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Synthesis and Hypoglycemic Activity of some Phthalimide Derivatives

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Abstract:

N-hydroxyethyl phthalimide (PE-I) was prepared by refluxing phthalic anhydride with ethanolamine. 3-phthalimidoethyl substituted benzoic acids (PE-II) were prepared by refluxing N-hydroxyethyl phthalimide with substituted benzoic acids. The corresponding acid chlorides (PE-III) were condensed with benzilidine-4-acetophenone derivatives to afford the 3-(phthalimidoethyl)-4-substituted cinnamoyl substituted benzanilides (PE-IV-1, 2, 4, 6, 9, PE-V-2, 4, 9). The compounds were screened for the *in vitro* DPP-IV inhibitory activity. The best three compounds in terms of the DPP-IV inhibition were subjected to *in vivo* studies in Streptozocin induced diabetic rats for the possible hypoglycemic activity.

Keywords: *Benzanilides, DPP-IV inhibitors, hypoglycemic activity.*

Introduction:

Diabetes mellitus is one of the major threats to worldwide healthcare, causing 100 million deaths with prevalence for type-2 diabetes.[1] Besides classical insulin-based treatment, several new approaches are emerging, including DPP-IV inhibitors.[2] These substances act by preventing the rapid degradation of GLP-1, an incretin hormone having multiple beneficial effects on glucose homeostasis.[3] Several incretin mimetics and DPP-IV inhibitors are undergoing late-stage clinical trials for the treatment of type 2 diabetes. These agents appear to have multiple mechanisms of action, including some or all of the following: enhancement of glucose-dependent insulin secretion; suppression of inappropriately elevated glucagon secretion; slowing of gastric emptying; and decreased food intake (i.e. appetite suppression) [4]. N-phenylphthalimide and its derivatives have been widely reported to possess beneficial pharmacological effects. They have been shown to be anticonvulsant, anti-inflammatory, hypolipidemic and inhibitors of dipeptidyl peptidase IV (DPP IV). In addition, tetrachlorophthalimide derived from thalidomide has been reported to possess potent hypoglycemic activity.[5] In view of the biological importance of anilides; some phthalimide compounds show hypoglycemic activities. The phthalimide compounds and their derivatives are reported to show various pharmacological activities.[6, 7] Further, benzoic acid derivatives are also available for clinical use in doses which lower the blood glucose level.[8] Looking at the importance of these types of compounds, it was planned to synthesize a new system incorporating both these units with potent pharmacological activity.

Materials and Methods:

All solvents, reagents and catalysts for synthesis were of synthetic grade and were used directly. The melting points were determined by open capillary method and were uncorrected. The purity of the compounds was confirmed by thin layer chromatography using Silica Gel glass plates as the stationary phase and suitable mobile phase. The mass spectra of compounds were recorded by Varian Inc, USA Model: 500 MS IT with 410 Prostar Binary LC at IIT mumbai. NMR spectra were recorded by Varian Mercury Plus 300 NMR spectrophotometer at University of Pune. IR spectra were recorded on FTIR_8300, KBr Press (Shimadzu) spectrophotometer at University of Pune. For in vitro studies, DPP-IV (Sigma aldrich) and p-Nitro aniline (Sigma aldrich) were used and sitagliptin was a generous gift from Merck India. For in vivo studies, Streptozocin (Sigma aldrich) was used to induce diabetes in rats. All the other chemicals used were of analytical grade. Male Wistar albino rats (150–180 g) of were used for the study. They were obtained from the animal house, college of pharmacy IPS

