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A multi-component reaction approach to the synthesis of potent antidiabetic agents five-membered iminosugars analogues

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Abstract: Some novel five-membered iminosugar analogues were efficiently synthesized via the MCRs of three components; amines, aldehydes and oxaloacetate to give 4-hydroxymethylpyrrolidines. Reduction of 3-hydroxy-4-carbomethoxy-3-pyrroliden-2-ones with Pd/C-catalyzed hydrogenation gave the corresponding *cis*-hydrogenated products at C_3/C_4 stereoselectively. Then following reductions of ester and amide functionalities with $LiAlH_4$ afforded 4-(hydroxymethyl)pyrrolidin-3-ols. Among the synthesized compounds 4-hydrazineyl-3-hydroxymethyl-2-(4-methoxyphenyl)-N-methylpyrrolidine and 3-hydrazineyl-4-hydroxymethyl-1-methylpyrrolidine were found to be the most promising α-glucosidase inhibitor with IC_{50} values of 1.12 and 1.17 mM, respectively.

Keywords: Iminosugar; pyrrolidine; one-pot reaction; stereoselective hydrogenation; antidiabetic.

1. Introduction

Polyhydroxylated piperidines and pyrrolidines, also known as iminosugars, are important class of compounds with interesting structures and immense biological significance, especially as glycosidase inhibitors,[1,2] thus making them important targets for organic synthesis. Among them, naturally occurring five-membered iminosugars such as 1,4-dideoxy-1,4-iminohexitol (iminohexitol) and 1-butyl-1,4-dideoxy-1,4-iminoarabinitol (iminoarabinitol). The synthetic analogues of iminohexitol and iminoarabinitol are known to show powerful glycosidase inhibition though stereochemical similarity between these iminosugars and the structure of hexoses or enzyme-bound intermediates.[3-7] As a result, several synthetic routes towards these pyrrolidine-based iminosugars have been reported in the literature.[1]

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Most of the synthetic routes to the skeleton of iminosugar analogues began with their carbohydrate analogues by taking advantage of the carbohydrates' many stereocenters. However, non-carbohydrate routes such as ring closing metathesis and consecutive reductive aminations are becoming popular,[8-11] since they exhibit an increase in stereoselectivity and are more efficient in introducing the amine moiety.[10] Recently, some synthetic approaches to these biologically active pyrrolidine-based iminosugars have been reported.[12,13] However, very few reports are known on the preparation of five-membered iminosugars through multi-component reactions (MCRs). In continuation to our ongoing research projects in our laboratory on pyrrolidine-type compounds,[14-19] we herein report on the synthesis and antidiabetic evaluation of 11 new pyrrolidine-based iminosugar analogues in search of new potential glucosidase inhibitors. In addition to the syntheses, all synthesized iminosugars were evaluated for α-glucosidase inhibitory activity.

2. Experimental

2.1. Chemical Material and Apparatus

High-resolution mass spectra were obtained from Agilent 1290 Infinity LC system coupled to Agilent 6520 Accurate-Mass Q-TOF. The ^{1}H and ^{13}C NMR spectra were registered in $CDCl_3$ with Joel Resonance ECZ400S [400 MHz (^{1}H) and 100 MHz (^{13}C)] using TMS as the internal standard. Analytical TLC was performed on silica gel 60 F_{254}, Merck (layer thickness 0.25 mm, Merck) and visualized with UV light and $KMnO_4$ as the detecting agent. All reagents and starting materials such as sodium diethyl oxaloacetate, amine and aldehyde were purchased from Sigma-Aldrich Co. and Merck Chemical Co.

*2.2. General Procedure for the Synthesis of Pyrrolidine Intermediates **2a-e*** (one-pot reaction)

A suspension of sodium diethyl oxaloacetate (10 mmol), amine (10 mmol) and aldehyde (10 mmol) dissolved in absolute EtOH (40 mL) were heated at reflux for 0.5-2 h. After cooling, the mixture was added onto ice-water and then acidified with 12M HCl until pH 2. The precipitate was filtered, washed with H_2O and Et_2O for removal of unreacted aldehyde to yield pyrrolidine intermediates (**a**-**e**).

*Ethyl 2-oxo-3-hydroxypyrrole-4-carboxylate (**2a**)*: Compound **2a** was previously reported (See Scheme 1)[20] White solid; 58%, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H 4.89 (2H, *s*, CH_2), 4.36 (2H, *q*, *J*= 7.2 Hz, CH_2), 1.35 (3H, *t*, *J*= 7.1 Hz, CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz), δ_C 166.5, 164.2, 151.3, 107.6, 66.1, 62.1, 14.2.

*Ethyl 2-oxo-3-hydroxy-1-methylpyrrole-4-carboxylate (**2b**)*: Compound **2b** was previously reported (See Scheme 1)[20] Yellowish solid; 57%, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H 4.31 (2H, *q*, *J*= 7.2 Hz, OCH_2), 3.96 (2H, *s*, CH_2), 3.07 (3H, *s*, NCH_3), 1.33 (3H, *t*, *J*= 7.2 Hz, CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz), δ_C 165.2, 164.1, 157.4, 107.6, 61.2, 48.1, 30.1, 14.3.

*Ethyl 2-oxo-3-hydroxy-1,5-dimethylpyrrole-4-carboxylate (**2c**)*: Yellowish solid; 68%, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H 4.28 (1H, *m*, NCH), 4.22 (2H, *m*, OCH_2), 2.90 (3H, *s*, CH_3), 2.09 (1H, *m*, CH_2-b), 1.95 (1H, *m*, CH_2-a), 1.27 (3H, *t*, *J*= 6.8 Hz, CH_3), 0.48 (3H, *d*, *J*=7.6 Hz, CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz), δ_C 188.4, 163.8, 156.5, 109.7, 60.2, 57.9, 26.4, 20.7, 13.8, 4.9.

