Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

Synthesis and Biological Evaluation of 2-Hydroxy Benzyl Hydrazides Congeners

Vipin Kumar Sharma[1], Rosaline Mishra[1*], Ajit Kiran Kaur[2], Neha Ronald William,[2] Md Quammuddin[1]

[1]Metro College of Health Science and Research, Greater Noida
[2]Accurate College of Pharmacy, Greater Noida
*Corresponding author Rosaline Mishra: Metro College of Health Science and Research, Greater Noida;

(Received: 16 September 2024 Revised: 11 October 2024 Accepted: 04 November 2024)

KEYWORDS
Hydrazide derivatives,antibacterial, antioxidant, molecular docking, spectral characterization.

ABSTRACT:
Since 2-hydroxy benzyl hydrazide congeners may have biological functions, much effort has been paid to their design and production. The antibacterial and antioxidant qualities of these molecules are improved via structural modification. This study focuses on the synthesis of new derivatives of 2-hydroxy benzyl hydrazide and their evaluation for antibacterial and antioxidant activities. using molecular docking via PyRx, five derivatives (C-1, C-2, C-3, C-7, and C-12) out of thirteen derivatives were identified with good affinity scores.These five derivatives were synthesized and subjected to physicochemical characterization, including solubility, melting point, and Rf values, as well as spectral analysis through infrared, nuclear magnetic resonance (NMR) and mass spectroscopy, .In vitro antioxidant activity was assessed using the DPPH assay, revealing that the IC50 Value of synthesized compounds (C-1; 223.87, C-2; 85.64, C-3; 162.18, C-7; 81.28 and C-12; 309.03µg/mL) and ascorbic acid (30.20 µg/mL) was found respectively. The radical scavenging activity (% RSA) or inhibition rates of compound code C-2 (85.64%), C-3 (86.49%) and C-7 (91.45%) shown good activity compared to the reference drug (Ascorbic acid; 93.58%). Based on their antioxidant potential, C-2, C-3, and C-7 were further evaluated for antimicrobial activity using the agar disc diffusion method against gram-positive Staphylococcus aureus and gram-negative Escherichia coli. Compound C-7 exhibited a largerzone of inhibition (2.0 cm for S. aureus and 2.1 cm for E. coli) compared to the standard drug ciprofloxacin (1.9 cm).The results demonstrate that the synthesized 2-hydroxy benzyl hydrazide derivatives possess magnificent antibacterial and antioxidant activity compared to standard medications, making them promising candidates for further therapeutic development.

1. INTRODUCTION

Congeners of 2-hydroxy benzyl hydrazide have drawn a lot of attention due to their variety of biological activities, which include antibacterial and antioxidant qualities [1-2]. The synthesis and design of these compounds are essential for both creating novel therapeutic agents to treat a range of ailments as well as for comprehending their biological behaviour. A distinct class of hydrazone derivatives known as 2-hydroxy benzyl hydrazide congeners is distinguished by the existence of a hydroxyl group linked to the benzyl ring [3]. It is well recognized that this structural motif has a major impact on the compounds' biological activity. In particular, the hydroxyl group has the ability to form hydrogen bonds and improve the compound's ability to interact with biological targets. These congeners are useful in medicinal chemistry because the hydrazide moiety can further modify the compound's biological profile by forming hydrazone connections [4-6].

Because oxidative stress plays a crucial role in the pathophysiology of many diseases, including cancer, cardiovascular problems, and neurological ailments, interest in the antioxidant capacity of 2-hydroxy benzyl hydrazide congeners has increased. Reactive oxygen species (ROS) and the body's capacity to counteract them through antioxidant mechanisms are out of

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

balance, which leads to oxidative stress [7-8]. Strong antioxidant compounds neutralize reactive oxygen species (ROS) to prevent oxidative damage and provide protection against oxidative illnesses. The capacity of 2-hydroxy benzyl hydrazide congeners to transfer electrons or hydrogen atoms, neutralizing ROS and averting cellular harm, is associated with their potency as antioxidants [9–10].

Derivatives of 2-hydroxy benzyl hydrazide have demonstrated promise as antibacterial agents in addition to their antioxidant capacity. The need for new antimicrobial medicines with unique modes of action arose from the rising incidence of infections resistant to antibiotics. Significant antibacterial action has been shown by hydrazone derivatives, particularly those with the 2-hydroxy benzyl hydrazide structure, against a variety of bacterial and fungal species [11]. The capacity of these substances to interfere with microbial enzyme systems or damage microbial cell membranes is frequently used to explain this behavior. The creation of such substances is especially crucial for tackling the problem of infections that are resistant to drugs and enhancing treatment results. [12-13].

As a crucial tool in the drug development process, in-silico investigations offer important insights into how molecules interact at the molecular level with biological targets [14]. By predicting a compound's binding affinity, stability, and possible activity, these computational methods can help in the design and development of new derivatives. For example, molecular docking experiments can mimic how 2-hydroxy benzyl hydrazide congeners interact to target proteins, offering insights into the possible mechanism of action [15].

2. MATERIALS AND METHODS

2.1 Materials

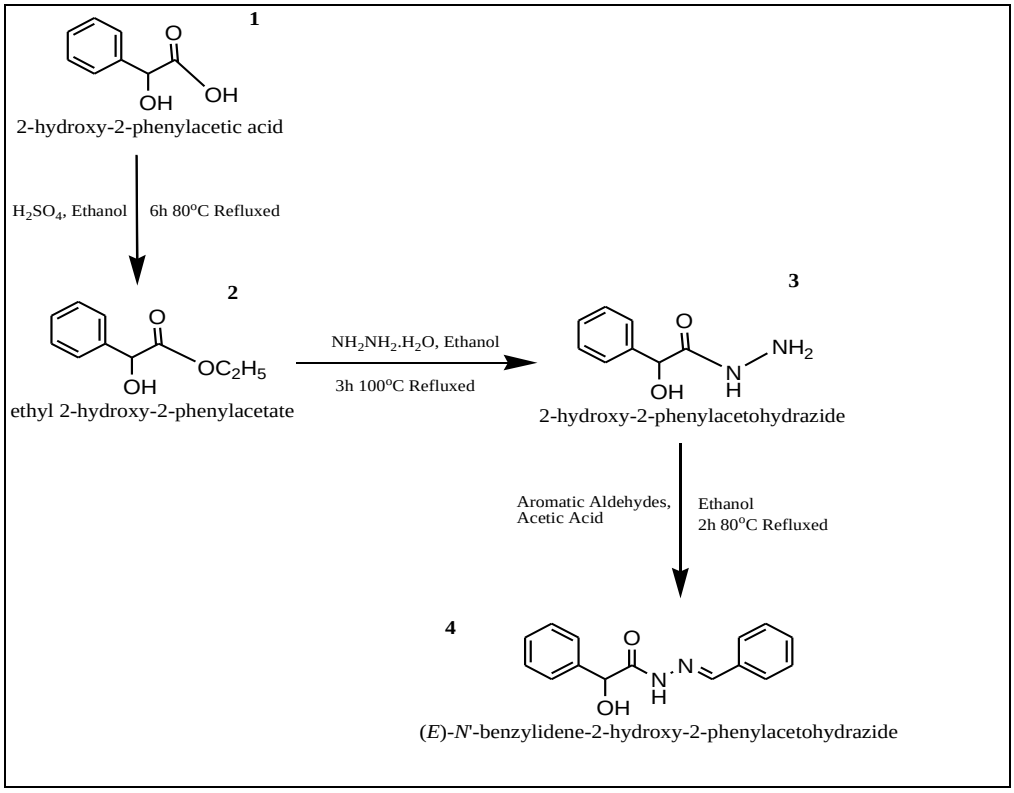
Drug sample and chemical reagents used in the synthesis of 2-Hydroxy Benzyl Hydrazide Congeners were procured from different reputed companies.

2.2 Method

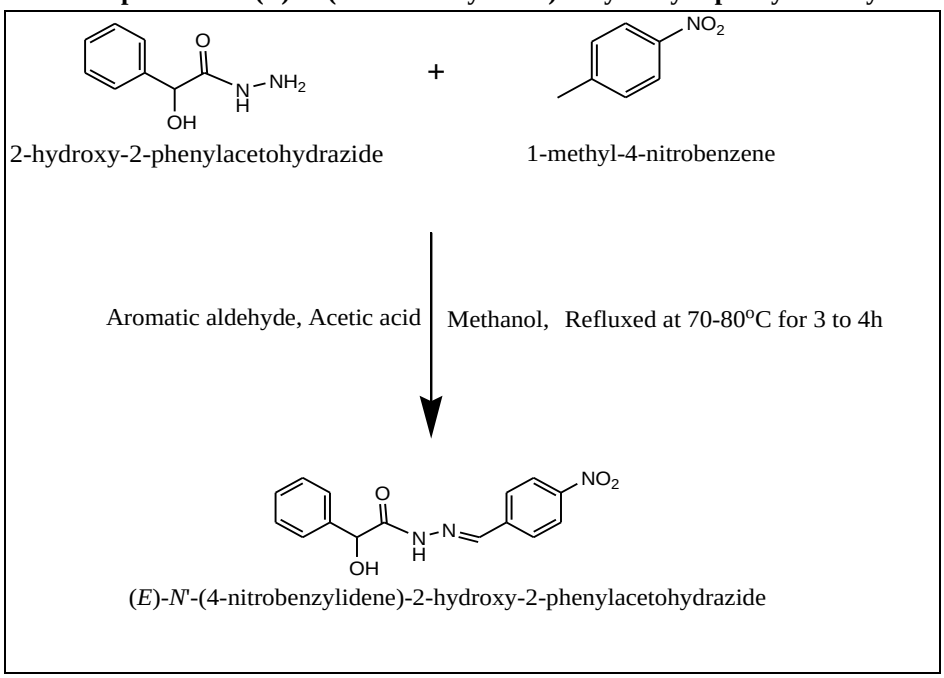
2.2.1General Procedure of Preparation of 2-Hydroxy Benzyl Hydrazide Congeners

- First take different derivatives of aromatic acids (ethanol and few drops of sulphuric acid) as dehydrating agent, were refluxed for 4 hours on water bath.
- The progress of the reaction was observed by TLC using Toluene/ ethyl acetate/ formic acid (5:4:1) as the mobile phase.
- After completion of reaction, a rotary evaporator was used to evaporate any excess alcohol.
- The residue was extracted out by using ether or ethyl acetate.
- Extracted liquid was evaporated and get 70 to 80% yield the title compounds.
- After that respective esters were mixed with the methanol and heated at 60·C then hydrazine hydrate (6 eq mol.) was added drop wise. The reaction mixture was refluxed for 6 h and the progress of reaction was observed by TLC using chloroform/methanol (9:1) as the mobile phase.
- When the reaction was completed, the reaction mixture was kept cooling down at room temperature and poured onto crushed ice and hydrazides were precipitate out that are filter out as well as re-crystalized with ethanol.
- Finally, Equimolar quantities of hydrazide and different aldehyde were refluxed in methanol for 3-4 hours with few drops of acetic acid. Once reaction completed compound poured into crushed ice, to get different derivatives of hydrazide product (compound code; C-1 to C-13).

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

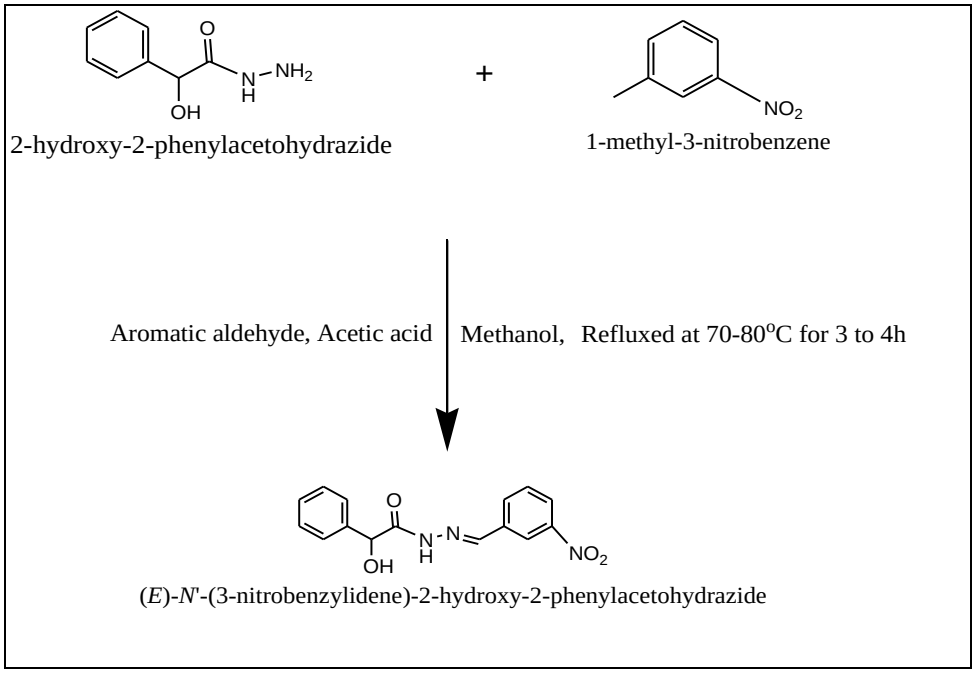


Scheme-1: Preparation of (E)-N-(4-nitrobenzylidene)-2-hydroxy-2-phenylacetohydrazide (Compound Code C-1)

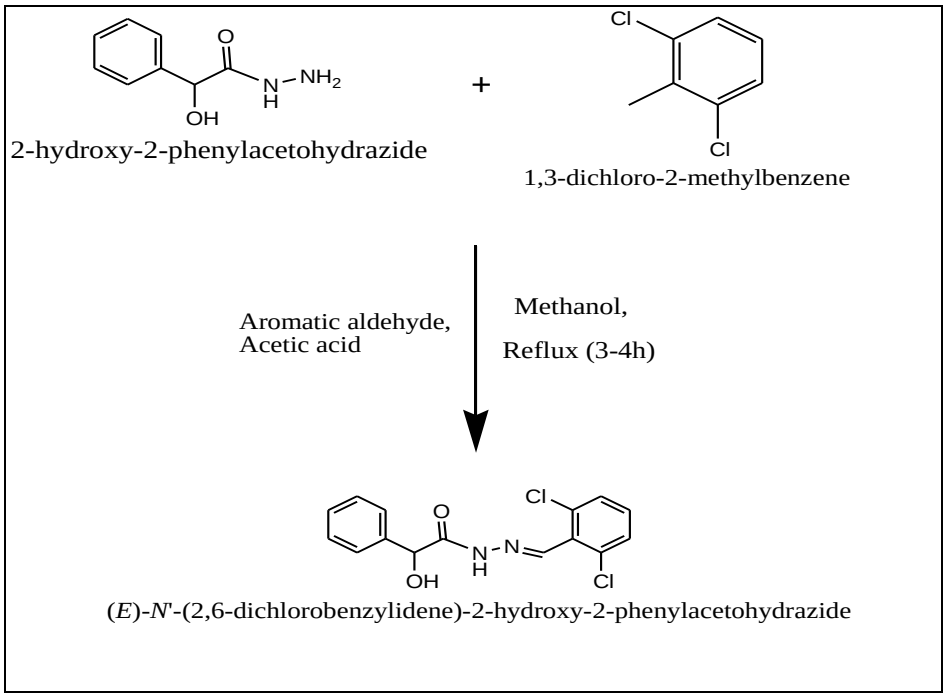


Scheme-2: Preparation of (E)-N-(3-nitrobenzylidene)-2-hydroxy-2-phenylacetohydrazide (Compound Code C-2)

