

Synthesis, Characterization and Molecular Docking of *E*)-1-((5-(4-Chlorophenyl) Furan-2-yl) Methylene)-2-(2,4-Dinitrophenyl) Hydrazine)

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ABSTRACT

In this study, a novel hydrazone derivative was synthesized through the condensation of 2,4-dinitrophenylhydrazine and 5-(4-chlorophenyl) furfural. The structural characterization of the synthesized compound was carried out using melting point analysis, infrared (IR) spectroscopy, and proton nuclear magnetic resonance (¹H-NMR) spectroscopy, confirming its purity and chemical integrity. Molecular docking studies were performed to evaluate the binding affinity of the synthesized hydrazone derivative with the active site of Escherichia coli K-12 (Protein Data Bank ID: 7NB9), yielding a binding energy of -5.74 kcal/mol, which is significantly stronger than the standard antimicrobial drug nitrofurantoin (-4.71 kcal/mol). Additionally, the compound was analyzed for its interaction with human serum albumin complexed with cis-9-octadecenoic acid (oleic acid) (PDB ID: 1GNI), demonstrating an even greater binding energy of -9.48 kcal/mol, surpassing that of nitrofurantoin (-4.56 kcal/mol). These findings suggest that the synthesized hydrazone derivative exhibits strong molecular interactions, highlighting its potential as a promising candidate for further drug development.

Keywords: Hydrazone, 2,4-Dinitrophenylhydrazine, Nitrofurantoin, Antimicrobial Activity, Molecular Docking Study

INTRODUCTION

Hydrazone derivatives, identified by the functional group (-C=N-NH-), are a type of chemical substance formed by the condensation of hydrazines with carbonyl-containing compounds such as aldehydes and ketones [1]. These derivatives have emerged as a focal point in modern research due to their diverse applications in chemical synthesis and pharmaceutical advancements. Their ability to undergo straightforward designing bioactive molecules [2, 3]. Hydrazone compounds have received a lot of attention due to their broad range of biological activities, which include antimicrobial, anticancer, antihypertensive, anticonvulsant, analgesic, anti-inflammatory, and antimalarial effects [3, 4]. In this investigation, a novel hydrazone compound was synthesized through the reaction of 5-(4-chlorophenyl) furfural with 2,4-dinitrophenylhydrazine (DNPH). The resultant product was comprehensively analyzed using melting point assessments and a variety of spectroscopic methods to validate its composition and structural integrity [5, 6]. Molecular docking is a computer tool in molecular modeling that is used to develop drugs and analyze molecular interactions. It involves making atomic-level predictions about the preferred binding orientations and affinities of small molecules (ligands) and macromolecular target structures. This approach expedites the process of creating drugs and contributes to the production of more potent medicinal products [7]. The synthesized compound is subjected to molecular docking analysis to compare its binding affinity with that of nitrofurantoin, a well-known antimicrobial agent by binding to the active site of Escherichia coli K-12 (PDB ID: 7NB9) in the target protein [8, 9]. Human serum albumin, the best prevalent plasma protein, serves as a primary human transporter for a number of intrinsic and extrinsic substances that are present in blood. Consequently, the study of compounds based on albumin binding is crucial, considering that this affinity is connected with the bioavailability of numerous physiologically active substances [10]. For these reasons, we also used molecular docking to

bind the synthesized compound and nitrofurantoin to the active site of a human serum protein complexed with cis-9-octadecenoic acid (oleic acid) (PDB ID: 1GNI) in the target protein. These findings shed light on how this substance is transported through the bloodstream and ultimately eliminated by the kidney [8, 10].

