed. John Wiley and Sons, Inc., New Jersey, U.S.A., 2007, pp.1411, 425, 1780-1783.

26-Ingold, C.K. (Eds.). Structure and mechanism in organic chemistry , 2nd ed., Cornell University Press, USA, 1969. pp. 1129-1131.

27- Jerry, M. (Eds.). Advances organic chemistry , 4th ed., 1992; pp. 378.

28- John McMurry (Eds.). Organic Chemistry , 7th ed. ,Thomson Learning. Inc., U. S. A., 2008, Chapter 21, pp.794-811.

29- Robert, M. Silverstein, Francis X. Webster and David, J. Kiemle(Eds.). Spectrometric Identification of Organic Compounds , 7th ed. John Wiley and Sons, Inc. U.S.A., 2005, PP. 72-108.

30- Robert Kiss, Mark Sandor, and Ferenc A Szalali. (2012), Public web service for drug discovery, J Cheminform, 4(Suppl 1):P17.

31- Garavito, R.M., Malkowski, M.G. , DeWitt, D.L, Prostagl. & Other Lip. Med., 2002, 68(69),129–152.

32- Professor, Computer Graphics Laboratory, Department of Pharmaceutical Chemistry, University of California, 600 16th Street, San Francisco, CA 94143-2240, USA.

33- Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J. ,Adv. Drug Deliv. Rev., March 2001.46 (1-3), 3–26.

34- Lipinski, C.A., Drug Discovery Today: Technologies, December 2004, 1 (4), 337–341.

35- Oprea, T.I., Davis, A.M., Teague, S.J., Leeson , P,D., J Chem Inf Comput Sci,. 2001, 41 (5), 1308–15.

36- Leeson, P.D., Springthorpe, B. , Nat Rev Drug Discov., November 2007, 6 (11), 881–90.

37- Leo, A., Hansch, C., Elkins, D., Chem Rev., 1971, 71 (6),525–616.