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Research Article

Computational and Pharmacokinetic Investigation of Some Heterocyclic Amide Derivatives as Cyclooxygenase Inhibitors: An *In-Silico* Approach

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ABSTRACT

The two most significant as well as historically important non-steroidal and anti-inflammatory medications (NSAIDs), aspirin and ibuprofen, are frequently used to treat fever, pain, and inflammation. By blocking the activity of cyclooxygenase (COX), it can prevent the production of prostaglandin. In an effort to examine the physiochemical and biological properties of some heterocyclic amide derivatives and quantum mechanical computations have been used to analyze the compounds. To clarify the thermochemical, molecular orbital, and equilibrium geometrical features in the gas phase, density functional theory (DFT) with the B3LYP/6-31G basis set has been used. Binding affinities and modes of heterocyclic amide analogs have been investigated on human cyclooxygenase (COX-1 and COX-2) proteins (6Y3C and 5F19) using molecular docking as well as nonbonding interactions. Results from geometry and thermochemical analysis support the chemical sustainability of all the structures. Most of the compounds exhibited a significant affinity for binding to the receptor protein (5F19) than the standard drugs aspirin and ibuprofen. The improved pharmacokinetic features of certain derivatives with reduced acute oral toxicity were revealed by ADMET prediction. Overall, four heterocyclic amide analogs 3-6 were found to be more efficient in inhibiting COX-2 (5F19) than COX-1 (6Y3C), suggesting that they may be useful as COX-2-related inflammation drug candidates.

Key words: Cyclooxygenase (COX), Heterocyclic amide derivatives, Molecular Docking, ADMET.

INTRODUCTION

Aspirin has been used as a medication to alleviate fever and inflammation for more than a century. The biological action of aspirin has been investigated previously.[1-3] Additionally, Ibuprofen is also one of the widely utilized and most frequently prescribed NSAID.[4,5] Both aspirin and ibuprofen are inclusive inhibitor of cyclooxygenase-1 (COX-1) and also Cyclooxygenase-2 (COX-2).[6,7] Aspirin's anti-platelet effect has generally had beneficial results when administered at low doses to treat coronary artery disease, pregnancy-induced high blood pressure, and other conditions in angiotensin-sensitive primigravida.[8] On the contrast, ibuprofen may have fewer anti-inflammatory activities than certain distinct NSAIDs, but it has a significant anti-pyretic and analgesic performance.[9] Ibuprofen is a medication used to treat moderate discomfort, fever, and irritation, which are caused by the body's generation of prostaglandins. Prostaglandins play a crucial part in the development of pain, fever and inflammation.[10] However, some complications of aspirin, such as gastrointestinal damage and oxidative stress, are very dangerous.[11] Clinical research demonstrated that COX-2 selective drugs with anticancer activity have low gastrointestinal symptoms. Nitric oxide (NO)-releasing moiety can be added to aspirin to minimize its negative effects on the gastrointestinal tract.[12] Besides, along with advantages of ibuprofen, it also has some drawbacks. It raises the risk of liver, renal, and cardiac failure. At low dosage, the drug does not seem to raise the risk of a heart attack; however, the risk may rise at higher dosage.[10] The prolonged use of NSAIDs, such as Ibuprofen may cause substantial gastro-intestinal (GI) toxicity.[13] In order to improve the safety profile of NSAIDs, drug design techniques based on chemical modification have been developed. A study of the literature revealed that modifying the carboxylic function of some typical NSAIDs increased their anti-inflammatory performance and lowering their ulcerogenic effect.[14] According to past studies, several ibuprofen analogues comprising 1,2,4-triazole and 1,3,4-thiadiazole heterocyclic cores show greater anti-inflammatory effect and have low GI toxicity.[13,15] Therefore, developing new heterocyclic compounds that effectively suppress COX-2 rather than COX-1 may be preferred.[16] In order to create novel drug candidates with superior therapeutic qualities, it is a typical method in pharmaceutical enquiries to use well-known drugs as lead compounds. Less anti-inflammatory and ulcerogenicity action are displayed by the pro-drugs compared to the parent drugs.[17] Aspirin and ibuprofen, among other NSAIDs, have free carboxylic acid groups that interact with the cyclooxygenase active site residues in crucial ways.[18] The screening of aspirin and ibuprofen, free carboxylic groups appear to be primary cause of the reduced topical irritating action.[19] In order to increase their analgesic action and reduce their ulcerogenic effects, aspirin and ibuprofen have been transformed into a variety of heterocyclic amide analogues.[20,21] So, developing new heterocyclic amide analogues along with enhanced cyclooxygenase (COX) and anti-cancer activity, as well as fewer gastrointestinal side effects, would be a sensible option. Recent research has demonstrated that heterocyclic derivatives with aspirin and ibuprofen have COX activity.[22] In this

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study, we report the findings of assessments of few heterocyclic amide analogs utilizing thermodynamic, molecular orbital, electrostatic potential, geometric, FT-IR, UV-Vis, molecular docking, non-bonding interactions, and ADMET prediction methods. The most of the derivatives revealed improved binding affinities, interactions, and thermal and chemical stability. In biological processes, hydrogen bonding is important because it influences thermodynamic and structural stability of the system.[23-25]

MATERIALS AND METHODS

Structures of aspirin (1), ibuprofen (2) and heterocyclic amide derivatives (3-6)

In this research, aspirin (1), its 2-acetoxybenzoyl amides with heterocycles (3, 4) and ibuprofen (2), its 2-(4-isobutylphenyl) propionoyl amides with heterocycles (5, 6) are selected for thermochemical, spectral, biological and toxicological studies in Figure 1. These compounds were already synthesized by the research group Ibrahim *et al.*[22] Structurally, all investigated compounds (3-6) contain N-[5- ethyl -1,3,4-thiadiazole] and N-[5-tri fluoro methyl-1,3,4-thiadiazole] groups which are connected with phenyl acetate and 2-(4-Isobutyl phenyl)-propionate via amide functional group in heterocyclic amides with aspirin (3, 4) and heterocyclic amides with ibuprofen (5, 6) respectively (Figure 1).

DFT based optimization

The fundamental geometry of aspirin (1) (CID 2244) and ibuprofen (2) (CID 3672) were collected from online structural database PubChem.[26] By using these structures, the required molecules 3-6 were drawn in the Gauss View (5.0) application.[27] The compounds are subjected to structural optimization after being saved separately. The B3LYP hybrid density functional technique in DFT (density functional theory) was applied in this study.[3] The basis set 6-31G was used where optimization in a core i5 laptop required several days. These optimal structures were retrieved and used appropriately during molecular docking. It was emphasized that optimized structures have more precise binding scores compared to non-optimized molecules.

Thermodynamic properties, molecular orbital's and chemical reactivity descriptors

The optimal structures were utilized to predict a number of thermodynamic parameters employing GaussView and WebMD.[29] The distinct characteristics of lowest unoccupied molecular orbital (LUMO) and also the highest occupied molecular orbital (HOMO) both contribute to their inherent reactivity. The values of HOMO and LUMO were computed and converted into electron Volt. The following formulae have been applied to study the molecular orbital features as well as compute the global chemical reactivity;[30] energy gap, $\Delta\varepsilon = \varepsilon LUMO - \varepsilon HOMO$; ionization potential, $I = -\varepsilon HOMO$; electron affinity, $A = -\varepsilon LUMO$; electronegativity, $\chi = (I+A)/2$; chemical potential, $\mu = -(I+A)/2$; hardness, $\eta = (I-A)/2$; electrophilicity, $\omega = \mu^2/2\eta$; softness, $S = 1/\eta$. All chemical reactivity identifiers are compared with aspirin (1) and ibuprofen (2), a commonly employed non-steroidal anti-inflammatory drug. The DOS plots are generated by GaussSum 3.0. Additionally, the molecular electrostatic potential (MEP) was reported in an online WebMO demo server performed on same degree of DFT and MEP calculation.[31,32]

ADMET and Lipinski rule

These heterocyclic amide derivatives **(3-6)** were searched in the SwissADME (http://www.swissadme.ch) online database using their structural characteristics and chemical descriptors.[33] Utilizing the web service *AdmetSAR* (http://lmmd.ecust.edu.cn/admetsar2), pharmacokinetic criterion associated to drug absorption, distribution, metabolism, and toxicity (ADMET) were estimated.[34] "Simplified molecular input line entry system (SMILES)" strings and structure data files both were used in the conversion process.