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

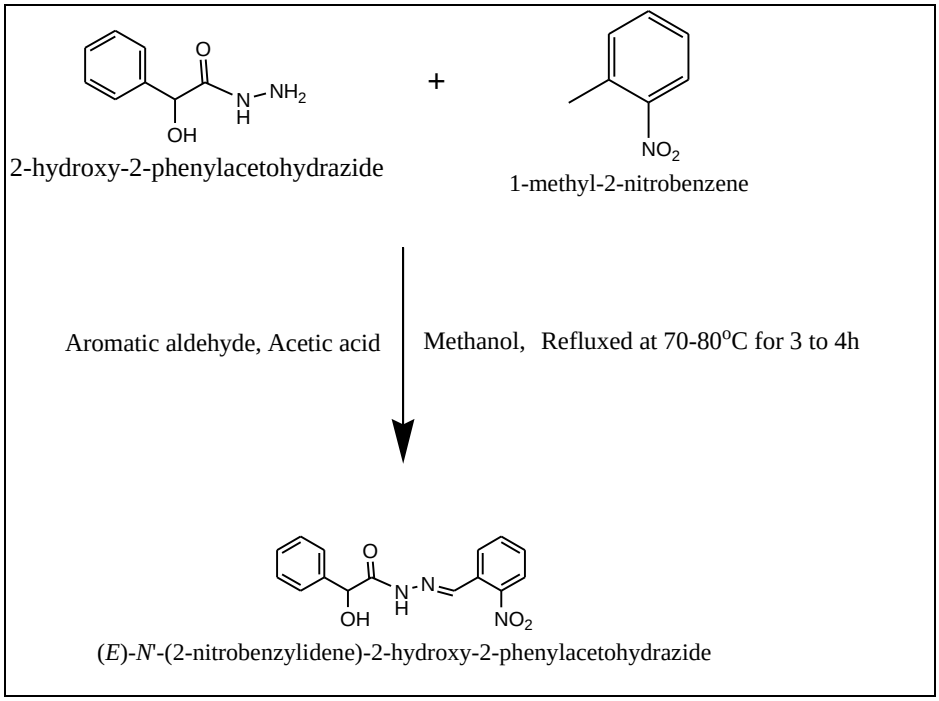


Scheme-3: Preparation of (E)-N-(2,6-dichlorobenzylidene)-2-hydroxy-2-phenylacetohydrazide (Compound Code C-3)

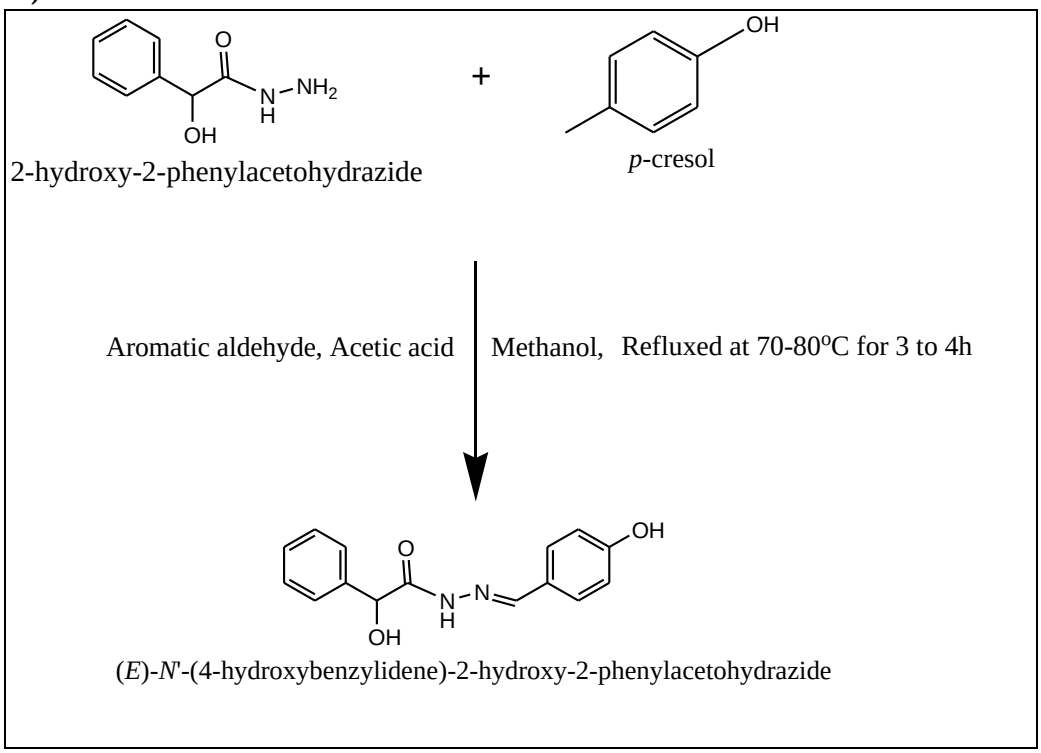


Scheme-4: Preparation of (E)-N-(2-nitrobenzylidene)-2-hydroxy-2 -phenylacetohydrazide(Compound Code C-7)

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

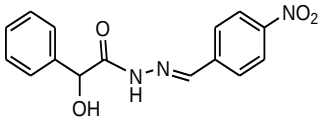
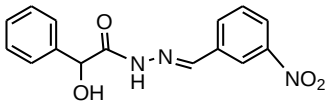
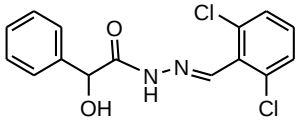
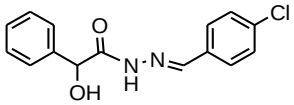
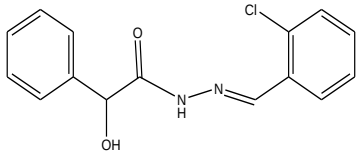
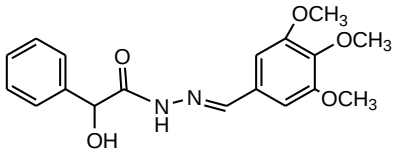
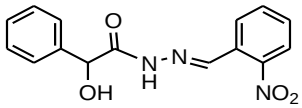
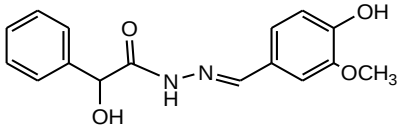
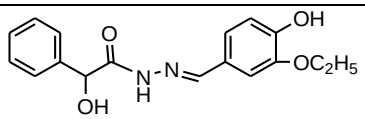


Scheme-5: Preparation of (E)-N-(4-hydroxybenzylidene)-2-hydroxy-2-phenylacetohydrazide(Compound Code C-12)

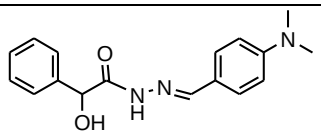
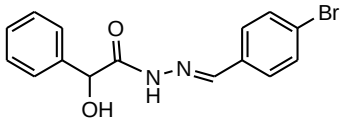
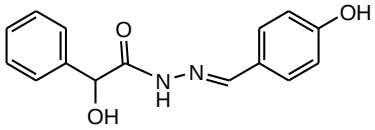
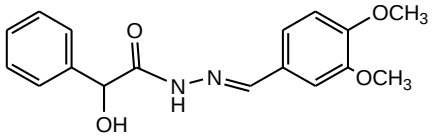


Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

Table 1. List Of Derivatives of 2-Hydroxy Benzyl Hydrazide

Compound Code	2D Structure	IUPAC Name
C-1		(E)-N'-(4-nitrobenzylidene)-2-hydroxy-2-phenylacetohydrazide
C-2		(E)-N'-(3-nitrobenzylidene)-2-hydroxy-2-phenylacetohydrazide
C-3		(E)-N'-(2,6-dichlorobenzylidene)-2-hydroxy-2-phenylacetohydrazide
C-4		(E)-N'-(4-chlorobenzylidene)-2-hydroxy-2-phenylacetohydrazide
C-5		(E)-N'-(2-chlorobenzylidene)-2-hydroxy-2-phenylacetohydrazide
C-6		(E)-N'-(3,4,5-trimethoxybenzylidene)-2-hydroxy-2-phenylacetohydrazide
C-7		(E)-N'-(2-nitrobenzylidene)-2-hydroxy-2-phenylacetohydrazide
C-8		(E)-N'-(4-hydroxy-3-methoxybenzylidene)-2-hydroxy-2-phenylacetohydrazide
C-9		(E)-N'-(3-ethoxy-4-hydroxybenzylidene)-2-hydroxy-2-phenylacetohydrazide

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

C-10		(E)-N'-(4-(dimethylamino)benzylidene)-2-hydroxy-2-phenylacetohydrazide
C-11		(E)-N'-(4-bromobenzylidene)-2-hydroxy-2-phenylacetohydrazide
C-12		(E)-N'-(4-hydroxybenzylidene)-2-hydroxy-2-phenylacetohydrazide
C-13		(E)-N'-(3,4-dimethoxybenzylidene)-2-hydroxy-2-phenylacetohydrazide

2.2.2Molecular Docking

Molecular Docking is used to design the compounds and predict of binding interaction between the macromolecule and ligands-based structures. Protein and ligand are prepared for docking process, which is done by using PyRx software.

2.2.3Physicochemical and Spectral Characterization of Synthesized Compounds

2.2.3.1 Solubility

The solubility of synthesized compound was tested in different solvents having polar, semi polar, and non-polar characteristics.

2.2.3.2 TLC Study

Using Silica gel GF_{254} (type 60) pre-coated Aluminium sheets from Merck, thin-layer chromatography (TLC) was used to determine the completion of reactions and the purity of the compounds.

2.2.3.3 Melting Point

In open capillary tubes, melting points were calculated using the Stuart/SMP3 melting point apparatus version 5.0.

2.2.3.4Infra-Red Spectroscopy

IR(KBr) spectra υ,cm-1 were recorded using Thermo Scientific™ Nicolet™ iS™10 FT-IR Spectrometer in Pioneer company for pharmaceutical industry-Sulaimani-Kurdistan-Iraq.

2.2.3.5NMR Spectroscopy

^{13}C-NMR spectra were recorded on Bruker FT–NMR spectrophotometer 400, using deuterated dimethyl sulfoxide (DMSO-$_{d6}$) and deuterated chloroform ($CDCl_3$) as internal standards. Chemical shifts were expressed in δ ppm.

2.2.3.6Mass Spectroscopy

MS was run, using Agilent Technology (HP), GC/MS model 5973 network mass selective detector. The values were

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

expressed as m/z.

2.2.4 *In-Vitro* Evaluation of Antioxidant and Antimicrobial Activity

2.2.4.1 *In-Vitro* Evaluation of AntioxidantActivity:

A methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH)was used as a reagent for the spectrophotometric assay withmodifications.3 mM solutions of hydrazide derivatives were prepared using DMSO. 1 mLof 0.1 mM methanolic solution of DPPH was added to a 3 mLsolution of the compound and the mixture was shakenvigorously using a vortex mixer. Absorbance was read againsta blank at 517 nm after incubation of the reaction mixtures for
60 min in the dark at room temperature. Ascorbic acid was used as a reference compound. The radicalscavenging activities were expressed as the inhibition percentage and were calculated using the formula:

$$\text{Antioxidant activity } (\%) = \frac{\text{A0} - \text{A1}}{\text{A0}} \times 100$$

where A_0 is absorbance of the control (blank, without compound)and A_1 is the absorbance of the compound [16,17].

2.2.4.2 *In-Vitro* Evaluation of Antibacterial Activity

The antibacterial activity of our tested compoundswas determined using the agar disc diffusion assay,while a 24h bacterial culture was spread on thesurface of the Mueller-Hinton agar plate. A disc ofsterile 6mm filter paper was saturated with 10 mM ofsolution of the synthesized compounds in dimethylsulfoxide (DMSO). After 1 h of diffusion, the Petri dishes wereincubated at 37^0C for 24 h and the diameter of thezone of inhibition was measured and compared with that of the ciprofloxacin reference disc [18].

3. RESULT AND DISCUSSION

A series of2-Hydroxy Benzyl Hydrazide derivative weresynthesized. Compounds code C-1, C-2, C-3, C-7 and C-12 were synthesized and evaluated by spectral parameters. The obtained results were depicted in this section.

3.1 Molecular Docking Analysis

After examining molecular interaction findings produced from docking experiments with various medications, we observed that derivates of 2-hydroxy benzyl hydrazide interacts with proteins in some way. The final intermolecular energy and creation of hydrogen bonds during the interaction between medications and receptor molecules might all be utilized to assess molecular docking data. During blind docking, the ligand was found to interact and form conventional and a carbon hydrogen bond. The results of docking analysis are depicted in table 3 and table 3.1.

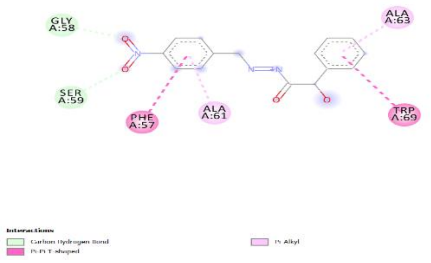
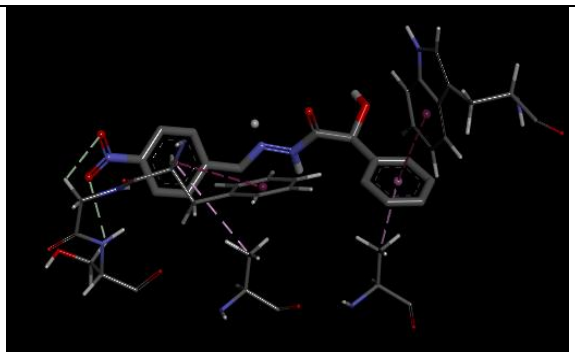
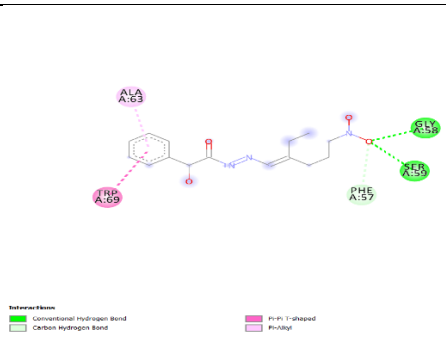
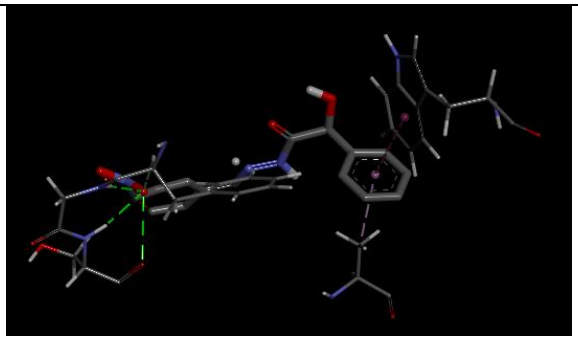
Table 2.Molecular Docking Results

Compounds Code	Affinity Score, kcal/mol	Interactions			
		Carbon Hydrogen Bond	Pi-Alkyl	Pi-Pi T-Shaped	Conventional Hydrogen Bond
C-1	-5.9	GLYA:58,SERA:59	ALAA:61,ALAA:63	PHEA:57,TRPA:69	--
C-2	-6.3	GLYA:58,SERA:59	ALAA:63	TRPA:69	PHEA:57
C-3	-5.9	--	ALAA:61,ALAA:63	PHEA:57,TRPA:69	--