*Ethyl 2-oxo-5-ethyl-3-hydroxy-1-methylpyrrole-4-carboxylate (**2d**)*: Yellowish solid; 53%, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H 4.28 (2H, *q*, *J*=7.2 Hz, OCH_2), 4.10 (1H, *q*, *J*= 6.4 Hz, NCH), 2.93 (3H, *s*, CH_3), 1.37 (3H, *d*, *J*= 6.4 Hz, CH_3), 1.28 (3H, *t*, *J*= 7.2 Hz, CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz), δ_C 164.4, 164.0, 156.0, 112.0, 60.2, 54.1, 26.2, 16.6, 13.8.

*Ethyl 2-oxo-3-hydroxy-1-methyl-5-(4-methoxyphneyl)pyrrole-4-carboxylate (**2e**)*: Compound **2e** was previously reported (See Scheme 1)[20] Yellow solid; 58%, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H 7.08 (2H, d,

J= 9.6 Hz, Ar), 6.88 (2H, *d*, *J*= 9.6 Hz, Ar), 4.98 (1H, *s*, CH), 4.18 (2H, *q*, *J*= 7.2 Hz, OCH_2), 3.82 (3H, *s*, OCH_3), 2.81 (3H, *s*, NCH_3), 1.17 (3H, *t*, *J*= 7.2 Hz, CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz), δ_C 165.3, 163.7, 159.9, 157.8, 128.8, 126.5, 114.3, 112.9, 62.2, 61.0, 55.4, 27.6, 14.0.

*2.3. Synthesis of Ethyl 2-oxo-3-hydroxyaminopyrrolidine-4-carboxylate (**5**)*

To a solution of pyrrolidine **2a** (1.5 g, 8.82 mmol) in CH_2Cl_2 (100 mL) was added $NH_2OH.HCl$ (0.74 g, 10.59 mmol), $NaHCO_3$ (1.07 g, 12.71 mmol) and Na_2SO_4 (1.8 g, 12.71 mmol). The mixture was stirred at room temperature for 24 h, filtered and evaporated to furnish the hydroxyamino pyrrolidine **5** as white solid in a yield of 95%. White solid. ^{1}H-NMR (D_2O, 400 MHz) δ_H 3.95 (2H, *m*, OCH_2), 4.62 (2H, *s*, NCH_2), 1.05 (3H, *t*, *J*= 6.8 Hz, CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ_C 175.6, 166.2 158.0, 104.3, 67.1, 60.0, 48.5, 13.3.

*2.4. General Procedure for the Synthesis of Pyrrolidine Intermediates (**8a-e**)*:

$NH_2NH_2.H_2O$ (5.8 mmol) was added to a mixture of ethyl 2-oxo-3-hydroxy-5(substituted)-pyrrole-4-carboxylate (**2a-e**) (2.9 mmol), AcOH (2.9 mmol) and EtOH (15 mL). The mixture was heated at reflux for 60 minutes. For **8a-d,** the reaction mixtures were evaporated under reduced pressure, extracted with EtOAc, washed with H_2O, dried over $MgSO_4$ and evaporated to afford the products. For **8e**, the resulting precipitate was filtered and washed with EtOH to give a white precipitate product **8e**.

*Ethyl 2-oxo-3-hydrazinylpyrrolidine-4-carboxylate (**8a**)*: Yield: 95%; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 4.66 (2H, *s*, NCH_2), 3.98 (2H, *q*, *J*= 7.2 Hz, OCH_2), 3.12-3.96 (3H, *brs*, $NHNH_2$), 1.12 (3H, *t*, *J*= 7.2 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 173.8, 164.7, 160.3, 98.3, 67.0, 58.2, 15.2.

*Ethyl 2-oxo-3-hydrazinyl-1-methylpyrrolidine-4-carboxylate (**8b**)*: Yield: 74%; ^{1}H NMR (400 MHz, DMSO-D_6) δ_H 3.96 (2H, *q*, *J*= 7.0 Hz, OCH_2), 3.74 (2H, *s*, CH_2), 2.86 (3H, *s*, NCH_3), 1.11 (3H, *t*, *J*= 7.1 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C170.08, 166.69, 164.90, 93.90, 57.74, 48.63, 30.24, 15.37.

*Ethyl 2-oxo-3-hydrazinyl-1,5-dimethylpyrrolidine-4-carboxylate (**8c**)*: Yield: 70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 4.16 (2H, *q*, *J*= 7.0 Hz, OCH_2), 3.99 (1H, *q*, *J*= 6.4 Hz, CH), 2. 90 (3H, *s*, NCH_3), 1.30 (3H, *d*, *J*= 6.4 Hz, CH_3), 1.24 (3H, *t*, *J*= 7.1 Hz, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 165.83, 163.57, 157.18, 112.96, 59.63, 55.39, 27.26, 18.01, 14.53.

*Ethyl 2-oxo-5-ethyl-3-hydrazinyl-1-methylpyrrolidine-4-carboxylate (**8d**)*: Yield: 91%; ^{1}H NMR (400 MHz, CD_3OD) δ_H 4.21-4.09 (2H, *m*, OCH_2), 4.15 (1H, *t*, *J*= 7.2 Hz, CH), 2.91 (3H, *s*, NCH_3), 2.12-2.08 (1H, *m*, CH_2-a), 1.87-1.84 (1H, *m*, CH_2-b), 1.26 (3H, *t*, *J*= 7.1 Hz, CH_3), 0.47 (3H, *t*, *J*= 7.3 Hz, CH_3). ^{13}C NMR (100 MHz, CD_3OD) δ_C 171.65, 164.23, 163.87, 109.74, 58.53, 58.27, 26.33, 21.03, 14.16, 13.64.

