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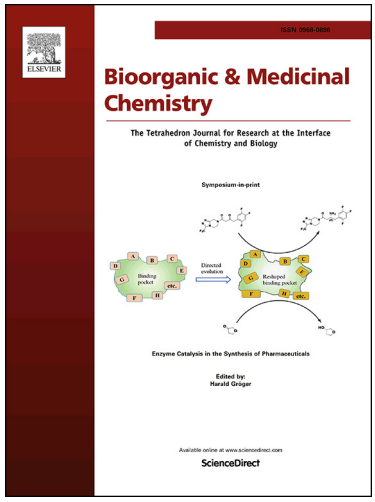

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Synthesis, Spectroscopic Characterization, Molecular Docking and Theoretical Studies (DFT) of N-(4-aminophenylsulfonyl)-2-(4-isobutylphenyl) propanamide having Potential Enzyme Inhibition Applications

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Abstract

A mutual prodrug (**1**) of ibuprofen and sulphanilamide has been synthesized with dual activity and improved toxicity profile. The synthesized compound has been characterized by elemental analysis, FT-IR, ^{1}HNMR, ^{13}CNMR and ESI-MS. The molecular geometry of the compound (**1**) was optimized using density functional theory (DFT/B3LYP) method with the 6-311G(d, p) basis sets in ground state. Geometric parameters (bond lengths, bond angles, torsion angles), vibrational assignments, chemical shifts and thermodynamics of the compound (**1**) has been calculated theoretically and compared with the experimental data. Comparative AutoDock study of compound (**1**) with cyclooxygenase enzymes (COX-1 and COX-2) were performed involving docking for possible selectivity of our prodrug within the two Cox enzymes. The highest binding affinities of -8.7 Kcal/mol and -8.1 Kcal/mol has been obtained for COX-1 and COX-2 enzymes respectively. Compound (**1**) exhibited enhanced anti-inflammatory, anti-ulcer and free radical scavenging activities as compared with the parent drugs. Based on various *in vitro* and *in vivo* tests it is suggested that the Compound (**1**) is more active than the parent drugs. Moreover, LD_{50} of compound (**1**) is higher than parent drug i.e. ibuprofen and sulphanilamide suggesting that the synthesized compound is much safer than its parent analogous.

Keywords: Ibuprofen, Sulfanilamide, Antibiotics, Anti-inflammatory drugs, AutoDock, DFT

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1. Introduction

Treatment of fever and inflammation dates back to centuries (~ 400 B.C.) when the Greek physician Hippocrates prescribed an extract from willow bark and leaves.[1] It was about 17th century when the active ingredient of willow bark salicin was identified and acetylsalicyclic acid (aspirin) was introduced into the market by Bayer in 1899. But the mechanism of action of anti-inflammatory and analgesic agents such as aspirin was unclear until 1971 when John Vane discovered the mechanism of action of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) thereby increasing our ability to develop novel anti-inflammatory therapies.[2] It is well known phenomenon that infections usually also result in inflammation. Therefore, it is common clinical practice that non-steroidal anti-inflammatory drugs (NSAIDs) are concomitantly prescribed with anti-infective.[3] Thus Non-steroidal anti-inflammatory drugs (NSAIDs) form a group of compounds which serve the purpose of fixing/inhibiting cyclooxygenase and thereby arresting prostaglandin production through arachidonic acid metabolism.[4]

Generally NSAIDs such as aspirin, ibuprofen and diclofenac exhibit nonselective COX inhibition but these are most widely prescribed NSAIDs to relieve short term fever, pain and inflammation.[5] The characteristic feature of these traditional nonselective COX inhibitor NSAIDs was the presence of a carboxylic acid (COOH) functional group.[6] In the early 1990s the second isoform of COX was discovered, providing a novel target to develop anti-inflammatory agents with superior safety profiles compared to traditional NSAIDs.[7,8] Consequently, selective COX-2 inhibitors (coxibs) based on a diarylheterocyclic ring template as in celecoxib and rofecoxib were developed.[9,10]

Scientists are taking interest in determination of the structural and spectroscopic properties of compounds using both experimental techniques and theoretical methods to have better understanding of phenomenon at molecular level. Moreover, autodock studies of targeted compound give us a clear picture of interaction of a drug with enzyme of interest thus saving time that has been spent in past for *in vitro* experiments.[11] Density functional theory (DFT) is a powerful tool in theoretical modeling and with recent advancement in computational facilities it been able to have great accuracy in reproducing the experimental values for the geometry, dipole moment, vibrational frequency, etc.[12-19] Molecular docking is one of the common tools used in computer-aided drug designing (CADD) which is widely used in studying the binding affinity of a ligand to a target protein and also in understanding the possible mode of action by which the ligand inhibits the target protein in disease treatment.[20] A comparison of the experimental and theoretical studies can be very useful in

making correct assignments and understanding the basic phenomenon and hence establishment of molecular structure-activity relationship.

