Molecular synthesis, structural elucidation, charge transfer interaction, vibrational investigation and molecular docking studies of ethoxy substituted ketothiophene-thaizole derivative

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Abstract: The molecular structure of the novel ketothiophene-thaizole derivative was synthesized. Optimized parameters of the compound was investigated by using density functional theory calculation using Beckes three-parameter exchange functional in combination with the Lee Yang Parr correlation Gaussian 09 program package with the standard 6-311G basis software set. Reactivity of the compound was predicted by the existence of hydrogen bonded intramolecular interactions and the hyperconjugative energy transfer leading to the stabilization of the system. Molecular docking scores reveal good binding affinity and the inhibition activity of the molecule against PDB : 4QP1 as a crystal structure of empty hepatitis A virus and the protein structure of heparan sulfate lyase HepC mutant from Pedobacter heparinus (PDB code: 4MMI) obtained from the protein data bank.

Keywords: Ketothiopene derivative, ethoxy substitution, thiazole ring, Density functionl theory, molecular docking.

Introduction:

Despite existing therapies for various ailments, emerging diseases or disorders need more selective treatments. Therefore the runaway progress of drug discovery and development is inevitable. In medicinal chemistry, nitrogen and sulfur based heterocycles, including thiophene and thiazoles, are regarded as a fundamental platform for constructing newer entities for various diseases. Thiophene is a 5-membered ring having sulfur atom, thiazole is a five membered ring having both nitrogen and sulphur. A heterocyclic ring, which serves as the central component of pharmacological compounds, is a widespread occurrence in both natural and synthesized medications. One of the effective strategies utilized in the process of discovering novel drugs is the hybrid architecture of bioactive pharmacophore compounds [1]. Thiophenes clubbed with other thiazole have recently been favored as scaffolding for the creation of new promising bioactive compounds [2]. Thiophene along with the thiazole and carbonyl group possesses antibacterial, antifungal activities, anttibercular[3], anticancer activities [4], anti-oxidant, anti-inflammatory [5].

In the process of doing research of new heterocyclic compounds with expected biological activities, we reported the synthesis of new ketothiophene-thiazole derivatives, viz.(4-amino-2-(ethoxyphenylamino)thiazol-5-yl)(thiophene-2-yl)methanone (AETM), The optimized structure of the compound was analyzed by the density functional theory (DFT) calculation using Becke's three-parameter exchange functional in combination with the Lee-Yang-Parr correlation (B3LYP) Gaussian 09 program package with the standard 6-311G basis software set. Docking studies were carried out to find its antiviral and antibacterial activities using the Hex 8.0 software and visualized by Discovery Studio 3.5.

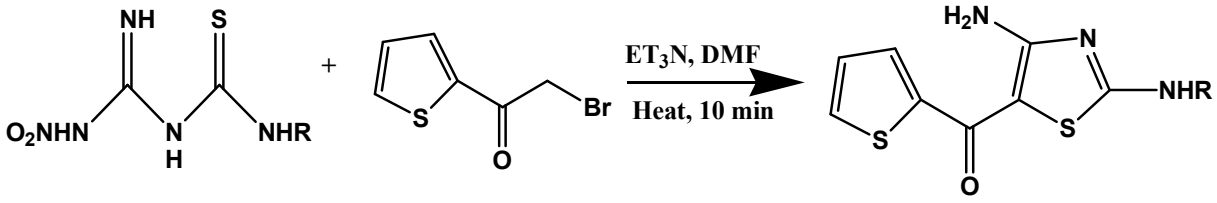
EXPERIMENTAL SECTION

Methodology

It was experimented with AR grade chemicals, wihout purification. FT-NMR spectra were documented with the Bruker Avance 400 spectrometer(four hundred MHz for ^{1}H and ^{13}C NMR spectra), mass spectrometer on Agilent 6520(QTOF) positive mode ESI-MS and Nicolet 400 FTIR spectrometer. Uncorrected melting point was determined using digital melting point apparatus. The Density Functional Theory (DFT) calculations were performed using Gaussian-09 B3LYP/6-311G(d,p) basis set.