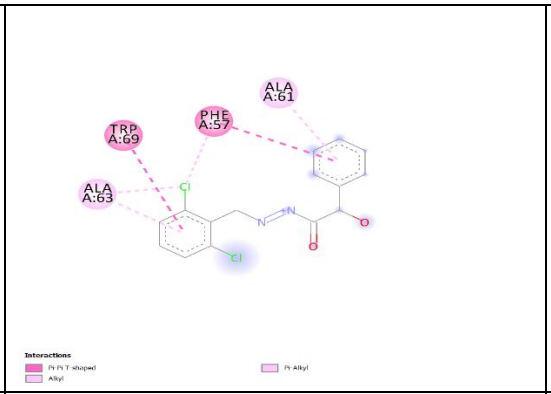
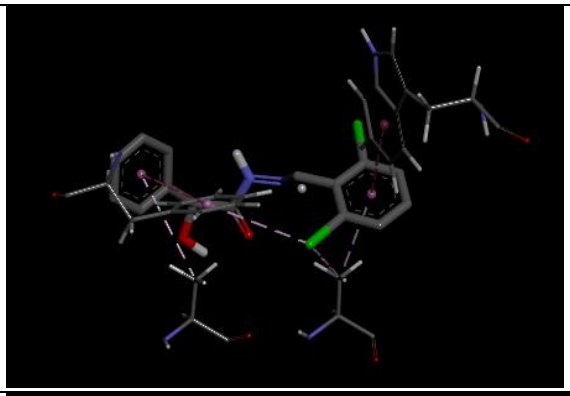
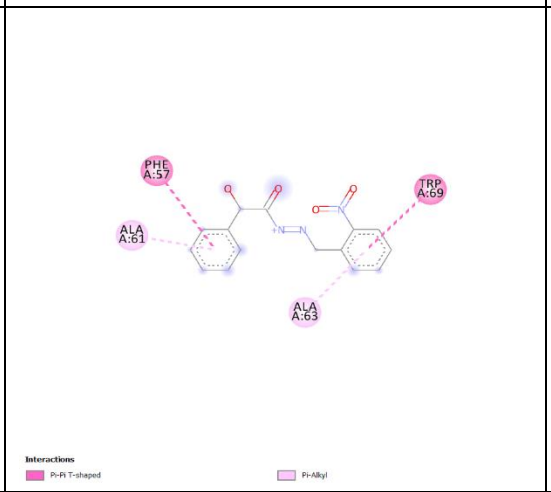
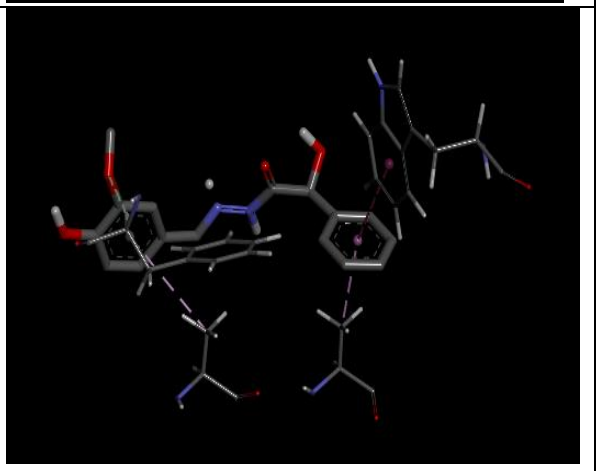
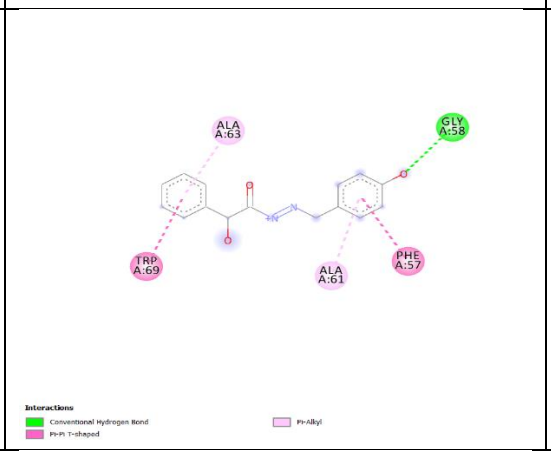
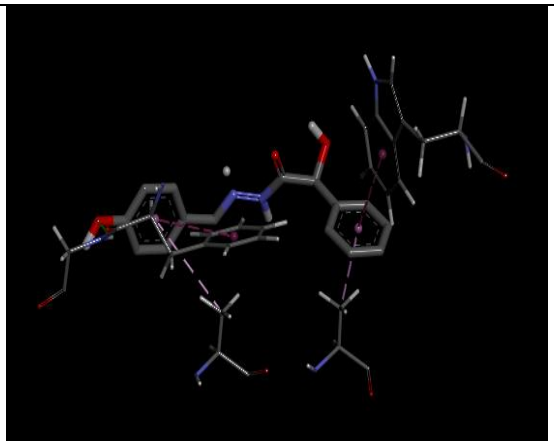
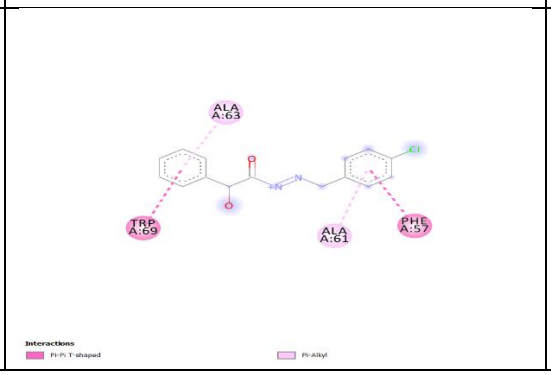
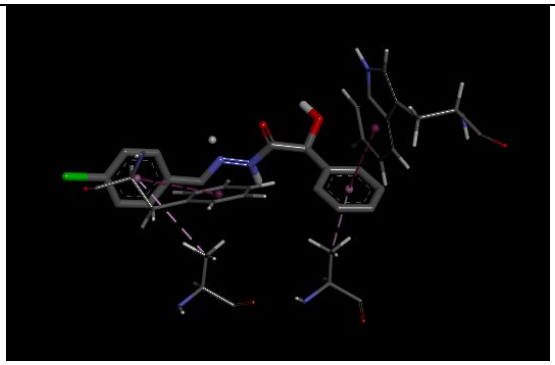
Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

C-7	-5.9	--	ALAA:61,ALAA:63	PHEA:57,TRPA:69	--
C-12	-5.8	--	ALAA:63,ALAA:61	PHEA:57,TRPA:69	GLYA:58
C-4	-5.5	--	ALAA:61,ALAA:63	PHEA:57,TRPA:69	--
C-5	-5.7	--	PHEA:57, ALAA:63	TRPA:69	PROA:66,PHEA:67
C-6	-5.4	PHEA:57	ALAA:61,ALAA:63	TRPA:69	GLYA:58,SERA:59
C-8	-5.6	PHEA:57	ALAA:61,ALAA:63	TRPA:69	--
C-9	-5.6	PROA:66,ASNA:65	ALAA:61,ALAA:63	PHEA:57,TRPA:69	--
C-10	-5.5	SERA:59	ALAA:61,ALAA:63	PHEA:57,TRPA:69	--
C-11	-5.3	--	ALAA:61,ALAA:63	TRPA:69	--
C-13	-5.7	--	ALAA:61,ALAA:63	TRPA:69	--

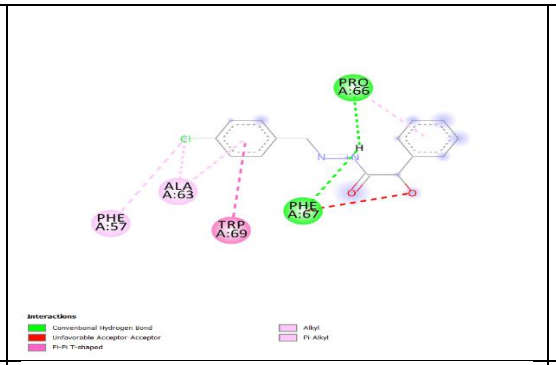
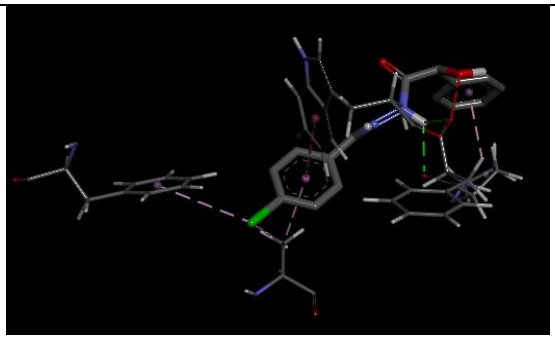
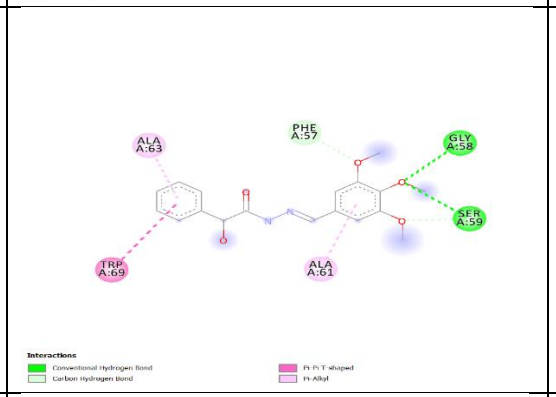
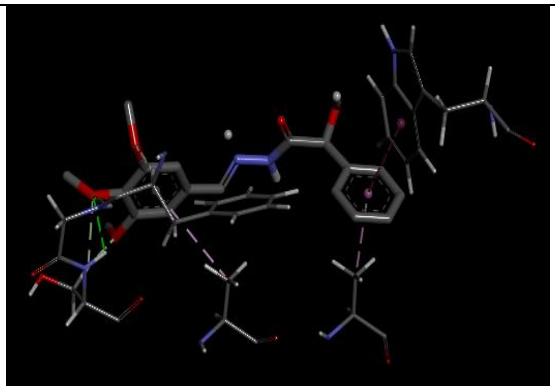
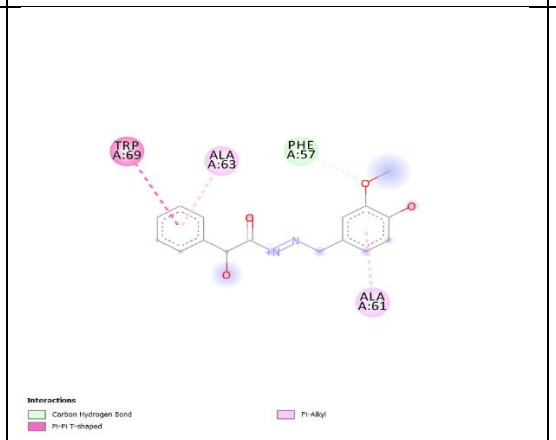
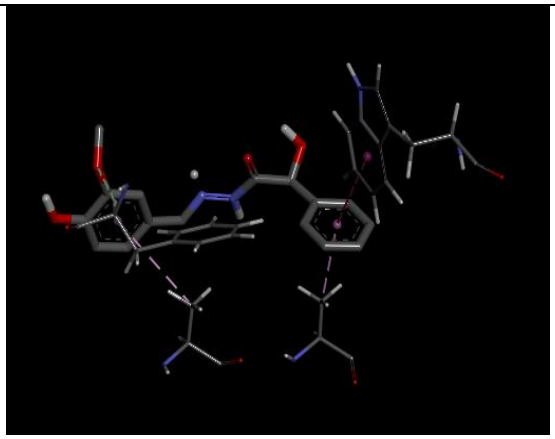
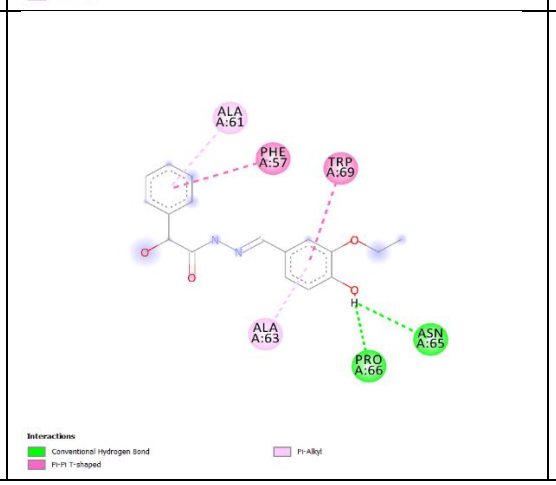
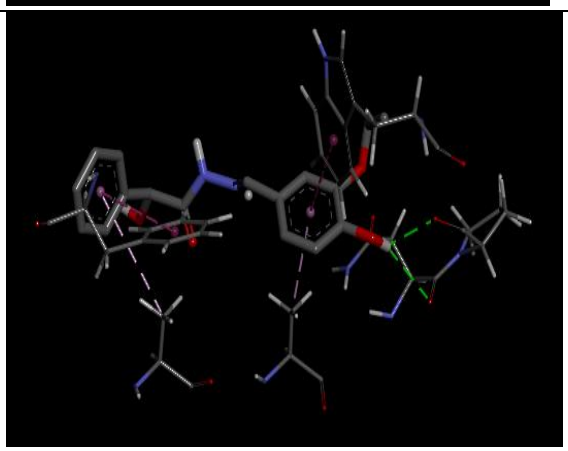
Table 3Interpretation of Docking ResultUsing Visualization Via Discovery Studio

Compound Code	2D structure	3D structure
C-1		
C-2		

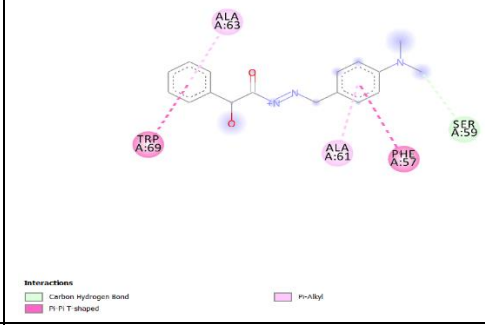
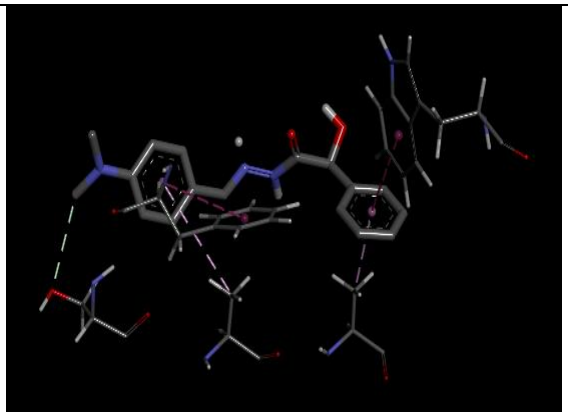
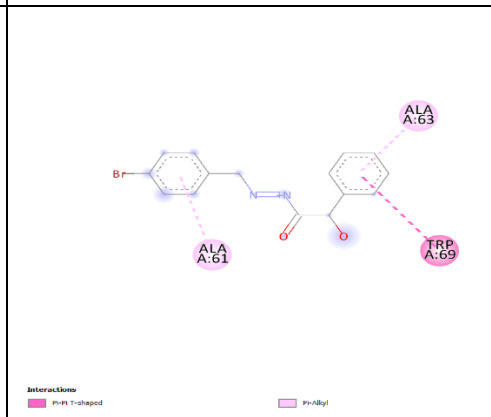
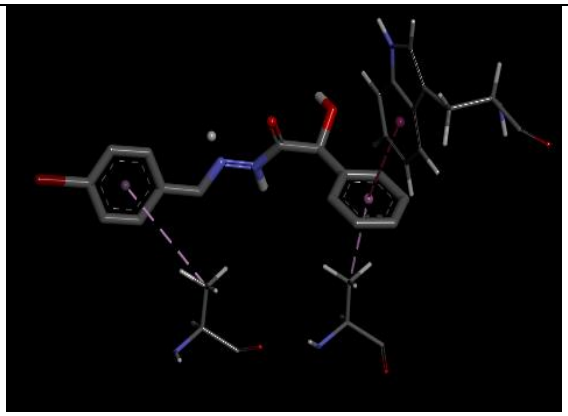
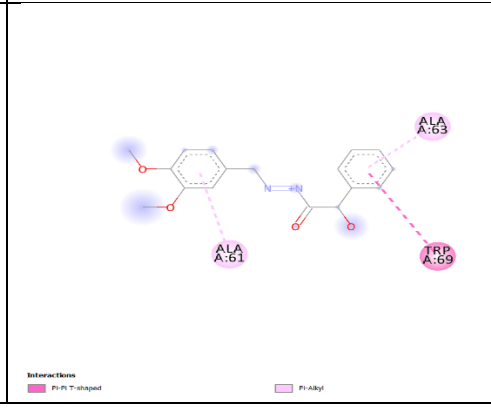
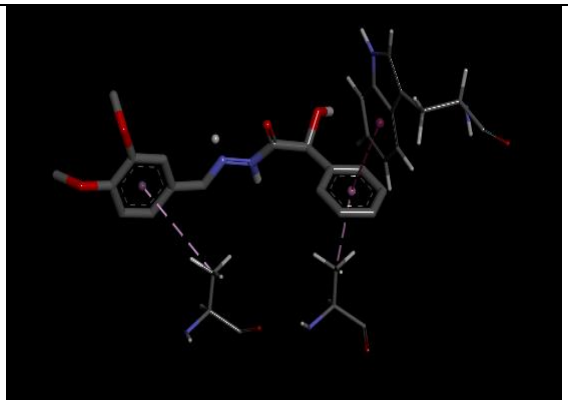
Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

C-3		
C-7		
C-12		
C-4		

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

C-5		
C-6		
C-8		
C-9		

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

C-10		
C-11		
C-13		

3.2 Physiochemical Characterizationof Synthesized Compounds

The synthesized compounds were subjected to physiochemical characterization and the results obtained are given as below in Table 4.