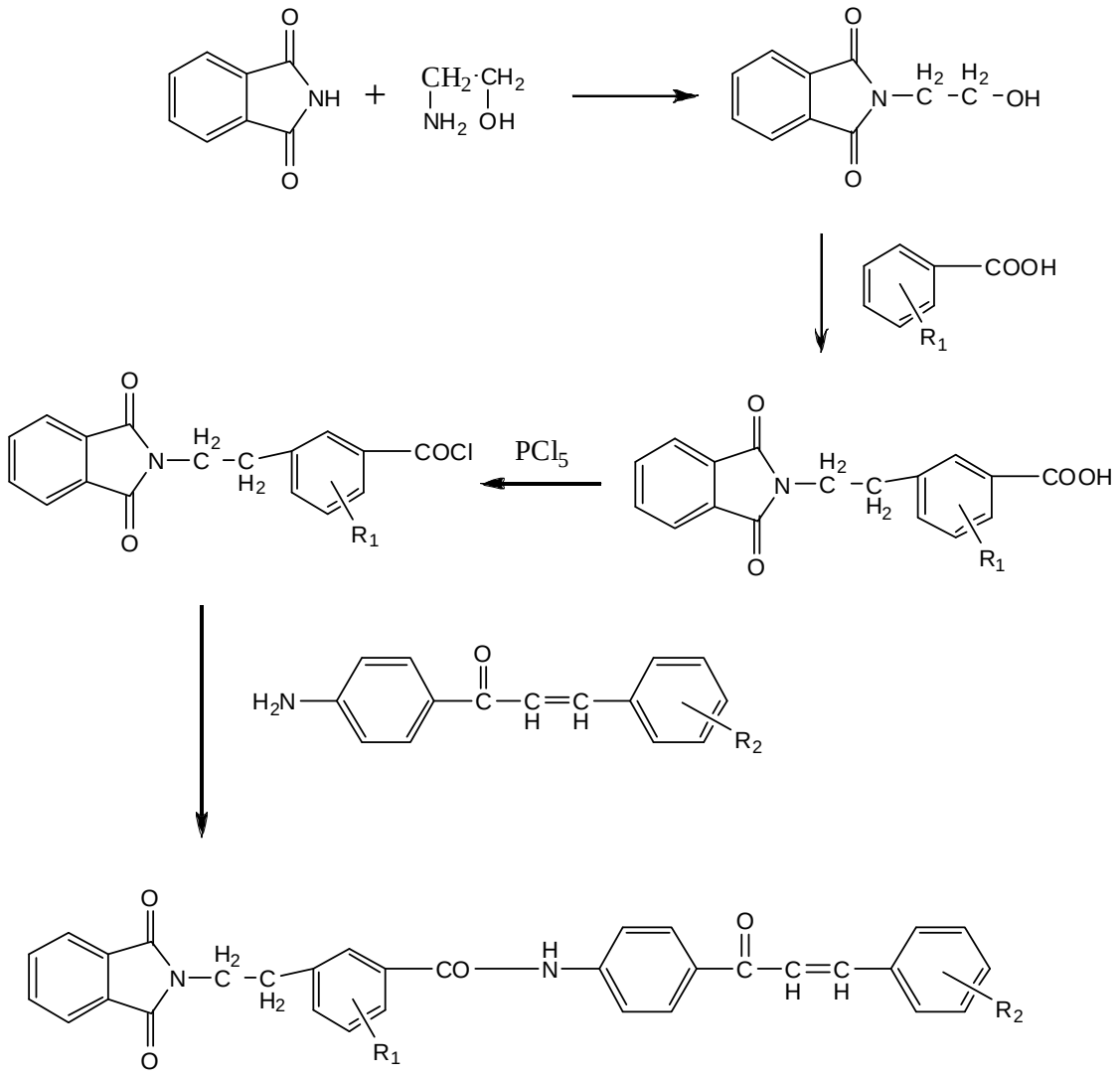
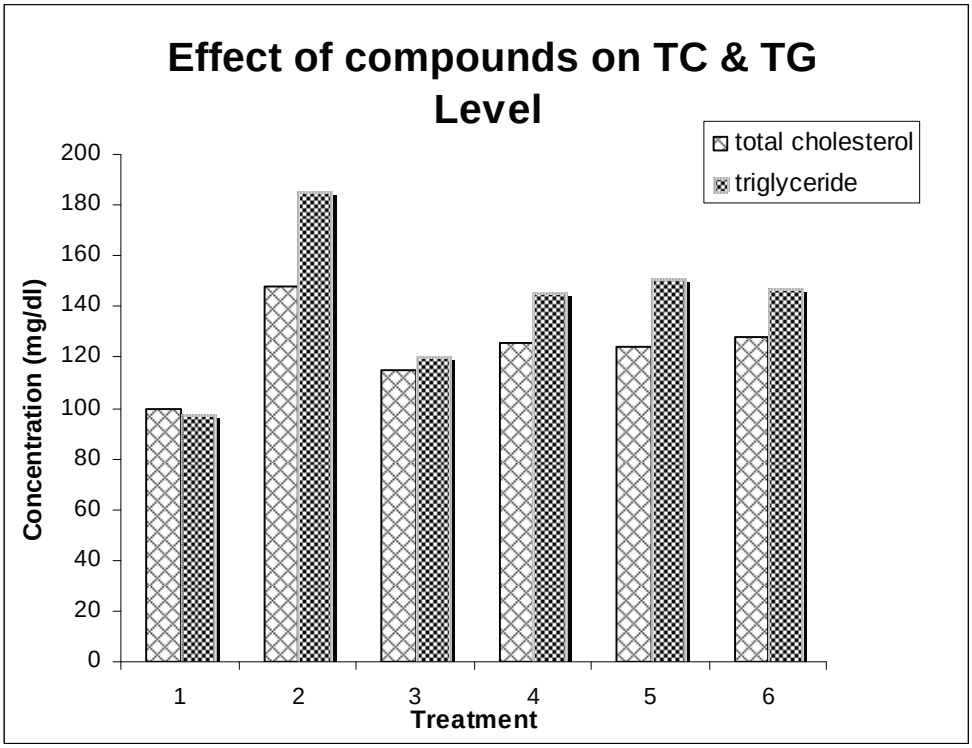


