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LETTERS

Design, synthesis, ADME prediction and anti-hyperglycemic evaluation of new alkoxyimino-substituted phenyl carboxylic acids as potent α-glucosidase inhibitors

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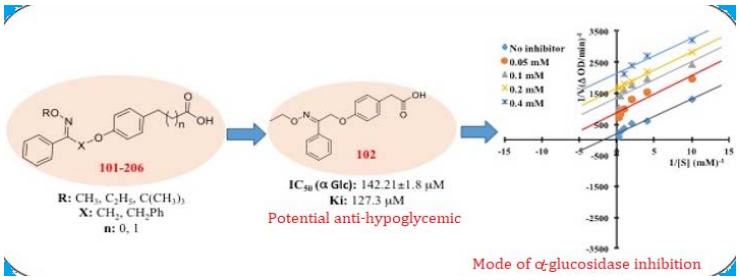
ABSTRACT



In an attempt to further explore the role of substituted carboxylic acid derivatives as antidiabetic agent, a series of alkoxyimino-substituted carboxylic acid derivatives (**101-206**) were designed, synthesized and evaluated for their inhibitory potential against a-amylase and a-glucosidase enzyme. Among all the tested compounds, **102** & **105** has displayed the most potent activity against α-glucosidase with the IC_{50} of 142.21±1.8 μM and 182.83±2.43 μM respectively, as compared to the standard drug acarbose (136.89±1.67 μM). Based on the inhibition percentage, the inhibition activity of **102** and **105** on α-glucosidase had higher potential than α-amylase. The mode of binding interactions between the α-glucosidase enzyme and the compound **102** was established to be uncompetitive using kinetic analysis. The predicted drug-likeness properties (Lipinski parameters and in silico ADME properties) of the compounds revealed their suitability as potential drug candidate.

Keywords: Carboxylic acid derivatives; Anti-hyperglycemic; α-glucosidase inhibition; ADME properties; Kinetic analysis.

INTRODUCTION

Diabetes is a major health issue that has reached alarming levels: today, nearly half a billion people are living with diabetes worldwide. Diabetes is one of the fastest growing global health emergencies of the 21st century. In 2019, it is estimated that 463 million people have diabetes and this number is projected to reach 578 million by 2030, and 700 million by 2045.[1] Type 2 diabetes is a heterogeneous disease resulting from a dynamic interaction between defects in insulin secretion and insulin action.[2] Such a deficiency results in increased concentrations of blood glucose, which in turn lead to long term complications like retinopathy, nephropathy, neuropathy and cardiovascular diseases. Achieving normoglycemia, and to reduce insulin resistance, thereby improving metabolic control, are the major goals of anti diabetic therapy. Attenuation of the postprandial hyperglycemia by inhibition of the intestinal carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase is a medically practical treatment to achieve the glycemic control.[3-4] α-amylase is a prominent enzyme found in the pancreatic juice and saliva, which breaks down large insoluble starch molecules into absorbable molecules.[5] On the other hand, mammalian -glucosidase in the mucosal brush border of the small intestine catalyzes the end step of digestion of starch and disaccharides that are abundant in human diet.[6] Inhibitors of α-amylase and α-glucosidase delay the breaking down of carbohydrates in the small intestine and diminish the postprandial blood glucose excursion.[7]

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Due to non-specific inhibition of these enzymes, the use of commercial inhibitors such as acarbose, voglibose, miglitol etc. is associated with the excessive accumulation of undigested carbohydrates in the large intestine, resulting in significant adverse gastrointestinal (GI) side effects.[8-10] Therefore, moderate α-amylase inhibition with potent α-glucosidase inhibitory activity may offer desirable therapeutic strategy that could slow down the availability of dietary carbohydrate substrate for glucose production in the gut.[11-12]

Several research groups have reported substituted carboxylic acid derivatives in materials studies[13] as well as potent anti diabetic compounds.[14-15] Lakshminarayana et al reported isochroman carboxylic acid derivatives as protein tyrosine phosphatase 1B inhibitor,[16] while another report by Negoro et al evaluated them as GPR 40 agonist.[17] Lozano et al reported them as aldose reductase (AKR1B1) inhibitors.[18] As a result of the multifactorial complexity of chronic diseases, the current therapeutic arsenal and the old-fashioned "one-molecule, one-target" model seem not so effective.[19] The design of new chemical entities (NCE) with two or more balancing bioactivities for the treatment of complex diseases would be very advantageous.

In order to further explore the role of these molecules, we designed and synthesized a novel series of alkoxyimino-substituted carboxylic acid derivatives, containing different carboxylic and oxime terminal fragments **(101-206)**. The synthesized derivatives were evaluated for their α glucosidase and α amylase inhibitory potential, and their mode of action was described by means of kinetic evaluation.

EXPERIMENTAL

MATERIAL AND METHOD

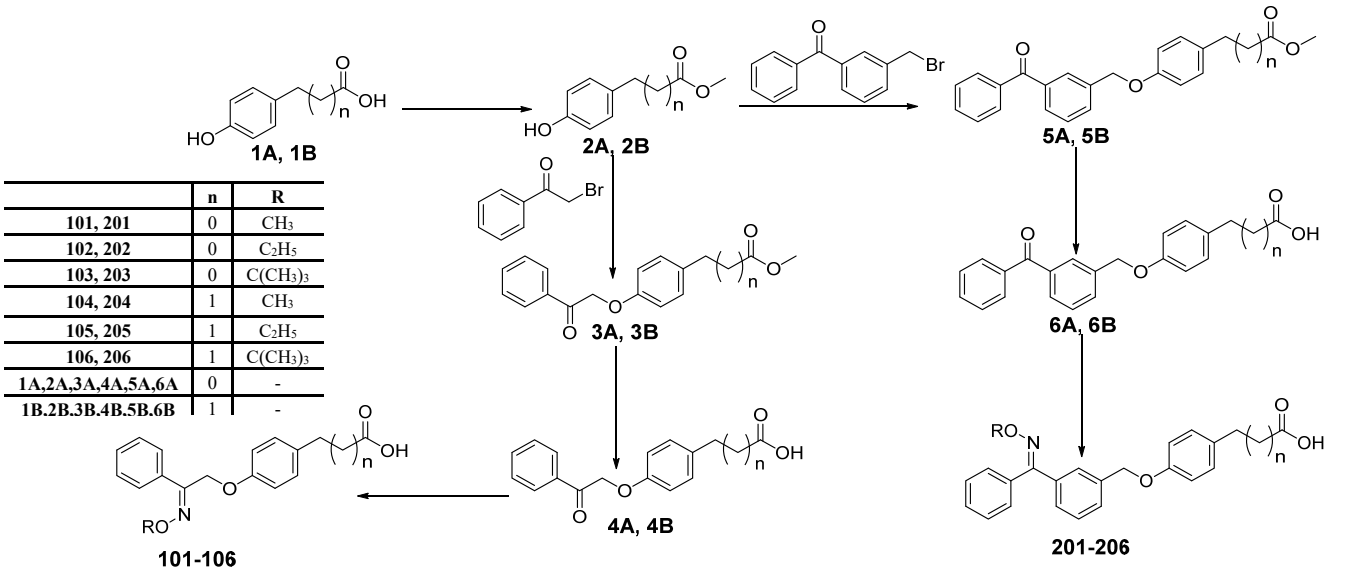
All organic solvents and common reagents were procured from the Merck India Ltd. Reactants and Reagents were procured from Aldrich Chemical Company, Inc. USA. TLC was carried out on commercially available flexible TLC silica gel (silica gel 60 F254) plates (E Merck, Germany). TLC, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy confirmed the purity of all organic compounds. ^{1}H NMR spectra were recorded in Bruker Spectrospin Avance 300 instrument operating at 400 MHz in CDCl3 or DMSO using TMS as an internal standard. The chemical shifts are reported in parts per million (d) downfield from TMS and coupling constants are reported in Hertz (Hz). Melting points were determined on a Buchi melting point B-450 instrument. MS spectra were recorded on a Qstar (Applied biosystem) ESI-MS mass spectrometer.