Table 4.Physicochemical Characteristics of Synthesized Compounds

COMPOUND CODE	Molecular Formula	Molecular Weight	% Yield
C-1	$C_{15}H_{13}N_3O_4$	299.28 g/mol	70

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

C-2	$C_{15}H_{13}N_3O_4$	299.28 g/mol	75
C-3	$C_{15}H_{12}N_2O_2Cl_2$	323.17 g/mol	73
C-7	$C_{15}H_{13}N_3O_4$	299.28 g/mol	79
C-12	$C_{15}H_{14}N_2O_3$	270.28 g/mol	78

3.3 Solubility Profile

Solubility of compounds checked in chloroform, methanol, water, ethanol and DMSO. The solubility profile of synthesized compounds were presented in table 5.

Table 5. Solubility profile of Synthesized Compounds

Compound Code	**Solvents**				
	Chloroform	**Methanol**	**Water**	**Ethanol**	**DMSO (Dimethyl Sulfoxide)**
C-1	Soluble	Soluble	Soluble	Insoluble	Soluble
C-2	Insoluble	Soluble	Insoluble	Insoluble	Soluble
C-3	Soluble	Soluble	Soluble	Soluble	Soluble
C-7	Soluble	Soluble	Soluble	Soluble	Soluble
C-12	Soluble	Soluble	Insoluble	Insoluble	Soluble

3.4 Thin Layer Chromatography (TLC)

Using Silica gel GF_{254} (type 60) pre-coated Aluminium sheets from Merck, thin-layer chromatography (TLC) was used to determine the completion of reactions and the purity of the compounds. The Rf value of synthesized compounds is depicted in Table 6 and the TLC plates are shown in figure 1.

Table 6. R_f values of Synthesized Compounds

S. No.	**Compound Code**	**R_f Value**
1	**C-1**	0.57
2	**C-2**	0.60
3	**C-3**	0.58
4	**C-7**	0.61
5	**C-12**	0.59

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

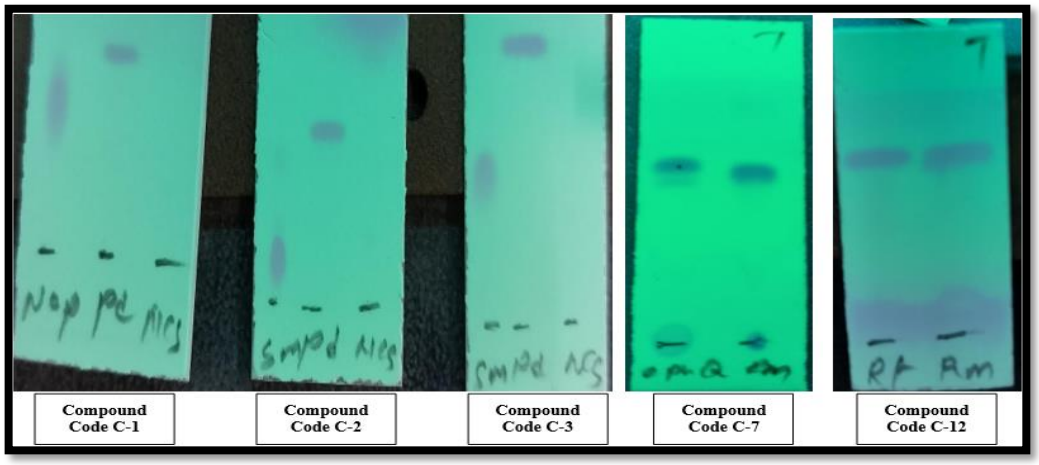
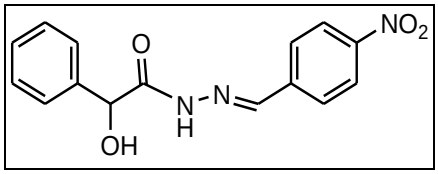


Figure 1. Photographic Representation of TLC

3.6Spectral Characterization of Synthesized Compounds

The spectral characterization of synthesized derivatives was conducted by constructing IR, NMR and MS spectra.IR(KBr) spectra υ,cm^{-1} were recorded. Out of 13 compounds, only 5 compounds have found to be suitable and hence, the spectral analysis was done for these five derivatives.

3.6.1 Spectral Characterization ofCompound Code C-1



(E)-N'-(4-nitrobenzylidene)-2-hydroxy-2-phenylacetohydrazide

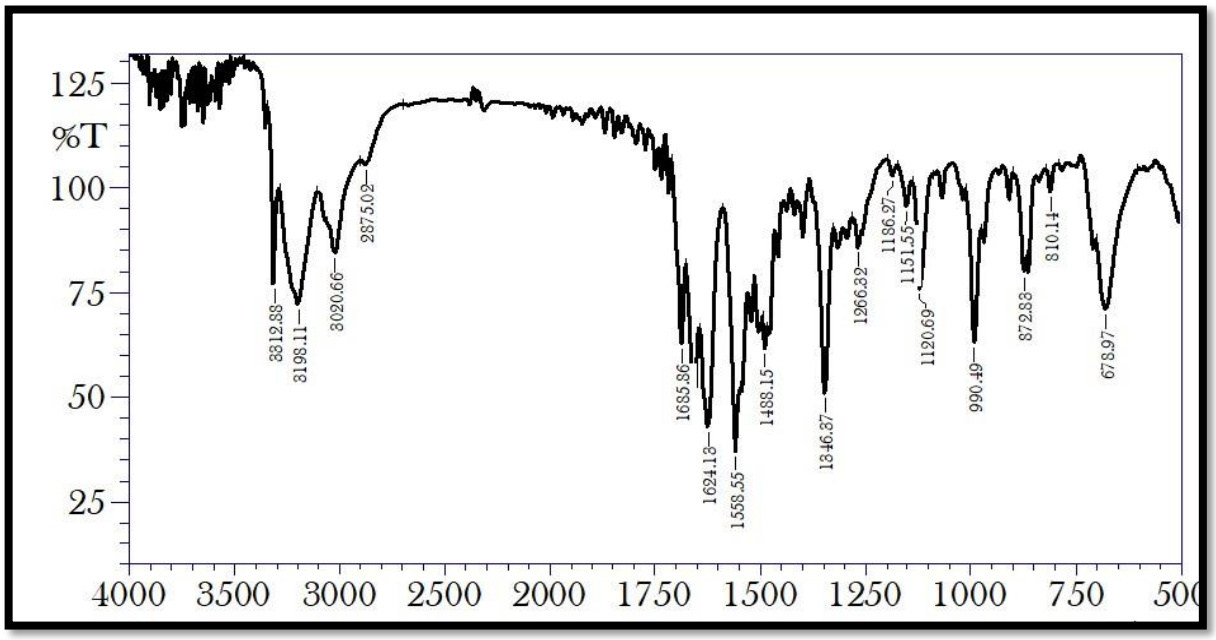


Figure 2. IR spectra of compound Code C-1

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.76 (s, 1H), 8.29 – 8.23 (m, 2H), 7.97 – 7.92 (m, 2H), 7.84 (s, 1H), 7.45 – 7.37 (m, 2H), 7.36 – 7.28 (m, 2H), 5.57 – 5.52 (m, 1H), 4.35 (d, *J* = 4.6 Hz, 1H).
^{13}C NMR (125 MHz, DMSO d_6) δ 168.75, 148.36, 146.18, 138.38, 136.44, 128.35, 128.20, 127.98, 126.24, 124.50, 71.98.

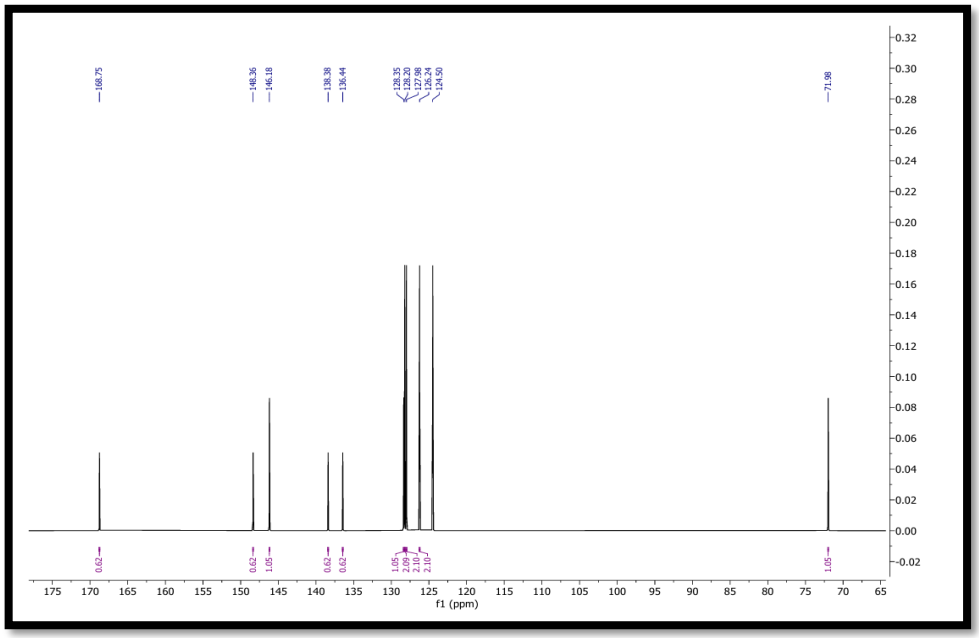


Figure 3.^{1}H NMR Spectra of compound codeC-1

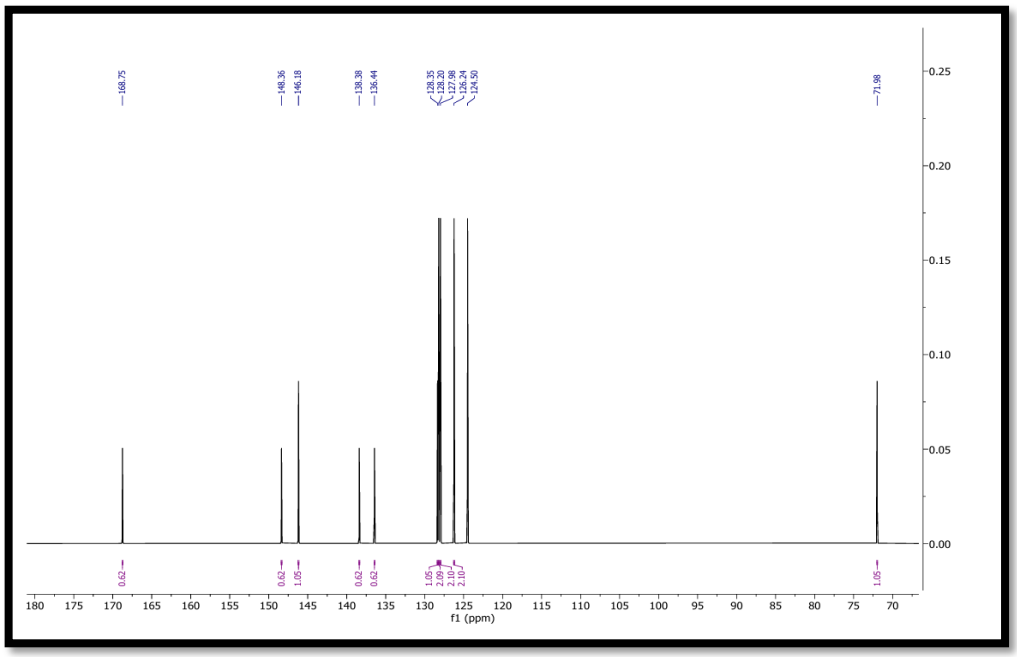


Figure 4.^{13}C NMR spectra of compound code C-1

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

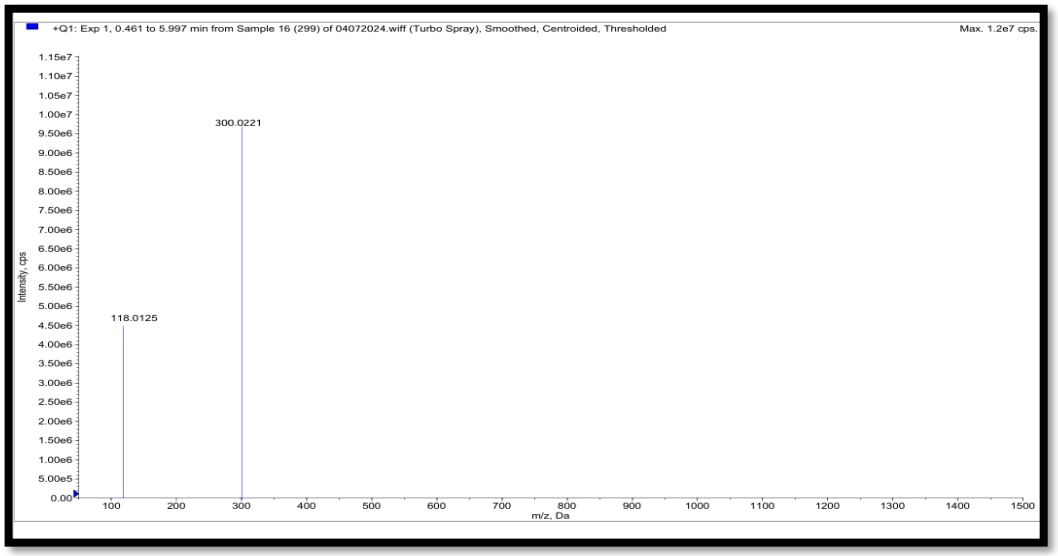
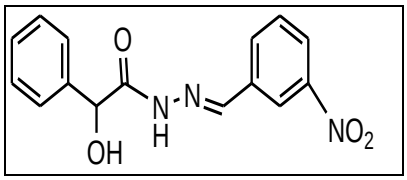


Figure 5.Mass spectra of compound code C-1

3.6.2 Spectral characterization ofcompound code C-2



(E)-N'-(3-nitrobenzylidene)-2-hydroxy-2-phenylacetohydrazide

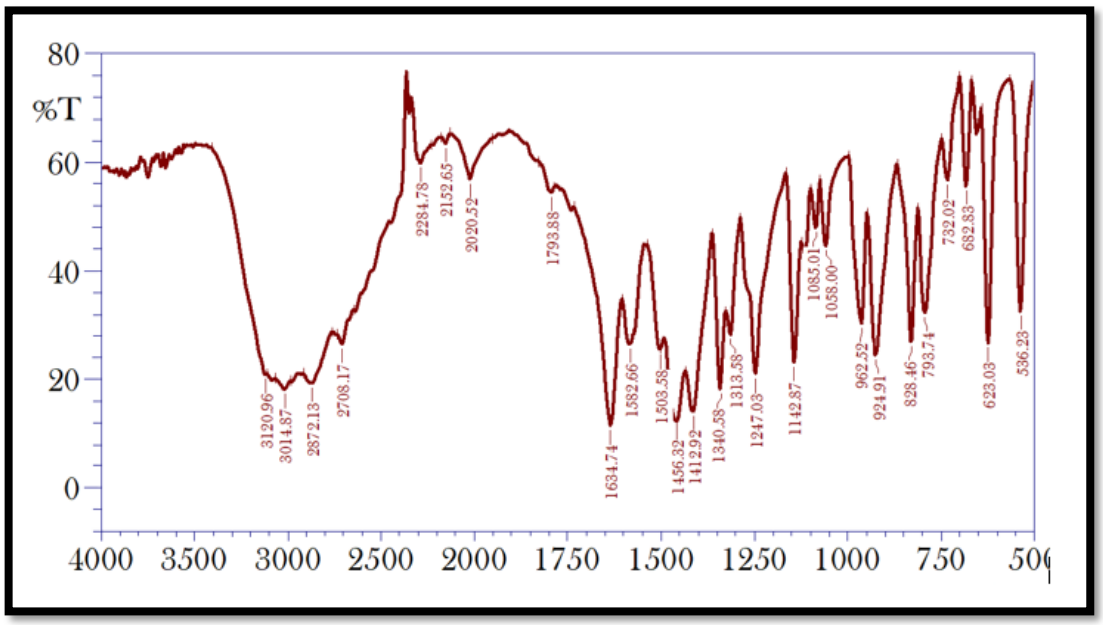


Figure 6. IR spectra of compound code C-2

^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.88 (s, 1H), 8.47 (t, *J* = 2.2 Hz, 1H), 8.26 (ddd, *J* = 8.4, 2.1, 1.2 Hz, 1H), 8.09 (s,

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

1H), 8.06 – 8.00 (m, 1H), 7.70 (dd, *J* = 8.4, 7.3 Hz, 1H), 7.45 – 7.37 (m, 2H), 7.36 – 7.27 (m, 2H), 5.57 – 5.52 (m, 1H), 4.35 (d, *J* = 4.6 Hz, 1H).

