A Facile Synthesis of Pyrrolidine-based Iminosugars as Potential Alpha-Glucosidase Inhibitors

MUHAMAD ZULFAQAR BACHO¹, MOHD FAZLI MOHAMMAT², ZURINA SHAAMERI², AGUSTONO WIBOWO³, FIRDAUS KAMARULZAMAN⁴ and AHMAD SAZALI HAMZAH²*

¹Faculty of Applied Sciences, Universiti Teknologi Mara (UiTM), 40450 Shah Alam, Selangor Darul Ehsan, Malaysia.
²Organic Synthesis Laboratory, Institute of Science, Universiti Teknologi MARA (UiTM), 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia.
³Faculty of Applied Sciences, Cawangan Pahang, Universiti Teknologi Mara (UiTM), 26400 Bandar Jengka, Pahang, Malaysia.
⁴Natural Products Division, Forest Research Institute Malaysia (FRIM), Kepong, 52109 Selangor Darul Ehsan, Malaysia.
*Corresponding author E-mail: asazali@uitm.edu.my

http://dx.doi.org/10.13005/ojc/360214

(Received: October 14, 2019; Accepted: April 01, 2020)

ABSTRACT

A multifaceted approach comprising MCR (multicomponent reaction), amination and stereoselective reduction reactions was used to synthesize new pyrrolidine-based iminosugars. The key step of this strategy involves the construction of a highly functionalised pyrroldine ring skeleton through MCR approach. Subsequently, amination and reduction reactions to the ring skeleton provide a quick access to new pyrrolidine-based imino sugars. The iminosugars were then tested against alpha glucosidase activity in which one compound (4-((4-methoxyphenyl)amino)pyrrolidin-3-ol), was found to be the most potent at low dosage.

Keywords: Iminosugars, Pyrrolidine, Alpha-glucosidase, Antidiabetic.

INTRODUCTION

Diabetes mellitus (DM) is a continuously rising chronic metabolic disorder and has become a vital problem to the world population¹. Over time, this disease has escalated the number of health issues among patients commonly to the type 2 diabetes (T2D)². T2D happens by an abnormal postprandial increase of blood glucose level due to failed insulin production or action³. One way to overcome this calamity is by using alpha-glucosidase inhibitors, an approach to prevent postprandial hyperglycemia since they can act as competitive inhibitors of small intestinal brush-border alpha-glucosidases⁴,⁵. This glucosidase can inhibits the enzymes and delay the conversion of polysaccharide into absorbable monosaccharides (glucose and fructose)⁶. Some alpha-glucosidases such as acarbose, voglibose
and miglitol that are currently available in the market prove the effectiveness of this approach (Fig. 1). Due to the limited number of these commercially available inhibitors, new alternative synthetic methods are thus required to combat this disease effectively.

For the past decade, many groups have reported the synthesis of pyrrolidine-based iminosugars. Zhang and co-workers reported the synthesis of iminosugars using D-glucose as its main precursor. The target molecule (3S,4S)-3-((R)-1-2-dihydroxyethyl)pyrrolidine-3,4-diol was obtained in 10 steps with 24% yield. Doddi and co-workers reported the synthesis of azasugars utilizing pyrrolidine skeleton followed by regiospecific amination, ring closing metathesis, and diastereospecific dihydroxylations as the key reactions. These sugar molecules however, were found to have moderate inhibition against glycosidase enzyme. In addition other synthetic strategies of pyrrolidine-based iminosugars have also been reported.

In continuation of our work on the five membered heterocycle system, we now report the synthesis of pyrrolidine based iminosugars in short steps via multi component reaction (MCR), amination, stereoselective reduction.

**EXPERIMENTAL**

**General**

Infrared spectra (IR) was recorded on NICOLET 6700 FT-IR using diamond with ATR. NMR spectra was recorded on JEOL Resonance ECZ400 [400 MHz (1H) and 100 MHz (13C)] using TMS as the internal standard. The molecular weight of synthesized compound was recorded on Agilent Technologies model: 6500 Accurate-Mass Q-TOF LC/MS, Thermo Scientific Orbitrap Fusion Tribid Mass Spectrometer and GC-MS Agilent Technologies 7893 Autosampler (GC System). Elemental analysis was performed by Flash 2000 Organic Elemental Analyzer. Melting point was run by Stuart SMP30. Analytical TLC was performed on silica gel 60 F254, Merck (layer thickness 0.25 mm, Merck) and visualized with UV light and KMnO4 as the detecting agent.

**General procedure for the synthesis of pyrrolidine-based iminosugar intermediates (4a-k).**

A mixture of compound 1 (1 equiv) and aldehyde (1 equiv) together with an equimolar amount of amine in ethanol was refluxed towards completion (0.5 – 2 hours). Iced-water was added to the mixture after cooling and HCl was then added dropwise to pH 1. Filter the solid while appear. Traces aldehyde in the crude product was washed with water and ether to give (4a-k).

**Ethyl 4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylate (4a)**

White solid; 60%; m.p. 106-109°C. IR (ATR) ν/cm⁻¹: 3344 (OH), 2986 (NH, amide), 1782 (C=O, ester), 1687 (C=C), 1670 (C=O, amide), 1302 (C-N); 1H-NMR (400 MHz, CDCl₃): δ 4.91-4.85 (2H, s, CH₂), 4.39-4.32 (2H, q, J= 7.2 Hz, CH₂), 1.38-1.31 (3H, t, J= 7.1 Hz, CH₃); 13C-NMR (100 MHz, CDCl₃): δ 166.54 (COH), 164.23 (C=O) 151.29 (C=O), 116.09 (quat. C), 66.26 (OCH₂), 62.09 (CH₂), 14.34 (CH₃); Anal. Calcd. for C₇H₉NO₄: C, 49.12; H, 5.30; N, 8.18; O, 37.39. Found: C, 49.30; H, 4.65; N, 7.04; O, 39.01; GCMS m/z (EI, + ve): found 172.00 ([M]+), C₇H₇NO₄ calculated 172.06.

