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Research Article

Synthesis, Identification, and In-vitro Antidiabetic Evaluation of 2,4,5-Trisubstituted Imidazole Derivatives

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Abstract

To offer new imidazole compounds powerful to reduce hyperglycemia by inhibiting the enzymes δ-amylase and α-glucosidase, a new series of trisubstituted imidazole derivatives 4(a-d) were prepared by using the Radziszewski reaction of 4,4'-dimethoxybenzil, different aromatic aldehydes and ammonium acetate in glacial acetic acid as a catalyst. All newly synthesized compounds were identified by various spectral data and were examined for purity by thin-layer chromatography. Then all prepared compounds were screened for them in *vitro* α-amylase and α-glucosidase inhibitory activities using acarbose as a standard reference at different concentrations (50-250 µg/mL). The findings showed that all the synthesized derivatives have good to excellent inhibitory potential against α-amylase ranging between 51% to 90% compared with the reference drug acarbose ranging from 32% to 63%. Among the series compounds, 4f was the most potent. The α-glucosidase inhibition assay showed the reference drug acarbose exhibited the highest α-glucosidase inhibition (66%) followed by compounds 4b, 4d, and 4a (55%), (52%) and (51%) respectively at their highest concentrations (250 µg/mL). Therefore, these new imidazole derivatives have the potential to develop a new inhibitory activity.

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Introduction

Diabetes mellitus, a chronic endocrine disease, effects protein, lipid, carbohydrate, electrolyte, and water metabolism. It describes hyperglycemic metabolic disorders, which occurs when the level of blood sugar rises due to either a lack of insulin production by the pancreas or a failure of cells to respond to the insulin produced. As a result, decreasing postprandial hyperglycemia is a therapeutic approach for diabetes [1]. This is achieved by inhibiting carbohydrate hydrolyzing enzymes such as alpha-amylase and alpha-glucosidase which are important in carbohydrates metabolism [2]. Long-chain carbohydrates are broken down by α-amylase, while starch and disaccharides are converted to glucose by α-glucosidase. In diabetic patients, pancreatic a-amylase inhibitors retard carbohydrate digestion, reducing glucose absorption and lowering postprandial blood glucose levels [3].As a result, inhibiting α-amylase and α-glucosidase and noteworthy substances has been established as a beneficial and practical approach to decreasing postprandial hyperglycemia levels. Miglitol ,Voglibose, and acarbose are a few antidiabetics commercial drugs now available for treating α-glucosidase and α-amylase catalyst restraint in diabetes mellitus [4]. As an all-inclusive answer, no one therapy is feasible. Anyway, these drugs are not recommended for long-term usage due to their side effects. So, scientists are searching for more secure, single, and ordinary inhibitors with fewer side effects that can be used to treat diabetes. [5]. Some medicinal compounds' antidiabetic efficacy has been documented *in vitro* and *in vivo* [6].

Heterocyclic compounds containing imidazole moieties play a vital role in biological processes and are widespread as natural products due to its presence in bioactive compounds, synthetic intermediates, and pharmaceuticals; thus, researchers have focused on the methods for their synthesis and applications. Their derivatives are involved in a variety of vital natural products like alkaloids nucleic acid bases (RNA) and DNA [7], hormones [8], and vitamins[9]. They also have antiviral [10], antifungal [11], antibacterial [12], antioxidant [13], anticonvulsant [14], anti-inflammatory [15], and anticancer properties [16], as well as inhibitors of mammalian 15-LOX, [17], and B-Raf kinase[18] , β-lactamase inhibitors, heme oxygenase inhibitors, and antiaging agents [19- 21]. Various methods for their synthesis have been used, including a typical one-pot synthesis from a 1,2-dione, an aromatic aldehyde, and NH_4OAC using zeolites HY/silica gel [22], ionic liquids[23] , catalyst-free under microwave irradiation [24], and traditional refluxing in AcOH which is a good confirmed approach for the preparation of imidazole derivatives [25] generally , all of the above methods have their own advantages . The current study involved the synthesis, and characterization of new tri-imidazole and the study of their effect to reduce hyperglycemia.

Experimental section

Materials and instrumentation

Chemicals and solvents that were used in this work were purchased from different manufacturers, such as Aldrich, Hi-Media and directly used without extra purification. All reactions were monitored by (TLC) thin-layer chromatography. Visualization of spots on TLC plates was done by heating plates coated with KMnO4 stain. FT.IR spectra were registered as KBr disk using SHIMADZU FTIR-8400S. Melting points were measured by using Stuart SMP 30 capillary melting Electro thermal analyzer. ^1H NMR spectra was measured in deuterated dimethyl sulfoxide (DMSO-d_6) with Bruker Bio Spin at 500 MHz. Mass spectra were measured by Agilent technology (HP) instrument (EI, 70 eV).

preparation of 2,4,5- trisubstituted imidazole derivatives 4a–d

A mixture of 4,4'-dimethoxybenzil (1mmol), aromatic aldehyde (1mmol), NH_4OAC, (4mmol) with glacial acetic acid (15 mL) as solvent and catalyst in 50 mL round-bottom flask, the mixture was reflex at 120°C for 6–h h until the reaction was completed. The progress of the reaction was monitored by TLC. A sufficient amount of cold water is added to the reaction vessel, followed by the addition of ammonium hydroxide solution drop by drop with stirring to obtain the solid, then the product was filtered and washed well with deionized water to remove any remnants of base and salts, the product was dried, and recrystallized from hot ethanol. Confirmation of all structures was done by mass and NMR spectra, as explained below:

3-[(4,5-bis(4-methoxyphenyl)-1H-imidazol-2-yl)] phenol (4a): $C_{23}H_{20}N_2O_3$. Color**:** off-white powder, yield 80 %. m.p.; 110-111◦C; FT-IR υmax cm^{-1} = 3302(N–H), 3055(C-H, arom.)1654 (C=N). ^{1}HNMR (500 MHz, DMSO-d6) δ ppm: 12.43 (s, 1H, NH), 9.54 (s, 1H, OH), 7.55 – 7.48 (m, 2H), 7.45 (d, J = 8.2 Hz, 4H), 7.26 (t, J = 7.9 Hz, 1H), 6.96 (d, J = 7.7 Hz, 4H), 6.79 (dd, J = 7.9, 2.5 Hz, 1H), 3.79 (s, 6H). MS(EI) (m/z) = 372$[M]^+$.

2-[(4-chlorophenyl)-4,5-bis(4-methoxyphenyl)]-1H-imidazole (4b): $C_{23}H_{19}ClN_2O_2$ White powder, yield 82 %. m.p.; 116-118 ◦C; FT-IR υmax cm^{-1} = 3398 (N–H), 3097(C-H, arom.),1654 (C=N), 640 (C–Cl). ^{1}H NMR (500 MHz, DMSO-d6) δ ppm; 12.61 (bs, 1H, NH), 8.09 (d, J = 8.6 Hz, 2H), 7.96 (d, J = 8.5 Hz, 1H), 7.88 (d, J = 8.9 Hz, 1H), 7.57 (d, J = 8.6 Hz, 1H), 7.54 (d, J = 8.7 Hz, 2H), 7.46 (d, J = 8.2 Hz, 3H), 7.14 (d, J = 9.0 Hz, 2H), 3.88 (s, 6H). MS(EI) (m/z) = 390 $[M]^+$.

4-[(4,5-bis(4-methoxyphenyl)-1H-imidazol-2-yl)]-3-methoxyphenol (4c): $C_{24}H_{22}N_2O_4$ yellow colored powder, yield 85 %. m.p.; 123-125 ◦C; FT-IR υmax cm^{-1} = 3448(N–H), 3088(C-H, arom.),1651(C=N), 1245 (C–O) .^{1}HNMR (500 MHz, DMSO-d6) δ ppm;12.28 (bs, 1H, NH), 9.25 (bs, 1H, OH), 7.87 (d, J = 8.8 Hz, 1H), 7.61 (d, J = 2.0 Hz, 1H), 7.50 (dd, J = 8.2, 2.0 Hz, 1H), 7.43 (d, J = 8.4 Hz, 3H), 7.14 (d, J = 6.8 Hz, 1H), 6.94 (d, J = 8.1 Hz, 3H), 6.85 (d, J = 8.2 Hz, 1H), 3.86 (s, 3H), 3.77 (s, 6H). MS(EI) (m/z) = 402$[M]^+$.