^{13}C NMR (125 MHz, DMSO *d*$_6$) δ 168.73, 147.41, 145.30, 138.38, 134.80, 132.03, 129.55, 128.35, 128.20, 127.98, 124.21, 121.78, 71.98.

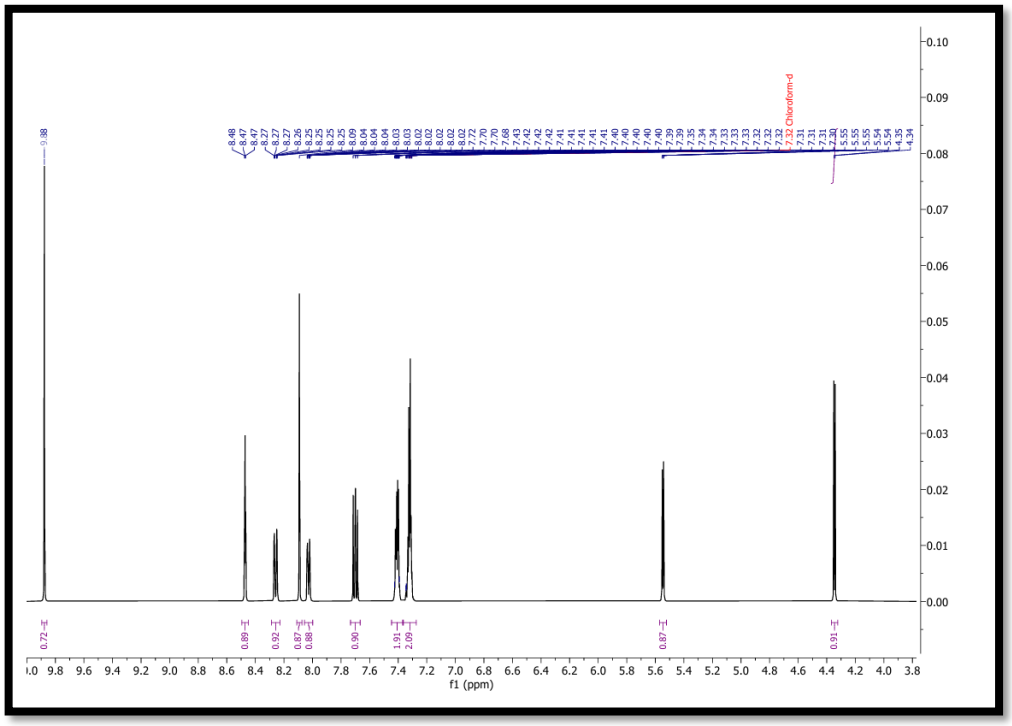


Figure 7. ^{1}H NMR spectra of compound code C-2

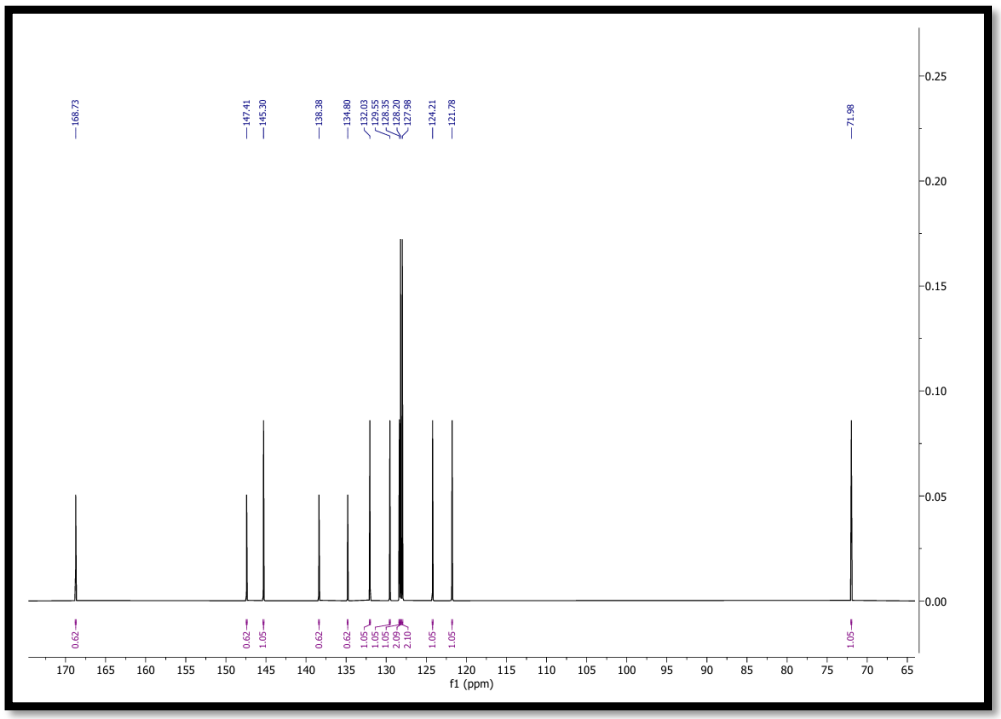


Figure 8.^{13}C NMR spectra of compound code C-2

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

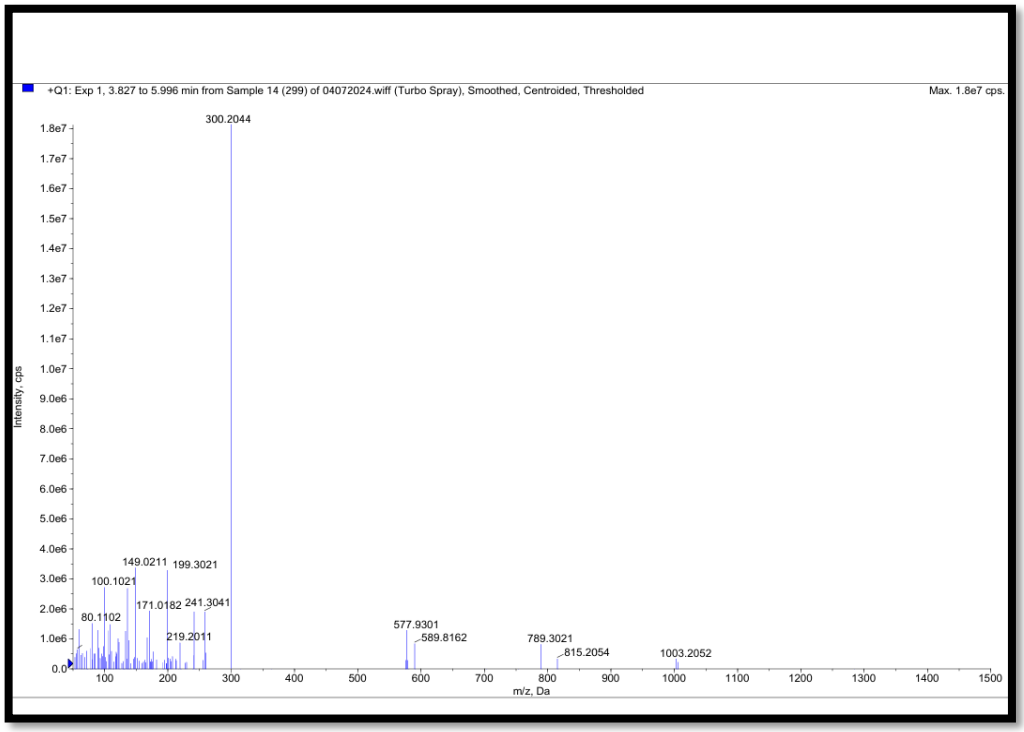
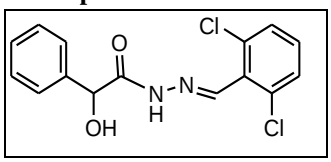


Figure 9. Mass spectra of compound code C-2

3.6.3 Spectral Characterization of Compound CodeC-3



(E)-N'-(2,6-dichlorobenzylidene)-2-hydroxy-2-phenylacetohydrazide

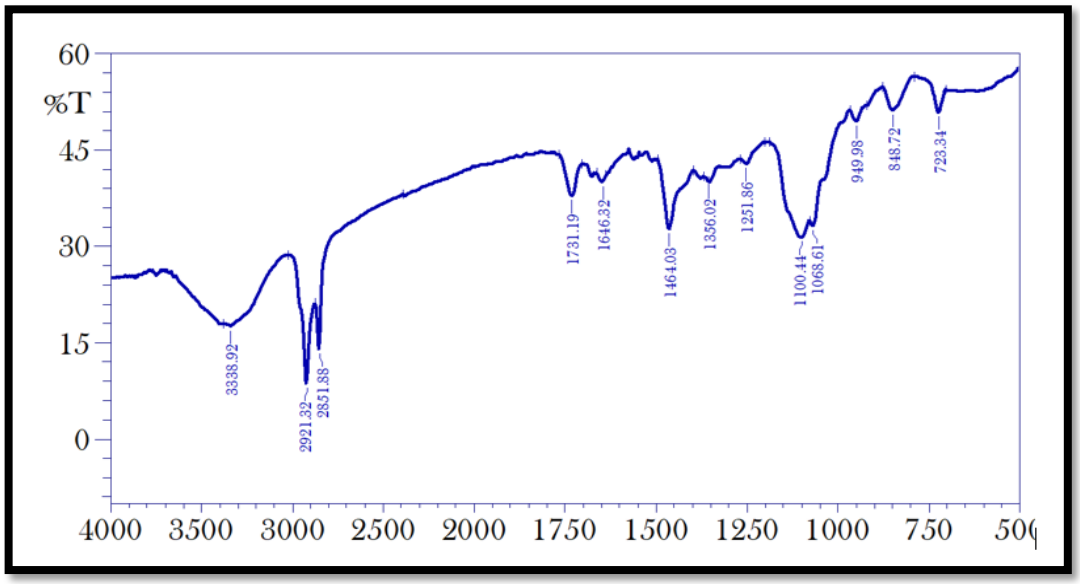


Figure 10. IR spectra of compound code C-3

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.76 (s, 1H), 7.92 (s, 1H), 7.59 – 7.54 (m, 2H), 7.45 – 7.37 (m, 2H), 7.36 – 7.28 (m, 2H), 6.75 – 6.69 (m, 2H), 5.55 (dd, *J* = 4.5, 0.8 Hz, 1H), 4.35 (d, *J* = 4.6 Hz, 1H), 2.92 (s, 4H).
^{13}C NMR (150 MHz, DMSO *d6*) δ 168.75, 152.08, 146.24, 138.38, 128.35, 128.20, 127.98, 127.20, 124.45, 111.94, 71.98, 40.31.

