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SYNTHESIS, SPECTRAL CHARACTERIZATION AND BIOLOGICAL SCREENING OF NOVEL THIOSEMICARBAZONES WITH MOLECULAR DOCKING STUDIES

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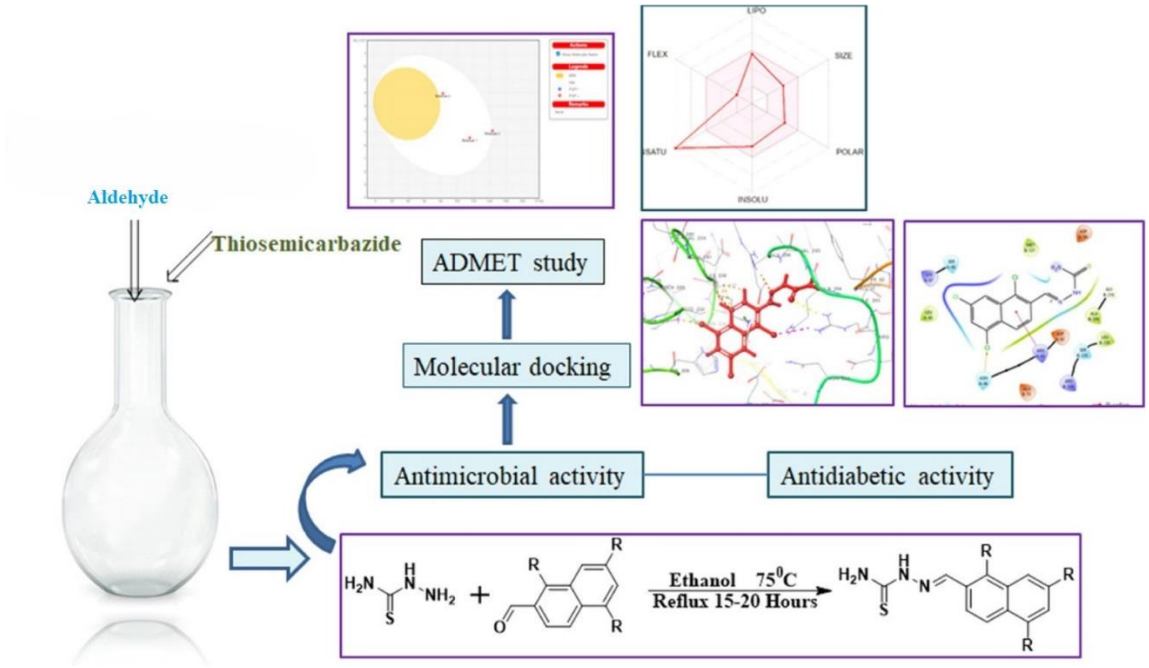
Abstract:
Thiosemicarbazones were synthesized by reacting carbonyl compounds with thiosemicarbazide, the prepared thisemicarbazones such as (2*E*)-2-[(5,7-dichloronaphthalen-2-yl)methylidene]-*N*-phenylhydrazine-1-carbothioamide were characterized using diverse spectral techniques, such as UV–Vis and FT-IR. The synthesized compounds were subsequently evaluated for their antibacterial properties against Gram-positive Bacillus subtilis and Gram-negative Escherichia coli, using ciprofloxacin as a reference, as well as for their antidiabetic activities. For the evaluation of anti-diabetic activity, Acarbose served as the reference. Molecular docking results indicated that C-1 exhibited superior performance against Alpha-glucosidase proteins, evidenced by their lowest binding energies (-8.7 kcal/mol) compared to other ligands. These findings suggest that C-1 is promising candidates for further research and development by pharmaceutical companies to explore additional biological activities.

Keywords: Organic Synthesis, Thiosemicarbazones, Molecular docking, Anti-bacterial, Anti-oxidant

Graphical Abstract



1. Introduction

Thiosemicarbazones typically act as bidentate ligands through azomethine nitrogen and thione/thiolate sulfur atoms, but they can coordinate tridentate if an additional coordination site is nearby. Despite significant advances in antimicrobial therapies, infections caused by bacteria and fungi persist as major global health threats due to increasing drug resistance. Thiosemicarbazones and their derivatives have garnered significant attention from medicinal chemists for their potential biological activity [1]. Significant efforts are underway to elucidate the structure-activity relationship of thiosemicarbazones, which are renowned for their diverse biological applications. These compounds are well known for their vast chemical [2] and biological applications including anticancer [5–6], antibacterial [3–4], antiviral [9–10] antifungal, activities [7–8], and anti-HIV [11] carry the potential to enter semi-permeable membrane [12]. Thiosemicarbazones are used to induce oxidative cleavage of DNA strands [13].

