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Research Article

SYNTHESIS AND EVALUATION OF FIBRATES FOR ANTI-DIABETIC AND LIPID LOWERING ACTIVITY

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ABSTRACT

Low circulating levels HDLC and high circulating levels of TG levels constitutes a major risk factor for cardiovascular disease: These are also frequently observed among patients with type II Diabetes mellitus (DM). The principal hypolipidimic agents currently in use majorly are fibrates, a PPAR α agonist. Among oral hypoglycemic agents thiazolidinedione, PPAR γ agonist, effective in reducing elevated blood sugar levels. In the present study we are reporting a systematic synthesis of fibrate analogues PPAR α agonists and a PPAR α-γ dual agonist. Synthesis of PPAR α-γ dual agonist is done by hybridisation approach by combining of lead molecules of both class of drugs namely, fibrate and Thiazolidinedione for dual agonist activity (PPAR α-γ). A series of fibrate analogues is synthesized by forming a scaffold(26) in scheme6. A series of ten derivatives (28a to 28j) is synthesized by reacting scaffold (26) and different amines. PPAR α-γ dual agonist is synthesized by merging pharmacophores of fibrate and thiazolidinedione. In scheme-7; 2, 4 thiazolidinedione is synthesized and scaffold (32) is synthesized from 2, 4 thiazolidinedione. Further in scheme-7 compound (33) is synthesized form scaffold 32. Compounds 28e and 33 are undertaken for blood TG lowering effect and blood glucose lowering effect by using respective Preicugent commercial assay kits. Screening was undertaken for blood triglyceride lowering effect and blood glucose lowering effect for 28e and 33.

INTRODUCTION

Elevated circulating levels of low density lipoprotein cholesterol (LDLc) levels indicates a major risk factor for coronary artery disease and there exists a wealth of clinical data supporting the use of the low density lipoprotein cholesterol (LDLc) lowering statins in an increasingly wide range of patients.

More recently, the roles of both low circulating levels of high density lipoprotein cholesterol (HDLc) and high circulating levels of triglycerides (TG) as cardiovascular disease risk factors have come in to focus. There is a growing level of confidence in the potential of drugs that lower triglycerides (TG) or raise high density lipoprotein cholesterol (HDLc) and can play an important part in future Cardiovascular Drug Therapy.

The principal agent in current use for raising high density lipoprotein cholesterol (HDLc) and lowering triglyceride are “Fibrates” and “Nicotinic Acid”. The fibrates are known to increase up to 20% in high density lipoprotein cholesterol (HDLc) and decrease triglycerides TG ends by 40%.[1]

Epidemiological studies have shown that type II diabetes is associated with 2 to 4 fold increased risk of Coronary Heart Disease (CHD) and a 2-3 fold increased risk of Ischemic Stroke.[2] Type II diabetes mellitus is chronic and complex metabolic disorder that affects a big part of population. Approximately 12 million people diagnosed with diabetes and over 90% people were diagnosed with type II among them.

This metabolic disease, which is characterized by progressiv e insulin secretory dysfunction and insulin resistance at major target tissues such as skeletal muscle, liver and adipose tissue.[3] Pathophysiology of disease includes metabolic defects in liver, pancreatic β cells, adipose tissue, and skeletal muscle. The wide range of pathological conditions are also associated with type-II diabetes mellitus such as dyslipidemia (hypertriglyceridemia, decreased serum HDLc, increased plasminogen activator inhibitor-1 {PAI-}, abnormal fibrinolytic system and obesity).

Therapy for type II diabetes mellitus has been targeted for improving hyperglycemic control through combination of oral agents such as sulfonylureas, biaguanides, thiazolidinediones along with diet and exercise etc.

