

Synthesis, Characterization and Alpha Glucosidase Inhibition Activity of New Phthalimide Derivatives

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Abstract

Three of intermediate imide compounds were synthesized through reacting of phthalic anhydride with glycine (compound 2a), and tetrachlorophthalic anhydride with glycine, (S)-2-[(*tert*-Butoxycarbonyl)amino]-3-aminopropionic acid (compounds 2b,c) respectively in dry toluene with azeotropic removal of water using Dean-stark apparatus then carboxyl functional group activated by refluxing with thionyl chloride, the resulted acid chloride (compounds 3a-c) were reacted with different amine (5-flourouracil, 4-chloroaniline, 4-bromoaniline, 2-amino thiazole, and pyrrolidine) (compounds 4a-e), the compounds (5a-j) consider as end products while the compounds (5k-o) required further reaction to deprotect aliphatic amine this was achieved by treating the compounds with trifluoro acetic acid (TFA) to remove *tert*-Butoxycarbonyl group (compounds 6a-e).

The alpha glucosidase inhibitory activity of some synthesized compounds (5a, 5f, 6a) was evaluated against alpha-glucosidase enzyme extracted from *Saccharomyces* bacteria, p-nitrophenol glucopyranoside (pNPG) was used as substrate and the standard was acarbose.

All these test compounds show excellent inhibitory activity according to IC₅₀ values which is ranging from (4.61-7.32 M).

Keywords: Antidiabetic, Synthesis, Phthalimide, IC₅₀.

تصنيع، تشخيص والتقييم الاولي لدراسة النشاط التثبيطي الى الفا كلوكوسايديس بواسطة مركبات الفثاليميد الجديدة

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الخلاصة

تم تحضير ثلاثة من المنتجات الوسيطة ايميد عن طريق تفاعل أنهيدريد الفثاليميد مع جلايسين (2a) و أنهيدريد رباعي الفكين (الفتاليميد مع الجلايسين)، [(S)-2- (تترت-بوتوكسيكاربونيل) أمينو] -3-أمينوبروبيونيك أسيد (2b,c) على التوالي في التولوين الجاف مع إزالة أزيوتروبيا من الماء باستخدام جهاز ديان-ستارك ثم مجموعة وظيفية الكربوكسيل تفعيلها عن طريق ارتداد مع كلوريد الثيونيل، وكان رد فعل حمض كلوريد (3a-c) مع أمين مختلفة (ه) فلوروراسيل، 4-كلورانيلين، 4-برومانيلين، 2-أمينو ثيازول، وبيروليدين (4a-e)، والنواتج الناتجة تعتبر المنتجات النهائية (5a-j) في حين أن المركبات (5k-o) تتطلب المزيد من التفاعل ل ديبروتيك أمين الأليفاتية التي تحققت من خلال معالجة المركبات مع حامض ثلاثي فلوروالخلك لإزالة مجموعة بيوتوكسي كاربونيل الثلاثية (6a-e). تم اختبار نشاط المثبطة ألفا غلوكوزيداز لبعض المركبات المركبة (5a, 5f and 6a) باستخدام الانزيم-α غلوكوزيداز من ساكارومييسز سيريفيسياي و-p نيتروفينول غلوكوبيرانوسيد (بينب) المستخدم كركيزة و أكاربوس تستخدم كمعيار. كل مركبات الاختبار هذه تظهر مثبط ممتاز وفقا لقيم نصف التركيز المثبط القصوى التي تتراوح من (4,61 – 7,32 مولاري). الكلمات المفتاحية:- مضاد للسكري، تحضير، فثاليميد، التركيز الكبلي.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder with heterogeneous etiologies (genetic and environmental factors), it is characterized by disturbance in the metabolisms of carbohydrate, fat and protein resulting from defects in insulin secretion, insulin action or both⁽¹⁻³⁾.

Insulin is a peptide hormone produced by beta cells of the pancreas⁽²⁾. It has two

essential functions: (1) insulin stimulates glucose uptake and lipid synthesis; (2) insulin inhibits the breakdown of lipids, proteins and glycogen, and also inhibits the glucose biosynthesis pathway (gluconeogenesis)⁽⁴⁻⁶⁾. There are two main types of diabetes, type-1 and type-2, and also what is termed gestational diabetes that affects females during pregnancy. Type-1 diabetes is also known as insulin-dependent diabetes⁽⁷⁾.

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In type-2 diabetes, the body does not produce enough insulin for proper functioning or the cells do not react to insulin (insulin resistance)⁽⁸⁾. As type 2 diabetes is a progressive disease, intensification of therapy is normally required over time, traditional treatment algorithms often fail to address the progressive nature of the disease. Furthermore, current therapeutic agents may also be associated with wide range of various effects: increased risk of hypoglycemia (sulphonylureas and insulin), weight gain (sulphonylureas, thiazolidinediones and insulin), and gastrointestinal intolerance (metformin), which represent barriers to maximum glycemic control⁽⁹⁻¹¹⁾.

One of the current therapies is Alpha-glucosidase inhibitors. α -Glucosidase is the key enzyme which catalyzes the final step in the digestion of carbohydrates in mammals. Hence, α -glucosidase inhibitors can suppress the liberation of D-glucose of oligosaccharides and disaccharides from dietary complex carbohydrates and prolong glucose absorption, resulting in decrease post-prandial plasma glucose levels and retard post-prandial hyperglycemia^(12, 13).

Consequently, α -glucosidase inhibitors have been approved for clinical use in the management of type 2 diabetes, as well as the treatment of obesity such as acarbose and miglitol^(14, 15).

