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Synthesis, Characterization and Preliminary ά - Glucosidase Inhibition Study of 4-(1,3-dioxoisoindolin-2-yl)butanamide Derivatives

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Abstract
Two series of*4-(1, 3-dioxoisoindolin-2-yl)butanamide* derivatives were synthesized under Schotten-Baumann conditions. The first series consists of *4-(1, 3-dioxoisoindolin-2-yl)butanamide*(5a-e), and the second one 4-(4,5,6,7-tetrachloro-1,3-dioxoisoindolin-2-yl)butanamide (5f-j). The structures of the synthesized compounds were characterized by IR, ^{1}H NMR and ^{13}C NMR. The *in vitro* antidiabetic activity by using *α*-glucosidase from *Saccharomyces cerevisiae* was done for selected analogues (5a-e, 5f). All tested compounds were found to be the most potent antidiabetic agent when compare with standard compound (acarbose) depending on calculated IC50 values (5.44- 9.227).

Keywords-Antidiabetic, GABA, (1,3-dioxoisoindolin-2-yl)butanamide, α-glucosidase inhibitor

1. INTRODUCTION

Diabetes is a chronic illness requiring continuous medical care with multifactorialrisk-reduction strategies beyond glycemic control. [(1)]Type 2 diabetes mellitus (T2DM) is a condition characterized by insulin resistance and a relative deficiency of insulin secretion [(2)].The relative insulin deficiency leads to chronic hyperglycemia and multiple disturbances in carbohydrate, protein and fat metabolism including:

- ß-islet cell dysfunction, failure of response to insulin signaling and increased islet cell apoptosis
- α-cell dysfunction with elevated glucagon levels
- Resultant disorders of hepatic gluconeogenesis and insulin resistance with elevated glucose production
- Muscle cell insulin resistance with decreased glucose uptake
- Kidney adaptation with altered gluconeogenesis and increased glucose reabsorption via increased sodium glucose transporter protein activity
- Diminished incretin hormonal production or resistance
- Maladaptive cerebral hormonal responses to insulin and appetite
- Increased lipolysis with elevated free fatty acids [(3)].

Mortality is increased among individuals with T2DM compared to the non-diabetic population. The main cause of the increased mortality is macrovascular disease (e.g. ischaemic heart disease, stroke, and ischaemia of the lower extremities) [(4)]. The early identification and optimal management of people with type 2 diabetes is therefore critical [(5)].Modern medical research suggests that improving lifestyle can effectively control and prevent the occurrence of diabetes and its complications [(6)].The Diabetes Diagnosis and Treatment Guideline issued by the American Diabetes Association in 2009 stated that exercise prescription is an important treatment on improving lifestyle, and effective exercise can prevent and control the occurrence of type 2 diabetes. Moderate-to-vigorous intensity of aerobic exercise and moderate resistance training can decrease blood glucose and dyslipidemia indicators in patients with type 2 diabetes[(7)].

In spite of the underscored importance of lifestyle measures in diabetes therapy, most diabetics cannot escape the value of pharmacotherapy to achieve target glucose concentrations. Different oral hypoglycemics have been in use to aid in maintenance of blood glucose level at the requisite threshold in diabetics through distinct mechanisms [(8)].

Sulfonylureas and the nonsulfonylureasecretagogues establish normoglycemia by upregulating endogenous insulin secretion; alpha-glucosidase inhibitors work by delaying intestinal carbohydrate absorption [(9)]. Patients with inadequate glycemic control often require additional combination therapy or treatment with newer anti-diabetic agents or insulin to achieve the desired glycemic target levels [(10)]. Dipeptidyl peptidase-4 (DPP-4) is the enzyme responsible for inactivating the incretin hormones glucagon-like peptide 1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP), two hormones that play important roles in glucose homeostasis. Inhibition of dipeptidyl peptidase 4 is a promising new approach for the treatment of type 2 diabetes. DPP-4 inhibition results in increased blood concentration of the incretin hormones GLP-1 and GIP. This causes an increase in glucose-dependent stimulation of insulin secretion, resulting in a lowering of blood glucose levels [(11)].

2. 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 GF_{254} (type 60) pre-coated Aluminum sheets, Merck (Germany) and the eluent used is Chloroform: methanol (85:15)

Glacial acetic acid: ethyl Acetate: methanol (0.1:3:1)to run TLC.

B. HPLC was performed 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) (υ,cm^{-1}) were recorded using (Biotech engineering management FTIR-600, UK).

Furthermore, ^{1}H-NMR spectra*:* was recorded on (Bruker, Germany NMR Spectrometer 300 MHz, Avance III 300 spectrometer) with tetramethylsilane (TMS) as an internal standard, DMSO used as a solvent for samples measurement, (δ=ppm) and coupling constant in Hz.