4-[(4,5-bis(4-methoxyphenyl)-1H-imidazol-2-yl)] benzoic acid (4d): $C_{24}H_{20}N_2O_4$ Color: pale yellow colored powder, yield 79 %. m.p.; 220-222 ◦C; FT-IR υmax cm^{-1} = 3483 (N–H), 3005 (C-H, arom.),1647 (C=N), 1253 (C–O). ^{1}H NMR (500 MHz, DMSO-d6) δ ppm: 12.77 (s, 1H, COOH), 12.48 (bs, 1H, NH), 8.21 (d, J = 8.3 Hz, 3H), 8.05 (d, J = 8.3 Hz, 3H), 7.48 (d, J = 8.3 Hz, 6H), 3.79 (s, 6H). MS(EI) (m/z) = 400$[M]^+$.

Enzymatic evaluation

Anti-α-amylase assay

The inhibitory activity of the α-Amylase enzyme was determined using the reference method [26] .A test tube containing (250 µL) of tested compound solution with various concentrations (50 - 250 µg/mL), (250 µL) of [1% (w/v)] starch solution and (250 µL) of (1U/mL) α-amylase solution. A (500 µL) of dinitro salicylic acid (color reagent) was added to the mixture after it had been incubated at 20°C for 3 minutes to stop the enzymatic process. A (250 µL) of α-amylase was added immediately to the mixture after it had been kept in hot water. The mixture was heated at 85°C for 15 minutes. Then, the solution was removed from the heating process and allowed to incubation for 5 minutes at room temperature. A (4500 mL) of distilled water was added to give a total volume of (6000 µL). The absorbance was determined at 540 nm using spectrophotometry. The control was prepared without test sample. Acarbose was utilized as reference drug, and the % inhibition was determined by equation (1)

% inhibition = [(Abs control- Abs sample) / Abs control] ×100…… eq. (1)

where Abs mean the absorbance.

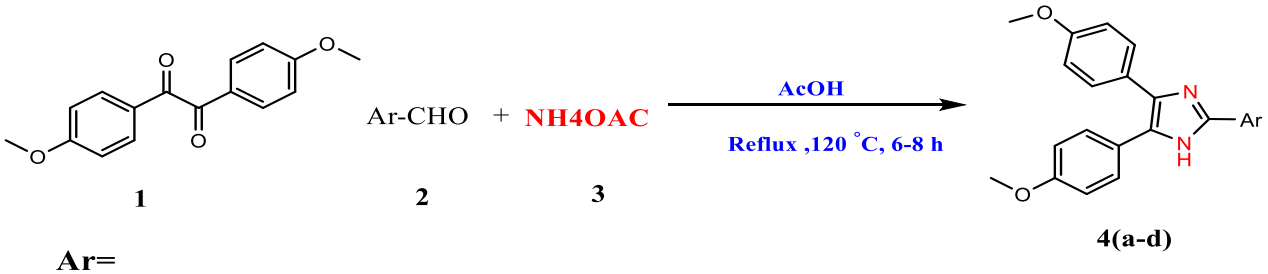
Anti- α-glucosidase assay

The inhibitory activity of α-glucosidase was examined using the described method [27] . Briefly, (35 µl) of phosphate buffer solution, (31 µL) of tested compound solution with different concentrations (50-250 µg/mL), and (18 µL) of [4-nitrophenyl-α-D glucopyranoside *(p-NPG)*] as a substrate were added to each 96-well plate and incubated in 37 °C for 5 minutes, (16 µL) of α-glucosidase (0.15 U/mL) which dissolved in sodium phosphate was added to each well to give a total volume of (100µl). A (100 µL) of sodium carbonate (200 mM) was added to the mixture to spot the reaction. A microplate reader was used to measure absorbance at 405 nm. The experiment was done in triplicate. The control was prepared without any tested compound. Acarbose was used as a standard reference drug for this assay. The %inhibition was calculated using equation (1).

Results and Discussion

Preparation and Spectral characterization of imidazole (4a-d) derivatives

Imidazole derivatives 4(a-d) were synthesized from 4,4'-dimethoxybenzil (**1**), different aromatic aldehyde (**2**) and NH_4OAC (**3**) in the presence of glacial acetic acid A_COH as catalyst and solvent for (6-8) h, giving the pure products with moderate to good yield, after recrystallization from hot ethanol (Table1), glacial acetic acid acts as a mild acid when used as a catalyst in organic synthesis and can activate carbonyl group (C=O) leading to increase the reactivity of the carbonyl compounds (Scheme 1); also, simple work-up, clean reaction profiles, inexpensive, readily available, stable under normal pressures and temperatures of the catalyst, make this methodology a valuable contribution to the current processes in the field of 2,4,5- tri-substituted imidazoles synthesis.



1 **2** **3** **4(a-d)**

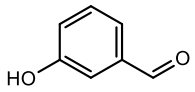
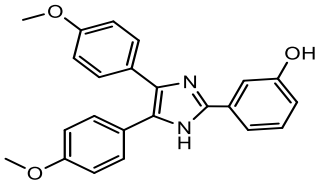
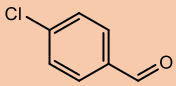
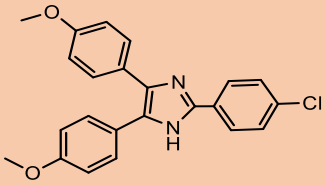
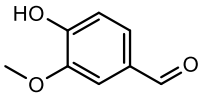
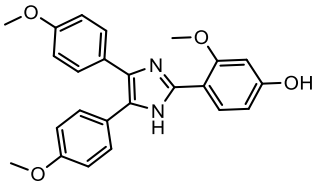
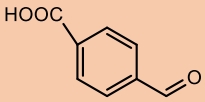
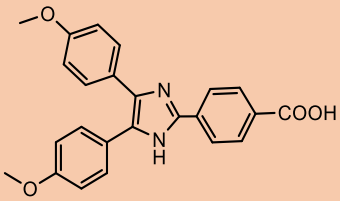
Ar=

a= 3-OHPh , **b**=4-ClPh, **c**=3-OCH_3, 4-OHPh, **d**= 4-COOHPh

Scheme 1 Synthesis of 2,4,5-tri substituted imidazole 4 (a-d)

Several techniques, such as FT-IR, ^{1}H-NMR, and mass spectroscopy, were used to characterize the four new compounds. (Figures 1-12). The FT-IR spectroscopy data give good evidence that all compounds have been successfully synthesized by appearing of characteristic ν (C=N) and (N-H) bands typically for all 4a-dg at (1647-1654) and (3302-3483) cm^{-1} respectively and disappearance of the strong bands which belong to C=O group in carbonyl compounds. The characteristic proton peak (NH-in imidazole ring) was detected in the ^{1}H NMR spectra for all compounds that promoted the synthesis of imidazole ring by the absence (NH_2) resonance, as well as the appearance of a broad singlet ranging from 12.28 to 12.77 ppm regions which belong to the resonance of the imidazole ring (–NH). The full spectral data (FT-IR, ^{1}HNMR and MS) and melting points of all imidazole derivatives are provided in the Experimental section.

Table 1 Synthesis of 2,4,5-trisubstituted imidazole 4(a-d) by using glacial acetic acid.