Thiosemicarbazone and its derivatives have been utilized to quantify metal ions in pharmaceutical and environmental samples, including blood, soil, water, synthetic mixtures, standard alloys, and food samples such as leafy vegetables and medicinal leaves, *etc.*, [14] Additionally, various research groups have demonstrated that the cyclic derivatives of thiosemicarbazone exhibit potent and effective biological properties. Thiosemicarbazones serve as fundamental precursors for synthesizing a variety of nitrogen- and sulfur-containing heterocyclic compounds, including imidazoles, coumarins, thiazolidinediones, triazoles, and thiazoles, many of which exhibit significant biological activity [15–17]. These derivatives have been found to possess a range of biological properties, such as antifungal, anti-inflammatory, and antibacterial activities [18–20].

Thiosemicarbazones hold significant value in medicine, serving as treatments for cancer [22–23], allergies [21], hypertension [25], fibrinogen receptors [26 inflammation [24], HIV infections [27], schizophrenia [26], hypnotics [30], bacterial infection [28–29],] for the treatment of pain [31] and Inhibitors of bacterial DNA gyrase B. Notable examples include imidacloprid, a vital insecticide, and ritonavir, a renowned anti-HIV drug.

2. Material and methods

2.1. Rational drug design

A large number of thiosemicarbazones drugs are in practice to treat different disease like allergies, cancer, inflammation,hypertension, schizophrenia , HIV infections, bacterial infection , hypnotics,

fibrinogen receptors for the treatment of pain. With a focus on the significant biological activities of thiosemicarbazones in medicinal chemistry, our study endeavors to synthesize novel derivatives with enhanced efficacy and safety. Employing a rational design approach (Scheme 1), we formulated the target molecules to fulfill this objective.

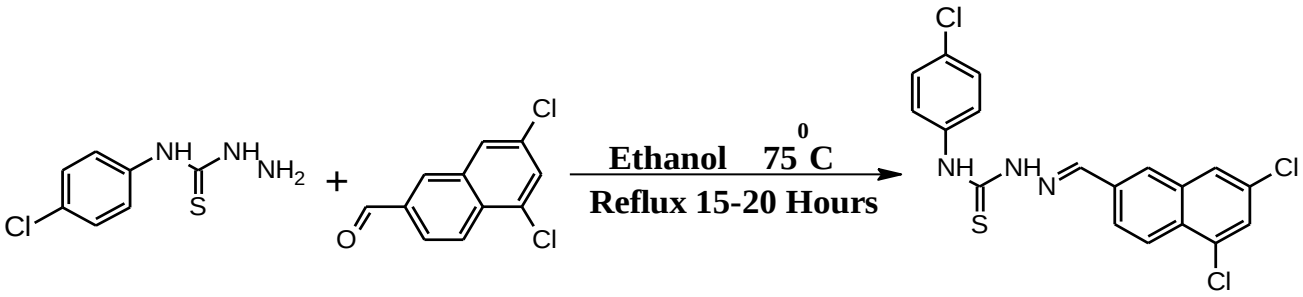
2.2. Chemistry

The materials and chemical reagents utilized in this study were sourced from Merck, Sigma Aldrich, Macklin, and Daejung. TLC sheets from Merck facilitated reaction monitoring, while chromatograms were examined using a UV-lamp (Spectroline) at 254nm and 365nm wavelengths. Melting points were determined using a digital melting point apparatus (Stuart), and FT-IR spectra were obtained using a Bruker OPUS 7.518 spectrophotometer. All chemicals were of analytical grade and employed without additional purification.

2.3. Synthesis of thiosemicarbazones

2.3.1. Synthesis of (2*E*)-2-[(5,7-dichloronaphthalen-2-yl)methylidene]-*N*-phenylhydrazine-1-carbothioamide

In a round-bottom flask, 20 mL of ethanol and 2.5 mmol of *N*-(4-chlorophenyl) hydrazinecarbothioamide were stirred for 20 minutes with a drop of concentrated HCl. A solution of 2.5 mmol of 5,7-dichloronaphthalene-2-carbaldehyde, in 20 mL ethanol was added in above reaction mixture and stirred for 24 hours. On addition of 5,7-dichloronaphthalene-2-carbaldehyde, a colour change was observed. The reaction's progress was monitored using TLC with ethyl acetate and petroleum ether (3:7) as the mobile phase.