*Ethyl 2-oxo-3-hydrazinyl-1-methyl-5-(4-methoxyphneyl)pyrrolidine-4-carboxylate (**8e**)*: Yield: 86%; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.03 (2H, *d*, *J*= 8.5 Hz, CHAr), 6.81 (2H, *d*, *J*= 8.2 Hz, CHAr), 4.93 (1H, *s*, NCH), 4.07 (2H, *q*, *J*= 7.0 Hz, OCH_2), 3.74 (3H, *s*, OCH_3), 2.74 (3H, *s*, NCH_3), 1.08 (3H, *t*, *J*= 7.1 Hz, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 164.90, 164.08, 159.89, 157.03, 128.82, 126.13, 114.22, 112.92, 62.29, 60.92, 55.34, 27.64, 14.03.

2.5. Synthesis of 2-oxo-5(4-methoxyphneyl)-1-methylpyrrolidine-2,3-dione (**11**)

Pyrrolidine **2e** (0.20 g, 0.77 mmol) was dispersed in 10% aq. HCl solution (10 mL) and heated under reflux for 7 hours which was gradually dissolved to give a brownish solution. The reaction mixture was then cooled and concentrated to dryness. The crude product was triturated with Et_2O and extracted with CH_2Cl_2 and water. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo to give the pure product pyrrolidinone intermediate **11** in a yield of 75% . ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H 7.11 (2H, *d*, *J*= 8.8 Hz, Ar), 6.93 (2H, *d*, *J*= 8.8 Hz, Ar), 4.74 (1H, *dd*, *J*= 7.4 Hz, 3.2 Hz, NCH), 3.79 (3H, *s*, OCH_3), 3.17-3.10 (1H, *dd*, *J*= 20.4 Hz; *J*=7.4 Hz, H_{4a}), 2.87 (3H, *s*, NCH_3), 2.53

(1H, *dd*, *J*= 20.4 Hz, *J*=3.2 Hz, H_{4b}). ^{13}C-NMR ($CDCl_3$, 100 MHz), δ_C 198.07, 160.04, 159.3, 130.0, 127.6, 114.8, 57.8, 55.3, 40.9, 29.6,

*2.6. General procedure for the Synthesis of Iminosugars (***4a-e, 7, 10a-e, 12***):*

To a stirred solutions of **2a-e**, **5** and **8a-e** (6 mmol) in EtOH (40 mL) were added catalytic amount of Pd-C (10% wt.) (0.90 g) and AcOH (0.2 mL). The reactions were stirred vigorously under H_2 atmosphere for 24 h and then filtered through Celite. After removal of the solvent, the crude products were submitted to the next steps without purification. The crude products were dissolved in dry THF and were added slowly into solution of $LiAlH_4$ (excess) in a 50 mL round bottom flask. The mixtures were heated at 90 ºC for 4-6 h and then cooled to 0 °C. The reaction mixtures were quenched by the addition of distilled H_2O, and the mixtures were filtered through Celite and concentrated in vacuo to give the pure products iminosugar analogues **(4a-e, 7, 10a-e, 12)** as solids after recrystallization or purification by column chromatography.

*3-hydroxy-4-hydroxymethylpyrrolidine (**4a**)*: Compound **4a** was previously reported (See Scheme 2)[20] White solid; 90%. HRESI-MS *m/z*: $[M+H]^+$ 118.0674 (Calc. for $C_5H_{12}NO_2$, 118.0862). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 3.52-3.40 (3H, *m*, CHOH and CH_2OH), 3.41-3.25 (4H, *m*, 2 x $NHCH_2$), 1.53 (1H, *m*, CH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ_C 70.6, 64.8, 60.3, 59.8, 46.2.

*3-hydroxy-4-hydroxymethyl-1-methylpyrrolidine (**4b**)*: Compound 4**b** was previously reported (See Scheme 2)[20] Brown solid; 90%. HRESI-MS *m/z*: $[M]^+$ 131.0908 (Calc. for $C_6H_{13}NO_2$, 131.0941). ^{1}H-NMR (CD_3OD, 400 MHz) δ_H 4.35 (1H, *dt*, *J*= 5.2, 4.4 Hz, CHOH), 3.77 (1H, *dd*, *J*= 8.0, 11.2 Hz, OCH_2-a), 3.60 (1H, *dd*, *J*= 6.8, 11.2 Hz, OCH_2-b), 3.52-3.40 (3H, *m*, CHOH and CH_2OH), 3.01 (1H, *dd*, *J*= 5.6, 10.4 Hz, NCH_2-a CHOH), 2.79 (1H, *dd*, *J*= 8.0, 9.2 Hz, NCH_2-a), 2.44 (1H, *m*, NCH_2-b CHOH), 2.41 (1H, *m*, NCH_2-b), 2.34 (3H, *s*, NCH_3) and 1.61 (1H, *m*, CH). ^{13}C-NMR (CD_3OD, 100 MHz) δ_C 71.3, 64.2, 60.3, 57.5, 45.3, 41.6.