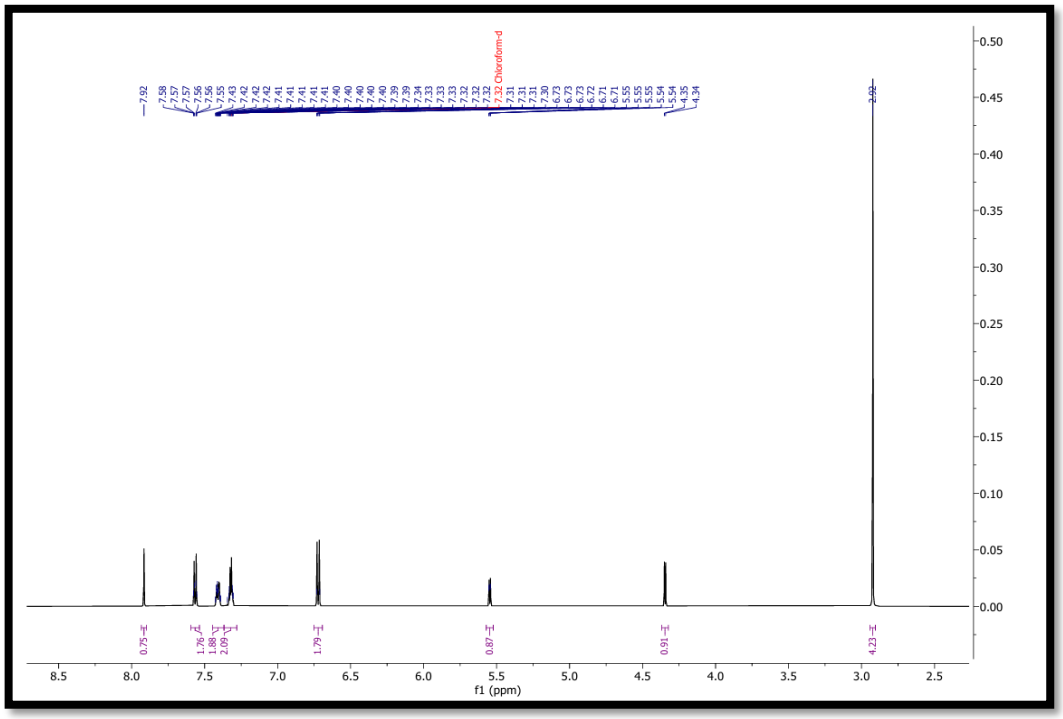


Figure 11.^{1}H NMR spectra of compound code C-3

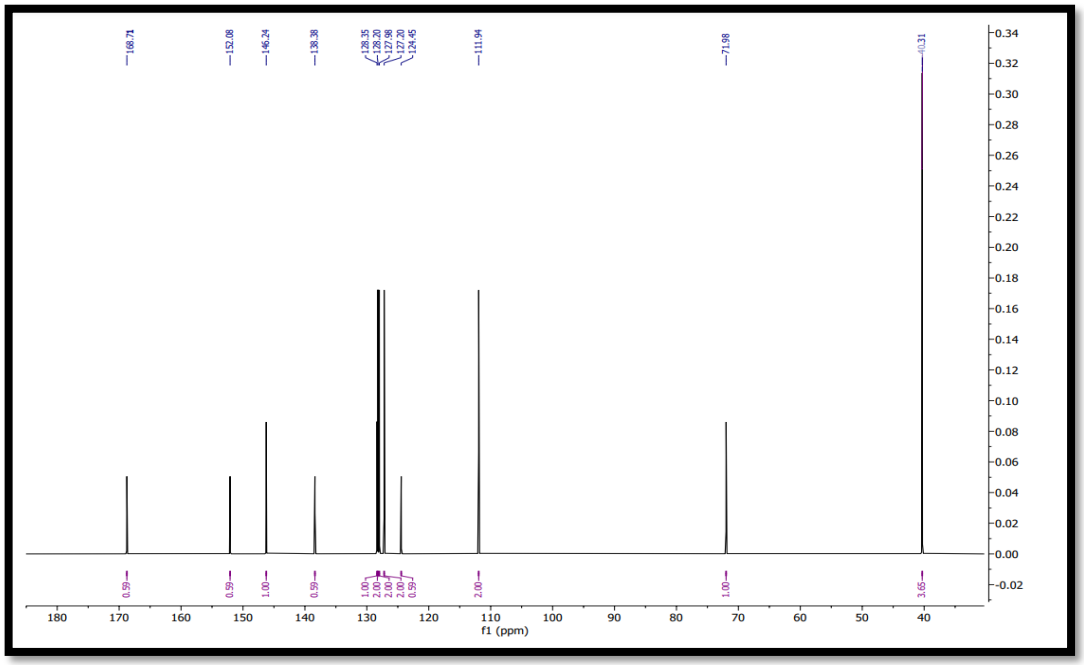


Figure 12.^{13}C NMR spectra of compound codeC-3

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

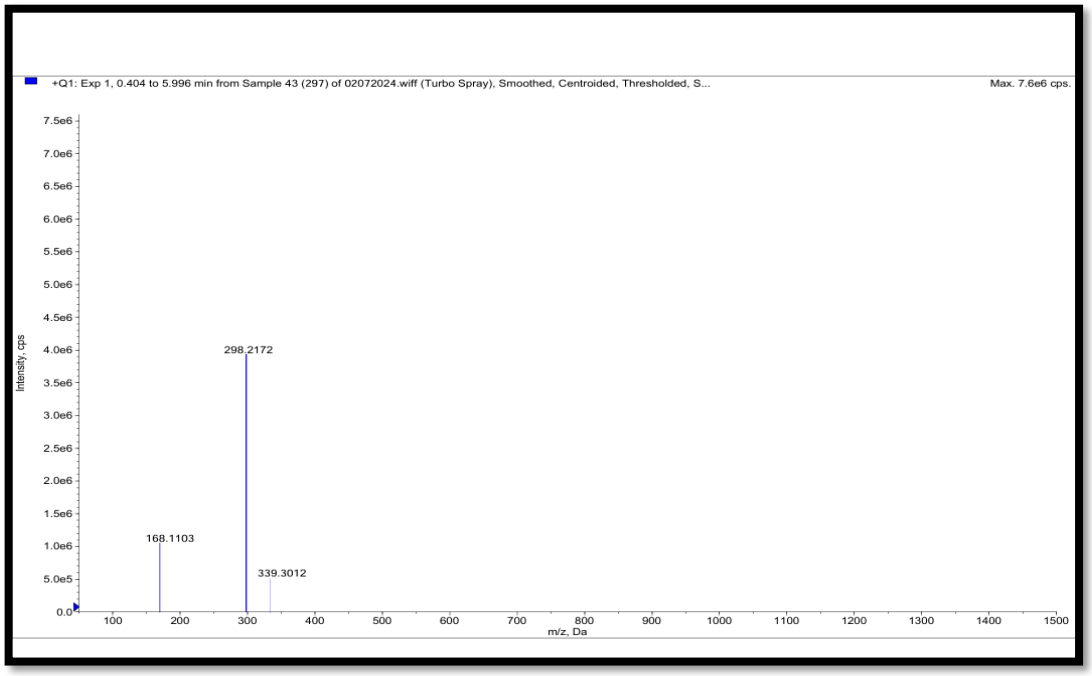
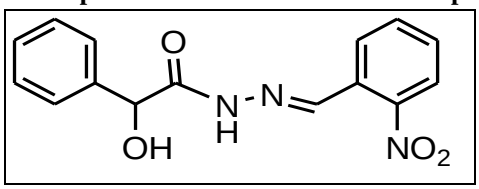


Figure 13.Mass spectra of compound code C-3

3.6.4 Spectral Characterization of Compound Code C-7



(E)-N'-(2-nitrobenzylidene)-2-hydroxy-2-phenylacetohydrazide

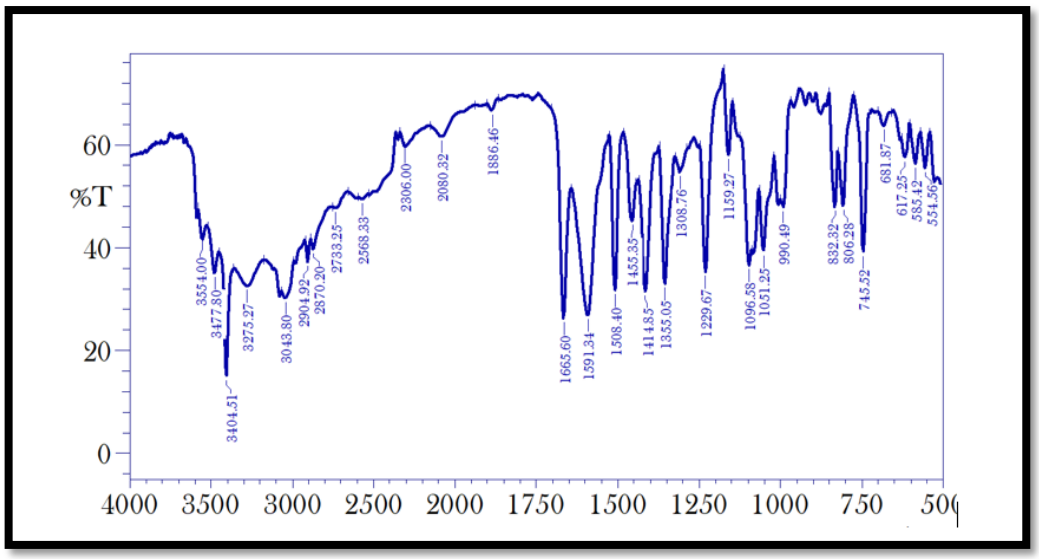


Figure 14. IR spectra of compound code C-7

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.90 (s, 1H), 8.43 (s, 1H), 7.66 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.50 (dd, *J* = 6.9, 1.6 Hz, 1H), 7.44 – 7.37 (m, 3H), 7.36 – 7.28 (m, 3H), 5.55 (dd, *J* = 4.5, 0.8 Hz, 1H), 4.35 (d, *J* = 4.6 Hz, 1H).
^{13}C NMR (125 MHz, DMSO d_6) δ 168.80, 143.87, 138.38, 134.15, 132.16, 131.84, 128.92, 128.68, 128.46, 128.35, 128.20, 127.98, 71.98.

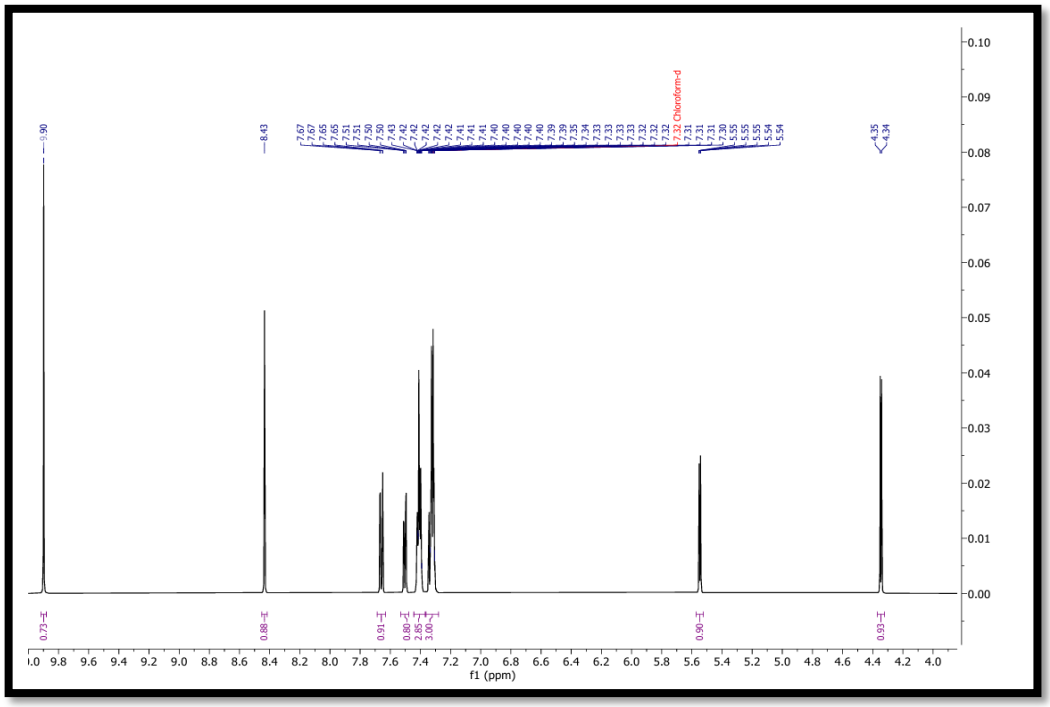


Figure15.^{1}H NMR spectra of compound code C-7

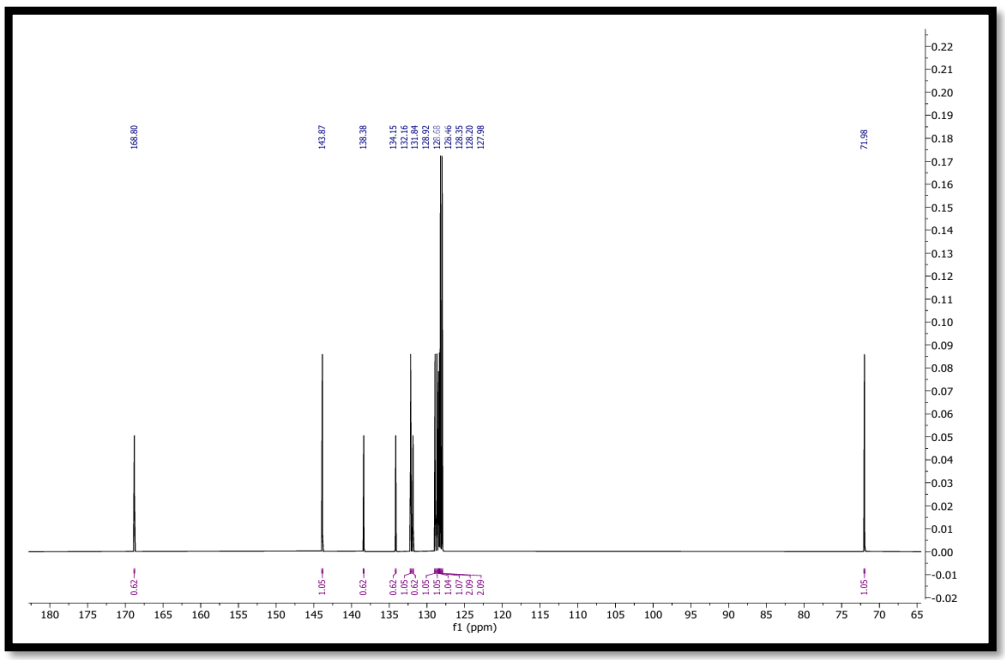


Figure 16.^{13}C NMR spectra of compound code C-7

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

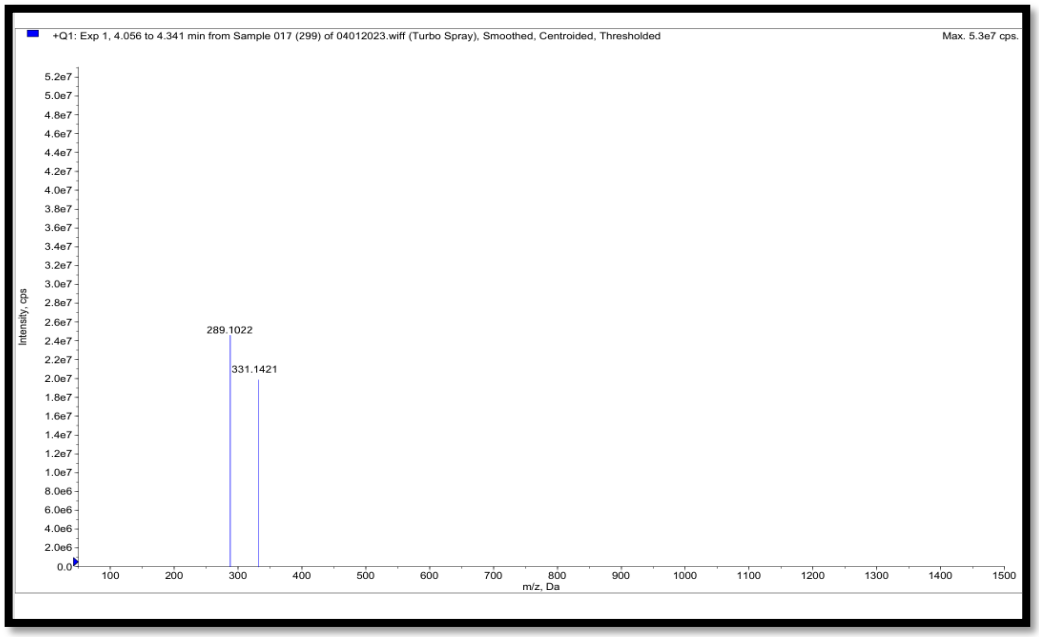
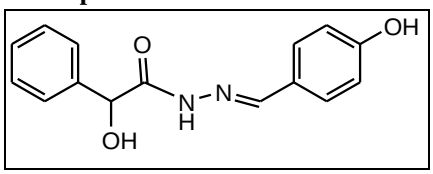


Figure 17. Mass spectra of compound code C-7

3.6.5 Spectral Characterization of Compound CodeC-12



(E)-N'-(4-hydroxybenzylidene)-2-hydroxy-2-phenylacetohydrazide

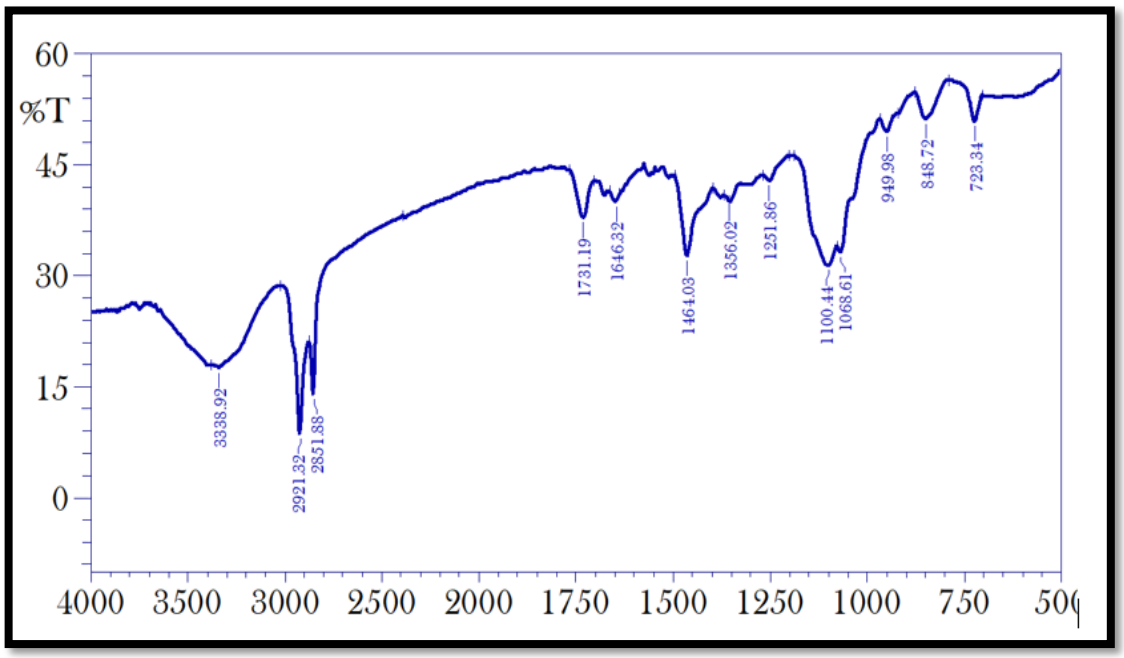


Figure 18. IR spectra of compound code C-12

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.72 (s, 1H), 8.27 (s, 1H), 7.45 – 7.37 (m, 2H), 7.36 – 7.28 (m, 3H), 7.24 – 7.16 (m, 2H), 6.88 (d, *J* = 8.7 Hz, 1H), 5.55 (dd, *J* = 4.5, 0.8 Hz, 1H), 4.35 (d, *J* = 4.6 Hz, 1H), 4.11 (q, *J* = 6.2 Hz, 2H), 1.42 (d, *J* = 12.6 Hz, 1H).
^{13}C NMR (125 MHz, DMSO d_6) δ 168.75, 150.59, 147.98, 145.12, 138.38, 128.35, 128.20, 127.98, 126.08, 121.69, 115.14, 111.27, 71.98, 64.74, 14.73.