Fig 1: Scheme of Synthesis of 3-(phthalimidoethyl)-4-substituted cinnamoyl substituted benzanilides

Academy, Indore, India. Experiments were complied with the rulings of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) New Delhi, India (Registration No: 0367/01/C/CPCSEA) and the study was permitted by the Institutional Ethical Committee of the college of pharmacy IPS Academy, Indore, India.

In the present work, 3-phthalimidoethyl-4-substituted cinnamoyl substituted benzanilides were synthesized and evaluated for their hypoglycemic potential. The N-ethylation of phthalimide with 2-amino ethanol yielded N-ethyl phthalimide which on treatment with various acids, yielded 3-phthalimidoethyl substituted benzoic acids. These acids were then treated with PCl_5 to yield 3-phthalimidoethyl substituted benzoyl chlorides. The benzoyl chlorides thus obtained were further treated with substituted benzilidine 4 amino acetophenone derivatives which in turn were prepared by the reaction of acetophenone with various benzaldehyde derivatives. The reaction of benzoyl chlorides with



1: Control, 2: diabetic control, 3: sitagliptin, 4: PE-IV-2, 5: PE-V-4, 6: PE-V-9

Fig 2: Effect of synthesized compounds on TC and TG levels

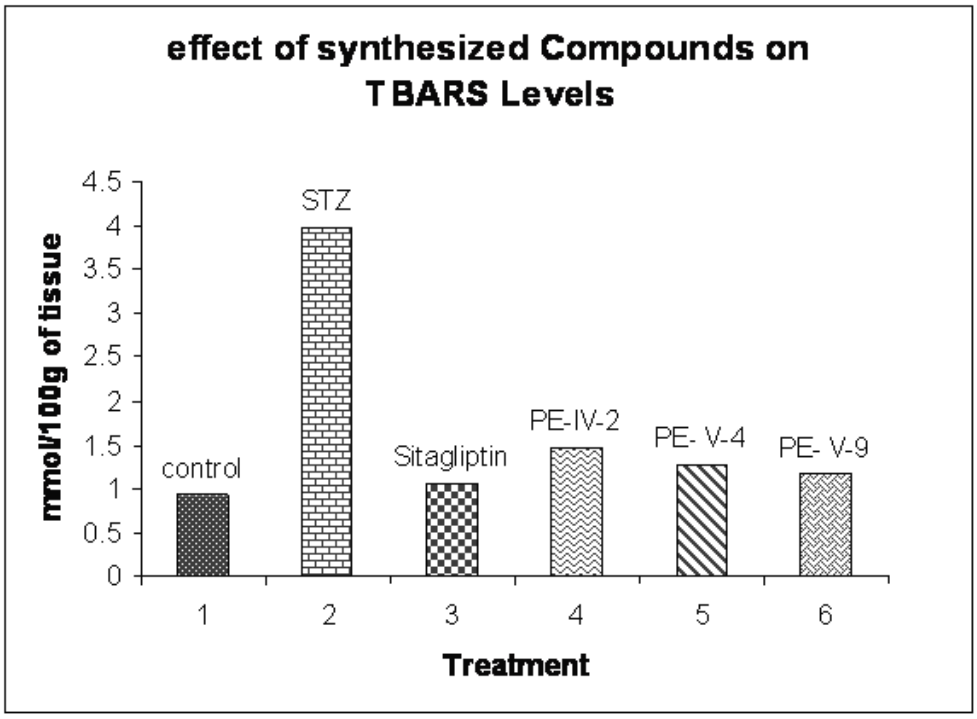


Fig 3: Effect of synthesized compounds on TBARS levels

Table 1: IC_{50} values of the synthesized compounds

S.No.	Compounds Code number	IC_{50} value (µg/ml)
1	PE-IV-1	78
2	PE-IV-2	20.1
4	PE-IV-4	24.1
6	PE-IV-6	>100
9	PE-IV-9	23.4
12	PE-V-2	34.9
14	PE-V-4	19.0
16	PE-V-9	18.8
18	Sitagliptin	18.5

benzilidine-4-amino acetophenone derivatives gave the final compounds which are 3-(phthalimidoethyl)-2/4-substituted cinnamoyl substituted benzanilides.

Synthesis of N- Hydroxyethylphthalimide (PE_I):

Phthalic anhydride and 2-Amino ethanol were heated together in an oil bath at 100^0c for 2 hours. The content was cooled and dilute HCl was added slowly with constant stirring. A white mass was separated out. This mass was recrystallized using ethanol, Yield 82%, M.P. 133-136^0C, IR 3447 (OH stretching), 3058 (C-H stretching), 1776 (C=O cyclic), 1607 (aromatic hydrocarbon), 1426 (C-N stretching in primary aromatic ring).