*5-ethyl-3-hydroxy-4-hydroxymethyl-1-methylpyrrolidine (**4d**)*: Brown solid; 85%. HRESI-MS *m/z*: $[M]^+$ 159.9179 (Calc. for $C_8H_{17}NO_2$, 159.1254). IR (ATR) ν/cm^{-1}: 3539 (stretching, OH), 3124-3024 (stretching, CH aliphatic), 1476-1347 (bending, CH aliphatic), 1043 (stretching, C-O). ^{1}H-NMR (CD_3OD, 400 MHz) δ_H 4.53 (1H, *dd*, *J*= 5.6, 9.2 Hz, CHOH), 3.83 (1H, *dd*, *J*= 6.0, 10.8 Hz, OCH_2-b) 3.79 (1H, *dd*, *J*= 6.0, 10.8 Hz, OCH_2-a), 3.46 (1H, *dd*, *J* = 3.2, 11.6 Hz, NCH_2-b), 3.13 (1H, *dd*, *J* = 6.0, 12.0 Hz, NCH_2-a), 2.80 (3H, *s*, NCH_3), 2.54 (1H, *dt*, *J* = 7.6, 13.2 Hz, NCH), 1.86 (2H, *sx*, *J*= 7.2 Hz, CH_2), 1.07 (3H, *t*, *J*= 7.2 Hz, CH_3). ^{13}C-NMR (CD_3OD, 100 MHz) δ_C 71.4, 69.2, 62.9, 56.2, 46.1, 41.2, 21.1, 10.4.

*3-hydroxy-4-hydroxymethyl-1-methyl-5(4-methoxypheneyl)pyrrolidine (**4e**)*: IR (ATR) ν/cm^{-1}: 3652 (stretching, OH), 3211-3179 (stretching, CH Ar), 3110-2967 (stretching, CH aliphatic), 1658 and 1559 (stretching, C=C Ar), 1505-1226 (bending, CH aliphatic), 1282 and 1070 (stretching, C-O-C), 1054 (stretching, C-O). Brown solid; 78%. HRESI-MS *m/z*: $[M]^+$ 237.1249 (Calc. for $C_{13}H_{19}NO_3$, 237.1359). ^{1}H-NMR (CD_3OD, 400 MHz) δ_H 7.24 (2H, *d*, *J*= 8.4 Hz, Ar-CH), 6.87 (2H, *d*, *J*= 8.4 Hz, Ar-CH), 3.80 (1H, *m*, CHOH), 3.76 (3H, *s*, OCH_3), 3.47 (1H, *dd*, *J*= 4.0, 10.8 Hz, OCH_2-b), 3.36 (1H, *dd*, *J*= 6.4, 10.8 Hz, OCH_2-a), 3.32 (1H, *m*, NCH), 3.13 (1H, *t*, *J*= 7.6 Hz, NCH_2-b), 2.71 (1H, *d*, *J*= 9.2 Hz, NCH_2-a), 2.22 (1H, *m*, CH), 2.07 (3H, s, NCH_3). ^{13}C-NMR (CD_3OD, 100 MHz) δ_C 159.5, 132.3, 129.0, 113.6, 73.5, 62.6, 55.6, 54.3, 49.4, 39.2.

*3-hydroxyamino-4-hydroxymethylpyrrolidine (**7**):* Brown solid; 70.4%. HRESI-MS *m/z*: $[M]^+$ 132.0428 (Calc. for $C_5H_{12}N_2O_2$, 132.0893). IR (ATR) ν/cm^{-1}: 3556 (stretching, OH), 3426 (stretching, NH), 3097-2991 (stretching, CH aliphatic), 1460-1367 (bending, CH aliphatic), 1069 (stretching, C-O). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 3.52-3.40 (2H, *m*, CH_2OH), 3.41-3.25 (4H, *m*, 2x$NHCH_2$), 2.6 (1H, *m*, HONHCH), 1.35 (1H, *m*, CH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ_C 61.2, 33.0, 31.8, 29.2, 26.0, 22.6.

*3-hydrazinyl-4-hydroxymethylpyrrolidine (**10a**)*: Pale yellow solid; 93%. HRESI-MS *m/z*: $[M]^+$ 131.0853 (Calc. for $C_5H_{13}N_3O$, 131.1053). IR (ATR) ν/cm^{-1}: 3545 (stretching, OH), 3394 (stretching,

NH), 3097-2976 (stretching, CH aliphatic), 1463-1333 (bending, CH aliphatic), 1074 (stretching, C-O). 1H-NMR (D_2O, 400 MHz) δ_H 3.35-3.19 (2H, *m*, CH_2OH), 3.21-3.01 (4H, m, 2 x NCH_2), 1.28 (1H, *m*, CH). ^{13}C-NMR (D_2O, 100 MHz) δ_C 70.2, 63.4, 59.2, 58.7, 44.5.

3-hydrazinyl-4-hydroxymethyl-1-methylpyrrolidine ***(10b)***: White solid; 90%. HRESI-MS (negative mode) *m/z*: $[M-H]^-$ 144.0791 (Calc. for $C_6H_{14}N_3O$, 144.1142). IR (ATR) ν/cm^{-1}: 3547 (stretching, OH), 3394 (stretching, NH), 3083-2974 (stretching, CH aliphatic), 1462-1330 (bending, CH aliphatic), 1059 (stretching, C-O). ^{1}H-NMR (CD_3OD, 400 MHz) δ_H 3.85-3.90 (2H, *m*, CH_2OH), 3.80 (1H, m, NH_2NHCH), 3.70-3.50 (4H, m, 2 x NCH_2), 2.80 (3H, *s*, NCH_3), 1.80 (1H, *m*, CH). ^{13}C-NMR (D_2O, 100 MHz) δ_C 71.2, 68.8, 62.1, 59.4, 55.9, 53.4.

