RESEARCH
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DESIGN, MOLECULAR MODELING, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NEW NSAIDS DERIVATIVES WITH POSSIBLE COX-2 SELECTIVITY

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ABSTRACT
Background: Effective treatment strategies are required to address the numerous health issues brought on by inflammation. A famous group of drugs used to treat inflammations includes nonsteroidal anti-inflammatory drugs (NSAIDs). Additionally, their indiscriminate inhibition of cyclooxygenase (COX) leads to many undesirable outcomes. The use of selective COX-2 inhibitors has shown promise in reduction of these hazards without sacrificing therapeutic efficacy.
Aim: Creating, synthesizing and evaluating new NSAID compounds that may be COX-2 selective was the objective of this paper. Predicting their binding affinities and modes of interaction with COX-2 enzyme as well as the use of molecular modeling to predict these with synthesized derivatives.
Methods: The synthesis of amide derivatives was done by combining cysteine, aspartic acid and tyrosine with the already-approved NSAID such as diclofenac, mefenamic acid and indomethacin. The structures of these derivatives were characterized after their synthesis through formation of amide bonds via spectroscopic methods. In molecular modelling research carried out, molecules were docked using the Molecular Operating Environment (MOE) program to assess ligand-receptor interaction.
Results: Binding affinities of synthesized NSAID derivatives differed. Through docking studies, this was evident in compound 2 having the highest S score (-8.40), which meant a remarkable COX-2 enzyme binding. The compound with the lowest RMSD value (1.36) was compound 6 which indicated a favorable binding geometry. Particular interactions between amino acids were found which emphasized hydrogen bonding with important residues like Leu 517 and Arg 106.
A comparison of the synthesized compounds with the well-known COX-2 inhibitors celecoxib and diclofenac revealed possible therapeutic benefits.
Conclusion: The developed NSAID compounds exhibited encouraging COX-2 binding affinities and interaction patterns and pointing to possible COX-2 selectivity. To evaluate these compounds' potential therapeutic uses, more research is needed to determine their pharmacokinetic profiles and in vitro and in vivo anti-inflammatory properties.
KEYWORDS: NSAIDs, COX-2 selectivity, drug design, molecular modeling, anti-inflammatory activity, rational drug design, structure-activity relationship (SAR), spectroscopic analysis, synthetic routes, computational chemistry, in vitro assays, pharmacokinetics

1. INTRODUCTION

The body uses inflammation as a defense mechanism against viruses, damaged cells and irritants, among other adverse stimuli. But if this reaction gets out of control or lasts longer than necessary, it causes inflammatory conditions like rheumatoid arthritis, asthma and even some types of cancer (Bindu et al., 2020). There is no doubt that nonsteroidal anti-inflammatory drugs (NSAIDs) are important to use for managing pain, fever and inflammation (Sharma et al., 2019). It is their efficacy not only that this comes from

the fact that they block cyclooxygenase enzymes, which cannot be synthesized into prostaglandins and thromboxane from arachidonic acid without these enzymes (Mohsin & Irfan, 2020). Some of the most essential ones include COX-1 and COX-2 isoforms with COX-2 being highly involved in initiation of inflammatory responses (Ju et al., 2022).

However, long term usage of these drugs has been associated with a number of side effects. These reactions include ulcers in the gut as well as damage to the kidney brought about by inhibiting naturally occurring COX-1 enzyme (Rouzer & Marnett, 2020). In this regard, specific COX-2 inhibitors have been developed in order to lower these consequences (Sharma et al., 2019). Celecoxib as well as rofecoxib were some of the earliest selective COX-2 inhibitors given a nod for commercial purposes. Nonetheless, apprehensions were raised over their application connecting them with heart-related problems (Carullo et al., 2017). It is therefore still very imperative to look out for fresh NSAIDs having an advanced safety profile but not restricted selectivity against onlyCOX-2(Mohsin et al., 2022).