However it was found that phthalimide, tetrachlorophthalimide derivatives exhibited potent α -glucosidase inhibition and the structure activity relationship studies show the following:

1. A phthalimide or tetrachlorophthalimide moiety connected to a variously substituted phenyl ring by an alkyl chain shows promising alpha glucosidase inhibitory activity (Scheme 1).
2. Studies revealed the importance of the distance between the phthalimide ring and the phenyl moiety.
3. Presence of an electron withdrawing group (NO_2 , CF_3 etc.) at the (R_3) position was more potent.
4. Tetrachlorophthalimide skeleton is a useful non-sugar-type sugar mimic pharmacophore; it is characterized by high lipophilicity which could influence their pharmacokinetic properties and biological activity⁽¹⁶⁾.

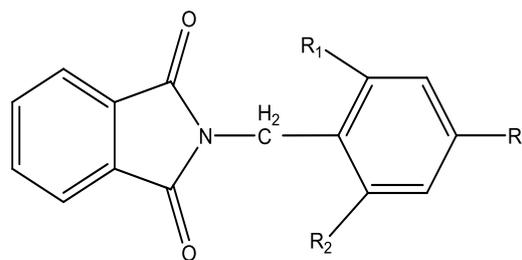


Figure 1. Phthalimide moiety connected to substituted phenyl ring by an alkyl chain

Materials and Methods

All chemicals and solvents used during synthesis were of analytical grade and used without further purification. Completion of reactions and the purity of compounds were ascertained by

- A. Thin-layer chromatography (TLC), using Silica gel GF₂₅₄ (type 60) pre-coated Aluminum sheets, Merck (Germany) and the eluent used is
 1. Chloroform : methanol (85:15)
 2. Glacial acetic acid: ethyl Acetate: methanol (0.1:3:1) to run TLC.

- B. High performance liquid chromatography was performed at the Lebanese university / Lebanon (PF4370) for the final compounds in order to ensure complete purity of compounds. Melting points were determined using Stuart SMP3 melting point apparatus in open capillary tubes, and are uncorrected. Fourier-Transform Infrared spectroscopy (FTIR), (KBr disc) (ν, cm^{-1}) were recorded using (Biotech engineering management FTIR-600, UK) at College of Science- Al-Mustansiriya University, and the College of Pharmacy- Al-Mustansiriya University.

Furthermore, The elemental microanalysis of the synthesized compounds was done using (Elementar vario MICRO cube instrument, Germany) in the University of Mustansiriya-College of Pharmacy. Proton-NMR spectra and (^{13}C NMR): were recorded on (Bruker, Germany NMR Spectra 300 MHz, Avance III 300 spectrometer) with tetramethylsilane (TMS) as an internal standard, dimethyl sulphoxide used as a solvent for samples measurement, ($\delta = \text{ppm}$) and coupling constant in Hertz, which was run in (Lebanese University)-Lebanon.

Chemical synthesis

Synthesis of *N,N*-phthaloyl glycine (compound 2a)

A mixture of phthalic anhydride (6.23 gm, 42.29 mmol), glycine (3.57 gm, 47.61 mmol) and triethyl amine (0.7 mL) in dry toluene (250 mL) was heated under reflux for 4

While azeotropic removal of water using Dean-Stark apparatus⁽¹⁷⁾.

The reaction mixture was concentrated at reduced pressure, added ethyl acetate to the residue, washed the organic phase with dilute HCl (1N) to eliminate the unreacted triethylamine, dry over MgSO₄, concentrated to yield the N-phthaloyl glycine (compound 2a) as a solid compound (percent yield 90%)^(18, 19).

Compound 2a is White crystalline solid; Melting point: 193-195°C; IR (KBr), (ν, cm⁻¹): 3200-2500 O-H str., 2993 and 2885 asym. and sym. Str. of CH₂, 1772 and 1726 (C=O) Str. of phthalimide, 1728 (C=O) Str. of carboxylic acid consolidated with (C=O) Str. of phthalimide, 1466 O-H bend, 1215 C-O str. v., 736 Out of plane aromatic bend cm⁻¹.

Synthesis of N,N-tetrachloro phthaloyl Amino Acids [glycine , (S) - 2 - [(tert - Butoxycarbonyl) amino] - 3 - aminopropionic acid (BOC Dap OH)] (compounds 2b and 2c)

A mixture of tetrachloro phthalic anhydride (12.09 gm, 42.29 mmol), glycine or BOC Dap OH (47.61 mmol) and triethyl amine (0.7 mL) in dry toluene (250 mL) was heated under reflux for 4 h while azeotropic removal of water using Dean-Stark apparatus⁽¹⁷⁾.

The reaction mixture was concentrated at reduced pressure, added ethyl acetate (20 ml) to the residue, washed the organic phase with dilute HCl (10 ml) (1N) to eliminate the unreacted triethylamine, dried over MgSO₄, concentrated to yield the N,N-tetra chloro phthaloyl glycine, BOC Dap OH (compounds 2b and 2c) as a solid compounds (percent yield 95 % and receptively 88%).

Compound 2b is yellowish crystalline solid; Melting point: 201-203°C; IR (KBr), (ν, cm⁻¹): 3300-2500 O-H str., 3059 (C-H) Str. of aromatic, 2939 and 2870 (C-H) asym. and sym. Str. of CH₂. 1774 and 1724 (C=O) Str. of Tetrachloro phthalimide, 1724 (C=O) Str. of carboxylic acid consolidated with (C=O) Str. of Tetrachlorophthalimide, 1523 and 1442 (C=C) aromatic Str., 1442 O-H bend, 1373 C-N Str., 1296 C-O str., 736 Out of plane aromatic bend cm⁻¹.

2c yellow to white crystalline solid; Melting point: 180-183°C; IR (KBr), (ν, cm⁻¹): 3483-2500 O-H str, 2978 (C-H) asym. Str. of CH₂, 1778 and 1716 (C=O) Str. of Tetrachlorophthalimide, 1717 (C=O) Str. of carboxylic acid consolidated with (C=O) Str. of Tetrachlorophthalimide, 1581 and 1431 (C=C) aromatic Str., 1396 C-N Str., 1199 C-O str. v., 736 Out of plane aromatic bend cm⁻¹.