3-hydrazinyl-4-hydroxymethyl-1,5-dimethylpyrrolidine ***(10c)***: White solid; 95%. HRESI-MS MS (negative mode) *m/z*: $[M+H]^+$ 159.0902 (Calc. for $C_7H_{17}N_3O$, 159.1377). IR (ATR) ν/cm^{-1}: 3531 (stretching, OH), 3392 (stretching, NH), 3103-2981 (stretching, CH aliphatic), 1468-2981 (bending, CH aliphatic), 1073 (stretching, C-O). ^{1}H-NMR (CD_3OD, 400 MHz) δ_H 3.84 (1H, *dd*, *J*= 7.2, 10.8 OCH_2-b), 3.72 (1H, *dd*, *J*= 5.2, 10.8 Hz, OCH_2-a), 3.58 (1H, *q*, *J*= 7.2 Hz, NH_2NHCH), 3.55 (1H, *m*, NCH_2-b), 3.41 (1H, *dd*, *J*= 4.8, 11.6 Hz NCH_2-a), 2.33 (1H, *m*, NCH), 2.27 (3H, *s*, NCH_3), 1.70 (1H, *m*, CH), 0.94 (1H, *d*, *J*=7.2 Hz, CH_3). ^{13}C-NMR (CD_3OD, 100 MHz) δ_C 72.4, 69.2, 63.9, 60.4, 55.9, 46.2, 9.24.

5-ethyl-3-hydrazinyl-4-hydroxymethyl-1-methylpyrrolidine ***(10d)***: White solid; 90%. HRESI-MS (negative mode) *m/z*: $[M+K-2H]^-$ 210.9579 (Calc. for $C_8H_{17}N_3OK$, 210.1014). IR (ATR) ν/cm^{-1}: 3531 (stretching, OH), 3393 (stretching, NH), 3113-2970 (stretching, CH aliphatic), 1466-1285 (bending, CH aliphatic), 1058 (stretching, C-O). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 3.66 (1H, *dd*, *J*= 4.8, 10.8 OCH_2-b), 3.52 (1H, *dd*, *J*= 5.2, 10.8 OCH_2-a), 3.44 (1H, *m*, NH_2NHCH), 3.24 (1H, *m*, NCH_2-b), 3.10 (1H, *m*, NCH_2-a), 2.90 (3H, s, NCH_3), 2.01 (1H, *m*, CH), 2.19 (1H, *q*, *J*= 5.6, NCH), 1.75 (2H, qt, *J*=7.2 Hz, CH_2), 1.06 (1H, t, *J*=7.2 Hz, CH_3). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ_C 72.4, 69.2, 63.9, 60.4, 55.9, 39.8, 25.1, 9.29.

3-hydrazinyl-4-hydroxymethyl-1-methyl-5-(4-methoxypheneyl)pyrrolidine ***(10e)***: Brown solid; 80%. HRESI-MS (negative mode) *m/z*: $[M]^-$ 251.1182 (Calc. for $C_{13}H_{21}N_3O_2$, 251.1639). IR (ATR) ν/cm^{-1}: 3532 (stretching, OH), 3391 (stretching, NH), 3211-3174 (stretching, CH Ar), 3119-2957 (stretching, CH aliphatic), 1659 and 1559 (stretching, C=C Ar), 1469-1286 (bending, CH aliphatic), 1282 and 1073 (stretching, C-O-C), 1055 (stretching, C-O). ^{1}H-NMR (CD_3OD, 400 MHz) δ_H 7.31 (2H, *d*, *J*= 8.8 Hz, Ar-CH), 6.87 (2H, *d*, *J*= 8.8 Hz, Ar-CH), 4.21 (1H, *m*, NHCH), 3.75 (3H, *s*, OCH_3), 3.60 (1H, *dd*, *J*= 4.4, 11.6 Hz, OCH_2-b), 3.39 (1H, *dd*, *J*= 6.0, 11.6 Hz, OCH_2-a), 3.01 (1H, *d*, *J*= 10.8 Hz, NCH_2-b), 2.91 (1H, *d*, *J*= 9.6 Hz, NCH_2-a), 2.50 (1H, *dd*, *J*= 6.0, 11.2 Hz, CH), 2.06 (3H, s, NCH_3). ^{13}C-NMR (CD_3OD, 100 MHz) δ_C 159.5, 131.8, 129.2, 113.6, 72.9, 71.5, 64.5, 60.0, 59.7, 54.4, 39.2.

3-hydroxy-5(4-methoxypheneyl)pyrrolidine ***(12)***: Pale brown solid; 85%. HRESI-MS (negative mode) *m/z*: $[M]^-$ 207.0912 (Calc. for $C_{12}H_{17}NO_2$, 207.1265). IR (ATR) ν/cm^{-1}: 3670 (stretching, OH), 3211-3173 (stretching, CH Ar), 3109-2980 (stretching, CH aliphatic), 1659 and 1560 (stretching, C=C Ar), 1497-1237 (bending, CH aliphatic), 1283 and 1072 (stretching, C-O-C), 1068 (stretching, C-O). ^{1}H-NMR (CD_3OD, 400 MHz) δ_H 7.27 (2H, *d*, *J*= 8.8 Hz, Ar-CH), 6.84 (2H, *d*, *J*= 8.8 Hz, Ar-CH), 4.39 (1H, *m*, CHOH), 4.29 (1H, *m*, NCH), 3.74 (3H, *s*, OCH_3), 3.50 (1H, *dd*, *J*= 6.8, 10.4 Hz, NCH_2-b), 3.50 (1H, *dd*, *J*= 7.2, 9.6 Hz, NCH_2-a), 2.52 (1H, *dt*, *J*= 7.6, 14.0 Hz, CH_2-b), 2.38 (1H, *m*, CH_2-a), 2.04 (3H, *s*, NCH_3). ^{13}C-NMR (CD_3OD, 100 MHz) δ_C 159.5, 131.2, 128.7, 113.5, 70.9, 68.6, 65.4, 54.4, 44.8, 39.2.