Scheme 1. Scheme for synthesis of (2E)-N-(4-chlorophenyl)-2-[(5,7-dichloronaphthalen-2-yl)methylidene]-N-phenylhydrazine-1-carbothioamide

2.4. General Experimental Methods

Column chromatography utilized silica gel (300–400 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), while thin layer chromatography (TLC) employed TLC silica gel 60 F254 plates measuring 0.2 mm with dimensions of 200 × 200 nm. Visualization of spots occurred under UV light at wavelengths of 254 nm and 360 nm.

2.4.1. Characterization of (2*E*)-2-[(5,7-dichloronaphthalen-2-yl)methylidene]-*N*-phenylhydrazine-1-carbothioamide

M.P: 241˚C **%age Yield:** 54 %. **IR (solid cm^{-1}):** 1042 (-C=S), 1651 (-C=N), 3241 (-NH_2), 3421 (-NH-), 673-857 (Ar).

2.5. Pharmacological Study

2.5.1. Antimicrobial Activity

The study assessed the antimicrobial efficacy of synthesized compounds using the agar disc diffusion method outlined by Kadri et al. [49]. Four bacterial strains were tested as (*Escherichia coli ATCC 25,922, Staphylococcus aureus ATCC 25923, Micrococcus luteus NCIMB 8166,* and *Pseudomonas aeruginosa ATCC 2785*3) and two fungal strains (*Candida albicans ATCC 90,028* and *Candida krusei*

ATCC 6258) respectively. Microbial inoculums were adjusted to OD600 for bacteria and OD540 for yeasts, then streaked onto Muller–Hinton (MH) and Sabouraud (SB) agar plates. Sterile filter discs were impregnated with 10 µL of product dissolved in 10% solvent, and tetracycline (10 mg/mL) and amphotericin B (10 mg/mL) served as reference antibiotics. Incubation at 37°C for 24 hours allowed assessment of antibacterial activity by measuring the inhibition zone around each disc, with triplicate assays conducted for accuracy.

2.5.2. Antibacterial Activity

The newly prepared thiosemicarbazones were screened for anti-bacterial test *in-vitro* against *Bacillus subtilis* and *Escherichia coli*. Menichetti *etal.,* method of Agar disc diffusion [51] was utilized for this activity. Bacteria were initially cultured in agar nutrient and incubated at 37°C for one day. Subsequently, a hearty blend of approximately 10–5 CFU/mL of the bacterial suspension was spread on an agar plate pre-prepared with agar medium within a Laminar flow cabinet. Different concentrations of 1.0, 5.0, 10.0, and 20.0 µg/mL in 0.1% DMSO were added to assess the antibacterial properties of compounds C-1 and WS-2. Filter paper discs containing the sample compounds were then placed in a petri dish, alongside a standard drug, Ciprofloxacin (30 µg/mL), for comparison. These preparations were incubated at 37°C for 24 hours, with tests conducted in duplicate.

2.6. Computational Study

2.6.1. Molecular Docking Simulation

We used Molegro Virtual Docker 6.0 (MVD), Schrodinger, and biological resources including PubChem and PDB (Protein Data Bank) for the current investigation. The individual global database containing structural information on biological macromolecules is the Protein Data Bank (PDB), which was established in 1971 at Brookhaven National Laboratories (BNL). It contains structural information about macromolecules that was gathered using NMR, X-ray crystallography, and other methods. ChemDraw is a powerful, all-purpose chemical drawing and graphics programme developed by Labs to help scientists calculate chemical properties, design molecules, processes, and schematic diagrams quickly and easily, and produce professional reports and presentations. Using Molegro Virtual Docker 6.0, the docking observations were examined. The results showed hydrogen bonds, tight contact, and hydrophilic and hydrophobic interactions. Docking allows a scientist to employ multiple scoring systems to anticipate the strongest binders while digitally exploring a library of chemicals. It examines the manner in which two compounds have strong binding affinities with the antibacterial proteins of *E. coli* and *B. subtilis*. For the visualization of protein ligand interaction and two dimensional structure of ligand Schrodinger software was used.

