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Synthesis, DFT Computational Studies and Biomolecular Interactions of 4-(2-fluorophenyl)thiosemicarbazide

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Herein, we report the synthesis of a thiosemicarbazide derivatives, namely 4-(2-fluorophenyl) thiosemicarbazide from the reaction between 2-fluorophenyl isothiocyanate and hydrazine hydrate. The isolated solid compound was elucidated from micro-elemental analysis and IR spectroscopy. The structure of the molecule in the ground state was calculated using density functional theory (DFT) method and basic set of 6-311G (*d,p*) was used to calculate the energy gap ($\triangle E_{gap}$), hardness (η), softness (σ) and the global electronegativity (χ). Its electrostatic potential mapping and frontier orbital energy analysis were also discussed. The interaction of the molecule with selected proteins are investigated using SwissTargetPrediction database.

Keywords: Synthesis, thiosemicarbazide, DFT, docking studies

I. INTRODUCTION

Thiosemicarbazide derivatives (R-NH-C(=S)-NH-NH_2) is one of the most important classes of mixed hard-soft nitrogen-sulphur donor ligands, which having four donor atoms. In fact, the presence of hard nitrogen and soft sulphur atoms are sterically available at a time to bind with receptors such as metal ions [1] and amino acid residues [2]. Hence, their capability is a concern of interest due to their biological potential as an antioxidant [3] anti-inflammatory [4], anti-cancer [5] and anti-bacterial [6].

In order to explore their preliminary potential biological activities, the main aim of the present work is to synthesis as well as predict the biological interactions between 4-(2-fluorophenyl) thiosemicarbazide and selected proteins. The analyses include the structural elucidation of the isolated compound such as elemental analysis, Fourier transform-infra red

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(FT-IR) spectroscopic, band gap calculations as well as their molecular electrostatic potential (MEP). Additionally, molecular docking studies for the 4-(2-fluorophenyl) thiosemicarbazide also have been performed to find the possible interaction with potential proteins.

II. EXPERIMENTAL SECTION

A. Synthesis of 4-(2-fluorophenyl) thiosemicarbazide

Compound 2-fluorophenyl isothiocyanate (1.53 g, 0.01 mol) was dissolved in ethanol 85% (50 ml). To this solution, hydrazine hydrate (0.320 ml, 0.01 mol) was added dropwise with constant stirring over an hour using a mechanical stirrer. During this time, a white precipitate which formed was filtered, washed with cold ethanol and recrystallized after drying. The white precipitate was kept in a desiccator. The chemical equation is shown in Scheme 1.

B. Computational method

Optimized structure of 4-(2-fluorophenyl) thiosemicarbazide was done with GaussView 5.0.9 [7]and Gaussian 09 software package programme [8]. One of the density functional theory (DFT) method, named Becke, 3-parameter, Lee-Yang-Parr (B3LYP) [9][10] was selected as method for studied their highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO) orbital and molecular electrostatic potential (MEP) analysis. In calculations, 6-311G (*d,p*) [11] was selected as basic set. All calculations were optimized at the singlet ground state (S_0) in vacuum. Determination of its several key factors of biological activity which are the energy gap ($\triangle E_{gap}$), hardness (η), softness (σ) and the global electronegativity (χ) were calculated by using Eqs. 1 - 4 as similar equation as reported, previously [12].

$$\triangle E_{gap} = E_{LUMO} - E_{HOMO} \tag{1}$$

$$\eta = \frac{E_{LUMO} - E_{HOMO}}{2} \tag{2}$$

$$\sigma = 1/\eta \tag{3}$$

$$\chi = -\frac{E_{LUMO} - E_{HOMO}}{2} \tag{4}$$

C. Virtual screening of potential target in human

Screening of 4-(2-fluorophenyl) thiosemicarbazide potential interaction with proteins in human was done by screening the compound in the www.swisstargetprediction.ch database. The prediction is based on similarity in 3-dimensional structure of ligands that are known to be interacted with the protein [13]. Proteins with the highest probability value were chosen for docking analysis.