Synthesis of the compound (4-amino-2-(4-ethoxyphenyl)aminothiazol-5yl)(thiophene-2-yl)methanone(AETM)

1 p-ethoxyphenyl-3-(N-nitroguanidine)thiourea (1 mmol) in DMF (2 ml) was added to the mixture of 2-(2-bromoacetyl)ketothiophene in DMF(2 mL). The reaction mixture was kept in a waterbath and the temperature was maintained to 80-85°C for five minutes. To this triethylamine (0.15 ml, 1mmol) was brought and heating become persevered for every other 10 minutes(Scheme 1). This aggregate was cooled and poured into ice cold water with stirring. The yellow-orange precipitate was filtered, washed with water and dried. The crude sample was recrystallized from methanol-water (2:1).



Scheme1: Synthetic Route of the titled compound

Synthesis of (4-amino-2-(4-ethoxyphenyl)aminothiazol-5 yl)(thiophene-2-yl)methanone

The orange yellow precipitate obtained was recrystallized using 2:1 ethanol-water solution. Yeild 78%, melting point: 144-147°C, Molecular weight: 345.06, Chemical formula: $C_{16}H_{15}N_3O_2S_2$. Elemental analysis of carbon, hydrogen, nitrogen, oxygen and sulphur found as: 55.63, 4.38, 12.16, 9.26, 18.56; Determined: 55.60, 4.35, 12.15, 9.24, and 18.52. FTIR(KBr) spectrum consists of bands at 3588.89 cm^{-1}, 3611.23 cm^{-1} (ν_{N-H}), 1714.86cm^{-1} ($\nu_{C=O}$), 3247.52 cm^{-1}(aromatic ν_{C-H}), 3058.90cm^{-1}, 3144.27cm^{-1} (aliphatic ν_{C-H}). ^{1}H NMR: (400 MHz, DMSO-d_6) δ7.83 (d, 1H, J=1.6 Hz, H-1 of thiophene), δ7.22 (t, 1H, J=5.2 Hz, H-2 of thiophene), δ7.83 (d, 1H, J=1.2 Hz, H-3 of thiophene) δ 7.17 (s, 2H, H-4), δ7.55 (d, 2H, J=2.8 Hz, 2ArH), δ6.95 (d, 2H, J=1.6 Hz, 2ArH), δ 4.05 (s, 2H,1.2 Hz), δ1.34 (t, 3H, 4.5 Hz), δ 10.22, (s, 1H, H-5). ^{13}C (75MHz, DMSO-d_6) 135.88, 129.0, 133.7, 144.3, 180.7, 137.3, 142.0, 159.1, 132.1, 121.3, 115.2, 152.0, 64.6, 14.8. ESI-MS MH^+ (346.07).

RESULTS AND DISCUSSION

Charactarization of the synthesized (4-amino-2-(4-ethoxyphenyl)aminothiazol-5 yl)(thiophene-2-yl)methanone(AETM)

The functional groups in the synthesized compounds were analyzed with the support of FTIR spectroscopic method. It was found that the absorption bands at 3588.89 cm^{-1}, 3611.23 cm^{-1} are due to the vibration of two N-H groups. The carbonyl group stretching vibration is specified at 1714.86 cm^{-1} and aromatic C-H stretching vibration is absorbed at 3247.52 cm^{-1}(aromatic ν_{C-H}). The C-H aliphatic stretching vibration is found at 3058.90cm^{-1}, 3144.27cm^{-1}. The ^{13}C NMR spectrum shows fourteen peaks, two of which arise from two-carbon each, thus accounting for all the sixteen carbons.