Pharmacologically and commercially important NSAIDs like aspirin, ibuprofen, naproxen, indomethacin, flurbiprofen have been well studied both kinetically and structurally.[6-10] But another distinct class of compounds, the sulphanilamide group, was not given proper attention for time long. Keeping in view such a necessity, design and synthesis of mutual prodrugs (PD) involving anti-infectives and NSAIDs has been of interest. This design carries the benefits of a prodrug, which are: better lipophilicity, better bio-availability, reduced toxicity and a sustained release effect. Keeping in view of above mentioned facts we have synthesized a novel prodrug of ibuprofen and sulphanilamide, N-(4-aminophenylsulfonyl)-2-(4-isobutylphenyl) propanamide, (**1**). The synthesized compound (**1**) has been characterized by instrumental techniques and molecular geometry was optimized using density functional theory (DFT/B3LYP) method with the 6-311G(d,p) basis sets in ground state. Geometric parameters (vibrational assignments, chemical shifts) has been calculated theoretically and compared with the experimental data. Comparative AutoDock study of compound (**1**) was also conducted with cyclooxygenase enzymes (COX-1 and COX-2) involving docking for possible selectivity of our prodrug within the two Cox enzymes. The highest binding affinities of -8.7 Kcal/mol and -8.1 Kcal/mol has been obtained for COX-1 and COX-2 enzymes respectively. The compound was also subjected to *in vitro* analysis and the results showed enhanced anti-inflammatory, anti-ulcer and free radical scavenging activities as compared with the parent drugs. To the best of our knowledge, no experimental, computational and *in vitro* study on the compound (**1**) has been published in the literature yet. Furthermore, the presented data can be helpful in context of the further studies of prodrugs of ibuprofen and its derivatives.

2. Results and Discussion

2.1 Synthesis

Synthesis of the title compound (**1**) was carried out by converting ibuprofen to its acid chloride by use of oxalyl chloride followed by coupling with sulphanilamide through an $–NH_2$ group as shown in Scheme 1. There are two possible products as a result of the coupling reaction. We have isolated the compound (**1**) by column chromatography. (detail in supporting information)

2.2 Computational analysis

2.2.1 Quantum chemical calculations

All the calculations were performed by using Gaussian 09 package [21] and Gauss-View molecular visualization software [22] on the personal computer without restricting any symmetry. The structure was optimized by density functional theory (DFT)/B3LYP [23,24] method with 6-311G(d,p) as basis sets. From the optimized geometry of the molecule (Figure 1), vibrational assignments and chemical shifts of the title compound have been calculated theoretically and compared with the experimental data. Besides, the frontier molecular orbital (FMOs), molecular electrostatic potential (MEP), Mulliken population analysis and thermodynamic properties of the compound were also investigated by theoretical calculation results.

2.2.2 Vibrational spectra

Theoretical harmonic frequencies had been calculated using density functional theory (DFT/B3LYP) method with the 6-311G(d,p) basis sets in ground state. Scaling factors of 0.982 [25] was used for B3LYP/6-311G(d,p) levels. Theoretical FT-IR spectra of the compound (Figure 2) showed that the results are in agreement with experimental. (supporting information)

2.2.3 NMR spectra

The ^{1}H and ^{13}C NMR spectra of the compound (**1**) recorded using TMS as an internal standard and chloroform ($CDCl_3$) solvent. Chemical shifts of hydrogen and carbon atoms were determined from the obtained spectra. GIAO ^{1}H- and ^{13}C- chemical shift calculations had been carried out using the B3LYP methods with 6-311G(d,p) as basis set for the optimized geometries. The ^{1}H and ^{13}C NMR chemical shifts were converted to the TMS scale by subtracting the calculated absolute chemical shielding of TMS ($\delta = \Sigma o - \Sigma$), where δ is the chemical shift, Σ is the absolute shielding and Σo is the absolute shielding of TMS, whose values are 31.64 and 193.08 ppm for B3LYP/6-311G(d,p), respectively. To make comparison with experimental observations, we presented correlation graphs in Figure 3 based on the calculations (correlation coefficients of 0.9994 for ^{1}H NMR and 0.9984 for ^{13}C NMR, respectively).

22.2.4 Quantum-chemical studies

2.2.4.1 HOMO and LUMO analysis

Frontier molecular orbitals (FMO) i.e. highest occupied molecular orbital (HOMO) and lowest lying unoccupied molecular orbital (LUMO) are important ones in a molecule. The HOMO energy characterizes the ability of electron giving while LUMO energy characterizes the ability of electron accepting. Molecular stability depends on the gap between HOMO and LUMO energy levels [26]. The distributions of the HOMO and LUMO orbitals computed at the B3LYP/6-311G(d, p) levels for the compound (**1**) shown in Figure 4. The calculations indicated that the compound (**1**)

has 76 occupied molecular orbitals and the value of the energy separation between the HOMO and LUMO are -6.3085 and -1.1793 eV for at the same levels. By using HOMO and LUMO energy values for a molecule, electronegativity, chemical hardness and chemical softness were calculated as follows:

$\chi = (I+A)/2$(electronegativity), $\eta = (I-A)/2$ (chemical hardness), $S = 1/2\ \eta$ (chemical softness). Where I and A are ionization potential and electron affinity; $I = -E_{HOMO}$ and $A = -E_{LUMO}$, respectively [27]. The HOMO and LUMO energies, the energy gap (ΔE), the ionization potential (I), the electron affinity (A), the absolute electronegativity (χ), the absolute hardness (η) and softness (S) for molecule had been calculated at the same levels and the results are given in Table 1.

