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Evaluation of Some Synthesized Novel Substituted Phthalimide Derivatives as Potent Antidiabetic Agents

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Tetrachlorophthalimide derivatives were synthesized and their ability to inhibit α-glucosidase was investigated *in vitro* as well as *in vivo* in streptozotocin-induced diabetic rats. The purity of synthesized compounds were confirmed by spectral and elemental analysis. Then the compounds were biologically screened on streptozotocin-induced diabetic rats compared with standard drug (miglitol). Tetrachlorophthalimide derivatives were potent inhibitors of yeast α-glucosidase (IC_{50} at 50 μM). Administration of S-5, S-2 and SE-II-13 orally at the dose of 250 mg/kg body weight resulted in significant ($p < 0.001$) reduction in blood glucose levels. The body weights were significantly ($p < 0.001$) reduced in streptozotocin-induced diabetic rats when compared to normal rats. The present study reveals that out of all synthesized compounds, N-(2,4-dinitrophenyl) tetrachlorophthalimide could be a representative of a new group of α-glucosidase inhibitors and exhibit significant anti-hyperglycemic effect.

Key Words: Tetrchlorophthalimide, Streptozotocin, Miglitol, Diabetes.

INTRODUCTION

Non-insulin dependent diabetes mellitus (NDDM) (often referred as diabetic type 2) is one of the greatest challenges confronting the human beings. This metabolic disease is a growing public health problem affecting *ca.* 194 million people world wide according to the World Health Organization and this member is projected to be 366 million by 2030. The survey reported that it is affecting nearly 10 % of the population every year[1]. The prevalence of these diseases is increasing steadily and adequate treatment is often expensive or unavailable[2]. Therefore the human population worldwide appears to be in the midst of an epidemic of diabetes[3]. In modern medicine no satisfactory effective therapy is still available to cure diabetic mellitus[4].

The treatment of diabetes mellitus in clinical practice has been confined to use of oral hypoglcemic agents and insulin, the former being reported to be endowed with characteristics profiles of serious side effects like, hypoglycaemia, nephropathy, neuropathy, retinopathy, polyurea, polydypsia, ketonuria, *etc.*[5].

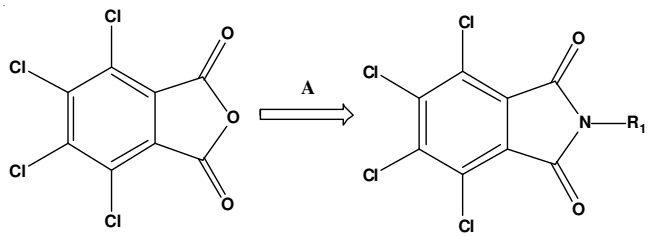
These metabolic disturbances are predisposing factors for cardiovascular, hepatic and renal complications[6]. Though the pharmaceutical drugs like, sulfonylureas, biguanides, thiazolidines, α-glucosidase inhibitor, DPP-IV inhibitors, *etc.* are used for the treatment of diabetes type 2, but these are either too expensive or contraindication[7]. Neither insulin nor these modern pharmaceuticals have been shown to modify the course of diabetic complications[8]. Alternative strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed[9] because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of the modern therapies for many rural population in developing countries. α-Glucosidase is one of the brush border membrane enzymes which play a role in the final steps of digestion of starch. These are enzymes involved in breaking down complex carbohydrates such as starch and glycogen these catalyze the cleavage of individual glucosyl residues from various glycoconjugates including α- or β-linked polymers of glucose. During the last few decades, there has been widespread interest in α-glucosidase (EC 3.2.1.20) because of its important role not only in carbohydrate digestion, but also in the processing of glucoproteins and glycolipids. The enzyme is also involved in a variety of metabolic disorder and other diseases such as diabetes, viral attachment and cancer formation[10,11]. Now-a-days, many α-glucosidase inhibitors[12,13] have been reported, such as acarbose and voglibose from microorganisms and 1-deoxynojirimycin isolated from plants. However, they are largely confined to glycosidic derivatives. α-Glucosidase catalyzes the final step

in the digestive process of carbohydrates. Its inhibitors can retard the uptake dietary carbohydrates and suppress postprandial hyperglycemia and could be useful to treat diabetic and/or obese patients[14]. Phthalimide derivatives have been widely reported to possess beneficial pharmaceutical effects like, antiviral[15], antifertility[16], antibacterial[17], anti-HIV[18], anticonvulsant[19], analgesic[20], antiinflammatory[21] and hypoglycemic[22] activities. Tetrachloro phthalimide derivatived from thalidomide has been reported to be α-glucosidase inhibitor[23]. This impetus to us to investigate the α-glucosidase inhibitory activity of derivatives of N-phenyltetrachloro phthalimide against yeast and steptozotocin-induced diabetic rats.