Method for molecular docking

To forecast the binding conformational changes and interactions between the ligands and protein binding sites, molecular docking programs are utilized. There have been various molecular docking programs developed over the last 40 years. Today, many of the well-known programs are still used, allowing scientists to determine how

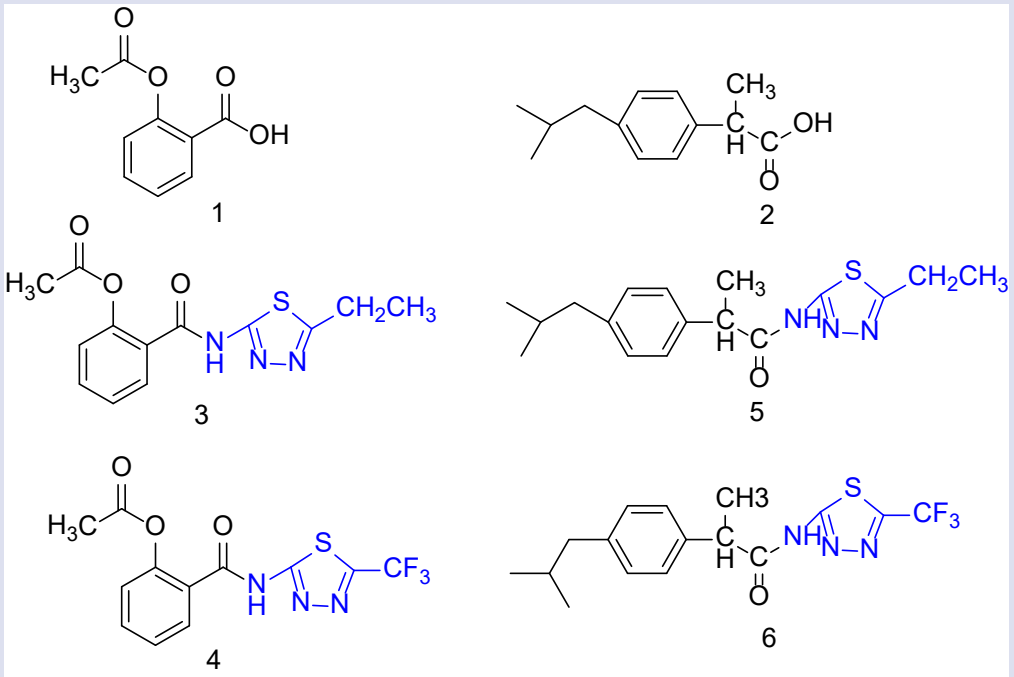

Figure 1: Structures of standard drugs aspirin (**1**), ibuprofen (**2**) and heterocyclic amide derivatives (**3-6**).

chemicals interact with one another.[35] Docking was performed on optimized structures 1-6 (Section 2.1). From the RCSB proteins data bank, two significant proteases 3D crystal structure of cyclooxygenase-1 (6Y3C) and cyclooxygenase-2 (5F19), were also downloaded (PDB).[30,36] Water and extraneous hetero-atoms from 6Y3C and 5F19 were eliminated by directing PyMOL (Version 1.7.4) application,[37] and pdb file format was saved. The energy of each ligand was minimized using the Swisspdb program (Version 4.1.0) software.[38] The ligands are stored in pdbqt format after energy minimization. Having all the drugs and proteins were in usable formats, we utilized PyRx (Version 0.8) autodock vina for conducting molecular docking.[39] By loading the ligands and proteins, auto docking was performed after the box size was fixed to the highest dimension level (using a software command). The required non-bond interactions were computed once docked complexes were accessed in the Discovery Studio 4.1 (client).[40]

Prediction of "Quantitative Structure-Activity Relationship (QSAR)".

In computational drug design, "Quantitative structure-activity relationships (QSAR)" is described by correlating it to drug-allied eight descriptions from ChemDes (http://www.scbdd.com/chemdes/), a web based platform for the molecular descriptor computation.[41] On the ground of statistical and mathematical correlations, QSAR were used to establish linkages between the physicochemical properties of chemical compounds and their biological functions.[42,43]

RESULTS AND DISCUSSION

Structures of standard drugs aspirin (1), ibuprofen (2) and heterocyclic amide derivatives (3-6), after optimization

The biological functionality of molecules is greatly influenced by their structure, conformation, and interactions with receptor proteins.[31] The determination of any molecular stable configuration is crucial in computational chemistry. In the beginning, the standard NSAIDs drugs aspirin (1), ibuprofen (2) and conformations of heterocyclic amide derivatives (3-6), are determined. All tested structures of 1-6 were obtained using DFT/B3LYP functionality and 6-31G basis level as shown in Figure 2. All molecules have nearly identical symmetry, according to their optimized structures. All further computer assisted studies are developed on top of these optimized stable structures.

Thermodynamic studies

Thermochemical calculations are used to predict the reaction kinetics and chemical durability of the final products. Free energy and enthalpy are primarily correlated with the absorption or emission of energies during such chemical process and the high stability of a molecule.[44,45] The free energy value plays a notable role in research on the easy potential for binding with other compounds in where both the sign and magnitude of a compound represent its distinctive properties. Larger values relate to the more possible bindings while the negative sign indicates the spontaneous binding.[30,46,47] There is no external energy required for bindings because all of the compound's free energy and enthalpy values are negative (Table 1). In comparison to the other amide derivatives, the higher electronegative atoms fluorine and oxygen contained in compounds 4 and 6 also have a higher energy value. Compared to the analogue 4 and 6, other compounds exhibit less binding interactions. Greater energy (free or internal energy) and enthalpy readings with a negative sign are symbol of the better thermodynamic properties of molecules 4 and 6 compared to other amide derivatives. In this study, enthalpy values of compounds 4 and 6 are -1549.134 Hartree and -1557.036 Hartree respectively where that of compound 5 is -1298.678 Hartree which is the third highest enthalpy value. On the contrarily, compounds 4 and 6 have significantly greater free energy values, measuring -1549.206 Hartree and -1557.119 Hartree respectively, because of the presence of CF_3 and C=O groups in their chemical structures. A molecule's dipole moment value is vital in describing its electrical properties as it influences the amount of intermolecular interactions that occur. Greater value of the dipole moment reveals a more polar character.[48-50] In Table 1, the reasonably high dipole moment values of compounds 4 and 6 measuring 7.861 Debye and 7.662 Debye respectively which show the molecules' strong

