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Synthesis and Evaluation of *In-Vitro* Anti-Diabetic Potential of Novel Derivatives of Semicarbazide

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ABSTRACT:

Semicarbazide derivatives have garnered a lot of interest because of their wide variety of biological effects and function as building blocks in numerous families of important pharmacological molecules. Type 1 diabetes usually affects kids or teenagers, while type 2 diabetes mellitus usually affects middle-aged and older adults for long period of time due to poor food and lifestyle choices. The present research was based on the synthesis and evaluation of *in-vitro* anti-diabetic potential of novel derivatives of semicarbazide.To design, synthesize the novel derivatives of semicarbazide. The novel derivatives of semicarbazide were synthesized and characterized based on physicochemical properties i.e., melting point, Rf value, FTIR, NMR and Mass spectroscopy methods. They were also evaluated for the in-vitro anti-diabetic potential of synthesized novel derivatives i.e., estimation of DPPH Radical Scavenging Assay and Estimation of α-amylase. In results, the Rf values for C1 to C6 were found to be 0.65, 0.67, 0.71, 0.69, 0.66, and 0.72, respectively. The α-Amylase inhibition is an important feature for estimation of anti-diabetic potential. At 800µg/ml, the α-Amylase inhibition was estimated as 45.34±0.11%, 63.45±0.21%, and 91.35±0.46%, in water, semi-carbazide derivatives and acarbose, respectively. In conclusion, all the synthesized semi-carbazide derivatives demonstrated s significant antioxidant activity and α-amylase inhibition when compared with control. On the basis of physicochemical parameters i.e., physical state, melting point, FTIR, NMR, mass studies, the derivatives were confirmed for the novel derivatives of semi-carbazides. Further research may carried-out to confirm its mode of action and optimum doses required in hypoglycemic action.

Keywords: Synthesis, Semi-Carbazide, Anti-Diabetic Potential, A-Amylase, DPPH

1. Introduction

The word comes from the Greek "diabetes," which means "siphon" or "to pass through," and the Latin word "mellitus," which means "sweet[1][2]. Looking through the historicaldocuments, it appears that Apollonius of Memphis coined the term "diabetes" in the year 250–300 BC [3]. Significant progress has been made throughout the years, and various innovations and efficient management techniques have been created [4]. Both types are usually brought on by decreased insulin function or production. Type 1 diabetes usually affects kids or teenagers, while type 2 diabetes mellitus usually affects middle-aged and older adults for long period of time due to poor food and lifestyle choices. Type 1 and Type 2 have quite different etiologies, which have different origins, symptoms, and treatment approaches [5].

Semicarbazide derivatives have garnered a lot of interest because of their wide variety of biological effects and function as building blocks in numerous families of important pharmacological molecules [6][7][8]. Semicarbazides condense and react with various ketones or aldehydes to form important compounds like semicarbazones and thiosemicarbazones [9]. A thiosemicarbazone is created when a sulfur atom is substituted for an oxygen atom in a semicarbazone. When these thiosemicarbazones come into contact with metallic cations, they bind and create chelating ligands [10]. It is a white substance made of urea that dissolves in water.

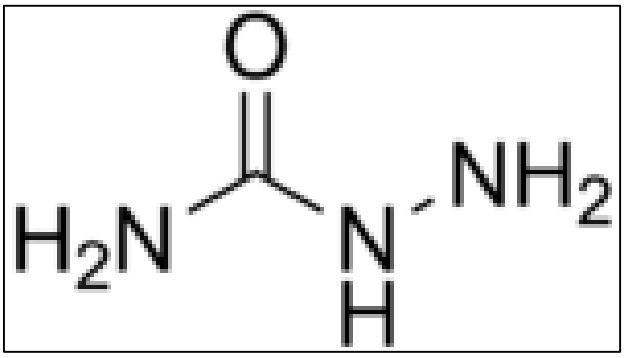


Fig 5. Semi-carbazide

IUPAC: Aminourea
Molecular formula:OC (NH_2) (N_2H_3)
Molar mass:75.08 g/mol
Melting point- 96ºC

Certain 1–5 hydrazones and semicarbazones also demonstrated anticonvulsant properties. According to the maximal electroshock seizure (MES) screen, aryl semicarbazones offered better defense[11][13][13]. The reaction that provides enhanced safety is the presence of a more electronegative group at the para position of the aryl ring. Vibrational spectroscopy is among the most helpful research methods for comprehending the connection between molecular structure and electronic states [14].

2. Materials and Methodology

Experimental Requirements

Isatin, semicarbazide, glacial acetic acid, ethanol, sulfuric acid, distilled water and paraffin. Weight balance, RBM, condenser, thermometer, and pH meter.

Synthesis of novel derivatives of semicarbazide

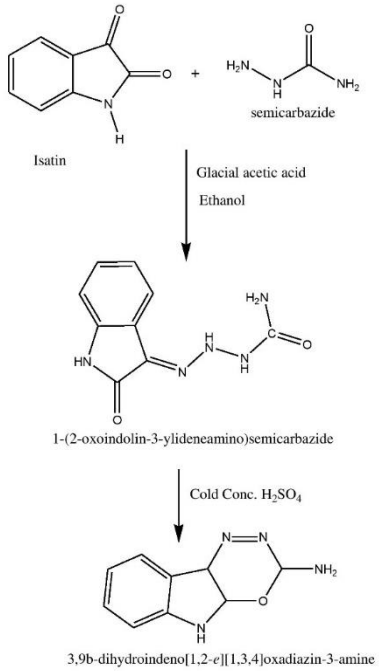


Fig 6. Scheme for synthesis of semicarbazide derivatives

All the 6 novel derivatives will be synthesized using above scheme. These will be evaluated for physiochemical parameters i.e.,melting point, RF value, FTIR, NMR & Mass spectroscopy and anti-diabetic activity further.

Procedure of synthesis (C1)

Upon reaction of semi-carbazide with 5-methylindoline-2,3-dione, in presence of glacial acetic acid and ethanol produced the intermediate moiety. That on further reaction with sulfuric acid produced the final product.

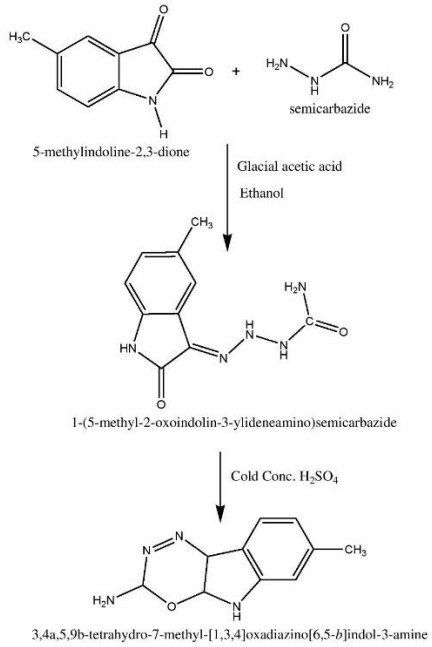


Fig 7. Synthesis of semi-carbazide derivative (C1)

Procedure of synthesis (C2)

In the presence of glacial acetic acid and ethanol, 5-bromoindoline-2,3-dione upon reaction with semi-carbazide produced the intermediate of semi-carbazide; which on reaction with cold sulfuric acid produced the final derivatives (C2).

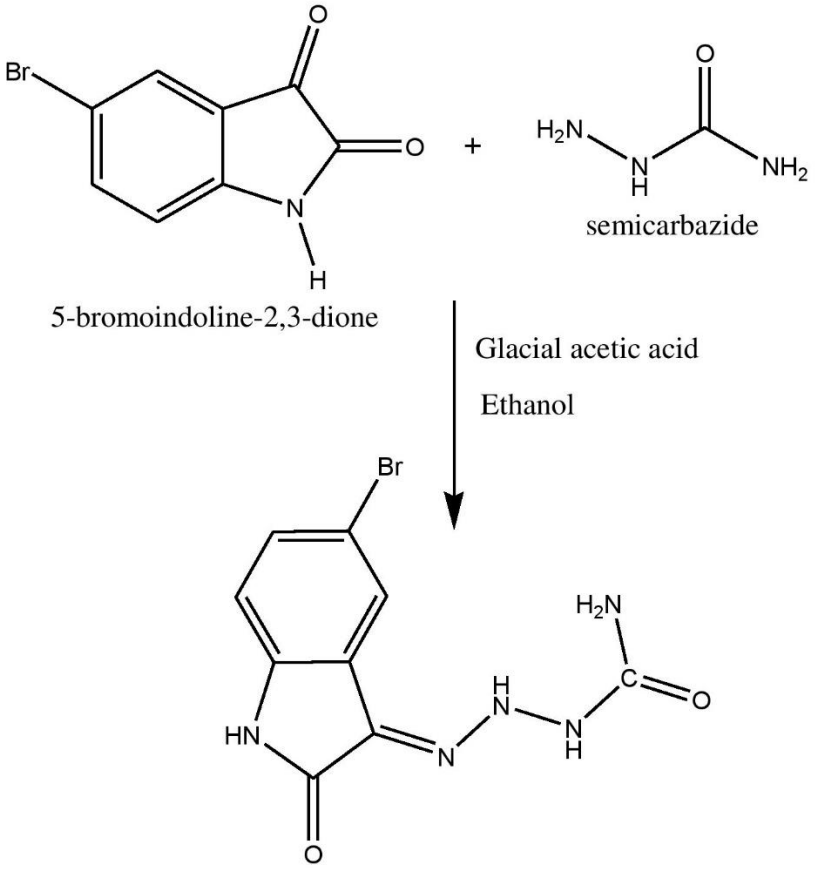


Fig 8. Synthesis of semi-carbazide derivative (C2)

Procedure of synthesis (C3)

In the presence of glacial acetic acid and ethanol, 5-iodoindoline-2,3-dione upon reaction with semi-carbazide produced the intermediate of semi-carbazide; which on reaction with cold sulfuric acid produced the final derivative.