EXPERIMENTAL

Materials

All of the chemicals were bought from Sigma Aldrich, Loba Chemie, and Across Belgium, along with analytical-grade solvents. Thin-layer chromatography (TLC) was performed on silica gel plates 60 GF254 and the spots were detected by exposure to iodine vapors or UV254 lamp. A Stuart SMP-10-Barloword apparatus was used to detect the melting point by use an open glass capillary tube. IR spectra were recorded by the KBr pellet method in a Bruker FT-IR spectrometer and the nuclear magnetic resonance (NMR) spectra were recorded on Jeol ECA 500 MHz in DMSO-d₆.

Synthetic Procedure

A solution containing (0.198 g, 1 mmol) of 2,4-dinitrophenylhydrazine and (0.206 g, 1 mmol) of 5-(4-chlorophenyl) furfural in 10 mL of ethanol was supplemented with 0.5 mL of concentrated sulfuric acid. After stirring the mixture for an hour at room temperature, it was cooled in an ice bath, precipitate formed was filtered, dried and crystallized from ethanol [11].

COMPUTATIONAL METHODOLOGY

Molecular Docking Studies

Molecular docking simulations are performed to select the active binding site of the protein, where Molecular Operating Environment

(MOE) software was used [12]. The ChemOffice tool known as “Chem & Bio Draw 12.0” was used to show the correct 2D orientation of the chemical structures of the selected compound, and the MOE was used to minimize the amount of energy molecule. To perform docking simulations in MOE, molecules with the lowest energy were chosen. By searching the Protein Data Bank repository, the 3D structure of the receptor protein was downloaded as a format with the PDB ID of 7NB9 for antibacterial activity and 1GNI for higher biological potential. In order to successfully prepare the target protein according to the standard protocol, it was necessary to release the co-crystallized complex, as well as delete the water molecules. It has been demonstrated that the compound and protein can be docked in the most efficient manner by taking advantage of the docking approach made possible by the MOE docking, a maximum five conformations of

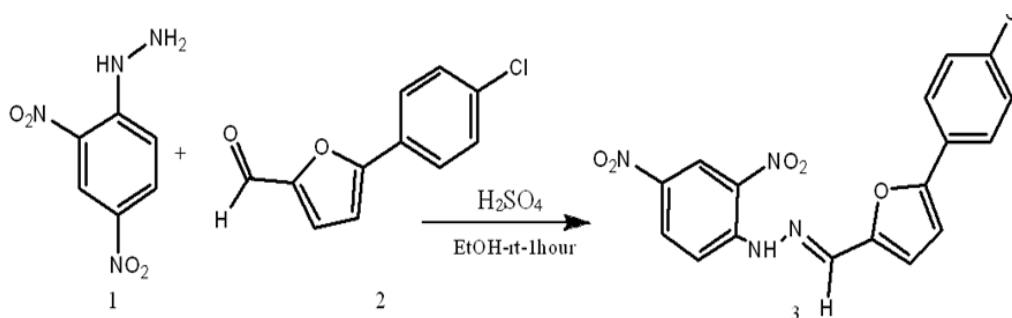
each ligand and reference drug (nitrofurantoin) were investigated while performing the docking procedure.

Investigated 3D and 2D ligand-receptor interactions and demonstrated binding on a bitter ligand with low S and RMSD ~2 and demonstrated binding between receptor residues and both the reference molecule or drug.

Results and Discussion

Synthesis and Characterization

Scheme 1 demonstrates how the target compound was created. Compound **3** was produced when 2,4-dinitrophenylhydrazine **1** and 5-(4-chlorophenyl) furfural **2** reacted at room temperature with concentrated sulfuric acid.



SCHEME 1

The spectroscopic data (^1H NMR and IR) for *E*-1-((5-(4-chlorophenyl) furan-2-yl) methylene)-2-(2,4-dinitrophenyl) hydrazine compound **3** demonstrated good agreement with the recommended structures. In FT-IR, the hydrazine was characterized by the existence of a band at 1614-1586 cm^{-1} equivalent to $\text{C}=\text{N}$ stretching and a band at 3395-3274 cm^{-1} equivalent to $\text{N}-\text{H}$ stretching Figure 1.