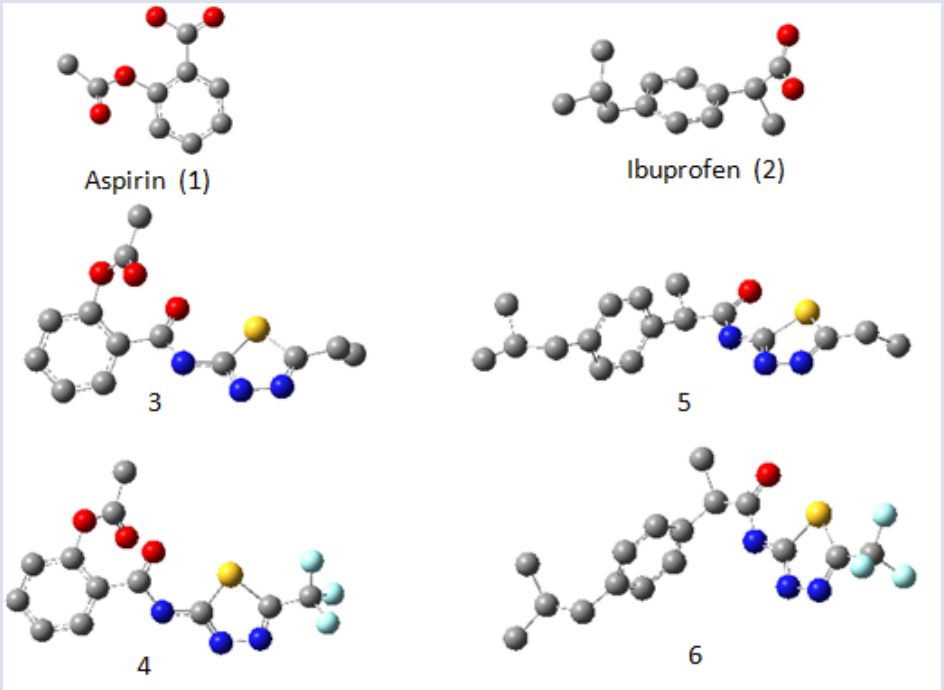

Figure 2: Structures of standard dugs aspirin (**1**), ibuprofen (**2**) and heterocyclic amide derivatives (**3-6**). (Only backbones are presented).

Table 1: Molecular formula (MF), molecular weight (MW), enthalpy, free energy (Hartree) and dipole moment (Debye) of standard drugs aspirin (1), ibuprofen (2) and heteocyclic amide derivatives (3-6).

Mol.	MF	MW	Dipole moment	Internal Energy	Enthalpy	Free energy
1.	$C_9H_8O_4$	180.16	5.302	-648.365	-648.352	-648.404
2.	$C_{13}H_{18}O_2$	206.28	2.113	-656.267	-656.250	-656.311
3.	$C_{13}H_{13}N_3O_3S$	291.33	3.316	-1290.771	-1290.752	-1290.821
4.	$C_{12}H_8F_3N_3O_3S$	331.27	7.861	-1549.154	-1549.134	-1549.206
5.	$C_{17}H_{23}N_3OS$	317.45	2.684	-1298.678	-1298.654	-1298.734
6.	$C_{16}H_{18}F_3N_3OS$	357.39	7.662	-1557.061	-1557.036	-1557.119

Mol. = molecule (compound); 1 = Aspirin, 2 = Ibuprofen, MF= Mol. Formula, MW=Mol. Wt.

Table 2: Energy (eV) of HOMO-LUMO, gap, hardness (η), softness (S), chemical potential (μ), electronegativity (χ) and electrophilicity (ω) of standard drugs aspirin (1), ibuprofen (2) and heterocyclic amide derivatives (3-6).

Mol.	εHOMO	εLUMO	Gap (Δε)	η	S	μ	χ	ω
1.	-7.674	-2.149	5.525	2.763	0.362	-4.412	4.412	3.523
2.	-6.694	-0.947	5.747	2.874	0.378	-3.821	3.821	2.540
3.	-6.773	-1.867	4.906	2.453	0.408	-4.325	4.325	3.813
4.	-7.439	-2.446	4.993	2.497	0.400	-4.943	4.943	4.892
5.	-6.607	-2.211	4.396	2.198	0.455	-4.909	4.909	4.422
6.	-6.977	-2.161	4.816	2.408	0.415	-4.569	4.569	4.335

Mol = molecule (compound); 1 = Aspirin, 2 = Ibuprofen, LUMO = lowest unoccupied molecular orbital, HOMO = highest occupied molecular orbital.

Table 3: Drug-likeness properties of compounds 1-6.

Mol.	HBD	HBA	NBR	TPSA (Å²)	Log Po/w	Log S (mg/ml)	MW	Lipinski rule	
								Follow	Violation
1.	1	4	3	63.60	1.28	-1.85	180.16	5	0
2.	1	2	4	37.30	3.0	-3.44	206.28	5	0
3.	1	5	6	109.42	2.15	-4.30	291.33	5	0
4.	1	8	6	109.42	2.53	-4.38	331.27	5	0
5.	1	3	7	83.12	3.82	-5.88	317.45	5	0
6.	1	6	7	83.12	4.27	-5.95	357.39	5	0

binding affinities, hydrogen bond makeup, and binding interactions in drug protein complexes.[51] Due to the comparatively low dipole moments, compounds 3 and 5 exhibit less binding interactions. Hence, compounds 4 and 6 exhibited more binding affinity to receptor protein COX-1 (6Y3C) and COX-2 (5F19).

Chemical reactivity descriptors analysis

Frontier molecular orbital data are important in estimating the potential energy needed for chemical reactions. For many reactions, the HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) are significant.[52,53] Electronic absorption is linked to the shift from HOMO to LUMO.[54] The HOMO-LUMO gap's value affects the chemical properties of hardness, softness, potential, electronegativity, and electrophilicity.[55,56] Significant gap in HOMO-LUMO is associated with low molecular softness and increased kinetic stability as it is energetically inexpedient for one electron to move from HOMO to LUMO. Due to the ease of electron transition, a low HOMO-LUMO separation is linked to greater chemical softness and lower kinetic stability.[57] Here, in Table 2, HOMO-LUMO gap (Δε) of heterocyclic amide derivatives (3-6) is comparatively lower than aspirin (1) and ibuprofen (2) meaning them higher chemical softness and less kinetic stability. From Table 2 and Figure 3, compound 4 and 6 have energy gap values 4.993 eV and 4.816 eV respectively which are higher than that of other two analogues 3 and 5. Heterocyclic amide analogue 4 has hardness (η) as well as softness (S) values of 2.497 eV and 0.400 eV, respectively, whereas the analogue 6 has hardness (η) as well as softness (S) values of 2.408 eV and 0.415 eV, respectively. It should be mentioned that reduce the energy gap Δε, increases the chemical reactivity.[58,59] In this study, in Table 2 shows that all heterocyclic amide analogue 3-6 are comparativelay more reactive than that of the standard drugs aspirin (1) and ibuprofen (2). Figure 3 displays a DOS plot with HOMO-LUMO gaps (Δε) of 4 and 6.

Molecular orbital analysis

HOMO and LUMO orbital diagrams have been computed using DFT method. In general, "HOMO" signifies the highest probable electron immensity in the region of a molecule that an electrophile can mostly attack, whereas "LUMO" describes the absence of electrons in a circumstance where a nucleophilic or electron-withdrawing group can be added with ease.[60] Figure 4 shows that the alkyl chains of the examined compounds 3-6 as well as the N-[5-ethyl-1,3,4-thiadiazole] and N-[5-trifluoromethyl-1,3,4-thiadiazole] moieties include LUMO parts. In contrast, the HOMO part present on the phenyl acetate and 2-(4-Isobutyl phenyl)-propionate portion. Additionally, HOMO orbitals are more common in electronegative atoms, primarily oxygen atoms, in those locations. This work clearly demonstrated the conceptual understanding of HOMO and LUMO from frontier molecular orbitals after investigating their optimized structures.