Among oral hypoglycemic agents thiazolidinediones are high affinity ligands for Peroxisome Prolifrator Activated Receptors (PPAR) these receptors are highly conserved set of ligand activated transcription factors in the nuclear hormone receptor superfamily.[4]

These PPARs are group of nuclear receptor protein that functions as transcription factors regulating the expression of genes[5]. PPAR play essential roles in the regulation of cellular differentiation, development and metabolism (Carbohydrates, Lipids, Protein) and tumorigenesis of higher organisms.[6, 7]

PPARs are ligand activated transcription factors and control the gene expression by binding to specific response element (PPREs) within the promoter regions of several target genes. [8] More potent synthetic ligands, including fibrates and thiazolidinediones have been proven to be effective in the treatment of dyslipidemia and type 2 diabetes through activation of α and γ PPAR isoform, respectively.[10]

In many intervention studies, fibrates showed beneficial effects in preventing the progression of atherosclerotic lesions and cardiovascular events in both non-diabetic and diabetic patients. These effects are attributed to PPAR α activation, which enhance lipid catabolism, decreases triglyceride (TG) by induction of apo A-I, A-II. In more recent studies, PPAR α activation was found to exert anti-inflammatory effects in blood vessels.[11]

Fibrates such as Clofibrate, Benzfibrate, Finofibarte, Ciprofibrate, are generally effective in lowering elevated plasma level of triglycerides. They are well tolerated clinically and enjoy favorable safety profiles. A rare incidence of fibrate associated toxicities has been reported. The most pronounced contraindication is the liver and renal insufficiency. Fibrate toxicities include myalgia, myotonia, sporadiacrhabdomyolysis (may be aggravated by combination with statins), elevated transaminase and gallstone formation.[10]

In case of diabetes mellitus type-II results suggest that PPAR γ is the molecular target responsible for the antidiabetic activity of the thiazolidinedione class of agents.

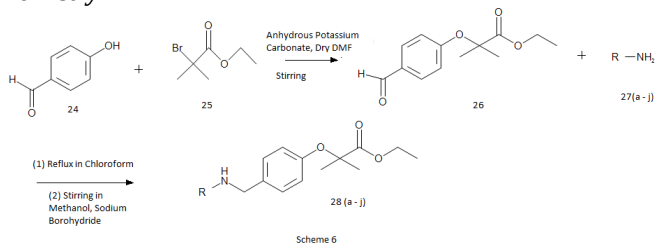
Thiazolidinediones or Glitazones enhance the sensitivity of target tissues to insulin and also reduce lipid and insulin levels in animal models of type II diabetics.

Useful forms of thiazolidinediones have been developed such as Ciglitazone, Pioglitazone, Rosiglitazone, Troglitazone. The TZD class of compounds is not without drawbacks, a number of TZDs have been dropped from development due to their unacceptable adverse effect profile.[12]

In the present study we are reporting a systematic synthesis of fibrate analogues PPAR α agonists and a PPAR α-γ dual agonist. Synthesis of PPAR α-γ dual agonist is done by merging of pharmacophores of both class of drugs namely fibrate and Thiazolidinedione pharmacophores for dual activity.

MATERIALS AND METHODS

Chemistry



Scheme 6

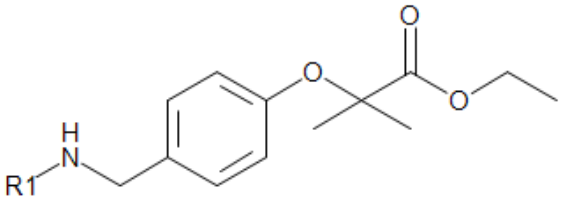
Preparation ofethyl 2-(4-formylphenoxy)-2-methylpropanoate

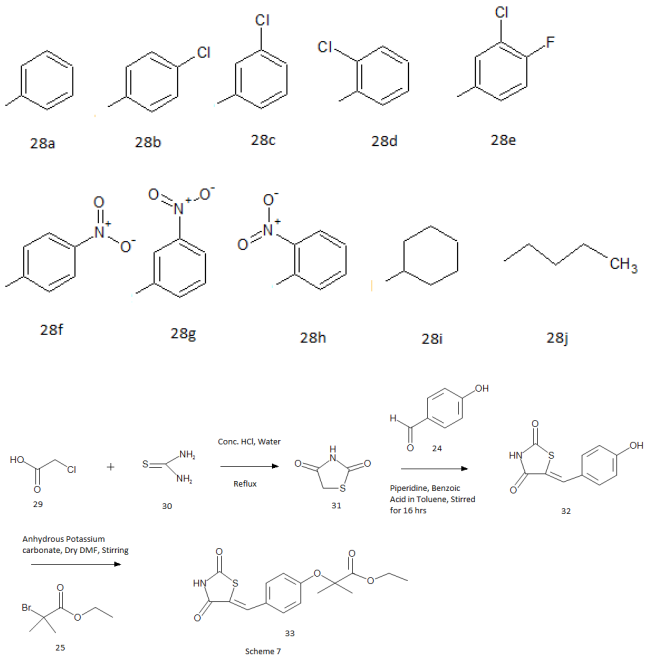
p-Hydroxybenzaldehyde 1.5 g (12.2 mmol) is dissolved in dry DMF (10 ml) and added to 100 ml RBF, in which is placed anhydrous potassium carbonate 10.25 g (73.6 mmol). The flask is fitted with a guard tube and mixture stirred for 1 hour. After stirring, ethyl α-bromoisobutyrate 2.39 g (12.2 mmol) is added and reaction further stirred for 16 hrs at 50°C. The mixture is poured in to 150 ml of water and extracted with 3 portions of 30 ml ethyl acetate. The combined organic layer is washed with 2% NaOH solution to remove traces of unreacted *p*-hydroxybenzaldehyde. The organic layer is dried over anhydrous Na_2SO_4, and solvent removed in vacuum to obtain the product. The reaction is monitored by TLC, using precoated silica gel GF 254 plates. Mobile phase is ethyl acetate: n-hexane (1:5).The b.p. is found to be 140 °C. [11]