Entry	Aldehyde	Product 4(a-d)	Time (h.)	Yield (%)
4a			6	80
4b			7	82
4c			6	85
4d			8	79

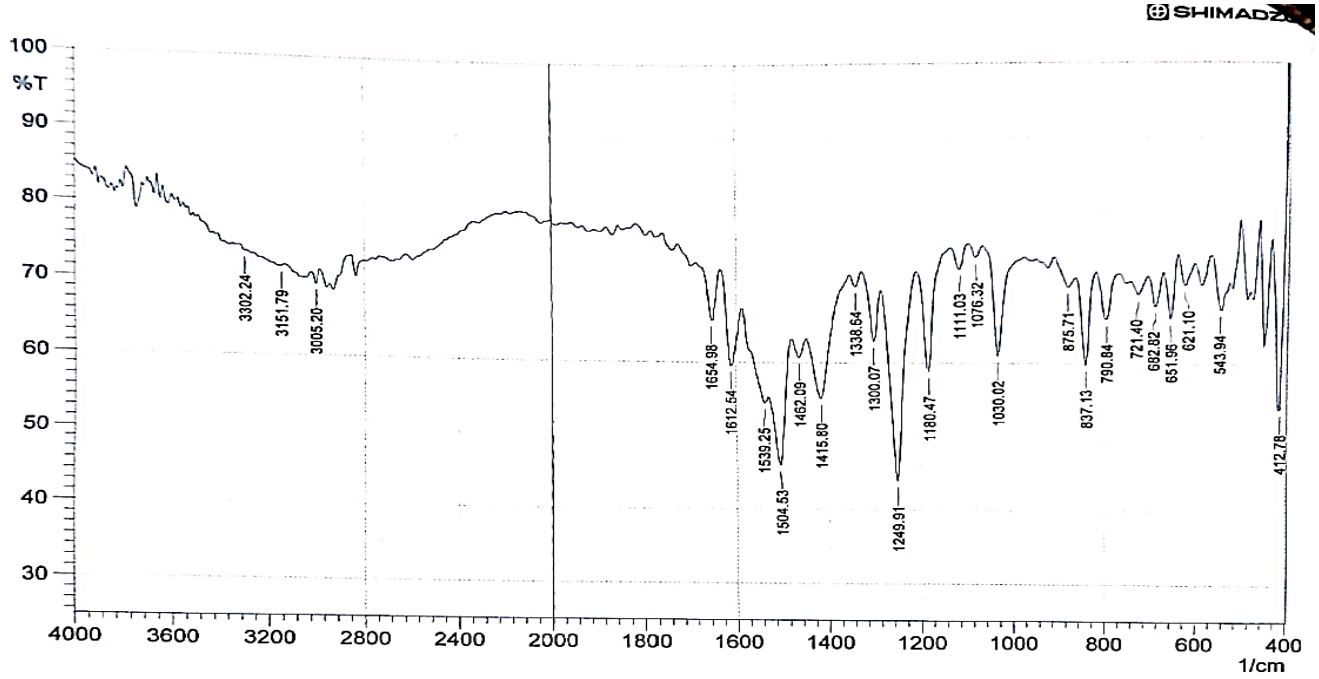


Figure (1): FT-IR spectrum of derivative (4c)