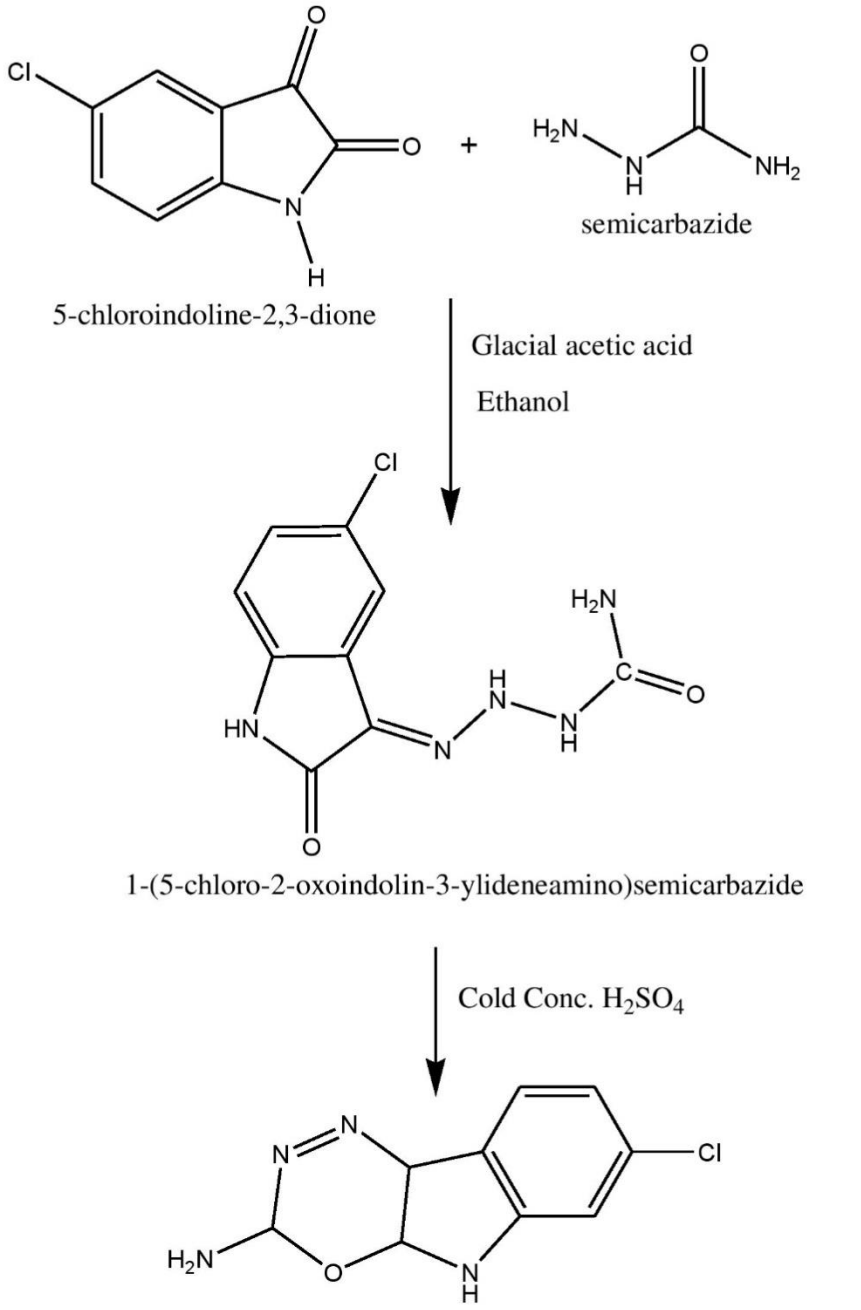


Fig 9. Synthesis of semi-carbazide derivative (C3)

Procedure of synthesis (C4)

In the presence of glacial acetic acid and ethanol, 5-fluoroindoline-2,3-dione upon reaction with semi-carbazide produced the intermediate of semi-carbazide; which on reaction with cold sulfuric acid produced the final derivative.

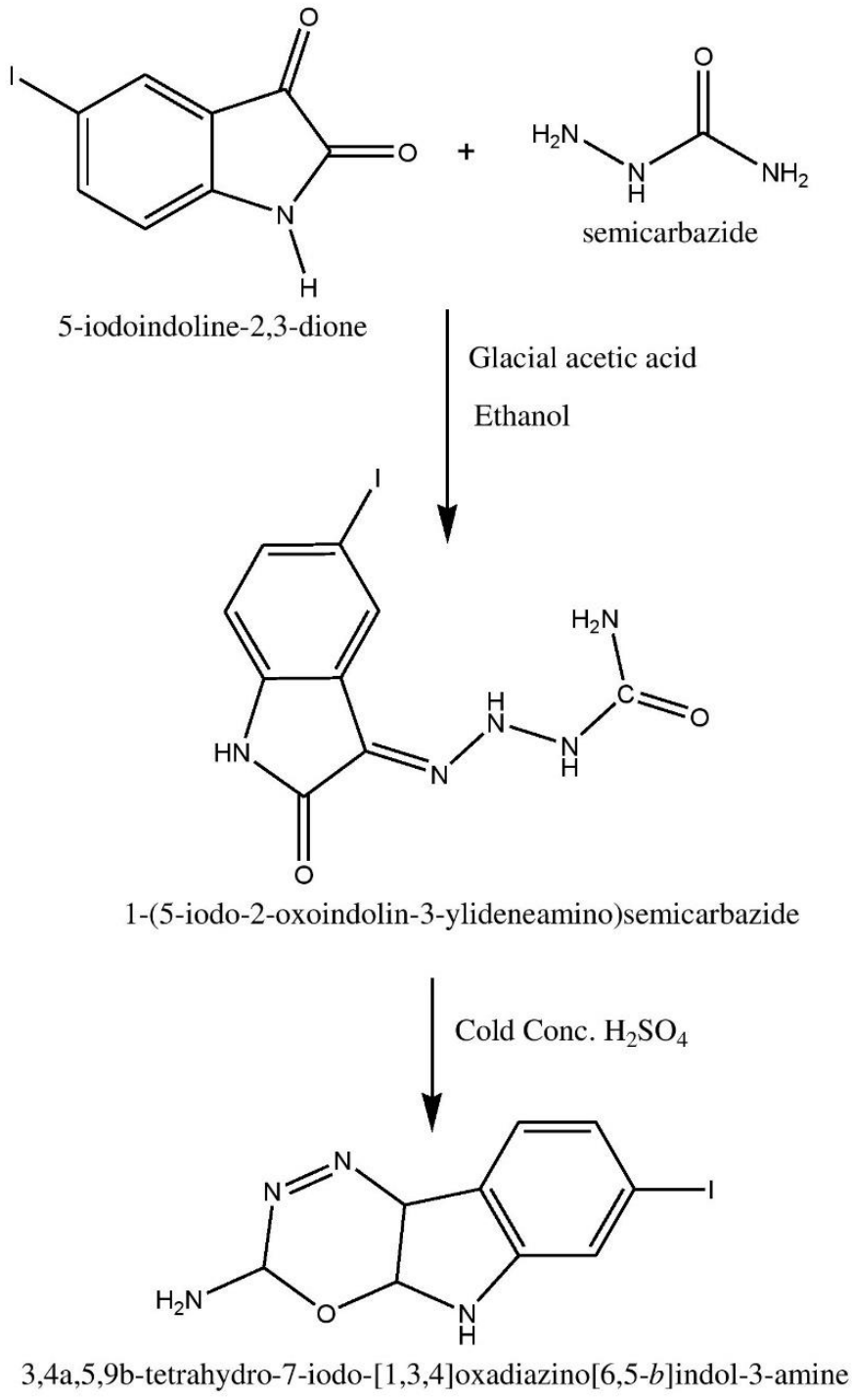


Fig 10. Synthesis of semi-carbazide derivative (C4)

Procedure of synthesis (C5)

In the presence of glacial acetic acid and ethanol, 5-nitroindoline-2,3-dione upon reaction with semi-carbazide produced the intermediate of semi-carbazide; which on reaction with cold sulfuric acid produced the final derivative (C5).