Synthesis of N,N-phthaloyl-acid chloride (compounds 3a, 3b and 3c)

N,N-phthaloyl glycine (Compound 2a), or N,N-tetra chloro phthaloyl glycine (compound 2b) or N,N-tetra chloro phthaloyl BOC Dap OH (compound 2c) (5 mmol) was placed in a 50 mL round-bottom flask and then thionyl chloride (5 mL) was added. Thionyl chloride was added dropwise over a period of 15 min. with cooling on ice bath. The mixture was refluxed for 8 hrs at 65 °C with continuous stirring and monitored by evolution of HCl gas (which is detected by changing the color of litmus paper into reddish when placed on the top of condenser) and changing the color of the solution. The reaction are often promoted by the addition of a drop of dimethylformamide (DMF)⁽²⁰⁾. The excess of thionyl chloride was removed under reduced pressure and the residue was re-dissolving in dry dichloromethane (10 ml) and was re-evaporated to give an oily residue. The resulting acyl chloride (Compounds 3a, 3b and 3c) were used directly for the next step⁽²¹⁾.

Synthesis of N-phthaloyl-amino acid amides (compounds 5a-o)

A solution of one amine derivatives (5-fluorouracil, or 4-chloroaniline, or 4-bromoaniline, or 2-aminothiazole, or Pyrrolidine) (compounds 4a-e, 5.5 mmol) were mixed with dry dichloromethane (15 ml) except for 4a and 4d using mixture of 5 ml DMF and 10 ml dichloromethane, then triethylamine (5 mmol, 0.5 ml) was added drop wise with stirring for 20 min. on ice bath and then, freshly prepared acid chloride of either N,N-phthaloyl- (Compounds 3a, 3b and 3c) were slowly dropped for 50 min. with continuous stirring on an ice bath, and stirring was continued at room temperature overnight. The reaction can be accelerated with a catalytic amount (2-3 drops) of pyridine, or N,N-dimethylaminopyridine (DMAP)⁽²²⁾. Solvents were removed under reduced pressure by using rotary evaporator. The resulting solid product was re-dissolved in ethyl acetate (10 ml) and washed with 5 % aqueous solution of sodium bicarbonate (20 ml), 5% HCl (20 ml) and distilled water (20 ml) and then dried over anhydrous magnesium sulphate [compounds 5a-o]^(18,19).

Deprotection of tert butyloxycarbonyl (N-Boc) of compounds (5k-5o)

The respective peptide (compounds 5k-5o) was dissolved in CH₂Cl₂ or in CH₂Cl₂:CH₃OH 9:1 (depending on solubility) and cooled to 0 °C. Trifluoroacetic acid (equal volume as the solvent) (10 ml) was added and the solution was allowed to warm to room temperature. After stirring at room temperature

until starting material was consumed (TLC monitoring), the solution was concentrated in vacuum. The solution washed with saturated aqueous NaHCO₃ solution (CH₃OH was added to assure solubility of the peptide), water, and brine solution. The combined organic layers were dry over MgSO₄, filtered, and evaporated in vacuum to yield the crude product in quantitative yield. In case of remaining protected peptide, the procedure was repeated. The reaction mixture was evaporated in vacuum and evaporated several times after adding CH₂Cl₂ to remove residual TFA and give the product in quantitative yield⁽²³⁻²⁵⁾.

In vitro evaluation of α -glucosidase inhibitory activity

The α -glucosidase activity of some tested compounds (5a, 5f and 6a) was determined according to the method described by Kim et al., using α -glucosidase enzyme extracted from *Saccharomyces* bacteria. The substrate solution p-nitrophenol glucopyranoside (pNPG) was prepared in 20mM phosphate buffer, and pH 6.9. 100 μ L of α -glucosidase (1.0U/mL) was pre incubated with 50 μ L of the different concentrations of the test compound (in DMSO) for 10min. Then 50 μ L of 3.0mM (pNPG) as a substrate dissolved in 20mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37°C for 20min and stopped by adding 2mL of 0.1M Na₂CO₃. The α -glucosidase activity was determined by measuring the yellow-colored para nitrophenol released from pNPG at 400 nm using UV-Visible spectrophotometer. The results were expressed as percentage of the blank control.

$$\% \text{ Inhibition} = \left[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{control}}} \right] \times 100$$

Acarbose uses as positive control, Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC₅₀) were determined graphically are shown in Figures (2-4)⁽²⁶⁾.

Result and discussion

Spectral data and chemistry

The synthesis of our compounds (intermediates and end products) is depicted in scheme 1.

The physical properties and the spectroscopic (IR, ¹H-NMR) data of the synthesized compounds:

5a2- (2- (5- fluoro -2 ,4 -dioxo -3 ,4-dihydropyrimidin -1 (2H) -yl) - 2 - oxoethyl) isoindoline -1 ,3- dione.

White solid ;yield 85.6%;Melting point: 267-270°C; IR (KBr), (ν ,cm⁻¹): 3132 NH str.v. of secondary amide of 5- fu, 3066 (C-H) Str. of aromatic, 2935and 2885(C-H) asym. Str. of CH₂, 1770 and 1724(C=O) Str. of phthalimide, 1662 (C=O) Str. of secondary amide, 1504 and 1431 (C=C) Str. of aromatic, 1246 C-F str.v., 813 Out of plane aromatic bendcm⁻¹

¹HNMR (300 MHz, DMSO): δ 1.12 (S, 2 H, CH₂), 7.8 (S, 1 H, CH), 7.8-7.9 (m, 4 H, Ar-H) 10.84 (S, 1 H, NH).