**Ethyl 4-hydroxy-1-methyl-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylate (4b)**

Yellowish solid; 40%; m.p. 139-141°C. IR (ATR) ν/cm⁻¹: 3400 (OH), 1780 (C=O, ester), 1648 (C=C), 1274 (C-N), 734; 1H-NMR (400 MHz, CDCl₃):...
Ethyl 2-ethyl-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylate (4c)

White solid: 12.93%; m. p. 146-148°C. IR (ATR) v/cm−1: 3305 (OH), 3176 (NH, amide), 1766 (C=O, ester), 1678 (C=C), 1639 (C=N, amide), 1454 (CH=CH), 1371 (CH=CH), 1304 (C=N): 1H NMR (400 MHz, CDCl3): δ 4.31-4.25 (m, 1H), 4.28-4.17 (m, 2H), 2.05-1.90 (m, 1H), 1.66 (td, J = 14.4, 7.3 Hz, 1H), 1.28 (tdd, J = 14.9, 7.2, 1.0 Hz, 3H), 0.85-0.69 (m, 3H); 13C NMR (100 MHz, CDCl3): δ 166.31 (COOH), 164.05 (CO), 151.28 (C=O), 112.47 (quat. C), 60.24 (OCH3), 54.37 (CH), 24.79 (CH3), 13.32 (CH3), 7.04 (CH); Anal. Calcd. for C16H15NO5: C, 55.10; H, 5.12; N, 3.37, O, 36.41; GCMS m/z (EI, +ve): found 331.10 ([M+H]+); C16H15NO5 calculated 331.10.

Ethyl 4-hydroxy-2-isopropyl-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylate (4d)

White solid: 29.57%; m. p. 165-167°C. IR (ATR) v/cm−1: 3318 (OH), 3177 (NH, amide), 2925 (C-H), 1768 (C=O, ester), 1722 (C=C), 1630 (N-C=O, amide), 1372 (CH=CH): 1H NMR (400 MHz, CDCl3): δ 4.31-4.22 (m, 2H), 4.19 (d, J = 2.8 Hz, 1H), 2.42 (dt, J = 13.9, 7.0, 2.8 Hz, 1H), 1.30 (s, 3H), 1.07 (d, J = 7.1 Hz, 3H), 0.59 (d, J = 6.8 Hz, 3H); 13C NMR (100 MHz, CDCl3): δ 167.85 (COOH), 164.13 (CO), 155.31 (C=O), 112.83 (quat. C), 60.28 (OCH3), 58.64 (CH), 29.01 (CH), 19.83 (CH3), 13.18 (CH3); Anal. Calcd. for C19H16NO5: C, 56.33; H, 7.09; N, 6.57; O, 30.01. Found: C, 55.30; H, 6.89; N, 6.18; GCMS m/z (EI, +ve): found 213.10 ([M]+); C19H16NO5 calculated 213.10.

Ethyl 4-hydroxy-5-oxo-2-(p-tolyl)-2,5-dihydro-1H-pyrrole-3-carboxylate (4e)

Light yellow solid: 28.04%; m. p. 153-156°C. IR (ATR) v/cm−1: 3303 (OH), 2982 (NH, amide), 1719 (C=O, ester), 1621 (C=O) 1556 (N=C=O, amide), 667 (Ar-CH3); 1H-NMR (400 MHz, CDCl3): δ 7.12 (s, 2H), 7.07 (s, 2H), 4.34 (q, J = 7.2 Hz, 2H), 4.14 (dd, J = 7.1, 1.6 Hz, 1H), 2.31 (d, J = 9.6 Hz, 3H), 1.36 (t, J = 7.1 Hz, 3H); 13C NMR (100 MHz, CDCl3): δ 170.59 (COH), 165.52 (C-O), 160.36 (C=O), 140.56 (aromatic C), 137.29 (aromatic C), 129.45 (CH-Ar), 127.14 (CH-Ar), 107.97 (quat. C), 64.11 (OCH3), 56.85 (CH), 21.25 (CH-Ar), 13.89 (CH3); Anal. Calcd. for C16H15NO5: C, 61.85; H, 5.88; N, 4.81; O, 27.46. Found: C, 55.10; H, 5.12; N, 3.37, O, 36.41; GCMS m/z (El, +ve): found 284.1 ([M + Na]+); C16H15NO5 calculated 284.09.

Ethyl 2-(4-cyanophenyl)-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylate (4f)

Yellow solid: 20.49%; m. p. 139-141°C. IR (ATR) v/cm−1: 3302 (OH), 2987 (NH, amide), 1728 (C=O, ester), 1591 (C=O), 1501 (N=C=O), 768 (Ar-CN); 1H-NMR (400 MHz, CD3OD): δ 7.76-7.61 (2H), 7.54-7.39 (2H), 5.37-5.22 (1H), 4.12-3.99 (2H), 1.17-1.00 (3H); 13C NMR (100 MHz, CD3OD): δ 167.39 (COOH), 165.66 (CO), 163.10 (C-O), 143.63 (quat. C), 132.14 (CH-Ar), 128.29 (CH-Ar), 118.43 (CN), 111.66 (quat. C), 110.38 (quat. C), 60.67 (CH3), 39.46 (CH), 12.94 (CH3); Anal. Calcd. for C19H14NO5: C, 61.76; H, 4.44; N, 10.29; O, 23.51. Found: C, 58.36; H, 4.31; N, 6.92; O, 30.41; GCMS m/z (El, +ve): found 274.2 ([M + 2H]+); C19H14NO5 calculated 274.09.