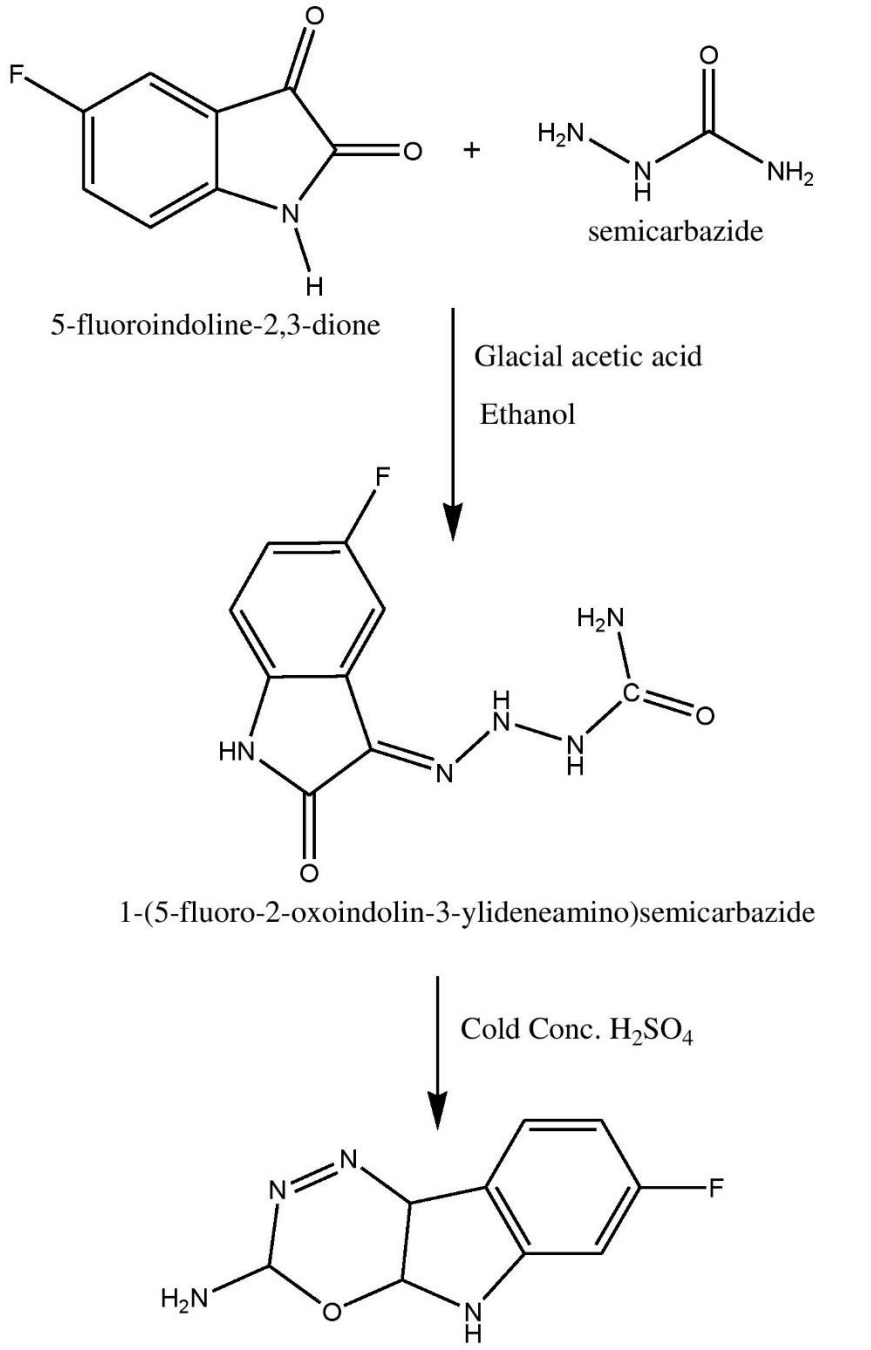


Fig 11. Synthesis of semi-carbazide derivative (C5)

Procedure of synthesis (C6)

5-Chloroindoline-2,3-dione upon reaction with semi-carbazide, in the presence o glacial acetic acid and ethanol produced the intermediate of semi-carbazide; which on reaction with cold sulfuric acid produced the final derivative.

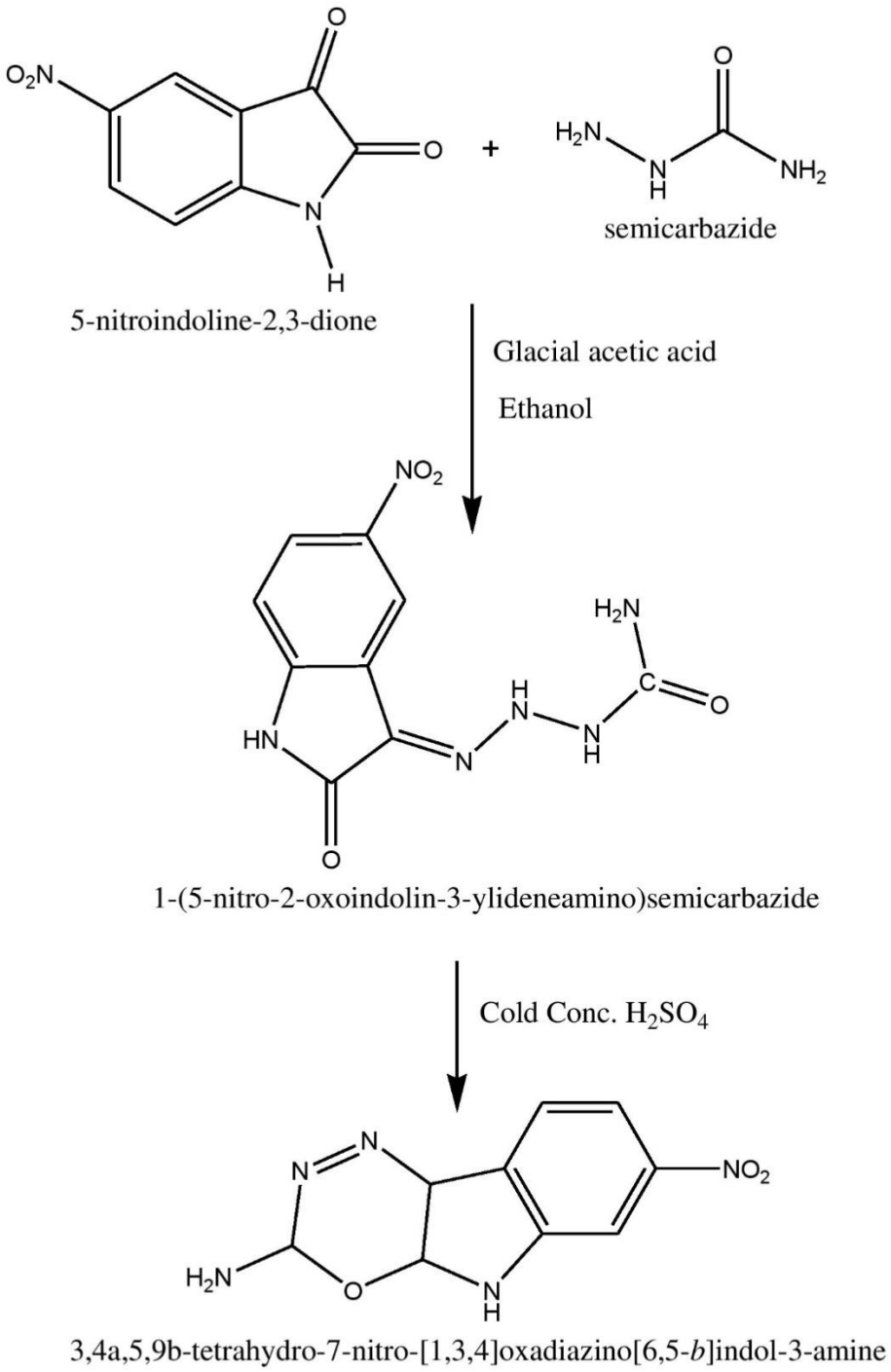


Fig 12. Synthesis of semi-carbazide derivative (C6)

A. Identification of physicochemical properties

- **Melting point**

The melting point of an organic compound was ascertained using Thiel's melting point tube. Finding a compound's melting point is the most crucial and direct way to differentiate one from another [15].

- **Rf value**

Thin layer chromatography, or TLC for short, is a technique in synthetic chemistry that uses a compound's variable Rf value to deduce the molecule's synthesis. It also helps to validate the reaction's advancement [16].

- **Infrared Spectroscopy**

One classifies the infrared spectrum as a vibrational-rotational spectrum. For solid compounds, the KBr pellet technique is utilized; for liquid compounds, the Nujol mull method is employed. It is a very useful document that provides details about the functional groups found in organic molecules. When electromagnetic radiation with a wavelength spanning from 500 cm-1 to 4000 cm-1 passes through a sample, the mechanism of bond stretching and bending occurs [17].

- **NMR Spectroscopy**

Proton NMR is the most widely utilized NMR method due to its high sensitivity and extensive characteristic information. The chemical shift (δ) range is 0–14 ppm. The test unknown compound's chemical shift was compared to TMS protons, which had an attribution of 0 ppm. However, the shift extends to the component [18] for the organic compound range δ 0 – 14.

- **Mass Spectroscopy**

An essential physico-chemical tool for determining the structures of chemicals found in natural goods, such as medicinal herbs, is mass spectrometry. The application of various physical techniques for sample ionization and ion generation based on mass to charge ratio (m/z) is the fundamental idea of mass spectrometry. Electrospray ionization, air pressure chemical ionization, electron ionization, chemical ionization, rapid atom bombardment, and matrix analysis laser desorption ionization are among the ionization techniques that are accessible. Compared to NMR, which has a sensitivity limit of the nanogram range and above, mass spectrometry has a high sensitivity with a detection limit of the fentogram. MS is a versatile analytical tool because to its sensitivity and versatility for hypenation with other chromatographic techniques [19].

B. Evaluation of anti-diabetic potential (in-vitro)

- **Estimation of DPPH Radical Scavenging Assay**

The 1,1′-diphenyl-1-picrylhydrazyl was used to test the derivatives' capacity to scavenge free radicals in vitro using the methodology described by Tariq et al. [76]. The assay involved reacting 0.4 ml of various doses of synthesized derivatives with 1.6 mL of 0.135 mM DPPH solution in 100% v/v methanol. After giving the reaction mixture a good vortex, it was kept at room temperature (for 30 min) in the dark. After two minutes, the mixture's absorbance was measured at 517 nm. Here, the concentration of the derivatives required to reduce the DPPH radical absorbance by 50% was determined.

- **Estimation of α-amylase**

The alpha-amylase assay was carried out using Odeyemi's [20] methodology. In a nutshell, 5µl of enzyme porcine pancreatic solution was mixed with 15µl of the derivative at varying concentrations (50µg/ml – 200µg/ml) (diluted in phosphate buffer) in a 96-well plate. The reaction was started by adding 20µl of starch solution and incubated for an additional 30 minutes at 37°C after 10 minutes of incubation at that temperature. After that, 10µl of 1M HCl and 75µl of iodine reagent were added to each well to halt the reaction. A positive control (acarbose, 64µg/ml) and a blank containing phosphate buffer (pH 6.9) in place of the derivatives were made. For every test sample, there was no starch control or enzyme control. At 580 nm, the absorbance was measured.

3. Results and Discussion

Synthesized derivatives

Newer derivatives of semi-carbazide were synthesized.Semicarbazide derivatives were found as a white, water-soluble solid with the chemical formula OC. It is a derivative of urea. Semicarbazide hydrochloride is a white, crystalline solid that is prism-like in shape.

Identification of physicochemical properties

Melting point determination

The novel derivatives of semi-carbazide were reported for the melting point as 140ºC, 112ºC, 164ºC, 184ºC, 156ºC and 164ºC for compounds C1 to C6, respectively.

Rf value (TLC)

Synthetic chemistry uses thin layer chromatography to verify the synthesis of a molecule based on its Rf value, which changes according to the substance. The Rf values for C1 to C6 were found to be 0.65, 0.67, 0.71, 0.69, 0.66, and 0.72, respectively.