General Procedure for synthesis of 2A and 2B:

A 100 mL RB flask fitted with a magnetic stirrer was charged with 10 mL Methanol. To the stirred solvent was added (4-hydroxy-phenyl)-carboxylic acid (1A,1B) (10mM). The resulting mixture was stirred for 10 minutes followed by addition of 2-3 drops of H_2SO_4 at room temperature and then put on reflux for 2-3 hours. Reaction was monitored by TLC. On completion reaction water was added and extracted with ethyl acetate. The organic layer was washed with water, $NaHCO_3$, brine, dried over Na_2SO_4, evaporated under reduced pressure obtained desired product (2A,2B).

Methyl 2-(4-hydroxyphenyl)acetate (2A): Off white solid; Yield: 92 %; m.p. 98-100°C; Rf = 0.50 (SiO_2, Hexane/Ethyl acetate=70/30); MS (EI): 167.12 m/z $[M + 1]^+$ Calcd for $C_9H_{10}O_3$; ^{1}H NMR (400 MHz, DMSO): δ ppm 9.12 (br s, 1 H), 6.99−6.95 (d, J=8.0Hz, 2 H, Ar-H), 6.72-6.75 (d, J=8.0 Hz, 2 H, Ar-H), 3.61 (s, 3H), 3.58 (s, 2 H)

Methyl 3-(4-hydroxyphenyl)propanoate (2B): Off white Solid; Yield: 90 %; m.p. 100-102°C; Rf = 0.60 (SiO_2, Hexane/Ethyl acetate=70/30); MS (EI): 181.10 m/z $[M + 1]^+$ calcd for $C_{10}H_{12}O_3$; ^{1}H NMR (400 MHz, DMSO): δ ppm 9.10 (br s, 1 H), 6.94−6.97 (d, J=7.2Hz, 2 H, Ar-H), 6.68-6.71 (d, J=7.2 Hz, 2H, Ar-H), 3.60 (s, 3H), 2.73-2.78 (t, J=4.8Hz, 6.8Hz, 2H), 2.59-2.62 (t, J=7.2Hz, 5.6 Hz, 2H)



	n	R
101, 201	0	CH_3
102, 202	0	C_2H_5
103, 203	0	$C(CH_3)_3$
104, 204	1	CH_3
105, 205	1	C_2H_5
106, 206	1	$C(CH_3)_3$
1A,2A,3A,4A,5A,6A	0	-
1B,2B,3B,4B,5B,6B	1	-

Scheme 1: Reagents and Conditions: (a) MeOH, H_2SO_4, Reflux, 2-4 h (b) K_2CO_3, DMF, RT, 24h (c) NaOH (1 eq), MeOH, THF, H_2O, RT, 15h (d) Pyridine (2 eq), EtOH, $RONH_2$.HCl Reflux, 15h

General Procedure for synthesis of 3A, 3B and 5A, 5B:

A 100 mL RB flask fitted with a magnetic stirrer was charged with (4-Hydroxy-phenyl)-carboxylic acid methyl ester (**2A,2B**) (5mM) in DMF, K_2CO_3 (15mM) was added portion-wise to the mixture under stirring for 10 minutes at 0ºC. To this was added 2-Bromo-1-phenyl-ethanone / (3-Bromomethyl-phenyl)-phenyl-methanone (5mM). The reaction mixture was then stirred for 24 hours at room temperature. The resulting mixture was then washed with water and extracted from ethyl acetate. The ethyl acetate layer was dried over anhydrous sodium sulfate and concentrated over rotary evaporator to obtain the product.

Methyl 2-(4-(2-oxo-2-phenylethoxy)phenyl)acetate (3A): Cream solid; Yield: 74 %; m.p. 130-132ºC; Rf = 0.60 (SiO_2, Hexane/Ethyl acetate=70/30); MS (EI): 285.12 m/z $[M + 1]^+$ Calcd for $C_{17}H_{16}O_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 7.52-7.55 (m, 2 H, Ar-H), 7.32-7.35 (m, 3H, Ar-H), 7.12−7.16 (d, J=8.0 Hz, 2 H, Ar-H), 6.82-6.86 (d, J=8.0 Hz, 2 H), 5.32 (s, 2 H), 3.61 (s, 3 H), 3.56 (s, 2 H).

Methyl 3-(4-(2-oxo-2-phenylethoxy)phenyl)propanoate (3B): Pale yellow solid; Yield: 74 %; m.p. 134-136ºC; Rf = 0.70 (SiO_2, Hexane/Ethyl acetate=70/30); MS (EI): 299.11 m/z $[M + 1]^+$ Calcd for $C_{18}H_{18}O_4$; ^{1}H NMR (400 MHz, DMSO): 8.10-8.13 (m, 2 H, Ar-H), 7.55-7.58 (m, 3H, Ar-H) , 7.12−7.16 (d, J=6.2Hz, 2H, Ar-H), 6.82-6.86 (d, J=6.2 Hz, 2 H, Ar-H), 5.24 (s, 2 H), 3.60 (s, 3 H), 2.73-2.78 (t, J=6.0Hz, 5.6Hz, 2 H), 2.59-2.62 (t, J=6.8Hz, 5.6Hz, 2H).

Methyl 2-(4-((3-benzoylbenzyl)oxy)phenyl)acetate (5A): Yellow oil; Yield: 65 %; Rf = 0.60 (SiO_2, Hexane/Ethyl acetate=50/50); MS (EI): 361.18 m/z $[M + 1]^+$ Calcd for $C_{23}H_{20}O_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 7.48-7.55 (m, 3H, Ar-H), 7.38-7.41 (m, 3H, Ar-H) , 7.35 (m, 1H, Ar-H), 7.25-7.28 (m, 2H, Ar-H), 7.14-7.17 (d, J=8.4Hz, 2H, Ar-H), 6.89-6.94 (d, J=8.2 Hz, 2H, Ar-H), 5.12 (s, 2 H), 3.62 (s, 3 H), 3.54 (s, 2 H).