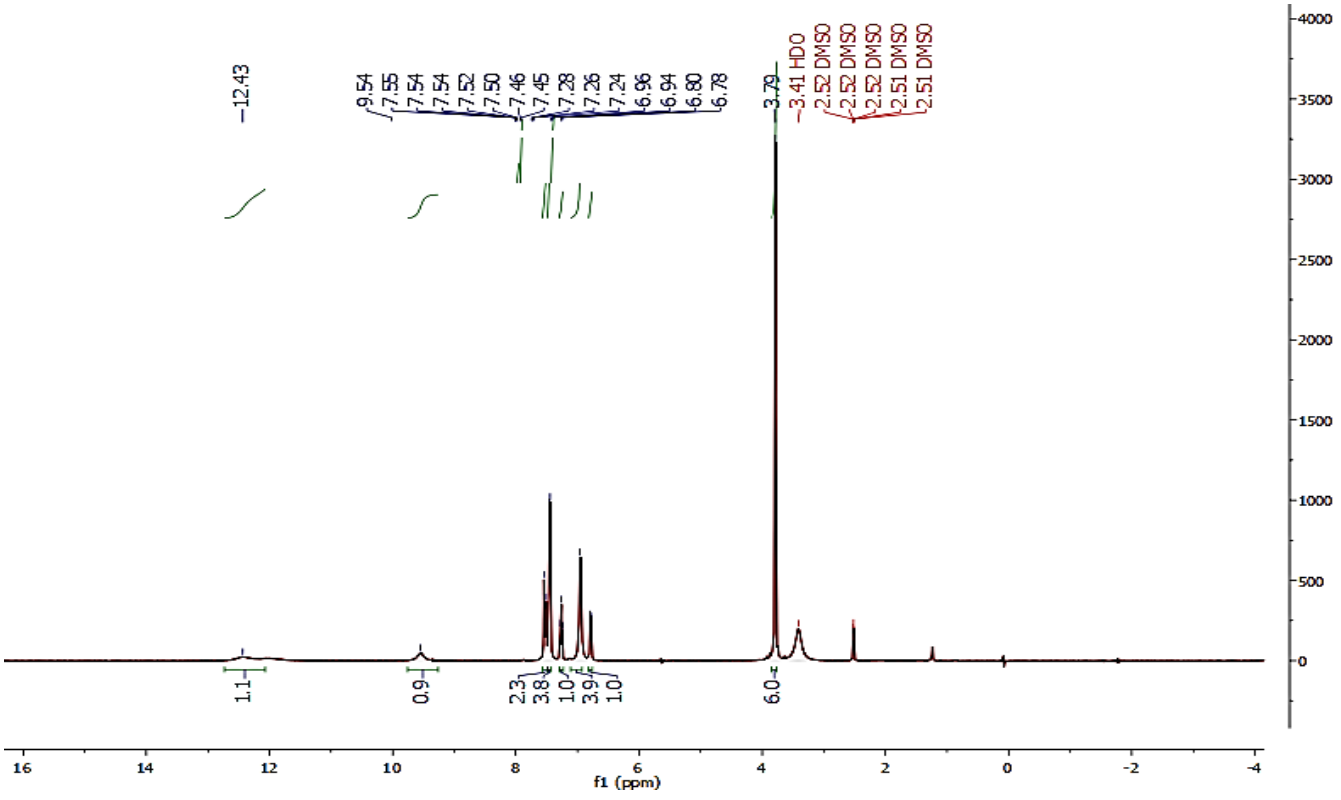


Figure (2): ^{1}HNMR (500 MHz, DMSO-d6) spectrum of derivative 4a

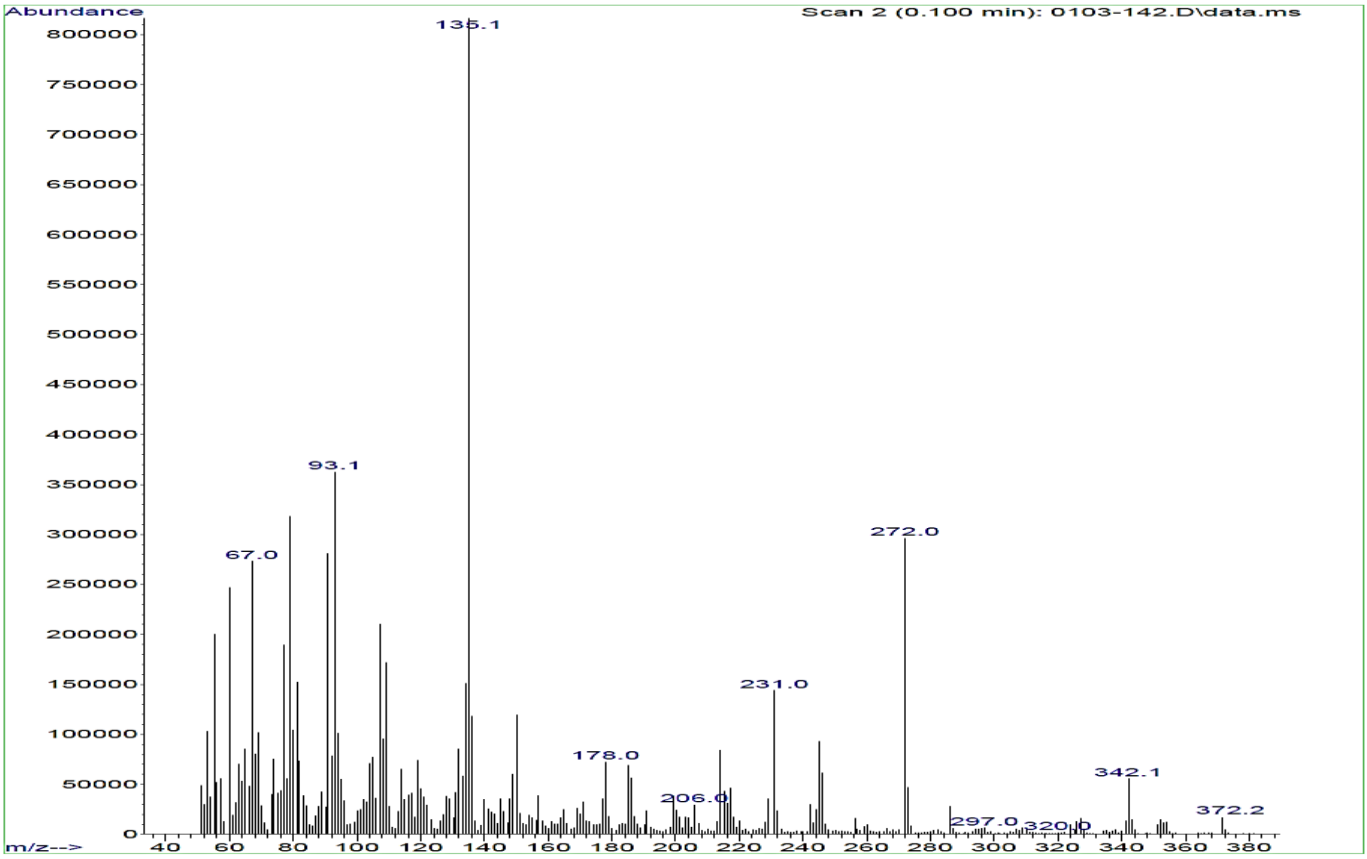


Figure (3): Mass spectrum of derivative 4a

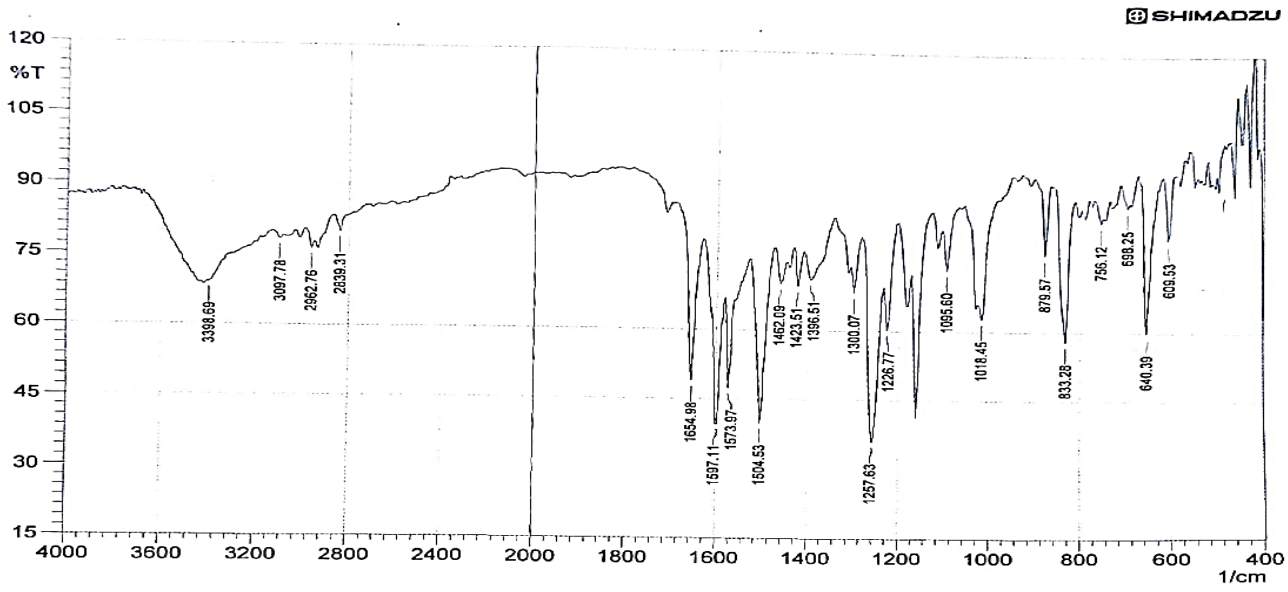


Figure (4): FT-IR spectrum of derivative 4b

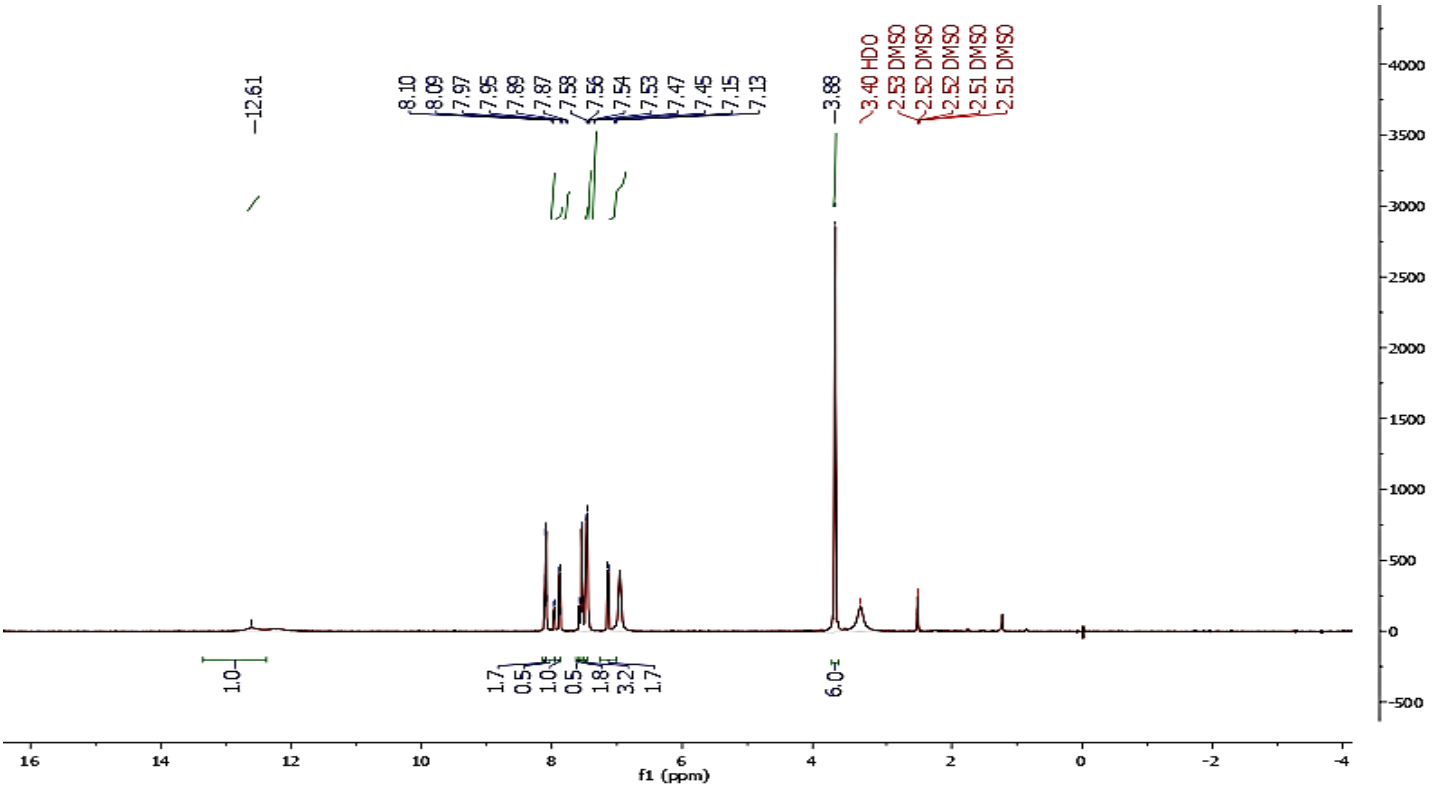


Figure (5): [1]HNMR (500 MHz, DMSO-d6) spectrum of derivative 4b

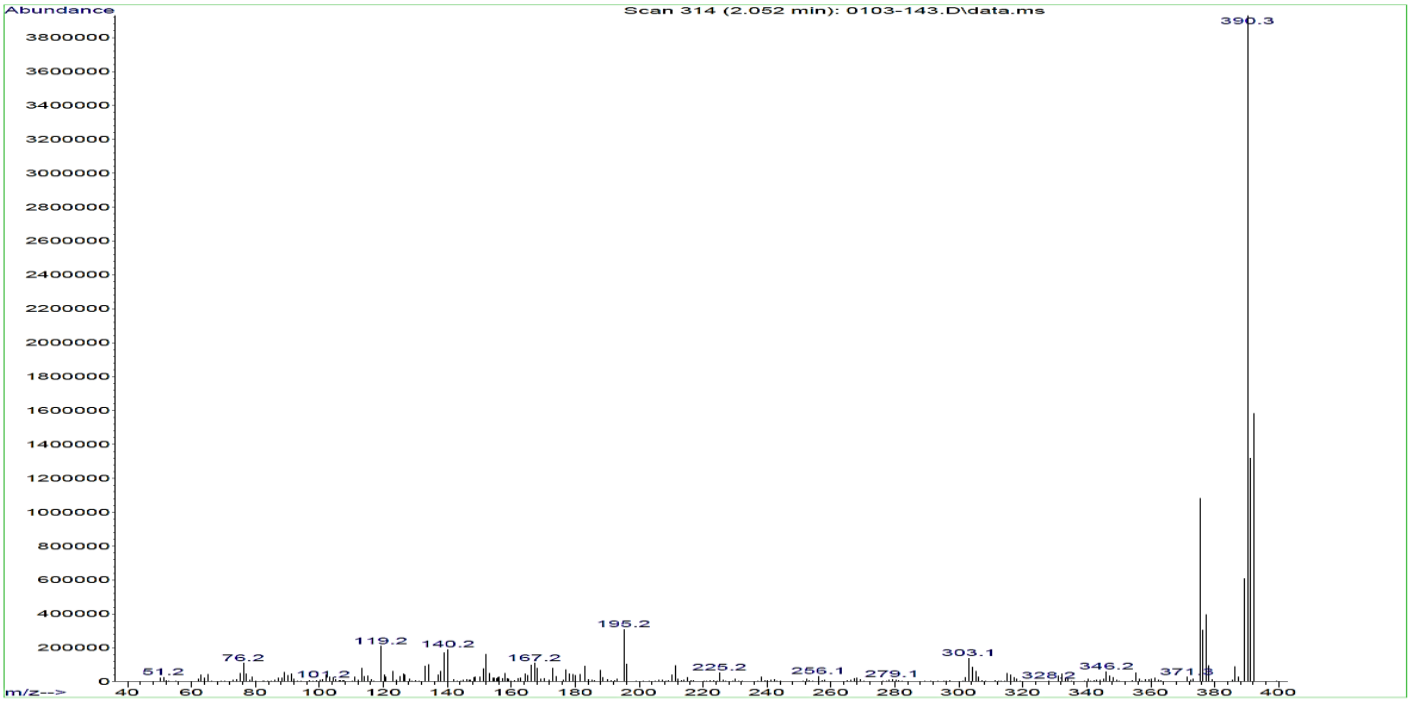


Figure (6): Mass spectrum of derivative 4b

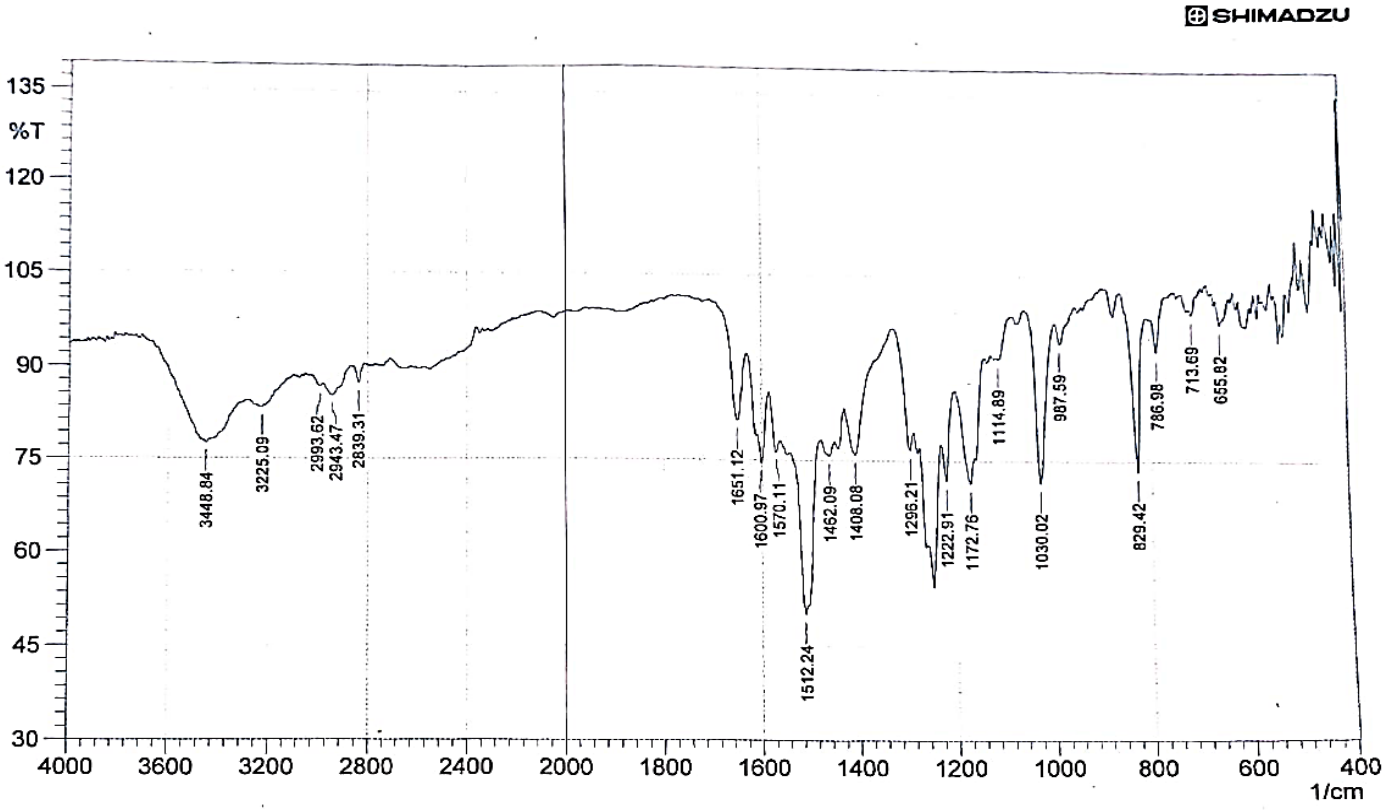


Figure (7): FT-IR spectrum of derivative 4c

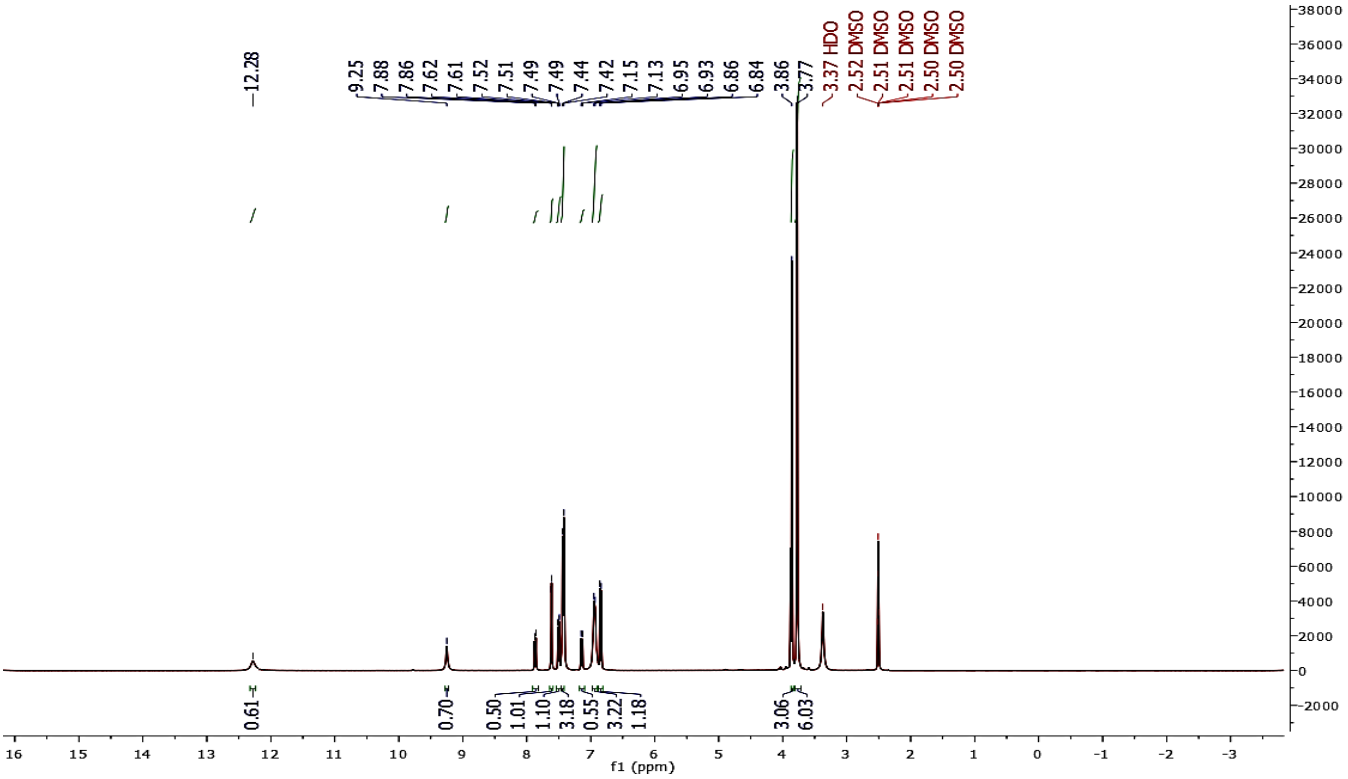


Figure (8): ^{1}HNMR (500 MHz, DMSO-d6) spectrum of derivative 4c

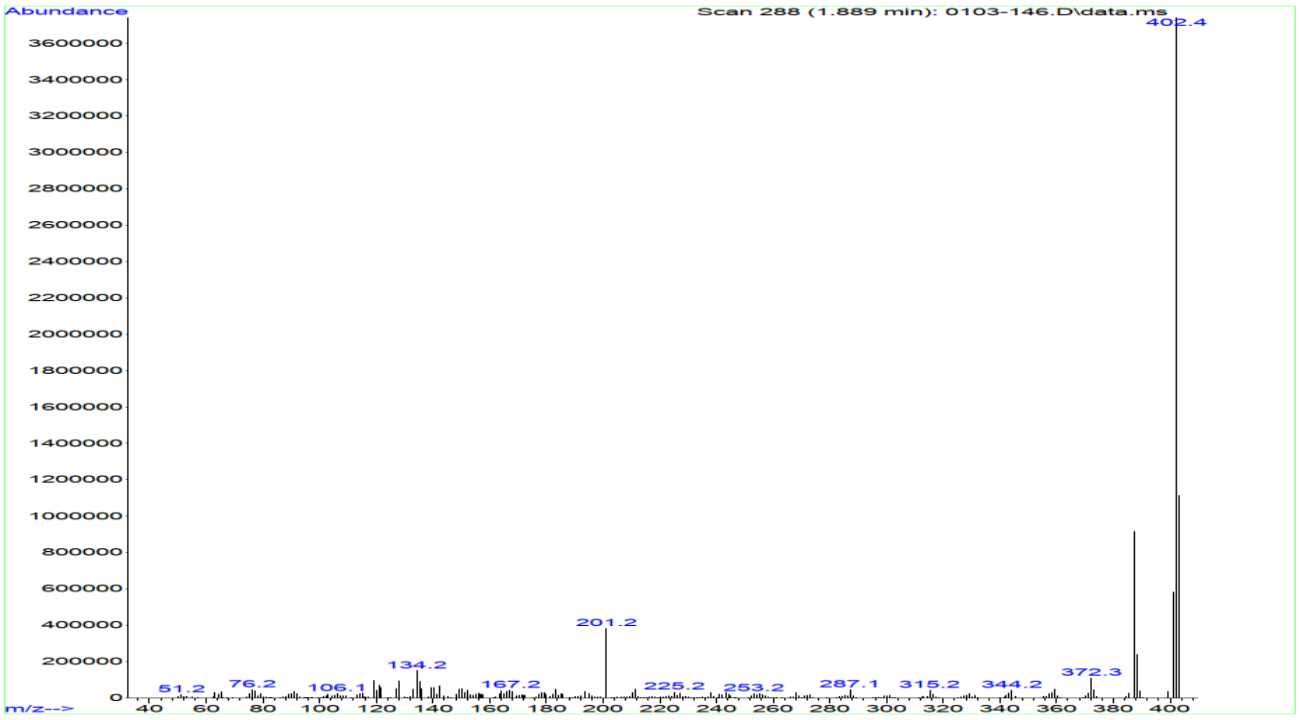


Figure (9): Mass spectrum of derivative 4c

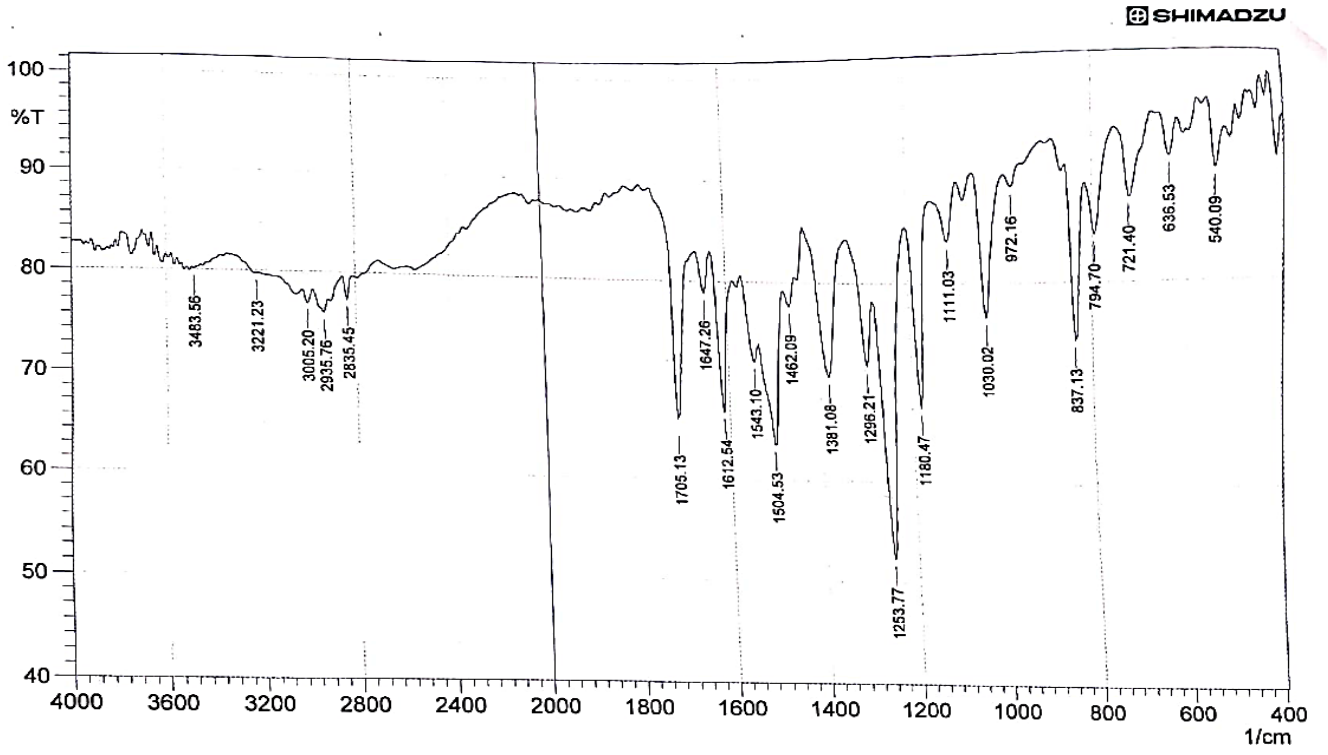


Figure (10): FT-IR spectrum of derivative 4d

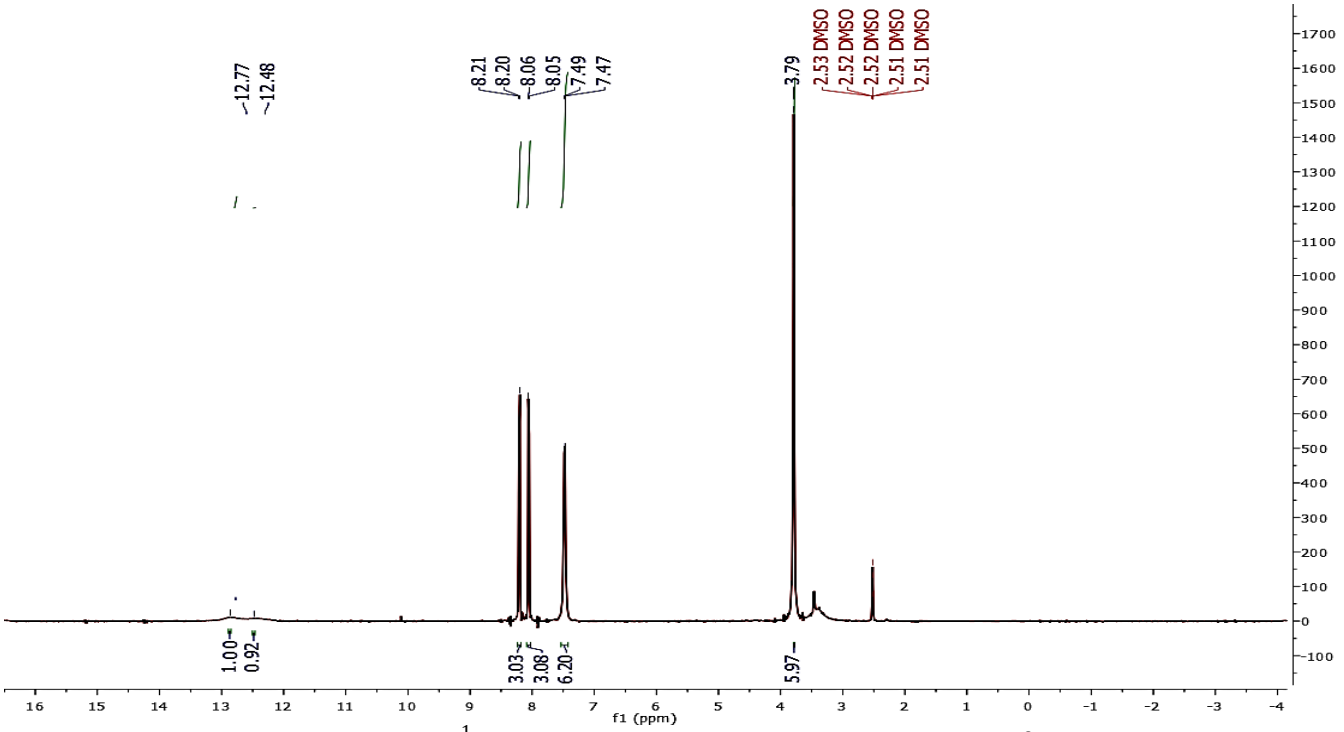


Figure (11): ^{1}HNMR (500 MHz, DMSO-d6) spectrum of derivative 4d