2.6.1.1. Preparation of Ligand Structure

Chem Draw 19.1 was used to draw the two-dimensional (2D) structures of the two molecules C-1 and WS-2) for docking and the interacting amino acid residues of the reference drug for in-depth docking. The Chem 3D 19.1 was used to convert these 2D structures into 3D structures. The final structures were then uploaded into the Molegro Virtual Docker 6.0 workspace for docking study. By utilizing the MDL (sdf/sd/mol/mdl) file format, which includes bonding formation, the molecule can be integrated into the MVD. The preparation of the compounds involved assigning bonds, charges, explicit hydrogen's, bond order and hybridization, and flexible torsion in ligands.

2.6.1.2. Preparation of protein

The RCSB PDB provided the structure of antibacterial proteins for *E. coli* (PDB: 2W6N and 4Z7M) and for B. subtilis (PDB: 3EX8 and 8I2D). The anti-diabetic protein 3WY1 was also downloaded in the PDB format from the protein data bank. Chloramphenicol and acarbose, the reference antibacterial and anti-diabetic drug respectively, and all of the developed compounds were imported into the Molegro Virtual Docker 6.0 workspace. After the protein was constructed, water molecules were

eliminated and bonds, bond orders, hydrogen atoms, and charges were assigned. The automated approach identified the binding cavities.

2.6.2. ADMET predictions

We used the SwissADME and Protox 3.0 web services, which are accessible at http://www.swissadme.ch/ and https://tox.charite.de/protox3/index.php?site=compound_input, to evaluate the ADME/T characteristics of the synthesized compounds. With the use of this computational tool, a wide range of physicochemical descriptors may be calculated, and ADME/T parameters, pharmacokinetic profiles, drug-likeness, and medicinal chemistry compatibility can all be estimated. In order to input the two-dimensional chemical structures of the alkaloids into the web servers for the predictive analysis, we translated them into the SMILES (Simplified Molecular Input Line Entry System) format.

3. Results and Discussion

3.1. Synthesis of compounds

The condensation reaction between *N-(4-chlorophenyl)hydrazinecarbothioamide with* 5,7-dichloronaphthalene-2-carbaldehyde *gives* (2*E*)-2-[(5,7-dichloronaphthalen-2-yl)methylidene]-*N*-phenylhydrazine-1-carbothioamide respectively in good yield. The elemental analysis of C, H, N, and S demonstrates close agreement between calculated and experimental data for the Schiff base, indicating its high purity, further affirmed by mass spectrometry. Infrared absorption bands are valuable tools for elucidating ligand coordination to metals.

3.2. Characterization of compounds by Spectroscopic analysis

The structure of the thiosemicarbazone was established using IR spectroscopy. The infrared spectrum of **C-1** were taken in 4000-400 cm^{-1} region.

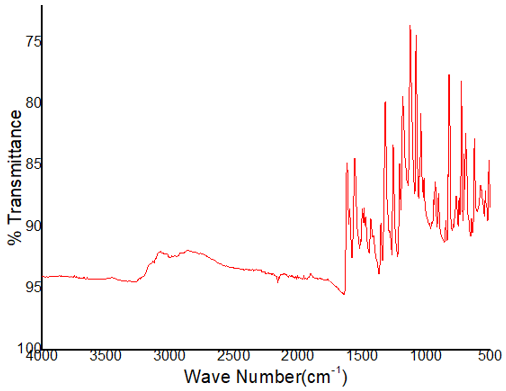


Figure 1: FTIR of Structure of (2*E*)-2-[(5,7-dichloronaphthalen-2-yl)methylidene]-*N*-phenylhydrazine-1-carbothioamide

For thiosemicarbazones (-NH) groups is represented by the bands from 3000-3500. Bands between 690 and 760 cm^{-1} indicate benzene ring. The bands on 1590 cm^{-1} shows (C=N), 1560 cm^{-1} shows (C=S), 3239 cm^{-1} shows (-NH_2) . The infrared spectra of TSCs showed a strong band at 1649-1595 cm^{-1} attributed to C=N group. The next strong band at 1590-1303 cm^{-1} is attributed to C=S group.

3.3. Biological evaluation

3.3.1. Antibacterial activity

The synthesized compounds (**C-1**) were examined against *Bacillus subtilis which is* Gram positive bacterium and *E.coli* that is Gram negative category of bacterium. The compounds showed special inhibitory activity in MIC values of 30 mg/mL for targeted bacterial strains. MIC values of compounds were presented in table 1. These compounds show good results but **C-1** showed excellent activity against *Bacillus subtilis*. *E.coli*.

