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New Barbiturate Derivatives as Potent *in vitro* α-Glucosidase Inhibitors

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Abstract

A series of new Schiff base derivatives were synthesized by reaction barbiturate derivatives (barbital and phenobarbital) with some aromatic aldehydes. Barbital and phenobarbital were treated with formaldehyde in ethanol as solvent to produce **a** and **b**. Then reaction compounds **a** or **b** with paratoluenesulfonylchloride in DCM and presence triethylamine to form (1,3(2H,4H)-diyl)bis(methylene)bis(4-methylbenzene sulfonate) barbiturate **1a** and **1b**. Nucleophilic substitution reaction of compound **1a** or **1b** with sodium amide or with hydrazine hydrate to form barbiturate derivatives contain free amino group**2a, 3a,2b** and **3b**. barbiturate derivatives which contain free amino or hydraznyl group reacted with some aromatic benzaldehyde to preparation final products **1-8** (Schiff base derivatives). The structures of the prepared derivatives were identified by many spectroscopic methods such as Mass, NMR, FTIR spectroscopy and the elemental analysis(C,H,N). The end products were evaluated *in vitro* α-glucosidase inhibitory activity. All Schiff base derivatives were showed α-glucosidase inhibition with IC_{50} values 110 ± 2.15, 197 ± 3.11, 38 ± 0.84, 64 ± 1.78, 119 ± 3.55, 204 ± 2.08, 32 ± 1.42, 81 ± 2.23 µM respectively, when compared to the standard drug acarbose (IC_{50} =787.27 ± 2.23 µM).

Keywords: α-Glucosidase inhibitors, Schiff base, Barbital, Phenobarbital, Diabetes, Acarbose**.**

1. Introduction

Inhibitors of α-Glucosidase are the drugs used to treat high blood sugar in type 2 diabetes [1,2]. Alpha-glucosidase inhibitors are used to maintain elevated glycemic control along with other insulin secretagogues [3]. Insulin resistance and/or absolute or relative deficiency of insulin secretions leads to hypo glycaemia, which is associated with diabetes [4,5]. In 2025, it is estimated that diabetes will affect about 300 million people worldwide and therefore there is an urgent need to develop improved treatments for this chronic disorder [6,7]. One of the ways to manage this disease is to control the activity of α-glucosidase. This enzyme is responsible for splitting α-1-4 bond in polysaccharides, oligo-saccharides and disaccharides into monosaccharide's mainly glucose [8-11]. Barbiturates are an inevitable class of medically heterocyclic compounds, and it's have a wide range of many biological activity and medicinal interest including sedative, antimicrobial, antihypertensive, anticonvulsant and anesthetic [12,13] antioxidants [14], hypnotics [15], anticancer [16-17] and tyrosinease inhibitors[18]. A review of literature also revealed that barbiturates shows anti-diabetic properties [19]. Acarbose is distinguished α-glucosidase inhibitors used for controlling of diabetes mellitus. Unfortunately, these distinguished and clinically used inhibitors have also numerous side effects. Subsequently, there is still needed to develop safer therapy. Despite of a broad spectrum of biological importance of barbiturates and Schiff base[20-22], it is occasionally evaluated for α-glucosidase activity. In this study, successfully prepared a series of new Schiff base compounds based on barbital and phenobarbital and determined *in vitro* α-glucosidase inhibitory activity.

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2. Experimental part

2.1. General consideration

All chemicals and solvents were obtained from Merck, BDH, Fluke Chemicals Companies and commercial sources were used without further purification. IR spectra was recorded by using FT-IR Bruker ALPHA FT-IR, Faculty of Science, University of Kufa. Elemental analysis was performed using a Perkin- Elmer 204E Instrument, University of Babylon. ^{1}H and ^{13}C NMR were obtained in DMSO on the Bruker spectrometer (300 MHz for ^{1}H NMR and 75 MHz for NMR ^{13}C, respectively), Mashhad University, Iran, Mashhad. Melting points were measured using Electro Thermal Melting Point, UK. Glass TLC 1020GS with silica gel 60, thickness 0.25, size 10x20cm. The chromatograms were visualized under UV 254-366nm and iodine. Measurements of pH were carried out by using pH-meter Hanna. Absorbance was recorded by appel spectrometer , Japan. Mass spectra were recorded on LC/MS/MS system, model CBM-20A, SHIMADZU, Japan, Mashhad University, Iran, Mashhad.