2.1. Chemical Synthesis

2.1.1 Synthesis of 4-(1,3-dioxoisoindolin-2-yl) butanoic acid (2a)

A mixture of phthalic anhydride 1a (6.23, 42.29 mmol), gamma amino butyric acid (GABA) (4.87g, 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. The reaction mixture was concentrated at reduced pressure, added ethyl acetate to the residue, washed the organic phase with dilute HCl (1N), dried over MgSO4, and concentrated to yield the 4-(1,3-dioxoisoindolin-2-yl)butanoic acid (2a) as a solid (90%). 2a White crystalline solid; Mp: 198-200°C; IR (KBr): n 3426, 2974, 2933, 1766, 1705, 1546, 1469 1369, 1192, 721 cm^{-1}.

2.1.2 Synthesis of 4-(4,5,6,7-tetrachloro-1,3-dioxoisoindolin-2-yl) butanoic acids (2b)

A mixture of tetrachlorophthalic anhydride 1b (12.09, 42.29 mmol), GABA (4.87g, 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. 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, dried over MgSO4, concentrated to yield the 4-(4,5,6,7-tetrachloro-1,3-dioxoisoindolin-2-yl)butanoic acids 2b a solid (78%)**.** 2b off white solid; Mp: 116-118°C; IR (KBr): n 3460, 3032, 2939, 1766, 1701, 1438, 1369, 1284, 721 cm^{-1}

2.1.3 Synthesis of 4-(1,3-dioxoisoindolin-2-yl)butanoyl chloride (3a, 3b)

4-(1,3-dioxoisoindolin-2-yl) butanoic acid (2a), or 4-(4,5,6,7-tetrachloro-1,3-dioxoisoindolin-2-yl) butanoic acids (2b) (5mmol) 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).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 3a, 3b was used directly for the next step.

2.1.4. 4-(1,3-dioxoisoindolin-2-yl)butanamide derivatives (5a-j)

A solution of one amine derivatives(5-fluorouracil, or 4-chloroaniline, or 4-bromoaniline, or 2-aminothiazole, or Pyrrolidine) (4a-e, 5.5 mmol) were mixed with dry dichloromethane (15 ml) except for 4a, 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. in ice bath and then, freshly prepared acid chloride of either 3a,3b were slowly dropped for 50 min. with continuous stirring in 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). 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 to give [5a-j].

5a 2-(4-(5-fluoro-2, 4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-oxobutyl)isoindoline-1,3-dione

Yellowish orange powder; yield 85%; Mp: 186-188°C; IR (KBr): n 3132, 3066, 2974, 1770, 1720, 1662, 1496, 1477, 1246, 810 cm^{-1}

^{1}HNMR (300 MHz, DMSO): δ 1.2 (s, 2 H, CH_2-NCO), 3.05 (2 H, CH_2-CH_2), 3.5 (2 H, CH_2-CO), 7.7 (m, 4 H, Ar-H), 7.78 (H, Ar-CH-CF), 10.54 (s, 1 H, NH).

5b 2-(4-oxo-4-(pyrrolidin-1-yl)butyl)isoindoline-1,3-dione

Yellow powder; yield 74%; Mp: 176-178°C; IR (KBr): n 3043, 2978, 2881, 1766, 1732, 1639, 1550, 1404, 725cm^{-1}

^{1}HNMR (300 MHz, DMSO):δ 1.16 (2 H, CH_2-NCO),1.8(2H,CH_2-CH_2), 1.9 (4H, CH_2-N), 3.2 (2 H, CH_2-CH_2), 3.5 (2H, CH_2-CO), 7.9 (m, 4 H, Ar-H).

5c 4-(1,3-dioxoisoindolin-2-yl)-N-(thiazol-2-yl)butanamide

Yellowish orange powder; yield 72%; Mp: 160-162°C; IR (KBr): n 3452, 3097, 2939, 2877, 1766,1732, 1608, 1550,1442, 723cm^{-1}

^{1}HNMR (300 MHz, DMSO): δ 1.18 (s, 2 H, CH_2-NCO), 3 (2 H, CH_2-CH_2), 3.13 (2 H, CH_2-CO), 7.28(H, Ar-H-S), 7.48(2H, Ar-H-NH), 7.84 (m, 4 H, Ar-H), 10.63 (s, 1 H, NH).

5d N-(4-chlorophenyl)-4-(1,3-dioxoisoindolin-2-yl)butanamide

Yellow powder; yield 81%; Mp: 172-174°C; IR (KBr): n 3360, 3097, 2951, 2889, 1789, 1732, 1666, 1647, 1543, 1489, 736 cm^{-1}

^{1}HNMR (300 MHz, DMSO):δδ 1.2 (2 H, CH_2-NCO), 3.05 (2 H, CH_2-CH_2), 3.34 (2 H, CH_2-CO), 7.3 (2 H, Ar-H-Cl), 7.55 (m, 2 H, Ar-H- NH), 7.85 (m, 4 H, Ar-H), 10.57 (S, 1 H, NH).