MEP ANALYSIS

The "molecular electrostatic potential (MEP)" map shows the complete charge of the electrons and nuclei as well as some information on the electro negativity, partial charge, dipolar moment, and reactivity of the molecules.[30,61] The reactive points for both electrophilic and nucleophilic assault in all optimized conformers were predicted by MEP.[62] By using the colors blue and red, it depicts a potential electrophilic and nucleophilic attack.[48] Green represents zero potential area, blue signifies the largest positive zone that is a convenient site

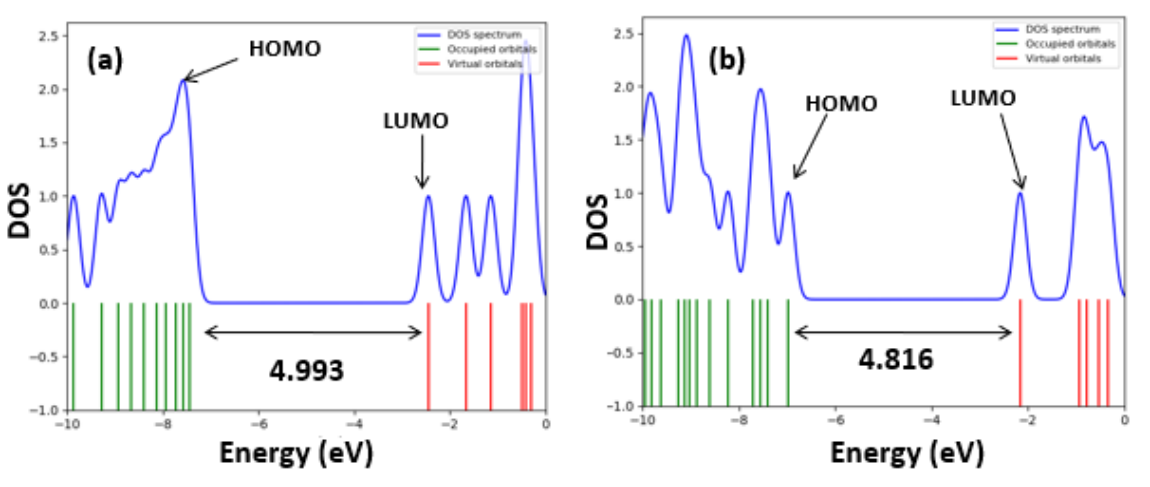

Figure 3: DOS plot and energy gap of HOMO-LUMO (a) Compound **4** and (b) Compound **6.**

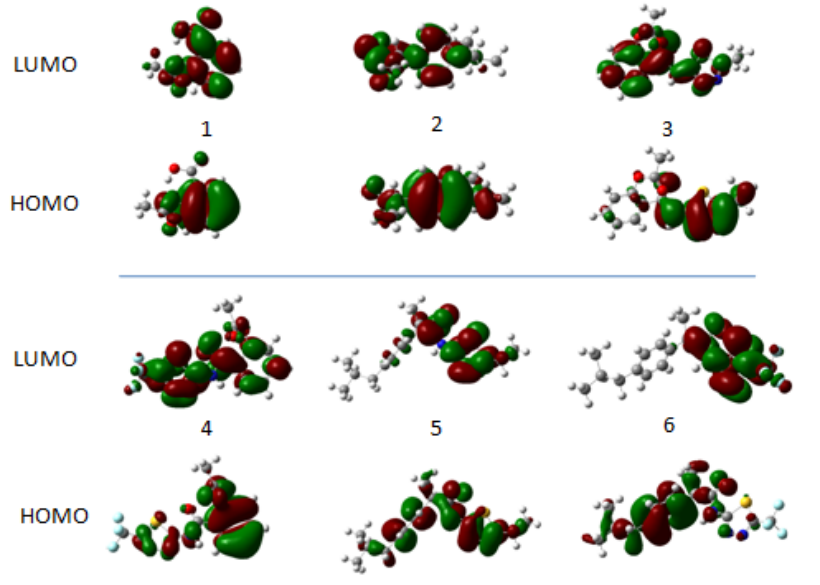

Figure 4: Molecular orbital (HOMO and LUMO) of standard drugs aspirin (**1**), ibuprofen (**2**) and heterocyclic amide derivatives (**3-6**), after DFT optimization.

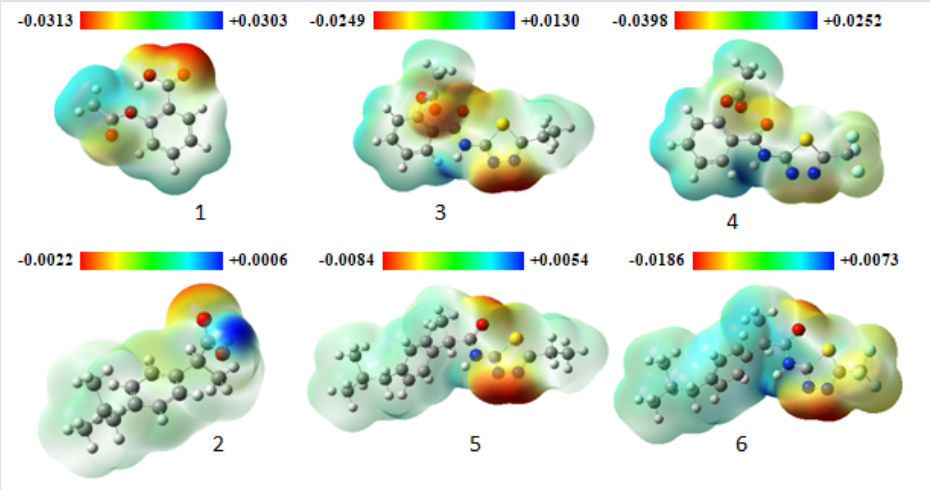

Figure 5: Molecular electrostatic potential (MEP) of aspirin (**1**), ibuprofen (**2**) and heterocyclic amide derivatives (**3-6**), after DFT optimization.

for nucleophilic strike, and red represents the maximum negative area which is favorable for electrophilic strike. According to the MEP map (Figure 5), regions with negative potential are just over electronegative atoms (oxygen and Nitrogen), whereas regions with positive potential values are just over hydrogen atoms. Here, among the heterocyclic amide derivatives 3-6, compound 4 exhibited the highest negative potentiality (-0.0398 a.u.) and also the highest positive potentiality (+0.0252 a.u.) whereas compound 3 showed the second highest negative (-0.0249 a.u.) and positive (+0.0130 a.u.) potentiality.

Vibrational frequency analysis

To analyse chemical anatomy that indicate the existence of various functional units in the molecule, vibration spectrum analysis is a crucial parameter.[63] Table S1 presents the FT-IR spectral data for heterocyclic amide analogues (3-6). According to Figure 6(a) and Table S1, all compounds have vibration frequencies in the range of 400–4000 cm^{-1}. For precision in understanding the investigational results and tentative vibrational assignments provided in [Figure 6(a)], the predicted FT-IR vibration wave numbers of the heterocyclic amide analogues have been multiplied by the scaling factor 0.9602 (https://cccbdb.nist.gov/vsfx.asp). The carbonyl (C=O) and N-H vibrational stretching for the amide functional group, which occur in the frequency ranges of 1690-1710 cm^{-1} and 3439-3470 cm^{-1}, respectively, indicate the presence of CO-NH functional group in all tested compounds (3-6). Again the carbonyl (C=O) group produces vibration stretching for the acetate functional group in the range of frequency of 1760-1765 cm^{-1}, which supports the existence of the C=O group in molecules 3 and 4. Here, the frequency of carbonyl vibration for the functional group with OCO-CH_3 is greater than that of the CO-NH amide group. Moreover, 3069 cm^{-1} and 3028 cm^{-1} stretching vibrations were found for C-H (ester group) in heterocyclic amide analogs (3, 4) whereas 3019 cm^{-1} and 3012 cm^{-1} were shown by C-H (aliphatic group) in heterocyclic amide derivatives (5, 6). Again, a frequency of C-H stretching observed at 3090-3110 cm^{-1} for aliphatic CH_3 functional group in 3 and 4. All of the heterocyclic amide derivatives (3-6) showed three separate stretching vibration for three aromatic double bond (C=C) at the range of 1480-1560 cm^{-1}, 1565-1595 cm^{-1}, and 1600-1620 cm^{-1} respectively.