2.2. Synthesis of 1,3-bis(hydroxymethyl) barbiturate (a, b): A mixture of barbital or phenobarbital (0.02 mol) and formaldehyde (0.04 mol) in ethanol (50 mL) with (2 mL) D.W. was stirred at 80 ºC for 6-7 hours. The stirring continued and the progress is monitored using TLC. Ethanol was evaporated at room temperature. The solid product was slurred in cold water (100 mL), stirred for 1 hour and then the solid product was separated by filtration. The crystalline product was washed with cold water (3X50 mL) and then dried in the oven, resulting in a pure product.

2.2.1. 1,3-bis(hydroxymethyl)barbital (a): It was prepared as a white crystalline, Chemical formula: $C_{10}H_{16}N_2O_5$; yield 92%; m p: 93-95 ºC; FTIR spectrum, ν, cm^{-1}: 3427 (OH), 1751, 1666 (C=O); ^{1}H NMR (300 MHz, DMSO-*d6*)δ ppm 6.47 (s, 2H,OH), 5.23 (s,4H,$2CH_2$), 1.96 (q,4H,$2CH_2$, *J*=7.2 Hz), 0.92 (t,6H, $2CH_3$, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO-*d6*)δ ppm 173.42, 151.51, 69.05, 58.63, 28.07, 10.22.

2.2.2. 1,3-bis(hydroxymethyl)phenobarbital (b): It was prepared as a white powder, Chemical formula: $C_{14}H_{16}N_2O_5$; yield 87%; m p: 87-89 ºC; FTIR spectrum, ν, cm^{-1}: 3511(OH), 1704,1664(C=O);^{1}H NMR (300 MHz, DMSO-*d6*): δ ppm 7.49-7.30 (m, Ar-H), 4.94 (s,4H, $2CH_2$), 2.14 (q, 2H, CH_2, *J*=7.2 Hz), 0.80 (t,3H, CH_3, *J*=7.2 Hz);^{13}C NMR (75 MHz, DMSO-*d6*): δ ppm 172.41, 151.29, 135.12, 130.10, 129.49, 128.40, 126.52, 123.17, 69.62, 60.28, 28.64, 10.28.

2.3. Synthesis of (1,3(2H,4H)-diyl)bis(methylene) bis(4-methylbenzenesulfonate) barbiturate (1a,1b)

To a solution of **a** or **b** (0.0122 mol) and p-toluenesulfonyl chloride (0.0244 mol) were refluxed in (50 mL) dichloromethane with (3.4 mL) triethylamine for 4-5 hours. The stirring continued and the progress is monitored using TLC. After reaction complete (50 mL) of water was added for extraction, the organic layer was separated and the solvent was evaporated at room temperature. The precipitate product was washed with water (3X20mL), dried in the oven to give desired compounds **1a** and **1b**.

2.3.1. 1,3(2H,4H)-diyl)bis(methylene)bis(4-methyl benzene sulfonate) barbital (1a) : It was prepared as a light white solid, Chemical Formula: $C_{24}H_{28}N_2O_9S_2$; yield 77% ; m p: 61-63 ºC; FTIR spectrum, ν, cm^{-1}: 3091(Ar-H), 1759, 1696(C=O; ^{1}H NMR (300 MHz, DMSO-*d6*): δ ppm 7.40 -7.23 (m, Ar-H), 4.57 (s, 4H, $2CH_2$), 2.43 (s, 6H,$2CH_3$), 1.96 (q, 4H,$2CH_2$, *J*=7.2 Hz), 0.84 (t, 6H, $2CH_3$, *J*=7.2 Hz); ^{13}C NMR (75 MHz, DMSO-*d6*): δ ppm 173.09, 151.80, 134.41, 131.35, 129.29, 129.13, 128.64, 125.62, 122.56, 68.34, 56.50, 28.98, 22.68,10.87.