Geometrical optimization

The compound (4-amino-2-(4-ethoxyphenyl)aminothiazol-5-yl)(thiophene-2-yl)methanone was optimized using B3LYP/6-311G(d,p) basis set function using Gaussian 09 package. The optimized structure of the focused compound is shown in (Figure 1). Their bond distance, angles and dihedral angles are also calculated.

Due to the attachment of the carbonyl group in thiophene and thiazole ring, there is a slight variation in the bond length and bond angle. The C-C-S bond angle in thiophene decreases to 110.73°, the C-S bond length falls

from 1.74 Aº to 1.725 Aº. The thiazole ring is more influenced by the addition of the C=O group. The bond distance of C-S rises from 1.728 to 1.7786 and 1.714 to 1.7486. C-C and C-N bond length increases to 1.396 and 1.316 respectively. There is a minor deviation of bond dimension to 1.39 Aº and 1.38 Aº and bond angle to 121º and 118º in the phenyl ring due to the attachment of ethoxy group.

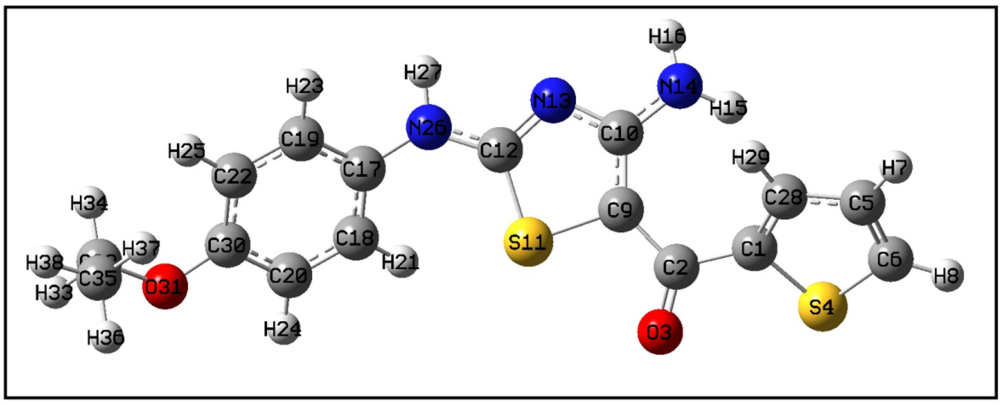


Figure 1: Optimized structure of the compound (4-amino-2-(4- ethoxyphenyl)aminothiazol-5 yl)(thiophene-2-yl)methanone

Natural bond orbital analysis

NBO analysis provides detailed descriptions of the various second-order interaction between filled orbitals of one system and the vacant orbitals of another subsystem, the natural bond orbital (NBO) calculations were performed using Gaussian 09 package at the DFT/B3LYP/6-311G* level. NBO analysis also used to elucidate the intermolecular interaction and delocalization of electron density within the molecule, larger E^2 value shows the intensive interaction between electron-donors and electron-acceptors and also the greater extent of conjugation of the whole system, the possible intensive interactions are given in (Table 1).

A very strong interaction has been observed between lone electron pair of S_4 and neighbour antibonding orbital of C_1-C_{28}, C_5-C_6 and the other lone pair of S_{11} and neighbour antibonding orbital of C_9-C_{10}, C_{12}-N_{13}, C_{18}-H_{21} with the occupancy of 1.98358 and 1.98114. The intramolecular hyperconjugative interaction in AETM is formed by the orbital overlap between σ (C-C), σ *, π (C-C), π*(C-C) bond orbitals which results in the intramolecular charge transfer (ICT) causing stabilization of the system. The hyperconjugative interaction of lone pair, LP (1) S_{11}> σ*C_{18} - H_{21} possibility of intramolecular interaction whose energy contribution is 0.70 kcal/mol with Vanderwall radii of 3.22 Aº.