2.7. Biological Assay

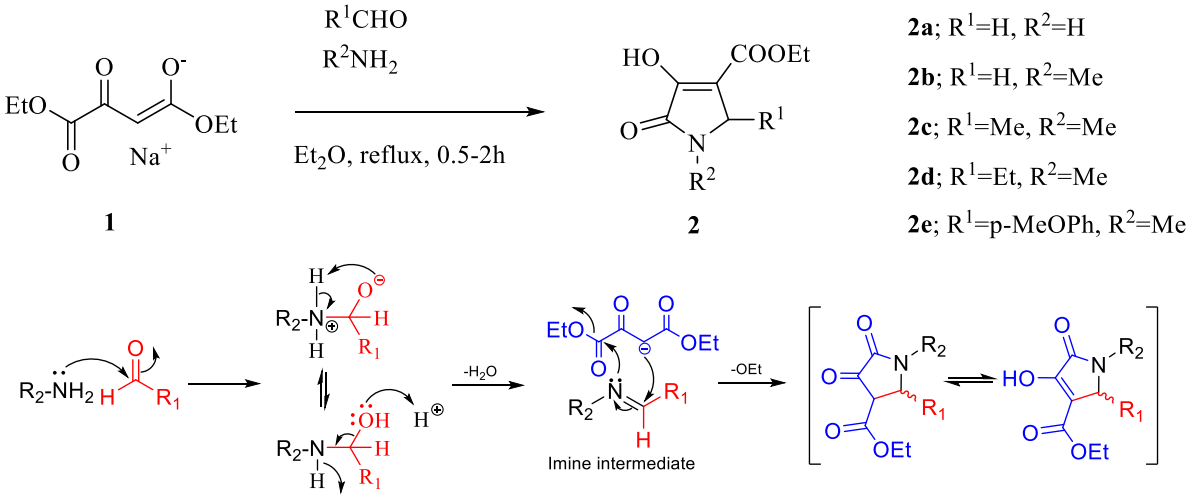
α-Glucosidase enzyme inhibitory activity assay was adopted from method as described by Elya at al. (2012) with slight modifications.[25]

3. Results and Discussion

3.1. Chemistry

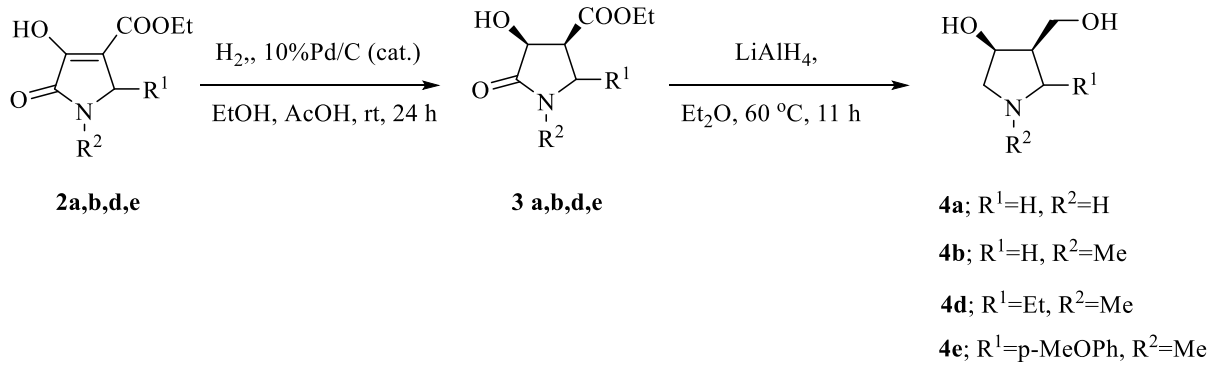
Synthesis of five-membered iminosugars analogues in this study are based on the modification of the core structures of glucosidase inhibitor iminohexitol and iminoarabinitol. Conventionally, synthesis of hydroxyprolines and pyrrolidines utilize carbohydrates as starting materials, due to the similarity of their structure.[21] In this work, we prepared all starting substances for targeted iminosugar analogues via multicomponent reaction strategy (MCRs) using readily accessible oxaloacetate, aldehyde and amine as starting material.[22]

In this study, five key intermediate ethyl 2-oxo-3-hydroxy-5(substituted)-pyrrole-4-carboxylates **(2a-e)** were prepared from MCRs of three components including diethyl oxaloacetate (ammonia and methylamine), aldehyde (formaldehyde, acetaldehyde, ethanaldehyde and *p*-methoxybenzaldehide) and amine precursors (Scheme 1). The reaction was performed in the EtOH under reflux condition as reported previously by Mohammad at al. (2009).[17] The MCRs begins with condensation of amine and aldehyde *via* nucleophilic addition to give a hemiaminal intermediate, followed by an elimination of water to yield the imine intermediate. Then the reaction continues with the formation of pyrrolidine ring *via* one-step addition and cyclization reaction of imine and diethyl oxaloacetate. In this route, the racemic mixture is formed, due to the direction of nucleophile attack can be achieved from both side of imine intermediate (Scheme 1). The product yield of this step is only moderate (57-68%) due to the presence of byproducts and side-products during the reactions.[23] The ^{13}C NMR spectral data show the presence of four carbon signals in the downfield region belong to amide and ester (around δ_C 163.0 – 167.0) and enolic alkene part (around δ_C 157.0, 106.0). The racemic excess at $C_{(5)}$ position was recognized from the presence of replication signals on their ^1H NMR spectrum.