Synthesis of 3- phthalimidoethyl substituted benzoic acids:

To the solution of substituted benzoic acid dissolved in ethanol was added with a hot solution of PE_I in ethanol in equimolar quantity slowly with constant stirring followed by concentrated HCl (5 drops) and the mixture was kept under reflux on a waterbath for 4 hours. On cooling, the separated solid was dried and crystallized from acetone. PE-II-1: yield 71.2%, M.P. 210-213^0C, I.R. 2858.60(CH_2-CH_2), 3045.36(CH=CH), 1711.10(acyclic C=O), 1620.56(carbonyl stretching), 3364 (N-H stretching), 1459(CH_2 bending).

Synthesis of 3-phthalimidoethyl substituted benzoyl chlorides:

To the solution of 3-phthalimidoethyl benzoic acid in dry benzene was added equimolar quantity of PCl_5 and heated on waterbath for 2.5 hours. Then the contents were cooled and the solvent was removed under vacuum to obtain the product.

Synthesis of benzilidine 4-amino acetophenone:

To compound 4-Aminoacetopenone (0.1 mol) dissolved in absolute ethanol (50 mL) was added a solution of aryl aldehyde (0.1 mol) in ethanol followed by triethyl amine (2 mL). The reaction mixture was refluxed on a water bath for about 5 hours. Ethanol was distilled off and the crude residue was treated with petroleum ether (60-80^0c). The product was crystallized from acetone. Yield 74.2%, M.P. 231-233^0C, IR 1720(C=O stretching), 3023 (CH=CH), 3380(N-H stretching).

Synthesis of 3-(phthalimidoethyl)-4-substituted cinnamoyl substituted benzanilides:

To the 3-Phthalimidoethyl-4-acetyl substituted benzanilides, dissolved in absolute ethanol, was added an equimolar quantity of benzilidine 4 amino acetophenone in ethanol and refluxed for about 5 hours. The solvent was removed and the compound was recrystallized with ethanol.

PE-IV-1: Yield 68.5%, M.P. 210-213^0C, I.R. 2858.60(CH_2-CH_2), 3045.36(CH=CH), 1711.10(acyclic C=O), 1620.56(carbonyl stretching), 3364 (N-H stretching), 1459(CH_2 bending). Chemical shift 1.98(CH_2) 2.58 (CH_2), 9.10 (NH), 7.33-8.10 (aryl H). M/Z – 499. 18 (100%), 500.18 (37.8%), 501.19 (6.3%).