Methyl 3-(4-((3-benzoylbenzyl)oxy)phenyl)propanoate (5B): Pale yellow oil; Yield: 65 %; Rf = 0.70 (SiO_2, Hexane/Ethyl acetate=50/50); MS (EI): 375.27 m/z $[M + 1]^+$ Calcd for $C_{24}H_{22}O_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 7.47-7.55 (m, 3H, Ar-H), 7.38-7.41 (m, 3H, Ar-H) , 7.34 (m, 1H, Ar-H), 7.28-7.31(m, 2H, Ar-H), 7.14-7.17 (d, J=7.2Hz, 2H, Ar-H), 6.89-6.94 (d, J=7.2Hz, 2H, Ar-H), 5.12 (s, 2 H), 3.61 (s, 3 H), 2.75-2.79 (t, J=4.8Hz, 5.2Hz, 2H), 2.50-2.52 (t, J=6.8Hz, 5.6Hz, 2H)

General Procedure for synthesis of 4A, 4B and 6A, 6B:

To a 100 mL RB fitted with a magnetic stirrer was added (4-Hydroxy-phenyl)-carboxylic acid methyl ester / [4-(3-Benzoyl-benzyloxy)-phenyl]-carboxylic acid methyl ester (5mM) in THF. To this reaction mixture was added 0.5 mL methanol and 0.5 mL distilled water and kept on stirring at RT. To this NaOH (5mM) was added and let at stirring for 15 hours at room temperature. Completion of reaction was monitored by TLC. The reaction mixture was transferred to RB and MeOH and THF evaporated. The resulting crude reaction mixture was then washed with water and ethyl acetate. The water layer was then acidified using diluted hydrochloric acid to a pH of 2-3. The water layer is then again extracted from ethyl acetate. The ethyl acetate layer was dried over anhydrous sodium sulfate and concentrated over rotary evaporator to obtain the product.

2-(4-(2-oxo-2-phenylethoxy)phenyl)acetic acid (4A): Amorphous solid; Yield: 95 %; m.p. 145-147ºC; Rf = 0.40 (SiO_2, Dichloromethane/Methanol=80/20); MS (EI): 271.10 m/z $[M + 1]^+$ Calcd for $C_{16}H_{14}O_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.25 (br s, 1H), 7.61-7.66 (m, 2H, Ar-H), 7.37-7.42 (m, 3H, Ar-H) , 7.12−7.16 (d, J=7.2Hz, 2H, Ar-H), 6.82-6.86 (d, J=7.2Hz, 2H, Ar-H), 5.31 (s, 2 H), 3.46 (s, 2 H).

3-(4-(2-oxo-2-phenylethoxy)phenyl)propanoic acid (4B): Amorphous powder; Yield: 87 %; m.p. 178-187ºC; Rf = 0.50 (SiO_2, Dichloromethane/Methanol=80/20); MS (EI): 386.12 m/z $[M + 1]^+$ Calcd for $C_{17}H_{16}O_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.20 (br s, 1 H), 8.10-8.13 (m, 2 H, Ar-H), 7.55-7.58 (m, 3H, Ar-H) , 7.12−7.16 (d, J=6.2Hz, 2H, Ar-H), 6.82-6.86 (m, J=6.2Hz, 2H), 5.24 (s, 2 H), 2.71-2.75 (t, J=4.8Hz, 5.6Hz, 2H), 2.56-2.58 (t, J=4.8Hz, 5.8Hz, 2H).

2-(4-((3-benzoylbenzyl)oxy)phenyl)acetic acid (6A): Pale yellow oil; Yield: 85 %; Rf = 0.40 (SiO_2, Dichloromethane/Methanol=80/20);MS (EI): 347.10 m/z $[M + 1]^+$ Calcd for $C_{22}H_{18}O_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.24 (br s, 1 H), 7.48-7.55 (m, 3H, Ar-H), 7.38-7.41 (m, 3H, Ar-H) , 7.35 (m, 1 H, Ar-H), 7.25-7.28 (m, 2 H), 7.14-7.17 (d, J=8.0Hz, 2H, Ar-H), 6.89-6.94 (d, J=8.2Hz, 2H, Ar-H), 5.12 (s, 2 H), 3.48 (s, 2 H).

3-(4-((3-benzoylbenzyl)oxy)phenyl)propanoic acid (6B): Yellow oil; Yield: 83 %; Rf = 0.45 (SiO_2, Dichloromethane/Methanol=80/20); MS (EI): 361.2 m/z $[M + 1]^+$ Calcd for $C_{23}H_{20}O_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.08 (br s, 1 H), 7.47-7.55 (m, 3H, Ar-H), 7.38-7.41 (m, 3H, Ar-H) , 7.34 (m, 1H, Ar-H), 7.28-7.31 (m, 2H, Ar-H), 7.14-7.17 (d, J=8.2Hz, 2H, Ar-H), 6.89-6.94 (d, J=8.2Hz, 2H, Ar-H), 5.12 (s, 2 H), 2.72-2.77 (t, J=5.6 Hz, 6.0Hz, 2H), 2.49-2.51 (t, J=4.8Hz, 6.2 Hz, 2H).

General Procedure for synthesis of 101-106 & 201-206:

To a stirred solution of [4-(2-oxo-2-phenyl-ethoxy)-phenyl]-carboxylic acid / [4-(3-Benzoyl-benzyloxy)-phenyl]-carboxylic acid (5mM) in ethanol (50mL) was added pyridine (10 mM), followed by addition of methoxyamine hydrochloride / ethoxyamine hydrochloride / o-(tert-butyl)hydroxylamine hydrochloride (5mM) at room temperature. The reaction mixture was then reflux for 15 hours followed by directly concentrating the mixture using rotary evaporator. Completion of reaction was monitored by TLC. Then the concentrated mixture was washed with water and extracted from ethyl acetate. The ethyl acetate layer was dried over anhydrous sodium sulfate and concentrated over rotary evaporator to obtain the product.

2-(4-(2-(methoxyimino)-2-phenylethoxy)phenyl)acetic acid (101): Light yellow oil; Yield: 90 %; Rf = 0.20 (SiO_2, Dichloromethane/Methanol=80/20); MS (EI): 300.12 m/z $[M + 1]^+$ Calcd for $C_{17}H_{17}NO_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.25 (br s, 1 H), 7.61-7.66 (m, 2H.Ar-H), 7.37-7.42 (m, 3H, Ar-H) , 7.12−7.16 (d, J=7.2Hz, 2H, Ar-H), 6.82-6.86 (d, J=7.2Hz, 2H, Ar-H), 5.19 (s, 2 H), 3.99 (s, 3H), 3.46 (s, 2H); ^{13}C NMR (400 MHz, DMSO): δ 176.20, 164.60, 155.81, 134.01, 131.0, 130.72, 128.45, 127.10, 126.43, 114.87, 81.28, 62.20, 40.12.

2-(4-(2-(ethoxyimino)-2-phenylethoxy)phenyl)acetic acid (102): Viscous oil; Yield: 85 %; Rf = 0.25 (SiO_2, Dichloromethane/Methanol=80/20);MS (EI): 314.12 m/z [M + 1]$^+$ Calcd for $C_{18}H_{19}NO_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.25 (br s, 1H), 7.61-7.66 (m, 2H, Ar-H), 7.37-7.42 (m, 3H, Ar-H) , 7.12−7.16 (d, J=8.2Hz, 2H, Ar-H), 6.82-6.86 (d, J=8.4Hz, 2H, Ar-H), 5.20 (s, 2H), 4.22-4.27 (q, 2H,), 3.46 (s, 2H), 1.29-1.31 (t, 3H,); ^{13}C NMR (400 MHz, DMSO): δ 176.20, 164.60, 155.81, 134.01, 131.0, 130.72, 128.45, 127.10, 126.43, 114.87, 81.28, 65.20, 40.12, 14.20.