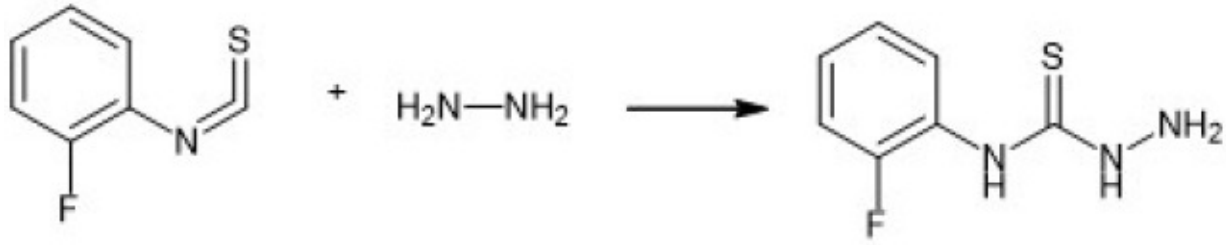

Figure 1. Chemical equation for 4-(2-fluorophenyl)thiosemicarbazide.

D. Molecular docking

The crystal structure of growth factor, FAD-linked sulfhydryl oxidase ALR was obtained from RCSB protein database (RSCB-PDB ID 3O55) for 4-(2-fluorophenyl)thiosemicarbazide molecular docking study in SystemDock (www.systemsdock.unit.oist.jp). The systems-Dock used machine learning algorithm to assess possible type interaction of compound on the selected protein as describe in [14]. The binding affinities is a negative logarithm of experimental dissociation/ inhibition constant value (pKd/pKi).

III. RESULTS AND DISCUSSION

A. Physico-chemical data and CHNS elemental analysis

The physical characterization of 4-(2-fluorophenyl)thiosemicarbazide is shown in Table 1. The compound was characterized according to colour, yield and melting point.

Micro-elemental analyses (C, H, N and S) data was used to calculate the percentage of carbon, hydrogen, nitrogen and sulphur elements for the synthesized compound. It was cleared that the experimental data is not much different with theoretical values (Table 1). Hence, the analytical data is well supported to the expected molecular formula of the compound.

B. FT-IR spectral analysis

Fourier FT-IR was used to identify functional groups presence in 4-(2-fluorophenyl)thiosemicarbazide. The spectrum is shown in Figure 2, while the data is listed in Table 2. Major functional groups were observed in 4-(2-fluorophenyl)thiosemicarbazide including v(N-H), v(N-H_2), v(C-N), v(N-N), v(C=S) and v(C-F) at 3442.18, both 3255.61 and 3163.64, 1279.59, 1030.41, 1176.93 and 1312.00 cm^{-1}, respectively. Band at around 2035 cm^{-1} due to stretching mode of isothiocyanates, v(N=C=S) [15] in this compound was dissappeared, while a new functional group, v(C-N) was observed at 1279.59 cm^{-1} confirming that the reaction was successfully synthesized [16] as shown in Figure 1. The present FT-IR result was comparable with it reported analogue, (E)-1-(2-fluorobenzylidene)

Table 1. Physico-chemical and microanalytical data for the compound.

Molecular formula	Colour	Percentage of yield (%)	Melt. Point (°C)	Found (Calculated) %C	%H	%N	%S
$C_9H_8N_3S_1F_3$	Light yellow	62.71	241.7	43.76 (44.82)	3.15 (3.34)	18.13 (17.42)	11.93 (13.29)

thiosemicarbazide [17].

C. Frontier molecular orbital analysis by DFT

The highest occupied molecular orbital energy (E_{HOMO}) is mainly associated with electron donating ability of molecule. Hence, high E_{HOMO} values indicate the tendency of electron transfer to acceptor protein. Whereas, the lowest unoccupied molecular orbital energy (E_{LUMO}) is the orbital that largely acts as the electron acceptor. The energy gap (E_{gap}) between HOMO and LUMO characterizes the molecular chemical stability [16]. As the E_{gap} becomes smaller, the molecules are expected to be more stable due to an electron are capable of being excited from HOMO to LUMO with small frequencies.