5bN-(4-chlorophenyl)-2-(1,3-dioxoisindolin-2-yl)acetamide

Light yellow needle solid ;yield 78%;Melting point: 180-183°C; IR (KBr), (ν ,cm⁻¹): 3267 NH str.v. of secondary amide, 3059(C-H) Str. of aromatic, 2947 and 2873 (C-H) asym. and sym.Str. of CH₂, 1774 and 1728 (C=O) Str. of phthalimide, 1666 (C=O) Str. of secondary amide, 1546 and1411(C=C) Str. of aromatic, 713Out of plane aromatic bend cm⁻¹

¹HNMR (300 MHz, DMSO): δ 4.3 (S, 2 H, CH₂), 7.06 (S, 1 H, NH), 7.35(m, 2 H, Ar-H), 7.6 (m, 2H, Ar-H-NH), 7.8-7.9 (m, 4 H, Ar-H-CONCO).

¹³CNMR d:10 (1C), 120 (4C), 125 (2C),129 (2C), 132 (2C), 135 (1C), 138 (1C), 165 (1C), 167(2C).

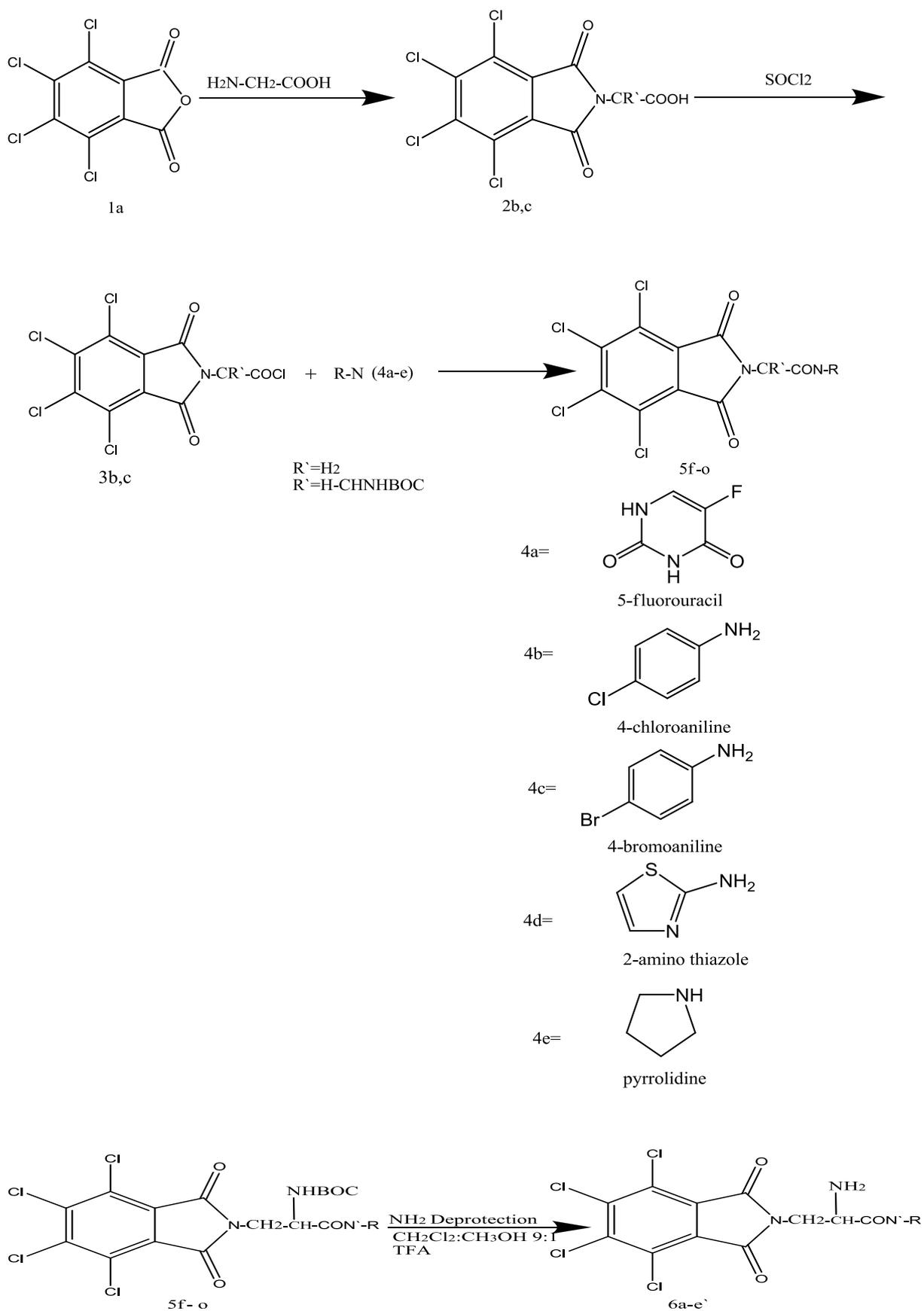
5cN-(4-bromophenyl)-2-(1,3-dioxoisindolin-2-yl)acetamide

Dark yellow solid; yield 87%;Melting point: 175-177°C; IR (KBr), (ν ,cm⁻¹): 3267NH str.v. of secondary amide, 3059(C-H) Str. of aromatic, 2939 and 2870 (C-H) asym. and sym.Str. of CH₂, 1774 and 1724(C=O) Str. of phthalimide, 1670(C=O) Str. of secondary amide, 1593 NH bend . 1543 and1411(C=C) Str.of aromatic, 713Out of plane aromatic bendcm⁻¹

¹HNMR (300 MHz, DMSO): δ 4.45 (S, 2 H, CH₂), 7.5-7.6 (m, 4H, Ar-H-Br), 7.8-7.9 (m, 4 H, Ar-H-CONCO), 10.5 (S, 1 H, NH).