TABLE I

SECOND ORDER PERTURBATION THEORY ANALYSIS OF THE TITLED COMPOUND

Donor(i)	Acceptor(j)	Energy($E^{2)}$	Occupancy
LP(2)O_3	σ *C_1-C_2	18.43	1.97534
LP(2)O_3	σ *C_2-C_9	16.35	1.97725
LP(2)S_4	π*C_1-C_{28}	21.91	1.98000
LP(2)S_4	π*C_5-C_6	23.91	1.98503
LP(1)S_{11}	σ *C_{18}-H_{21}	0.70	1.97502

$LP(2)S_{11}$	π^*C_9-C_{10}	13.23	1.77378
$LP(2)S_{11}$	π^*C_{12}-N_{13}	33.87	1.83009
$LP(1)N_{13}$	σ^*S_{11}-C_{12}	14.19	1.97856
$LP(1)N_{14}$	π^*C_9-C_{10}	41.29	1.77378
$LP(1)N_{26}$	π^*C_{12}-N_{13}	54.07	1.98143
$LP(1)N_{26}$	π^*C_{17}-C_{18}	33.55	1.97202

Atomic Charges

Atomic charge quantifies electronic shape discrepancies underneath atomic displacement. The uniformity in charge distribution is indicated by the smaller dipole moment (4.52 Debye). Biological activity of the titled compound increases due to the hyperconjugative interaction[6] of ethoxy group with the phenyl ring.

From the charge distribution diagram , we can find that the entire nitrogen atom poses negative charge and the nitrogen atom N_{14} retains more negative charge of (-0.4730)a.u due to high electronegativity (Figure 2,3). Maximum charge magnitude is observed in C_{10}(0.42089)a.u. All the hydrogen holds a positive charge, since the charge transfer is from carbon to hydrogen. Oxygen (O_3) conveys the charge of (-0.344405)a.u and the two sulphur conveys positive charge of S_4(0.320341)a.u and S_{11}(0.303157)a.u respectively (Table 2).

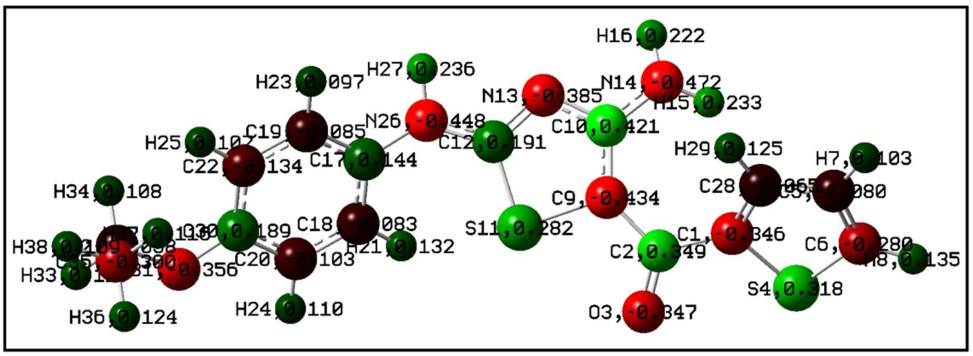


Figure 2: Mulliken atomic charge of the compound (4-amino-2-(4- ethoxyphenyl)aminothiazol-5 yl)(thiophene-2-yl)methanone

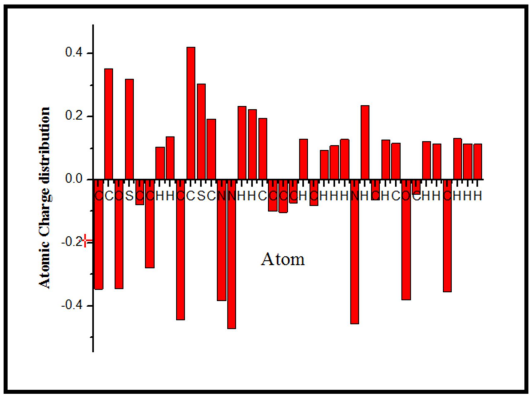


Figure 3: Charge distribution bar of the compound (4-amino-2-(4- ethoxyphenyl)aminothiazol-5 yl)(thiophene-2-yl)methanone