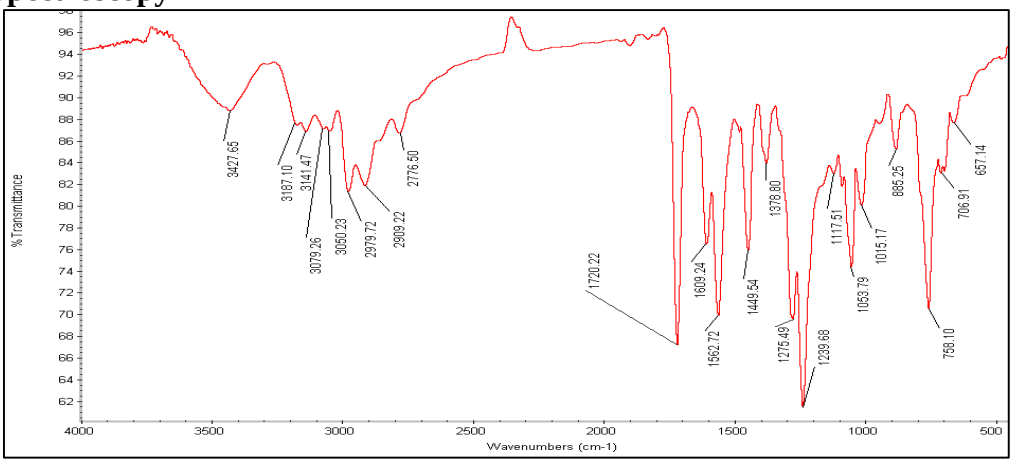
1. The physical characteristics of each synthesized semi-carbazide derivative, such as melting point and % yield, were examined.
2. The highest yielding samples, C5 and C6, were shown to be 65.20% and 66.44%, respectively. C1 had the lowest yield percentage, 61.34%.
3. Compound C4 was discovered to have the greatest melting point, measuring 184ºC. The compound with the highest melting point has the strongest density.
4. Significant differences in molecular weight were also observed in the produced derivatives of semi-carbazides.

The physical characteristics of every chemical were compiled in the table that follows.

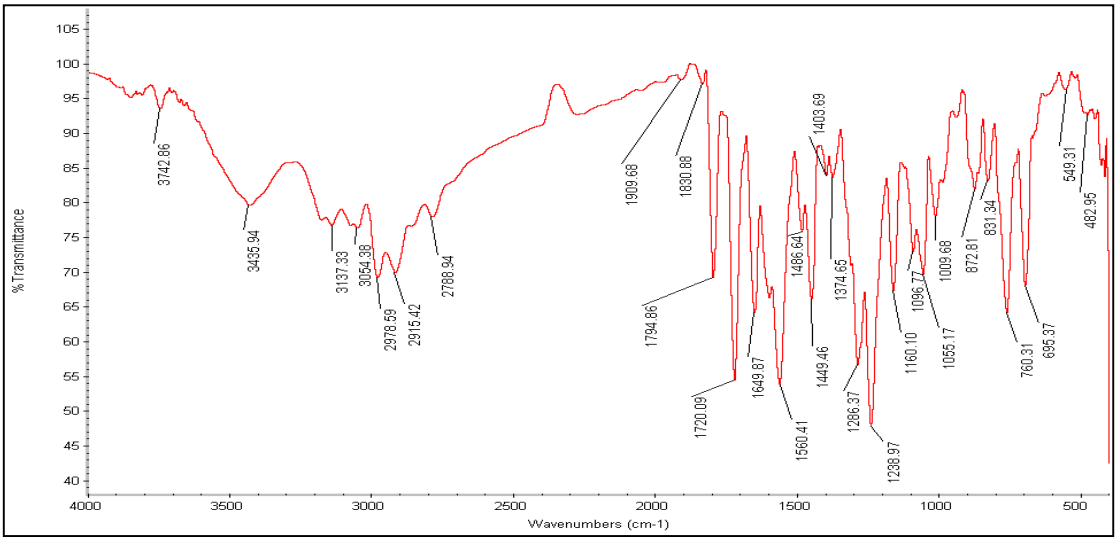
Table 1. Physicochemical properties of synthesized semi-carbazide derivatives

S. N.	Compound	Yield (%)	Rf Value	Melting point
1.	C1	61.34	0.65	140ºC
2.	C2	63.26	0.67	112ºC
3.	C3	62.56	0.71	164ºC
4.	C4	64.53	0.69	184ºC
5.	C5	65.20	0.66	156ºC
6.	C6	66.44	0.72	164ºC