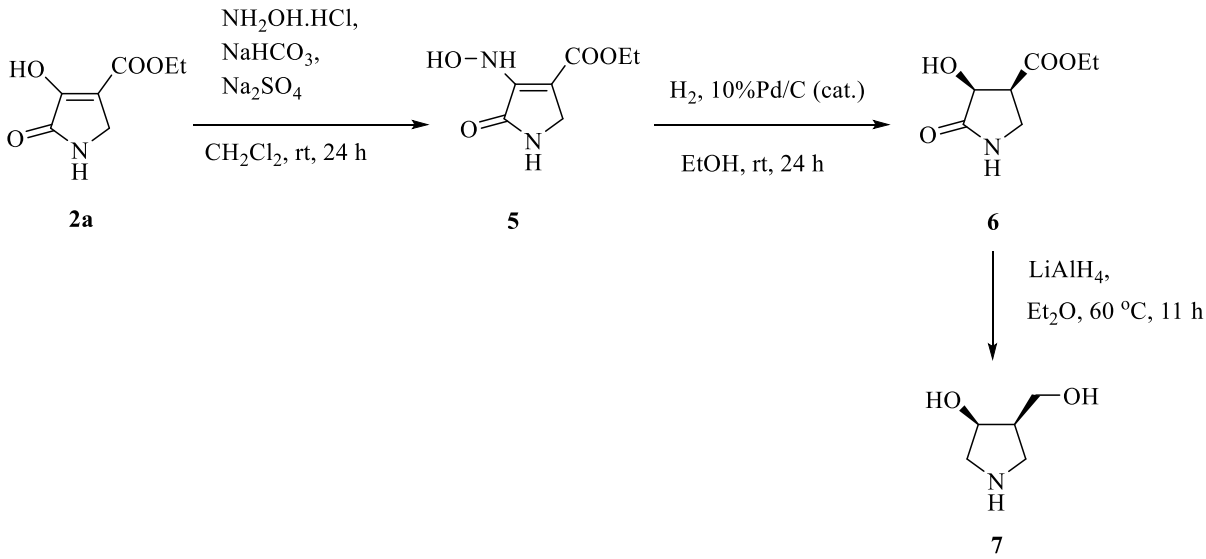
Scheme 1. Preparation of starting compounds **(2a-e)** and a proposed formation mechanism

Furthermore, Pd/C-catalyzed hydrogenation of **2a,b,d,e** in the presence of AcOH gave ethyl 2-oxo-3-hydrazinyl-5(substituted)-pyrrole-4-carboxylate (**3a,b,d,e**). Further reductions of amide and ester at using excess of $LiAlH_4$ in dry Et_2O lead to our desired products 3-hydroxy-4-hydroxymethyl-5(substituted)-pyrrolidines **(4a,b,d,e)** in good yield (75-90%). In the Pd/C-catalysis hydrogenation, hydrogen atom is always delivered in the same face of the alkene ("syn" addition), therefore the orientation of methine protons $H_{(3)}/H_{(4)}$ at **4a,b,d,e** are always in *cis*-configuration as show in Scheme 2. From this products, the important proton signals of **4a,b,d,e** were observed at around δ_H 1.28 (methine), δ_H 4.53 (oxygenated methine), δ_H 2.71 (methylene) and δ_H 3.47 (oxygenated methylene). The *cis*-configuration of protons at $C_{(3)}$ and $C_{(4)}$ was confirmed by the *J* coupling value of protons $H_{(3)}/H_{(4)}$ (^3J=5.6 Hz).[24] Those evident was supported by HRESI-MS experiments.



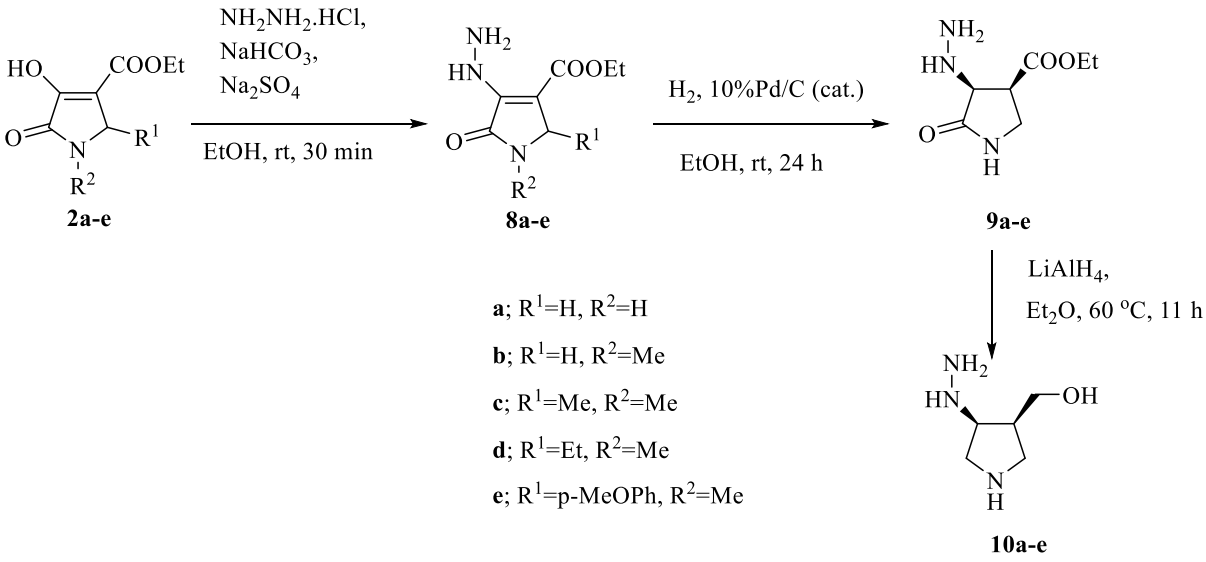
Scheme 2. Synthesis of iminosugar analogues **(4a,b,d,e)**