Table 2: Atomic Charge distribution of the compound (4-amino-2-(4- ethoxyphenyl)aminothiazol-5 yl)(thiophene-2-yl)methanone

Atom	Atomic charge distribution
C	-0.346953
C	0.352195
O	-0.344405
S	0.320341
C	-0.079146
C	-0.279619
H	0.104304
H	0.136096
C	-0.445205
C	0.420890
S	0.303157
C	0.193130
N	-0.383524
N	-0.473001
H	0.233544
H	0.223099
C	0.196289
C	-0.099860
C	-0.103443
C	-0.073180
H	0.128646

C	-0.081769
H	0.093009
H	0.108623
H	0.128778
N	-0.457604
H	0.235371
C	-0.064741
H	0.126457
C	0.116271
O	-0.380565
C	-0.045354
H	0.122607
H	0.114584
C	-0.356620
H	0.130343
H	0.114715
H	0.112536

HOMO-LUMO energy gap

HOMO-LUMO energy gap explains the chemical reactivity of the molecule. If the energy gap is less, it is more reactive. Thermal stability of the compound is related to the high energy gap and hardness of the molecule. It is found that the charge distribution of the HOMO level of the compound AETM is mostly localized on the thiophene ring and charge distribution of the LUMO level is delocalized throughout the thiazole ring. The energy gap is found to be less of -0.1366 a.u (Figure 4).

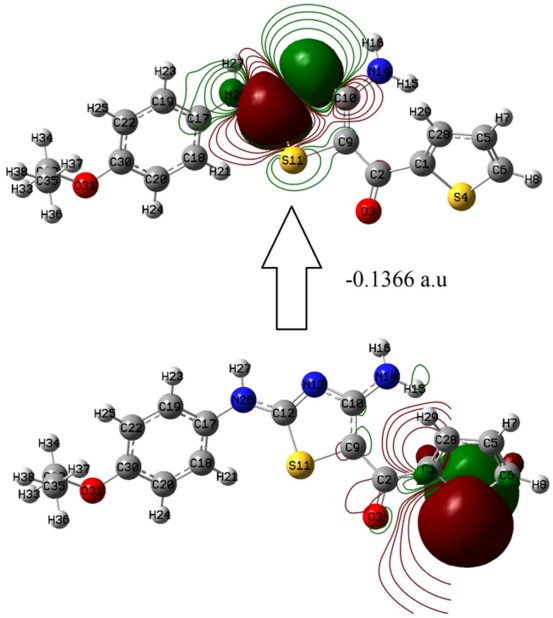


Figure 4: HOMO-LUMO energy diagram of the compound AETM

Vibrational assignment

The spectroscopic signature of the titled compound was performed by FT-IR spectra. B3LYP/6-311G method was used to calculate the theoretical vibrational frequency. The compound (4-amino-2-(4-ethoxyphenyl)aminothiazol-5 yl)(thiophene-2yl)methanone consists of 38 atoms, so it got 108 normal modes of vibrations. We could see a good agreement with the frequency calculated at DFT method using 6-311G basis set and experimental values.

The calculated band at 3611.23 cm^{-1} is consigned due to asymmetric NH stretching of NH_2 group and experimentally it is assigned at 3624.96 cm^{-1}. The calculated band at 3141.82 cm^{-1} is due the asymmetric C-H stretching of the phenyl ring and it is close to the experimental value of 3166.66 $cm^{-1.}$ Heterocyclic compounds containing C=O stretching vibration in the region of 1850-1550 cm^{-1}. In the title compound, the experimentally observed C=O stretching vibration around 1716.98 $cm^{-1,}$ which is in close to the calculated value 1714.86 cm^{-1}. The bands in the range of 1597.91 cm^{-1}, 1092.60 cm^{-1} are due to the C-H in-plane bending vibration. The bands in the range 946.73-478 cm^{-1} are assigned for C-H out of plane bending vibration. They are in good agreement with the experimental value. The C-S stretching vibration is likely in the region 710-685 cm^{-1}, which is closely connected to the calculated value 700.20 cm^{-1} and experimental value of 706.86 cm^{-1}.