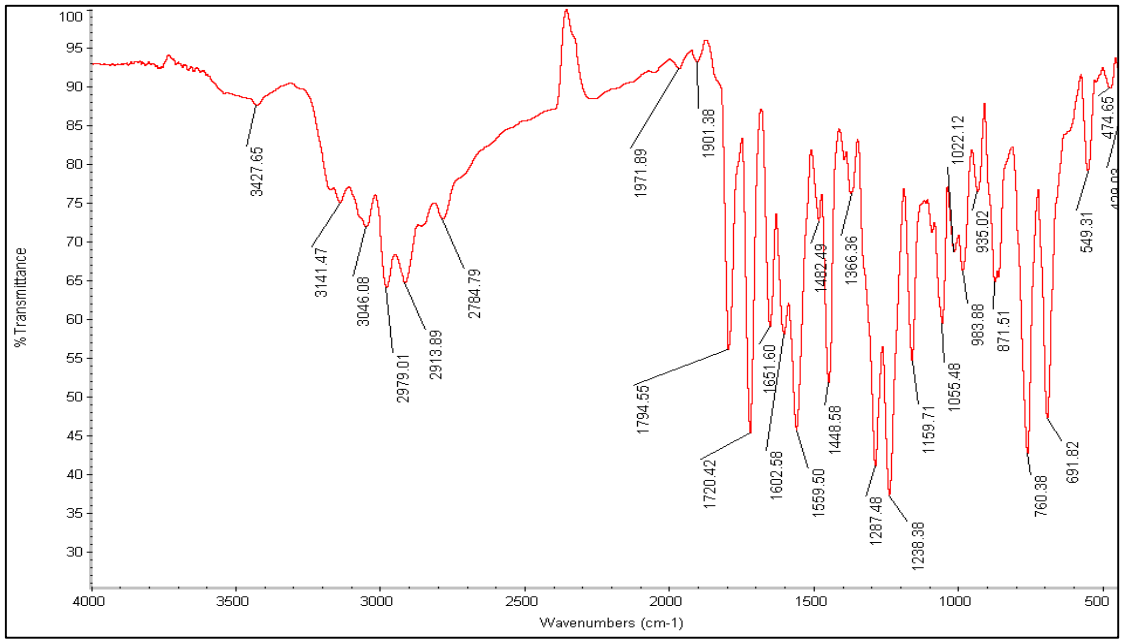
FTIR Spectroscopy



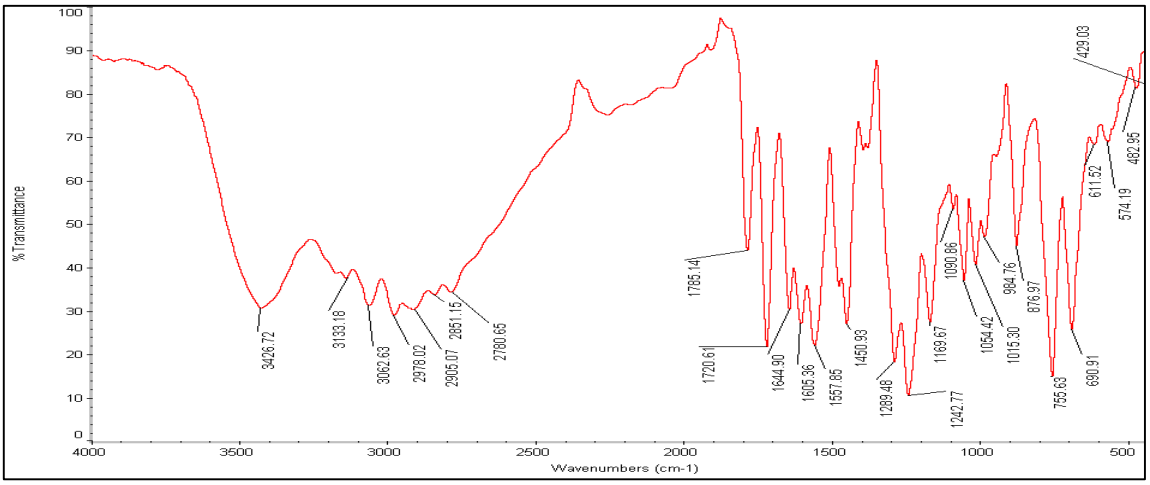
FTIRSpectrumof C1



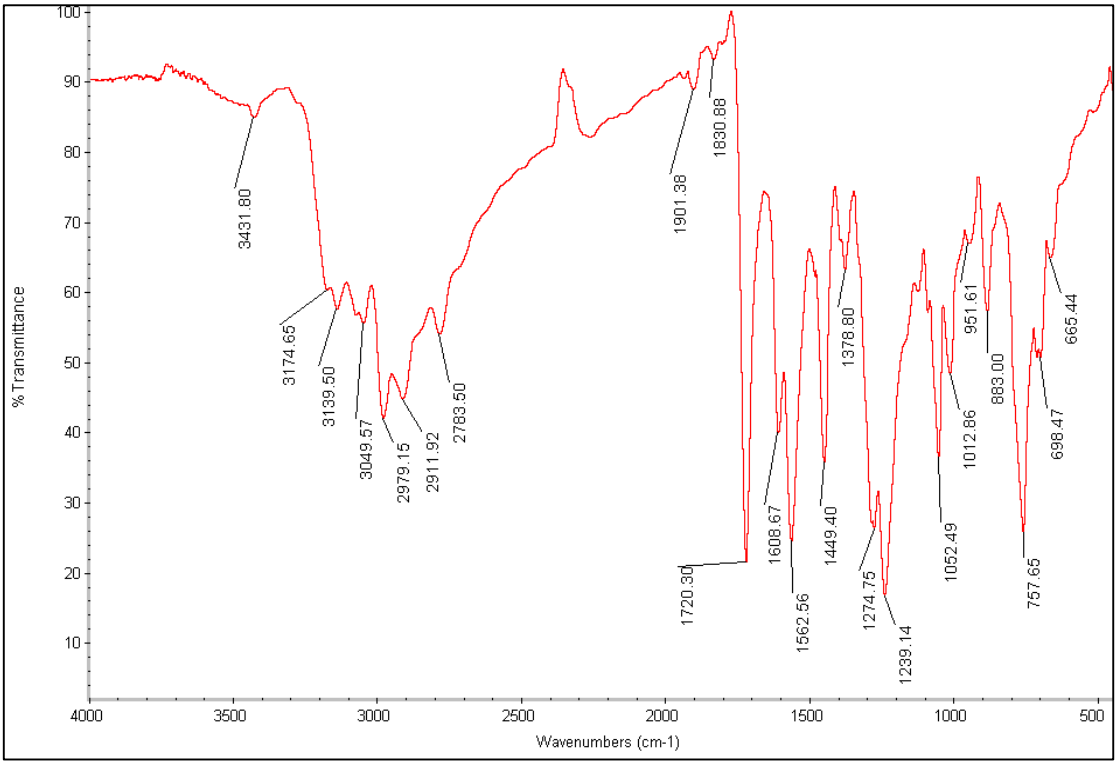
FTIR Spectrum of C2



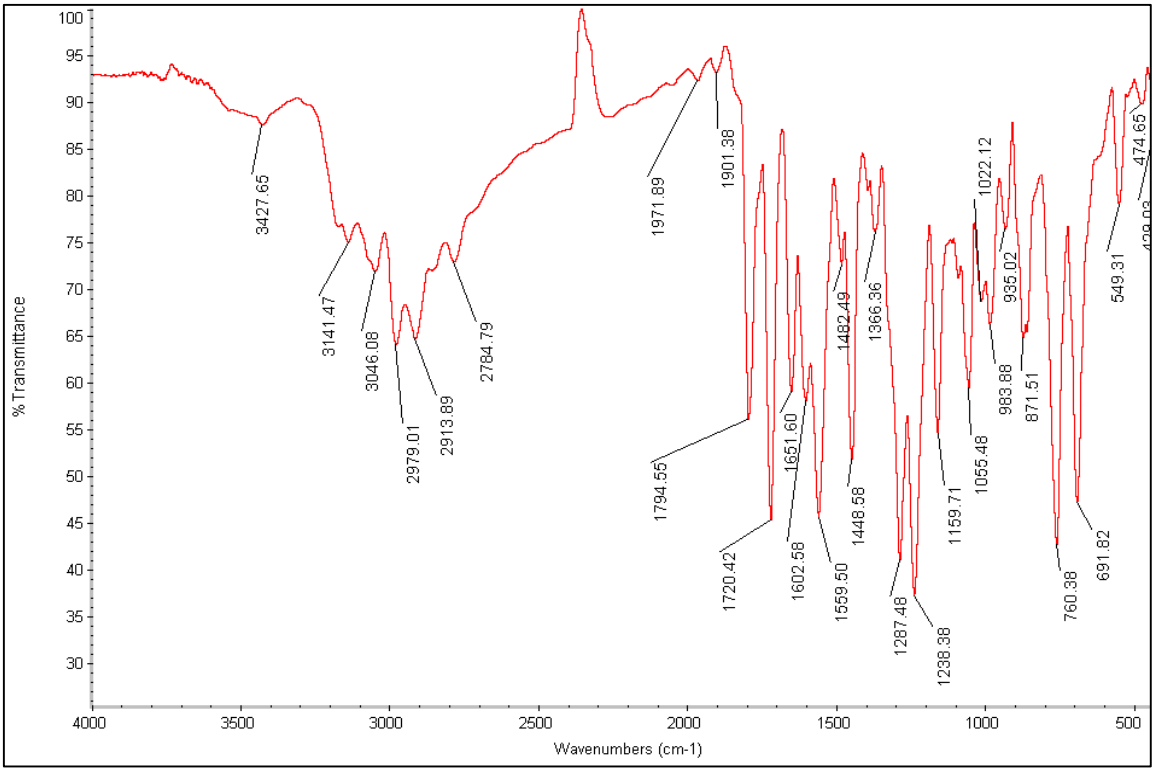
FTIR Spectrum of C3



FTIR Spectrum of C4

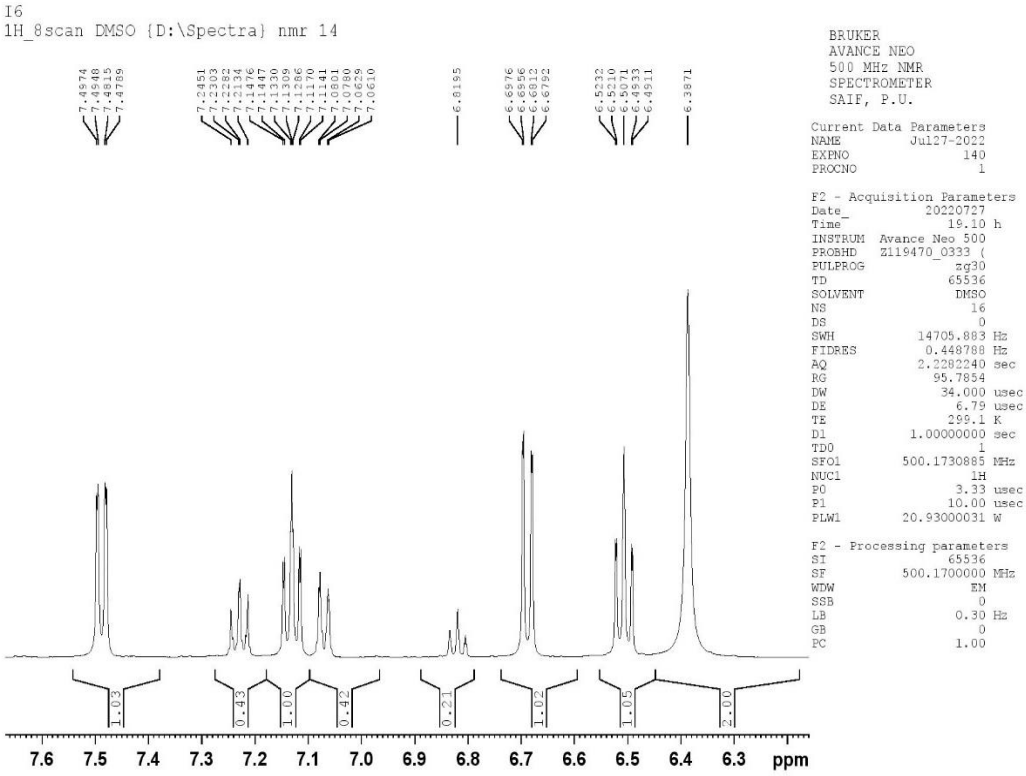


FTIR Spectrum of C5

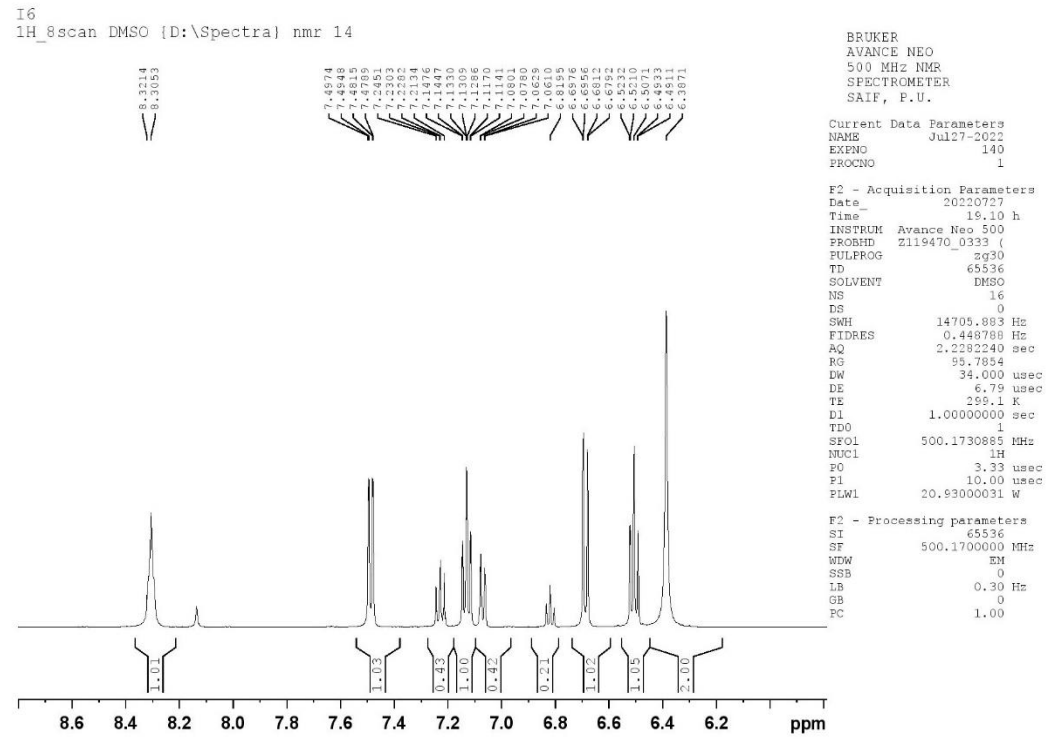


FTIR Spectrum of C6

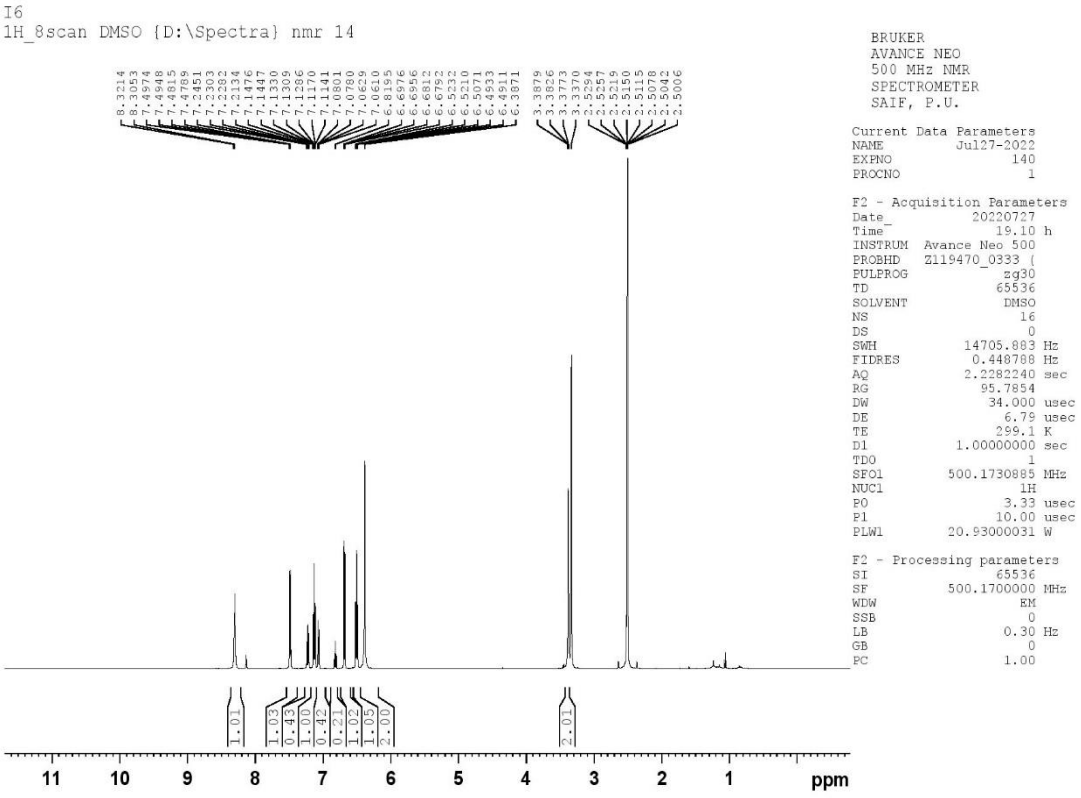