PE-IV-2: Yield 65.3%, M.P. 233-238^0C, I.R. 2868.24(CH_2-CH_2), 3020.63(CH=CH), 1710(acyclic C=O), 1604.83(carbonyl

Table 2: Acute toxicity studies of compound PE-IV-2

Group	Dose (mg/kg)	Log Dose (X)	No of animals used (n)	No of animals dead (n)	No. of animals survived (n)	Dead (%)	Corrected dead* (%)	Probit (y)
1	100	2.00	5	0	5	0	5	
2	200	2.30	5	2	3	40	40	4.75
3	250	2.39	5	2	3	40	40	4.75
4	500	2.69	5	3	2	60	60	5.25
5	1000	3.00	5	4	1	80	80	5.84
6	1500	3.17	5	5	0	100	100	

Table 3: Acute toxicity studies of compound PE-V-4

Group	Dose (mg/kg)	Log Dose (X)	No of animals used (n)	No of animals dead (n)	No. of animals survived (n)	Dead (%)	Corrected dead* (%)	Probit (y)
1	100	2.00	5	0	5	0	5	
2	200	2.30	5	1	4	20	20	4.16
3	250	2.39	5	2	3	40	40	4.75
4	500	2.69	5	3	2	60	60	5.25
5	1000	3.00	5	4	1	80	80	5.84
6	1500	3.17	5	5	0	100	100	

Table 4: Acute toxicity studies of compound PE-V-9

Group	Dose (mg/kg)	Log Dose (X)	No of animals used (n)	No of animals dead (n)	No. of animals survived (n)	Dead (%)	Corrected dead* (%)	Probit (y)
1	100	2.00	5	0	5	0	5	
2	200	2.30	5	1	4	20	20	4.16
3	250	2.39	5	1	4	20	20	4.16
4	500	2.69	5	3	2	60	60	5.25
5	1000	3.00	5	4	1	80	80	5.84
6	1500	3.17	5	5	0	100	100	

stretching), 3350(N-H stretching), 1448(CH_2 bending). Chemical shift 3.14 (CH_2), 3.9 (CH_2), 5.45 (OH), 7.1-8 (Ar-H). M/Z - 515.17 (100%), 516.18 (36.2%), 517.18(7.4%).

PE-IV-4: Yield 71.2%, M.P. 180-183^{0}C, I.R. 2889.24(CH_2-CH_2), 3050.31(CH=CH), 1728.26(acyclic C=O), 1640.25(carbonyl stretching), 3339.12(N-H stretching), 1448(CH_2 bending). Chemical shift 1.98(CH_2) 2.58 (CH_2), 9.10 (NH), 7.33-8.10

Table 5: Median lethal dose of the synthesized compounds

SL. NO.	TEST COMPOUNDS	LD_{50}
01	PE-IV-2	500
02	PE-V-4	500
03	PE-V-9	500

(aryl H). M/Z - 533.14(100%), 534.14 (36.2%), 535.14 (32.9%).

PE-IV-6: Yield 57.5%, M.P. 164-166^{0}C, I.R. 2865.65(CH_2-CH_2), 3031.38(CH=CH), 1730.56(acyclic C=O), 1609.39(carbonyl stretching), 3340(N-H stretching), 1458(CH_2 bending). Chemical shift 1.92 (CH_2), 2.65 (CH_2), 9.14 (NH), 7.33-8.05 (aryl H)
M/Z – 531.17 (100%), 532.17 (36.6), 533.17 (7.5).

PE-IV-9: Yield 67.1%, M.P. 190-192^{0}C, I.R. 2918.40(CH_2-CH_2), 1707.06(acyclic C=O), 1645(carbonyl stretching), 3362.04(N-H stretching), 1597.11(NO_2), 1491.02(CH_2 bending). Chemical shift 1.96(CH_2), 2.70 (CH_2), 4.49 (CH), 7.34-8.00 (aryl H) 9.2 (NH)
M/Z – 544.16 (100%), 545.17 (36.2%), 546.17 (7.6%).

PE-V-2: Yield 63.2%, M.P. 255-258^{0}C, I.R. 2844.34(CH_2-CH_2), 3026.36(CH=CH), 1707(acyclic C=O), 1600.89(carbonyl stretching), 3375(N-H stretching), 1470(CH_2 bending). Chemical shift 3.14 (CH_2), 3.9 (CH_2), 5.45 (OH), 7.1-8 (Ar-H). 558.22 (100%), 559.21 (38.4), 560.21 (8.2%).