EXPERIMENTAL

Melting points were determined with a Fisher-Johns melting point apparatus or the electrothermal digital melting point apparatus, model IA 9100 and are uncorrected. Column chromatography was carried out on silica gel (Merck Kieselgel 60, 70-230 mesh). Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet FT-IR spectrophotometer, model Impact 410. Solid samples were incorporated with potassium bromide to form a pellet. The elemental analysis of the synthetic compounds was performed on elemental analyzer, elemental Vario EL II/Carlo Ebra 1108 at sophisticated analytical instrumental facility, IICB Calcutta (W.B.) and results are within the limit of calculated values. ^{1}H NMR and ^{13}C NMR spectra were performed in deuterated chloroform ($CDCl_3$) or deuterated dimethyl sulfoxide (DMSO-d_6) with tetramethylsilane (TMS) as an internal reference on a bruker DRX-300 MHz at sophisticated analytical instrument facility, Indian Institute of Chemical Biology, Kolkata (W.B.) which operated at 200.13 MHz for ^{1}H and 50.32 MHz for ^{13}C nuclei.

Synthesis of N-phenylphthalimide derivatives[24]: About 0.05 mol (14.25 g) of phthalic anhydride (4,5,6,7-tetrachloroisobenzofuran-1,3-dione) and 0.05 mol of aniline derivatives was stirred and heated under reflux for 5 h in presence of 0.1 mol (6 mL) glacial acetic acid. The reaction mixture was checked by thin layer chromatography. When reaction was completed, stop the reflux and cool the reaction mixture. The precipitate of compounds (**S-1** to **S-7**); formed slowly by addition of water. This was filtered and washed well with water and recrystallized with 95 % ethanol (**Scheme-I**). The purity of synthesized compounds were ascertained by thin layer chromatography using methanol + acetonitrile mixture (4:6 v/v) and the structure of the product were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR and elemental analysis.



Reagents & Conditions: A = Aromatic amine; (a) Na/MeOH, Mg, reflux with stirring for 18 h; (b) Guanidine/MeOH, MS 3A, reflux for 24 h

Scheme-I:

N-(2-Nitrophenyl)tetrachlorophthalimide (S-1): Yield 82 %; m.p. 225-227 ºC; IR (KBr, ν_{max}, cm^{-1}): 3053 (aromatic C-H), 1789, 1731 (amide C=O), 1606 (aromatic-H), 1526 and 1343 (-NO_2 group), 783 (C-Cl str.); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.24-7.97 (3H, m, Ar-H) and 8.19 (1H, d, *J* = 8.2 Hz, Ar-H); ^{13}C NMR ($CDCl_3$) δ (ppm): 122.3, 122.8, 123.8, 130.5, 131.5, 133.5, 133.6, 134.8,147.5 and 166.8; MS$^+$ 405.8. Anal. calcd. (%): C, 41.42, H, 0.99, N, 6.90. Found: C, 41.42, H, 0.99, N, 6.99.

N-(3-Nitrophenyl)tetrachlorophthalimide (S-2): Yield 87 %; m.p. 255-257 ºC; IR (KBr, ν_{max}, cm^{-1}): 3092 (aromatic C-H), 1779, 1714 (amide C=O), 1606 (aromatic-H), 1537 and 1347 (-NO_2 group), 748 (C-Cl str.); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.82-8.0 (3H, m, Ar-H) and 7.69 (1H, s, Ar-H) ; ^{13}C NMR (DMSO-d_6) δ (ppm): 122.0, 122.7, 123.6, 130.3, 131.5, 133.0, 133.7, 134.9,147.8 and 166.6; MS$^+$ 405.7. Anal. calcd. (%): C, 41.42, H, 0.99, N, 6.90. Found: C, 41.40, H, 0.99, N, 6.99.