5d2-(1,3-dioxoisindolin-2-yl)-N-(thiazol-2-yl)acetamide

Black solid;yield 65%;Melting point: 165-167°C; IR (KBr), (ν ,cm⁻¹): 3479NH str.v. of secondary amide, 3047(C-H) Str. of aromatic, 2989(C-H) asym. Str. of CH₂, 1774 and 1732(C=O) Str. of phthalimide, 1612(C=O) Str. of secondary amide, 1544 NH b.v1519and 1469 (C=C) Str. of aromatic, 744Out of plane aromatic bendcm⁻¹



Scheme 1. Synthesis of phthalimide derivatives

¹HNMR (300 MHz, DMSO): δ 4.3 (S, 2 H, CH₂), 7.25(d, 1 H, Ar-H-S), 7.3(d, H, Ar-H-N), 7.8-7.9 (m, 4 H, Ar-H-CONCO), 10.2 (S, 1 H, NH).
5e2-(2-oxo-2-(pyrrolidin-1-yl)ethyl)isoindoline-1,3-dione

Off white needle shape solid; yield 68%; Melting point: 210-212 °C; IR (KBr), (ν , cm⁻¹): 3093(C-H) Str. of aromatic, 2978 and 2877 (C-H) asym. And sym. Str. of CH₂, 1774 and 1720(C=O) Str. of phthalimide, 1685 (C=O) Str. of amide, 1543 and 1446 (C=C) Str. of aromatic, 713 Out of plane aromatic bend cm⁻¹

¹HNMR (300 MHz, DMSO): δ 3.1-3.5 (m, 8H, CH₂), 4.35 (S, 2 H, CH₂-N), 7.8-7.9 (m, 4 H, Ar-H-CONCO).

5f 4,5,6,7-tetrachloro-2-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-oxoethyl)isoindoline-1,3-dione

White crystal; yield 88%; Melting point: 198-201 °C; IR (KBr), (ν , cm⁻¹): 3132NH str.v. of 5-fu ring, 3066(C-H) Str. of aromatic, 2978(C-H) asym. Str. of CH₂, 1774 and 1724(C=O) Str. of tetrachloro phthalimide, 1662(C=O) Str. of tertiary amide, 1549 NH bend 1477(C=C) aromatic Str., 1246C-F str.v., 806 Out of plane aromatic bend cm⁻¹

¹HNMR (300 MHz, DMSO): δ 1.37 (S, 2H, CH₂-N (CO)₂), 7.28 (S, 1H, CH-CF), 11.75 (S, 1 H, NH).

¹³CNMR d: 10 (1C), 125 (1C), 134 (5C), 143 (2C), 160 (1C), 166 (1C), 172 (1C), 185 (2C).

5g N-(4-chlorophenyl)-2-(4,5,6,7-tetrachloro-1,3-dioxoisindolin-2-yl)acetamide

White yellowish crystal; yield 77%; Melting point: 170-173 °C; IR (KBr), (ν , cm⁻¹): 3174NH str.v. of secondary amide, 2939 and 2862(C-H) asym. and sym. Str. of CH₂, 1778 and 1724(C=O) Str. of tetrachloro phthalimide, 1674(C=O) Str. of secondary amide, 1593 NH bend of secondary amide, 1562 and 1492 (C=C) Str. of aromatic, 821 Out of plane aromatic bend cm⁻¹

¹HNMR (300 MHz, DMSO): δ 1.12 (S, 2H, CH₂-N (CO)₂), 5.72 (S, NH). 7.3 (m, 2H, Ar-H-Cl), 7.45 (m, 2H, Ar-H-NH).

¹³CNMR d: 45 (1C), 120 (4C), 122 (2C), 130(5C), 132 (1C), 134(1C), 165 (1C), 169 (2C).

5h N-(4-bromophenyl)-2-(4,5,6,7-tetrachloro-1,3-dioxoisindolin-2-yl)acetamide

White crystal; yield 90%; Melting point: 158-160 °C; IR (KBr), (ν , cm⁻¹): 3390 NH str.v. of secondary amide, 3097 (C-H) Str. of aromatic, 2939 and 2862 (C-H) asym. and sym. Str. of CH₂. 1774 and 1720 (C=O) Str. of tetrachloro phthalimide, 1627 (C=O) Str. of secondary amide, 1550 NH bend of secondary amide, 1489 and 1469(C=C) Str. of aromatic, 744 Out of plane aromatic bend cm⁻¹

¹HNMR (300 MHz, DMSO): δ 1.21 (S, 2H, CH₂-N (CO)₂), 4.48 (S, NH). 7.51 (m, 2H, Ar-H-Br), 7.6 (m, 2H, Ar-H-NH).

¹³CNMR d: 45 (1C), 122 (4C), 128 (2C), 132(5C), 133 (1C), 162 (1C), 165 (2C).

5i 2-(4,5,6,7-tetrachloro-1,3-dioxoisindolin-2-yl)-N-(thiazol-2-yl)acetamide

Brown solid; yield 63%; Melting point: 148-150 °C; IR (KBr), (ν , cm⁻¹): 3421NH str.v. of secondary amide, 3039(C-H) Str. of aromatic, 2935(C-H) asym. Str. of CH₂, 1774 and 1716(C=O) Str. of tetrachloro phthalimide, 1685(C=O) Str. of secondary amide, 1585 NH bend of secondary amide, 1518 and 1419(C=C) Str. of aromatic, 952 Out of plane aromatic bend cm⁻¹

¹HNMR (300 MHz, DMSO): δ 1.21 (S, 2H, CH₂-N (CO)₂), 5.74 (S, NH), 7.4 (d, H, Ar-H-S), 7.6 (d, H, Ar-H-NH).

¹³CNMR d: 10 (1C), 125 (1C), 139 (4C), 143 (2C), 155 (1C), 157 (1C), 160 (1C), 165(1C), 170(2C).

5j 4,5,6,7-tetrachloro-2-(2-oxo-2-(pyrrolidin-1-yl)ethyl)isoindoline-1,3-dione

White color crystal; yield 60%; Melting point: 179-181 °C; IR (KBr), (ν , cm⁻¹): 3097(C-H) Str. of aromatic, 2947 (C-H) asym. Str. of CH₂, 1774 and 1716 (C=O) Str. of tetrachloro phthalimide, 1577 (C=O) Str. of tertiary amide, 1539 and 1473(C=C) aromatic Str., 736 Out of plane aromatic bend cm⁻¹

¹HNMR (300 MHz, DMSO): δ 1.2 (S, 2H, CH₂-N (CO)₂), 1.8 (m, 4H, beta CH₂), 1.95 (m, 4H, Alpha CH₂).