Preparation of ethyl 2-(4-(iminomethyl)phenoxy)-2-methylpropanoate Derivatives

Ethyl 2-(4-formylphenoxy)-2-methylpropanoate and different amines is used to prepare derivatives form 28a to 28j.

Ethyl 2-(4-formylphenoxy)-2-methylpropanoate 0.25 g (1.05 mmol) and amine is dissolved in chloroform (10 ml) and the resulting solution is heated under reflux for 5 hours. The solvent is removed under reduced pressure, and the residue is dissolved in methanol (10 ml), then sodium borohydride is added at 0 °C and the solution is stirred for 1 hour and then allowed to attain room temperature. The solvent is removed under reduced pressure, water is added and the solution is extracted with ethyl acetate. The organic layer is washed with saturated sodium bicarbonate solution and brine. The organic layer is dried over anhydrous sodium sulphate and the solvent is removed under reduced pressure. The residue is purified by preparative TLC using silica gel GF 254. (Refer table no. 4, page no. 28 for mobile phase description).





Preparation of2, 4-thiazolidinedione

In a 100 ml of RBF is placed 2 g (26.31mmol) of thiourea, 4.94 g (52.63 mmol) of monocholoroacetic acid, 5ml of water and 4.8 g (131.57 mmol) of hydrochloric acid. The reaction is refluxed for 10 hrs, cooled and poured into beaker. The pH of mixture is adjusted to 7 by adding sodium bicarbonate. The aqueous layer is extracted with 3 portions of 30 ml ethyl acetate. The combined organic layer is dried over anhydrous sodium sulphate and solvent removed in vacuum to get the product.

Preparation of5-(4-hydroxybenzylidene) thiazolidin-2-one

In a two necked 50 ml RBF is placed 1.04 g (8.5 mmol) of 4-hydroxy benzaldehyde, 1 g (8.5 mmol) of 2, 4-thiazolidinedione 0.04 g (0.5 mmol) of piperidine and 0.052 g (0.4 mmol) of benzoic acid, in toluene (5 ml) is stirred at 80 °C for 16 hours. Reaction mixture is cooled to room temperature and the yellow solid obtained is filtered. The solid is washed with methylene chloride and then with methanol/ methylene chloride (30:70) and dried in vacuum at 35 °C.

Preparation of 5-(4-(2-methyl-3-oxohexan-2-yloxy) benzylidene) thiazolidine-2,4-Dione

5-(4-hydroxybenzylidene) thiazolidin-2-one 0.25 g (1.1 mmol) is dissolved in dry dimethyl formamide (5 ml) and is placed in a 25 ml. RBF along with anhydrous potassium carbonate 1.25 g (9 mmol). The flask is fitted with a guard tube and mixture stirred for 1 hour. Ethyl-α-bromoisobutyrate 1.04 g (1.1 mmol) is added and reaction further stirred for 16 hrs at 50°C. The reaction mixture is poured in to 100 ml water and extracted with 3 portion of 20 ml ethyl acetate. The combined organic layer is dried over anhydrous Na_2SO_4. Ethyl acetate is removed under reduced pressure to get the product.