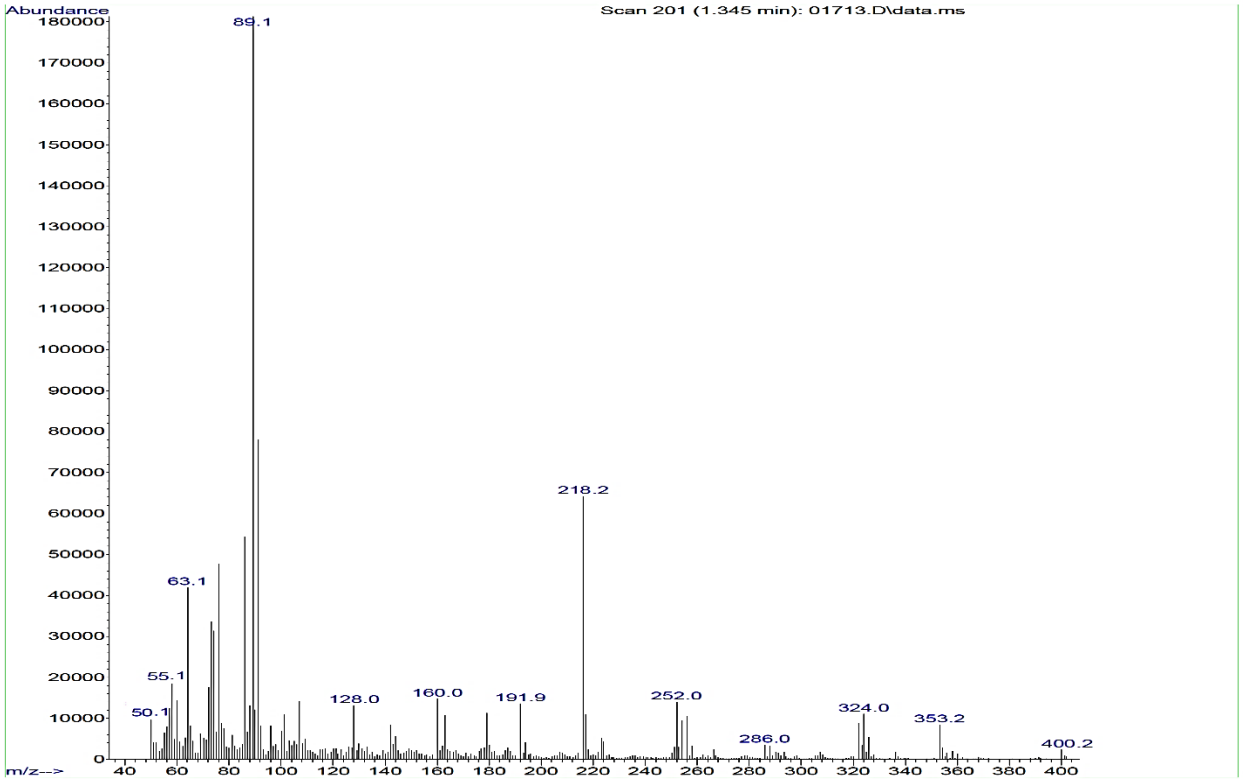


Figure (12): Mass spectrum of compound 4d

Evaluation of α-amylase and α-glucosidase Assy

The *in vitro* inhibitory activity of compounds (4a, 4b, 4c and 4d) was evaluated spectrophotometrically at 540 nm for α-amylase and 410 nm for α-glucosidase. The aim of diabetes therapy is to decrease postprandial hyperglycemia by inhibiting the digestive enzymes responsible for carbohydrate hydrolysis (alpha-amylase and alpha -glucosidase), glucose absorption is reduced. Inhibitors of these enzymes slow and prolong the digestion of carbohydrates, which in turn reduces the rate of glucose absorption and, as a result, reduces the increase in plasma glucose levels that occurs after a meal. In this study, inhibitory activity of tested compounds for α-amylase and α-glucosidase was examined and the findings are shown in Tables (2) and (3), α-amylase inhibitory studies demonstrated that all samples have excellent