6a 2-(2-amino-3-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-oxopropyl)-4,5,6,7-tetrachloroisindoline-1,3-dione

White solid; yield 70%; Melting point: 245-248 °C; IR (KBr), (ν , cm⁻¹): 3414NH str.v. of NH₂, 3132 NH str.v. of secondary amide, 2974 and 2881 (C-H) asym. and sym. Str. v. of CH₂., 1774 and 1720(C=O) Str. v. of tetrachloro phthalimide, 1583NH b.v. of secondary amide, 1477(C=C) Str. v. of aromatic, 1246C-F str.v., 1172C-N str.v. of NH₂, 736 Out of plane aromatic bend cm⁻¹

¹HNMR (300 MHz, DMSO): δ 1.16 (d, 2 H, CH₂), 3 (m, 1 H, CH-NH₂), 7.73(S, CH-CF) 10.3(S, 2 H, NH₂), 10.8 (S, H, NH).

6b 2-amino-N-(4-chlorophenyl)-3-(4,5,6,7-tetrachloro-1,3-dioxoisindolin-2-yl)propanamido

Green solid; yield 72%; Melting point: 166-169 °C; IR (KBr), (ν , cm⁻¹): 3414 NH str.v. of NH₂, 3150 NH str.v. of secondary amide, 3097 (C-H) Str. of aromatic, 2989 and 2904 (C-H) asym. and sym. Str. of CH₂., 1778 and 1735 (C=O) Str. of tetrachloro phthalimide, 1639 (C=O) Str. of secondary amide, 1616 NH bend

of NH₂, 1469 (C=C) Str. of aromatic, 1230 C-N str.v. of NH₂, 744 Out of plane aromatic bend cm⁻¹

¹HNMR (300 MHz, DMSO): δ1.16 (d, 2H, CH₂-NCO), 3.1 (m, H, CH-NH₂), 7.5(m, 2H, CH-Cl), 7.7(m, 2H, CH-NH), 5.71(S, 2 H, NH₂), 10.06(S, H, NH).

6c2-amino-N-(4-bromophenyl)-3-(4,5,6,7-tetrachloro-1,3-dioxoisindolin-2-yl)propanamide

White needle solid; yield 81%; Melting point: 170-172°C; IR (KBr), (ν, cm⁻¹): 3452 and 3300 NH str.v. of NH₂, 3150 NH str.v. of secondary amide, 3059 (C-H) Str. of aromatic, 2993 and 2877 (C-H) asym. and sym. Str. of CH₂, 1766 and 1732(C=O) Str. of tetrachloro phthalimide, 1708 (C=O) Str. of secondary amide, 1608 NH bend of NH₂, 1535 and 1469 (C=C) Str. of aromatic, 1396 C-N str.v. of NH₂, 725 Out of plane aromatic bend cm⁻¹

¹HNMR (300 MHz, DMSO): δ1.16 (d, 2H, CH₂-NCO), 3.16 (m, H, CH-NH₂), 7.45(m, 2H, CH-Br), 7.5(m, 2H, CH-NH), 5.71(S, 2 H, NH₂), 10.06(S, H, NH).

6d2-amino-3-(4,5,6,7-tetrachloro-1,3-dioxoisindolin-2-yl)-N-(thiazol-2-yl)propanamide

Brown solid; yield 59%; Melting point: 158-161°C; IR (KBr), (ν, cm⁻¹): 3421 NH str.v. of NH₂, 3124 NH str.v. of secondary amide 3024(C-H) str.v. of aromatic ring, 2962 and 2839 (C-H) asym. and sym. Str. of CH₂, 1789 and 1732(C=O) Str. of tetrachloro phthalimide, 1624(C=O) Str. of secondary amide, 1554 and 1469(C=C) Str. of aromatic, 1022 C-N str.v. of NH₂, 867 Out of plane aromatic bend cm⁻¹

¹HNMR (300 MHz, DMSO): δ1.18 (d, 2H, CH₂-NCO), 3 (m, H, CH-NH₂), 5.57(S, 2 H, NH₂), 7.5 (d, H, CH-S), 7.8 (d, H, CH-N), 8.83(S, H, NH).

6e2-(2-amino-3-oxo-3-(pyrrolidin-1-yl)propyl)-4,5,6,7-tetrachloroisindoline-1,3-dione

White needle shape solid; yield 62%; Melting point: 198-200 °C; IR (KBr), (ν, cm⁻¹): 3433 NH str.v. of NH₂, 2939 and 2881 (C-H) asym. and sym. Str. of CH₂, 1789 and 1735(C=O) Str. of tetrachloro phthalimide, 1639(C=O) Str. of amide, 1577 NH bend of NH₂, 1203 C-N str.v. of NH₂, 740 Out of plane aromatic bend cm⁻¹

¹HNMR (300 MHz, DMSO): δ1.16 (d, 2H, CH₂-NCO), 3 (m, 4H, CH₂-CH₂), 3.2 (m, 4H, CH₂-NCO), 3.5 (m, H, CH-NH₂), 5.57(S, 2 H, NH₂).

The fusion of amino acids with phthalic anhydride is a widely used methodology. For some amino acids good yields are obtained, but in some cases the conditions used are so drastic that racemization occurs. Furthermore, amino acids with functionalized side chains failed to

give the desired phthaloylated products. The extent of this racemization was limited by performing the reactions in boiling solvents and the presence of bases such as triethylamine. In such reactions, the medium should be kept neutral by slow distillation of the base and the water formed to allow cyclization of the intermediate phthalamic acids. Under prolonged heating, however, partial hydrolysis of the phthaloyl derivative to the intermediate phthalamic acid was sometimes observed⁽²⁷⁾.