5e N-(4-bromophenyl)-4-(1,3-dioxoisoindolin-2-yl)butanamide

Yellow powder; yield 88%; Mp: 167-169°C; IR (KBr): n 3360, 2943, 2881, 1766,1705, 1647, 1597, 1531,1481, 712cm^{-1}

^{1}HNMR (300 MHz, DMSO): δ 1.2 (2 H, CH_2-NCO), 3.05 (2 H, CH_2-CH_2), 3.65 (2 H, CH_2-CO), 7.45 (2 H, Ar-H-Br), 7.6 (2 H, Ar-H-NH), 7.83 (m, 4 H, Ar-H), 10.6 (S, 1 H, NH),

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

Mustard powder; yield 67%; Mp: 112-115°C; IR (KBr): n 3132, 3066, 2974, 1774, 1724, 1662, 1500, 1431, 1246, 813 cm^{-1}

^{1}HNMR (300 MHz, DMSO): δ 1.21 (s, 2 H, CH_2-NCO), 3 (2 H, CH_2-CH_2), 3.1 (2 H, CH_2-CO), 7.75 (H, Ar-CH-CF), 10.3 (s, 1 H, NH).

5g4,5,6,7-tetrachloro-2-(4-oxo-4-(pyrrolidin-1-yl)butyl)isoindoline-1,3-dione

White powder; yield 73%; Mp: 102-104°C; IR (KBr): n 2951, 2881, 1774,1708, 1620, 1550,1404, 736cm^{-1}

^{1}HNMR (300 MHz, DMSO):δ 1.25 (S, 2 H, CH_2-NCO), 3.3 (2 H, CH_2-CH_2), 3.46 (2 H, CH_2-CO), 4.1 (4H, CH_2-CH_2-N), 4.2 (4H, CH_2-N).

5h 4-(4,5,6,7-tetrachloro-1,3-dioxoisoindolin-2-yl)-N-(thiazol-2-yl)butanamide

Brown powder; yield 59%; Mp: 113-115°C; IR (KBr): n 3390, 3097, 2985, 1774, 1735, 1627, 1550, 1469, 1419, 965cm^{-1}

^{1}HNMR (300 MHz, DMSO): δ 1.12 (s, 2 H, CH_2-NCO), 3 (2 H, CH_2-CH_2), 3.1 (2 H, CH_2-CO), 6.85(H, Ar-H-S), 7.21 (2H, Ar-H-NH), 10.3 (s, 1 H, NH).

5i N-(4-chlorophenyl)-4-(4,5,6,7-tetrachloro-1,3-dioxoisoindolin-2-yl)butanamide

Pale yellow powder; yield 69%; Mp: 149-152°C; IR (KBr): n 3174, 2962, 2858, 1774,1712, 1647, 1597, 1527,1400, 736 cm^{-1}

^{1}HNMR (300 MHz, DMSO):δδ 1.16 (2 H, CH_2-NCO), 3 (2 H, CH_2-CH_2), 3.1 (2 H, CH_2-CO), 6.8 (s, 1 H, NH), 7.2 (2 H, Ar-H-Cl), 7.25 (m, 2 H, Ar-H- NH).

5j N-(4-bromophenyl)-4-(4,5,6,7-tetrachloro-1,3-dioxoisoindolin-2-yl)butanamide

Yellow powder; yield 75%; Mp: 130-132°C; IR (KBr): n 3224, 2943, 2877, 1774, 1712, 1647, 1593, 1523, 1489, 725 cm^{-1}

^{1}HNMR (300 MHz, DMSO): δ 1.18 (2 H, CH_2-NCO), 3 (2 H, CH_2-CH_2), 3.2 (2 H, CH_2-CO), 6.85 (s, 1 H, NH), 7.3 (2 H, Ar-H-Br), 7.4 (m, 2 H, Ar-H- NH).

2.2. In Vitro assay of α-glucosidase inhibitory activity

The *α*-glucosidase activity of selected compounds (5a-f) was determined according to the method described by Kim et al., using *α*-glucosidase from *Saccharomyces cerevisiae*. The substrate solution p-nitrophenolglucopyranoside (pNPG) was prepared in 20mM phosphate buffer, and pH 6.9. 100 *μ*L of *α*- glucosidase (1.0U/mL) was preincubated with 50 *μ*L of the different concentrations of the test compound (0001, 0.01, 0.1,1, 5, 10, 25, 50, 100 &200 uM) (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 0.1MNa_2CO_3.The *α*-glucosidase activity was determined by measuring the yellow-colored paranitrophenol released from pNPG at 400 nm. The results were expressed as percentage of the blank control. Acarbose were used as positive control,

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC50) were determined graphically.

3. RESULT AND DISCUSSIONS

3.1. Chemistry

The synthetic procedures for the target compoundswereillustrated in scheme (1). The Imides compound 2a and 2b were obtained by the reaction of anhydrides withgabaaminobutanoic acid to give compound 3a and 3b respectively. The reaction takes place by nucleophilic attack of an amino group at a carboxyl carbon atom of anhydride and azeotroping solvent typically used to remove the water formed during the reaction to drive the reaction to completion[12-14]. The acyl chloride derivatives compound 3a and 3b were prepared from the reaction of compound 2a and 2b with thionyl chloride respectively [15,16].