Ethyl 4-hydroxy-2-(4-methoxyphenyl)-1-methyl-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylate (4g)

Light Yellow: 58%; m. p. 154-156°C. IR (ATR) v/cm−1: 3105 (OH), 2927 (NH, amide), 1673.84 (C=O, ester), 1612 (C=C), 1512 (N=C=O), 776 (Ar-OMe); 1H-NMR (400 MHz, CDCl3): δ 7.07 (d, 2H), 6.86 (d, J = 6.9 Hz, 2H), 4.95 (s, 1H), 4.13 (s, J = 7.2 Hz, 2H), 3.80 (s, 3H), 2.79 (s, 3H), 1.13 (s, J = 7.1 Hz, 3H); 13C NMR (100 MHz, CDCl3): δ 165.94 (COOH), 159.95 (C=O), 157.87 (C=O), 128.80 (CH-Ar), 126.47 (aromatic C), 114.25 (CH-Ar), 112.12 (quat. C), 62.14 (OCH3), 61.06 (CH), 55.39 (OCH3), 27.65 (CH3-N), 14.04 (CH3); Anal. Calcd. for C17H15NO5: C, 61.85; H, 5.88; N, 4.81; O, 27.46. Found: C, 61.40; H, 5.81; N, 4.00; O, 28.69; GCMS m/z (El, +ve): found 293.10 ([M + 2H]+); C17H15NO5 calculated 293.13.

Ethyl 4-hydroxy-2-(4-methoxyphenyl)-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylate (4h)

Light yellow solid: 25.71%; m. p. 152-153°C. IR (ATR) v/cm−1: 3302 (OH), 3004 (NH, amide), 1685.55 (C=O, ester), 1612 (C-C), 1514.43 (N-C=O), 759 (Ar-OMe); 1H-NMR (400 MHz, CDCl3): δ 7.15 (dd, J = 6.6, 2.1 Hz, 2H), 6.84 (dd, J = 6.6,
2.1 Hz, 2H), 5.20 (d, J = 0.9 Hz, 1H), 4.15 (q, J = 7.0 Hz, 2H), 3.78 (s, 3H), 1.15 (t, J = 7.1 Hz, 3H); 

13C NMR (100 MHz, CDCl3): δ 170.70 (COH), 160.28 (C=O), 159.67 (C=O), 137.20 (aromatic C), 129.05 (CH-Ar), 114.32 (CH-Ar), 108.06 (quat. C), 64.17 (OCH3), 39.98 (CH), 13.89 (CH3); Anal. Calcd. for C17H17NO3: C, 58.14; H, 7.54; N, 6.16; O, 28.79. Found: C, 61.40; H, 5.81; N, 4.00; O, 28.79. GCMS m/z (EI, +ve): found 278.10 ([M + H]+), C17H17NO3 calculated 278.10.

**Ethyl 4-hydroxy-1-(2-hydroxyethyl)-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylate (4i)**

Light orange; 34.60%; m.p. 132-134°C. IR (ATR) ν/cm⁻¹: 3479 (-OH), 3100 (-CH-OH), 2920 (NH, amide), 1694 (C=O, ester), 1655 (C=N), 1513 (N=C=O); 1H-NMR (400 MHz, CDCl3): δ 4.26 (q, J = 7.2 Hz, 2H), 4.13 (s, 2H), 3.76-3.68 (m, 2H), 1.29 (t, J = 7.1 Hz, 3H); 13C NMR (100 MHz, CDCl3): δ 176.98 (COH), 165.88 (C=O), 163.69 (C=O), 167.51 (quat. C), 156.80 (C=O), 144.7 (CH), 138.69 (quat. C), 128.72 (aromatic C), 127.85 (quat. C), 113.79 (aromatic C), 110.92 (quat. C), 101.97 (quat. C), 60.32 (CH3); Anal. Calcd. for C19H17NO5: C, 59.42; H, 3.98; N, 5.32; O, 30.39. Found: C, 61.64; H, 4.92; N, 4.07; O, 29.37. GCMS m/z (EI, +ve): found 286.90 ([M + Na]+), C19H17NO5 calculated 286.07.

**General procedure for the synthesis of pyrrolidine-based iminosugar intermediates (5a-c)**

Mixtures of 0.01 mole of 2,3-dioxopyrrolidine (4a), 0.012 mole of amine, 0.016 mole of formic acid were heated at reflux for 16 hours. Each solution was concentrated by distillation to approximately 10 mL and diluted with water while still hot until faint turbidity appeared. The products which crystallized from the mixture upon cooling, were collected by filtration and purified by recrystallization in ethanol-water mixture.

**Ethyl 5-oxo-4-(phenylamino)-2,5-dihydro-1H-pyrrole-3-carboxylate (5a)**

Yellow solid; 21.55%; m.p. 59-62°C. IR (ATR) ν/cm⁻¹: 3396 (NH), 1700 (C=O, ester), 1675 (C=N), 1539 (N=C=O), 758 (NH-Ar); 1H NMR (400 MHz, CDCl3): δ 7.30 (t, 2H), 7.14 (t, J = 7.5 Hz, 1H), 7.08 (d, J = 7.3 Hz, 2H), 4.93 (s, 2H), 4.23 (q, J = 7.2 Hz, 2H), 1.25 (t, J = 7.1 Hz, 3H); 13C NMR (100 MHz, CDCl3): 167.51 (C=O), 163.89 (C=O), 137.85 (quat. C), 137.61 (aromatic C), 128.72 (aromatic C), 128.5 (quat. C), 121.87 (CH-Ar), 122.73 (CH-Ar), 111.83, (quat. C), 110.92 (quat. C), 101.97 (quat. C), 60.94 (OCH3), 14.28 (CH3); GCMS m/z (EI, +ve): found 247.10 ([M + H]+), C14H12NO4 calculated 247.11.