The ^1H NMR spectrum also shows two singlets at δ 8.85 ppm ($\text{N}=\text{C}-\text{H}$), D_2O exchangeable singlet assigned for ($\text{N}-\text{H}$) proton at 11.73 ppm, the furan proton, and two benzene rings in the 7.133–8.853 ppm range Figure 2 and Figure 3.

E-1-((5-(4-chlorophenyl) furan-2-yl) methylene)-2-(2,4-dinitrophenyl) hydrazine:

Yield 42%. Mp: above 300°C , analysis for $\text{C}_{17}\text{H}_{11}\text{ClN}_4\text{O}_5$ (MW, 386.75), Calculated : C,52.79; H,2.87; Cl,9.17; N,14.49; O,20.68; IR (KBr, cm^{-1}): 3395-3274 ($\text{N}-\text{H}$ stretching), 3088 (CH aromatic), 2929 (CH aliphatic), 1614 ($\text{C}=\text{C}$), 1586 ($\text{C}=\text{N}$), 1510 ($\text{N}=\text{O}$), ^1H NMR ($\text{DMSO}-d_6$, 500 MHz) δ (ppm): 11.73 (s, 1H, $\text{N}-\text{H}$), 8.85 (s, 1H, $\text{N}=\text{CH}$), 8.63 (s, 1H), 8.40 (d, $J = 10$ Hz, 1H), 8.03 (d, $J = 9.5$ Hz, 2H), 7.84 (d, $J = 8$ Hz, 2H), 7.54 (d, $J = 8$ Hz, 2H), 7.242 (d, $J = 3$ Hz, 1H), 7.133 (d, $J = 4$ Hz, 1H).

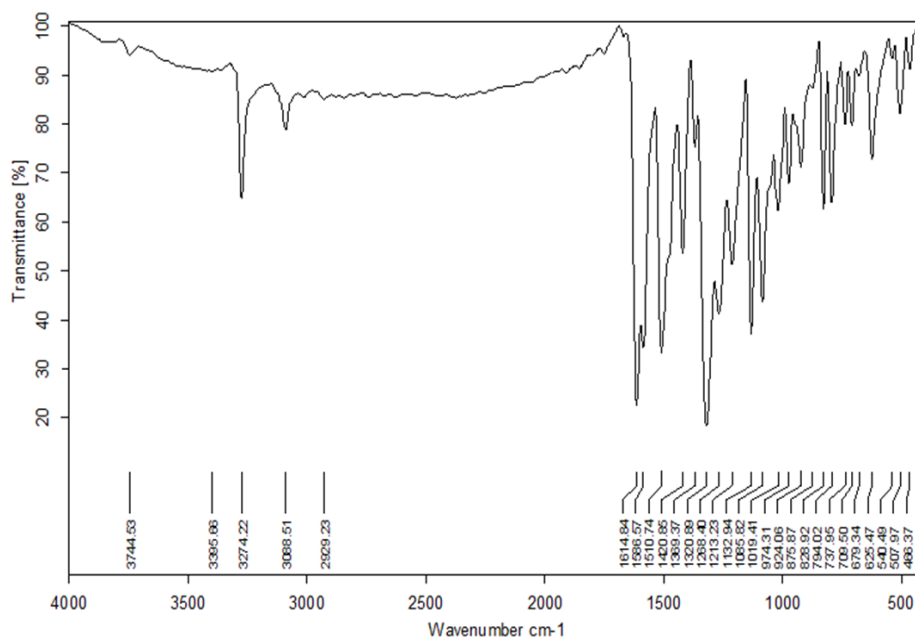


FIGURE 1: IR Spectrum of Compound 3

1H NMR spectrum of 1,2,3,4,5-pentachlorobenzene in CDCl₃. The main spectrum shows peaks from 7.0 to 8.9 ppm. An inset zooms in on the aromatic region from 7.1 to 8.9 ppm. The x-axis is labeled 'X : parts per Million : Proton' and the y-axis is 'abundance'.