2.3.2. Synthesis of (1,3(2H,4H)-diyl)bis(methylene) bis(4-methylbenzenesulfonate) phenobarbital (1b): It was prepared as a light white solid, Chemical Formula: $C_{28}H_{28}N_2O_9S_2$; yield 80%; m p: 98-100 ºC; FTIR spectrum, ν, cm^{-1}: 3099 (Ar-H),1699 (C=O);^{1}H NMR (300 MHz, DMSO-d_6) : δ ppm 7.69- 7.36 (m, Ar-H), 4.68 (s,4H, $2CH_2$), 2.44 (s, 6H, $2CH_3$), 2.05 (q,2H, CH_2, *J*=7.2 Hz), 0.86 (t,3H, CH_3, *J*=7.2 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm 172.58, 151.19, 136.22, 134.95, 133.45, 131.85, 129.38, 128.48, 126.61, 125.86, 69.97, 60.26, 28.98, 22.25, 10.16.

2.4. Synthesis of 1,3-bis(hydrazineylmethyl) barbiturate (2a,2b)

A mixture of **1a** or **1b** (0.0036 mol) and (0.0072 mol) (0.22 mL) of Hydrazine hydrate 99% were refluxed in (50 mL) of methanol for 7 hours. The reaction course was monitored by TLC. After reaction complete a (25 mL) of water was added for extraction. The mixture was evaporated to dryness and the residue was partitioned between $CHCl_3$ (3X15 mL). Subsequently, the organic phase was dried by adding anhydrous sodium sulfate and then filtered, the organic layer was evaporated. The precipitate product was washed with water (3X20 mL), then the residue was dried and purified by recrystallization from ethanol.

2.4.1. 1,3-bis(hydrazineylmethyl) barbital (2a) : It was prepared as a light white solid, Chemical formula: $C_{10}H_{20}N_6O_3$; yield 82%; m p: 112-114 ºC; FTIR spectrum, ν, cm^{-1}: 3380,3249 (NH, NH_2), 1762,1673 (C=O; ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 7.19 (s, 2H, NH), 5.45 (s,4H, $2CH_2$), 4.91 (s,4H, NH_2), 2.00 (q,4H, $2CH_2$, *J*=7.2 Hz), 1.00 (t,6H, $2CH_3$, *J*=7.2 Hz).^{13}C NMR (76 MHz, DMSO-d_6): δ ppm 172.75, 152.30, 68.18, 57.87, 27.93, 10.88.

2.4.2. 1,3-bis(hydrazineylmethyl)phenobarbital (2b): It was prepared as a light white solid, Chemical formula: $C_{14}H_{20}N_6O_3$; yield 79%; m p: 113-115 ºC; FTIR spectrum, ν, cm^{-1}:3353, 3255 (NH, NH_2),1697, (C=O); ^{1}H NMR (300 MHz, DMSO-*d6*): δ ppm 7.64 -7.49 (m,Ar-H), 7.19 (s,2H,2NH), 5.40 (s, 4H, $2CH_2$), 4.87 (s,4H, $2NH_2$), 2.09 (q,2H, CH_2, *J*=7.2 Hz), 0.83 (t,3H, CH_3, *J*=7.2 Hz); ^{13}C NMR (75 MHz, DMSO-d_6):δ ppm 172.58, 151.19, 135.08, 129.38, 128.48, 126.61,67.98, 60.26, 28.98, 10.16.

2.5. Synthesis of 1,3-bis(aminomethyl) barbiturate (3a,3b)

A mixture of **1a** or **1b** (0.0018 mol) and (0.0036 mol) of sodium amide were refluxed in (50 mL) of 2:6 dry DMF and chloroform for 5 hours. The reaction course was monitored by TLC. After reaction complete a (25 mL) of water was added for extraction. The mixture was evaporated to dryness and the residue was partitioned between $CHCl_3$ (3X15 mL). The organic layer was evaporated. The precipitate product was washed with water (2X20 mL), then the residue was dried and purified by recrystallization from ethanol.