2.2.5 Molecular electrostatic potential (MEP)

A molecule of electrostatic potential map provides vital information about the electron acceptor and electron-donor regions. Thus intramolecular and intermolecular hydrogen bonds can be conveniently calculated. The different values of the electrostatic potential at the surface are represented by different colors; red represents regions of most electro negative electrostatic potential, blue represents regions of most positive electrostatic potential and green represents regions of zero potential. The electrostatic potential increases in the order red < orange < yellow < green < blue. Figure 5 showed the molecular electrostatic potential maps of compound for B3LYP/6-311G(d, p) levels, where blue indicated the strongest attraction and red indicated the strongest repulsion. Here, the red regions settled on O1, O2, and O3 atoms while blue at N2 atom respectively. So, it can be estimated to be N2 atom electron-donor and O1, O2, and O3 atoms are electron-acceptor.

2.2.6 Mulliken population analysis

The Mulliken charge distributions of the compound (**1**) was calculated using B3LYP/6-311G(d, p) levels. The calculated charge for all atoms were shown in Figure 6. It is well-known that the Mulliken charges confirm the hydrogen bonding in the molecular structure of compound.

2.2.7 Thermodynamic properties

In order to determine thermodynamical properties of the compound (1), the standard thermodynamic functions, entropy (S_m^o) heat capacity ($C_{p,m}^o$) and enthalpy (H_m^o) based on the vibrational analysis at B3LYP/6-311G(d,p) level and statistical thermodynamics for the compound (**1**) were obtained. The entropies, standard heat capacities, and enthalpies increased at any temperature from 50 K to 900 K since increasing temperature causes an increase in the intensity of the molecular vibration and the populations of the excited vibrational states. Based on the calculated data of the compound (**1**), the correlation equations between the thermodynamic properties and

temperature T had been obtained which can be used for the further studies of the compound (**1**) (Figure 7).

$$S_m^o = -7E^{-5}T^2 + 0.3471T + 3.4273\ (R^2 = 0.9951) \quad (1)$$

$$C_{p,m}^o = -0.0001T^2 + 0.3543T - 0.6614\ (R^2 = 0.9924) \quad (2)$$

$$H_m^o = 0.0001T^2 + 0.0243T + 5.5404\ (R^2 = 0.9912) \quad (3)$$

2.3 Molecular docking studies

The molecular docking of compound (**1**) was done with two target proteins, COX1(1EQG) [28] and COX2 (1CX2) [29] downloaded from the protein data bank. The reference ligands were used to set-up the binding pocket before compound (**1**) was docked into the target protein. For, COX1, Ibuprofen which is the reference ligand in the co-crystallized protein gave the parameter used for the binding pocket where 25.995, 34.157 and 200.016 for the X, Y and Z axes within a radius of 16 Å while for COX-2 which had a selective inhibitor, SC 558 has its reference ligand in the co-crystallized protein gave rise to 24.306, 22.187 and 14.462 for the X, Y and Z axes within a radius of 15 Å as the parameters for its binding pocket.

Auto Dock Vina [30], which is a widely used molecular docking program combines both knowledge based and empirical scoring functions and whose docking score corresponds to binding energy in kcal/mol was used for the docking analysis. Density functional theory (DFT) method with the hybrid B3LYP employing the 6-311G(d,p) basis set, that has been shown to give quite satisfactory geometry results for organic systems [31, 32] using the Gaussian 09 package [21] was carried out to optimize the structure of compound (**1**). The optimized compound (**1**) structure (Fig. 1) was used as the input ligand in all the docking calculations. The docking result gave rise to 9 different poses (Tables 2 and 3). The first pose which had 0 has both the RMSD (Root Mean Square Distance) of its upper and lower boundaries was selected to determine the binding modes and molecular interactions (Figs 8 and 9) and this pose also had the highest binding affinity of -8.7 kcal/mol and -8.1 kcal/mol for both COX-1 and COX-2. Fig.8a shows the binding mode of PD in COX-1 with ibuprofen the reference ligand highlighted in yellow. The molecular interactions (Fig. 8b) of compound (**1**) in the binding pockets include hydrogen bonding with ARG 120, and alkyl bonding with PHE 518, ILE 523, VAL 349, ALA 527, LEU 531, LEU 359, VAL 116 and LEU 93. For COX-2, the binding mode (Fig. 9a) shows SC-558 highlighted in yellow. The molecular interactions (Fig 9b) of compound (**1**) in the binding pocket includes, hydrogen bonding with MET 522, carbon-hydrogen bonding with ALA 527, VAL 349 and SER 530; pi-cation bonding with ARG 120; pi-pi stacking with TRY 355 and GLY 526 and alkyl bonding with HIS 90, VAL 523, PHE 518,

LEU 351 and LEU 531. It is worthy to note that there are some common amino acids which compound (**1**) interacts with in both COX-1 and COX-2. These includes; ARG 120 and VAL 349. This may mean that compound (**1**) is a potential inhibitor for both COX-1 and COX-2.

Docking studies was also conducted with 5-LOX, AChE and BuChE (supporting information). Compound (**1**) exhibited the following binding affinities values for all three target proteins respectively -9.4, -9.1 and -10.5 kcalmol^{-1} for 5-LOX, AChE and BuChE respectively. Compound (**1**) showed highest binding affinity with BuChE. (Supporting Information)

2.4 Biological Activities

2.4.1 Anti-inflammatory and anti-ulcer activities

Percent inhibitions of edema by the ibuprofen, sulfanilamide and the compound (**1**) at 50 mg kg^{-1} dose were calculated. These result (Table 4) indicated a substantially enhanced anti-inflammatory activity of compound **1** compared to ibuprofen and sulphanilamide. Similar results has been obtained for anti-ulcer test where compound **1** showed substantially reduced ulceration compared to ibuprofen and sulphanilamide (Table 4). Experiments had also been carried out using ibuprofen and sulphanilamide at half dose as a control experiment. Again compound **1** was found to be more active compared to ibuprofen and sulphanilamide at half dose. The main cause of this enhanced anti-inflammatory and anti-ulcer activity may be due to the presence of polar groups (sulphonyl and amide) in middle and hydrophobic group (*p*-isobutyl and benzene) at one side of compound **1**.

