Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

Section A: Chemical Sciences

CODEN (USA): JCBPAT Research Article

Discovery of Potent of New Amino Acid Derivatives against Cycoloxgynase (Cox-2)

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Received: 20 April 2015**; Revised:** 10 May 2015**; Accepted:** 16 May 2015

Abstract: The present work aims to synthesize novel coumarine derivatives containing amino acid moiety. The synthesized compounds characterized with ^{1}H-NMR spectra. The X-Ray crystal structure do not discovered yet, so, the molecular structure of compounds was studied using semi empirical with PM3 molecular orbital theory. To discovery efficiency of synthesized compounds against COX-2 the docking study was preformed, the lowest energy of docked compound, which interacted with active site residues, perhaps could be making them possible selective inhibitors against (COX-2) and physiologically active.

Keywords: COX-2, docking and ADMET.

INTRODUCTION

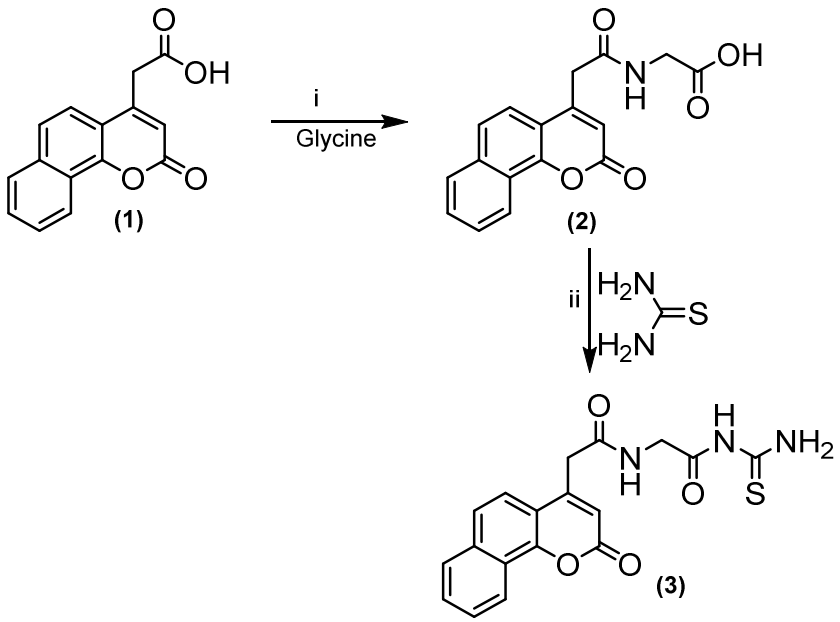
Non-steroidal anti-inflammatory drugs (NSAIDs) are widely employed in musculoskeletal disease, as well as their anti-inflammatory properties [1]. After widely evaluation, NSAIDs is efficacy in different clinical setting, and act as COX inhibitor (COX-1 and COX-2) through inhibiting the production of prostaglandins (PGs) [2-5].

The NASIDs suffer from a common toxicity of gastrointestinal drawback, due to inhibition non-selectively of cyclooxygenase enzyme [6-8], also, its display anti-microbial [9-11], ulcerogenic,

analgesic, anti-inflammatory, lipid peroxidation [12, 13], antitumor [14] and inhibitor formation of transthyretin amyloid fibril properties [15]. Also, the amino acid derivatives especially containing amide and thioamide moieties possess diverse biological activities, such as anti-inflammatory, anti-tumor and antimicrobial activity [16-21], CNS depressant, anticonvulsants [22], anti-inflammatory [23] and analgesic activities [24]. Hence, the present study aims to synthesis new series of coumarine acetic acid derivatives acting as new potent inhibitor against COX-2 with low toxicity effects, followed by molecular modeling to identify the structural features of these new series. The molecular docking was preformed, to predict the correct binding geometry for each ligand in the active site, which may be support that postulation, its active compounds may be act as a new NASIDs.

RESULTS AND DISCUSSION

Chemistry: The starting benzocoumarin-4-acetic acid **(1)** was easily prepared according to the previously reported procedure [25]. The compound **(1)** was fused with glycine to affording (2-(2-oxo-2H-benzo[h]chromen-4-yl) acetyl) glycine **(2)** [25], the N-carbamothioyl-2-(2-(2-oxo-2H-benzo[h]chromen-4-yl) acetamido) acetamide **(3)** was obtained via reaction compound (**2)** with thiourea (**Scheme 1**).



Scheme 1: Reagent and conditions:i-Gly./ 180-195°C, ii-thiourea/150-160°C.