Diaryl substituents attached to a central heterocyclic core ring are atypical of selective COX-2 inhibitors, according to Arora et al. (2020). There is increased COX-2 inhibition by some replacements like methyl sulfonyl groups. The activity of COX-2 inhibitors has been discovered in imidazo[1,2-a] pyridines and this has generated interest (Rayar et al., 2017). Knowing the structural basis for COX inhibition is required for designing better NSAIDs with enhanced efficacy and safety profiles (Mahboubi Rabbani & Zarghi, 2019).

In the case of arachidonic acid conversion into prostaglandins and thromboxanes, enzymatic action is carried out by COX enzymes; mostly inflammation related inducible enzyme known as COX-2 (Sharma et al., 2019). Apart from these compounds being anti-inflammatory agents, selective COX-2 inhibition reduces gastro-intestinal side effects that are a characteristic feature of non-selective NSAIDS (Bindu et al., 2020). By means of modulation of NF-κB pathway that results in decrease in production rate pro-inflammatory cytokines, functionally active molecules inhibit cox-2 pathway (Rouzer & Marnett, 2020).

The opportunity for selective inhibition can be realized through disparate architectures between the active site regions of COX-1 and COX-2 that will create nonsteroidal anti-inflammatory drug alternatives which are safer. While mainly catalyzing synthesis of prostaglandins within gastrointestinal tract, it gets activated into inflammatory processes whereby Cox 2 takes part in metabolism (Rayar et al., 2017).

In order to improve therapeutic outcomes and identify rational drug candidates, one must gain knowledge on the structural and functional characteristics of COX enzymes (Arora et al., 2020). Crystallographic investigations have been used to elucidate the mechanisms involved in COX inhibition as well as its interaction with NSAIDs (Mahboubi Rabbani & Zarghi, 2019). They are currently looking at diaryl and non-diaryl heterocyclic scaffolds that can lead to a new generation of NSAID drugs which have shown significant inhibitory properties against COX-2 and strong anti-inflammatory activities (Carullo et al., 2017).

The goal of this paper is to design and synthesize novel NSAIDs that are selective for COX-2. Amino acids such as cysteine (Cys), aspartic acid (Asp), and tyrosine (Tyr) were added to the parent NSAIDs, which included diclofenac (DF), mefenamic acid (MA) and indomethacin (ID), throughout the design process. These amino acids were selected because of their biological activity and potential interactions with COX-2 enzymes.

2. METHADOLOGY

2.1 Design

In order to develop novel NSAID derivatives that possibly is COX-2 selective, it was necessary to select certain amino acids for conjugation with the existing NSAIDs. For instance, this selection was based on previous studies indicating improved therapeutic profiles and enhanced COX-2 selectivity. To illustrate, we particularly focused on combination of diclofenac with tyrosine or mefenamic acid and indomethacin with various amino acids like cysteine, aspartic acid or tyrosine respectively. Similarly, these specific combinations were chosen consideration of their structural features and probable contact points with the COX-2 enzyme.‹

2.2 Chemical Synthesis

Synthesis of the NSAID derivatives required the formation of their carboxylic acid groups and the amine groups of their amino acids. For each derivative, bases like 4-dimethylaminopyridine (DMAP) or hydroxy benzotriazole (HOBT) were employed. Then Dicyclohexylcarbodiimide (DCC) or N,N'-diisopropylcarbodiimide (DIC) was used as a suitable coupling agent. The reactions ware made effective for coupling by performance of them at stoichiometric ratios of reactants and in appropriate solvent media. Column chromatography and recrystallization were the two methods that had to be employed to purify intermediates and end products (Shah et al., 2017). The sources for

diagrams representing complete synthesis procedures for each chemical (Shah et al., 2017).

● Cysteine in combination with mefenamic acid

This reaction occurs when the mefenamic acid carboxylic acid group and the amino group of cysteine interact during production.

●Aspartic acid with mefenamic acid

Also, this reaction occurs when the amino group of aspartic acid and the carboxylic acid group of mefenamic acid react.

●Tyrosine and indomethacin together

Additionally, occurs when the carboxylic acid group of indomethacin and the amino group of tyrosine react.

● Indomethacin coupled with cysteine

During the synthesis process, the amino group of cysteine and the carboxylic acid group of indomethacin interact.