2.4.3 Enzyme inhibition studies

2.4.3.1 In vitro 5-lipoxygenase (5-LOX) inhibition assay

In vitro anti-inflammatory activity of the compound (**1**) was evaluated by inhibition of 5-LOX as this enzyme is involved in an alternative pathway for processing of arachidonic acid resulting in increased production of pro-inflammatory and gastrotoxic leukotrienes [33]. Compound (**1**) exhibited higher inhibition (48.4%) than ibuprofen (42.5%) suggesting that compound (**1**) possesses improved activity. Experiments had also been carried out using ibuprofen and sulphanilamide at half dose as a control experiment. Again compound **1** was found to be more active compared to ibuprofen and sulphanilamide at half dose. The same results has also been proved by docking studies (supporting Information). Main cause of this activity is the presence of sulphonyl and amide group in the vicinity of ARG246 and ARG 370 which provide hydrogen bonding interactions. Presence of *p*-isobutyl group provides the cruicial pi-alkyl interactions with Val361,

Leu281 and phe286 within the pocket of enzyme. All these interactions together with others (Figure 4, supporting information) are responsible for better binding affinity with compound **1** compared with ibuprofen.

2.4.3.2 In vitro acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibition assays

Acetylcholine has been shown to exert an anti-inflammatory function by down-modulating the expression of pro-inflammatory cytokines [34]. Thus the expression of AChE, being responsible for acetylcholine hydrolysis, is modulated in inflammation. Generally the BuChE activities resemble those of AChE, therefore, the compounds under investigation were tested for their inhibitory activity against these enzymes. It was found (Table 5) that the inhibitory activity of the compound (**1**) was substantially less than that of ibuprofen against AChE and almost two times higher against BuChE (Table 6). This result suggests partially selective inhibition of BuChE by the compound (**1**) as compared with ibuprofen. Experiments had also been carried out using ibuprofen and sulphanilamide at half dose as a control experiment. Again compound **1** was found to be more active compared to ibuprofen and sulphanilamide at half dose. The same results has also been proved by docking studies which shows highest affinity of compound **1** with BuChE (supporting information). By comparing the experimental data and molecular docking studies structure-activity relationship can be deduced. Here again the sulphonyl, amide and *p*-isobutuyl groups of compound **1** fit into the right sized pocket of enzymes AChE and BuChE compared with ibuprofen. His381, Arg525 and Ala397 provides important pi-alkyl interactions in the case of AChE (Figure 5, Supporting Information) while phe526, try396 asn229 groups of BuChE interacts with *p*-isobutuyl, benzene and sulphonyl groups of compound 1 via pi-alkyl, pi-pi and hydrogen bonding respectively (Figure 6, Supporting Information).

2.4.4 Free radical scavenging activities

The free radical scavenging activity of the compound (**1**) was found (Table 7) to be nearly double than that of ibuprofen; this also suggests that the compound (**1**) should exhibit enhanced anti-inflammatory activity by suppressing the effect of reactive oxygen species.

2.4.5 Toxicity study

The LD_{50} (oral, rats) values were found to be sulfanilamide: 3500 mg kg^{-1}, ibuprofen: 630 mg kg^{-1} and compound (**1**): 4050 mg kg^{-1}. These results clearly indicate that the compound (**1**) safer than the parent drugs.

3. Conclusion

NSAIDs containing carboxylic groups can be covalently coupled with anti-infectives containing amino groups to produce mutual prodrugs with dual activity and improved toxicity profile. A novel compound (**1**), a mutual prodrug of ibuprofen and sulfanilamide, has been synthesized. Compound (**1**) geometry was optimized using density functional theory (DFT/B3LYP) method with the 6-311G(d, p) basis sets in ground state. Calculated geometric parameters (bond lengths, bond angles, torsion angles), vibrational assignments, chemical shifts and thermodynamics are in good agreement with the experimental data. Molecule was subjected to Comparative AutoDock study with cyclooxygenase enzymes (COX-1 and COX-2) involving docking for possible selectivity of compound (**1**) within the two Cox enzymes. The highest binding affinities of -8.7 Kcal/mol and -8.1 Kcal/mol has been obtained for COX-1 and COX-2 enzymes respectively. Compound (**1**) was found to have enhanced anti-inflammatory, anti-ulcer and free radical scavenging activities when compared with the parent drugs suggesting that the compound (**1**) is more active than the parent drugs while LD_{50} of compound (**1**) is higher than parent drug i.e. ibuprofen and sulphanilamide.