Synthesis of compound **7** was carried out by treatment of intermediate **2a** with $NH_2OH.HCl$ in the presence of a weak base $NaHCO_3$ at room temperature gave enamine-imine tautomerism products of ethyl 2-oxo-3-hydroxyamino-pyrrole-4-carboxylate and 2-oxo-3-hydroxyimino-pyrrole-4-carboxylate (**5**) (95%). This tautomerization was confirmed from the presence of additional signals belong to aliphatic methine (δ_C 48.5) and quaternary sp^2 carbon (δ_C 158.0), in which oxime product only exist as minor products. Further catalytic hydrogenations of **5** using in the presence of Pd/C and then reduction with $LiAlH_4$ lead to the targeting compound 3-hydroxyamino-4-hydroxymethyl-pyrrolidine (**7**) in the good yield (70.4%) (Scheme 3). In these reductions, the *cis*-orientation of methine protons $H_{(3)}/H_{(4)}$ was anticipated due to the utilization of stereoselective hydrogenation catalyst H_2 Pd/C. The structures of **5** and **7** were confirmed by NMR and HRESI-MS spectral data.



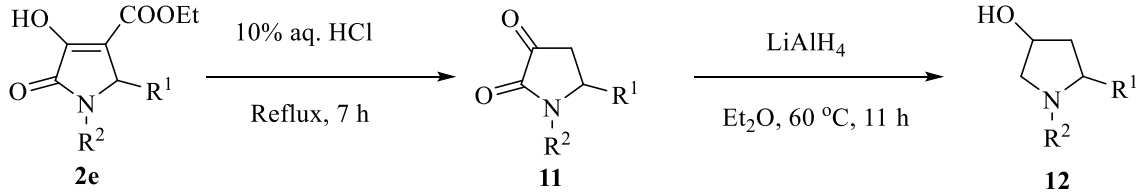
Scheme 3. Synthesis of 3-hydroxyamino-4-hydroxymethylpyrrolidine **7**.

For synthesis of iminosugars (**10a-e**), the transformation of hydroxyl group of **2a-e** into hydrazinyl functionalities have been done through the treatment with hydrazine hydrate in EtOH under reflux condition.[25] In this step, the appropriate products ethyl 2-oxo-3-hydrazinyl-5(substituted)-pyrrole-4-carboxylate **(8a-e)** were formed in moderate to good yields (70-95%). Further steps are continued with catalytic hydrogenations in the presence of Pd/C and reduction with $LiAlH_4$ respectively to give targeting 3-hydrazinyl-4-hydroxymethyl-5(substituted)-pyrrolidine **(10a-e)** in the excellent yields (80-95%) (Scheme 4). The characteristic signals in the skeleton of intermediates (**8a-e**) and targeting iminosugars (**10a-e**) have been confirmed by NMR and HRESI-MS.



Scheme 4. Synthesis of targeting iminosugar analogues (**10a-e)**

Synthesis of targeting iminosugar **12** was started with decarboxylation of key intermediate **2e** using 10% aqueous HCl under reflux condition which gave pyrrolidinone intermediate **11** in good yield (75%). Furthermore, direct reduction of ketone and amide groups in **11** using $LiAlH_4$ in the dry Et_2O at 60 °C lead to 3-hydroxy-5-(4-methoxyphneyl)-pyrrolidine (**12**). The structure of **12** was confirmed by the presence of two methylene groups and absence proton signals for ethyl ester group. The structure of **12** was also supported by HRESI-MS spectral data.



Scheme 5. Synthesis of 3-hydroxy-5-(4-methoxyphneyl)-pyrrolidine (**12)**

All synthesized iminosugars have been evaluated for their inhibitory potential against α-glucosidase as reported by Elya at al. (2012) with slight modification.[26] The tested compounds showed a variable degree of α-glucosidase inhibition with IC_{50} values ranging between 1.116 ± 1.010 mM and 2.938 ± 1.076 mM as compared with 1-deoxynojirimycin (DNJ) (IC_{50} = 0.558 ± 1.030 mM). Compound **10e** is found to be the most potent compound in the series (Table 2). This compound has hydrazine group at C-4 position and methoxybenzene group at C-2 position which seem to play an important role in the inhibition activity. Besides that, among of the 4-hydroxypyrrolidine analogues (**4a-e**), **4a** has the highest inhibitory activity because the compound highly mimics the structure of substrate, thus it fits well in the active site.

Table 2. α-Glucosidase inhibition studies of five-membered iminosugars

Compound	IC_{50} (mM)*
4a	1.404 ± 1.005
4b	2.938 ± 1.076
4d	1.800 ± 1.016
4e	1.660 ± 1.017
7	2.171 ± 1.365
10a	1.751 ± 1.006
10b	1.311 ± 1.003
10c	1.271 ± 1.859
10d	1.523 ± 1.006
10e	1.116 ± 1.010
12	1.169 ± 1.012
DNJ (control)	0.558 ± 1.030

*Data was expressed as mean ± S.D., n = 3.

As a conclusion, a diverse framework of pyrrolidine-based iminosugars analogues was successfully synthesized from MCRs strategy with simple procedure under mild conditions, short steps and good yields. The utilization of non-carbohydrate precursor for the synthesis of iminosugar have opened the diversity of the iminosugar skeleton for the variety of biological activity study. This study also demonstrated that the newly synthesized pyrrolidine-based iminosugar analogues had promising effects in inhibiting α-glucosidase and further optimization can be done to improve their activity.

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