The reaction of the acyl chloride 3a and 3b with nitrogen containing compound 4(a=5-fluorouracil, b=pyrrolidine, c=2-aminothiazole, d=4- chloroaniline or e=4-bromoaniline) respectively will give the final compounds 5a-j[13,14,17].The synthesized compounds have been identified and charactrized by their melting points, IR spectrometry and ^{1}HNMR.

3.2. In Vitro assay of α-glucosidase inhibitory activity

The *α*-glucosidase activity of selected compounds (5a-f) was determined according to the method described by Kim et al., using *α*-glucosidase from *Saccharomyces cerevisiae*.Acarbose uses as positive control, Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC50) were determined graphically .The percent of enzyme inhibition calculated from the following equation[18].

$$\% \text{ Inhibition} = \left[\frac{Abs_{(control)} - Abs_{(extract)}}{Abs_{(control)}} \right] \times 100$$

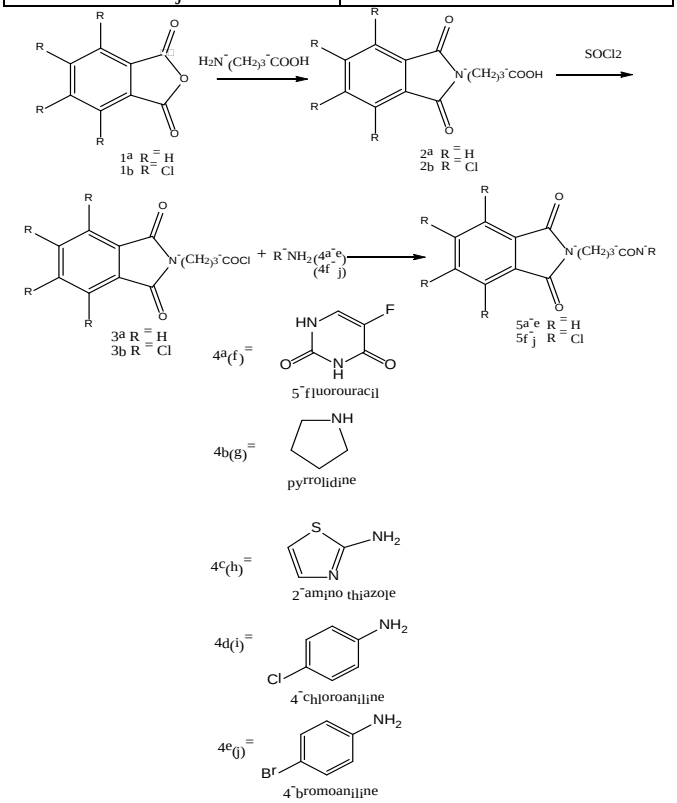
The percent of alpha glucosidase inhibition were plotting versus the concentrations as shown in Figure 1 to 6. The IC_{50} value for each compound were measure graphically as shown in table 1 and by comparing IC_{50} value with standard compound (acarbose). The result showed all the compounds have promising alpha glucosidase inhibition activity. Compound 5h showed the better inhibitory activity according to IC 50 value. Alpha glucosidase docking study using online docking website(https://mcule.com/dashboard/) Figure 7to 11.The result of docking study are comparable to the alpha glucosidase inhibition activity table 2 and reveal that the activity will be increased when the phthalyl moiety replaced by tetrachlorophthalyl moiety in addition to the reduce the decrease the size of butanamide substitution or ability to form hydrogen bondings.

Table 1: IC50 values of compounds (5a, 5f-j)

Compound	IC50
Acarbose (standard)	817.38 ± 6.27
5a	9.06
5f	5.688
5g	5.668
5h	5.44
5i	9.227
5j	7.153

Table 2: compounds affinity to alpha-glucosidase.

Compound	Affinity (Ki)
5a	**-6.8**
5f	-7.3
5g	-6.9
5h	-7.2
5i	-6.8
5j	-7.2



Scheme 1: Synthesis of 4-(1,3-dioxoisoindolin-2-yl)butanamide derivatives (5a-e) and4-(4,5,6,7-tetrachloro-1,3-dioxoisoindolin-2-yl)butanamide derivative (5f-j)