Table 1: Antibacterial action against *Bacillus subtilis* (gram +ve).

S#	*Samples*	*Bacterial Strains*	*Concentrations (mg/mL) used and zones of inhibition (mm) against each concentration*					
	Concentrations		*30*	*20*	*10*	*5*	*2.5*	*1.25*
1	*C-1*	*Bacillus subtilis.* (gram +*ve*)	*25*	-	-	-	-	-
		E. coli. (gram -*ve*)	*23*	-	-	-	-	-
		E. coli. (gram -*ve*)	*10*	-	-	-	-	-
3	*Control (DMSO)*	*Bacillus subtilis.* (gram +*ve*)	-					
		E. coli. (gram -*ve*)	-					
4	*AMP (concentration in 12.8 mg/mL)*	*Bacillus subtilis.* (gram +*ve*)	*30*					
		E. coli. (gram -*ve*)	*30*					

3.3.2. Antidiabetic Activity

Targeted synthesized derivatives of thiosemicarbazone (**C-1**) were evaluated against alpha-glucosidase. These derivatives showed excellent inhibitory potentials with excellent IC_{50} as compared to the standard drug acarbose. Derivative **C-1** was found the most potent among them. A limited structure–activity relationship was carried out, which mainly depends upon the number, nature, position, and electron donating/withdrawing effects of the substituent/s on the aryl ring. In case of antidiabetic activity, Acrabose was used as reference. Molecular docking result revealed that **C-1** displayed the finest enactment against the Alpha-glucosidase proteins as reinforced by its binding energy -8.7 kcal mol)

Table 2: alpha-glucosidase inhibition

Dose Conc.	**Blank**	**Control**	**R1**	**R2**	**C-R1**	**C-R2**	**C-R1/C**	**C-R2/C**	**C-R1/C* 100**	**C-R2/C* 100**	**Mean**	**SD**
Acrabose												
50	0.095	0.897	0.489	0.463	0.34166667	0.36766667	0.48319239	0.4244186	38.4192389	42.5418605	40.58405497	2.91513366
	0.091	0.678										
	0.093	0.639										
		0.575										
		0.63066667										
C-1												
50	0.085	0.897	0.626	0.691	0.20466667	0.04966667	0.26596195	0.07289641	16.6961945	6.38964064	11.3429176	7.38783416
	0.081	0.778										
	0.083	0.739										
		0.675										
		0.73066667										

Computational Study

3.3.3. Molecular Docking

We used PyRx 0.8 Ssetup, Schrodinger, and biological resources including PubChem and PDB (Protein Data Bank) for the current investigation. The individual global database containing structural information on biological macromolecules is the Protein Data Bank (PDB), which was established in 1971 at Brookhaven National Laboratories (BNL). It contains structural information about macromolecules that was gathered using NMR, X-ray crystallography, and other methods [1]. ChemDraw is a powerful, all-purpose chemical drawing and graphics programme developed by Labs to help scientists calculate chemical properties, design molecules, processes, and schematic diagrams quickly and easily, and produce professional reports and presentations. Using PyRx 0.8 Ssetup, the docking observations were examined. The results showed hydrogen bonds, tight contact, and hydrophilic and hydrophobic interactions. Docking allows a scientist to employ multiple scoring systems to anticipate the strongest binders while digitally exploring a library of chemicals[2]. It examines the manner in which two compounds have strong binding affinities with the antibacterial proteins of *E. coli* and *B. subtilis*. For the visualization of protein ligand interaction and two dimensional structure of ligand Schrodinger software was used.

The compounds were docked to the active site amino acids of Glucosamine-6-Phosphate (GlcN-6-P) as receptor proteins in order to explore their inhibiting potential. All the compounds against each receptor protein were explored as potential drug candidates on the basis of their binding affinity and root-mean square deviation values. All the compounds against the protein GlcN-6-P were found to be **C-1** (S-score −8.7 kcal/mol).

3.3.3.1. Preparation of Ligand Structure

Chem Draw 19.1 was used to draw the two-dimensional (2D) structures of the two molecules **C-1** for docking and the interacting amino acid residues of the reference drug for in-depth docking. The Chem 3D 19.1 was used to convert these 2D structures into 3D structures. The final structures were then uploaded into the PyRx 0.8 setup, workspace for docking study. By utilizing the MDL (sdf/sd/mol/mdl) file format, which includes bonding formation, the molecule can be integrated into the MVD. The preparation of the compounds involved assigning bonds, charges, explicit hydrogen's, bond order and hybridization, and flexible torsion in ligands [4].