Biological Evaluation

Lipid Lowering and Anti Diabetic Activity

Female wistar rats (150-220 grams) are purchased from Biovivo services (Kachohalli, Banglore, India). Institution Animals Ethics Committee has approved the experimental protocol (IAEC/NCP/21/09). Animals are housed in polypropylene cages. Paddy husk is provided as bedding material. Food and water is provided *ad libitum.* Rats are maintained on standard, pelleted rodent diet.

Eighteen female wistar rats are selected randomly and divided into 3 groups. Each group consists of 6 animals.

Fenofibrate18 mg/kg body weight of the rats. [20-22]

Two compounds **28e** and **33** are tested for lipid lowering and blood glucose lowering activity. Dose of test compounds is with respect to fenofibrate. Dose of fenofibrate in humans is 200 mg/day. This is extrapolated to ratsand a dose of 18 mg/kg is selected in rats.

Procedure

Animals are selected randomly and divided into 3 groups. Each group consists of 6 animals.

Group 1: Received fenofibrate 18 mg/kg body weight of rat in 0.4% starch suspension.

Group 2 to 3: Received the synthesized derivates 28e, 33 respectively at a dose of 18 mg/kg body weight of rat in 0.4% starch suspension.

Blood samples are collected before and after 4 hours of drug treatment by puncturing the retro orbital plexus under mild ether anesthesia. Blood triglycerides and blood glucose levels are estimated using respective commercial assay kit (Preicugent, Thane, India.). Blood triglyceride levels are determined in 10 µl serum samples with the glycerol phosphate oxidase, peroxidase GPO/POD enzyme method.

Subsequently blood glucose levels are determined in 10 µl serum samples with the glucose oxidase, peroxidase GOD/POD enzyme method.[20]

Blood samples are collected in centrifuge tubes and kept aside for clotting. After clotting the sample are centrifuged at 5000 rpm for 10 minutes and serum is separated which is used for biochemical estimations. Both the biochemical parameters: blood triglyceride levels and blood sugar levels is estimated separately by semi autoanalyser using respective commercial assay kits. (Preicugent, Thane, India.)

RESULT AND DISCUSSION

During the period of this project work, it was possible to successfully synthesize 11 derivatives, (**28a** to **28j** and **33**) which were characterized by melting point, TLC elemental analysis, IR and ^{1}H NMR.

*ethyl 2-[4-(anilinomethyl)phenoxy]-2-methylpropanoate**(28a):***oil , 1.731g, 5.55 mmol, 57.7% yield, ^{1}H NMR ($CDCl_3$): δ 1.22-1.29 (3H, t CH_3), 1.49 (6H, S, 2 CH_3), 4.18 (2H, q, CH_2), 4.29(2 H, S), 6.610 (2 H dtd, Ar), 7.25 (ddd, Ar). IR (neat) 2926.11 (C-H str), 1730.21 (ester C=O Str), 3421.83 (N-H Str).

*2.ethyl 2-{4-[(4-chloroanilino)methyl]phenoxy}-2-methylpropanoate**(28b):*** oil, 1.638 g, 4.73 mmol, 54.6%, yield, IR (neat) 2987.84, 2926.11, 2852.81 (C-H str.), 1734.06 (ester C=O str), 1600.97, 1506.46, 1467.88 (C=C Ar str.), 3406.40 (N-H str.), 1283.341016 (aralkyl C-O-C str.), 1141.90 (ester C-O str.), 756.12 (C-Cl str.).

*ethyl 2-{4-[(3-chloroanilino)methyl]phenoxy}-2-methylpropanoate**(28c):***oil, 1.971 g, 5.699 mmol, 65.7%, yield, IR (neat) 2922.25 (C-H str.), 1734.04 (ester C=O str), 3406.40 (N-H str.), 1599.04, 1508.38 (C=C Ar str.), 1236.41,

1022.31 (aralkyl C-O-C str.), 1141.90 (ester C-O str.), 763.84 (C-Cl str.).

*ethyl 2-{4-[(2-chloroanilino)methyl]phenoxy}-2-methylpropanoate**(28d)**:* oil, 1.695 g, 4.90 mmol, 56.5 % yield, IR (neat) 2921.66 (C-H str.), 1732.01, (ester C=O str), 34015.06, (N-H str.), 1560.44, 1456.06 (C=C Ar str.), 1292.03, 1020.99 (aralkyl C-O-C str.), 1117 (ester C-O str.).