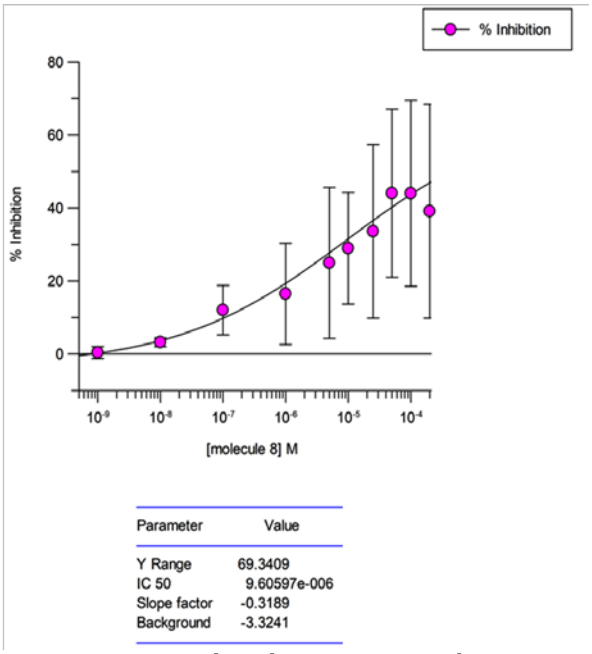
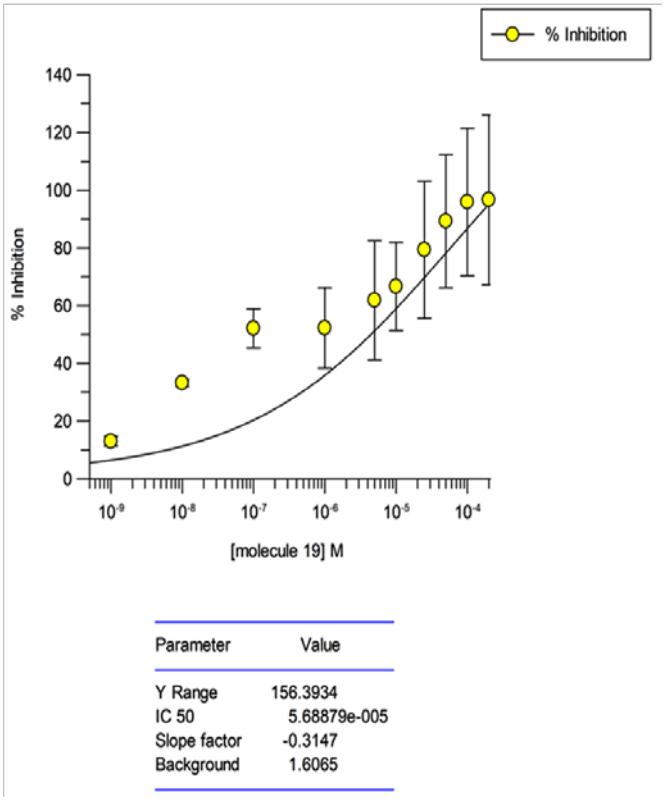
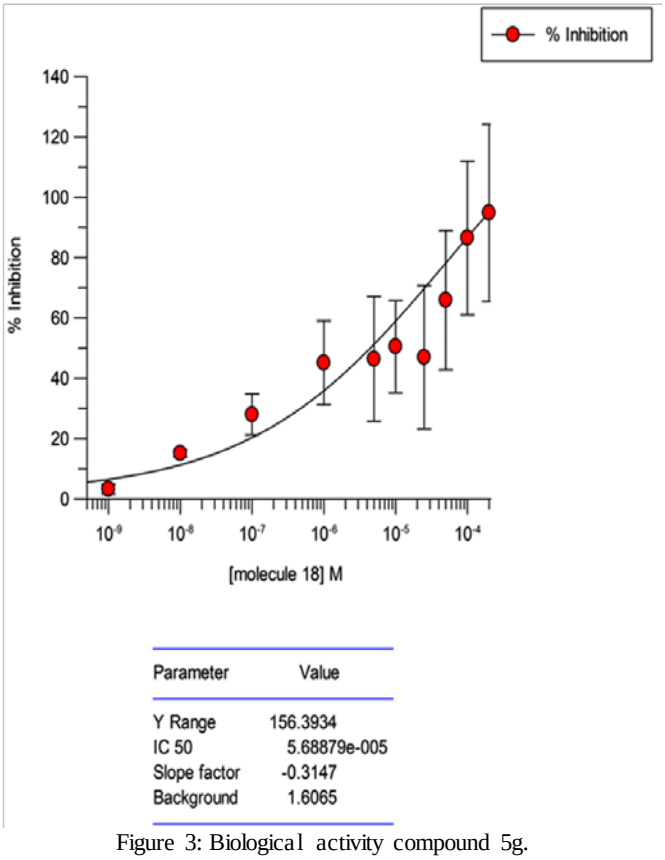


Figure 1: Biological activity compound 5a.