**Ethyl 4-((4-ethylphenyl)amino)-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylate (5b)**

Reddish brown oily; 36.06%; IR (ATR) ν/cm⁻¹: 3328 (NH), 2976 (NH, amide), 1665 (C=O, ester), 1517 (N=C=O), 1459 (CH3), 1356 (CH2), 759 (NH-Ar-CH3); 1H NMR (400 MHz, CDCl3): δ 7.13 (d, J = 8.5 Hz, 2H), 7.01 (d, J = 6.2 Hz, 2H), 4.93 (s, 2H), 4.23 (q, J = 7.2 Hz, 2H), 2.62 (q, J = 7.6 Hz, 2H), 1.28-1.19 (m, 6H); 13C NMR (100 MHz, CDCl3): δ 143.35 (C=O), 137.92 (C=O), 135.36 (quat. C), 128.12 (CH-Ar), 122.98 (CH-Ar), 119.530 (aromatic C), 113.79 (aromatic C), 110.92 (quat. C), 60.82 (OCH3), 28.39 (CH3), 15.59 (CH3), 14.29 (CH3); GCMS m/z (EI, +ve): found 275.10 ([M + H]+), C15H17NO3 calculated 275.14.
Ethyl 4-((4-methoxyphenyl) amino)-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylate (5c)

Light yellow solid; 25.05%; m.p. 70-71°C. IR (ATR) ν/cm⁻¹: 3327 (NH), 2982 (NH, amide), 1766 (C=O, ester), 1638 (C=C), 1517 (N=C=O), 755 (NH-Ar-OMe); ¹H NMR (100 MHz, CDCl₃) δ 7.68-7.63 (m, 2H), 7.12 (t, J = 7.1 Hz, 3H), 6.80 (qt, 1H), 6.67 (d, J = 7.3 Hz, 2H), 6.58 (d, J = 8.7 Hz, 2H), 4.59 (dd, J = 9.6, 0.9 Hz, 1H), 4.45-4.36 (m, 2H), 4.10 (qd, J = 7.1, 2.6 Hz, 2H), 3.78-3.72 (m, 1H), 2.54 (q, J = 7.6 Hz, 2H), 1.18 (q, J = 7.3 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 174.20 (C=O), 170.17 (C=O), 146.25 (aromatic C). GCMS m/z (EI, +ve): found 249.10 ([M + H]+); C₁₅H₁₆N₂O₅ calculated 249.12.

Ethyl 5-oxo-4-(phenylamino)pyrroline-3-carboxylate (6c)

Colorless oily; 58%; IR (ATR) ν/cm⁻¹: 3345.40 (NH), 3007.85 (NH, amide), 2980 (CH, aromatic), 1770.48 (ester), 1514 (N=C=O), 1452 (CH₂), 1376 (CH₂), 690 (NH-Ar); ¹H NMR (400 MHz, CDCl₃) δ 8.79 (d, J = 3.8 Hz, 1H), 8.20 (dd, J = 8.2 Hz, 2H), 4.60 (d, J = 10.1 Hz, 1H), 4.42 (dd, J = 10.1, 5.9 Hz, 1H), 4.34 (q, J = 4.1 Hz, 1H), 4.10 (qd, J = 7.1, 2.6 Hz, 2H), 3.78-3.72 (m, 1H), 2.54 (q, J = 7.6 Hz, 2H), 1.18 (q, J = 7.3 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 174.20 (C=O), 170.17 (C=O), 144.16 (aromatic C), 135.38 (aromatic C), 128.75 (CHAr), 113.87 (CH₂), 66.93 (OCH₃), 61.62 (CH₂), 55.85 (CH), 46.37 (CH), 16.01 (CH₁), 14.00 (CH₂); GCMS m/z (EI, +ve): found 277.10 ([M + H]+); C₁₅H₁₆N₂O₅ calculated 277.15.
140.32 (aromatic C), 115.27 (CH=Ar), 114.13 (CH=Ar), 66.91 (OCH3), 61.62 (CH3), 55.79 (OCH3), 46.38 (CH), 14.02 (CH3); GCMS m/z (EI, +ve): found 279.10 ([M + H]+), C9H14N2O4 calculated 279.13.

General procedure for the synthesis of pyrrolidine-based iminosugars intermediates (8a-e)

A stirred mixture of 4c-g (1 eq.) in CH2Cl2 (0.05 L) was added acetic acid (1 eq.) and NaBH4 (1.1 mol) at 0°C. After 0°C achieved, the mixture was stirred for one hour, then the mixture was stirred at room temperature upon completion (8 hours). The solvent was then removed from mixtures and was extracted with EtOAc. The organic layer was washed with highly saturated NaHCO3 solution. Trace of water was removed with anhydrous MgSO4 and concentrated in vacuo. The crude product was purified by column chromatography to give the hydroyx ester product (8a-e).

Ethyl-2-(4-cyanophenyl)-4-hydroxy-5-oxopyrrolidine-3-carboxylate (8d)

White solid; 82.73%; m.p. >231°C decomposed. IR (ATR) v/cm-1: 3200 (OH), 1675 (C=O, ester), 1476 (CH3), 637 (Ar-CN); 1H-NMR (400 MHz, CD3OD) δ 7.54 (d, J = 8.2 Hz, 2H), 7.51 (d, J = 8.2 Hz, 2H), 4.80 (d, J = 8.2 Hz, 1H), 4.58 (d, J = 9.1 Hz, 1H), 1.7 (t, J = 7.3 Hz, 2H), 2.88 (t, J = 7.1 Hz, 1H), 2.01 (t, J = 7.1 Hz, 3H); 13C NMR (100 MHz, CD3OD) δ 175.54 (C=O), 175.10 (C=O), 152.07 (aromatic C), 132.48 (CH=Ar), 129.96 (CH=Ar), 127.45 (CN), 110.78 (aromatic C), 73.92 (CHOH), 58.84 (OCH3), 43.97 (CH), 42.24 (CH), 11.85 (CH3); GCMS m/z (EI, +ve): found 297.00 ([M + Na]+), C9H14N2O4 calculated 297.08.