Molecular Docking

Molecular docking simulation has been carried out on the ethoxy substituted ketothiophene derivative in order to get the binding modes and binding affinities toward hepatitis A virus and Pedobacter heparinus. The protein ligand docking aims at predicting the predominant binding mode of a ligand with a protein of known three-dimensional structure. We get a ligand-protein complex with optimized conformation, possessing less binding free energy. Hex dock software has been used to cognizes protein and DNA structures in the PDB format. The above novel thiophene-thiazole compound was docked with the crystal structure of empty hepatitis A virus (PDB code: 4QPG) and the protein structure of heparan sulfate lyase HepC mutant from Pedobacter heparinus (PDB code: 4MMI) obtained from the protein data bank [7,8]. The ligand has been prepared for docking by minimizing its energy at a B3LYP/6- 311++G(d,p) level of theory and is analyzed whether it obeys the Lipinski rule of five (Table 3), which shows whether the chemical compound has biological activities. These ligand molecules were docked into the active site of the protein structure. The 2D pose of the compounds are visualized using the Discovery studio 3.5 visualizer. The less binding energy of the molecule indicates a greater interaction with the receptor protein.

Table 3: Lipinsky Rule for (4-amino-2-(ethoxyamino)thiazol-5-yl)(thiophene-2-yl)methanone

Compound	Molecular weight (< 500 D)	HB donor (< 5)	HB acceptor (< 10)	logP (< 5)	Molecular refractivity 40—130
AETM	345.00	3	5	4.16	95.17

The 2D diagram of the compound with the protein receptor (PDB: 4QPG) shows π-π interaction of His B:169 aminoacid with thiophene ring (Figure 5) and the protein receptor (PDB: 4MMI) shows electrostatic interaction of keto group attach to the thiophene with the aminoacid ASN A: 328, GLN A324, ALA A283, GLY A285, THR A325 (Figure 6). It was found that the binding score of the compound towards the protein receptor PDB: 4QPG is lower than the protein receptor (PDB: 4MMI) (Table 4). From this it was considered that the ethoxy substituted thiophene-thiazole compound shows more antiviral activity than antibacterial activity.

Table 4: Docking score and interaction of the compound with the protein 4QPG and 4MMI

Protein Receptor	Binding Energy (kJ/mol)	Active sites of interactions				
		π- σ interactions	π-cation interactions	π- π interactions	Electrostatic	Vanderwaals
4QPG	-331.90	-	-	HIS B169	ALA A61, HIS B169, LYS A62	GLU A56, LEU B171, GLY B170
4MMI	-303.56	-	-	-	ASN A328, GLN A324, ALA A283, GLY A285, THR A325	SER A321, LYS A278, MET A331, GLU A327