UV-Visible spectral analysis

As a standard, the molecular orbital study of attached aromatic ring systems, the UV-visible spectra analysis utilizing time-dependent density functional theory (TD-DFT) method ensures the balance between precision and computational cost. Table S2 and Figure 6(b) present each of the two distinctive electronic energy states from the analogs. In this work, the first electron transfers from ground state (S_0) to singlet (S_1) determines the kinetic stability and reactive sites. Compounds 3 and 4 exhibited large absorption bands at 350.38 nm and 346.49 nm including oscillator strengths of 0.0032and 0.0034, respectively. The maximum wave-length of the configurations is produced by charge transfer of electrons to excited state S_0 to S_1; (0.236) H-2→L, (0.646) H→L for 3 and [(0.310) H-1→L, (0.589) H→L, (0.148) H→L+1] for 4. The shifting of electrons between HOMO to LUMO is the main cause of both of the broad absorption spectra at 350.38 nm and 346.49 nm. In this research, it is found that all amide derivatives (3-6) have more reaction sites than that of the standard drugs aspirin (1) and ibuprofen (2), which is confirmed by their lower excitation energies corresponding to their HOMO-LUMO energy space, hence enhanced the reactivity and minimized the kinetic stability of molecules.[57]

Pharmacokinetics: drug-likeness studies

Appropriate drug-likeness in molecules should be searched out at the early stages of drug development as it affects a human's ADMET (absorption, distribution, metabolism, elimination, and toxicity), BBB permeability, and clearance. The Lipinski principles (rule of five) state that drug-like molecules must have five characteristics, such as a drug molecule should have – (i) less than 5 hydrogen bond donors (HBD), (ii) less than 10 hydrogen bond acceptors (HBA), (iii) more than three number of rotatable bonds (NBR), (iv) molecular mass (MW) less than 500 Daltons, and (v) octanol-water partition coefficient (log Po/w) is not greater than 5.[64] Table 3 summarizes the violations of the Lipinski rule, HBD, HBA, NBR, MW, log Po/w, log S, and heterocyclic amide derivatives (3-6) with aspirin (1) and ibuprofen (2). In this study, it is found that all tested amide analogues 3-6 follow Lipinski rule. These substances also have topological polar surface areas (TPSA) above 80 $Å^2$, which are greater than those of aspirin (1) and ibuprofen (2). A vital physicochemical factor for the development of innovative therapeutics is the partition factor of n-octanol with water (log Po/w). It is defined as the ratio of the non-ionized compound's concentration in the organic and aqueous portions at equilibrium. Here, all the investigated compounds 3-6 have higher lipophilicity and lower water solubility than standard drugs aspirin (1) and ibuprofen (2).

Pharmacokinetics: ADMET studies

"Absorption, distribution, metabolism, excretion, and toxicity (ADMET)" refers to the body's response to a drug that is investigated

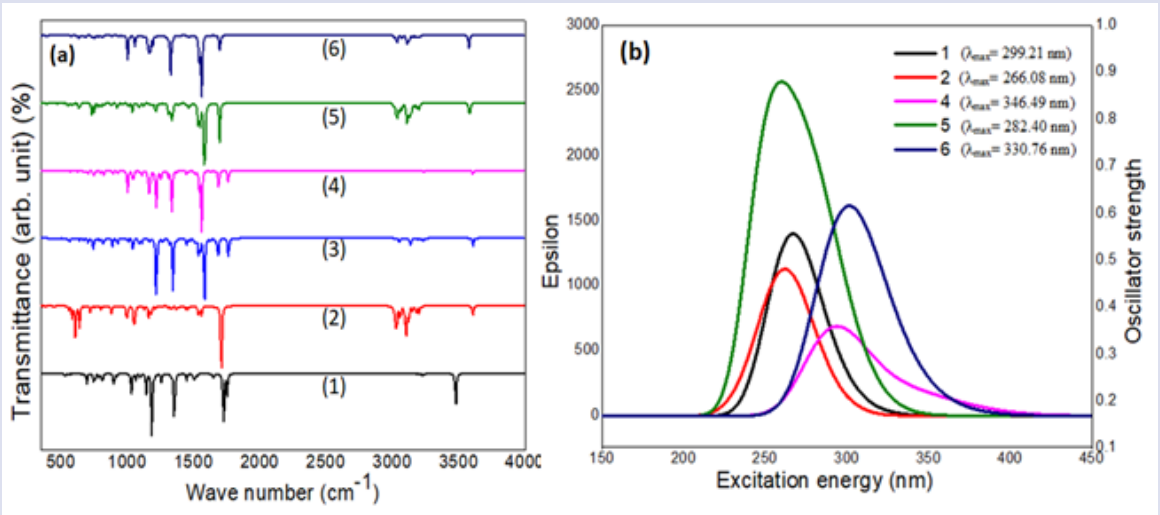

Figure 6: (a) FT-IR and (b) UV-Visible spectra of selected compounds.

in pharmacokinetics.[65] Thus, it's essential for early research and development of drugs in addition to ADMET testing, which is applied to assess all of a possible drug's aspects.[66,67] The *admetSAR* online database shows the *Admet* features of different heterocyclic amide analogues (3-6) in Table 4. According to *AdmetSAR* data (Table 4), all compounds 3-6 effectively respond to human intestinal absorption (HIA), so nothing can be removed from the body more rapidly through to the urinary and rectal systems.[34] Here, HIA values of compounds 3 and 4 are +0.899 and +0.916 respectively which are pretty close to aspirin (1) (+0.937). Whereas, HIA responses of compound 5 and 6 are +0.988 and +0.993 respectively which are higher than that of ibuprofen (2) (+0.937). All chemicals showed favorable human oral bioavailability (HOB) levels. Positive oral bioavailability in humans can cause health issues.[68] The colon cancer cell line Caco-2 is grown on permeable scaffolds, and the permeability coefficients throughout the monolayers are widely used to predict the administration of xenobiotics and other medications taken orally.[69,70] In our study, as opposed to aspirin (1) and ibuprofen (2), compounds 3, 4, and 6 showed positive C2P values, suggesting that these drugs are absorbed quickly in the human body. Blood-brain barrier (BBB) is a very restrictive boundary that controls the flow of chemicals, ions, and cells between the brain and other parts of the body. This barrier shields the brain's central nervous system (CNS) from poisons, infections, inflammation, injury, and disease.[71,72] Alarmingly, in our investigation, all heterocyclic amide derivatives 3-6 showed a positive reaction to BBB in the range of +0.974 to +1.000. Positive response to BBB is alarming and suggests that they will not pass BBB promptly.[68] P-glycoprotein can be inhibited by reducing ATP hydrolysis, obstructing drug-binding sites, or altering the stability of cell membranes.[73] The P-glycoprotein inhibition values for all of the analogues are negative, indicating that P-glycoprotein is not inhibited and that they cannot interfere with absorption, permeability, or retention.[73] The P-glycoprotein substrate often serves as an inducer based on its roles, and if it induces, it also decreases the drug's bioavailability.[74] In this research, CYP2C9 is a significant cytochrome P450 enzyme that was the target of compounds 3 and 4, which showed positive inhibition. Various toxic drug interactions involving the enzyme are affected by inhibition.[73,74] The risk of the long QT syndrome and other serious cardiac effects, such as sudden death, is increased by the fact that all of the drugs under investigation weakly suppress the "ether-a-go-go-related gene (hERG)" in human.[30,75] Carcinogens are substances or chemical mixtures that, in certain circumstances and during a protracted or intense exposure, have the ability to cause cancer in people.[76] In this investigation, the analogue 6 was carcinogenic while the compounds 3, 4, and 5 exhibited non-carcinogenic toxicity. Acute toxicity describes a substance's toxic effects on humans and other living things through various biochemical systems. Research of the risk of a substance's toxic effects must consider factors such as acute oral, skin, and inhalation rodent toxicity.[77] Except for 5, this exhibited the toxicity level II for acute oral, all other amide analogues exhibited acute oral toxicity in category III, making them relatively less dangerous. The median lethal dose for rat oral acute toxicity is a typical parameter used to categorize compounds in terms of the possible risk they pose to public health following initial consumption (LD50).[78,79] Here, the predicted LD_{50} for aspirin (1), ibuprofen (2), and all of the tested amide derivatives (3-6) ranged from 2.309-2.808 mol/Kg, which is quite dangerous.[79]