The HNMR proton of compounds (**3**) showed, the characteristics bands at 13.60 of imino group of thiourea and 5.98 ppm for H protons of amino group for thiourea group. The characteristic band for aromatic proton for naphthalene moiety at 7.35-7.86ppm, the bands at 6.49 ppm for imino group of glycine.

MOLECULAR MODELING STUDIES

Conformational Analysis: In trying to achieve better insight into the molecular structure of the most stable forms for compounds **(2,3)**, the conformational analysis of the target compounds has been performed using the MMFF94 force-field [26] (calculations in vacuo, bond dipole option for electrostatics, PolakeRibiere algorithm, RMS gradient of 0.01 kcal/ mol) implemented in MOE [27]. The most stable conformer for **2,3** were fully geometrical optimized with molecular orbital function PM3 semi-empirical *Hamiltonian* molecular orbital calculation MOPAC 7 package [28].

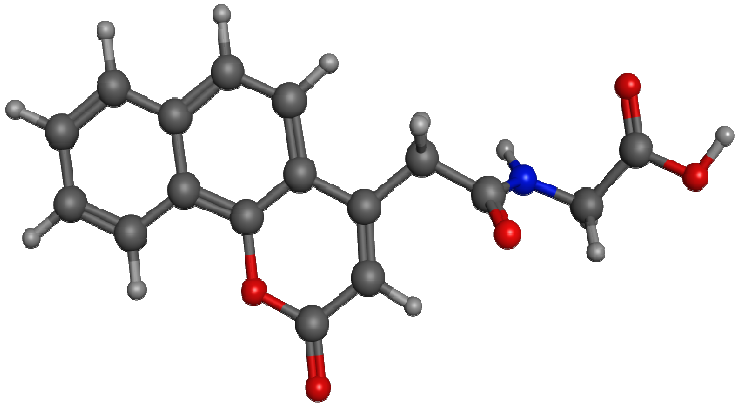


Figure 1: The Lowest energy conformers of the compound 2 at PM3 semi-empirical molecular orbital in rendering ball and stick.

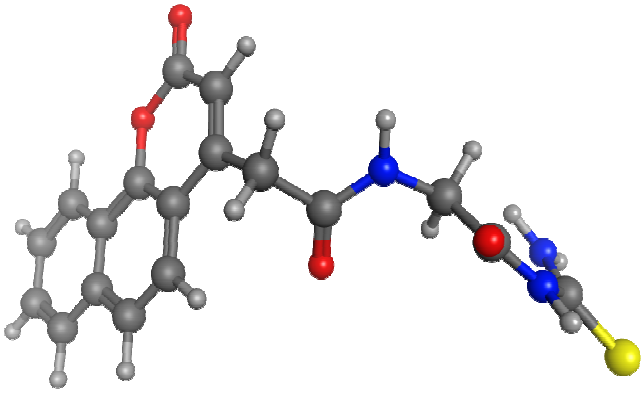


Figure 2: The Lowest energy conformers of the compound **3** at PM3 semi-empirical molecular orbital in rendering ball and stick.

The computed molecular parameters, total energy, electronic energy, heat of formation, the highest occupied molecular orbital (HOMO) energies, the lowest unoccupied molecular orbital (LUMO) energies and the dipole moment for studied compounds were calculated. **Table-1, Figure 1, 2**.

Table-1: The Optimized Calculations Energies at PM3 molecular orbital for 2, 3.

Cpd	*E*	*E-ele*	*HF*	*IP*	*HOMO*	*LUMO*	*μ*
2	-88666.5	-609149	-149.48	-8.92	8.92	-1.09	5.30
3	-97088.7	-725801	-69.752	-8.95	8.95	-1.29	8.51

E: Total energy (kcal/mol). ***E-ele***:Electronic energy (kcal/mol),***HF***: Heat of formation (kcal/mol), ***IP***: Ionization potential energy(kcal/mol), ***HOMO***: Highest Occupied Molecular Orbital(eV), ***LUMO***: Lowest Occupied Molecular Orbital(eV), **μ***: Dipole moment*(*Deby)*.

The lowest minimization energy for the structures **(2, 3)** were exhibited common arrangement of naphthalene ring in plane with pyran ring, as shown in **Figure1, 2**, the higher HOMO energy values show the molecule is a good electron donor, on other hand, the lower HOMO energy values indicate that, a weaker ability of the molecules for donating electron. LUMO energy presents the ability of a molecule for receiving electron.