2-(4-(2-(tert-butoxyimino)-2-phenylethoxy)phenyl)acetic acid (103): Pale yellow viscous oil; Yield: 83 %; Rf = 0.35 (SiO_2, Dichloromethane/Methanol=80/20); MS (EI): 342.16 m/z [M + 1]$^+$ Calcd for $C_{20}H_{23}NO_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.25 (br s, 1 H), 7.61-7.66 (m, 2H, Ar-H), 7.37-7.42 (m, 3 H, Ar-H) , 7.12−7.16 (d, J=8.0Hz, 2H, Ar-H), 6.82-6.86 (d, J=8.0Hz, 2H, Ar-H), 5.20 (s, 2 H), 3.46 (s, 2H), 1.52 (s, 9H); ^{13}C NMR (400 MHz, DMSO): δ 176.20, 164.60, 155.81, 134.01, 131.0, 130.72, 128.45, 127.10, 126.43, 114.87, 88.90, 81.28, 65.20, 40.12, 29.87.

3-(4-(2-(methoxyimino)-2-phenylethoxy)phenyl)propanoic acid (104): Light yellow oil; Yield: 91 %; Rf = 0.30 (SiO_2, Dichloromethane/Methanol=80/20);MS (EI): 314.2 m/z [M + 1]$^+$ Calcd for $C_{18}H_{19}NO_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.20 (br s, 1 H), 8.01-8.03 (m, 2H, Ar-H), 7.54-7.57 (m, 3 H, Ar-H) , 7.12−7.16 (d, J=7.2 Hz, 2H, Ar-H), 6.82-6.86 (d, J=7.2Hz, 2H, Ar-H), 5.20 (s, 2H), 3.57 (s, 3H), 2.71-2.75 (t, J=4.8Hz, 5.6Hz, 2H), 2.56-2.58 (t, J=5.6, 6.0Hz, 2H). ^{13}C NMR (400 MHz, DMSO): δ 173.20, 164.60, 154.21,134.67, 133.11, 129.75, 128.45, 126.20, 126.43, 113.64, 81.26, 62.70, 36.12, 33.95.

3-(4-(2-(ethoxyimino)-2-phenylethoxy)phenyl)propanoic acid (105): Light yellow oil; Yield: 84 %; Rf = 0.35 (SiO_2, Dichloromethane/Methanol=80/20);MS (EI): 328.4 m/z [M + 1]$^+$ Calcd for $C_{19}H_{21}NO_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.30 (br s, 1 H), 7.61-7.66 (m, 2H, Ar-H), 7.37-7.42 (m, 3H, Ar-H) , 7.12−7.16 (d, J=8.0Hz, 2H, Ar-H), 6.82-6.86 (d, J=8.2Hz, 2H, Ar-H), 5.20 (s, 2 H), 4.13-4.16 (q, 2H), 2.71-2.75 (t, J=5.6Hz, 6.0Hz, 2H), 2.56-2.58 (t, J= 4.8Hz, 6.2Hz, 2H), 1.29-1.31 (t, 3H); ^{13}C NMR (400 MHz, DMSO): δ 173.20, 164.60, 154.21, 134.67, 133.11, 129.75, 128.45, 126.20, 126.43, 113.64, 81.26, 78.50, 36.12, 33.95, 15.60.

3-(4-(2-(tert-butoxyimino)-2phenylethoxy)phenyl)propanoic acid (106): Transparent viscous oil; Yield: 80%; Rf = 0.40 (SiO_2, Dichloromethane/Methanol=80/20); MS(EI): 356.18 m/z [M + 1]$^+$ Calcd for $C_{21}H_{25}NO_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.28 (br s, 1 H), 7.61-7.66 (m, 2H, Ar-H), 7.37-7.42 (m, 3H, Ar-H) , 7.12−7.16 (d, J=7.2Hz, 2H, Ar-H), 6.82-6.86 (d, J=7.2Hz, 2H, Ar-H), 5.20 (s, 2H), 2.71-2.75 (t, J=5.2Hz, 6.8Hz, 2H), 2.56-2.58 (t, J=5.6Hz, 6.0Hz, 2H), 1.51 (s, 9H); ^{13}C NMR (400 MHz, DMSO): δ 173.20, 164.60, 154.21, 134.67, 133.11, 129.75, 128.45, 126.20, 126.43, 113.64, 87.89, 81.26, 36.12, 33.95, 31.30.

2-(4-((3((methoxyimino)(phenyl)methyl)benzyl)oxy)phenyl) acetic acid (201): Yellow oil; Yield: 92 %; Rf = 0.20 (SiO_2, Dichloromethane/Methanol=80/20); MS (EI): 376.15 m/z [M + 1]$^+$ Calcd for $C_{23}H_{21}NO_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.24 (br s, 1H), 7.45-7.52 (m, 3H, Ar-H), 7.36-7.40 (m,3H, Ar-H) , 7.33 (d, 1H, Ar-H), 7.25-7.28 (d, 2H, Ar-H), 7.14-7.17 (d, J=8.2Hz, 2H, Ar-H), 6.89-6.94 (d, J=8.2Hz, 2H, Ar-H), 5.12 (s, 2H), 3.88 (s, 3H), 3.48 (s, 2H); ^{13}C NMR (400 MHz, DMSO): δ 175.20, 158.81, 156.75, 136.75, 134.17, 133.10, 131.56, 130.89, 129.45, 129.40, 129.12, 129.01, 128.84, 127.89, 127,63, 121.90, 72.69, 63.07, 38.97.

2-(4-((3((ethoxyimino)(phenyl)methyl)benzyl)oxy)phenyl) acetic acid (202): Pale yellow oil; Yield: 89 %; Rf = 0.22 (SiO_2, Dichloromethane/Methanol=80/20); MS (EI): 390.16 m/z [M + 1]$^+$ Calcd for $C_{24}H_{23}NO_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.24 (br s, 1H), 7.45-7.52 (m, 3H, Ar-H), 7.36-7.40 (m, 3H, Ar-H) , 7.33 (m, 1H, Ar-H), 7.25-7.28 (m, 2H, Ar-H), 7.14-7.17 (d, J=8.0Hz, 2H, Ar-H), 6.89-6.94 (d, J=8.2Hz, 2H, Ar-H), 5.12 (s, 2H), 4.11-4.17 (q, 2H), 3.48 (s, 2H), 1.11-1.14 (t, 2H); ^{13}C NMR (400 MHz, DMSO): δ 175.20, 157.70, 156.75, 136.75, 134.17, 133.10, 131.56, 130.89, 129.45, 129.40, 129.12, 129.01, 128.84, 127.89, 127,63, 121.90, 72.69, 65.07, 38.97,16.21.