The HOMO, LUMO energies (eV) and their contour diagram of the 4-(2-fluorophenyl)thiosemicarbazide is represented in Figure 3. The HOMO orbital is mainly localized on the sulphur donor atom, while the LUMO orbital is mainly on the 2-fluorophenyl moiety, due to bearing one electron withdrawing fluorine atom. The E_{HOMO} adopting DFT/6-311G(*d,p*) method was found to be -5.469 eV and that corresponding to E_{LUMO} was observed as -0.560 eV to exhibit E_{gap} of 4.909 eV. The E_{gap} value indicates that the strong delocalication within the present molecule as comparable with previous compound, 1-(1-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-yl)ethylidene)thiosemicarbazide (E_{gap} = 4.039 eV) [16].

The chemically interactions can be explained by commonly concept HSAB (hard-soft-acid-base). Biological molecules, namely protein is known as soft molecules. Soft compound can interact easily with biological molecules. Hence, the biological activity is expected to be increased with the increase of softness value (σ) and decrease of hardness value (η). The experimental chemical softness and hardness of 4-(2-fluorophenyl)thiosemicarbazide was found to be 0.407 and 2.454 eV, respectively. These values are not much different with other heterocyclic thiosemicarbazide copper (I) complexes (σ = 0.358 0.951 eV, η = 0.715 1.902 eV) [18]. Interestingly, they found that the complexes showed good theoretical dock score with 8.006 kcal/mol and experimental inhibition zone of 20 mm for *C. Albicans*.

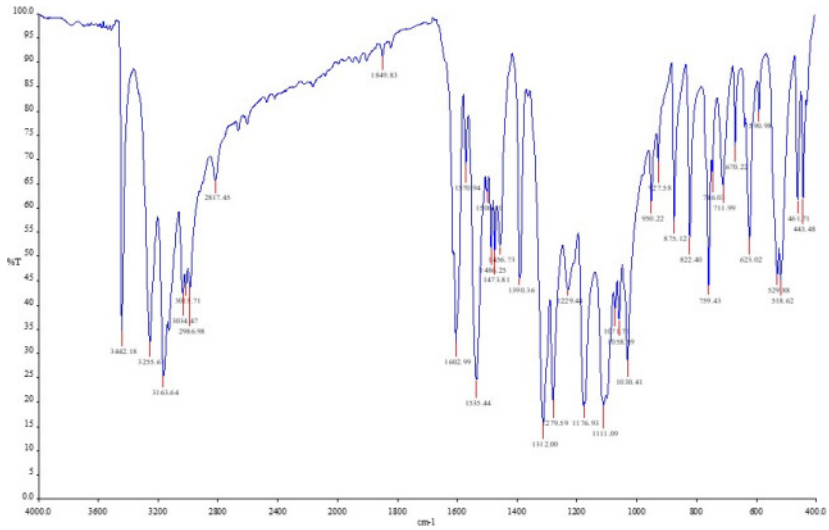

Figure 2. FT-IR spectrum of the 4-(2-fluorophenyl)thiosemicarbazide.

Table 2. FT-IR data of the compound.

Functional Group	Bands (wavelength, cm^{-1})
v(N-H)	3442.18
v(N-H_2)	3255.61. 3163.64
v(C-N)	1279.59
v(N-N)	1030.41
v(C=S)	1176.93
v(C-F)	1312.00

Another important parameter is electronegativity (χ). This parameter is related with freedom of electrons in the molecule. The smaller values show electrons in molecules are more freedom. The present electronegativity was found to be 3.015 eV, which lower than reported Cr(II) complexes derived from thiosemicarbazide (In a range of 3.476 - 4.179 eV). In addition, these complexes were showed moderate antibacterial activity with 8 -14 mm inhibition zone [19].

D. Molecular electrostatic potential (MEP) analysis

The molecular electrostatic potential (MEP) is the useful to visualize variably charged regions of a 4-(2-fluorophenyl)thiosemicarbazide molecule. Hence, the charge distributions can give the information about how the molecules interact with protein. In addition, determination the sites for electrophilic attack and nucleophilic reaction could also be identified.