NMR Spectroscopy



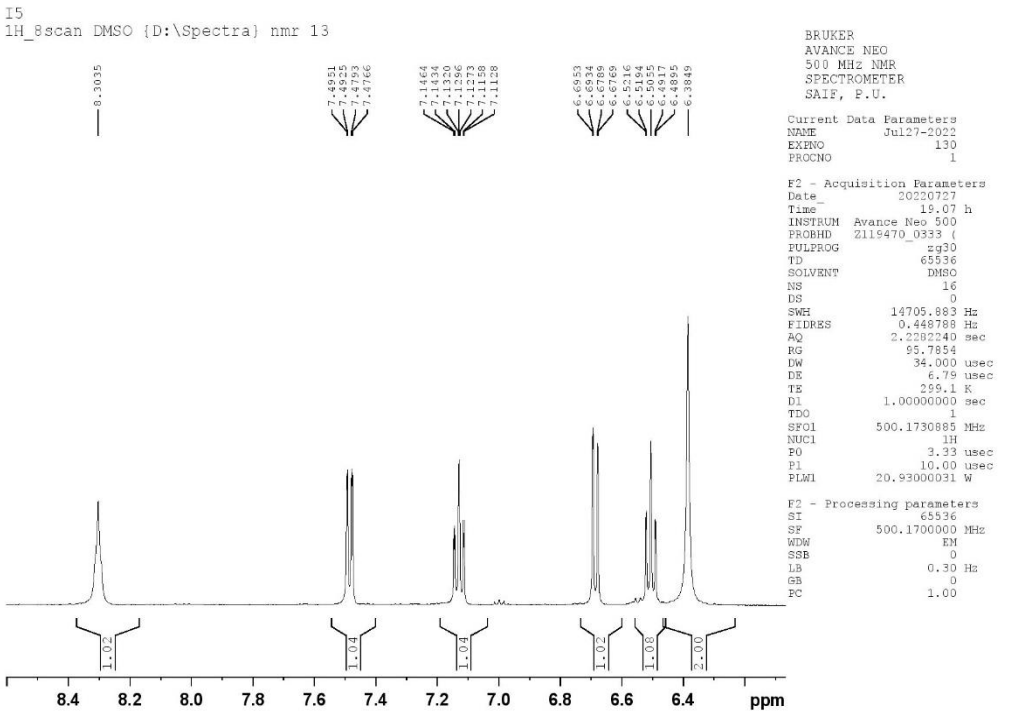
NMR Spectrum of C1



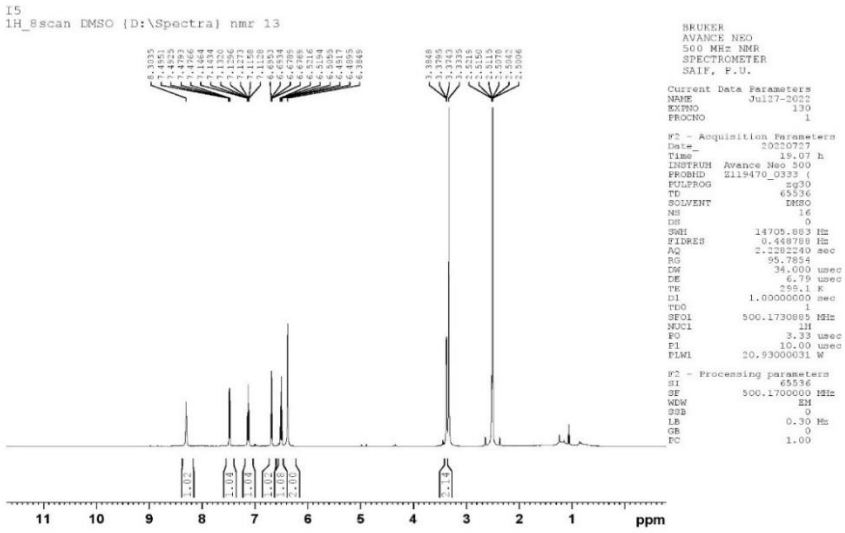
NMR Spectrum of C2



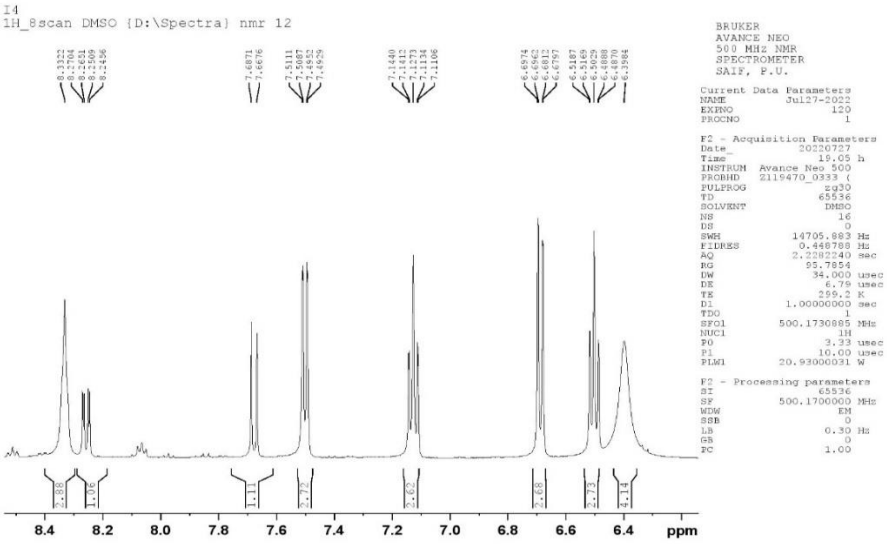
NMR Spectrum of C3



NMR Spectrum of C4



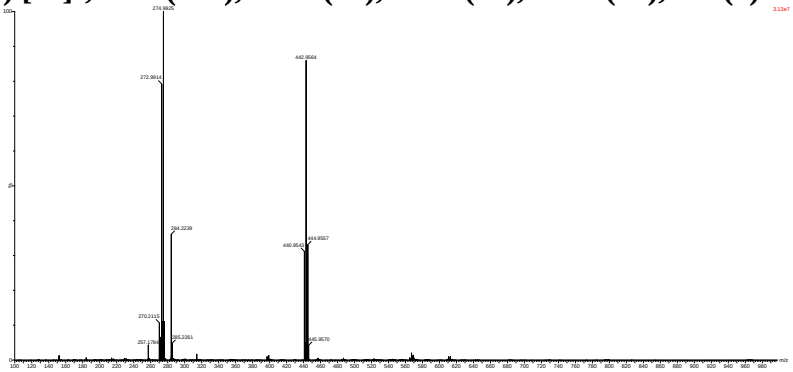
NMR Spectrum of C5



NMR Spectrum of C6

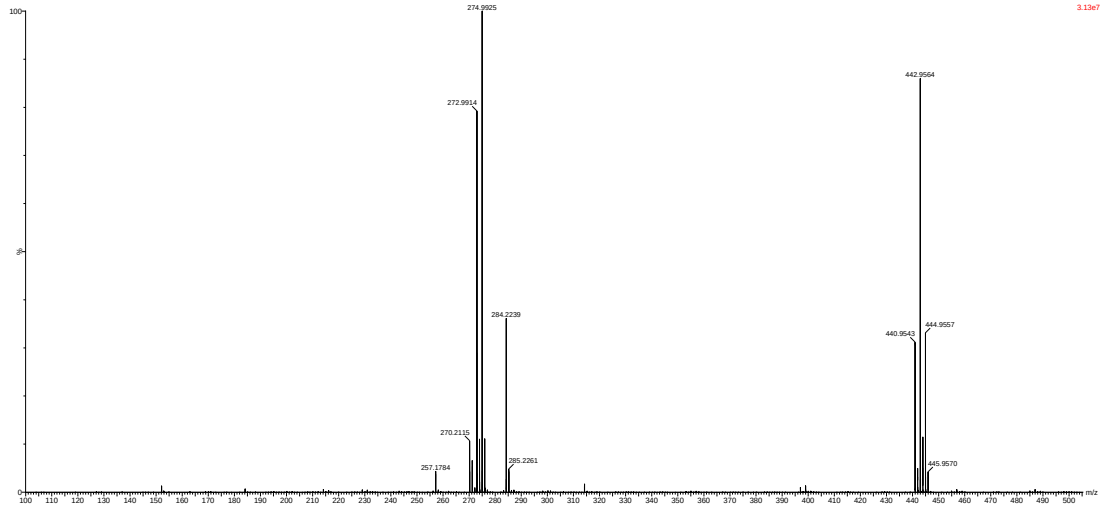
Mass Spectroscopy

C1-MS:m/z (%) [M]$^+$,274.9(100), 272.9(84), 284.3(32), 270.2(24), 257(9).