Ethyl-4-hydroxy-2-(4-methoxyphenyl)-1-methyl-5-oxopyrrolidine-3-carboxylate (8e)

Yellow solid; 99%; m.p. 54-55°C. IR (ATR) v/cm-1: 3017 (OH), 2889 (CH, aromatic), 1721 (C=O, ester), 1503 (N=C=O), 1473 (CH3), 688 (Ar-OMe); 1H-NMR (400 MHz, CD3OD) δ 7.19 (d, J = 8.7 Hz, 2H), 6.89 (d, J = 8.7 Hz, 2H), 4.80-4.75 (m, 1H), 4.55 (d, J = 7.3 Hz, 1H), 3.84-3.70 (m, 5H), 3.62 (t, J = 7.1 Hz, 1H), 2.69 (d, J = 7.3 Hz, 9H), 0.90 (t, J = 7.1 Hz, 3H); 13C NMR (100 MHz, CD3OD) δ 169.07 (C=O), 168.04 (C=O), 160.06 (aromatic C), 129.51 (CH=Ar), 126.86 (aromatic C), 113.55 (CH=Ar), 70.30 (CHOH), 61.83 (CH3), 60.22 (OCH3), 54.42 (OCH3), 51.32 (CH), 47.51 (CH), 52.39 (CH), 31.81 (CH3), 16.77 (CH2), 13.09 (CH2); LCMS m/z (EI, +ve): found 295.10 ([M + 2H]+), C15H19NO5 calculated 295.14.

General procedure for the synthesis of pyrrolidine-based iminosugars (7a-e) and (9a-e)

The crude products obtained were dissolved in dry tetrahydrofuran and were added
slowly to the lithium aluminium hydride solution (excess) in inert atmosphere. The mixtures were heated at 90°C towards completion (4-8 h) and then cooled to 0°C. The reaction mixtures were quenched by adding of distilled water, and the mixtures were filtered through Celite and concentrated in vacuo to give the crude products iminosugar derivatives. Compounds (7a-e) and (9a-e) as oil after purification by column chromatography.

4-(hydroxymethyl)pyrrolidin-3-ol (7a)

Reddish oily; 81.23%; IR (ATR) v/cm⁻¹: 3278 (CH-OH), 2927 (NH, stretch), 1654 (NH, bend), 1412 (CH₂), 1016 (C-O); ¹H-NMR (400 MHz, CD₂OD): δ 3.81-3.72 (m, 2H), 3.67 (q, J = 5.6 Hz, 3H), 3.58 (td, J = 11.5, 5.5 Hz, 2H), 1.78 (q, J = 5.6 Hz, 1H); ¹³C NMR (100 MHz, CD₂OD): δ 71.30 (CHOH), 64.32 (CH₂), 60.28 (CH₂OH), 59.69 (CH), 45.75 (CH); LCMS m/z (ESI-QTOF, +ve): found 119.0794 ([M + 2H]⁺), C₆H₁₂NO₂ calculated 119.0941.

4-(hydroxyethyl)-1-methylpyrrolidin-3-ol (7b):

Reddish oily; 64.27%; IR (ATR) v/cm⁻¹: 3254 (OH), 2948 (NH, stretch), 1654 (NH, bend), 1407 (CH₂), 1021 (C-O); ¹H-NMR (400 MHz, CD₂OD): δ 4.36 (td, J = 6.1, 3.8 Hz, 1H), 3.77 (dd, J = 11.0, 6.4 Hz, 1H), 3.60 (dd, J = 11.0, 7.3 Hz, 1H), 3.05 (q, J = 5.5 Hz, 1H), 2.84 (dd, J = 9.6, 7.8 Hz, 1H), 2.53-2.43 (m, 2H), 2.38 (s, 3H), 1.90-1.80 (m, 1H); ¹³C NMR (100 MHz, CD₂OD): δ 71.17 (CHOH), 63.87 (CH₃), 60.17 (CH₂OH), 57.42 (CH₂), 45.31 (CH₃-N), 41.56 (CH); LCMS m/z (ESI-QTOF, +ve): found 132.1044 ([M + H]⁺), C₈H₁₄NO₂ calculated 132.1019.

4-(phenylamino)pyrrolidin-3-ol (7c)

Yellow solid; 22.19%; IR (ATR) v/cm⁻¹: 3345 (OH), 2917 (NH, stretch), 2830 (CH, aromatic), 1599 (NH, bend), 1496 (CH₂), 1020 (C-O), 692 (NH-Ar); ¹H-NMR (400 MHz, CD₂OD): δ 7.05 (dd, J = 8.7, 7.3 Hz, 2H), 6.64 (dd, J = 7.8 Hz, 2H), 6.55 (tt, J = 7.1 Hz, 1H), 3.75 (dd, J = 5.5, 3.7 Hz, 2H), 3.70 (d, J = 5.9 Hz, 2H), 3.66 (s, 3H), 2.02-1.93 (m, 1H); ¹³C NMR (100 MHz, CD₂OD): δ 148.39 (aromatic C), 128.76 (CH-Ar), 116.50 (CH-Ar), 113.04 (CH-Ar), 61.37 (CH₂OH), 60.05 (CH₃), 53.76 (CHNH), 46.67 (CH), 29.43 (CH₃); LCMS m/z (ESI-QTOF, +ve): found 194.1199 ([M + 2H]⁺), C₁₃H₁₄NO₂ calculated 194.1414.

4-((4-ethylphenyl)amino)pyrrolidin-3-ol (7d)

Reddish oily; 38.5%; IR (ATR) v/cm⁻¹: 3243.34 (OH), 2915 (NH, stretch), 2845 (CH, aromatic), 1518 (NH, bend), 1455 (CH₂), 1019 (C-O), 820 (NH-Ar); ¹H-NMR (400 MHz, CD₂OD): δ 6.91 (d, J = 8.7 Hz, 2H), 6.59 (d, J = 8.7 Hz, 2H), 3.79-3.72 (m, 2H), 3.70 (d, J = 5.9 Hz, 2H), 3.65 (d, J = 3.7 Hz, 3H), 2.46 (q, J = 7.6 Hz, 2H), 1.98 (t, J = 5.5 Hz, 1H), 1.12 (t, J = 7.5 Hz, 3H); ¹³C NMR (100 MHz, CD₂OD): δ 146.20 (aromatic C), 132.72 (aromatic C), 128.08 (CH-Ar), 113.50 (CH-Ar), 61.39 (CH₂OH), 60.28 (CH₃), 60.06 (CH₃), 54.30 (CHNH), 44.66 (CH), 27.65 (CH₃), 15.32 (CH₃); LCMS m/z (ESI-QTOF, +ve): found 222.1514 ([M + 2H]⁺), C₁₃H₁₆NO₂ calculated 222.1727.