2-(4-((3-((tert-butoxyimino)(phenyl)methyl)benzyl)oxy) phenyl)acetic acid (203): Light yellow oil; Yield: 82 %; Rf = 0.30 (SiO_2, Dichloromethane/Methanol=80/20); MS (EI): 418.18 m/z [M + 1]$^+$ Calcd for $C_{26}H_{27}NO_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.24 (br s, 1H), 7.45-7.52 (m, 3H, Ar-H), 7.36-7.40 (m, 3H, Ar-H) , 7.33 (m, 1H, Ar-H), 7.25-7.28 (m, 2H, Ar-H), 7.14-7.17 (d, J=8.2Hz, 2H, Ar-H), 6.89-6.94 (d, J=8.2Hz, 2H, Ar-H), 5.12 (s, 2 H), 3.48 (s, 2 H), 1.50 (s, 9H); ^{13}C NMR (400 MHz, DMSO): δ 175.20, 157.70, 156.75, 136.75, 134.17, 133.10, 131.56, 130.89, 129.45, 129.40, 129.12, 129.01, 128.84, 127.89, 127,63, 121.90, 80.32, 72.69, 38.97,28.03.

3-(4-((3-((methoxyimino)(phenyl)methyl)benzyl)oxy)phenyl) propanoic acid (204): Pale yellow oil; Yield: 90 %; Rf = 0.30 (SiO_2, Dichloromethane/Methanol=80/20); MS (EI): 390.2 m/z [M + 1]$^+$ Calcd for $C_{24}H_{23}NO_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.08 (br s, 1 H), 7.45-7.52 (m, 3H, Ar-H), 7.36-7.40 (m, 3H, Ar-H) , 7.33 (m, 1H, Ar-H), 7.25-7.28 (m, 2H, Ar-H), 7.14-7.17 (d, J=8.0Hz, 2H, Ar-H), 6.89-6.94 (d, J=8.0Hz, 2H, Ar-H), 5.12 (s, 2 H), 3.88 (s, 3 H), 2.72-2.77 (t, J=4.8Hz, 5.6Hz, 2H), 2.49-2.51 (t, J=5.2 Hz, 6.0Hz, 2H); ^{13}C NMR (400 MHz, DMSO): δ 174.70, 157.70, 156.75, 136.75, 134.17, 133.10, 131.56, 129.91, 129.45, 129.40, 129.12, 129.01, 128.84, 127.89, 125.45, 121.90, 72.69, 36.37, 34.02.

3-(4-((3-((ethoxyimino)(phenyl)methyl)benzyl)oxy)phenyl) propanoic acid (205): Pale yellow oil; Yield: 87 %; Rf = 0.25 (SiO_2, Dichloromethane/Methanol=80/20); MS (EI): 404.12 m/z [M + 1]$^+$ Calcd for $C_{25}H_{25}NO_4$; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.08 (br s, 1 H), 7.45-7.52 (m, 3H, Ar-H), 7.36-7.40 (m, 3H, Ar-H) , 7.33 (m, 1H, Ar-H), 7.25-7.28 (m, 2H, Ar-H), 7.14-7.17 (d, J=8.2Hz, 2H, Ar-H), 6.89-6.94 (d, J=8.2Hz, 2H, Ar-H), 5.12 (s, 2H), 4.13-4.16 (q, 2H), 2.72-2.77 (t, J=4.8Hz, 6.2Hz 2H), 2.49-2.51 (t, 6.0Hz, 7.2Hz, 2H), 1.26-1.28 (t, 3H); ^{13}C NMR (400 MHz, DMSO): δ 174.70, 157.70, 156.75, 136.75, 134.17, 133.10, 131.56, 129.91, 129.45, 129.40, 129.12, 129.01, 128.84, 127.89, 125.45, 121.90, 72.69, 64.79, 36.37, 34.02, 16.23.

3-(4-((3-((tert-butoxyimino)(phenyl)methyl)benzyl)oxy) phenyl)propanoic acid (206): Yellow viscous oil; Yield: 85 %, Rf = 0.35 (SiO_2, Dichloromethane/Methanol=80/20); MS (EI): 432.34 m/z $[M + 1]^+$ Calcd for $C_{27}H_{29}NO_4$; ^1H NMR (400 MHz, DMSO): δ ppm 12.08 (br s, 1H), 7.45-7.52 (m, 3H, Ar-H), 7.36-7.40 (m, 3H, Ar-H), 7.33(m, 1H, Ar-H), 7.25-7.28 (m, 2H, Ar-H), 7.14-7.17 (d, J=8.4Hz, 2H, Ar-H), 6.89-6.94 (d, J=8.4Hz, 2H, Ar-H), 5.12 (s, 2 H), 2.72-2.77 (t, J=6.0Hz, 5.6Hz, 2H), 2.49-2.51 (t, J=4.8Hz, 5.6Hz, 2H), 1.52 (s, 9H); ^{13}C NMR (400 MHz, DMSO): δ 174.70, 157.70, 156.75, 136.75, 134.17, 133.10, 131.56, 129.91, 129.45, 129.40, 129.12, 129.01, 128.84, 127.89, 125.45, 121.90, 80.67, 72.69, 36.37, 34.02, 28.78.

Biological Assays

Materials:

Yeast α-glucosidase (EC 3.2.1.20), porcine pancreatic α-amylase (EC 3.2.1.1), paranitrophenyl α-D-glucopyranoside (pNPG) and acarbose were purchased from Sigma-Aldrich Chemical Company (Gillingham, Dorset, UK). Other chemicals were purchased from Fluka and Aldrich and used without further purification.

ADMET Prediction:

To analyze the potential of the new compounds as anti-hyperglycemic agents, their ADME (Absorption, Distribution, Metabolism and excretion) properties were predicted using QikProp program, v. 3.5 of the Schrödinger software.[20] This gave an estimate of the physicochemical properties and the bioavailability of the compounds. Parameters such as QP log S (predicted aqueous solubility), QP log Khsa (prediction of binding to human serum albumin), QPlogBB (predicted brain/ blood partition coefficient), QPPCaco (predicted apparent Caco-2 cell permeability in nm/s. Caco-2 cells is a model for the gut blood barrier), QPPMDCK (predicted apparent MDCK cell permeability in nm/s. MDCK cells are considered to be a good mimic for the blood–brain barrier) and HERG binding were calculated. The acceptability of the compounds based on the Lipinski's rule of five was also estimated from the results.[21]

α-Amylase Inhibition Assay:

This assay was carried out using a modified procedure of McCue and Shetty.[22] A total of compounds (100 µM – 1000 µM) was placed in a tube and 250 µL of 0.02M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5mg/mL) was added. This solution was preincubated at 25°C for 10 min, after which 250 µL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at timed intervals and then further incubated at 25°C for 10min. The reaction was terminated by adding 500 µL of di nitro salicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5mL distilled water and the absorbance was measured at 540 nm using spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water. The α-amylase inhibitory activity was calculated as percentage inhibition:

$$\% \text{ Inhibition} = [Abs_{control} - Abs_{compounds}/ Abs_{control}] \times 100$$

α-Glucosidase inhibition assay:

The effect of the test compounds on α-glucosidase activity was determined according to the method described by Kim et al., using α-glucosidase from *Saccharomyces cerevisiae*.[23] The substrate solution p-nitrophenyl glucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, and pH 6.9. 100µL of α-glucosidase (1.0 U/mL) was preincubated with 50µL of the different concentrations of the compounds for 10 min. Then 50µL of 3.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37∘C for 20 min and stopped by adding 2 mL of 0.1 M Na2CO3. The α-glucosidase activity was determined by measuring the yellow-colored paranitrophenol released from pNPG at 405 nm. The results were expressed as percentage of the blank control.