2.5.1. 1,3-bis(aminomethyl) barbital (3a) :It was prepared as a light white solid, Chemical formula: $C_{10}H_{18}N_4O_3$; yield 77%; m p: 65-67 ºC; FTIR spectrum, ν, cm^{-1}:3354, 3252 (NH_2), 1724, 1680(C=O); ^{1}H NMR (300 MHz, DMSO-*d6*): δ ppm 5.55 (s,4H, $2CH_2$), 4.63 (s,4H,NH_2), 1.99 (q,4H, $2CH_2$, *J*=7.2 Hz), 0.98 (t,6H, $2CH_3$, *J*=7.2 Hz); ^{13}C NMR (76 MHz, DMSO-d_6): δ ppm 173.05,152.49, 67.98, 58.17, 28.58, 9.98.

2.5.2. 1,3-bis(aminomethyl)phenobarbital (3b) : It was prepared as a light white solid, Chemical formula: $C_{14}H_{18}N_4O_3$; yield 79%; m p: 69-71 ºC; FTIR spectrum, ν, cm^{-1}: 3356, 3255 (NH_2), 1696(C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 7.81-7.47 (m, Ar-H), 5.54 (s,4H, $2CH_2$), 4.87 (s,4H,NH_2), 2.03 (q,2H, CH_2, *J*=7.2 Hz), 1.02 (t, 3H,CH_3, *J*=7.2 Hz); ^{13}C NMR (76 MHz, DMSO-d_6): δ ppm 172.11, 153.64, 135.77, 130.69, 129.71, 126.21, 64.42, 56.58, 28.95,10.75.

2.6. General method for Synthesis of Schiff base (1-8)

A mixture (0.0122 mol) of barbiturate derivatives which contain free amino or hydraznyl group with aromatic benzaldehyde (0.0244 mol) in methanol (40 mL) with some drops of glacial acetic acid was refluxed for 5-7 hours at 80ºC. The reaction course was monitored by TLC. Then it was extracted with (75 mL) of diethyl ether and then the organic phase was treated with (100 mL) of water. Subsequently, the diethyl ether phase was dried by adding anhydrous sodium sulfate and then filtered. The diethyl ether was evaporated and then the residue was dried and purified by recrystallization from ethanol.

2.6.1. 1,3-bis((2-((-4-hydroxybenzylidene)hydrazineyl)methyl)phenobarbital (1): It was prepared as a yellow solid, yield 88%; m p: 95-97 ºC; FTIR spectrum, ν, cm^{-1}: 3345 (OH),1707(C=O), 1646 (C=N) , 588(C=C); ^{1}H NMR (300 MHz, DMSO-*d6*): δ ppm 9.22 (s,2H,2OH), 8.31 (s,2H,2N=CH), 7.82-7.31 (m, Ar-H), 7.37 (s,2H,2NH), 5.70 (s,4H,$2CH_2$), 1.98 (q,2H, CH_2, *J*=7.2 Hz), 0.98 (t,3H, CH_3, *J*=7.2 Hz); ^{13}CNMR (75 MHz, DMSO-d_6): δ ppm 174.08, 154.52,151.80,143.27,136.47,134.87, 131.92, 129.21, 127.19, 124.97, 66.85, 57.07, 27.93, 10.44. Mass spectrum: *m*/*z* 528.3 $[M]^+$. Anal. Calc. for $C_{28}H_{28}N_6O_5$: C, 63.63; H, 5.34; N, 15.90. found: C, 63.61; H, 5.32; N, 15.88.