BIOLOGICAL SCREENING

Docking Studies: In brief, two isoforms of COX protein are known: COX-1, is responsible for the physiological production of prostaglandins, which is expressed in most tissues; and COX-2, is responsible for the increasing production of prostaglandins during process of inflammation, which is induced by endotoxins, cytokines and mitogens in inflammatory cells [29].

Recently, from analysis of X-ray co-crystal of arachidonic acid with COX-2 showed that, carboxylate coordinated with Tyr-385and Ser-530 [30], as well as the action of NSAIDs, through the interaction carboxylate group with Tyr-385 and Ser-530, which stabilize the negative charge of the tetrahedral intermediate [30], and demonstrated that, Tyr-385 and Ser-530 have a structural and functional evidence for the importance of them in the cheating of the ligands [30].

Molecular docking of the synthesized compounds into the active site of COX-2 was performed, in order to understanding the biological data on a structural basis, through rationalized ligand–protein interaction behavior. All calculations for docking experiment preformed with MOE 2008.10 [27]. The tested compounds were evaluated in silico (docking), using X-ray crystal structures of COX-2 (ID: 1PXX) [30].

Table-2: Binding Scores Derived from the MOE Docking Tools.

Cpd.	*d.G.*	*Int.*	*H.B.*	*E-ele.*	*Evdw.*
1	-9.817	-20.01	-10.90	-7.95	-10.14
2	-12.16	-23.61	-18.34	-8.14	-12.50

d.G.*:** free binding energy of the ligand from a given conformer, ***Int.: affinity binding energy of hydrogen bond interaction with receptor, ***H.B.:*** Hydrogen bonding energy between protein and ligand. ***E-ele:*** the electrostatic interaction with the receptor**,** ***Evdw:*** van der Waals energies between the ligand and the receptor.

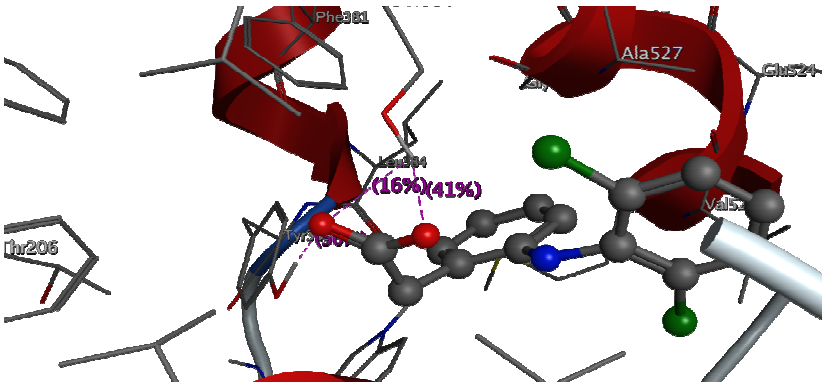


Figure 3: The reference molecule Docked into the active site of COX-2.

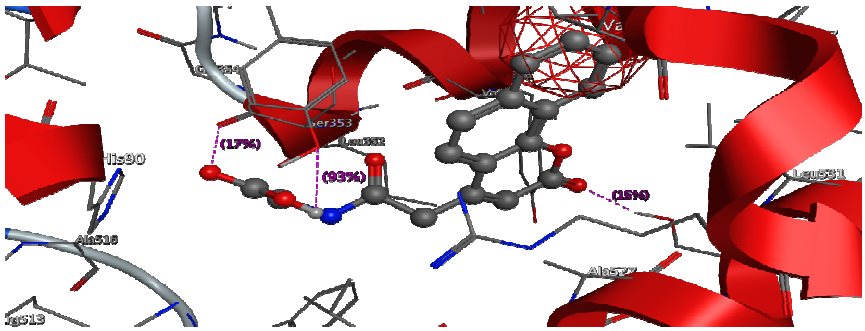


Figure 4: Interaction between ligand (**2**) and binding site of COX-2.(1PXX, PDB code), which pink dot lines represented hydrogen bonding interaction of ligand (**2**) with binding site. Ligand (**2**) are represented in ball and stick mode, which carbon atoms are colored in dark grey, oxygen in red, nitrogen in blue and sulfur in yellow. Hydrogen atoms of the amino acid residues and ligand were removed to improve clarity.