● Tyrosine combined with digoxin

Diclofenac's carboxylic acid group reacts with tyrosine's amino group during the synthesis process.

● Tyrosine combined with mefenamic acid

Mefenamic acid's carboxylic acid group reacts with tyrosine's amino group throughout the synthesis process.

2.3 Molecular Modeling

The Molecular Operating Environment (MOE) software, version 15, was used for molecular modelling investigations. After being precisely sketched in ChemDraw Professional, the chemical structures of the synthesized NSAID derivatives were imported into MOE. To maximize the conformations, the structures underwent three-dimensional protonation, partial charges were applied and energy minimization was carried out. After the target enzyme, cyclooxygenase-2 (COX-2), crystal structure was obtained from the Protein Data Bank (PDB code:). It was docked with the ligands that were synthesized. Docking simulations were performed to predict the ligand-receptor binding affinities and modes of interaction after the protein's active site was identified.

2.4 Synthesis Characterization

Some spectroscopic methods were used to characterize the derivatives of NSAIDs synthesized, including mass spectrometry (MS), infrared (IR) and nuclear magnetic resonance (NMR) spectroscopies. The chemical structures were confirmed and the purities assessed by NMR spectra. Infrared spectroscopy was used to identify the functional groups of these compounds. MS analysis revealed molecular weights of the substances, which validated that synthesis was effective.

2.5 Biological Evaluation

COX-2 selective inhibitors – COX-2 selective inhibitors are NSAID derivative that have been tested for their potential through a biological evaluation. Enzymatic techniques familiarly known as common enzymatic techniques were employed in this research to establish the inhibitory activity exhibited by drugs on COX-2. The derivatives' selectivity over COX-1 was determined, and IC50 values calculated for inhibition concentrations. Cell viability experiments were also performed for detecting cytotoxicity of substances thus revealing their safety in possible medical applications.

3. Results and Discussion

3.1 Docking of NSAID Derivatives

The binding affinities and pattern of interaction of synthetic NSAID derivatives with the COX-2 enzyme active site were ascertained using molecular docking experiments (Dunya et al., 2022). Heidarpoor et al. (2021) reported that the Molecular Operating Environment (MOE) program made the visualization and study of ligand-receptor interactions easier. Docking results indicated significantly different binding affinities of diverse derivatives as evidenced by individual S scores. This is exemplified by Compound 2 which had an S score of -8.40, highest among all compounds, indicating a strong affinity for COX-2 enzyme. On the other hand, compound 3 scored the lowest (-3.02). It means this compound binds relatively poorly (Table 1).

3.2 Analysis of RMSD Values

The natural ligand bound to the COX-2 active site and the docked ligands were compared structurally using root mean square deviation (RMSD) values, which were calculated (Leão et al., 2020). Reduced RMSD values indicate a more favorable binding geometry and a closer structural likeness. The compounds with the lowest RMSD values in our study—1.69 and 1.36, respectively showed close alignment with the native ligand. Compound 3, on the other hand, showed the greatest RMSD value of 2.39. This show a less ideal binding conformation (Table 1).

3.3 Identification of Amino Acid Interactions

Certain amino acid residues important in ligand binding inside the COX-2 active site were identified through analysis of the docking postures (Oniga et al., 2017). The finding of hydrogen bonding connections between the NSAID derivatives and significant amino acid residues allowed for the understanding of ligand-receptor interactions. As an illustration, Compound 1 formed hydrogen bonds with Arg 106 and continued to interact with Val 509. Compound 4 showed hydrogen bonding

with Ser 516, but compound 5 formed hydrogen connections with Arg 106 and additional interactions with Val 74. Compound 6 on the other hand showed interactions with Leu 517 (Table 1).

3.4 Comparison with Celecoxib and Diclofenac

In comparison with that of diclofenac and celecoxib, the docking results were compared. Celecoxib had an affinity to bind well and a structure resembling the native ligand as demonstrated by its low RMSD value (0.88) and high S score (-9.01). In contrast to celecoxib, diclofenac demonstrated less optimal binding properties with lower S score (-6.17) and slightly higher RMSD (1.12). The interaction of the synthesized NSAID derivatives and reference drugs was further visualized in terms of hydrogen bonds as well as other interactions within amino acid residues in order to elucidate the differences in their binding modes (Bello-Vargas et al., 2023) (Table 1).