to good inhibitory activity between 51% to 90% compared with the acarbose as a standard drug Table (1). The most potent active compound was 4d with 90% at highest concentration (250 mg/mL), which has substituent 4-COOH, while compounds 4a,4b and 4c with substituents 4-OH, 4-Cl and 4-$OH_{,3}OCH_3$, respectively exhibited 64% ,75% and 70% inhibitory activity.

In α-glucosidase assay, the results showed the reference drug acarbose exhibited the highest α-glucosidase inhibition (66%) followed by compounds 4b, 4d and 4a (55%), (52%) and (51%) respectively at their highest concentrations. Figures 1 and 2 show the percentage of α-amylase and α-glucosidase inhibition of the four tested compounds as a function of concentration compared with the acarbose.

Table 2: The percentage of inhibition for α-amylase using of 4a, 4b, 4c and 4d at different concentrations

Concentration (µg /ml)	% Inhibition 4a	% Inhibition 4b	% Inhibition 4c	% Inhibition 4d	% Inhibition acarbose
50	53	55	51	54	32
100	55	66	54	59	42
150	59	67	60	80	55
200	61	69	61	87	59
250	64	75	70	90	63

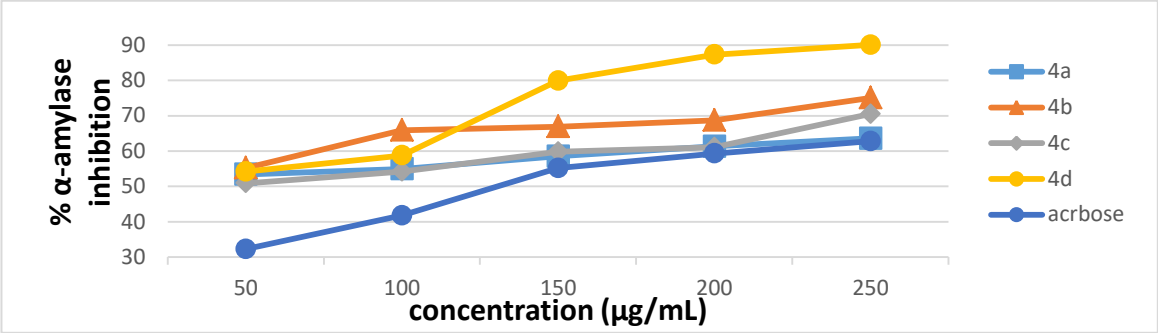


Figure (1) The % inhibition of the α-amylase enzyme by 4a-d and acarbose as a positive standard drug

Table 3: The percentage of inhibition for α -glucosidase using 4a, 4b, 4c and 4d at different concentrations

Concentration (µg /ml)	% Inhibition 4a	% Inhibition 4b	% Inhibition 4c	% Inhibition 4d	% Inhibition acarbose
50	24	26	22	23	43
100	26	31	24	26	47
150	32	36	28	29	54
200	43	47	34	43	61
250	51	55	38	52	66

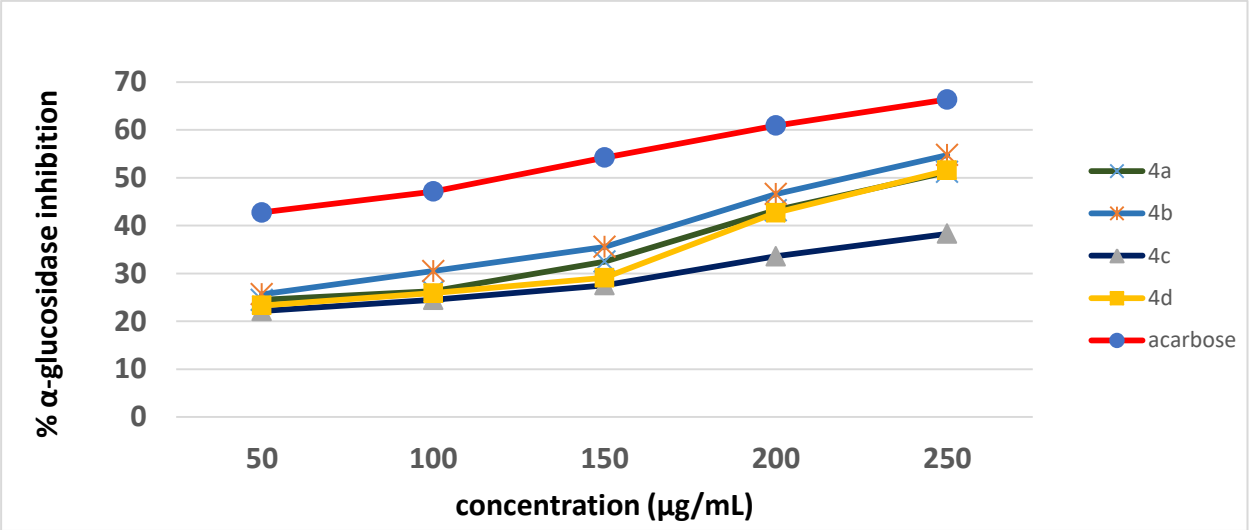


Figure (2) The % inhibition of the α -glucosidase enzyme by 4(a-d) and acarbose as a positive standard drug

The current investigation indicates that 4b and 4d may be effectively treat postprandial hyperglycemia. The tested compounds showed a slightly lower inhibition of α -glucosidase enzyme as compared to α -amylase enzyme. The activity of examined compounds against these enzymes may be because of the presence of free imino hydrogen inside imidazole ring [28] also, may be due to different substituent found in various position of imidazole derivatives are responsible for the antidiabetic inhibition activity[29]

Conclusion

In summary, a total of four new imidazole derivatives were prepared with good to excellent yields. Spectral analysis by Fourier - transform infrared spectroscopy (FT-IR), nuclear magnetic resonance (^{1}HNMR), and mass, among the tested compounds 4d showed the strongest effectiveness against α-amylase and α-glucosidase activity when compared to the other compounds in the presence of the reference drug spectrometry (EI) were used to characterize them. All of synthesized compounds were evaluated for anti-hyperglycemic activity by α-amylase and α-glucosidase inhibition using the *in vitro* method.

Reference

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