3.3.3.2. Preparation of protein

The RCSB PDB provided the structure of antibacterial proteins for *E. coli* (PDB: 2W6N and 4Z7M) and for B. subtilis (PDB: 3EX8 and 8I2D). The anti-diabetic protein 3WY1 was also downloaded in the PDB format from the protein data bank. Chloramphenicol and acarbose, the reference antibacterial and anti-diabetic drug respectively, and all of the developed compounds were imported into the PyRx 0.8 Ssetup, workspace. After the protein was constructed, water molecules were eliminated and bonds, bond orders, hydrogen atoms, and charges were assigned. The automated approach identified the binding cavities.

1.1.2 Results:

The three-dimensional (3D) structures of Glucosamine-6-Phosphate (GlcN-6-P) were retrieved from the PDB database as receptor or target proteins. A total of 2 compounds were used as ligands and explored for their binding interactions with the amino acids of the active sites of the selected proteins involved in hepatocellular carcinoma through molecular docking studies.

1.1.3 Interaction Analysis:

A PyRx virtual screening tool and BIOVIA Discovery Studio were used for the docking and visualization of 2D patterns of ligand–target protein interactions, respectively. The top drug candidates were predicted based on their binding affinities and RMSD values. PyRx displayed the occupancy of the binding pocket of the target molecule by the ligand via the conformation scores.

Among the ten synthesized compounds **C-1** is selected separately against each receptor protein on the basis of the minimum binding score and RMSD values (Table 1).
The Pi–sigma interactions (i.e., Pi–alkyl and Pi–sulfur) help to intercalate the drug in the binding pocket of the receptor as they are largely involved in the charge transfer.

Table 1. Binding scores of 2 compounds with Alpha-glucosidase protein as a receptor protein

Sr.No.	Ligand	Receptor	Binging Affinity(kcal/mol)	Interacting Amino Acids
01	**C-1**	Alpha-glucosidase	-8.6	GLY A:24, GLY A:426, THR A:428, THR A:426, HOH A:586, HOH A:591, LYS A:166, ARG A:115, HOH A:661, GLN A:150

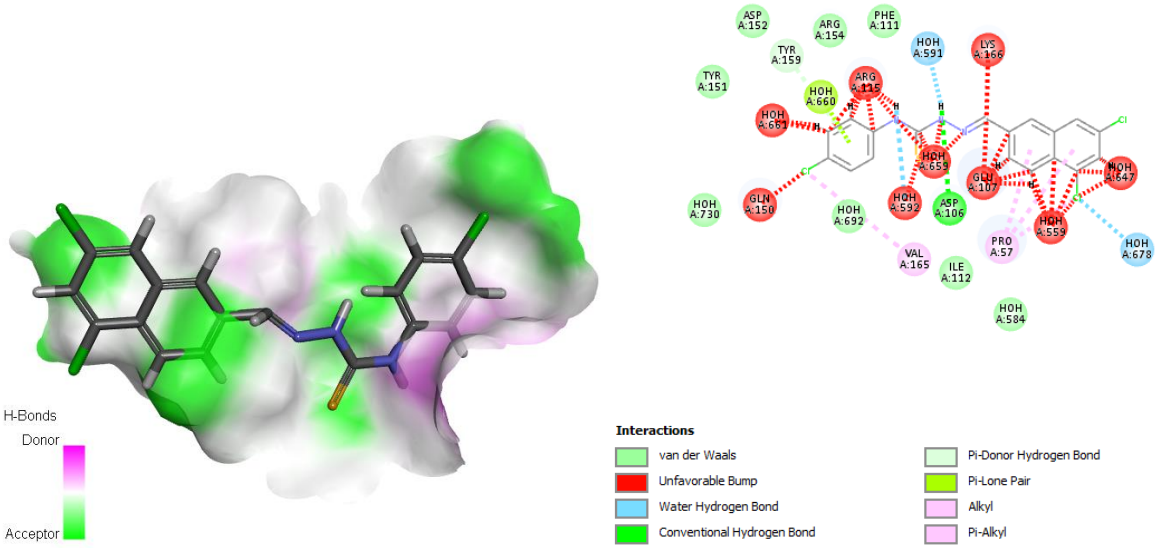


Figure Interaction (a) and binding pattern (b) of **C-1** with Alpha-glucosidase protein as a receptor.