The resultant data in Table 1 presents interactions of combined NSAID derivatives with particular enzyme amino acid residues, S scores and RMSD values as well as docking results with COX-2 enzyme. By employing their binding affinity and structural similarity to two established COX-2 inhibitors, celecoxib and diclofenac, this study examines the probable therapeutic potentials of these compounds as COX-2 inhibitors.

Compounds	**S score**	**RMSD**	**H bonds interaction with A.A residue**	**Other bonds interaction with A.A residue**
1	-7.86	1.91	Arg 106	Val 509, Arg 106
2	-8.40	1.69	Arg 106	-
3	-3.02	2.39	-	-
4	-6.23	1.71	Ser 516	-
5	-4.26	2.77	Arg 106	Val 74
6	-6.36	1.36	-	Leu 517
Celecoxib	-9.01	0.88	Arg 499	-
Diclofenac	-6.17	1.12	-	-

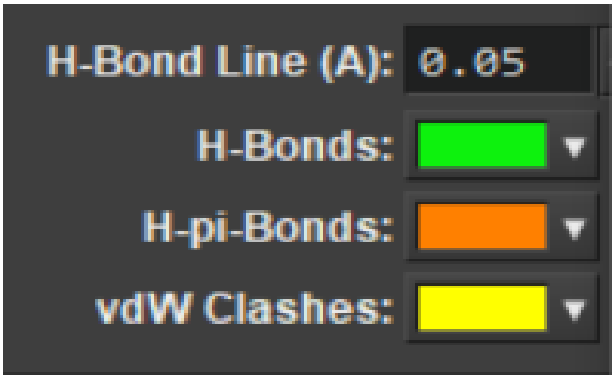

Figure (1): represent the type of bond in 3D picture

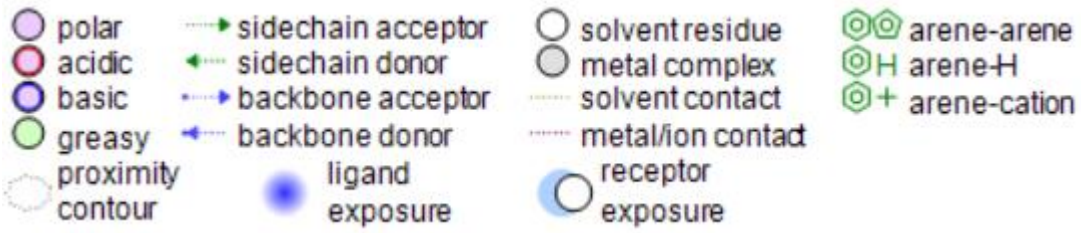

Figure (2): represent the type of bonds in 2D picture

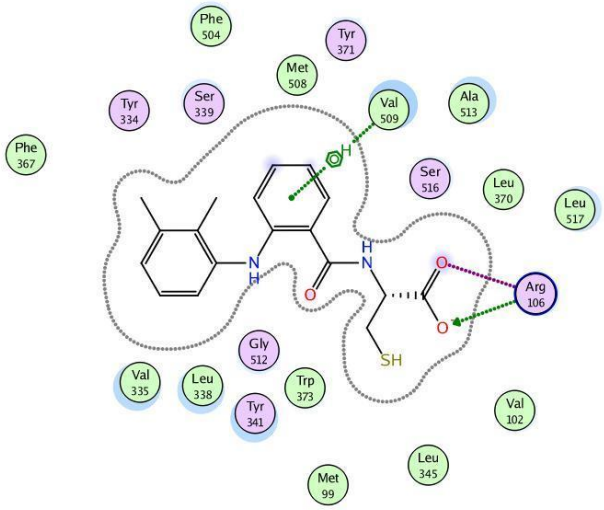

Figure (3): represent 2D pic

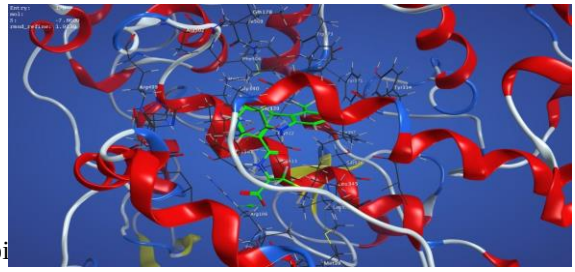

Figure (4): represent 3D pi

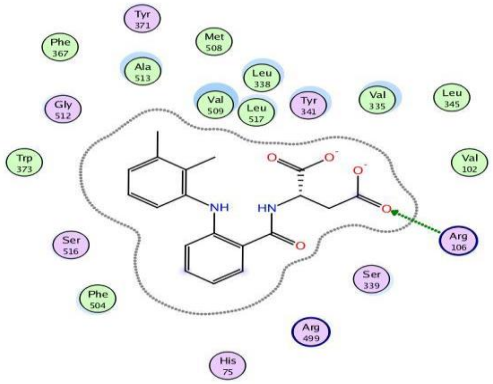

Figure (5): represent 2D picture of compound (2)

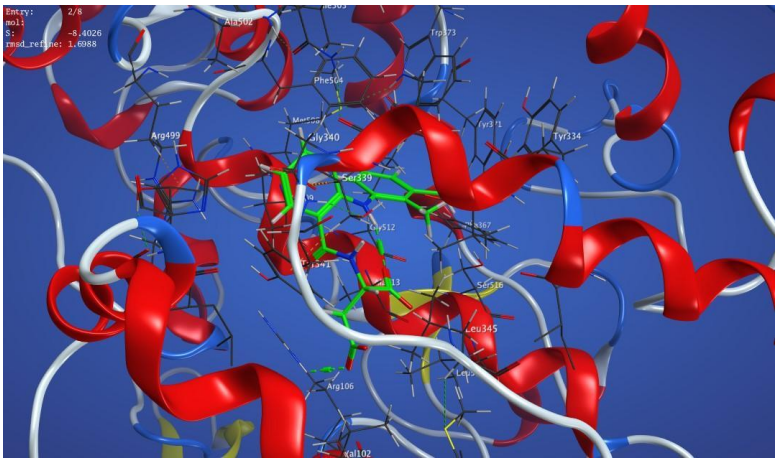

Figure (6): represent 3D picture of compound (2)

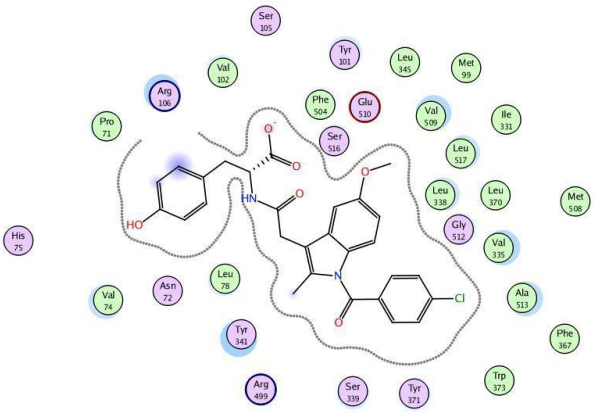

Figure (7): represent 2D picture of compound (3)

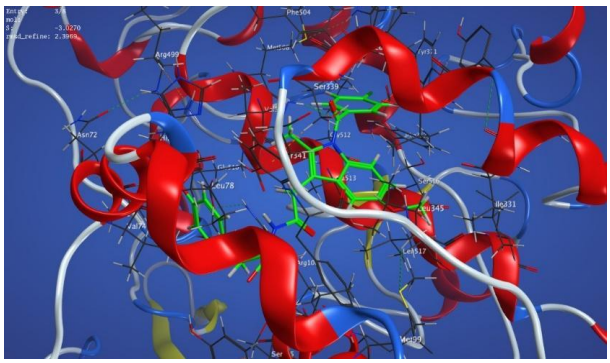

Figure (8): represent 3D picture of compound (3)