2.6.2. 1,3-bis(((-4-hydroxybenzylidene)amino) methyl)phenobarbital (2): It was prepared as a light

yellow solid, yield 87%; m p: 108-110 ºC; FTIR spectrum, ν, cm^{-1}: 3324(OH), 3077(Ar-H),1698 (C=O), 1611(C=N), 1595(C=C); ^{1}HNMR (300 MHz, DMSO-*d6*): δ ppm 9.01 (s,2H,2OH), 8.18 (s,2H, 2N=CH),7.80-7.32 (m,Ar-H), 5.56 (s,4H,2CH_2), 1.99 (q,2H, CH_2,*J*=7.2 Hz), 0.99 (t,3H, CH_3, *J*=7.2 Hz,); ^{13}C NMR (76 MHz, DMSO-d_6): δ ppm 173.76, 154.98, 151.70, 144.14, 135.88, 132.47, 130.07, 129.67, 127.55, 125.65, 123.59, 68.18, 59.12, 27.92, 10.18. Mass spectrum: *m*/*z* 498.02 [*M*]$^+$. Anal. Calc. for $C_{28}H_{26}N_4O_5$: C, 67.46; H, 5.26; N, 11.24; found: C, 66.38; H, 5.11; N, 11.01.

2.6.3. 1,3-bis((2-((-4-bromobenzylidene)hydrazineyl)methyl)phenobarbital (3): It was prepared as a light orang solid, yield 84%; m p: 88-90 ºC; FTIR spectrum, ν, cm^{-1}: 3190(NH), 1693(C=O), 1621 (C=N), 1589(C=C); ^{1}H NMR (300 MHz, DMSO-*d6*): δ ppm 8.20 (s,2H,2N=CH), 7.88-7.35 (m,Ar-H), 7.31 (s,2H,2NH), 5.61 (s,4H,2CH_2), 1.98 (q,2H, CH_2, *J*=7.2Hz), 1.01 (t,3H, CH_3,*J*=7.2 Hz); ^{13}C NMR (76 MHz, DMSO-*d*6): δ ppm 173.15, 151.51, 143.74, 135.89, 133.17, 130.68, 129.01, 126.95, 124.97, 122.54, 66.34, 58.37, 27.84, 10.67. Mass spectrum: *m*/*z* 654.2 [*M*]$^+$. Anal. Calc. for $C_{28}H_{26}Br_2N_6O_3$: C, 51.39; H, 4.01; N, 12.84. found: C, 51.35; H, 4.01; N, 12.82.

2.6.4. 1,3-bis((((-4-bromobenzylidene)amino) methyl)phenobarbital(4): It was prepared as a red solid, yield 82%; m p: 77-79 ºC; FTIR spectrum, ν, cm^{-1}: 3084(Ar-H),1705 (C=O), 1666(C=N), 1591 (C=C); ^{1}H NMR (300 MHz, DMSO-*d6*): δ ppm 8.83 (s,2H,2N=CH), 7.85-7.39 (m, Ar-H), 5.61 (s,4H, 2CH_2), 2.00 (q,2H, CH_2, *J*=7.2 Hz), 1.00 (t,3H, CH_3,*J*=7.2 Hz); ^{13}C NMR (76 MHz, DMSO-d_6): δ ppm 173.32, 151.41, 144.09, 135.94, 133.48, 131.99, 129.87, 128.11, 127.15, 125.26, 122.88, 67.79, 58.18, 27.57, 10.38. Mass spectrum: *m*/*z* 624.2 [*M*]$^+$. Anal. Calc. for $C_{28}H_{24}Br_2N_4O_3$: C, 53.87; H, 3.87; N, 8.97. found: C, 52.93; H, 3.78; N, 8.78.

2.6.5. 1,3-bis((2-((4-hydroxybenzylidene)hydrazineyl)methyl)barbital (5):It was prepared as a yellow solid, yield 85%; m p: 101-103 ºC; FTIR spectrum, ν, cm^{-1}: 3315(OH) ,1702(C=O), 1642 (C=N), 1596(C=C); ^{1}H NMR (300 MHz, DMSO-*d6*): δ ppm 9.22 (s,2H,OH), 8.31 (s,2H,2N=CH), 7.88-7.43 (m, Ar-H), 7.38 (s,2H,2NH), 5.70 (s,4H, CH_2), 1.98 (q,4H,2CH_2, *J*=7.2 Hz), 0.98 (t,6H, 2CH_3, *J*=7.2 Hz); ^{13}CNMR (76 MHz, DMSO-*d*6): δ ppm 172.75, 156.81,151.34, 143.77,130.41, 127.49, 123.38,68.17 ,57.87, 28.49,10.88. Mass spectrum: *m*/*z* 480.3 [*M*]$^+$. Anal. Calc. for $C_{24}H_{28}N_6O_5$; C, 59.99; H, 5.87; N, 17.49. found: C, 59.94; H, 5.84; N, 17.46.