Molecular docking: binding affinity and interaction with COX-1 and COX-2 main proteases

A key tool for structure-based novel drug development is molecular docking.[80] It was noticed that several heterocyclic amide derivatives with aspirin or ibuprofen showed cyclooxygenase inhibition activity and anti-inflammation properties were reported *in vitro*.[14,20,81] Encouraged by these results four new heterocyclic amide derivatives (3-6) with aspirin (1) and ibuprofen (2) were selected for molecular docking cyclooxygenase main proteases COX-1 (6Y3C) and COX-2 (5F19). Docking is utilized to search the binding attraction and inhibition of different heterocyclic amide derivatives (3-6) with aspirin (1) and ibuprofen (2) to COX-1 and COX-2 in Figure 8. Better negative values in binding properties denote well binding between drugs and proteins. As presented in Table 5, heterocyclic amide derivatives (3-6) showed the binding score to COX-1 (6Y3C) between -7.3 to -8.1 kcal/mol which are usually higher than that of aspirin (1) (-6.1 kcal/mol) and ibuprofen (2) (-7.1 kcal/mol).

Table 4: ADMET prediction data of compounds 1-6.

Mol.	Absorption			Distribution		Metabolism	Toxicity			
	HIA	HOB	C2P	BBB	P-GpI	CYP4502C9I	hERG	Carcinogen	AOT	RAT LD_{50} (mol/Kg)
1.	+0.937	+0.586	-0.612	+0.973	-0.748	+0.636	WI	NC (0.801)	III	2.343
2.	+0.937	+0.671	-0.588	+0.973	-0.555	-0.508	WI	NC (0.791)	III	2.470
3.	+0.899	+0.786	+0.683	+0.974	-0.715	+0.654	WI	NC (0.762)	III	2.529
4.	+0.916	+0.757	+0.619	+0.974	-0.784	+0.556	WI	NC (0.720)	III	2.808
5.	+0.988	+0.671	-0.735	+0.975	-0.980	-0.907	WI	NC (0.836)	II	2.639
6.	+0.993	+0.914	+0.931	+1.000	-0.979	-0.931	WI	C (0.555)	III	2.309

1 = Aspirin; 2 = Ibuprofen; HIA= Human intestinal absorption, HOB= Human oral bioavailability, C2P= CACO-2 permeability, BBB= Blood brain barrier, P-GpI = P-glycoprotein inhibitor, P- GpS = P-glycoprotein substrate, hERG = human Ether-a-go-go Related Gene, AOT = Acute oral toxicity, RAT = Rat acute toxicity, S = Substrate, NS = Non-substrate I = Inhibitor, NI = Non-inhibitor, WI= Weak inhibitor, NC = Non-carcinogen, C = Carcinogen, CYP3A4*I* = CYP3A4 Inhibition, CYP3A4*S* = CYP3A4 Substrate.

Table 5: Molecular docking score (binding energy) with COX-1 (6Y3C).

Ligand/Drug	Binding energy (kcal/mol)	No. of H bond	No. of Hydrophobic bond	No. of Vander Waal bond / Other Bond	Total bonds
Aspirin (1)	-6.1	4	2	0	6
Ibuprofen (2)	-7.1	3	9	0	12
3	-7.3	5	6	1	12
4	-7.8	5	3	2	10
5	-7.5	1	10	1	12
6	-8.1	6	9	1	6

*-6.0 kcal/mol is considered standard docking score.

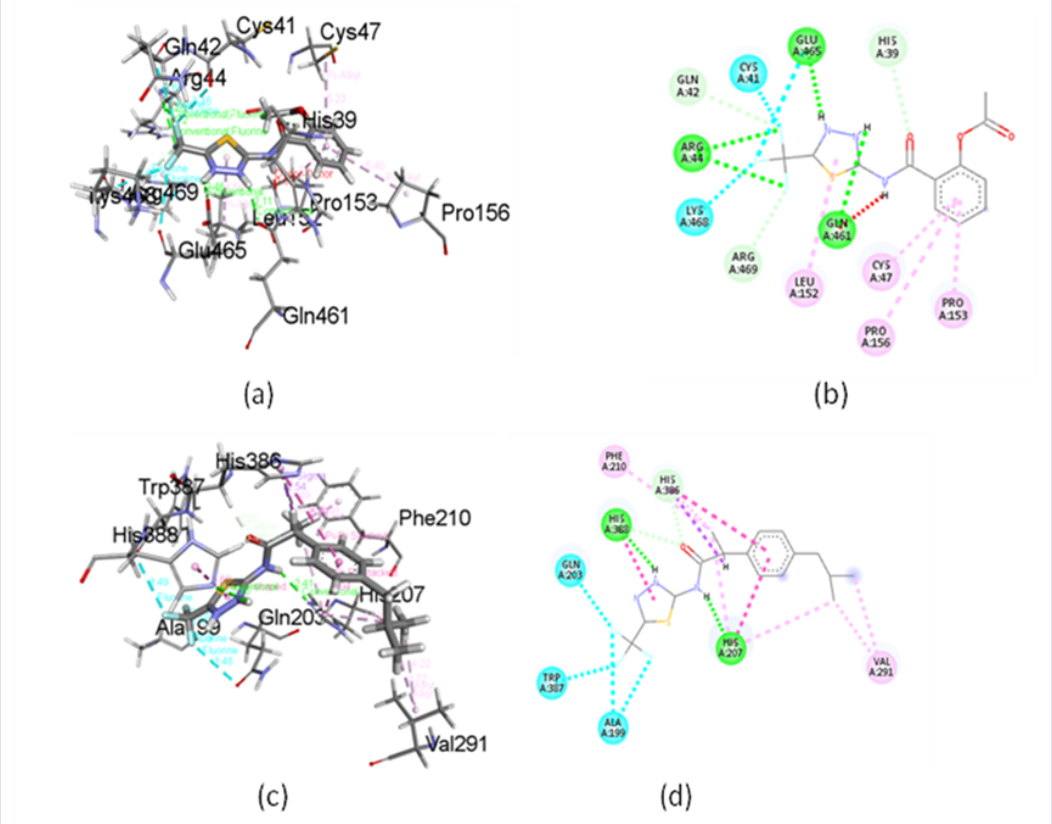

Figure 7: Selected non-bonding interactions of 5F19 with - (a) **4** (3D); (b) **4** (2D); (c) **6** (3D); (d) **6** (2D).