Alpha glucosidase inhibitory evaluation

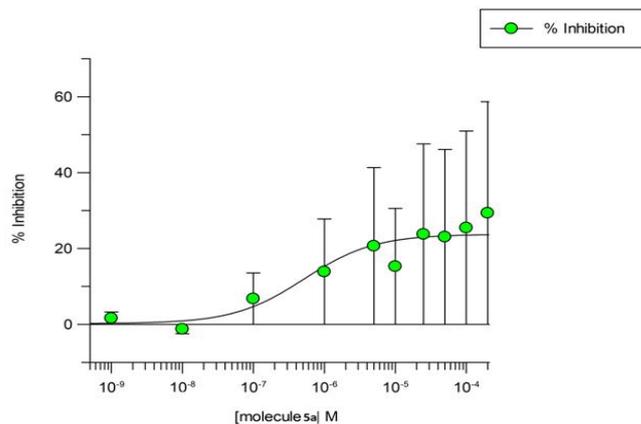


Figure 2. Alpha glucosidase inhibitory activity of compound 5a.

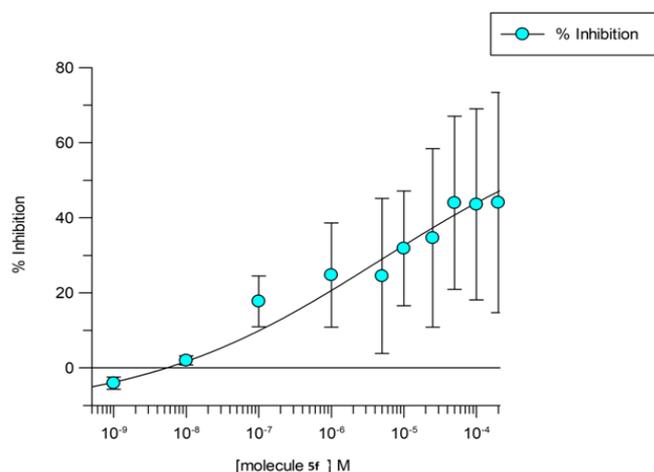


Figure 3 . Alpha glucosidase inhibitory activity of compound 5f.

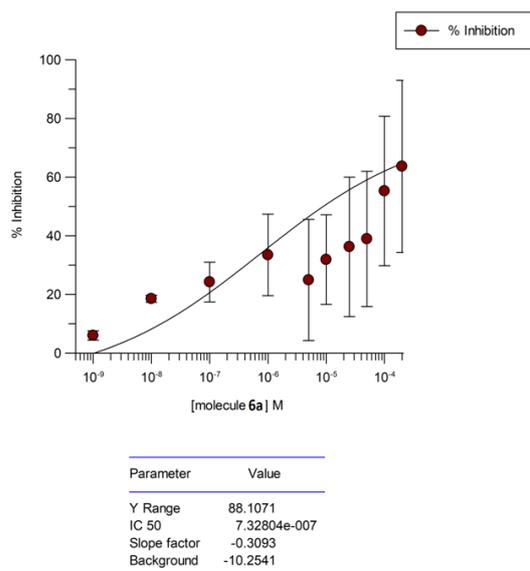


Figure 4. Alpha glucosidase inhibitory activity of compound 6a.

During the last years, considerable attention have been devoted to the creation of α -glucosidase inhibitors, which can be classified into sugar mimicking and non-sugar types according to their structural features⁽²⁸⁻³⁰⁾. Sugar-mimicking α -glucosidase inhibitors have been extensively studied, including: Acarbose, miglitol and voglibose have been clinically used to inhibit small intestinal α -glucosidase enzymes, such as α -glucosidase and glucoamylase⁽³¹⁾.

The synthesized compounds (5a, 5f, 6a) IC₅₀ values: 5.18, 4.6, 7.3 respectively accordingly these compounds have excellent alpha-glucosidase inhibitory activity in comparison with standard compound acarbose (IC₅₀ = 817.38 ± 6.27 M),

These results could be attributed to generation new binding site with a hydrophobic pocket in the active site of enzyme, the secondary amine in 5- fluoro uracil ring bind by an ionic bond, aromatic rings by hydrophobic bonds while oxygen, nitrogen, chlorine and fluoride atoms bind through hydrogen bonds.

Conclusion

The procedures for synthesis the target compounds was successfully achieved; the purity and structural formulas for the synthesized compounds were characterized and identified by melting points, Rf values, FT-IR spectroscopy, ¹H-NMR, ¹³C-NMR spectroscopy.

The preliminary evaluation of alpha-glucosidase inhibitory activity of some compounds was done, which includes compounds 5a, 5f and 6a and the results

indicate that these compounds have considerable antidiabetic activity with very useful affinity and IC₅₀ values.

References

1. American Diabetes Association. Classification and diagnosis of diabetes. Sec. in Standards of Medical Care in Diabetes 2016. Diabetes Care 2016; 39(Suppl.1):S13–S22
2. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014; 37(Suppl.1): S81–S90
3. “Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus” (Accessed 25 January 2016) Retrieved from <http://www.who.int>.
4. Joshi, S.R.; Parikh, R.M.; Das, A.K. Insulin—History, biochemistry, physiology and pharmacology. J. Assoc. Physicians India 2007;(55), S19–S25.
5. Newsholme, P.; Cruzat, V.; Arfuso, F.; Keane, K. Nutrient regulation of insulin secretion and action. J. Endocrinol. 2014;(221), R105–R120. [CrossRef] [PubMed]
6. Sonksen, P.; Sonksen, J. Insulin: Understanding its action in health and disease. Br. J. Anaesth. 2000;(85), 69–79. [CrossRef] [PubMed]
7. Rewers, M.; Ludvigsson, J. Environmental risk factors for type 1 diabetes. Lancet 2016, 387, 2340–2348. [CrossRef]
8. Reimann, M.; Bonifaci, E.; Solimena, M.; Schwarz, P.E.; Ludwig, B.; Hanefeld, M.; Bornstein, S.R. An update on preventive and regenerative therapies in diabetes mellitus. Pharmacol. Ther. 2009;(121), 317–331. [CrossRef] [PubMed]
9. Inzucchi SE, Bergenstal RM, Buse JB, et al. Management of Hyperglycemia in Type 2 Diabetes, 2015; A Patient-Centered Approach: Update to a Position Statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2015;38(1):140-149. doi:10.2337/ dc14-2441.
10. Handelsman Y, Bloomgarden ZT, Grunberger G, et al. American Association Of Clinical Endocrinologists And American College Of Endocrinology – Clinical Practice Guidelines For Developing A Diabetes Mellitus Comprehensive Care Plan – 2015 — Executive Summary. EndocrPract. 2015; 21(4):413-437. doi:10.4158/EP15672.GL.
11. American Diabetes Association. Pharmacologic Approaches to glycemic treatment. Sec. 8. In Standards of Medical Care in Diabetes 2017. Diabetes Care. 2017;