PE-V-4: Yield 72.0%, M.P. 280-283^{0}C, I.R. 2960.14(CH_2-CH_2), 3059.11(CH=CH), 1714.26(acyclic C=O), 1650.98(carbonyl stretching), 3346.12(N-H stretching), 1456.63(CH_2 bending). Chemical shift 1.97 (CH_2), 2.64 (CH_2), 7.33-8.05 (aryl H), 9.15 (NH). M/Z – 576.18 (100%), 577.18 (38.6%), 578.18 (32.2%).

PE-V-9: Yield **65.0**%, M.P. **175-178**^{0}C, I.R. 2960.14(CH_2-CH_2), 3059.11(CH=CH), 1714.26(acyclic C=O), 1650.98(carbonyl stretching), 3346.12(N-H stretching), 1456.63(CH_2 bending). Chemical shift 1.91 (CH_2), 2.61 (CH_2), 7.30-8.10 (aryl H), 9.17 (NH). M/Z – 587.21 (100%), 588.21 (38.4%), 589.21 (8.5%).

Biological activity:

(i)In vitro studies:

The enzyme used for the in vitro studies was DPP-IV (Sigma) (3.4.14.5) as the target protein. Gly-Pro-p-nitroanilde was used as substrate for the enzyme. All the synthesized compounds were 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 0.1 M Tris, pH 8.0 at 37 °C. Then, the enzymatic reaction mixture composed of 100 µl DPP-IV (0.1 U/ml), 99 µl of 5 mM 0.5 mM Gly-Pro-pNA, and 1 µl (10 mM/ml DMSO) of test compound was incubated at 37 ^{0}C for 15 min. The inhibitory activity of each test compound was determined by measuring the remaining activity of DPP-IV. The enzymatic activity was measured by the amount of the released product, p-nitroaniline that was detected by spectrophotometer at the wavelength of 405 nm.

(ii) In vivo study:

Acute toxicity studies:

Acute Toxicity is the adverse effects occurring within a short time of administration of a single dose of substance or multiple doses given within 24 hours.

In the Assessment and evaluation of toxicological characteristics of a substance, acute toxicity determination by oral, intra-peritoneal or any other route of administration is usually an initial step. It provides information on health hazards likely to arise when a short – term exposure by the dose given. Acute toxicity experiment is more informative and useful than an experiment designed solely to determine the LD_{50} Value. LD_{50} (Median Lethal dose).

Table 6: Food and liquid intake by the rats over the period of experimentation

Group	Dose (Mg/kg)	Body Weight (g)		Food intake (g/rat/Day)	Liquid intake (ml/rat/ day)
		Initial	Final		
I (Normal (0.9 % NaCl w/v))	-	163.4±1.1**	188.32±2.4**	15.14±2.5**	19.40±0.98**
II Diabetic control (STZ)	40	180.2±3.5	160.12±2.2	30.20±2.1	30.14±1.2
III Standard control (Sitagliptin)	25	173.1±3.4*	181.13±2.4**	20.42±1.4*	29.07±1.2*
IV Test control (PE-IV-2)	250	164.3±2.9*	163.56±3.2**	18.41±1.9*	21.08±1.2**
V Test control (PE-V-4)	250	169.9±2.8**	171.1.3.2**	19.23±3.5**	22.01±3.2**
IV Test control (PE-V-9)	250	167.±1.8*	173.±3.2**	20.81±1.4*	24.34±1.2*

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 (STZ).

Dose is expressed as weight (g, mg) of the test substance per unit weight of test animal i.e. mg/kg body weight. So, LD_{50}- Administration is a statistically derived single dose of a substance that can be expected to cause death in 50% of animals when administered by the oral or in the peritoneal dose.

LD_{50} Concept was introduced by Trevan (1927) for standardizing insulin, diphtheria toxin and extract of Digitals. Therapeutic Index - LD_{50} standing alone convey less information than dose in the ration of lethal to the effective dosage (LD_{50}: ED_{50}) , a quantity that is known as the therapeutic index. Dosage is a general term comprising the dose, this frequency and the duration of dosing. Dose-response is the relationship between the dose and the proportion of a population sample showing a defined effect. Dose – effect is the relationship between the dose and the magnitude of a defined biological effect either in an individual or in a population sample. On the basis of essential important of toxicological testing, the present study was carried out to evaluate the median Lethal dose (LD_{50}) of the various synthesized compounds.