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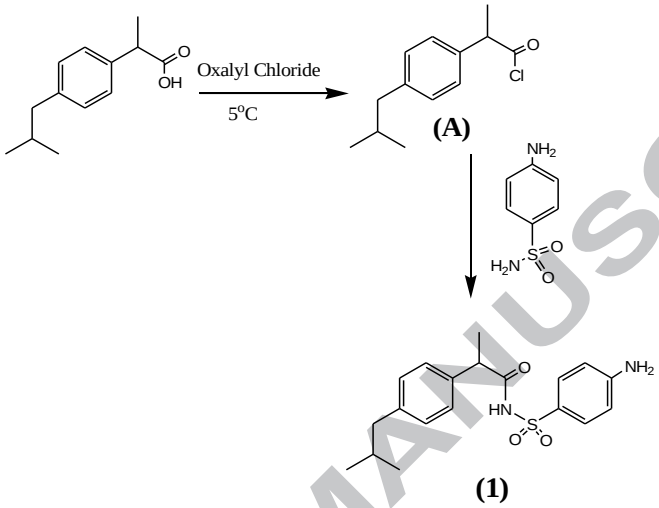
4. References

1. Vane JR, *J Physiol Pharmacol.* 2000; 51: 573–586.
2. Xiaoxia Z, Wenchao L, Alkebaier A, Tao C, Peng P. *Mol. Med. Rep.*, 2017; 16: 8619-8626.
3. Supakanya W, Amaraporn W, Katie M, Janani R, *Aging & Disease*, 2018; 9: 143-150.
4. Otto JC, Smith WL, *Lipid Mediate cell signal* 1995; 12: 139-156.
5. Inotai A, Hanko B, Meszaro A, *Pharmacoepidemiol. Drug Saf.* 2010; 19: 183–190.
6. Martin ACM, Christopher WM, Amode RT, Jerad AH, Charles DM, Sports Medicine, 2017; 3: 36.
7. Talley JJ, *Prog. Med. Chem. Res.* 1999; 36: 201–234.
8. Black WC, *Annu. Rep. Med. Chem.* 2004; 39: 125–138.
9. Andrew CF, Hong XD, Carolyn AL, Robert EK, Kevin KCL, Sarah JF, Christopher JOD, *J. Med. Chem.*, 2017; 60: 6480-6515.
10. Prasit P, Wang Z, Brideau C, et al. *Bioorg. Med. Chem. Lett.* 1999; 9: 1773–1778.
11. Proft FD, Geerlings P, *Chem. Rev.* 2001; 101: 1451–1464.

12. Fitzgerald G, Andzelm J, *J. Phys. Chem.* 1991; 95: 10531–10534.
13. Ziegler T, *Pure Appl. Chem.* 1991; 63: 873–880.
14. Andzelm J, Wimmer E, *J. Chem. Phys.* 1992; 96: 1280–1303.
15. Scuseria GE, *J. Chem. Phys.* 1992; 97: 7528–7530.
16. Dickson RM, Becke AD, *J. Chem. Phys.* 1993; 99: 3898–3905.
17. Johnson BG, Gill PMW, Pople JA, *J. Chem. Phys.* 1993; 98: 5612–5626.
18. Oliphant N, Bartlett RJ, *J. Chem. Phys.* 1994; 100: 6550.
19. Zhang Y, Guo ZJ, You XZ, *J. Am. Chem. Soc.* 2001; 123: 9378.
20. Seliman AAA, Altaf M, Onawole AT, et al. *J. Organomet. Chem.* 2017; 848: 175–183.
21. Frisch MJ, Trucks GW, Schlegel HB, Gaussian 03, Revision E.01, Gaussian Inc., Wallingford, CT; 2004.
22. Dennington R, Keith T, Millam J, Gauss View, Version 4.1.2, Semichem Inc., Shawnee Mission, KS; 2007.
23. Becke AD, *J. Chem. Phys.* 1993; 98: 5648.
24. Ditchfield R, Hehre WJ, Pople JA, *J. Chem. Phys.* 1971; 54: 724–728.
25. Dennington I, Keith RT, Millam J, et al. GaussView, Semichem, Inc., Shawnee Mission, KS; 2003.
26. Fleming I, Frontier Orbitals and Organic Chemical Reactions, Wiley, London; 1976.
27. Pearson RG, *Proc. Natl. Acad. Sci.* 1986; 83: 8440–8841.
28. Selinsky BS, Gupta K, Sharkey CT, et al. *Biochemistry* 2001; 40: 5172–5180.
29. Kurumbail RG, Stevens AM, Gierse JK, et al. *Nature* 1996; 384: 644–8.
30. Trott O, Olson A, *J. Comput. Chem.* 2010; 31: 455–461.
31. Wiberg KB, *J. Comput. Chem.* 2004; 25: 1342–1346.
32. Haroon M, Akhtar T, Yousuf M, et al, *J. Mol. Struct.*, 2018; 1167: 154-160.
33. Ellman GL, Courtney KD, Andres V, et al. *Biochem. Pharmacol.* 1961; 7: 88-95.
34. Brand-Willams W, Cuvelier ME, Berset C, *Food Sci.Technol.* 1995; 28: 25-30.



Scheme 1. Synthesis of mutual prodrug of ibuprofen and sulfanilamide (**1**).