2.6.6. 1,3-bis((((-4-hydroxybenzylidene)amino) methyl)barbital(6): It was prepared as a light red solid, yield 88%; m p: 89-91 ºC; FTIR spectrum, ν, cm^{-1}:3342(OH),1722 (C=O),1645(C=N), 1599 (C=C); ^{1}H NMR (300 MHz, DMSO-*d6*): δ ppm 9.22 (s,2H, 2OH), 8.61 (s,2H,2N=CH), 7.75-7.39 (m, Ar-H), 5.46 (s,4H,2H_2), 1.99 (q,4H, 2CH_2, *J*=7.1Hz), 0.97 (t,6H, CH_2, *J*=7.2 Hz); ^{13}C NMR (76 MHz, DMSO-*d6*): δ ppm 173.05,155.08,151.32,143.44, 131.48, 128.58, 125.27, 67.98, 58.17, 28.41, 9.98. Mass spectrum: *m*/*z* 450.1 [*M*]$^+$. Anal. Calc. for $C_{24}H_{26}N_4O_5$: C, 63.99; H, 5.82; N, 12.44. found: C, 62.92; H, 5.52; N, 12.23.

2.6.7. **1,3-bis((2-((-4-bromobenzylidene)hydrazineyl)methyl)barbital(7):**It was prepared as a brouwn solid, yield 84%; m p: 91-93 ºC; FTIR spectrum, ν, cm^{-1}: 3091(Ar-H),1697(C=O),1615 (C=N),1597(C=C); ^{1}H NMR (300 MHz, DMSO-*d6*): δ ppm 8.29 (s,2H,2N=CH), 7.90-7.39 (m, Ar-H), 7.41 (s,2H,2NH), 5.5746 (s,4H, 2CH_2), 1.96 (q,4H, 2CH_2, *J*=7.2 Hz), 0.98 (t,6H, 2CH_3, *J*=7.2 Hz); ^{13}C NMR (76 MHz, DMSO-*d6*): δ ppm 172.82, 152.58, 143.64, 133.54, 130.87, 128.76, 125.33, 68.04, 58.07, 28.98, 10.64. Mass spectrum: *m*/*z* 606.0 [*M*]$^+$. Anal. Calc. for $C_{24}H_{26}Br_2N_6O_3$: C 47.54; H 4.32; N, 13.86.found: C 47.51; H 4.31; N, 13.83.

2.6.8. 1,3-bis(((4-bromobenzylidene)amino) methyl)barbital(8): It was prepared as a nutty solid, yield 77%; m p: 74-76ºC; FTIR spectrum, ν, cm^{-1}: 3090(Ar-H), 1708(C=O), 1663(C=N),1589(C=C); ^{1}H NMR (300 MHz, DMSO-*d6*): δ ppm 8.17 (s,2H,2N=CH), 7.87-7.49 (m, Ar-H), 5.67 (s,4H, 2CH_2), 1.97 (q,4H, 2CH_2, *J*=7.2 Hz), 0.99 (t,6H, 2CH_3, *J*=7.2 Hz); ^{13}C NMR (76 MHz, DMSO-*d6*): δ ppm:172.24,152.19,143.87,133.71,129.47,127.91,124 .15,68.58,57.77,28.98,10.89. Mass spectrum: *m*/*z* 575.9 [*M*]$^+$. Anal. Calc. for $C_{24}H_{24}Br_2N_4O_3$: C, 50.02; H, 4.20; Br, 27.73; N, 9.72. found: C, 49.17; H, 4.06; N, 9.09.