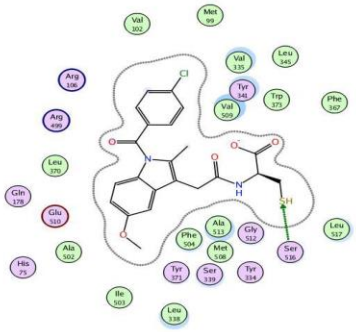

Figure (9): represent 2D picture of compound (4)

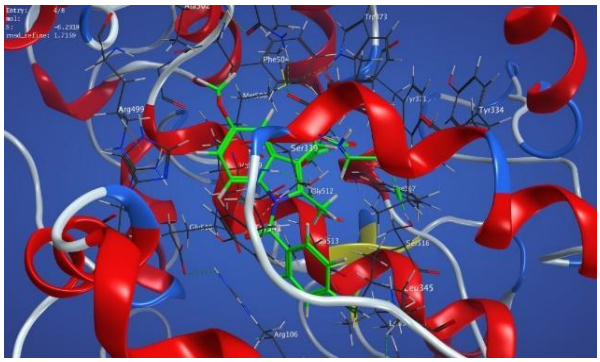

Figure (10): represent 3D picture

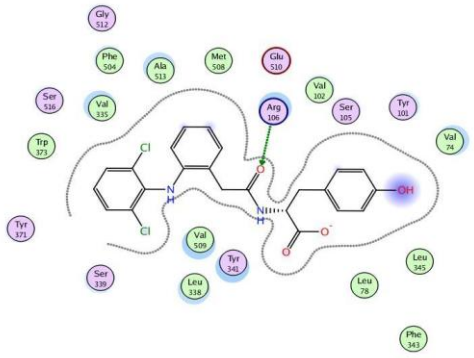

Figure (11): represent 2D picture of compound (5)

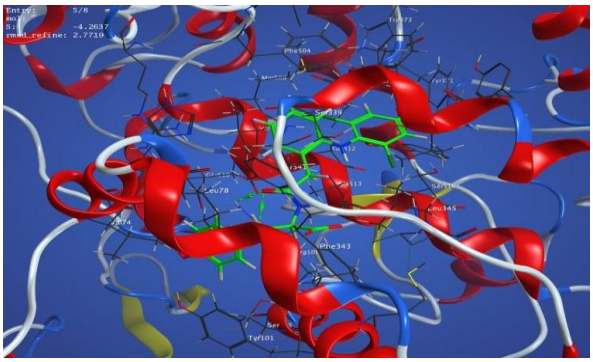

Figure (12): represent 3D picture of compound (5)

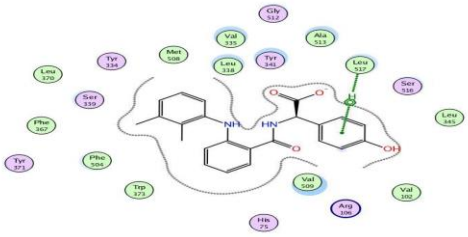

Figure (13): represent 2D picture of compound (6)

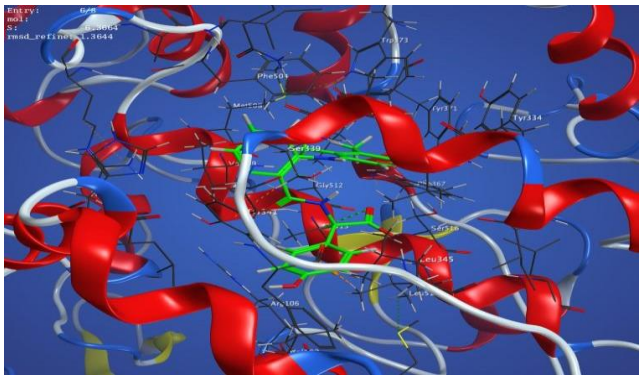

Figure (14): represent 3D picture of compound (6)