Percentage inhibition is calculated as

$$\% \text{ Inhibition} = [Abs_{control} - Abs_{compounds}/ Abs_{control}] \times 100$$

Mode of inhibition of α-glucosidase was determined using the compound with the lowest IC50 according to the modified method described by Ali *et al*.[24] A series of diluted inhibitor solutions was prepared, at constant PNPG concentration (1mM). The inhibition rates were measured by the above method with different concentrations of α-glucosidase (0.00, 0.35, 0.7, and 1.05 U/mL).

The inhibition type of the enzyme was assayed by Lineweaver–Burk and Dixon plots.[25-26] A series of diluted inhibitor solutions was prepared, at constant α-glucosidase concentration (1.0 U/mL). The inhibition rates were measured by the above method with different concentrations of PNPG (0.1, 0.25, 0.5, 1, 2.5, 5, and 10 mM).

Results and Discussion

CHEMISTRY

The synthetic methodology employed to develop target compounds is summarized in **Scheme 1**. 4-hydroxy phenyl carboxylic acid derivatives were esterified in methanol using acid catalyst. The methyl ester was then further reacted with 2-Bromo-1-phenyl-ethanone / (3-Bromomethyl-phenyl)-phenyl-methanone in the presence of DMF/K_2CO_3. As obtained, methyl esters were further hydrolyzed back to acid using NaOH in THF/MeOH/H_2O. [4-(2-oxo-2-phenyl-ethoxy)-phenyl]-carboxylic acid / [4-(3-Benzoyl-benzyloxy)-phenyl]-carboxylic acid were coupled with methoxyamine hydrochloride / ethoxyamine hydrochloride / o-(tert-butyl)hydroxylamine hydrochloride in pyridine and ethanol to afford the substituted derivatives (**101-106** and **201 to 206**) with an approximate yield of 70-85%. All the compounds were fully characterized by ^1H NMR, ^{13}C NMR and mass spectroscopy.

Computational Analysis of Drug-Likeness

The drug like properties of carboxylic acid derivatives (**101-206**) was assessed through ADME (Adsorption, Distribution, Metabolism, and Excretion) profiling using QikProp, v. 3.5.[20] In order to further explore drug-like properties of compounds, the lipophilicity, expressed as the octanol/water partition coefficient as well as other theoretical calculations such as molecular size,

Table 1: Predicted ADME properties, Lipinski parameters for drug likeness of the synthesized derivatives

Compound	MW	Donor HB	Accpt HB	logP (o/w)	n-rotB	MV	TPSA	% Abs	Rule of Five
101	299.32	1	5.45	3.52	8	1028.21	78.27	88.45	0
102	313.35	1	5.45	3.89	9	1085.41	77.56	91.01	0
103	341.40	1	4.45	4.92	9	1178.87	73.92	100	0
104	313.35	1	5.45	3.86	9	1090.31	78.98	89.17	0
105	327.37	1	5.45	4.23	10	1147.75	78.31	91.74	0
106	355.43	1	4.5	5.26	10	1240.93	74.66	86.16	1
201	375.42	1	5.45	5.15	9	1260.25	78.20	85.97	1
202	389.45	1	5.45	5.552	10	1324.59	77.58	88.34	1
203	417.50	1	4.5	6.64	10	1425.51	74.04	95.85	1
204	389.45	1	5.45	4.84	10	1231.44	78.14	96.14	0
205	403.47	1	5.45	5.59	11	1344.84	77.36	87.94	1
206	431.53	1	4.5	6.96	11	1483.08	74.4	96.83	1

MW: Molecular weight; Accpt HB: Number of hydrogen-bond acceptors (O and N atoms); Donor HB: Number of hydrogen-bond donors (OH and NH groups); logP (o/w): Octanol-water partition coefficient; *n*-rotb: No of Rotatable Bonds; MV: Molecular Volume; TPSA: Topological polar surface area; % ABS: Percentage of absorption (% Abs = 109 − [0.345 × TPSA])

the number of hydrogen bond acceptors and donors, TPSA and percentage of absorption were analysed as shown in **Table 1**.

The results showed that most of the compounds complied with Lipinski's rule **(Table 1)**, with compound **203** and **206** depicting a slightly higher octanol water partition coefficient. All the analogues have shown logP values less than 5.5 which demonstrate good membrane permeability. TPSA, which ranges from 74 to 79 Å and found to be less than 140 Å, which is a very useful parameter for the transport of drug molecule. Finally summarizing the physicochemical properties of these analogs we could conclude that most of the synthesized derivatives obey the rule-of- five of Lipinski rule[21] and also meet all criteria for good orally active compound.

ADME Profiling of Synthesized Derivatives

To predict the drug-likeness of the designed compounds, a few indicators of their pharmacokinetic profiles were predicted using QikProp, v. 3.5.[20] About 45 physically significant descriptors and pharmacologically relevant properties of these compounds were predicted. In the present study some of these important properties were considered and are reported in **Table 2**. Among calculated parameters, QPPCaco, QP log BB, QP Log Khsa, and QPPMDCK mainly account for the ability of the compound's distribution inside the body. Most of the compounds showed moderate permeability for both in vitro Caco-2 cells and in vitro MDCK cells. Predicted *In vivo* blood-brain barrier penetration demonstrated that all the compounds have low absorption into the CNS and this indicates these compounds have less capability to cross the CNS. The amount of protein binding of the drugs is an important consideration. The predicted values of QPlogKhsa for all compounds indicate their strong binding with plasma protein and showed maximum skin permeability. The predicted ADME properties revealed that all compounds fulfill drug-like criteria and could be considered as good candidate for drug development.

Table 2: Predicted ADME properties for synthesized derivatives **(101-206)**

Compound	Log S (-6.5 / 0.5)	Log K (serum protein binding) (-1.5 / 1.5)	Log BB (-3.0 / 1.2)	HERG (K^+ Channel blockade) (concern below -5)	CACO-2 permeability (<25 poor, >500 great)	MDCK permeability (<25 poor, >500 great)
101	-4.145	-0.154	-1.130	-4.438	191	105
102	-4.393	-0.045	-1.169	-4.420	202	111
103	-5.257	0.402	-1.064	-4.218	240	134
104	-4.590	-0.037	-1.307	-4.655	163	88
105	-4.847	0.074	-1.347	-4.644	171	93
106	-5.719	0.525	-1.239	-4.449	204	112
201	-6.100	0.414	-1.235	-5.548	216	120
202	-6.476	0.545	-1.310	-5.589	217	121
203	-7.538	1.026	-1.233	-5.538	252	141
204	-4.636	0.324	-1.142	-3.880	191	105
205	-5.549	0.580	-1.247	-4.381	200	110
206	-7.894	1.143	-1.376	-5.624	224	124

Log S: Aqueous Solubility; Log K: Serum protein binding; Log BB: Blood Brain Barrier; HERG: K^+ Channel

α-Amylase Inhibitory Activity

The new class of synthesized alkyl carboxylic acid derivatives **(101-206)** was screened for their potency of α-amylase inhibitory activity with acarbose taken as a standard drug.