4-((4-methoxyphenyl)amino)pyrrolidin-3-ol (7e)

Brown solid; 70.28%; m.p. 84°C; IR (ATR) v/cm⁻¹: 3252 (OH), 2912 (NH, stretch), 2830 (CH, aromatic), 1508 (NH, bend), 1462 (CH₂), 1033 (C-O), 823 (NH-Ar); ¹H-NMR (400 MHz, CD₂OD): δ 6.71 (dd, J = 6.4, 2.3 Hz, 2H), 6.67-6.62 (m, 2H), 3.80-3.72 (m, 2H), 3.71-3.68 (m, 2H), 3.67 (s, 3H), 3.64 (d, J = 5.5 Hz, 2H), 3.55 (q, J = 5.0 Hz, 1H), 1.97 (q, J = 5.6 Hz, 1H); ¹³C NMR (100 MHz, CD₂OD): δ 61.17 (CH₂), 60.44 (CH₂), 60.14 (CH₂), 55.41 (CH), 54.85 (CH), 44.66 (CH); LCMS m/z (ESI-QTOF, +ve): found 224.1326 ([M + 2H]⁺), C₁₃H₁₄NO₂ calculated 224.1519.

5-ethyl-4-(hydroxymethyl)pyrrolidin-3-ol (9a)

Yellow oil; 99%; IR (ATR) v/cm⁻¹: 3250 (OH), 1549 (NH, bend), 1410 (CH₂), 1345 (CH₂), 1019 (C-O); ¹H-NMR (400 MHz, CD₂OD): δ 4.19-4.13 (m, 1H), 3.62-3.49 (m, 2H), 3.29-3.27 (m, 1H), 3.02-2.83 (m, 3H), 2.06-1.89 (m, 2H), 1.00 (t, J = 7.5 Hz, 3H); ¹³C NMR (100 MHz, CD₂OD): δ 73.75 (CHOH), 62.57 (CH), 61.20 (CH₃), 55.32 (CH), 52.78 (CH₂OH), 27.19 (CH₃), 10.30 (CH₃); LCMS m/z (ESI-QTOF, +ve): found 146.1161 ([M + H]⁺), C₇H₁₄NO₂ calculated 146.1175.

4-(hydroxymethyl)-5-isopropylpyrrolidin-3-ol (9b)

Yellow oil; 99%; IR (ATR) v/cm⁻¹: 3293 (OH), 2919 (NH, stretch), 2850 (CH), 1506 (NH, bend), 1408 (CH₂), 1019 (C-O); ¹H-NMR (400 MHz, CD₂OD): δ 4.27 (s, 1H), 3.61 (dd, J = 11.0, 4.6 Hz, 1H), 3.45 (dd, J = 11.0, 6.4 Hz, 1H), 3.10 (d, J = 3.2 Hz, 2H), 2.89 (dd, J = 8.7, 6.4 Hz, 1H), 2.15-2.00 (m, 1H), 1.97-1.89 (m, 1H), 1.03 (t, J = 6.9 Hz, 6H); ¹³C NMR (100 MHz, CD₂OD): δ 73.24 (CHOH), 61.67 (CH₂), 52.73 (CH), 52.54 (CH₂OH), 31.70 (CH), 18.80 (CH₃), 18.69 (CH₃); LCMS m/z (ESI-QTOF, +ve): found 160.1315 ([M + H]⁺), C₇H₁₄NO₂ calculated 160.1332.
4-(hydroxymethyl)-5-(p-toly)pyrrolidin-3-ol (9c)

Yellow solid; 68.27%; m.p. exceed 270°C decomposed. IR (ATR) ν/cm⁻¹: 3676 (OH), 3565 (NH, stretch), 2850 (CH aromatic), 1574 (NH, bend), 1420 (CH), 843 (Ar-CH₃), 1H-NMR (400 MHz, CD₂OD): δ 7.28 (d, J = 8.2 Hz, 2H), 7.13 (d, J = 7.8 Hz, 2H), 4.28 (s, 1H), 3.72 (d, J = 7.8 Hz, 1H), 3.62 (s, 1H), 3.49 (s, 1H), 2.97 (d, J = 13.3 Hz, 2H), 2.30 (d, J = 4.6 Hz, 3H), 2.11-2.00 (1H); ¹³C NMR (100 MHz, CD₂OD) δ 140.71 (aromatic C), 135.60 (C=O), 128.33 (CH-Ar), 126.56 (CH-Ar), 72.10 (CHOH), 68.27% (CH), 66.90 (CH), 57.00 (CH), 56.20 (CHOH), 48.31 (CH); C₁₂H₁₇NO₂ calculated 221.363.

Determination of alpha-glucosidase inhibition activity

The alpha-glucosidase enzyme inhibitory activity assay was adopted from the method described by Lee et al., with slight modification. The inhibition rate of alpha glucosidase was determined at 37°C in phosphate buffer solution (pH 6.5). The reaction mixtures containing 10 μl of sample, 20 μl of alpha glucosidase enzyme, 20 μl of water and 40 μl of buffer solution were mixed in microtiter plate. The mixtures were pre-incubated at 37°C for 10 minutes. Then, 10 μl of substrate in buffer solution were added, and the incubation time was prolonged to an additional 30 minutes. The absorption at 405 nm was measured instantly after 30 min of incubation with microplate spectrophotometer and the inhibition activity was calculated by the equation described by Lee et al.