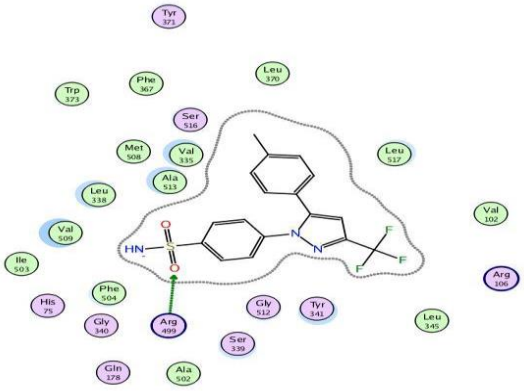

Figure (15): represent 2D picture of celecoxib

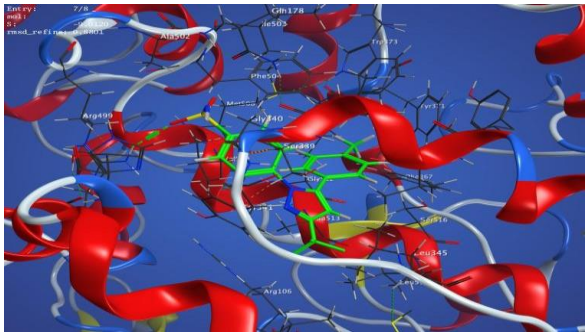

Figure (16): represent 3D picture of celecoxib

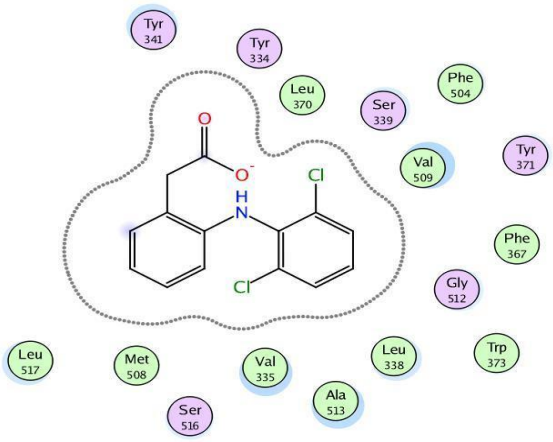

Figure (17): represent 2D pictu

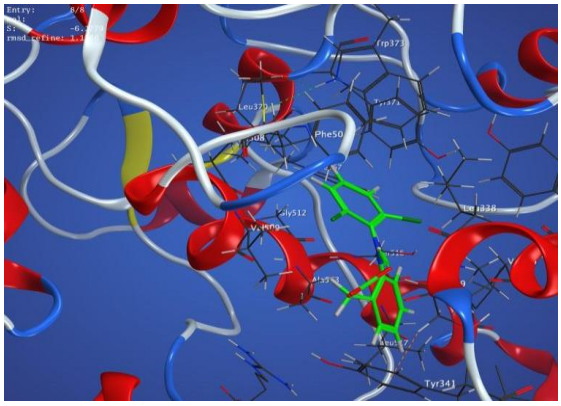

Figure (18): represent 3D picture of Diclofenac

4. CONCLUSION

Designing, creating and testing of new NSAID compounds with COX-2 selectivity was the target of this paper. 6 variants were made by combining various amino acids with mefenamic acid, indomethacin and diclofenac. The docking studies exhibited the different binding affinity levels and COX-2 interaction patterns of the synthesized compounds. Compounds 2 and 6 were characterized with a high S score (−8.40 and −6.36 respectively) as well as low RMSD values (1.69 and 1.36 decades respectively), which led to significant binding affinity and favorable structural alignment with active site COX-2 in these inhibitors. Identification of specific amino acid residues participating in ligand binding highlighted the molecular interactions underlying COX-2 inhibition process. In addition, comparative analysis with well-known celecoxib and diclofenac for COX-2 inhibitors indicated possible therapeutic efficacy of synthetic derivatives thereof... Other investigations are needed to confirm their inhibitory effects on COX-2 through in vitro experiments, pharmacokinetic studies among other tests for them to be used as safe alternatives to anti-inflammatory drugs that we have today in the market place.

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