Chemical structure: ClC1=CC(Cl)=CC(Cl)=C1Cl

Peak list (ppm):

- 8.861
- 8.856
- 8.572
- 8.402
- 8.383
- 8.378
- 8.044
- 8.024
- 7.840
- 7.824
- 7.537
- 7.520
- 7.199
- 7.191
- 7.122
- 7.114
- 3.608
- 2.490

352

Computational Methodology

Docking Studies

The antibacterial activity of the compound **3** was studied using molecular docking simulation studies and compared to the standard antibacterial nitrofurantoin. Using MOE, the compound **3** and the standard drug (nitrofurantoin) were bound to the active site of *Escherichia coli* K-12 (PDB ID: 7NB9) in the target protein, to show the antibacterial activity of compound **3** and compare it with nitrofurantoin. After that, we used MOE to bind both compound **3** and

nitrofurantoin to the target protein's active site of a human serum protein complexed with *cis*-9-octadecenoic acid (oleic acid) (PDB ID: 1GNI), in order to understanding compound **3** transporting in blood and compare it with nitrofurantoin.

First to Compare Activity

This docking investigation was performed using the 3D crystal structure of *Escherichia coli* K-12 (PDB ID: 7NB9) generated by: Day. M.D., Jarrom. D., Grainger. A.I., Parr. R.J., Hyde. E.I., White. S.A. (2001). The crystal form of the target protein is shown in the Figure 4.

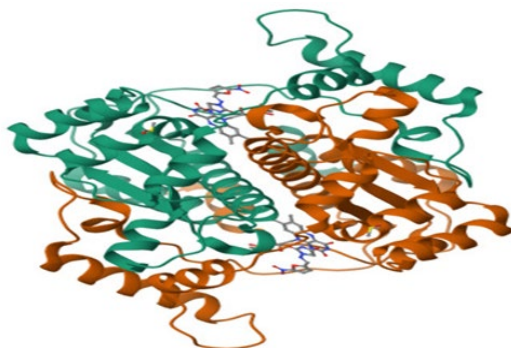


FIGURE 4: The Crystal Form of the Target Protein (PDB ID: 7NB9)

Putative binding sites for the target protein 7NB9 were evaluated indicating that the compound **3** was bound to the active binding site of the target protein. The active site of the target protein was well occupied by the tested compound.

A figure 5 showing docking results for the tested compound and the receptor protein representing a cardboard surface representation, and a 2D as well as 3D diagrammatic representation of the amino acid residues associated in the bonding reactions (e.g., hydrogen bonds are

shown with green colored dotted lines. Binding interaction of tested compound with the target protein showed conventional hydrogen bond between oxygen in nitro group with ARG35.

The tested compound was observed to bind to the active site of the target protein of 1NGI with a binding energy score of -5.74 kcal/mol. And it was calculated that nitrofurantoin binds to the active site of the target protein with a binding energy of -4.71 kcal/mole which was lower than that of the tested compound.

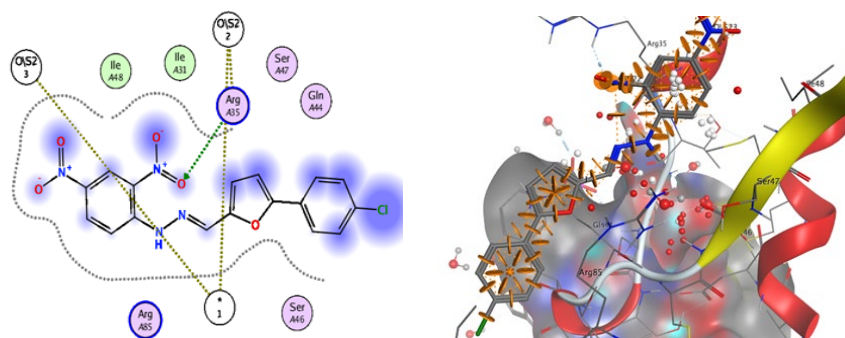


FIGURE 5: 2D and 3D of Ligand Interaction between Compound **3** and Protein 7NB9