N-(4-Nitrophenyl)tetrachlorophthalimide (S-3): Yield 87 %; m.p. 280-282 ºC; IR (KBr, ν_{max}, cm^{-1}): 3082 (aromatic C-H), 1777, 1723 (amide C=O), 1610 (aromatic-H), 1528 and 1496 (-NO_2 group), 745 (C-Cl str.); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.80 (2H, m, Ar-H), 7.90-8.03 (2H, m, Ar-H) and 8.36-8.43 (2H, m, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 123.6, 124.1, 127.7, 131.4, 134.9,138.2, 138.9 and 166.4; MS$^+$ 405.98. Anal. calcd. (%): C, 41.42, H, 0.99, N, 6.90. Found: C, 41.41, H, 0.99, N,6.91.

(Phenyl)tetrachlorophthalimide (S-4): Yield 73 %; m.p. 230-232 ºC; IR (KBr, ν_{max}, cm^{-1}): 3066 (aromatic C-H), 1782, 1712 (amide C=O), 757 (C-Cl str.); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.35-7.55 (5H, m, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 123.4, 127.4, 128.0, 128.8, 131.5, 131.9, 134.7 and 167.5. MS$^+$ 360.98. Anal. calcd. (%): C, 46.58, H, 1.40, N, 3.88. Found: C, 46.58, H, 1.10, N, 3.98.

N-(2,4-Dinitrophenyl)tetrachlorophthalimide (S-5): Yield 85 %, m.p. 163-165 ºC; IR (KBr, ν_{max}, cm^{-1}): 3108 (aromatic C-H), 1770, 1631 (amide C=O), 1492 (-NO_2 group), 757 (C-Cl str.); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.33-8.63 (2H, d, *J* = 9.4 Hz, Ar-H), 9.08 (1H, m, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 119.7, 123.3, 128.62, 129.3, 135.1, 136.5, 139.2, 145.5, 149.8 and 161.3. MS$^+$ 451.88. Anal. calcd. (%): C, 37.28, H, 0.67, N, 9.32. Found: C, 37.28, H, 0.67, N, 9.32.

N-(4-Chloro-2-nitrophenyl)phthalimide (S-6): Yield 76 %; m.p. 230-232 ºC; IR (KBr, ν_{max}, cm^{-1}), 3046 (aromatic C-H), 1786, 1725 (amide C=O), 1535 (-NO_2 group) and 783 (C-Cl str.); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.49 (1H, m, Ar-H), 7.70-7.97 (2H, m, Ar-H); ^{13}C NMR ($CDCl_3$) δ (ppm): 122.7, 124.2, 127.4, 131.4, 130.3, 132.0, 132.4,135.2, 141.7 and 166.4; MS$^+$ 439.38. Anal. calcd. (%): C, 38.18, H, 0.69, N, 6.36. Found: C, 38.19, H 0.69, N 6.35.

N-(2-Chloro-4-nitrophenyl)phthalimide (S-7): Yield 69 %, m.p. 291-292 ºC; IR (KBr, ν_{max}, cm^{-1}): 3096 (aromatic C-H), 1786, 1725 (amide C=O), 1534 (-NO_2 group) and 721 736 (C-Cl str.); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.55 (1H, d, *J* = 8.7 Hz, Ar-H), 7.81-7.99 (2H, m, Ar-H); ^{13}C NMR ($CDCl_3$) δ (ppm): 121.9, 122.7,124.3, 126.6, 127.3, 132.2, 132.5, 144.7, 145.4 and 166.4. MS$^+$ 439.37. Anal. calcd. (%): C 38.18, H 0.69, N 6.36. Found: C 38.18, H 0.69, N 6.36.