3.4. ADME study

Because the process of designing and developing new drugs is time-consuming and costly, particularly when evaluating the pharmacokinetic profile of the compound experimentally, computational approaches to optimize pharmacokinetic and toxicity properties facilitate the effective and efficient progression of discovery leads to drug candidate's molecules. In fact, a competent computational method can provide the same information as an experimental result—rather than necessarily producing outcomes identical to those of experimentation (Ranjith & Ravikumar, 2019).
Molecular weight, the number of hydrogen bond donors and acceptors, proportion Csp3, and TPSA (Å) were examples of the physicochemical qualities. Lipophilicity and solubility were the other two important factors that are tracked for advantageous medication development (Lohohola et al., 2021).
Molecule **C-1** is highly absorbed through the gastrointestinal tract, and the pharmacokinetics investigation showed that none of the molecule was BBB permeant. Table 7 summarizes the expected physicochemical characteristics and pharmacokinetic features of the reference drug and synthesized compounds.
The ideal range for each property was shown by the pink area in the bioavailability radar. Lipophilicity: −0.7 to +5.0 in XLOGP3, Size: MW ranging from 150 to 500 g/mol, Polarity: TPSA ranging from 20 to 130 Å2. Solubility: log S should not exceed 6. Saturation: a minimum of 0.25 percent of the carbons in the sp3 hybridization maximum flexibility: nine rotatable bonds (Tripathi,

Ghosh, & Talapatra, 2019). Due to significant instauration, both of the synthesized compounds are predicted in figure 13 of the bioavailability Radar to not be orally accessible.

For drug discovery and development, the BOILED-Egg model provides a fast, impulsive, effective, and noisy way to predict passive GI absorption. The molecules in the white region are more likely to be absorbed by the GI tract, while those in the yellow area (yolk) are more likely to permeate the brain (Yadav & Mohite, 2020). As figure 14 illustrates, all compounds were present in the white zone and absorbed in the GI.

By Lipinski's standards, the reference drug and compound **C-1** satisfied the druglikeness requirements. The first of five rules that describe tiny molecules based on physicochemical property profiles, such as Molecular Weight (MW) less than 500, MLOGP ≤ 4.15, N or O ≤ 10, and NH or OH < 5, is the Lipinski filter (Pfizer). The compound C2 showed two violations and both compounds are highly bioavailable as shown in table 8.

The compounds' toxicity analysis showed that although they were active in hepatotoxicity and immunotoxicity, they were inactive in cardiotoxicity and cytotoxicity. The reference drug was inactive in all the categories of toxicity.

Table 7: In silico predicted physicochemical properties and pharmacokinetics of synthesized compounds compared with reference

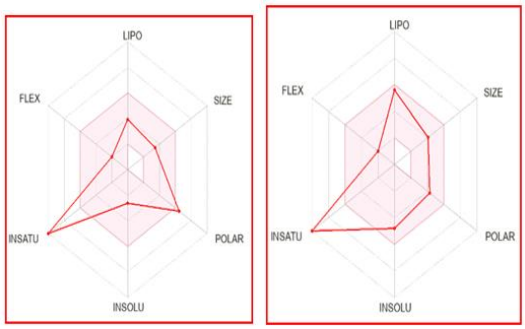
Sr. No	Molecule name	No. H-bond donor	No. H-bond acceptors	Fraction Csp3	TPSA	Lipophilicity	Water solubility	GI absorption	BBB permeant	Log K_p
1	Chloramphenicol	3	5	0.36	115.38	-0.26	-2.32	High	No	-7.46
2	**C-1**	2	1	0	82.5	5.04	-4.82	High	No	-5.25

Table 8: Druglikeness of synthesized compounds compared with reference

Sr. No	Molecule name	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability score
1	Chloramphenicol	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
2	**C-1**	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55

Table 9: Toxicity of compounds and reference drug

Sr.No:	Molecule name	Hepatotoxicity	Neurotoxicity	Cardiotoxicity	Immunotoxicity	Cytotoxicity
1	Chloramphenicol	Inactive (0.70)	Inactive (0.80)	Inactive (0.53)	Inactive (0.99)	Inactive (0.64)
2	**C-1**	Active (0.54)	Active (0.66)	Inactive (0.82)	Active (0.70)	Inactive (0.54)