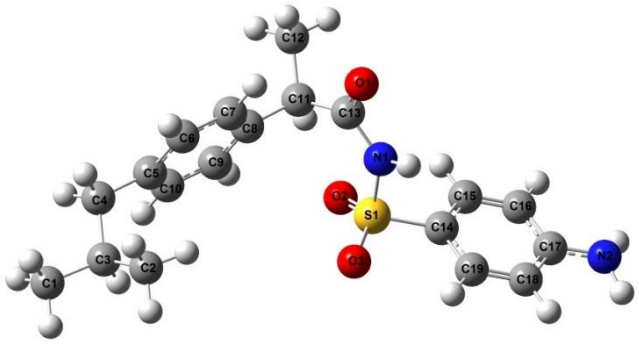


Figure 1: Optimized structure of compound (**1**)

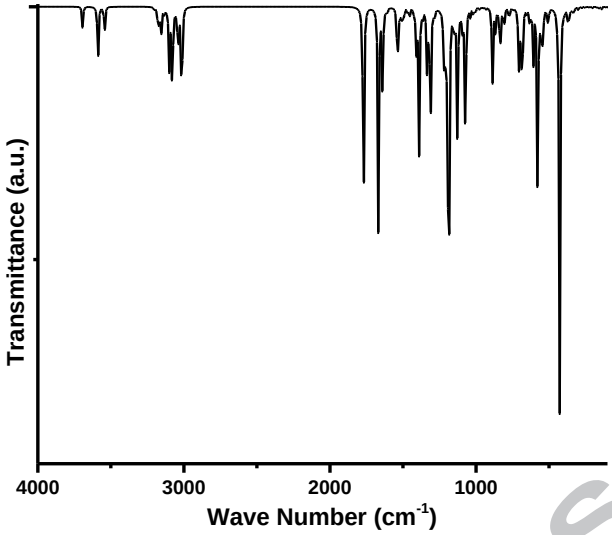


Figure 2: Theoretical FT-IR Spectrum of compound (**1**)

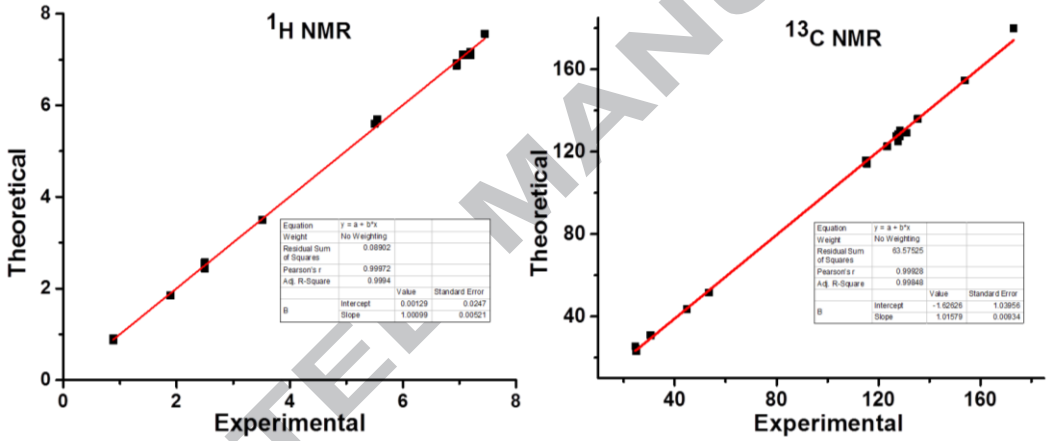


Figure 3: Correlation graphics of calculated and experimental chemical shifts of compound (**1**)

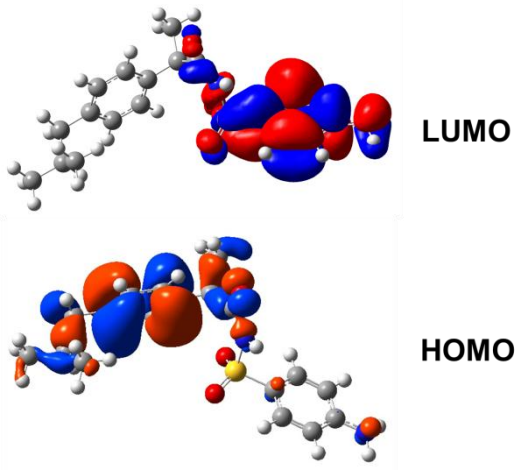


Figure 4: The HOMO and LUMO molecular orbital diagram

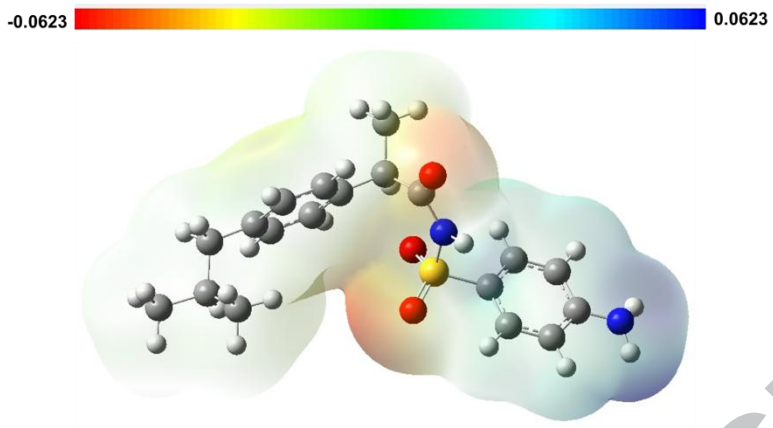


Figure 5: Molecular electrostatic potential (MEP) map of compound (**1**) in gas phase.