RESULTS AND DISCUSSION

Synthesis of pyrrolidine-based iminosugar derivatives

The synthetic strategy used in this study is outlined in Scheme 1. The pyrrolidine based iminosugar derivatives were synthesized in three or four steps linear synthetic route (Scheme 1) starting with the multicomponent reaction (MCR), amination, reduction of olefinic bond followed by simultaneous reductions of both the ester and amide (for compounds 7c-e using 4 steps strategy) (Scheme 1).
According to Mohammat et al., the 2,3-dioxopyrrolidine skeleton 4 can be obtained through a multicomponent reaction by reacting sodium diethyl oxaloacetate with the same molar concentrations of aldehydes 3 and amines 2 at reflux condition in moderate yields (Scheme 2).

Subsequently, compounds 5a-c were treated with acetic acid in the presence of Pd/C catalyst which underwent high diastereoselective hydrogenation at the olefinic bond of the pyrrolidine skeleton yielding 6a-e as cis-configured isomers. Reductions of the enolic tautomers 4a and 4b by hydrogenation were performed under neutral or acidic conditions in ethanol for 3 hours. The mechanism of the catalytic reduction of these compounds was proposed by Mohammat 2015. This strategy furnished the cis-hydroxy esters 6a and 6b in with moderate to high yields (38% and 98%, respectively). Similarly, stereoselective reduction of enamine tautomers 5a-c via syn hydrogenation was also performed under neutral condition for 24 h but the yields obtained were low (31-36%). Adding acetic acid as the catalyst to the reaction condition reduced the reaction time, but the yields were not improved.

The catalytic hydrogenation of 5a-c is formed via the chelation ring formation as proposed by Harada and Matsumoto. The six-membered chelated ring structure with the catalyst is present in the intermediate, the hydrogen atom preferentially attacked from the less hindered side to afford compounds 6c-e. The reduction of enamine was found to be low in yield under acidic and neutral conditions due to the presence of nitrogen atom of the amine derivatives which poisoned the palladium catalyst and degraded the selectivity of the catalyst. Similar observation reported by Xie et al., during hydrogenation of enamines and imines. Compounds 7a-e were obtained by reduction of amide and ester via excess LiAlH4 to give the target molecule.

![Scheme 2. Synthesis of 2,3-dioxopyrrolidines via a multicomponent reaction](image)

The crude product was then acidified and filtered to give the desired products, 4a-k. However, for the synthesis of compound 4a an excess of amount of ammonia is required. The amination of compound 2,3-dioxopyrrolidines 4a of the keto group at C-3 gave derivatives of different 4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylic acid esters 5a-c. These attempts were refluxed in ethanol for 12 and 24 h but the yield obtained were low (21-26%). Jourdan et al., reported similar observation during the amination of 4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylic acid esters molecule. The formic acid was added to favor the 4a in keto form, however the yields 5a-c were similar.

![Scheme 3. Synthesis of the Pyrrolidine-based iminosugars (9a-e)](image)
Alternatively, 2,3-dioxo-4-carboxy-pyrrolidines (4c-g) underwent stereoselective reduction using NaBH₄/AcOH to specifically yield 8a-e as trans-configured analogues in good yields. The mechanism and steric effect in this type of reduction reaction was proposed by Mohammat 2015 as shown in Fig.2. It was predicted throughout the reaction, compounds 4 tautomerized into both the enol and keto forms; however the keto analogues are more stable due to reaction being catalyzed by an acid. In terms of diastereoselection, the bulky C-5 substituent noticeably contributed towards the steric factor and this gave only the thermodynamically-stable trans product (8a-e)⁹. Therefore, the hydride was transferred from the less hindered part of P; away from the C-5 substituent forcing the hydroxy group to be in trans-position to that of the ethyl ester⁹. Then, the amide and ester functionalities of compounds 8a-e were reduced further with an excess LiAlH₄ to give compounds 9a-e in moderate to high yields.

Fig. 2. Mechanistic reduction

Biological activity of pyrrolidine-based iminosugar derivatives

Ten iminosugars and their respective intermediates were synthesized to evaluate their α-glucosidase inhibitory activity in comparison with deoxynojirimycin (DNJ) as the positive control, as shown in Table 1. The inhibition percentages against glucosidase of these were tested on two different concentrations at 1.0 mM and 5.0 mM. The inhibition percentages in 1.0 mM and 5.0 mM of DNJ were found to be 59.38±1.22 and 80.75±0.38, respectively and it was regarded as a positive control. In general, the present or increase in the inhibition activity is closely associated with the aryl substituents bearing either electron donating groups (-OMe and CH₃) or an electron withdrawing group (-CN). Among the ten synthesized iminosugars, 7e gave the highest inhibition of 67.5±0.5 at 1.0 mM followed by 9c with 17.9 ± 3.5 and 7c with 4.3±0.7. Compound 7e gave better inhibition activity at 1.0 mM concentration towards glucosidase as compared to DNJ. The presence of methoxy-containing arylamine at C-3 position somehow contributes towards the formation of iminosugars as potential glucosidase inhibitors. Further modification at the pyrrolidine skeleton is thus to enhanced the bio activity of the imino sugar while maintaining the presence of the p-methoxy arylamine at the C-3 position.

| Table 1: Alpha-Glucosidase inhibition studies of synthesized compounds |
|---------------------------------------------------------------|
| Iminosugars | 1.0mM | 5.0mM |
|----------|-------|-------|
| 5b       | NT    | NT    |
| 6e       | NT    | NT    |
| 7a       | NI    | NI    |
| 7b       | NI    | NI    |
| 7c       | 4.3 ± 0.7 | 16.2 ± 1.1 |
| 7d       | NI    | 5.7±0.7|
| 7e       | 67.5 ± 0.5 | NI    |
| 9a       | NI    | NI    |
| 9b       | NI    | NI    |
| 9c       | 17.9 ± 3.5 | 80.9 ± 0.3 |
| 9d       | NI    | 16.5 ± 1.8|
| 9e       | NI    | NI    |
| DNJ      | 59.38±1.22 | 80.75±0.38 |

% Inhibition determined at 1.0 mM and 5.0 mM concentration of compound
NI: No Inhibition                 NT: Not Tested

CONCLUSION

In conclusion, ten pyrrolidine-based iminosugars were synthesized in three or four steps utilizing MCR as the key step. One compound, 7e (4-((4-methoxyphenyl)amino)pyrrolidin-3-ol) demonstrated a strong potent inhibitory activity at 1.0 mM concentration against alpha-glucosidase test. In addition, the presence of a methoxy group at C-3 plays an important role in the inhibition and that further structural variations at the nitrogen atom of the skeleton using different amine derivatives could improve the alpha glucosidase activity.