Second to Increase Activity

This docking examination was achieved using the 3D crystal structure of human serum albumin complexed with *cis*-9-

OCTADECENOIC acid (oleic acid) (PDB ID: 1GNI) generated by Petitpas, I., Gruene, T., Bhattacharya, A. A., Curry, S. (2001). The crystal form of the target protein is shown in the Figure 6.

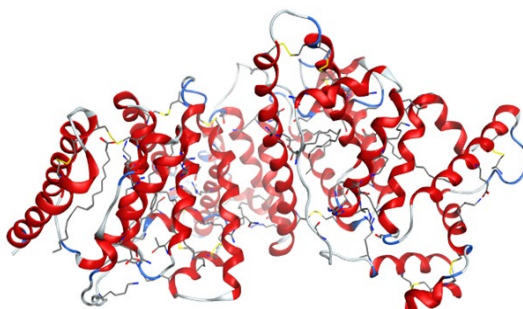


FIGURE 6: The Crystal Form of the Target Protein (PDB ID: 1GNI)

Putative binding sites for the target protein 1GNI, indicated that the tested compound was attached to the target protein's active binding site. The tested chemical occupied the target protein's active site well. Figure 7 illustrates the docking results for the tested compound and the receptor protein using a cardboard surface representation. It also shows a 2D and 3D diagrammatic representation of the amino acid residues involved in the bonding reactions, such as hydrogen bonds, which are indicated by green dotted lines. Binding interaction of tested compound with the target protein showed conventional hydrogen

bond between oxygen in nitro group with TYR 161. The other amino acid residues GLY 189 which were involved in another hydrogen bond interactions with tested compound. And the last binding interaction of tested compound with the target protein is hydrogen bond with SER 193. The tested compound was observed to bind to the active site of the target protein of 1GNI with a binding energy score of -9.48 kcal/mol. And it was calculated that nitrofurantoin binds to the active site of the target protein with a binding energy of -4.56 kcal/mole which was lower than that of the tested compound.

Table 1: Amino Acid Residue in Active Site of Escherichia Coli K-12 (PDB ID: 7NB9) and Human Serum Albumin Complexed with cis-9-OCTADECENOIC Acid (Oleic Acid) (PDB ID: 1GNI)

Receptor	Residue
7NB9	GLU24 ALA25 ARG27 GLU28 ILE31 ALA34 ARG35 ALA36 THR37 SER38 SER39 PHE42 GLN44 CYS45 SER46 SER47 ILE48 ARG50 ARG85 ASP107 THR108 MET111 ARG114 LEU115 VAL116 ARG117 MET123 TYR138 ILE142 ARG145 HIS146 TYR150 GLU153 PHE156 PHE 157 ARG160 TYR161 LEU182 ASP183 GLU184 ASP187 GLU188 LYS190 ALA191 SER192 ALA194 LYS195 GLN196 LEU198 LYS199 SER202 LUE203 PHE206 GLY207 ALA210 PHE211 TRP214 ALA215 ARG218 LEU219 GLN221 ARG222 PHE223 PRO224 LEU 238 VAL241 HIS242 CYC245 ARG257 LEU260 AL261 ILE264 LYS 274 SER287 HIS288 ILE290 ALA291 GLU292 VAL293 GLU294 ASN295 ASP296 GLU297 MET298 ARG336 PRO339 ASP340 TYR341 SER342 VAL344 LEU347 ARG348 PRO384 LUE387 ILE388