- 40(Supplement 1):S64-S74. Doi : 10 .2337 / dc1 7-S011.
12. Kajimoto, T.; Node, M. Inhibitors against glycosidases as medicines. *Curr.Top.Med. Chem.* 2009, (9), 13–33.
 13. Tundis, R.; Loizzo, M. R.; Menichini, F. Natural Products as alpha-Amylase and alpha-Glucosidase Inhibitors and their hypoglycemic Potential in the treatment of Diabetes: An Update. *Mini-Rev.Med. Chem.* 2010 ;(10), 315–331.
 14. Ross, S. A.; Gulve, E. A.; Wang, M. H. Chemistry and biochemistry of type 2 diabetes. *Chem. Rev.* 2004;(104), 1255–1282.
 15. Chiasson, J. L.; Rabasa-Lhoret, M. Prevention of type 2 diabetes Insulin resistance and beta-cell function. *Diabetes* 2004;(53), S34–S38.
 16. Neelottama K. ,Darpan K. Recent advances and future prospects of phthalimide derivatives. *Journal of Applied Pharmaceutical Science*, March, 2016; Vol. 6 (03), pp. 159-171, DOI: 10.7324/JAPS.2016.60330, ISSN 2231-3354.
 17. Satzinger, G.; Forsch, A. *Drug Res.* 1994;(44), 261.
 18. Cyril O. U., Didier M., Johan W., Gerhard K. E. Synthesis and anticonvulsant activity of N,N-phthaloyl derivatives of central nervous system inhibitory amino acids. *Arch. Pharm. Pharm. Med. Chem.* 2001;(334), 323–331.
 19. VARALA R. A Facilesynthesis of biologically active phthalimides&itsanalogues -A study.Indian institute of chemical technology hyderabad-500 007 India march2013International E – Publication.
 20. (a) Bosshard, H. H.; Mory, R.; Schmid, M.; Zollinger, H. *Helv. Chim.Acta* 1959, 42, 1653–1658. (b) Bruckner, R. “Advanced Organic Chemistry, Reaction Mechanisms”; Harcourt/Academic: San Diego; 2002; p 239.
 21. Mehdi S.Sohrab A. Synthesis of Benzoxazinone Derivatives: A New Route to 2-(N – Phthaloylmethyl) - 4H -3 ,1-benzoxazin-4-one. *Molecules* 2004;(9), 705-712. ISSN 1420-3049.
 22. Ragnarsson, U.; Grehn, L.”Novel amine chemistry based on DMAP-catalyzed acylation”. *Acc. Chem. Res.*; 1998; (31), 494–501.
 23. Sebastian Hart wig, Mary M. Nguyen and Stefan Hecht, “Exponential growth of functional poly(glutamic acid) dendrimers with varying Stereochemistry” *The Royal Society of Chemistry*, 2010.
 24. Khan Farhan A. ;Jaywant .Phopase and Ulrich Jordis, “Surprise in the Lithium Hydroxide Hydrolysis of a NXO-Compound” *EcoSo-13*, 2009.
 25. Yu Liu, Bruno L. Oliveira, Joao D. G. Correia, Isabel . Santos, Isabel S., Bernhard S. and Roger A., “Syntheses of bifunctional 2,3-diamino propionic acid based chelators as small and strong tripod ligands for the labelling of bimolecule with ^{99m}Tc” *University of Zurich, Institute of Inorganic Chemistry, Winterthurerstr . 190, 8057 Zurich, Switzerland.*
 26. Y.-M. Kim, Y.-K.Jeong, M.-H.Wang, W.-Y. Lee, and H.-I.Rhee, “Inhibitory effect of pine extract on α -glucosidase activity and postprandial hyperglycemia,” *Nutrition*, 2005; vol. 21, no. (6), pp. 756–761.
 27. Q. Zeng, Z. Liu, B. Li, and F. Wang. Mild and effective N-phthaloylation of amino acids, *Amino Acids*, 2004; 27: 183–186 DOI 10.1007/s00726-004-0109-1.
 28. Yuasa, H.; Izumi, M.; Hashimoto, H. Thiasugars: Potential Glycosidase Inhibitors. *Curr.Top.Med. Chem.* 2009; (9), 76–86.
 29. Ogawa, S.; Yuasa, H. Hot Topic: The medicinal chemistry of glycosidase Inhibitors. *Curr.Top.Med. Chem.* 2009; (9), 1–2.
 30. Ogawa, S.; Kanto, M. Design and Synthesis of 5a Carbaglycopyranosylamine glycosidase inhibitors. *Curr.Top.Med.Chem.* 2009; (9), 58–75.
 31. Hakamata, W.; Kurihara, M.; Okuda, H.; Nishio, T.; Oku, T. Design and Screening Strategies for alpha-glucosidase inhibitors based on enzymological Information. *Curr.Top.Med. Chem.* 2009; (9), 3–12.