As shown in the **Figure 1**, the synthetic compounds revealed either weak or moderate inhibitory properties, which are desirable on the basis of their lower susceptibility for possible development of the gastrointestinal side effects.[27]

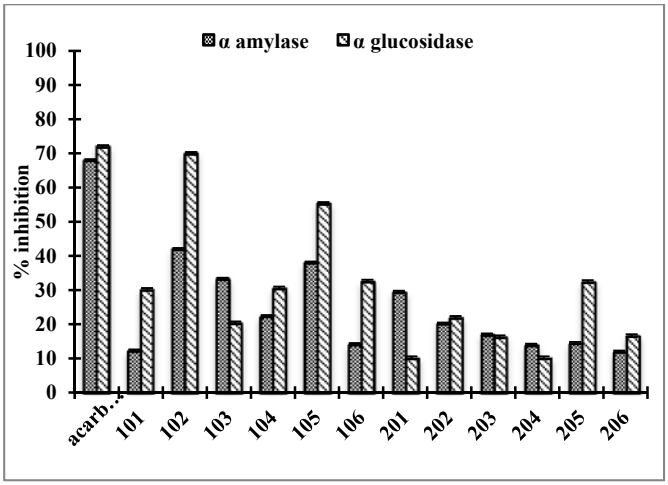


Figure 1: Inhibitory activity of synthetic compounds **(101-206)** and acarbose against porcine pancreatic α-amylase and yeast alpha glucosidase at 200 μM concentrations.

Comparing the results obtained herein, it is clear that the compounds of the series **(101-106)** are more potent in comparison with the other series **(201-206)**. The difference in the activity lies in the introduction of additional benzene ring along with spacer which leads to decreased flexibility which may not allow the molecule to bind to enzyme. Among the first series, compound **102** with one methyl spacer acid derivative and ethyl on oxime terminal, demonstrate good inhibitory action against alpha amylase with 42% inhibition. Although compound **105** also showed moderate inhibitory action, but the activity decreases with the increase in the chain length.

α-Glucosidase Inhibitiory Activity and its Kinetic Characterization

α-Amylase and α-glucosidase are the well-known enzymes that play a key role in the management of hyperglycaemia-linked type 2 diabetes.[28,29] To determine the potential application of the synthesized derivatives for the treatment of diabetes;[30] their inhibition towards α-glucosidase was also determined. As shown in **Figure 1**, modifications of the carbon chain length of acidic terminal along the rigidity of compounds revealed interesting relationship. As we introduced the benzene ring along with spacer, leading to increased rigidity of the compounds **(201-206),** decrease in the inhibitory activity was observed. The activity of compounds with one carbon chain acid and ethyl oxime (R= C_2H_5, **202**) was better than the rest of the compounds in the series. Among all the compounds, compound **102** exhibited the excellent alpha glucosidase inhibition activity with the IC_{50} value of 142.21±1.8 μM which is comparable with the standard drug acarbose (136.89±1.67 μM). Similarly compound **105** also exhibited potent activity with IC_{50} value of 182.83±2.43 μM. Rest of the compounds showed moderate to weak inhibitory potency (>200 uM) against the enzyme α-glucosidase. It is evident from the above results that compound **102** and **105** has excellent alpha glucosidase inhibitory activity and has significantly higher inhibitory potential than alpha amylase.

We further selected the most potent compound **102** for kinetic analysis to elucidate the mode of enzyme inhibition. Graphical analysis of the reciprocal Lineweaver–Burk plot and Dixon plot showed unchanged slopes with increasing intercepts (higher Km) at higher concentration of the inhibitor **102**, suggesting uncompetitive mode of inhibition (**Figure 2**). Thus, this compound inhibits by interfering with the formation of the α-glucosidase-PNPG inhibitor (ESI) complex in an uncompetitive manner. The inhibition constant for the inhibitor binding with the enzyme substrate complex (Kis) was obtained from a plot of the vertical intercept (1/Vm) versus inhibitor concentration, which was found to be 127.3 μM.

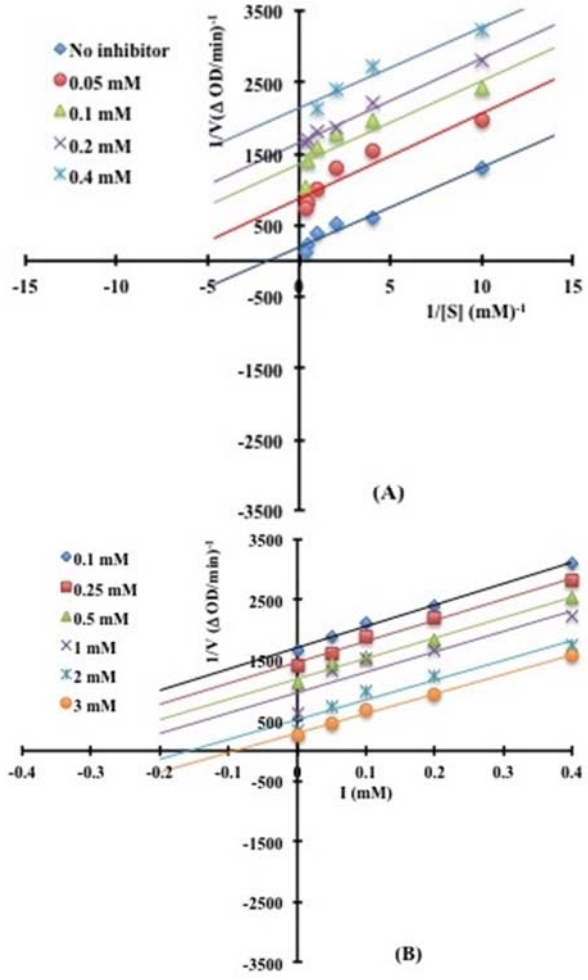


Figure 2: The Lineweaver-Burk (A) and Dixon plot (B) of α-glucosidase inhibition by compound 102

CONCLUSION

A new class of alkoxyimino-substituted carboxylic acid derivatives was designed, synthesized and their inhibitory potential against α-amylase and α-glucosidase enzymes were evaluated. All the synthesized compounds were well characterized by ^{1}H NMR, ^{13}C NMR, and mass spectrometry. SAR studies indicate that introduction of phenyl spacer and increase in the carbon chain length of terminal oxime decrease the enzyme inhibitory activity. Among the synthesized derivatives, compound **102** having one carbon chain terminal carboxylic acid and **105** having two-carbon chain carboxylic acid with ethyl oxime terminal were found to be the most active in the α-amylase inhibition assay. These compounds exhibited the most potent activity against α-glucosidase enzyme with IC_{50} values of 142.21±1.8 μM and 182.83±2.43 μM respectively. Further kinetic analysis of compound **102** reveals its uncompetitive mode of inhibition. ADME determination showed that all compounds obeyed the Lipinski's rule to become a ''drug like" molecule. Hence, these compounds could be promising hits for further development of new anti-diabetic drugs.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES AND NOTES