The MEP map of molecule 4-(2-

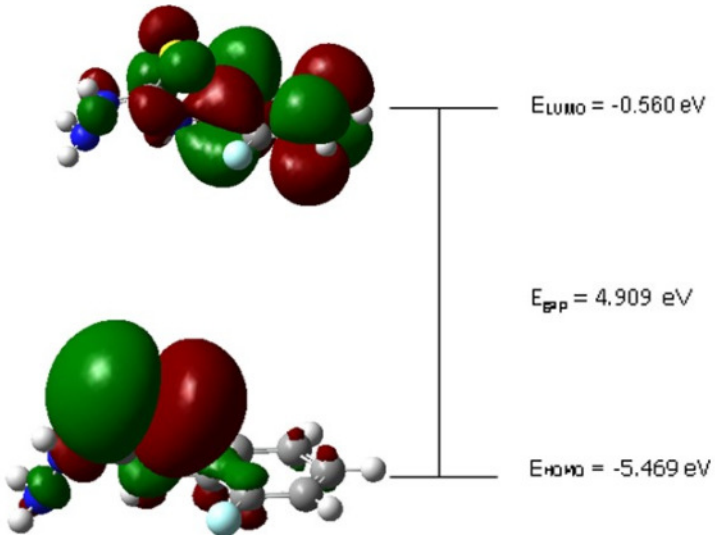

Figure 3. Molecular orbital E_{HOMO}, E_{LUMO} and E_{gap}(eV) for the 4-(2-fluorophenyl)thiosemicarbazide.

Table 3. The calculated quantum chemical parameters of the 4-(2-fluorophenyl)thiosemicarbazide.

E_{HOMO} (eV)	E_{LUMO} (eV)	E_{GAP} (eV)	η (eV) hard	σ(eV) soft	χ (eV)
-5.469	-0.560	4.909	2.454	0.407	3.015

fluorophenyl)thiosemicarbazide is shown in Figure 4. The electrophilic reactivity is shown by the red (negative) regions and nucleophilic reactivity is shown by the blue (positive). In Figure 4, the region for electrophilic attack (red) is mainly localized on the sulphur atom of thiosemicarbazide group. The region for nucleophilic reactivity of the molecule is mainly localized on the surface of hydrogen atoms bound to nitrogen of thisemicarbazide group. It is expected that both hydrogen and sulphur atoms could interact either with positively or negatively charged part of the protein. Therefore, 4-(2-fluorophenyl)thiosemicarbazide has potential to combine with the protein on its surface by the interaction of the imine ($-NH_2$) or thione (CS) moities, which may be responsible for the bioactivity.

E. Virtual Screening and molecular docking

4-(2-fluorophenyl)thiosemicarbazide was used for screening using SwissTargetPrediction database to obtain potential interaction targeted proteins in human. This compound retrieved 15 hits of targeted protein where the list was ranked from higher to lower probability value (Figure 5). The probability value represented the similarity in 3-dimensional structure of known ligands that are similar / homologous to

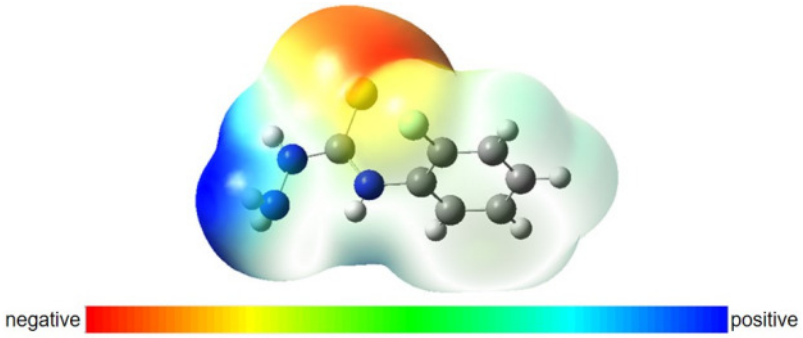

Figure 4. Molecular electrostatic potential mapping of the molecule.