2.7. α-Glucosidase inhibition assay[23]

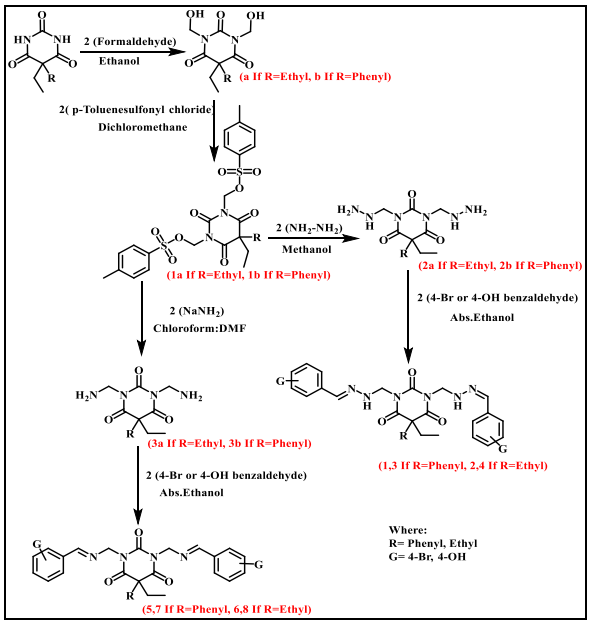
All synthesized Schiff base derivatives (1-8) where evaluated *in vitro* α-Glucosidase enzyme inhibitory

activity. Whereas used acarbose drug as positive control and (p-nitrophenyl glucopyranoside) as substrate. Enzyme was prepared in buffer saline solution of potassium phosphate (pH 7.4, 0.01M), and Schiff base derivatives (1-8) were dissolved in ethanol: distal water (1:3) (10% final concentration). 125 µL of potassium phosphate buffer, 25 µL of end products was added in several concentrations, and (25 µL 0.2 Unit/mL) of α-Glucosidase enzyme solution was added and incubated for 10 min at 37 ºC. After the incubation, the substrate (p-nitrophenyl glucopyranoside) (25 µL, 0.5 µM) was added to the mixture and incubated at 37 ºC for 30 min. Finally, the absorbance was measured at 405 nm by using spectrophotometer. The enzymatic reaction was stopped by adding 100µlof 200 µM Na_2CO_3. IC_{50} values were calculated and inhibition percentage for each compound was calculated by using the following formula: % Inhibition=[Abs control - Abs sample / Abs control] X 100%

3. Results and discussion

3.1. Chemistry

The desired substituted Schiff base based barbiturates derivatives (barbital and phenobarbital) **1-8** were prepared in good to very good yields 76–88% according to the procedure given in the **Scheme 1**.The preparation of **1-8** was achieved from reaction of barbital or phenobarbital with formaldehyde in ethanol to yield 1,3-bis(hydroxymethyl) barbiturates and **a** and **b**. The structure of prepared compounds were confirmed by ^{1}H NMR ,^{13}C NMR and IR spectra. Their IR spectrum contained, a broad absorption bands due to stretching vibrations of the hydroxyl group in the region 3427–3511 cm^{-1}. NMR spectroscopy in DMSO-*d6* of the solid precipitate present after evaporation of ethanol occurred. In our spectral analyses, there were clear differences in the spectra of barbital and phenobarbital for the NH signals ~11.25 ppm [24] was disappearance in products and appearance new peak (a singlet at at 5.23 and 4.94 ppm) of **a** and **b** compounds respectively due to methylene protons and (a singlet at 6.42 and 6.37 ppm) due to hydroxyl protons. The ^{13}CNMR spectrum of compounds **a** and **b** shows that there are additional signals at (69.49 , and 69.05 ppm) due to methylene carbon. 1,3-bis(hydroxymethyl)barbiturates were mix with p-toluenesulfonyl chloride in dichloromethane as solvent in the presence of tri ethylamine to produce **1a,1b**. Nuchliophilic substitution reaction between **1a** or **1b** with hydrazine hydrate (99%) in methanol or with sodium amid in mixture formed from chloroform and DMF to produce a new barbiturates derivatives with free NH_2 group. Their IR spectra contained a new absorption bands due to vibrations stretching of the NH_2 in the range 3255–3380 cm^{-1}. The ^{1}H NMR spectra of **2a**–**2b** showed signals from the two NH_2 protons (a singlet at δ 4.63–4.91 ppm) and proton NH group (a singlet at δ 7.21–733. ppm). Imine derivatives **1-8** were synthesized by reaction of barbiturates derivatives with free amino group and corresponding substituted benzaldehyde in presence glacial acetic acid. The ^{1}HNMR spectrum of compounds **1-8** showed the absence for the amines proton at(δ 4.63–4.91 ppm)and showed that there is a new signal in range (a singlet at δ 8.2-8.4 ppm) for the protons of imine group N=CH. The ^{13}CNMR spectrum of Schiff base compounds showed that there is appearance a new signal of the carbon imine group N=CH at 144-145 ppm.