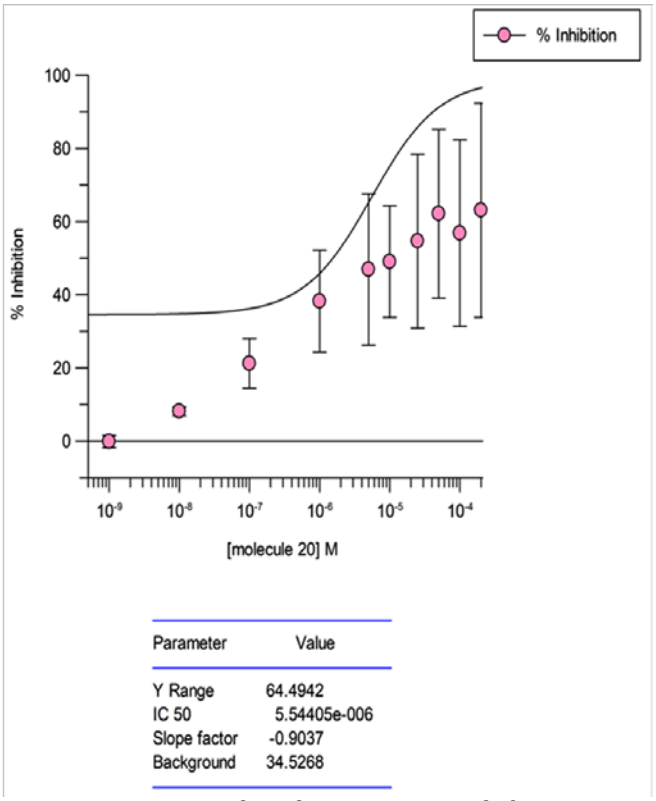
Parameter	Value
Y Range	156.3934
IC 50	5.68879e-005
Slope factor	-0.3147
Background	1.6065

Figure 2: Biological activity compound 5f.



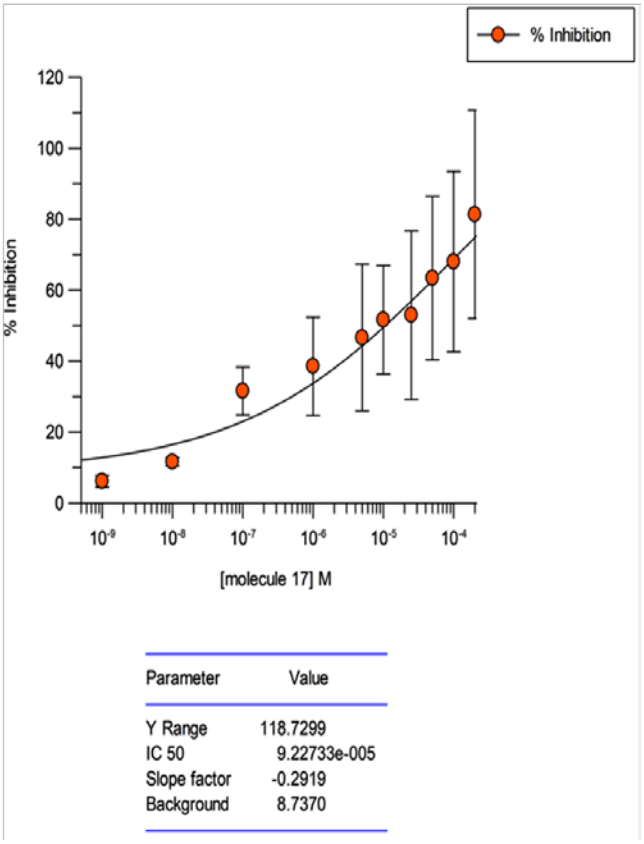
Parameter	Value
Y Range	156.3934
IC 50	5.68879e-005
Slope factor	-0.3147
Background	1.6065

Figure 3: Biological activity compound 5g.



Parameter	Value
Y Range	64.4942
IC 50	5.54405e-006
Slope factor	-0.9037
Background	34.5268

Figure 4: Biological activity compound 5h.



Parameter	Value
Y Range	118.7299
IC 50	9.22733e-005
Slope factor	-0.2919
Background	8.7370

Figure 5: Biological activity compound 5i.

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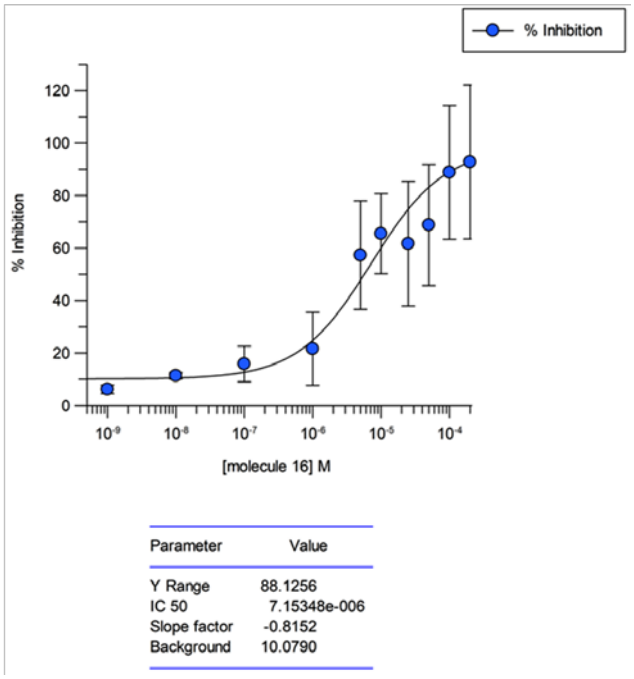