ACKNOWLEDGMENT

The authors wish to thank the Institute of Science (IOS), UiTM Shah Alam, Malaysia for its generous support and the Ministry of Education (MOE), Malaysia for the financial support under the Fundamental Research Grant Scheme (600-IRMI/FRGS 5/3 (109/2019).

Conflict of Interests

The authors declare that there is no conflict of interests related to the publication of this paper.
REFERENCES

1. Prasad, P.; Surajit, M.; Sudhir, K. T.; Santosh, A., *Eur. J. Nutr.*, 2015.

2. Sivaprasad, K.; Sujatha, S.; Srinivas, U.; Jaya, S. A.; Shubham D.; Hasitha, S. A.; Yogeeswari, P.; Dileep K. S.; Bathini N. B.; Krishna, S. E., *Bioorg., Med. Chem. Lett.*, 2017, 27, 2818–2823.

3. Clifford, J. B.; Caroline, D., *Br J Cardiol.*, 2003, 10, 128-36.

4. Lucassen, P.; Rutten, G.; Lucassen, C. V. W.; Rutten, P.; Van, G. W. C., *Cochrane Database Syst. Rev.*, 2005, 2.

5. Erica, C. S.; Nathália, C. G. Y.; Alcindo, A. D. S.; Fernando, C. R., *Synthesis*, 2017, 49, 4869–4875.

6. Doddi, V. R.; Yashwant D. V., *European J. Org. Chem.*, 2007, 33, 5583–5589.

7. Mohammat, M. F.; Shaameri, Z.; Hamzah, A. S., *Molecules*, 2009, 14(1), 250–256.

8. Mohammat, M. F.; Mansor, N.S.; Shaameri, Z.; Hamzah, A. S., *J. Korean Chem. Soc.*, 2015, 59(1), 31–35.

9. Zhang, E.; Bai, P. Y.; Sun, W.; Wang, S.; Wang, M. M.; Xu, S. M., *Carbohydr. Res.*, 2016, 434, 33–36.

10. Kotkar, S. P.; Chavan, V. B.; Sudalai, A., *Org. Lett.*, 2007, 9(6) 1001-1004.

11. Masakazu, S.; Zhangyong, H.; Liang, P. H.; Stephen, M. D.; Lisa, J. W. H.; William, A. G.; Chi-Huey., *J. Am. Chem. Soc.*, 2007, 6, 14811–14817.

12. En-Lun, T.; Sih-Yu, C.; Ming-Hsun, Y.; Shih-Chi, W.; Ting-Ren, R. C.; Wei-Chieh, C., *Bioorg. Med. Chem.*, 2008, 16(24), 10198–10204.

13. Shaameri, Z.; Ali, S. H. S.; Mohammat, M. F.; Yamin, B.M.B.; Hamzah, A. S., *J. Heterocycl. Chem.*, 2009, 0, 1208–1212.

14. Lee, S.; Lin, H.; Chen, C., *Phytochemistry.*, 2008, 69, 2347–2353.

15. Metten, B.; Kostermans, M.; Baelen, G. V.; Smet, M.; Dehaen, W., *Tetrahedron.*, 2006, 62(25), 6018–6028.

16. Southwick, P. L.; Hofmann, G.H., *J. Org. Chem.*, 1963, 28(5), 1332–1336.

17. Jourdan, F.; Kaiser, J. T.; Lowe, D. J., *Synth. Commun.*, 2006, 35, 2453–2466.

18. Augustin, M.; Jeschke, P., *J. prak. Chem.*, 1987, 329, 599-606.

19. Madhav, R.; Richard, F. D.; Southwick, P. L., *J. Heterocycl. Chem.*, 1973, 10, 25-28.

20. Ikemoto, N.; Tellers, D.M.; Dreher, S.D.; Liu, J; Huang, A.; Rivera, N. R.; Njolito, E.; Hsiao, Y.; Williams, J. C.; Williams, J.M.; Armstrong, J. D.; Yongkui, S.; Mathre, D.J.; Grabowski, E. J.; Tillyere, R. D., *J. Am. Chem. Soc.*, 2004, 126(10), 3048–3049.

21. Richard F. B.; Mark D. B.; Durstl, H. D., *J. Am. Chem. Soc.*, 1970, 70(1), 1968–1970.

22. Smaliy, R. V.; Aleksandra A. C.; Nataliya A. S.; Sergey A. Y.; Aleksandr A. Y.; Aleksandr I. L.; Alina O. G.; Aleksandr N. K., *J. Fluor. Chem.*, 2015, 180, 257–264.

23. Tungler, B. A.; Tarnai, T.; Hegedîs, L.; Fodor, K.; Mathe, T., *Platinum Metals Rev.*, 1998, 42(3), 108–115.

24. Mitsuru, F.; Tadashi, O.; Yoshhide, N.; Yuriko, T., *Chem. Pharm. Bull.*, 1979, 27(9), 2223-2225.

25. Marcuzzano, P; Patrick, B. O.; James, B. R., *Organometallics.*, 2003, 22(6), 1177–1179.

26. Xie, J. H.; Zhu, S. F.; Zhou, Q. L., *Chem. Rev.*, 2011, 111(3), 1713–1760.

27. Goti, A.; Cicchi, S.; Cacciarini, M.; Cardona, F.; Fedi, V.; Brandi, A., *European J. Org. Chem.*, 2000, 21, 3633–3645.