Assay for yeast α-glucosidase inhibitory activity: As widely used, the commercially available α-glucosidase from baker's yeast (Sigma, G5003) was selected as the target protein in this study using *p*-nitrophenyl α-D-glucopyranoside (Sigma, N1377) as the substrate. All the synthesized compounds dissolved in DMSO. The reason for this choice for solvent lies in that most of the test compounds were soluble and maintained stable in DMSO. The enzyme and the substrate were dissolved in 0.07 M potassium phosphate buffer with pH 6.8. Then, the enzymatic reaction mixture composed of 100 μL α-glucosidase (0.1 U/mL), 99 μL of 5 mM substrate and 1 μL (10 mM/mL DMSO) of test compound was incubated at 37 ºC for 0.5 h. The inhibitory activity of each test compound was determined by measuring the remaining activity of α-glucosidase at the concentration of 50 μM. The enzymatic activity was measured by the amount of the released product, *p*-nitrophenol, that was detected by spectrophotometer at the wavelength of 415 nm. For all test compounds, the inhibition assay was performed in duplicate[25].

***In vivo* evaluation of hypoglycemic potential:** The present work is to explore the scientific basis of the utility of tetrachlorophthalimide derivatives for ameliorating hyperglycemia in streptozotocin induced diabetic rats.

α-Glucosidase from baker's yeast (type I), from *B. cereus*, *p*-nitrophenyl α-D-glucopyranoside (PNP-G), sucrose, maltose and 1-deoxynojirimycin.HCl and streptozotocin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade and were obtained from the following indicated commercial sources. Thiobarbituric acid, nitro blue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH) (Loba Chemie, Mumbai, India), 5,5-dithio *bis*-2-nitro benzoic acid (DTNB),

Animals and ethical approval: Studies were carried out using Wistar albino rats (150-180 g) of male sex were used. They were obtained from the animal house, College of Pharmacy IPS Academy, Indore, India. The animals were grouped and housed in polyacrylic cages (38 cm × 23 cm × 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 °C) with dark and light cycle (14/10 h) and relative humidity (55-70 %). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment.

Induction of experimental diabetes: After 1 week of acclimatization, the rats were subjected to a 16 h fast. Diabetes was induced with a single i.p. injection of streptozotocin (STZ) at a dose of 40 mg/kg body weight. The streptozotocin was freshly dissolved in citrate buffer (0.01 M, pH 4.5)[26]. The injection volume was prepared to contain 1.0 mL/kg. After 5 days, blood glucose levels were measured and the animals with a concentration of more than 225 mg/dl were taken for the investigation[27,28].

Experimental design: In the experiment, a total of 36 rats (6 normal; 30 streptozotocin diabetic rats) were used. The rats were divided into six groups of six animals each. Group I: (normal control) received normal saline solution (0.9 % NaCl w/v, 5 mL/kg); Group II: (hyperglycemic control) hyperglycemic rats were administered streptozotocin (40 mg body wt., *i.e.*) once before the treatment; Group III: (standard control) received streptozotocin with miglitol as reference standard (25 mg/kg); Group IV: (test control 1) streptozotocin treated hyperglycemic rats were administered with 250 mg/kg body wt. of test sample S-2 for 14 days. Group V: (test control 2) streptozotocin treated hyperglycemic rats were administered with 250 mg/kg body wt. of test sample **S-5** for 14 days. The effect of synthetic samples of phthalimide derivative on streptozotocin induced diabetic rats were determined by measuring blood glucose levels, food and fluid intake amount and changes in body weights[29,30]. After 14 days of treatment, all the rats were decapitated after fasting for 16 h. The animals were dissected and a drop of blood from the heart was used for the estimation of blood glucose.

Measurement of blood glucose levels procedure: Groups of 6 diabetic and non-diabetic rats were used per experiment. Following 16 h fast, the rats (diabetic and non-diabetic) were given orally, *via* a stomach tube, corn starch (0.5 g/100 g body wt.) as a suspension with or without drug miglitol (25 mg/kg body wt.) and test samples (250 mg/kg body wt.). Drug miglitol was supplied by Glenmark Pharmaceutical Mumbai (M.H.). (Group-III standard control with drug miglitol and remaining five groups without drug only take test samples and normal saline solution) The starch sources used (partially purified food sources) and their composition (% protein, carbohydrates, fat and dietary fiber, respectively) was as follows: corn (9; 78; 2.8; 2.8); starch was cooked with water for 20 min, dried at 45 °C and ground into powder. Blood samples were collected from the tail tip into tubes pre washed with heparin (400 u/mL) and 0.1 mM NaF. Blood samples were withdrawn from each rat prior to starch loading and at 30, 60, 90, 120 and 180 min following the starch intubations. At the beginning of the experiment and at 5-day intervals, body weight and blood glucose levels with respect to time (30, 60, 90,120,180 min) were measured. Blood samples were obtained by tail-vein puncture of the normal and streptozotocin -induced diabetic rats on day zero (0), day 5, day 10 and on day 15. Blood glucose levels were determined using a glucometer. (One Touch Ultra blood glucose monitoring system from Lifescan, Johnson and Johnson Company, Milpitas, CA).