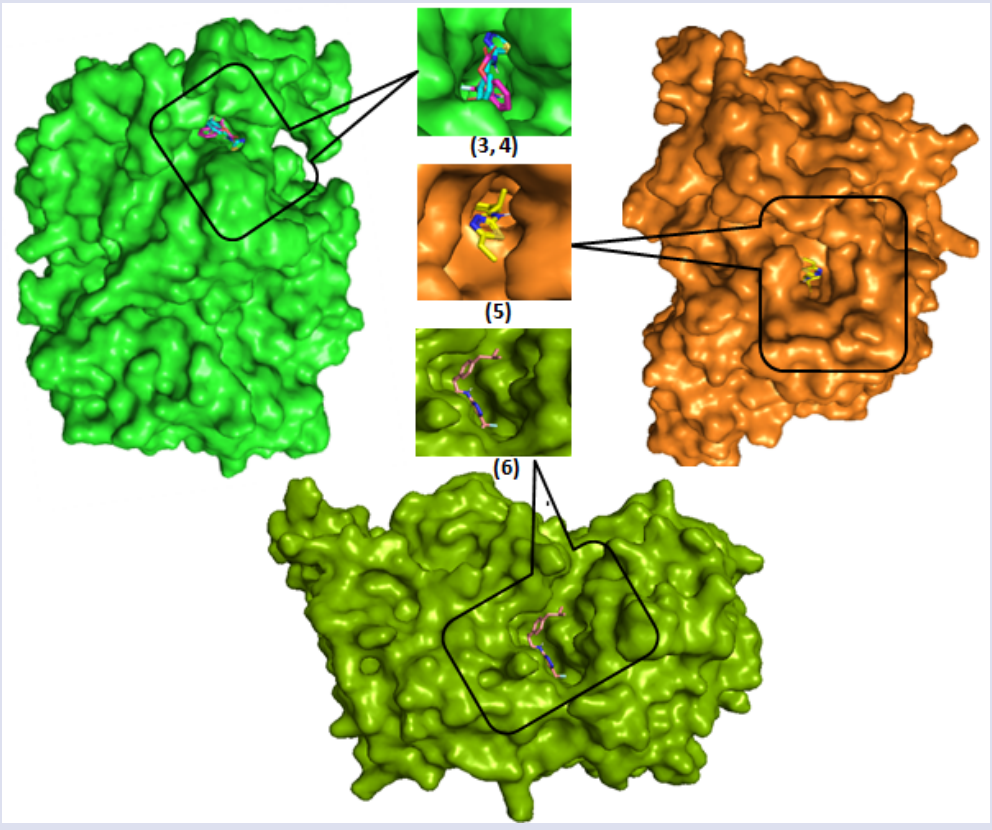

Figure 8: Docked conformer of heterocyclic amide derivatives (3-6) with receptor protein 5F19.

Table 6 Interaction of drugs with amino acid (AA) residues in compounds-6Y3C complex.

Drug	Hydrogen bond			Hydrophobic bond			Vander Waal bond / another Bond		
	Interacting residue of amino acid	Distance (Å)	Type of Interaction	Interacting residue of amino acid	Distance (Å)	Type of Interaction	Interacting residue of amino acid	Distance (Å)	Type of Interaction
Aspirin (1)	HIS43	2.76964	H	LEU152	4.67661	PA	Absent		
	GLN44	2.10788	H	PRO153	5.38319	PA			
	GLN461	1.96	H						
	TYR39	2.86344	Chy						
Ibuprofen (2)	HIS43	2.89483	H	LEU152	4.75065	A	Absent		
	GLN42	2.90891	Chy	CYS36	4.12221	A			
	GLN461	3.15411	Pd	CYS47	4.36176	A			
				PRO153	4.42007	A			
				PRO153	4.86434	A			
				PRO156	4.89836	A			
				TYR39	4.86891	PA			
				LEU152	5.20329	PA			
				PRO153	5.45431	PA			
3	HIS388	2.40101	H	ALA202	4.89479	APS	HIS207	5.03186	PS
	HIS207	2.42829	H	PHE210	4.45428	APS			
	TYR385	2.93235	H	ALA202	3.71266	A			
	HIS386	2.89114	Chy	TYR385	4.94655	PA			
	HIS388	2.84733	CHy	TRP387	4.69438	PA			
				TRP387	4.71295	PA			
4	GLN203	2.79855	H	HIS207	4.59558	PPTSh	ALA199	2.67341	X
	GLN203	2.02491	H	ALA202	4.24801	APS	TRP387	2.95147	X
	HIS386	2.7513	H	ALA202	4.89888	PA			
	HIS388	2.90356	H						
	THR206	2.58412	H						
5	ASP135	2.97241	H	PRO153	5.02453	A	TYR136	5.44465	PS
				PRO156	4.28999	A			
				PRO40	4.93213	A			
				CYS41	3.71119	A			
				CYS47	4.45997	A			
				LEU152	5.08602	A			
				TYR136	4.37022	PA			
				CYS36	4.41816	PA			
				CYS47	4.70223	PA			
				PRO153	4.33698	PA			
6	LYS157	2.57734	H	CYS36	2.92386	PS	ASP135	3.04706	Pa
	LYS157	2.98862	H	CYS36	3.74177	A			
	LYS157	2.66592	H	PRO156	4.48276	A			
	SER154	1.98175	H	CYS41	3.88848	A			
	SER154	2.87863	H	CYS47	4.88294	A			
	VAL155	2.96669	H	LEU152	4.55985	A			
				CYS47	4.55034	PA			
				PRO153	4.40174	PA			
				PRO156	4.11783	PA			

Note: TRP = Tryptophan, ASP = Aspartic acid, GLU = Glutamic acid, LEU = Leucine, THR = Threonine, ASN = Asparagine, GLN = Glutamine, PHE = Phenylalanine, ILE = Isoleucine, ARG = Arginine, VAL = Valine, SER = Serine, PRO = Proline, GLY = Glycine, HIS = Histidine, LYS = Lysine, CYS = Cysteine, MET = Methionine.

Note: H = conventional hydrogen bond, A = alkyl, PA = pi-alkyl, PC = Pi-cation, Pa = pi-anion, X = Halogen bond, CHy = Carbon Hydrogen Bond, Pd = Pi-donor, PS = Pi-sigma, PSu = Pi-sulfur, PPS = Pi-Pi stacked, PPTSh = Pi-Pi T-shaped, APS = Amide-Pi Stacked.

Table 7: Molecular docking score (binding energy) with 5F19.

Ligand/Drug	Binding energy (kcal/mol)	No. of H bond	No. of Hydrophobic bond	No. of Vander Waal bond / Other Bond	Total bonds
Aspirin (1)	-6.3	0	3	0	3
Ibuprofen (2)	-6.7	2	7	0	9
3	-7.7	7	7	1	15
4	-9	7	4	4	15
5	-8.1	2	12	1	15
6	-8.2	4	10	4	18

*-6.0 kcal/mol is considered standard docking score.

Table 8 Interaction of drugs with amino acid (AA) residues in compounds-5F19 complex.

Drug	Hydrogen bond			Hydrophobic bond			Vander Waals bond / Another Bond		
	Interacting residue of amino acid	Distance (Å)	Type of Interaction	Interacting residue of amino acid	Distance (Å)	Type of Interaction	Interacting residue of amino acid	Distance (Å)	Type of Interaction
Aspirin (1)	Absent			VAL523	2.80099	PS	Absent		
				LEU352	4.97098	PA			
				ALA527	4.94464	PA			
Ibuprofen (2)	GLY45	2.86006	H	ARG44	4.66322	A	Absent		
	CYS41	1.96422	H	CYS36	4.11642	Alkyl			
				PRO156	4.64807	A			
				PRO153	4.21568	A			
				PRO156	4.55801	A			
				HIS39	4.58285	PA			
				PRO153	4.55863	PA			
3	TYR130	2.9761	H	TYR130	5.54785	PPTSh	TYR130	5.3275	PS
	GLN461	2.09444	H	ARG44	4.16764	A			
	GLY45	2.41901	H	CYS36	4.8491	PA			
	ARG44	2.35917	H	CYS47	4.25768	PA			
	VAL46	2.36176	Chy	PRO153	4.64992	PA			
	PRO153	2.95303	CHy	ARG44	5.10625	PA			
	TYR130	3.12836	Pd	VAL46	5.04706	PA			
4	ARG44	2.71697	H	LEU152	4.56295	PA	CYS41	3.16268	X
	ARG44	2.61837	H	CYS47	5.22974	PA	GLN42	3.59678	X
	GLU465	2.4602	H	PRO153	4.19087	PA	GLU465	3.41582	X
	GLN461	2.11349	H	PRO156	5.46343	PA	LYS468	3.62829	X
	HIS39	2.74574	CHy						
	GLN42	2.65536	CHy						
	ARG469	2.71101	CHy						
5	ARG120	2.05167	H	VAL349	5.1135	A	ARG120	3.47233	PC
	TYR355	2.11191	H	LEU359	4.11821	A			
				LEU531	5.11838	A			
				VAL523	4.42195	A			
				TYR115	5.03218	PA			
				TYR355	5.41631	PA			
				TYR385	5.22032	PA			
				PHE518	4.80395	PA			
				VAL349	4.19848	PA			
				ALA527	3.48444	PA			
				LEU531	5.32917	PA			
				VAL116	4.48261	PA			
6	HIS207	2.41056	H	HIS386	2.54462	PS	ALA199	2.89573	X
	HIS388	2.95676	H	HIS207	4.04132	PPT	ALA199	3.19283	X
	HIS386	1.99763	Chy	HIS386	5.27227	PPT	GLN203	3.48423	X
	HIS388	2.4252	CHy	HIS388	4.67565	PPTSh	TRP387	3.48993	X
				VAL291	3.72248	A			
				VAL291	4.21809	A			
				HIS207	4.99172	PA			
				HIS207	4.58844	PA			
				PHE210	4.33802	PA			
				HIS386	4.49248	PA			