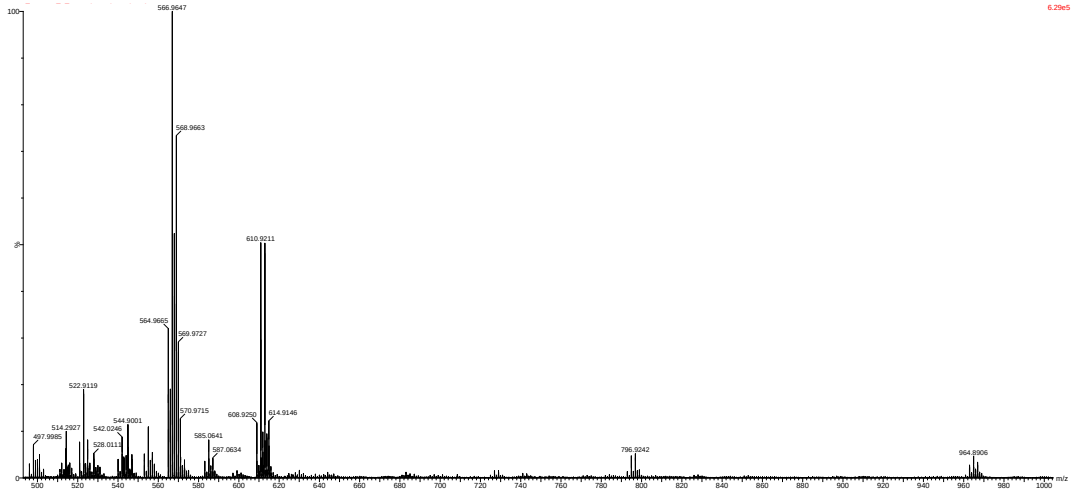
Mass Spectrum of C1

C2-MS:m/z (%) [M]$^+$, 274.9(100), 272.9(84), 284.3(32), 270.2(24), 257(9).



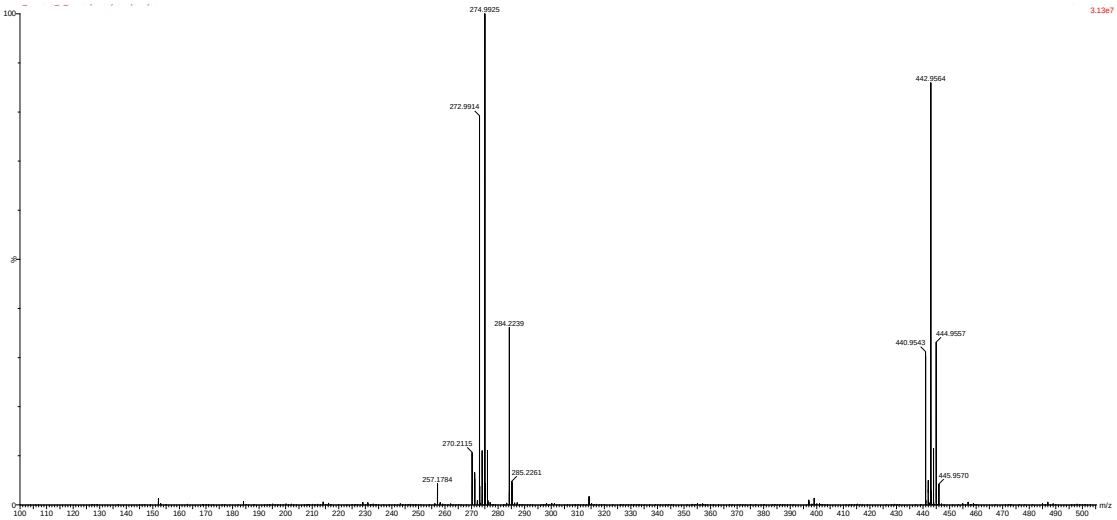
Mass Spectrum of C2

C3-MS:m/z (%) $[M]^+$, 566.9(100), 568.9(76), 564.9(35), 569.9(29), 570.2(14).



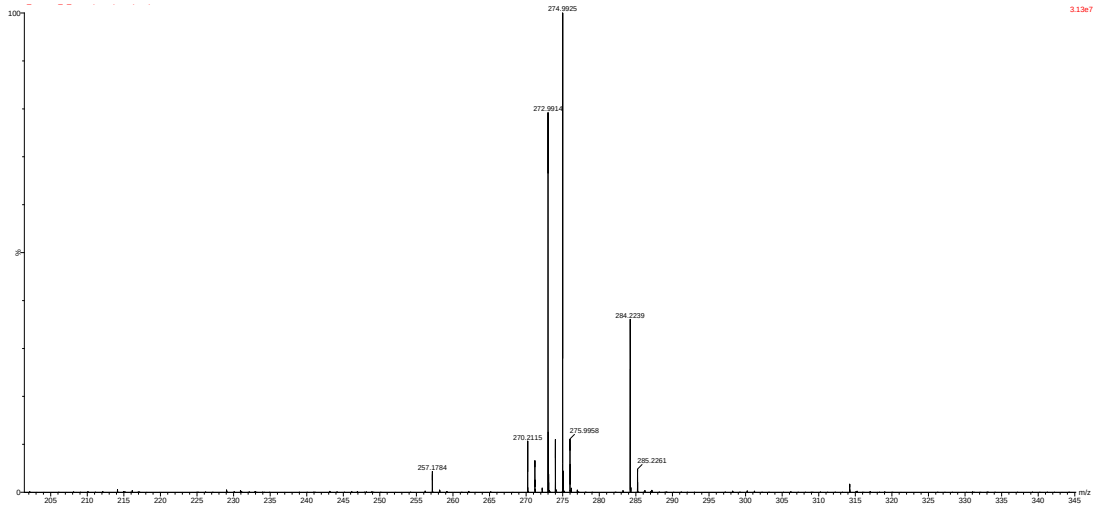
Mass Spectrum of C3

C4-MS:m/z (%) $[M]^+$, 274.9(100), 272.9(84), 284.3(33), 270.2(24), 257(9).



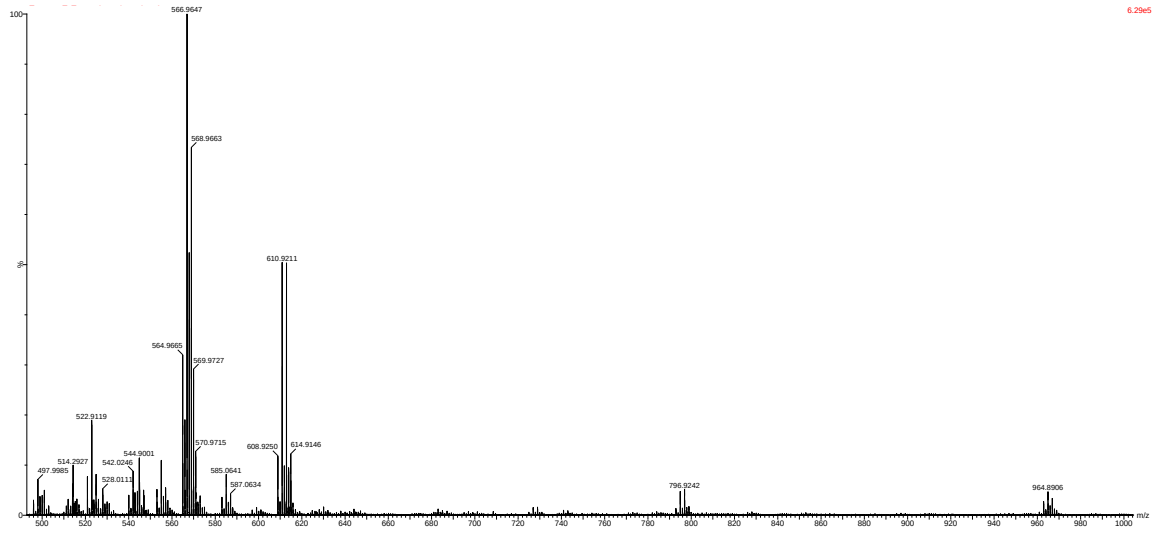
Mass Spectrum of C4

C5-MS:m/z (%) $[M]^+$, 274.9(100), 272.9(82), 275.9(13), 270.2(11), 557.2(6).



Mass Spectrum of C5

C6s-MS:m/z (%) $[M]^+$, 566.9(100), 568.9(76), 564.9(35), 569.9(29), 570.2(14).



Mass Spectrum of C6

Evaluation of anti-diabetic potential (in-vitro)

Determination of DPPH Scavenging capacity

In determination of DPPH scavenging activity, novel semi-carbazide derivatives were tested for different concentrations i.e., 200µg/ml, 400µg/ml and 800µg/ml and was compared with ascorbic acid.

The novel semi-carbazide derivatives (800µg/ml) showed the maximum % inhibition (DPPH Scavenging assay) as 64.18±0.29% which was significant when compared with placebo/water. Moreover, ascorbic acid showed highest percent inhibition. The antioxidant action might be due to blockage or suppression of free radical formation.