Test Substances:-

The pure synthesized compound of phthalimide derivatives were dissolved in minimum volume of CMC suspension of DMSO (10%) and diluted with distilled

Table 7: Effect of the synthesized compounds on glucose level in STZ induced rats

Group	Dose mg/kg	Blood glucose level 0 day	Blood glucose level 5 day	Blood glucose level 10 day	Blood glucose level 15 day
I (Normal (0.9 % NaCl w/v))	-------	84.1±1.2 **	85.0±1.4**	85.3±2.0**	85.2±2.1**
II Diabetic control (STZ)	40	312.18±2.0	312.32±19	312.31±22.1	312.20±21.8
III Standard control (Sitagliptin)	250	312.6±4.1*	242.6±3.5**	146.23±2.7**	106.14±6.4**
IV Test control (PE-IV-2)	250	327.41±3.5*	271.21±5.9**	167.15±5.8**	114.24±8.6**
V Test control (PE-V-4)	250	320.35±3.2*	282.42±5.6**	184.46±6.3**	123.86±14.6**
IV Test control (PE-V-9)	250	318.27±2.8*	256.71±4.2**	159.35±3.3**	112.43±12.3**

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 (STZ).

water and administered as per the requirement of oral dose of administration.

Experimental procedure:-

Swiss albino mice of either sex weighing between 20-22g were used for the acute toxicity study. They were maintained under standard environmental conditions and were fed with standard pellet diet with water *ad-libitum*. The animals were divided into different groups. They were kept on fasting for 18 hr before the commencement of experiment. The solution of synthesized compounds administered orally at various dose levels (50-3500 mg/kg, body weight) in different groups of 10 mice in each group. LD_{50} value was determined by the method of Litchfiled and Wilcoxon and the observed percentage (%) mortality was converted to probit by referring to the approximate table. The values thus obtained were plotted against the corresponding log dose. Before plotting the 0% death and 100% death were corrected. (Results were fitted with straight line after regression analysis of the probit dose corresponding to probit 50% value was found to be the LD_{50}). The values of log dose of synthesized compounds were plotted against the probit values giving the exponential curve after regression analysis of the probit dose corresponding to probit 50% value was found as LD_{50}.

Evaluation of Hypoglycemic Potential:

Male Wistar albino rats (150–180 g) of were used for the study. They were obtained from the animal house, college of pharmacy IPS Academy, Indore, India. The animals were grouped and housed in polyacrylic cages (38 × 23 × 10 cm) with not more than six

Table 8: Total cholesterol and triglyceride concentrations in the synthesized compounds

Group	Dose (mg/kg)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
I Normal (0.9 % NaCl (w/v)	-	100.0±5.7**	97.55±2.33**
II Diabetic control (STZ)	40	148.12±12.7	185.67±12.45
III Standard control (Sitagliptin)	25	115.0±6.1**	120.12±8.46**
IV Test control PE-IV-2	250	125.32±3.4*	145.74±6.48*
V Test control PE-V-4	250	124.19±6.1*	150.79±7.6*
VI Test control PE-V-9	250	128.0±4.5**	147.32±11.53**

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 (STZ).

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 one week of acclimatization, the rats were subjected to a 16 h fasting. Diabetes was induced with a single intraparitonial injection of Streptozotocin (STZ) at a dose of 40-mg/kg body weight. The STZ was freshly dissolved in citrate buffer (0.01 M, pH 4.5). 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.

Experimental Design:

In the experiment, a total of 36 rats (6 normal; 30 STZ 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 STZ (40mg/kg body wt), once before the treatment.

Group III: (Standard control) received STZ with sitagliptin as reference standard (25 mg/kg).

Table 9: TBARS levels in synthesized compounds

S.No.	Treatment	Absorbance 532 nm	Concentration Mmol/100gm of tissue
1	I Normal (0.9 % NaCl w/v)	0.118	0.92
2	II Diabetic control (STZ)-	0.495	3.98
3	III Standard control (Sitagliptin)	0.135	1.06.
4	IV Test control (PE-IV-2)	0.186	1.47
5	V Test control (PE-V-4)	0.161	1.27
6	VI Test control (PE-V-9)	0.148	1..16

Group IV: (Test control 1) STZ treated hyperglycemic rats were administered with 250 mg / kg body wt of test sample PE-IV-2 for 14 days.