Chloramphenicol C-1

Figure 13: Bioavailability radar

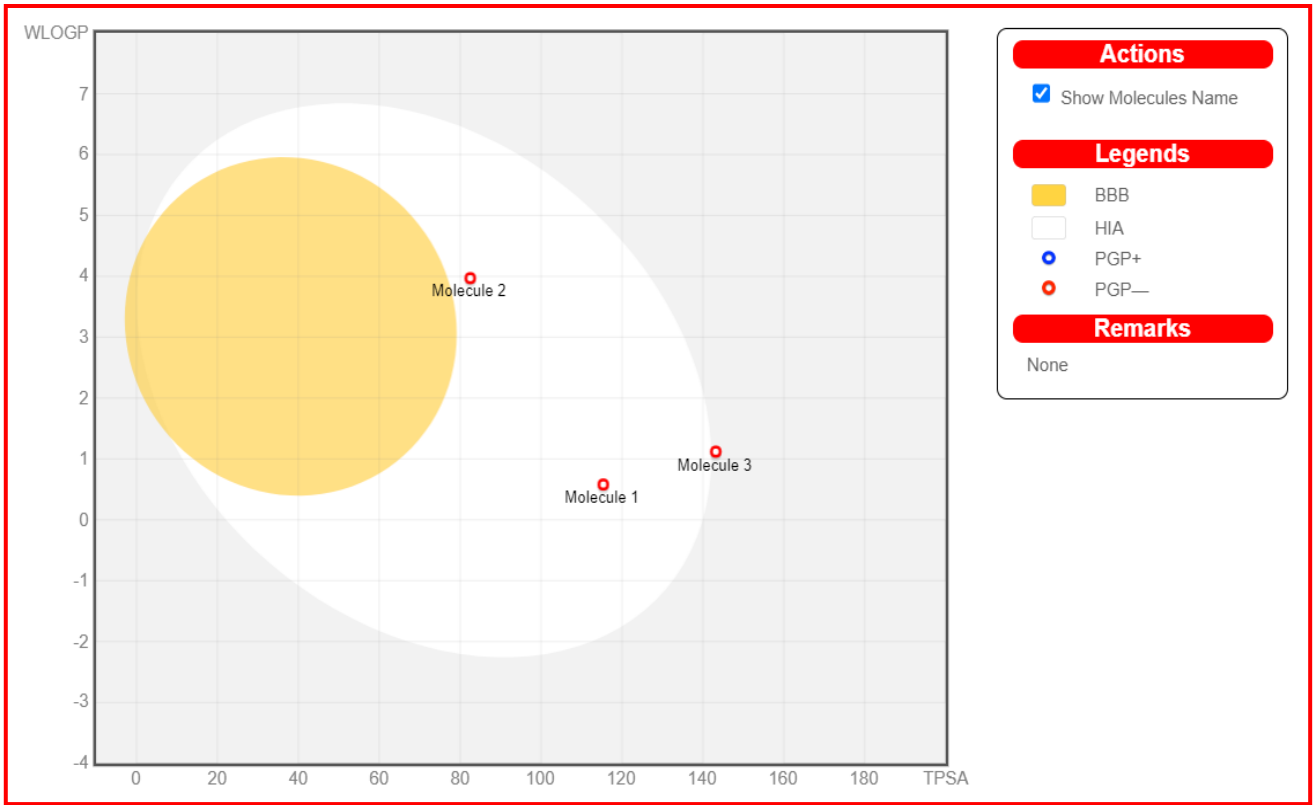


Figure 14: The BOILED egg Model prediction of GI absorption and BBB penetration by using swiss ADME

4. Conclusions

In short, we have successfully prepared a novel set of thiosemicarbazones using different carbonyl compounds by condensation reaction between *N-(4-chlorophenyl)hydrazinecarbothioamide with* 5,7-dichloronaphthalene-2-carbaldehyde *gives* (2*E*)-2-[(5,7-dichloronaphthalen-2-yl)methylidene]-*N*-phenylhydrazine-1-carbothioamide in good yield. The compounds were assessed for antibacterial and antidiabetic activity. All compounds were active against *Bacillus subtilis* and *Escherichia coli* and compounds both of these **C-1** appeared as good antibacterial agents (MIC; 1 µg/mL). This article supports to catch probable imminent directions on advancement of more effective and explicit analogues of the thiosemicarbazones for numerous biological targets. In case of antidiabetic activity, Acrabose was used as reference. Molecular docking result revealed that C-1 displayed the finest enactment against the Alpha-glucosidase proteins as reinforced by its lowest binding energy -8.7 kcal mol respectively)

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