Note: TRP = Tryptophan, ASP = Aspartic acid, GLU = Glutamic acid, LEU = Leucine, THR = Threonine, ASN = Asparagine, GLN = Glutamine, PHE = Phenylalanine, ILE = Isoleucine, ARG = Arginine, VAL = Valine, SER = Serine, PRO = Proline, GLY = Glycine, HIS = Histidine, LYS = Lysine, CYS = Cysteine, MET = Methionine.

Note: H = conventional hydrogen bond, A = alkyl, PA = pi-alkyl, PC = Pi-cation, Pa = pi-anion, X = Halogen bond, CHy = Carbon Hydrogen Bond, Pd = Pi-donor, PS = Pi-sigma, PSu = Pi-sulfur, PPS = Pi-Pi stacked, PPTSh = Pi-Pi T-shaped.

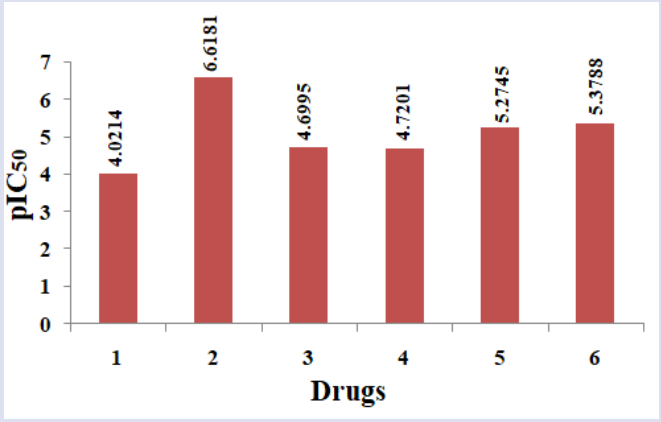

Figure 9: pIC_{50} studies of the compounds **1-6.**

In the next stage, heterocyclic amide analogues (3-6) with ibuprofen (2) were docked with another cyclooxygenase-2 protease (5F19) and the resulted binding energies are presented in Table 7. Here, amide derivatives (3-6) showed the binding score to COX-2 (5F19) between -7.7 to -9.0 kcal/mol which are also higher than that of aspirin (1) (6.3 kcal/mol) and ibuprofen (2) (-6.7 kcal/mol).

Halogen bonds, hydrogen bonds, and hydrophobic interactions are types of non-covalent interactions that are connected to the binding structures under analysis (Table 6 and Table 8). However, the binding affinity can substantially increase in the existence of strong hydrogen bonds with a range of less than 2.3Å.[53,82,83] Additionally, the fundamental characteristics of the ligand-protein complex are non-covalent interactions. Because these interactions alter the binding affinity, improve drug efficacy, and stabilize the compounds at the object site.[84,85] The complexes with 6Y3C and 5F19 under analysis involve non-covalent interaction such as halogen bonds, hydrogen bonds, and hydrophobic interactions. In addition, the halogen bond serves as a key component of both biological and chemical systems and is comparable to the hydrogen bond.[49] In this investigation, almost all of the heterocyclic amide derivatives have conventional hydrogen bonds. On the other hand, some essential carbon hydrogen bonds are shown with HIS386, HIS388 residues in 3 as COX-1 inhibition whereas, VAL46, PRO153 residues in 3, HIS39, GLN42, ARG469 residues in 4, and HIS386, HIS388 residues in 6 are shown as COX-2 inhibition. Important halogen bonds are found with ALA199, TRP387 residues in 4 as COX-1 inhibition, whereas, CYS41, GLN42, GLU465, LYS468 residues in 4, and ALA199, ALA199, GLN203, TRP387 in 6 are found as COX-2 inhibition. Furthermore, π-systems are crucial for biological processes like ligand-protein recognition. A useful portion of the binding score in the living system is provided by π-bonds.[46,51] In our analysis, all compounds displayed Pi-Alkyl attractions with certain significant amino acid residuum. A further useful interaction of Pi-Pi-T-shaped is found with HIS207 residue in 3 as COX-1 inhibition, whereas, TYR130 residue in 3 and HIS207, HIS386, HIS388 residues in 6 are found as COX-2 inhibition. In some of these derivatives, interactions involving the Pi-anion, Pi-cation, Pi-donor, Pi-Pi-stacked, Pi-Sulfur, and Amide-Pi-stacked are also seen. Here, some selected interactions of the selected drugs have been also shown in Figure 7.

pIC_{50} studies

In order to calculate pIC_{50} values, multiple linear regression (MLR) equation is applied.

Here, pIC_{50} (Activity) = −2.768483965 + 0.133928895 × (Chiv5) + 1.59986423 × (bcutm1) + (− 0.02309681) × (MRVSA9) + (− 0.002946101) × (MRVSA6) + (0.00671218) × (PEOEVSA5) + (− 0.15963415) × (GATSv4) + (0.207949857) × (J) + (0.082568569) × (Diametert)

The connection between one dependent variable (pIC_{50}) as well as a number of independent variables (descriptors Chiv5, bcutm1, MRVSA9, MRVSA6, PEOEVSA5, GATSv4, J, and Diametert) is investigated using a multiple linear regression equation. From Table S2 and Figure 9, it has been found that the pIC_{50} ranges from 4.0214 to 6.6181. It is mentioned that the range of pIC_{50} for standard drugs is between 4.0 and 10. Among the heterocyclic amide analogue (3-6), according to the investigated pIC_{50} values, compound 6 is considered the best drugs. In overall, compound 6 exhibited most potential drug activities among the all investigated heterocyclic amide derivatives (3-6).

CONCLUSION

In this work, the physicochemical and prostaglandin inhibitory properties of heterocyclic amide derivatives were investigated. The structures 3-6 were supported by equilibrium geometry, vibrational frequency, and UV-Visible spectral calculations. All of the compounds under investigation showed thermal stability, and exhibited lower gaps of HOMO-LUMO and greater softness than the parent molecules. Most of the analogues have higher binding affinities and non-bond interactions than the parent compounds. Compared to COX-1 (6Y3C), all heterocyclic amide analogues 3-6 have better binding affinities with the COX-2 (5F19) protein. Additionally, the presence of the polar acetate group (-$OCOCH_3$) and the substitution of the alkyl chain by CF_3, in the 4-5F19 complex result in a higher binding affinity than other compounds. All of the tested heterocyclic amide analogues have better pharmacokinetic characteristics, and exhibited acute oral toxicity in the III category except compound 5 (category II), suggesting that they have better oral absorption properties than standard drugs ibuprofen (2) and aspirin (1). As per pIC_{50} studies, compound 6 showed the best drug activity. In light of the aforementioned study, this work may be useful in developing potential COX-2 inhibitors.

CONFLICTS OF INTEREST

Author declares no conflicts of interest.

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