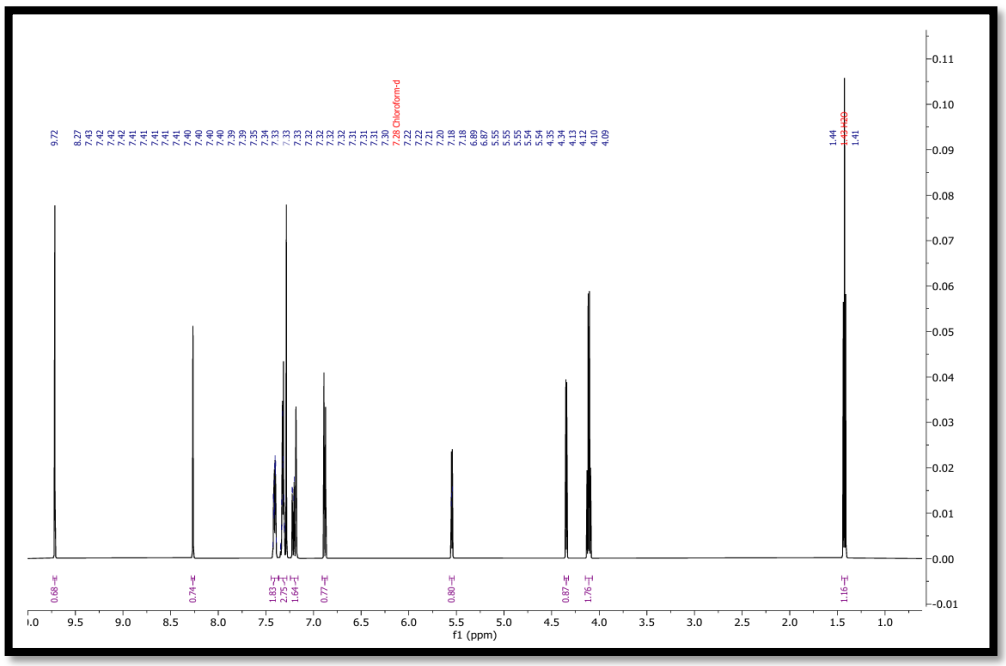


Figure 19.^{1}H NMR spectra of compound code C-12

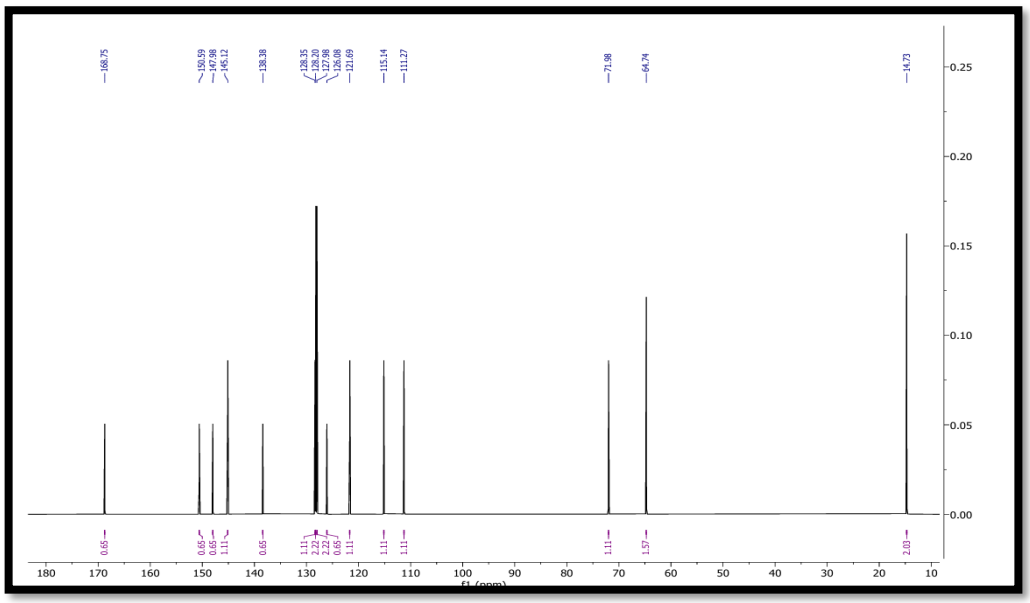


Figure 20.^{13}C NMR spectra of compound code C-12

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

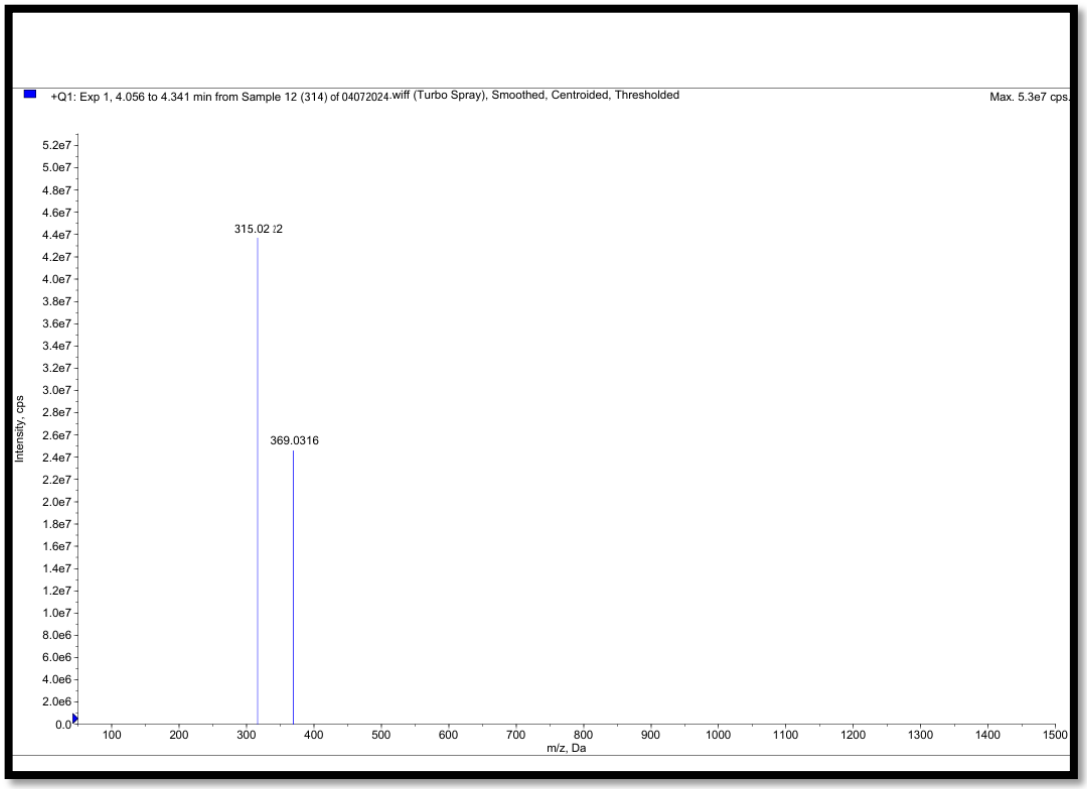


Figure 21. Mass spectra of compound code C-12

3.7In-vitro Evaluation of Antioxidant Activity

Synthesized compounds (C-1, C-2, C-3, C-7&C-12) were used to analyse the antioxidant activity using DPPH assay and the results are shown in table (7-13) and figure (22-28).

Table 7.DPPH Radical Scavenging Activity of Compound Code C-1

% Radical Scavenging Activity of Compound Code C-1		
Concentration (µg/mL)	**%RSA**	**IC_{50}(µg/mL)**
20	21.49	Log IC_{50}= 2.35
40	29.64	
60	36.59	
80	44.82	
100	52.94	IC_{50}=223.87 µg/mL
200	60.94	
300	66.92	
400	74.59	

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

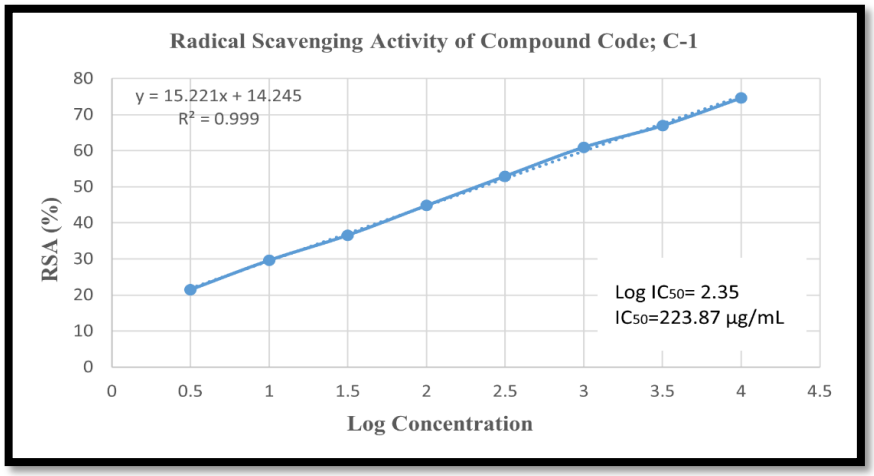


Figure 22. %RSA & IC_{50} Value of Compound Code C-1

Table 8. DPPH Radical Scavenging Activity of Compound Code C-2

% Radical Scavenging Activity Compound Code C-2		
Concentration (µg/mL)	**%RSA**	**IC_{50}(µg/mL)**
20	11.48	Log IC_{50}= 2.29
40	23.54	
60	32.98	
80	44.68	
100	54.74	IC_{50}=194.98 µg/mL
200	64.82	
300	11.48	
400	23.54	

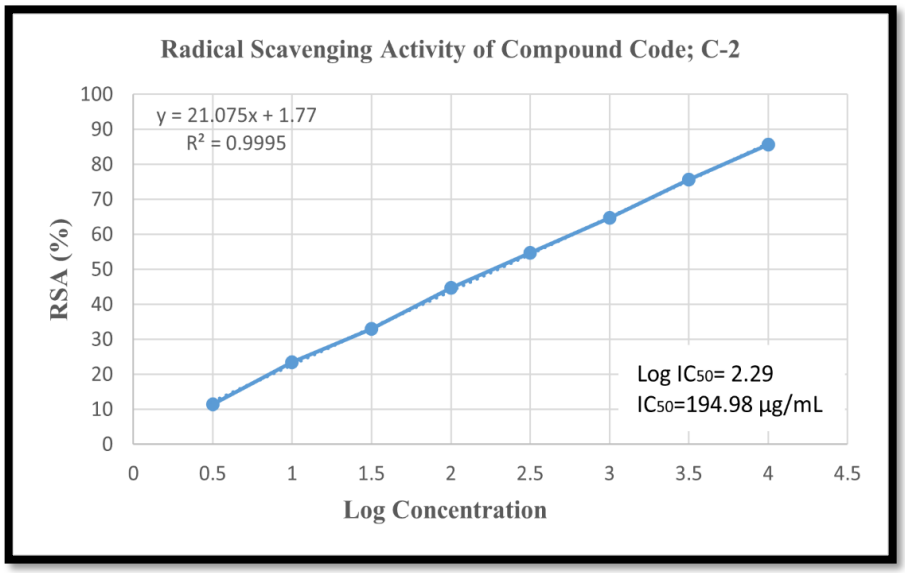


Figure 23. %RSA & IC_{50} Value of Compound Code C-2

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

Table 9. DPPH Radical Scavenging Activity of Compound Code C-3

% Radical Scavenging Activity Compound Code C-3		
Concentration (µg/mL)	**%RSA**	**IC_{50}(µg/mL)**
20	12.49	Log IC_{50}= 2.21
40	25.94	
60	35.57	
80	44.52	
100	56.48	IC_{50}=162.18 µg/mL
200	67.95	
300	77.12	
400	86.49	

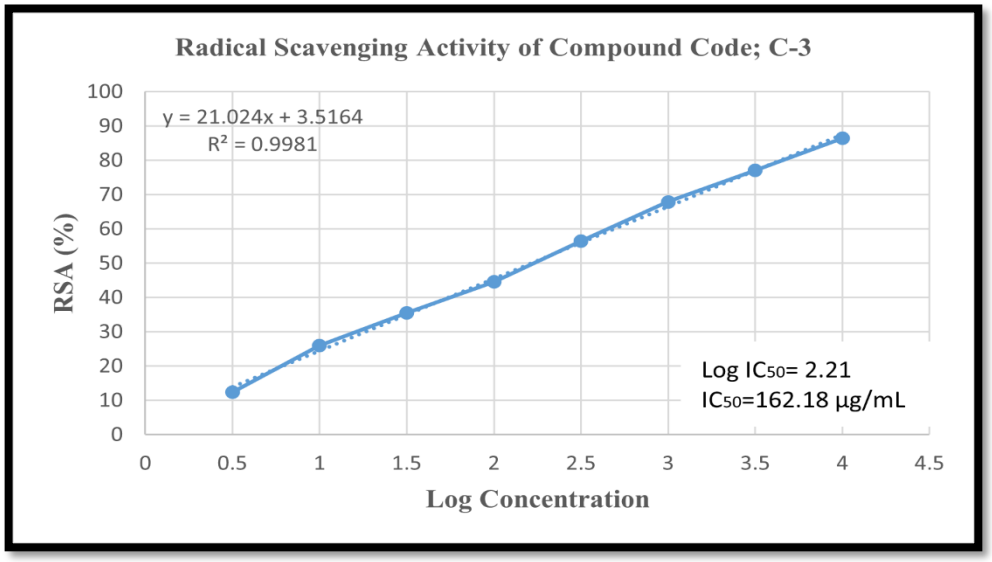


Figure 24. %RSA & IC_{50} Value of Compound Code C-3

Table 10. DPPH Radical Scavenging Activity of Compound CodeC-7

% Radical Scavenging Activity Compound Code C-7		
Concentration (µg/mL)	**%RSA**	**IC_{50}(µg/mL)**
20	20.59	Log IC_{50}= 1.91
40	32.23	
60	41.36	
80	52.64	
100	62.38	IC_{50}=81.28 µg/mL
200	72.59	
300	82.56	
400	91.45	

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

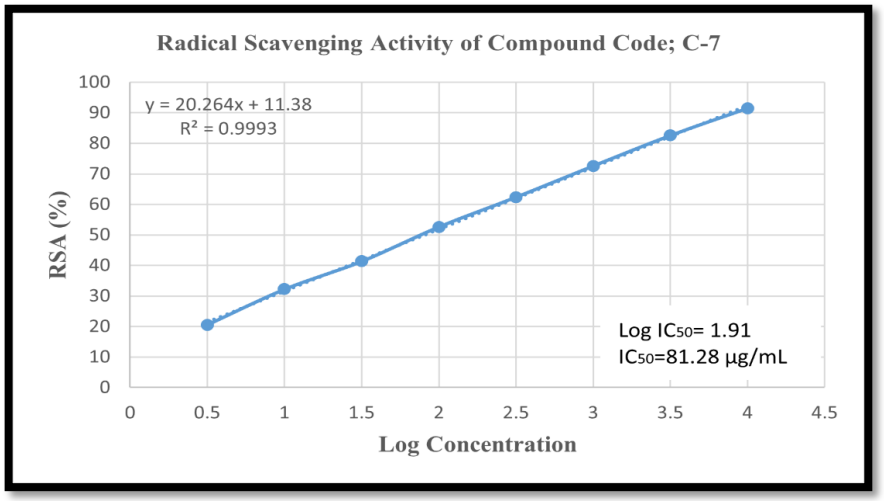


Figure 25. %RSA & IC_{50} Value of Compound Code C-7

Table 11. DPPH Radical Scavenging Activity of Compound Code C-12

% Radical Scavenging Activity Compound Code C-12		
Concentration (µg/mL)	**%RSA**	**IC_{50}(µg/mL)**
20	19.49	Log IC_{50}= 2.49
40	27.64	
60	34.68	
80	42.33	
100	50.49	IC_{50}= 309.03 µg/mL
200	58.73	
300	64.42	
400	72.94	