4-(2-fluorophenyl)thiosemicarbazide. Top five proteins with highest probability values were chosen for docking analysis microtubule associated tau protein (MAPT), muscle blind-like protein (MBNL) family, growth factor, FAD-linked sulfhydryl oxidase augmenter of liver regenerations (GFER), tyrosinase (TYR) and L-dopachrome tautomerase (DCT), respectively.

Docking analysis were conducted in Systems-Dock database shows all proteins except MAPT have possible docking site with high binding affinities (docking score; pKd/pKi) although lower than the native ligands (Figure 6). Out of five compound, GFER (PDB ID 3O55) has the highest docking score with 3.358 pKd/pKi although lower than the proteins native ligand, FADs score at 7.16 pKd/pKi. GFER is an oxidoreductase which responsible for rapid regeneration of liver cells [20]. Upon visual inspection on the docking site in 3D structure, the thiosemicarbazide derivative shown to be competing with FAD docking site (Figure 7-A-G). A total of 6 amino acid residues at the docking site form an interaction with the thiosemicarbazide (Table 1), while still less than FAD which bind to 14 amino acid residues. The compound competing with FAD to form interaction with Leu105, Arg23 and Glu24 (Table 4). This indicate potential biological application of the synthesized compound as disturbance agent of biomolecular pathways and cellular physiology.

IV. CONCLUSION

A 4-(2-fluorophenyl)thiosemicarbazide was successfully prepared and spectroscopically characterized via CHNS micro-elemental analyses and IR. The DFT based chemical reactivity descriptors of the present compound may be used to predict its biological activities. The values of quantum chemical parameters were showed that the compound have a potential to be a biologically active compound. In addition, the biomolecular interaction of 4-(2-fluorophenyl) thiosemicarbazide was studied by molecular modelling technique. It is concluded that the present molecule linked as similar pattern as native ligand, flavin adenine dinucleotide

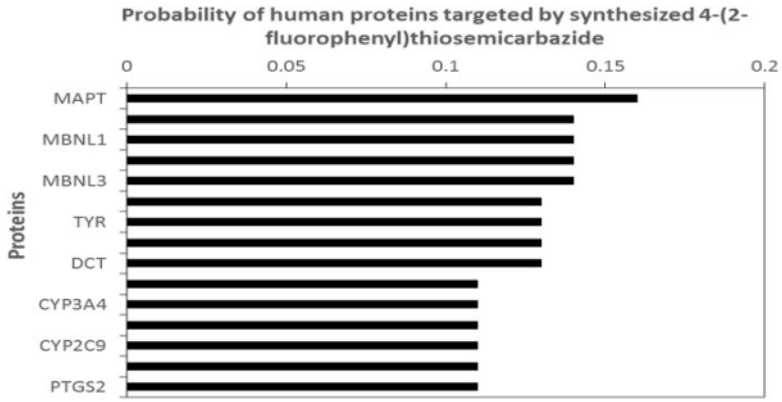

Figure 5. List of potential proteins in human to be targeted by synthesized thiosemicarbazide derivatives. The protein abbreviation is based on GeneCard ID or UniProt ID.

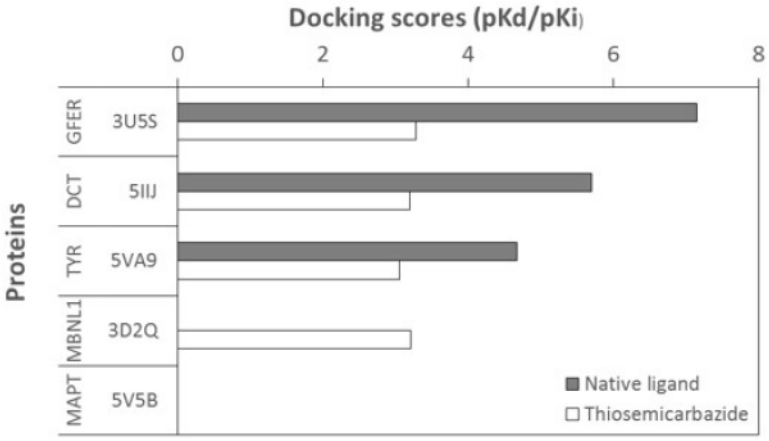

Figure 6. Binding strength of thiosemicarbazide derivatives interaction with human proteins in comparison with their native ligands.