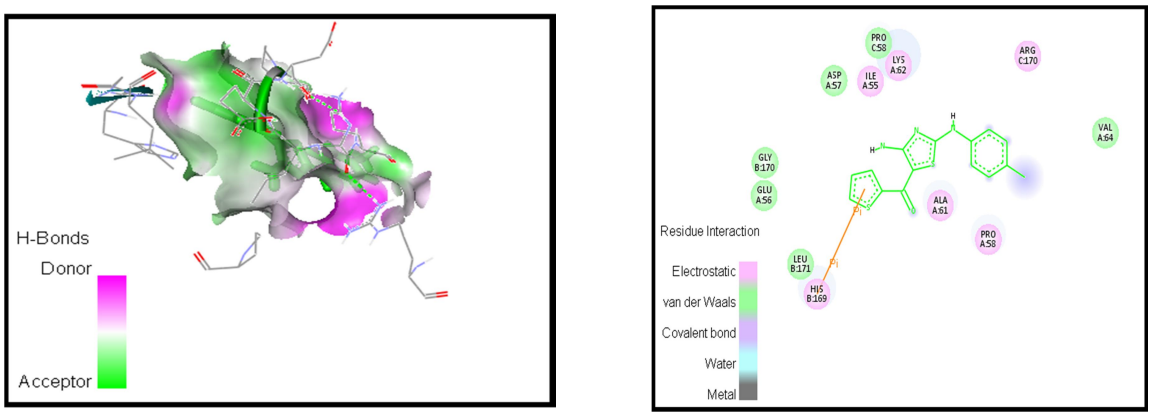


Figure 5: 3D and 2D representation of the compound (4-amino-2-(ethoxyamino)thiazol-5 yl)(thiophene-2-yl)methanone and protein receptor 4QPG

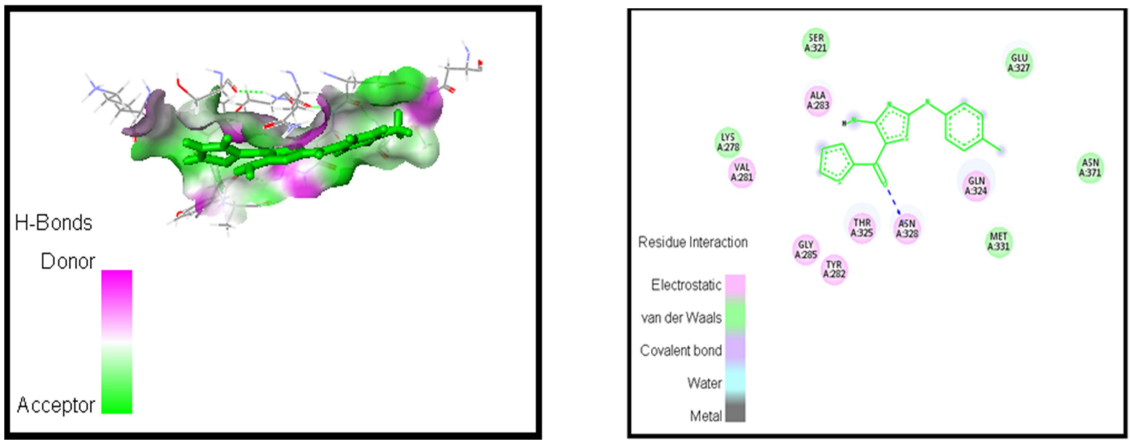


Figure 6: 3D and 2D representation of the compound (4-amino-2-(ethoxyamino)thiazol-5 yl)(thiophene-2-yl)methanone and protein receptor 4MMI

Conclusion:

We have developed successful synthetic methods for ketothiophene-thiazole analog of (4-amino-2-(ethoxyphenylamino)thiazol-5-yl)(thiophene-2-yl)methanone and characterized them by IR, UV, ^{1}H and ^{13}C NMR, and mass spectrometry. The theoretical optimized data were analyzed by using density functional theory calculation using Becke's three-parameter exchange functional in combination with the Lee Yang Parr correlation Gaussian 09 program package with the standard 6-311G basis software set of the compound AETM. It shows that the compound has less HOMO-LUMO energy gap and found to be more reactive, which is reflected in the biological activities. Reactivity of the compound was also predicted by the existence of hydrogen bonded intramolecular interactions and the hyperconjugative energy transfer leading to the stabilization of the system. Molecular docking simulation of AETM on the protein receptors PDB code (4QPG and 4MMI) shows that the compound has more antiviral activity than antibacterial activity.

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Conflict of interests: The authors declare that they have no conflict of interest.

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