Figure 6: Biological activity compound 5j

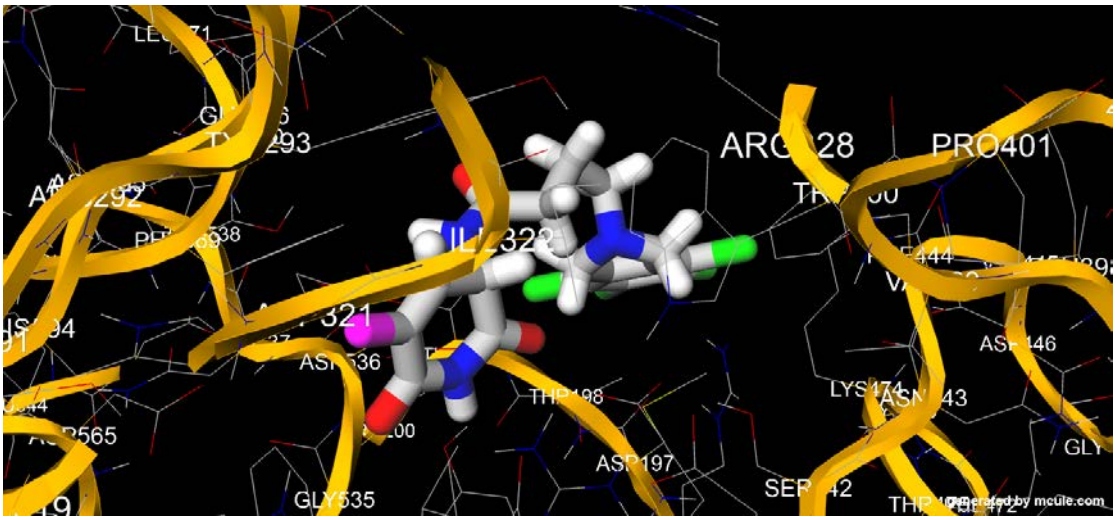

Figure 7: molecular docking of compound 5f

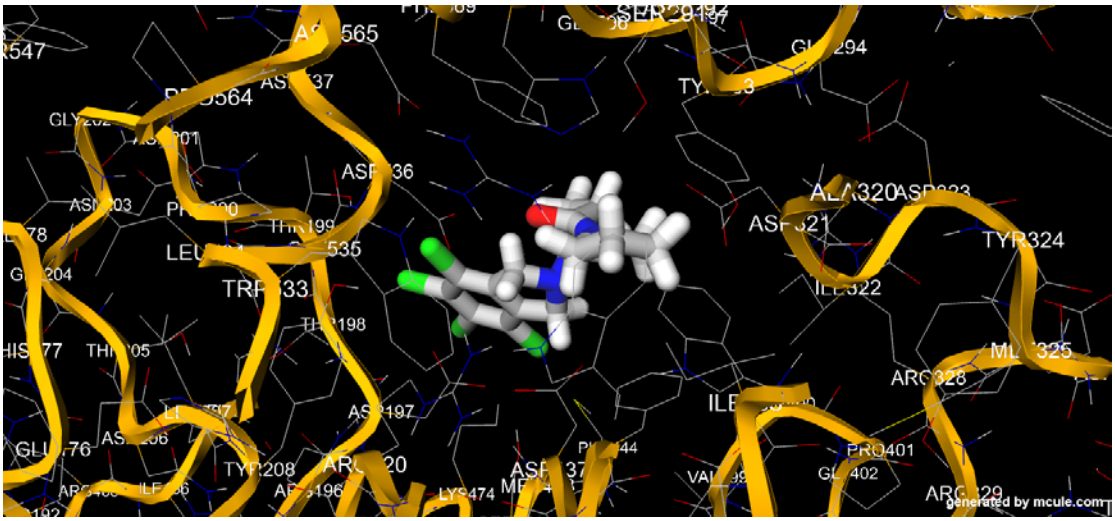

Figure 8: molecular docking of compound 5g

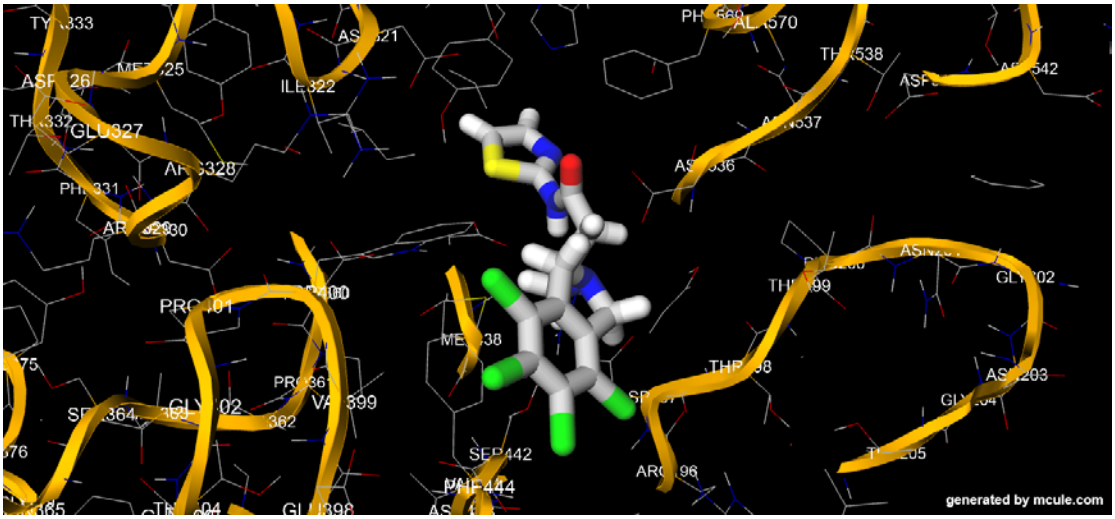

Figure 9: molecular docking of compound 5h

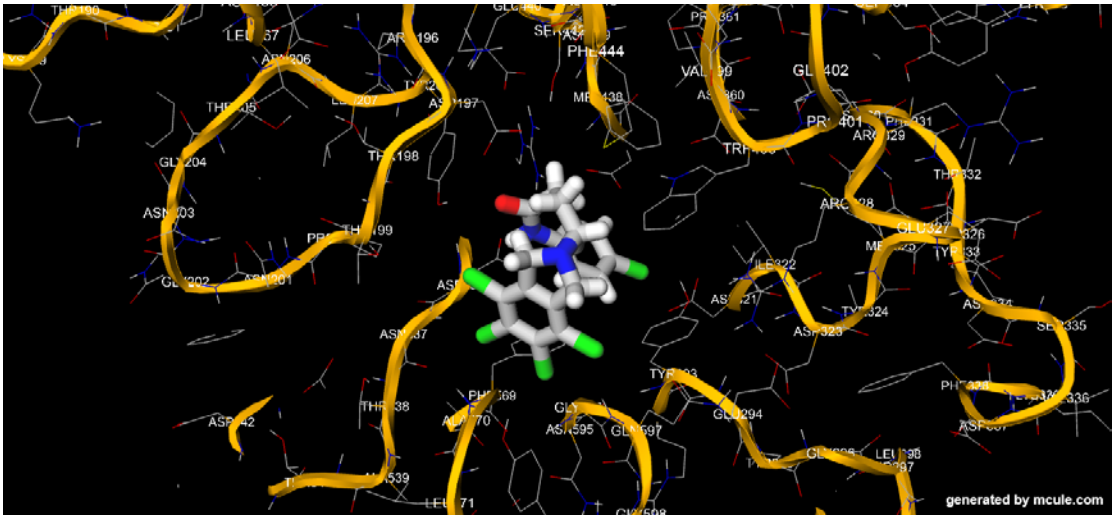

Figure 10: molecular docking of compound 5i

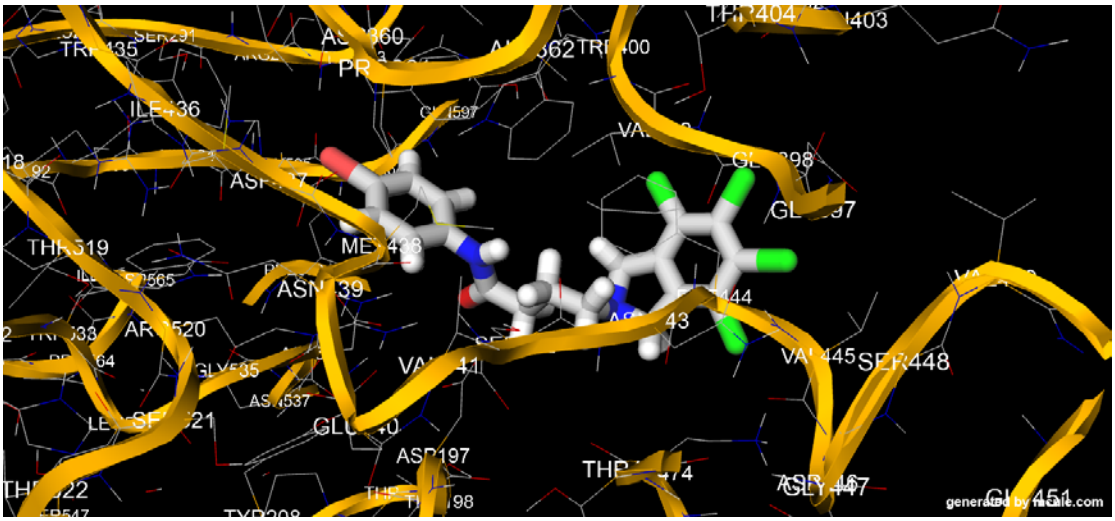

Figure 11: molecular docking of compound 5j

4. CONCLUSION

The compounds **5a-f** were found to be 88 to 150 fold more potent alpha-glucosidase inhibitor as compare to the standard acarbose. The results suggest that 4-(1,3-dioxoisoindolin-2-yl)butanamid derivatives are interesting lead molecules for further studies.More extensive work is still needed to confirm the preliminary results and mode of action to design compounds.

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