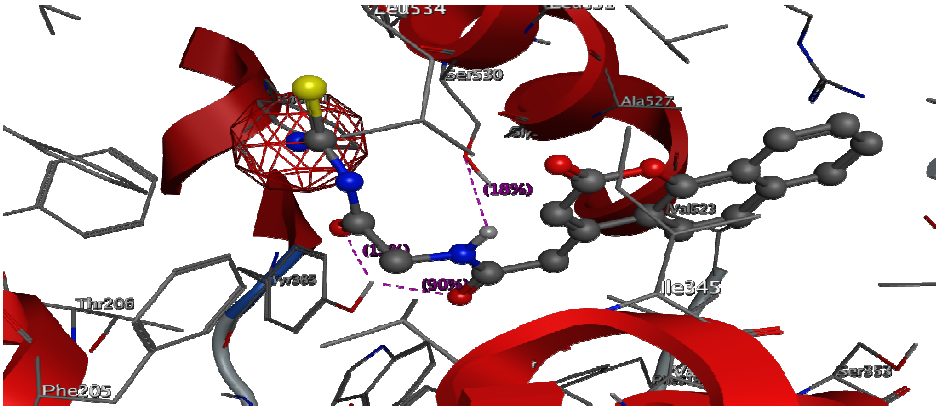


Figure 5: Interaction between ligand (**3**) and binding site of COX-2. (1PXX, PDB code), which pink dot lines represented hydrogen-bonding interaction of ligand (**3**) with binding site. Ligand (**3**) are represented in ball and stick mode, which carbon atoms are colored in dark grey, oxygen in red, nitrogen in blue and sulfur in yellow. Hydrogen atoms of the amino acid residues and ligand were removed to improve clarity.

The tested compounds docked into active sites of enzyme COX-2. The active site of the enzyme was defined to include residues within a 10.0 Å radius to any of the inhibitor atoms. MOE scoring function of the most stable docking model for tested compounds applied to evaluate the binding affinities between the inhibitors complexes with (COX-2) active site, **Table-2**. The complexes were energy-minimized with an MMFF94 force field [31] until the gradient convergence 0.05 kcal/mol reached. The most active compounds docked successfully into the COX-2 active site.

From **Figures 3-5, Table-2** the following results can be drawn: the compounds **2** and **3** were arranged with binding pocket in perpendicular mode by adjusting of glycyl in compound **2** thiourea in compound **3** fragments in a perpendicular position with a phenyl ring of Tyr-385**.**

ADMET factors profiling: Oral bioavailability was considered playing an important role for the development of bioactive molecules as therapeutic agents. Many potential therapeutic agents fail to reach the clinic because their ADMET (absorption, distribution, metabolism, elimination and toxic) Factors. Therefore, a computational study for prediction of ADMET properties of the molecules was performed for tested compounds **2, 3**, by determination of topological polar surface area (TPSA), a calculated percent absorption (%ABS) which was estimated by Zhao *et al*. equation [32], and ‘‘rule of five’’ [33]: the chemical compound could be an orally active drug in humans, if no more than one violation of the following rule:

a. C log P (partition coefficient between water and octanol < 5,

b. Number of hydrogen bond donors sites ≤ 5,

c. Number of hydrogen bond acceptors sites ≤ 10,

d. Molecular weight <500 and molar refractivity should be between 40 and 130.

In addition, the total polar surface area (TPSA) is another key property linked to drug bioavailability, the passively absorbed molecules [34] with (TPSA>140) have low oral bioavailability.

Table-3: Pharmacokinetic parameters important for good oral bioavailability of compounds **2,3.**

CPD	*HBD*	*HBA*	*CLogP*	*V*	*Vol.*	*TPSA*	*%ABS*	*Log S*	*mr*
Ref.	2	5	6.255	1	450.51	58.2	88.92	-5.267	79.32
2	2	6	2.0	0	275.32	39.23	90.98	-4.70	79.46
3	4	7	1.1	0	298.15	36.12	93.96	-6.27	74.13

TPSA*:* Polar surface area **(A^2)**, %**ABS***:* Absorption percentage, ***Vol****:* Volume (A^3), ***HBA****:* Number of hydrogen bond acceptor, ***HBD****:* Number of hydrogen bond donor, ***V:*** Number of violation from Lipinski’s rule of five. ***Log P:*** Calculated lipophilicity, ***Log S****:* Solubility parameter, ***mr:*** Molar Refractivity.