Group V: (Test control 2) STZ treated hyperglycemic rats were administered with 250 mg / kg body wt of test sample. PE-IV-4 for 14 days.

Group VI :(Test control-3) STZ treated hyperglycemic rats were administered with 250 mg / kg body wt of test sample. PE-IV-9 for 14 days.

The effect of synthetic samples of Phthalimide derivatives on STZ induced diabetic rats were determined by measuring blood glucose levels, food and fluid intake amount and changes in body weights. After 14 days of treatment, all the rats were decapitated after fasting for 16 h. and the blood from the heart was used for the estimation of Blood glucose levels and Total Cholesterol and triglycerides (TC & TG).

Tissues (liver and kidney) were removed and cleared off blood. They were immediately transferred to ice-cold containers containing 0.9% NaCl and homogenized in 0.1N Tris-HCl buffer (pH 7.4), and used for the estimation of thiobarbituric acid reactive substances (TBARS).

Measurement of blood glucose levels:

*Procedure**:***

Groups of 6 diabetic or nondiabetic rats were used for experiment. Following a 16-hr fast, the rats (diabetic and nondiabetic) were given orally, via a stomach tube, corn starch (0.5 g/100 g body wt) as a suspension with or without drug sitagliptin (25 mg/ kg body wt). Drug was supplied by Merck pharmaceuticals Ltd. Goa. Group I and Group II were not administered with any drug. Group-III standard control with drug

sitagliptin and remaining five groups were given phthalimide derivatives 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.

At the beginning of the experiment and at 5-day intervals, body weight and blood glucose levels with food and liquid intake were measured. Blood samples were obtained by tail-vein puncture of the normal and STZ-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).

Estimation of total cholesterol and triglycerides:

Hypercholesterolemia and hypertriglyceridemia occur in streptozotocin diabetic rats and significant increase observed in our experiment was in accordance to these studies. Under normal circumstances, insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides (Taskimen et al., 1987). However, in insulin deficient subject; it fails to activate the enzyme and causes hypertriglyceridemia. MEBT and tolbutamide showed in a similar way by increased insulin production in STZ induced hyperglycemic animals and lowered the triglyceride levels by activation of enzyme lipoprotein lipase.

Determination of in vivo antioxidants

Thio barbituric acid reactive substance assay (TBARS):

The concentration of lipid peroxidation increases in the kidney of diabetic rats and an increased level of TBARS is an index of lipid peroxidation. In the present work, the TBARS levels in diabetic control animals were high in plasma, due to increased lipid peroxidation. In diabetic rats treated with the synthesized compounds and sitagliptin, the TBARS levels were low which may be due to the free radical scavenging action of the compounds.

Results and Discussion:

In the present work, the 3-phthalimidoethyl 4-substituted cinnamoyl substituted benzanilides were synthesized and characterized by spectral data. The synthesized compounds were then evaluated for their *in-vitro* DPP-IV inhibitory activity. Most of the compounds exhibited significant DPP-IV inhibitory activity. The compounds PE-IV-2, PE-V-4 and PE-V-9 exhibited the best DPP-IV inhibitory activity. As the compounds PE-IV-2, PE-V-4 and PE-V-9 exerted good DPP-IV inhibitory activity, they were further subjected to the *in-vivo* hypoglycemic activity using the streptozocin induced hyperglycemic rats. The compounds were further evaluated for their antihyperlipidemic properties using total cholesterol and triglyceride lowering potential. The level of TC and TG was found to be lower in the rats treated with PE-IV-2, PE-V-4, PE-V-9 and sitagliptin than those without any treatment. In PE-IV-2, PE-V-4, PE-V-9 and sitagliptin treated diabetic rats; the TBARS levels were low which may be due to the free radical scavenging action of the compounds. Thus, in the present study the phthalimide derivatives have shown good *in-vitro* DPP-IV inhibitory activity and *in-vivo* hypoglycemic activity. The structure activity relationships reveal that the activating group in the R_2 position and a deactivating group in R_1 resulted in compounds with better hypoglycemic activity. The compounds devoid of activating group at R_2 exert moderate activity when the strongly activating group is present at R_1.

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