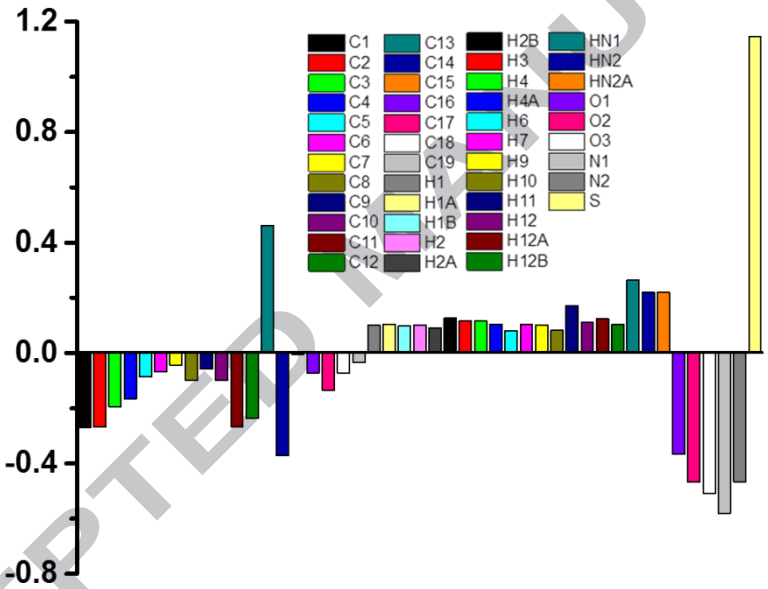


Figure 6: The Mullikan charges diagram of compound (**1**)

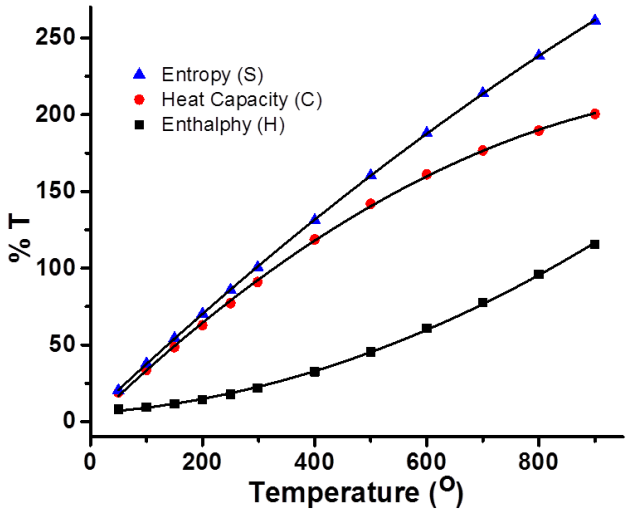


Figure 7: Thermodynamic Properties of compound (**1**)

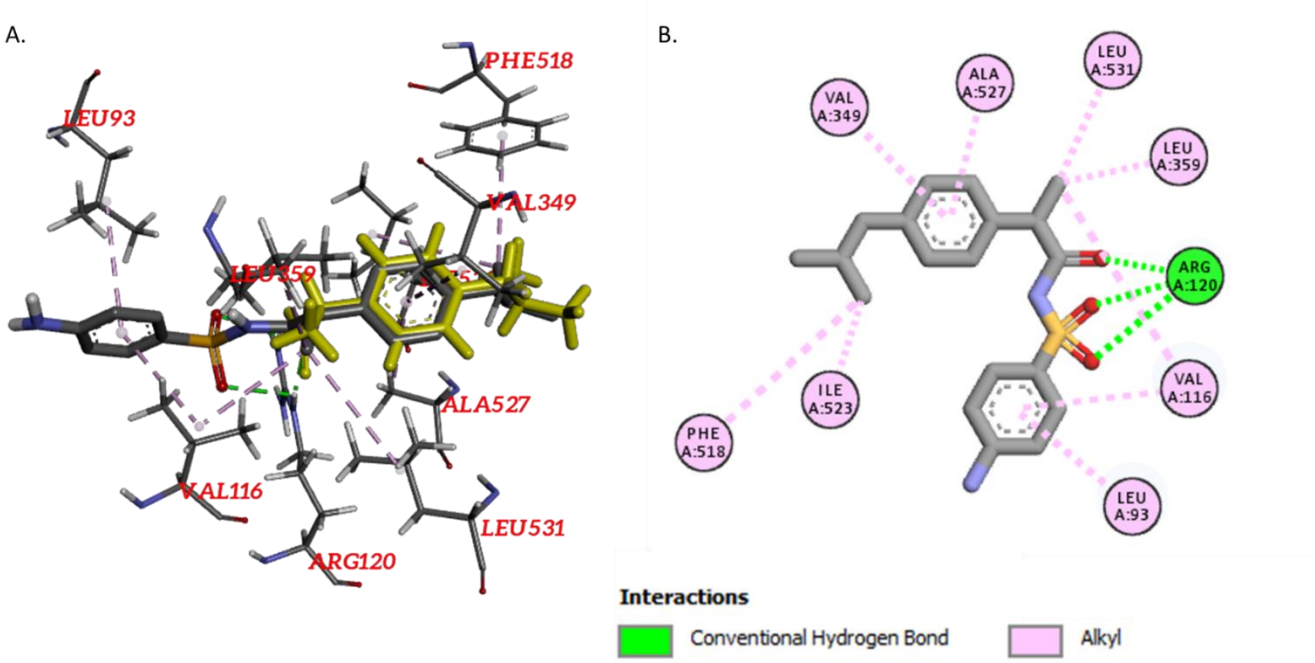


Figure 8. The binding mode(A)* and molecular interactions(B) of the title compound in COX2 (PDB ID: 1EQG) *the native ligand is in yellow