RESULTS AND DISCUSSION

In vitro studies of the compounds of the tetrachlorophthalimide derivatives were compared (Table-1) with standard drug miglitol which indicated that compound **S-2** and **S-5** having a better α-glucosidase inhibitory activity as compare

TABLE-1
IN VITRO EVALUATION OF SYNTHESIZED COMPOUNDS

Compounds code No.	% Inhibition at 50 μM
S-1	60.5 ± 5.4
S-2	69.3 ± 0.6
S-3	59.4 ± 4.7
S-4	61.4 ± 4.9
S-5	73.1 ± 6.6
S-6	63.1 ± 7.8
S-7	58.0 ± 4.3
Standard (migilitol)	86.3 ± 0.6

TABLE-2
EFFECT OF SYNTHESIZED COMPOUNDS ON GLUCOSE LEVEL IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

Group	Dose (mg/kg)	Glucose level (mg/dl) after taking food (min)							
		0 day	5th day					10th day	15th day
			30	60	90	120	180	180	180
Normal (0.9 % NaCl w/v)-Gr-I	-	84.1±1.2**	85.0±1.4**	85.3±2.0**	85.2±2.1**	84.1±1.2**	85.4±1.4**	84.2±1.2**	85.1±1.4**
Diabetic control (Streptozotocin)-Gr-II	40	85.0±1.4**	312.18±2.0	312.17±2.1	312.20±1.8	312.18±2.0	312.22±1.9	312.18±2.0	312.22±1.9
Standard control (miglitol) Gr-III	25	85.3±2.0**	312.19±1.0	330.13±2.2	252.10±1.6	212.15±2.1	103.20±1.5	100.15±2.1	95.20±1.5
Test control **S-2**; Gr-IV	25	86.3±1.0**	308.12±1.7	320.17±2.5	272.20±2.2	230.18±1.9	118.22±2.0	110.22±2.0	107.16±2.0
Test control **S-5**-Gr.-V	40	85.0±1.4**	312.18±2.0	312.17±2.1	262.20±1.8	220.18±2.0	107.22±1.9	104.34±1.3	98.22±1.9
Test control SE-II-13 Gr.-VI	25	85.3±2.0**	312.19±1.0	330.10±2.2	252.10±1.6	234.15±2.1	122.20±1.5	115.56±2.5	112.20±1.5

Values are mean ± SEM, 6 animals in each group (n = 6); *$p < 0.05$; Values are considered statistically significant; *$p < 0.05$, **$p < 0/01$, When compared to diabetic control (Streptozotocin)

to other synthesized molecules and moderate inhibitory activity as compare to miglitol. *In vivo* studies of synthesized compounds (**S-2** and **S-5**) showed the hypoglycemic activity in streptozotocin induced diabetic rats (Table-2). It showed that **S-2** and **S-5** inhibited yeast α-glucosidase enzyme as like as miglitol. This observation suggested that a decrease in electron diversity of the N-phenylphthalimide moiety would result in an increase in α-glucosidase inhibitory activity. While the presence of a NO_2 group at the 2- and 4- position (**S-5**) resulted in the most active α-glucosidase inhibitor among the N-phenyltetrachlorophthalimide derivatives.

Conclusion

It may be concluded from the results of the present investigation that tetrachlorophthalimide derivatives possessed α-glucosidase inhibitory activity as compare to standard drug miglitol and can be useful for treatment of diabetes. If the molecule, N-(2,4-dinitrophenyl)tetrachlorophthalimide (**S-5**) further evaluated in the terms of clinical trails and toxicity study in human volunteers it may be produced good α-glucosidase inhibitor in human subject.

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