(FAD) in growth factor, augmenter of liver regenerations (GFER). The results of molecular modelling studies indicate that the interaction occurred through N-H hydrogen bond with tyrosine play essential role in the binding with docking score of 3.358 pKd/pKi.

V. ACKNOWLEDGEMENT

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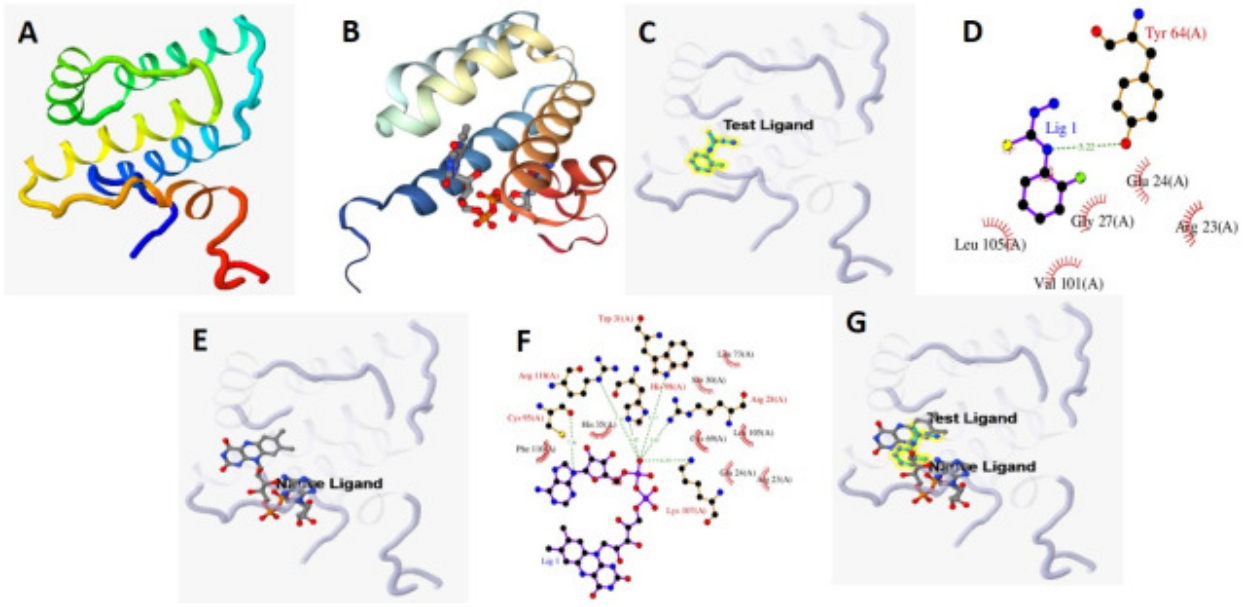

Figure 7. Docking simulation between growth factor, FAD-linked sulfhydryl oxidase ALR, PDB ID: 3O55 (**A**) and 4-(2-fluorophenyl)thiosemicarbazide (**C**) shows the synthesized compound dock at the same binding site as FAD-linked sulfhydryl oxidase ALR (**B** and **E**), the native compound (**E** and **G**). (**G**) Merge 3D view of synthesized compound and FAD at GFER docking site.2D views of GFER docking result for native ligand (**D**) and 4-(2-fluorophenyl)thiosemicarbazide (**F**).

4-(2-fluorephenyl) thiosemicarbazide	FAD (native ligand)
LEU_{105}	LEU_{105}
ARG_{23}	ARG_{23}
GLU_{24}	GLU_{24}
TYR_{64}	-
LY_{27}	-
VAL_{101}	-

Table 4. Potential amino acid residues form an interaction with the 4-(2-fluorophenyl)thiosemicarbazide and native ligand (FAD). In comparison, FAD interacted with 14 residues at the GFER docking site, although only 3 out of 14 amino acid residues are listed in this table.

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