Scheme 1: Synthetic protocol for Schiff base derivatives of barbital and phenobarbital.

3.2. α-Glucosidase inhibition

The second part of the work was determined α-glucosidase inhibition of target compounds. All the synthesized compounds **1-8** were evaluated for their *in vitro* α-glucosidase enzyme inhibition activity. All the members of the series **1-8** exhibited a potent α-glucosidase inhibition with IC_{50} values **(Fig.1.)** 110 ± 2.15, 197 ± 3.11, 38 ± 0.84, 64 ± 1.78, 119 ± 3.55, 204 ± 2.08, 32 ± 1.42, 81 ± 2.23 respectively. All

tested compounds were found to be more active than the standard drug, acarbose (IC_{50}=787.27 ± 2.23 µM). Compounds **7, 3** having a phenyl ring was substituted with bromo atom at para position[25-27] and attached to N-H group was found to be the highest active member of the series with an IC_{50} value 32 ± 1.42, 38 ± 0.84 µM. However the activity decreased when the phenyl ring was substituted with hydroxyl group at para position and without N-H group[28], as observed in compounds **2** and **6** (IC_{50} = 197 ± 3.11, 204 ± 2.08 µM). The presence of strong electron withdrawing group on phenyl ring and have N-H group was found to increase the α-glucosidase inhibitory activity, as observed for compounds **3** and **7**.

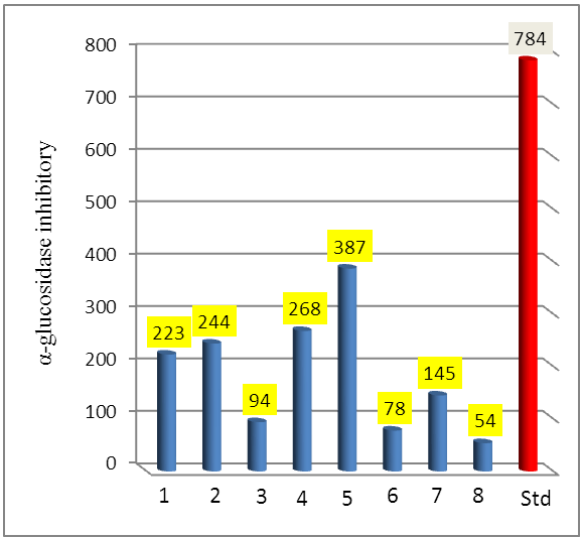


Fig.1. IC_{50} α-glucosidase inhibition of compounds (1-8)

4. Conclusions

In summary, a new series of barbiturates were synthesized **1-8** has been evaluated *in vitro* as inhibitors of α-glucosidase enzyme. The synthesized compounds showed excellent inhibition activity. These results indicate that inhibitory activity of the imine derivatives increase when structure contain substituted aryl with a withdrawing group (bromo group) in addition to the presence of a free N-H of hydrazine group which could easily form a salt link with the acidic part of α-glucosidase enzyme. Overall results suggest that substituted Schiff base based on barbiturates derivatives may be used as potential candidates in the search for anti-diabetic drugs.

5. References

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