The % inhibition of DPPH Scavenging assay was observed as follow-

Table 2. Determination of DPPH Scavenging capacity of semi-carbazide derivatives

Concentration (µg/ml)	**DPPH Scavenging capacity (% Inhibition)**		
	Water	**Semi-carbazide derivatives**	**Ascorbic acid**
200µg/ml	29.23±0.54	34.34±0.24	47.36±0.18

400µg/ml	28.46±0.33	43.23±0.12	68.24±0.22
800µg/ml	31.22±0.30	64.18±0.29	96.45±0.67

Data were depicted in Mean±S.D

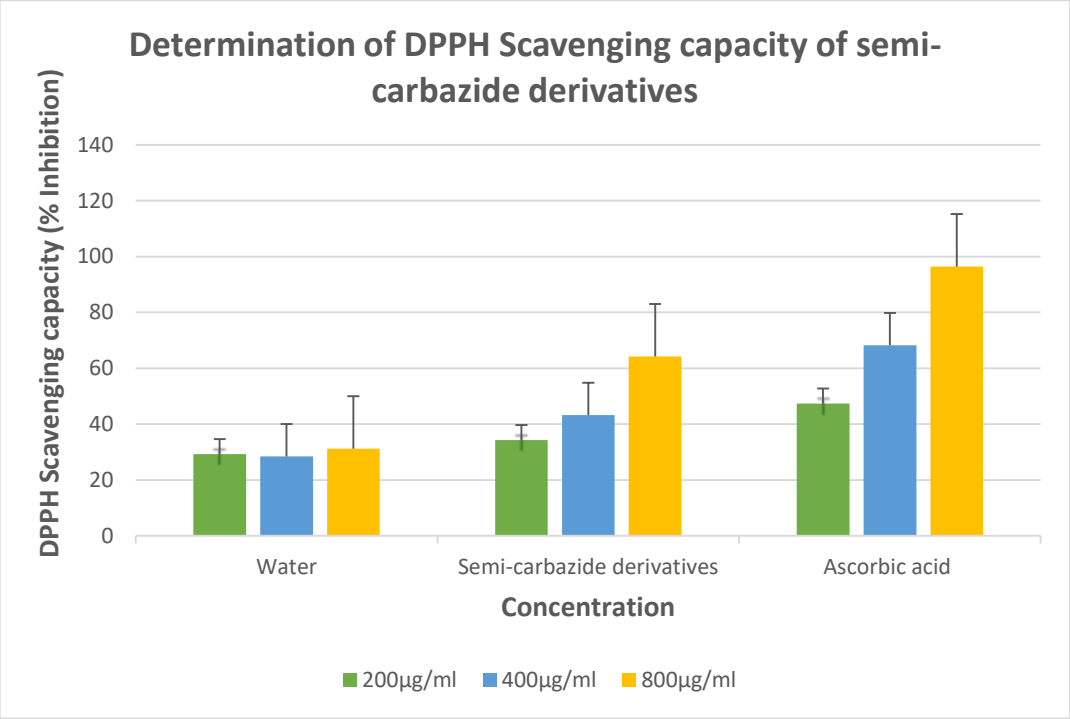


Fig 13. Determination of DPPH Scavenging assay

Estimation of α-amylase

In α-Amylase inhibition (% Inhibition), water, novel semi-carbazide derivatives and acarbose were estimated for different concentrations i.e., 200µg/ml, 400µg/ml and 800µg/ml. The % inhibition of novel semi-carbazide derivatives was compared with acarbose.

The α-Amylase inhibition is an important feature for estimation of anti-diabetic potential. At 800µg/ml, the α-Amylase inhibition was estimated as 45.34±0.11%, 63.45±0.21%, and 91.35±0.46%, in water, semi-carbazide derivatives and acarbose, respectively. Therefore, it can be easily observed that synthetic moieties showed an effective α-Amylase inhibition when compared with water.

Table 3. Estimation of α-amylase inhibition of semi-carbazide derivatives (800µg/ml)

Concentration (µg/ml)	**α-Amylase inhibition (% Inhibition)**		
	Water	**Semi-carbazide derivatives**	**Acarbose**
200µg/ml	26.21±0.35	38.24±0.35	61.29±0.24
400µg/ml	38.24±0.30	49.53±0.35	74.34±0.52
800µg/ml	45.34±0.11	63.45±0.21	91.35±0.46

Data were depicted in Mean± S.D

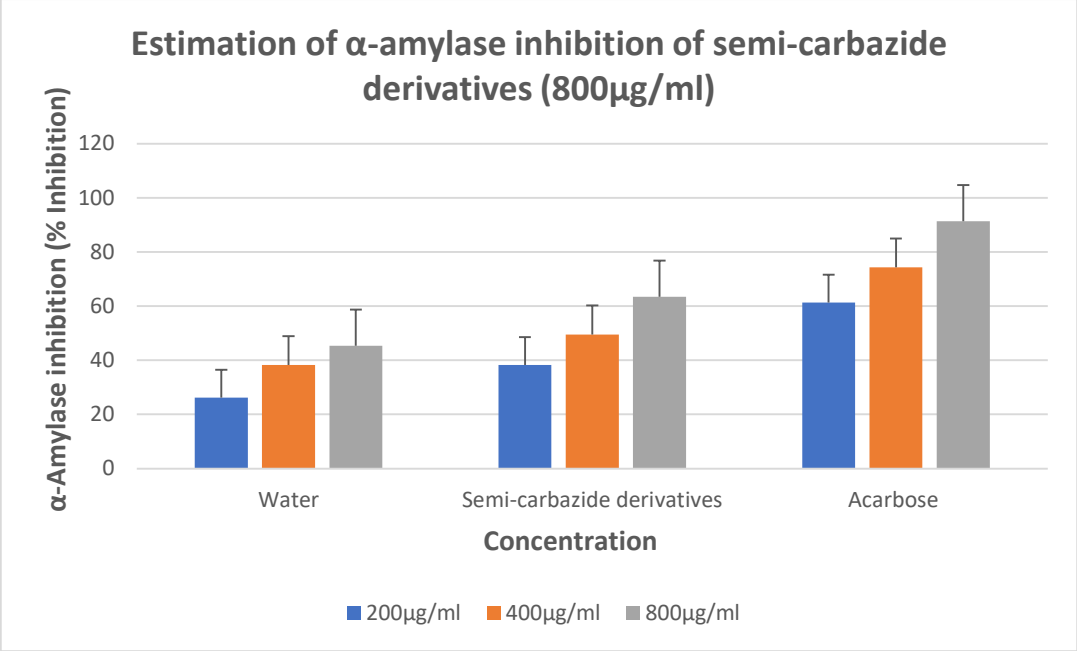


Fig 14. Estimation of α-amylase inhibition of semi-carbazide derivatives (800µg/ml)

The substances underwent both in vitro and in vivo screening for anti-diabetic efficacy. The anti-diabetic activity of 4m in In-vivo method 4a was found to be modest, comparable to that of the conventional medication, glibenclamide.The reference medication, acarbose, has similar mild anti-diabetic activity to that of In-vitro method 4a, 4e, and 4mhave [21]. The amylase inhibition assay was used to investigate the synthetic N-carbamoyl pyrazolines' antidiabetic efficacy, and the compounds' percentage inhibitions were calculated in comparison to standard acarbose23. Table 1 displays the excellent suppression of amylase that all the compounds demonstrated when compared to conventional acarbose. While compounds 6c, 6d, 6e, and 6i exhibit good antidiabetic action in comparison to conventional acarbose, compounds 6j and 6b from the series have more strong antidiabetic activity. It is clear from the discussion above that all of the synthesized derivatives of N-carboamylpyrazoline have the potential to have biological effects and to be valuable in the development of novel medications [22].The anti-diabetic effect of all synthesized compounds has demonstrated the same binding mechanism.

It was discovered that compounds containing an electron-withdrawing moiety on the thiophene ring have remarkable anti-diabetic properties. As a result, substances having substitutions for bromo, nitro, and chloro (4f, 4a, and 4e) were essential to their anti-diabetic effects. Additionally, a molecule (4b) consisting of a sulphonic acid moiety exhibited intriguing action. Conversely, the anti-diabetic effect is eliminated by molecules bearing an electron-donating group on the thiophene ring. As a result, compounds (4c and 4d) substituted with the CH3 and C2H5 moiety would not be favorable for anti-diabetic action. Compound 4f has generally exhibited the highest activity when compared to the other synthesized compounds, with a level of activity comparable to that of mainstream medications. Here, the anti-diabetic effect of synthetic compounds was assessed using Glibenclamide as a reference medication [23].

4. Conclusion

In conclusion, all the synthesized semi-carbazide derivatives demonstrated s significant antioxidant activity and α-amylase inhibition when compared with control. On the basis of

physicochemical parameters i.e., physical state, melting point, FTIR, NMR, mass studies, the derivatives were confirmed for the novel derivatives of semi-carbazides. The synthesized semi-carbazide derivatives have potential for future clinical investigations in the treatment of DM. Additional research is required to determine the specific mechanism by which synthetic moieties exhibit its antidiabetic properties. Semi-carbazide derivatives may serve as a promising synthetic reservoir of antioxidants suitable for application in diabetes mellitus. Ultimately, the antidiabetic effect of the synthetic derivatives may be attributed to its antioxidant properties and its ability to enhance the effectiveness of insulin. Confirmation of the mechanism of action requires molecular investigations to determine the specific receptor subtypes it targets and to explore methods for enhancing its binding efficiency.

Semi-carbazide derivatives are promising drug for the management of diabetes mellitus. The current study shown that semi-carbazide derivatives have enormous biological potential when compared with acarbose and ascorbic acid.

Research suggests that Semi-carbazide derivatives are an effective synthetic molecule in the management of diabetes but it's mode of action is still unknown. So further research may carried-out to confirm its mode of action and optimum doses required in hypoglycaemic action.Subsequently, a suitable dosage form will be formulated to enhance patient adherence and the extent to which the drug is absorbed and available for use in the body.

5. References

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