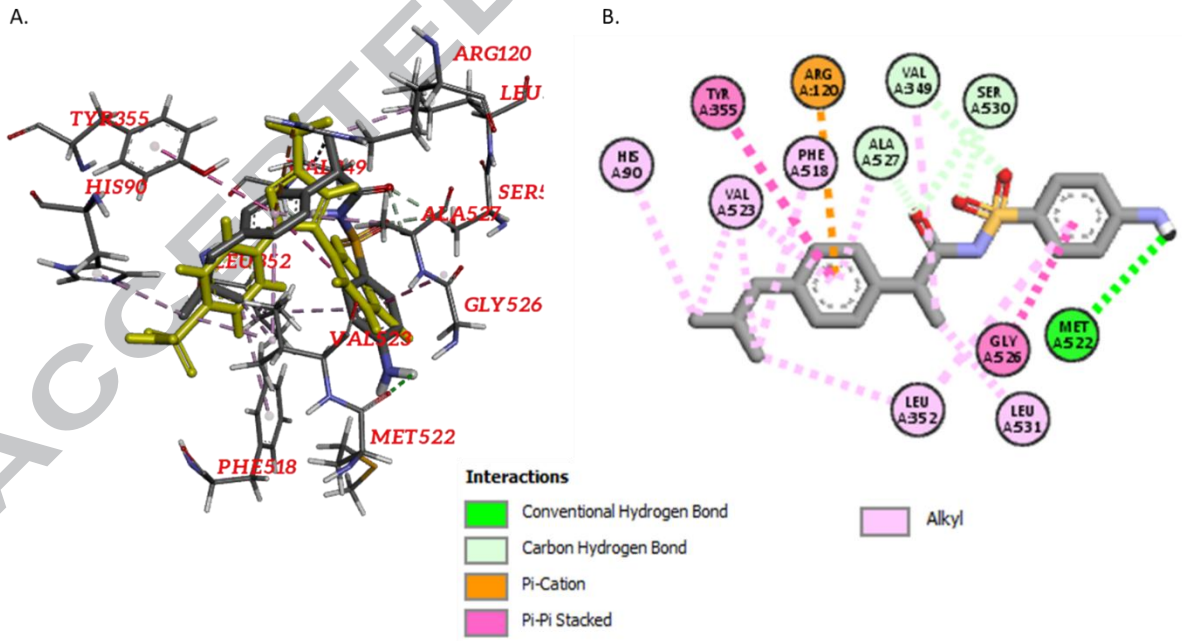


Figure 9: The binding mode(A)* and molecular interactions(B) of the title compound in COX2 (PDB ID: 1CX2) *the native ligand is in yellow

Table 1: The calculated frontier orbital energies, electronegativity, hardness, and softness of compound using B3LYP/6-311G(d, p) level.

Parameters	**B3LYP/6-311G(d, p)**
E_{HOMO}	-6.3085
E_{LUMO}	-1.1793
I (eV)	6.3085
A (eV)	1.1793
χ *(eV)*	3.7437
η *(eV)*	2.5646
S (eV)	0.1949

Table 2. Molecular docking results of compound (**1**) in COX-1

Poses	**Binding Affinity (kcal/mol)**	**rmsd/ub**	**rmsd/lb**
1	-8.7	0	0
2	-8.6	2.107	1.553
3	-8.6	9.115	3.223
4	-8.4	3.221	2.62
5	-8	8.912	4.505
6	-8	3.286	2.406
7	-7.9	10.319	6.734
8	-7.9	9.024	6.944
9	-7.9	7.349	4.156

Table 3. Molecular docking results of compound (**1**) in COX-2

Poses	**Binding Affinity (kcal/mol)**	**rmsd/ub**	**rmsd/lb**
1	-8.1	0	0
2	-8	2.267	1.095
3	-7.3	5.467	2.838
4	-7.3	5.816	2.61
5	-7	14.335	12.809
6	-6.9	14.497	11.266
7	-6.9	5.022	1.894
8	-6.6	14.748	12.79
9	-6.5	13.941	12.362

Table 4: Anti-inflammatory and Anti-Ulcer assay

Compound	**Anti-Inflammatory**	**Anti-Ulcer**
	% Inhibition	**% Ulcer Area**
Ibuprofen	45±0.14	35±0.18
Sulphanilamide	10±0.07	10±0.09
50%Ibuprofen+50%Sulphanilamide	23.23±0.11	20±0.12
Compound **1**	75±0.16	8±0.04

Table 5: Acetylcholinesterase assay

Compound	**Concentration of the Solution used in Assay (mM)**	**% Inhibition**	**IC_{50} (µ mol)**
Ibuprofen	0.5	61.19±0.04	235.11±0.17
Sulphanilamide	0.5	10.19±0.05	145.24±0.13
50%Ibuprofen+50%Sulphanilamide	0.5	40.23±0.07	------
Compound **1**	0.5	47.21±0.11	< 600

Table 6: Butyrylcholinesterase assay

Compound	**Concentration of the Solution used in Assay (mM)**	**% Inhibition**	**IC_{50} (µ mol)**
Ibuprofen	0.5	35.51±0.15	< 600
Sulphanilamide	0.5	5.19±0.07	138.79±0.09
50%Ibuprofen+50%Sulphanilamide	0.5	21.68±0.06	------
Compound **1**	0.5	65.88±0.36	214.41±0.05

Table 7: DPPH activity

Compound	**Concentration of the solution used in assay (mM)**	**Inhibition (%)**	**IC_{50} (µmol)**
Ibuprofen	0.5	11.33±0.11	<500
Quercetin	0.25	93.21±0.97	16.96±0.14