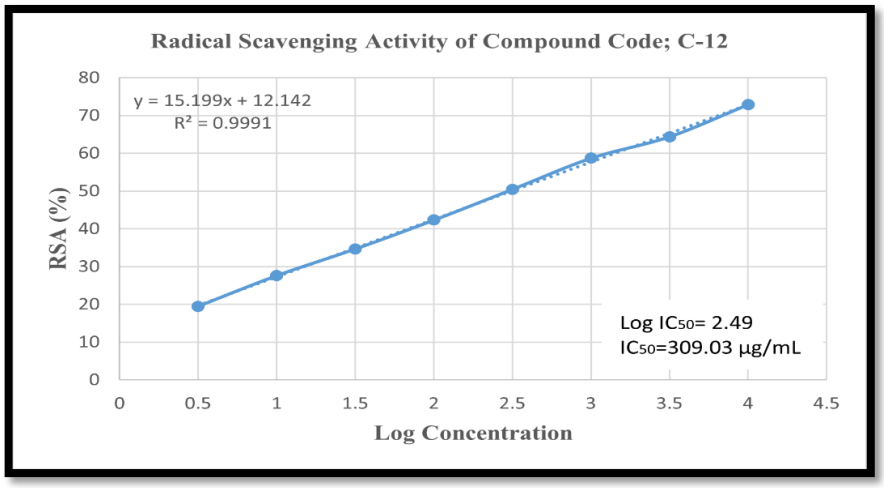


Figure 26. %RSA & IC_{50} Value of Compound Code C-12

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

Table 12. DPPH Radical Scavenging Activity of Ascorbic Acid

% Radical Scavenging Activity Ascorbic Acid		
Concentration (µg/mL)	**%RSA**	**IC_{50}(µg/mL)**
20	33.49	Log IC_{50}= 1.48
40	40.16	
60	49.25	
80	58.49	
100	69.42	IC_{50}= 30.20 µg/mL
200	78.55	
300	86.65	
400	94.22	

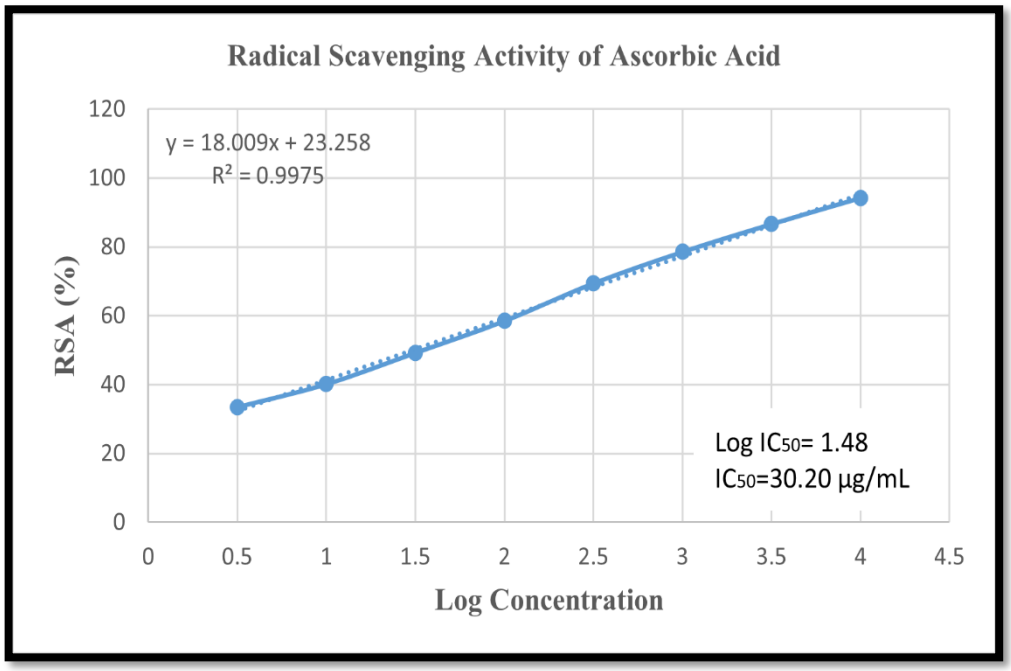


Figure 27. %RSA & IC_{50} Value of Ascorbic Acid

Table 13. Comparative DPPH Radical Scavenging Activity of Ascorbic Acid and Synthesized Compounds

Concentration (µg/ml)	**(%) RSA of C-1**	**(%) RSA of C-2**	**(%) RSA of C-3**	**(%) RSA of C-7**	**(%) RSA of C-12**	**(%) RSA of Ascorbic Acid**

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

20	21.49	11.48	12.49	20.59	19.49	33.49
40	29.64	23.54	25.94	32.23	27.64	40.16
60	36.59	32.98	35.57	41.36	34.68	49.25
80	44.82	44.68	44.52	52.64	42.33	58.49
100	52.94	54.74	56.48	62.38	50.49	69.42
200	60.94	64.82	67.95	72.59	58.73	78.55
400	66.92	75.63	77.12	82.56	64.42	86.65
800	74.59	85.64	86.49	91.45	72.94	94.22
IC_{50}(µg/mL)	223.87	194.98	162.18	81.28	309.03	30.02

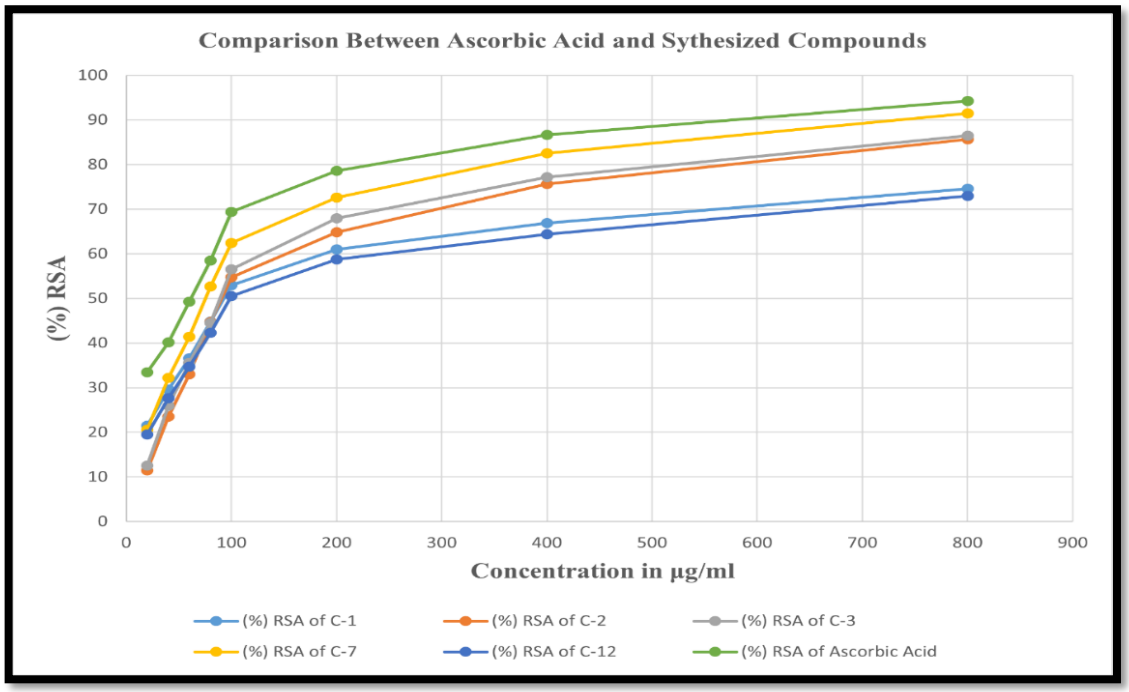


Figure 28. DPPH Radical Scavenging Activity of Synthesized Compounds in Comparison with Ascorbic Acid

3.8In-vitro Evaluation of Antibacterial Activity

The antibacterial activity of synthesized compoundswas determined using the agar disc diffusion assay and the zone of inhibition are shown in table 14 ang figure 29.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

Table 14. Comparative Antibacterial Activity of Ciprofloxacin and Synthesized Compounds

Compounds	**Inhibition Zone Diameter/cm**		
	C-2 (A1)	**C-3 (A2)**	**C-7 (A3)**
Staphylococcus aureus (Gram +) **33591**	1.8 cm	2.1 cm	2.0 cm
Escherichia coli (Gram -) **11775**	1.9 cm	1.7 cm	2.1 cm
Ciprofloxacin Standard	1.9 cm		

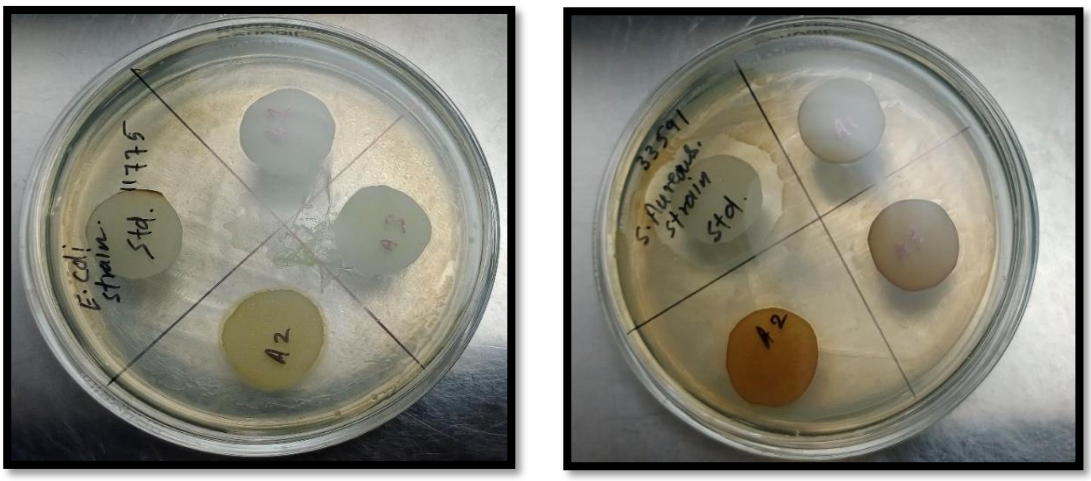


Figure 29.Zone of inhibition of Compound Code C-3, C-7 and C-12 Against Bacteria After 24 H of Incubation at 37 °C

4.CONCLUSION

Derivatives of 2-hydroxy benzyl hydrazide have attracted interest because of their possible medical uses, which include antibacterial and antioxidant capabilities. There are good chances to create novel bioactive chemicals through the design and synthesis of these congeners. Predicting their biological efficacy and comprehending their molecular interactions are made easier by in-silico research. This work aims to synthesize new derivatives of 2-hydroxy benzyl hydrazide and evaluate their antibacterial and antioxidant properties. Using PyRx, molecular docking was carried out. Docking results showed that five derivatives (compound codes C-1, C-2, C-3, C-7, and C-12) out of thirteen had good affinity scores. These five derivatives were then synthesized and subjected to physicochemical characterization, which included measurements of their solubility, melting point, and R_f value. Additionally, they underwent spectral characterization, which included infrared, mass, and nuclear magnetic resonance spectroscopy. Additionally, *in vitro* antioxidant and antimicrobial properties were examined.

By employing the DPPH assay to measure in-vitro antioxidant activity, the IC_{50} Value of ascorbic acid (30.20 µg/mL) and synthesized compounds (C-1; 223.87, C-2; 85.64, C-3; 162.18, C-7; 81.28 and C-12; 309.03µg/mL) which are calculated by linear regression analysis. the radical scavenging activity (% RSA) of

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

compound code; C-2=85.64%, C-3=86.49% and C-7=91.45% was found better compared to reference drug i.e., ascorbic acid=94.22. On the basis of antioxidant efficacy of compound code; C-2, C-3 and C-7, these compounds are further evaluated for in-vitro antimicrobial activity using agar disc diffusion assay against gram positive and gram-negative bacteria, *Staphylococcus aureus* and *Escherichia coli*,using the standard drug ciprofloxacin. The zone of inhibition of C-7 compound against both gram-positive and gram-negative bacteria was measured to be 2.0 cm and 2.1 cm, respectively. This is larger than the standard drug's (ciprofloxacin-1.9 cm) zone of inhibition.

According to the results of this study, the derivatives of 2-hydroxy benzyl hydrazide was effectively synthesized and shown magnificent antibacterial and antioxidant activity compared to the standard medication.

REFERENCE

1. Ozdemir, A., Turan-Zitouni, G., Kaplancikli, Z. A., & Tunali, Y. (2009). Synthesis and biological activities of new hydrazide derivatives. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *24*(3), 825-831.
2. Popiołek, L. (2017). Hydrazide–hydrazones as potential antimicrobial agents: overview of the literature since 2010. *Medicinal Chemistry Research*, *26*, 287-301.
3. Moussa, Z., Al-Mamary, M., Al-Juhani, S., & Ahmed, S. A. (2020). Preparation and biological assessment of some aromatic hydrazones derived from hydrazides of phenolic acids and aromatic aldehydes. *Heliyon*, *6*(9).
4. Angelova, V., Karabeliov, V., Andreeva-Gateva, P. A., & Tchekalarova, J. (2016). Recent developments of hydrazide/hydrazone derivatives and their analogs as anticonvulsant agents in animal models. *Drug development research*, *77*(7), 379-392.
5. Sedová, P., Buffa, R., Silhár, P., Kovářová, L., Vágnerová, H., Bednařík, J., ... & Velebný, V. (2019). The effect of hydrazide linkers on hyaluronan hydrazone hydrogels. *Carbohydrate polymers*, *216*, 63-71.
6. Teneva, Y., Simeonova, R., Valcheva, V., & Angelova, V. T. (2023). Recent advances in anti-tuberculosis drug discovery based on hydrazide–hydrazone and thiadiazole derivatives targeting inhA. *Pharmaceuticals*, *16*(4), 484.
7. Nurkenov, O. A., Ibrayev, M. K., Satpaeva Zh, B., Dauletzhanova Zh, T., & Seilkhanov, T. M. (2018). Synthesis and study of antioxidant activity of hydrazone and thiosemicarbazidebased on N-morpholinoacetic acid hydrazide. *Bulletin of the Karaganda University. Chemistry series*, *1*(89), 22-26.
8. Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., ... & Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Frontiers in physiology*, *11*, 694.
9. Boulebd, H., Zine, Y., Khodja, I. A., Mermer, A., Demir, A., & Debache, A. (2022). Synthesis and radical scavenging activity of new phenolic hydrazone/hydrazide derivatives: Experimental and theoretical studies. *Journal of Molecular Structure*, *1249*, 131546.
10. Narang, R., Narasimhan, B., & Sharma, S. (2012). A review on biological activities and chemical synthesis of hydrazide derivatives. *Current medicinal chemistry*, *19*(4), 569-612.
11. Popiołek, L., & Biernasiuk, A. (2016). Design, synthesis, and in vitro antimicrobial activity of hydrazide–hydrazones of 2-substituted acetic acid. *Chemical biology & drug design*, *88*(6), 873-883.
12. Popiołek, L., & Biernasiuk, A. (2017). Synthesis and investigation of antimicrobial activities of nitrofurazone analogues containing hydrazide-hydrazone moiety. *Saudi Pharmaceutical Journal*, *25*(7), 1097-1102.
13. Kumar, D., Judge, V., Narang, R., Sangwan, S., De Clercq, E., Balzarini, J., & Narasimhan, B. (2010). Benzylidene/2-chlorobenzylidene hydrazides: Synthesis, antimicrobial activity, QSAR studies and antiviral evaluation. *European Journal of Medicinal Chemistry*, *45*(7), 2806-2816.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(6), 562-593 | ISSN:2251-6727

14. Jabalia, N., Kumar, A., Kumar, V., & Rani, R. (2021). In silico approach in drug design and drug discovery: an update. *Innovations and Implementations of Computer Aided Drug Discovery Strategies in Rational Drug Design*, 245-271.
15. Molecular docking studies, for instance, can simulate the binding of 2-hydroxy benzyl hydrazide congeners to target proteins, providing information on their potential mechanism of action.
16. Dharmendra Kumar, Pramod Kumar Sharma, 2021. Anthelmintic, antimicrobial and antioxidant activity of different part of indian origin Salvadora persica. Jour. of Med. P'ceutical & Alli. Sci. V 10 - I 3, 1088 P-2728-2736. DOI: 10.22270/jmpas.V10I3.1088
17. Kumar, D., & Sharma, P. K. (2023). Wound Healing, Anti-inflammatory and Antioxidant Potential of Quercetin Loaded Banana Starch Nanoparticles. *Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents)*, *22*(4), 230-235.
18. Tiwari, C., Tomer, J., & Kumar, D. (2024). Liposomal Drug Delivery: Progress, Clinical Outlook, and Ongoing Challenges. *Recent Advances in Drug Delivery and Formulation: Formerly Recent Patents on Drug Delivery & Formulation*, *18*(3), 157-169.