Table 2: The Result Obtained from Docking with 7NB9 and 1GNI

Receptor	Ligand	Atom of Compound	Atom of Receptor	Induced Receptor Residue	Type of Interaction Bond	Distance	E(kcal/mol)
7NB9	Co-Crystalized Ligand	O4 6	N	ILE48	H-acceptor	3.29	-1.8
	(Reference Ligand)	O2' 32	NE	ARG35	H-acceptor	3.04	-2.8
		O1P47	NH ₂	ARG35	Ionic	3.18	-3.4
		6-ring	6-ring	FMN301	pi-pi	2.37	-0.0
		6-ring	6-ring FMN	FMN301	pi-pi	2.41	-0.0
		6-ring	6-ring FMN	FMN301	pi-pi	2.37	-0.0
		6-ring	6-ring FMN	FMN301	pi-pi	2.41	-0.0
	Nitrofurantoin (Reference Antibacterial)	O5 1	NH ₂	ARG 35	ionic	3.07	-4.0
		N4 3	NE	ARG 35	ionic	3.19	-3.3
		N4 3	NH ₂	ARG 35	ionic	3.04	-4.2
1GNI		O1 5	NE	ARG 35	ionic	3.56	-1.7
		5-ring	CA	ALA 36	pi-H	4.22	-0.6
	Compound 3	O 34	NE	ARG 35	H-acceptor	3.33	-0.8
	Co-Crystalized Ligand	O1 2	NH ₂	ARG117	H-acceptor	3.23	-5.2
	(Reference Ligand)	O1 2	NH	ARG117	Ionic	3.54	-1.7
		O1 2	NH ₂	ARG117	Ionic	3.23	-3.1
		C18 50	5-ring	HIS146	H-pi	3.77	-0.9
	Nitrofurantoin (Reference Antibacterial)	O 19	NH ₂	ARG117	H-acceptor	3.06	-2.6
	Compound 3	O 35	OH	TYR161	H-acceptor	3.24	-1.0
		O 35	CA	GLY189	H-acceptor	3.59	-0.7
		O 38	OG	SER 193	H-acceptor	3.24	-0.8

Table 3: The Result of S-Score and RMSD Value that Obtained from Docking with 1GNI in Active Site and 7NB9

Smiles	S-Score	RMSD
7NB9		
Clc1ccc(-c2oc(C=N/Nc3c([N+](=O)[O-])cc([N+](=O)[O-])cc3)cc2)cc1	-5.7498	1.5854
O=[N+](O-)]c1oc(C=N/N2C(=O)NC(=O)C2)cc1	-4.7142	1.9055
1GNI		
Clc1ccc(-c2oc(C=N/Nc3c([N+](=O)[O-])cc([N+](=O)[O-])cc3)cc2)cc1	-9.48005	1.269957
O=[N+](O-)]c1oc(C=N/N2C(=O)NC(=O)C2)cc1	-4.56212	1.014861

Conclusion

Spectroscopic methods (IR and ¹H NMR) and melting point were used to analyze the synthesized compound. In addition, the synthetic compound was tested for its *in-silico* molecular docking analysis using Escherichia coli K-12 (PDB ID: 7NB9) and active site of a human serum protein complexed with cis-9-octadecenoic acid (oleic acid) (PDB ID: 1GNI). With a binding energy score of -5.74 kcal/mol, our compound demonstrated excellent binding potential to Escherichia coli K-12 (PDB ID: 7NB9), surpassing the binding energy of the

standard drug nitrofurantoin (-4.71 kcal/mol). Furthermore, when it binds to cis-9-OCTADECENOIC acid (oleic acid), it has a binding energy score of -9.48 kcal/mol, which is higher than that of nitrofurantoin (-4.56 kcal/mol) (PDB ID: 1GNI).

This excellent binding potential suggests that the synthesized hydrazone derivative has antibacterial properties with good binding to human serum protein which will help us to gain greater awareness of the pharmacokinetics of our compound. This approach is a first step in the development of novel medications that may eventually be employed as antibacterial drug with better biological activity.

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