All calculated descriptors were performed using MOE Package [27], and their results were disclosed in **Table-3**. Our results revealed that, the C log P (factor of the lipophilicity was less than 5.0 [35], hydrogen bond acceptors between **(6, 7)**, hydrogen bond donors between **(2, 4)** and molar refractivity values in rang (~74-79), these data show these compounds fulfill Lipinski’s rule. Also, the absorption percent between (~ 90, 93%). The HOMO and LUMO of a molecule play important

roles in intermolecular interactions [36], through the interaction between the HOMO of the drug with the LUMO of the receptor and vice versa. The interactions were stabilized inversely with energy gap between the interacting orbital's. Increasing HOMO energy and decreasing LUMO energy in the drug molecule lead to enhanced stabilizing interactions, and hence, binding with the receptor. Furthermore, the global and local chemical reactivity descriptors for molecules have been defined **Table-3**.

CONCLUSION

In the present work aimed to the development of novel COX-2 inhibtor molecules, containing amino acid pharmacophore. Systematic structure based virtual screening of the synthesized compound library identified for the synthesized compounds as putative COX-2 binders. The results point that, 4-(2-(2-oxo-2H-benzo[h]chromen-4-yl) acetyl)-5-thioxoimidazo-lidin-2-one (**3**) considers suitable inhibitor against COX-2 with farther modification in the future.

EXPERMINTAL

Instrumentation and materials: Melting points taken on a Griffin melting point apparatus and are uncorrected. Thin layer chromatography (Rf) for analytical purposes was carried out on silica gel and developed. Benzidine, ninhydrin, and hydroxamate tests used for detection reactions. The HNMR spectra were observed on a Varian Genini-300 MHz spectrometer and chemical shifts (δ) are in ppm. The mass spectra recorded on a mass spectrometer HP model MS–QPL000EX (Shimadzu) at 70 eV. Elemental analyses (C, H, N) were carried out at the King abd ulaziz university-KSA.

SYNTHESIS

N-carbamothioyl-2-(2-(2-oxo-2H-benzo[h]chromen-4-yl) acetamido) acetamide (3): A mixture of thiourea (0.01 mol) and 2-(2-oxo-2H-benzo[h]chromen-4-yl) acetic acid (0.01 mol) was fused at 180°C in an oil bath for 15 min. The fused mass was dissolved in ethanol and poured onto cold water; the solid obtained was recrystallized from ethanol to give compound (**3**). The product was chromatographically homogeneous by iodine and benzidine development. Brown crystal : yields=73%; Rf= 0.55 (CHCl3/EtOH=3/1); mp: 170-72, IR (KBr cm-1) ν; 3343 broad band (NH,NH_2), 3016(CH-arm), 2905 (CHali), 1730(CO), 1614 (CONH) cm-1; ^{1}H NMR (300 MHz, CDCl3) δ =13.60(1H, NH), 7.35–7.86 (m, 7H- Ar-H), 6.49 (d, 2H-NHCSNH_2), 3.89(2H,2H, CH_2-coumarine), 2.36 (2H,2H, CH_2-Gly), Anal./Calcd. For $C_{18}H_{15}N_3O_4S$ (369): C (58.53%), H (4.06%), N (11.38%). Found: C (58.51); H (4.09); N, (11.58).

MOLECULAR MODELING STUDY

Generation of Ligand and Enzyme Structures

Selection of COX structures: Docking study was carried out for the target compounds into (COX-2, ID: 1PXX) using MOE, 10. The crystal structure of the (COX-2) complexes with a selective inhibitor of COX-2 in co-crystallized form in the active site of the receptor. From X-ray crystal structure studies of the COX-2enzyme, the mouse enzyme is expected to be very similar to the human, and can be used as model for human (COX-2) enzyme.

Preparation of Small Molecule: Molecular modeling of the target compounds were built using MOE, and were minimized their energy with PM3 through MOPAC. Our compounds were introduced into the (COX-2) binding sites according to the published crystal structures of reference molecule bound to kinase.

STEPWISE DOCKING METHOD

MOE Stepwise: The crystal structures of the (COX-2) with reference inhibitor molecule was used, Water and inhibitor molecule was removed, and hydrogen atoms were added. The parameters and charges were assigned with MMFF94x force field. After alpha-site spheres were generated using the site finder module of MOE. The optimized 3D structures of molecules were subjected to generate different poses of ligands using triangular matcher placement method, which generating poses by aligning ligand triplets of atoms on triplets of alpha spheres represented in the receptor site points, a random triplet of alpha sphere centers is used to determine the pose during each iteration. The pose generated was rescored using London dG scoring function. The poses generated were refined with MMFF94x forcefield, also, the solvation effects were treated. The Born solvation model (GB/VI) was used to calculate the final energy, and the finally assigned poses were assigned a score based on the free energy in kJ/mol.

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