1. IDF Diabetes Atlas, **2019**, 9th edition
2. A.J. Scheen. Treatment of Type 2 Diabetes. *Acta. Clin. Belg*. **2003,** 58(5), 318-24.
3. H. Lebovitz. Alpha-glucosidase inhibitors. *Curr. Ther. Diabetes.* **1997**, 26, 539– 551.
4. E. Standl, O. Schnell. Alpha-glucosidase inhibitors cardiovascular considerations and trial evaluation. *Diab. Vasc. Dis. Res.* **2010**, 9, 163–169.
5. A.F. Afifi, E. M. Kamel, A. A. Khalil, M. A. Foaad, E. M. Fawziand, M. Houseny. Purification and characterization of α-amylase from *penicillium olsonii* under the effect of some antioxidant vitamins. *Global J. Biotech. Biochem.* **2008**, 3(1), 14–12.
6. V. Manohar, N. A. Talpur, B. W. Echard, S. Lieberman, H. G. Preuss, Effects of a water-soluble extract of maitake mushroom on circulating glucose/insulin concentrations in KK mice. *Diab. Obes. Metab*. **2002**, 4(1), 43–48.
7. Y. I. Kwon, E. Apostolidis, K. Shetty, Evaluation of pepper (*Capsicum annuum*) for management of diabetes and hypertension. *J. Food Biochem.* **2007**, 31(3), 370–385.
8. S. Christudas, L. Gopalakrishnan, P. Mohanraj, K. Kalyamoorthy, P. Agastian. Alpha-glucosidase inhibitory and antidiabetic activities of ethanolic extract of *Pisonia alba* span leaves. *Int. J. Integr. Biol*. **2009**, 6, 41–45.
9. Z. Madar, A. Hazan. Effect of miglitol and acarbose on starch digestion, daily plasma glucose profiles and cataract formation. *J. Basic Clin. Physiol. Pharmacol*. **1993**, 4, 69.
10. N. Asano. Glycosidase inhibitors: update and perspectives on practical use. *Glycobiol.* **2003**, 13, 93–104.
11. W. Creutzfeldt. Effects of the alpha-glucosidase inhibitor acarbose on the development of long-term complications in diabetic animals: pathophysiological and therapeutic implications. *Diabetes Metab. Res. Rev.* **1999**, 15, 289–296.
12. J. Shinde, T. Taldone, M. Barletta, N. Kunaparaju, B. Hu, S. Kumar, J. Placido, S. W. Zito. Alpha-glucosidase inhibitory activity of *Syzygium cumini* (Linn.) Skeels seed kernel in vitro and in Goto-Kakizaki (GK) rats. *Carbohydr. Res.* **2008,** 343, 1278–1281.
13. T. Nagaveni, K. Mahadevan, R. Naik, T.S. Kumara. Synthesis of blue light emitting 5-carboxylicacid-2-arylsubstituted benzimidazoles as photosensitizers for dye-sensitized solar cells. *J. Mater. NanoSci*, **2020**, 7(1), 24-28.
14. S. Thakral, V. Singh. 2,4-Dichloro-5-[(N-aryl/alkyl)sulfamoyl] benzoic Acid derivatives: In vitro antidiabetic activity, molecular modeling and In silico ADMET screening. *Med. Chem*. **2019**, 15, 186-195.
15. A.N. Choudhary, M.S. Kohli, A. Kumar, A. Joshi. Synthesis of Tryptoline-3-Carboxylic Acid Derivatives A Novel Antidiabetic Agent. *J. Young Pharm.* **2011**, 3(2), 132–137.
16. N. Lakshminarayana, Y. Rajendra Prasad, L. Gharat,, A. Thomas, P. Ravikumar, S. Narayanan, B. Gopalan, Synthesis and evaluation of some novel isochroman carboxylic acid derivatives as potential anti-diabetic agents. *E. J. Med. Chem.* **2009**, *44(8), 3147–3157.*
17. N. Negoro, S. Sasaki, S. Mikami, et.al. Discovery of TAK-875: A Potent, Selective, and Orally Bioavailable GPR40 Agonist. *ACS Med. Chem. Lett.* **2010**, 1, 6, 290–294.
18. A. Colin-Lozano, S. Estrada-Soto, F. Chavez-Silva, et.al. Design, Synthesis and in Combo Antidiabetic Bioevaluation of Multitarget Phenylpropanoic Acids. *Molecules.* **2018,** *23*, 340.
19. Y. Li, X. Qiang, L. Luo, X. Yang, G. Xiao, Y. Zheng, Z. Cao, Z. Sang, F. Su, Y. Deng. Multitarget drug design strategy against Alzheimer's disease: Homoisoflavonoid Mannich base derivatives serve as acetylcholinesterase and monoamine oxidase B dual inhibitors with multifunctional properties. *Bioorg. Med. Chem.* **2017**, *25*, 714–726.
20. QikProp, Version 3.5, Schrodinger, LLC: New York, NY, **2012.**
21. A.A. Lipinski, F. Lombardo, B. W. Dominy, P. Feeney. *J. Adv. Drug Deliv. Rev*. **2001**, 46, 3.
22. P. P. McCue, K. Shetty. Inhibitory effects of rosmarinic acid extracts on porcinepancreatic amylase *in vitro*. *A. Pacific J. Clin. Nutr*. **2004**, 13, 101–106.
23. Y.M. Kim, Y.K. Jeong, M.H. Wang, W.Y. Lee, H.I. Rhee. Inhibitory effect of pine extract on *α*-glucosidase activity and postprandial hyperglycemia. *Nutrition*. **2005**, 21, 756– 761.
24. H. Ali, P. J. Houghton, A. Soumyanath. *α*-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. *J. Ethnopharmacol*. **2006**, 107, 449–455.
25. P. M. Gowri, A.K. Tiwari, A. Z. Ali, J. M. Rao. Inhibition of alpha-glucosidase and amylase by bartogenic acid isolated from *Barringtonia racemosa Roxb*. seeds. *Phytother. Res*. **2007**, 21, 796–799.
26. M. Dixon. The determination of enzyme inhibitor constants. *Biochem*. **I953**, 55, 170–171.
27. A. Levetan. Oral antidiabetic agents in type 2 diabetes. *Curr. Med. Res. Opin.* **2007**, 23, 945–952.
28. C. J. Bailey. New approaches to the pharmacotherapy of diabetes. In: J.C. Pickup and G. William, ediors. Textbook of diabetes. 3 ed, vol. 2. Oxford, UK: Blackwell Science; **2003**, pp 73.1–73.21.
29. R. Dabur, B. Sharma, A. Mittal. Mechanistic approach of anti-diabetic compounds identified from natural sources: Anti-diabetic natural compounds and their mode of action. *Chem. Biol. Lett.*, **2018**, 5(2), 63-99.
30. S. Sekar, K.V. Pugalendi, B. Kishnan, K. Kavasseri Ganesan. Evaluation of anti-hyperglycemic effect of Gracilaria corticata extract in Normal and Streptozotocin-induced Diabetic rats. *Chem. Biol. Lett.*, **2015**, 2(1), 6-11.