*ethyl 2-{4-[(3-chloro-4-fluoroanilino)methyl]phenoxy}-2-methylpropanoate**(28e)**:* oil, 1.401 g, 3.850 mmol, 46.7% yield, IR (neat) 2922.25, 2850.88 (C-H str.), 1734.06 (ester C=O str), 3402.54 (N-H str.), 1610.61, 1508.38 (C=C Ar. Str.)1226.77 (aralkyl C-O-C str.), 1139.97 (ester C-O str.)

*ethyl 2-methyl-2-{4-[(4-nitroanilino)methyl]phenoxy}propanoate**(28f)**:* Solid, 1.596 g, 4.481 mmol, 53.2 % yield, IR (neat) 2934.49, 2858.34 (C-H str.), 1743.61 (ester C=O), 3402.12(N-H str.), 1563.44(N=O Str.), 1466.43 (C=C), 1265.47, 1029.87 (aralkyl C-O-C str.), 1113.02 (ester C-O str.).

*ethyl 2-methyl-2-{4-[(3-nitroanilino)methyl]phenoxy}propanoate**(28g)**:* Solid, 1.671 g, 4.69 mmol, 55.7 % yield, IR (neat) 2921.66, 2857.36 (C-H str.), 1735.60 (ester C=O str), 3404.06 (N-H Str.), 1531.93(N=O str.), 1460.04 (C=C Ar str.), 1238.94, 1017.41(aralkyl C-O-C str.), 1142.47 (ester C-O str.).

*ethyl 2-methyl-2-{4-[(2-nitroanilino)methyl]phenoxy}propanoate**(28h)**:* oil, 1.83 g, 5.13 mmol, 61 % yield, IR (neat) 2962.76 (C-H str.), 1735.61 (ester C=O str), 3479.70 (N-H str.), 1431.23 (C=C Ar str.), 1508.38, 1346.36 (NO_2), 1259.56, 1020.38 (aralkyl C-O-C str.), 1101.39 (ester C-O str.).

*ethyl 2-methyl-2-{4-[(cyclohexylimino)methyl]phenoxy}propanoate**(28i)**:* oil, 2.01 g, 6.33 mmol, 67.71% yield, IR (neat) 2962.76, 2928.04, 2852.81 (C-H str.), 1734.06 (ester C=O str), 1653.05 (C=N str.), 1608.69, 1508.38, 1448.59 (C=C Ar str.), 1261.49, 1024.24 (aralkyl C-O-C str.), 1139.97(ester C-O str.).

*ethyl 2-methyl-2-{4-[(butylimino)methyl]phenoxy}propanoate**(28j)**:* oil, 1.653g, 5.672 mmol, 55.1% yield, IR (neat) 2962.76, 2928.04, 2852.81 (C-H str.), 1734.06 (ester C=O str), 1653.05 (C=N str.), 1608.69, 1508.38, 1448.59 (C=C Ar str.), 1261.49, 1024.24 (aralkyl C-O-C str.), 1139.97(ester C-O str.).

*ethyl 2-{4-[(Z)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}-2-methylpropanoate**(33)**:*oil, 1.992 g,5.974 mmol, 66.4 % yield, ^{1}H NMR ($CDCl_3$): δ 1.20-1.25 (3H, t CH_3), 1.65(6H, S, 2 CH_3), 4.19 (2H, q, CH_2), 6.89 – 6.91 (2 H dtd, Ar), 7.38 (ddd, Ar). IR (neat) 3421.83 (NH str.), 2924.18 (C-H), 1737.9 (ester C=O str), 1599.04, 1465.95 (C=C Ar str.), 1251.84, 1022.34 (aralkyl C-O-C str.) 1180.47, 1141.90 (ester C-O-C str).

Biological Activity

Lipid Lowering Activity

Animal : Albino wistar rats
Weight :150 – 220 mg
Vehicle :0.4 % Starch solution
Strength :25 mg/ ml

Table 8 Effect of fibrates analogues on blood triglyceride levels in albino wistar rats.

Animal group	Compoud	Dose (mg/kg)	Blood Triglyceride Level (mg/dl) ± SEM.		% Triglyceride Level changed
			Before drug treatment	4 hrs after drug treatment	
1	Finofibrate	18	133.00 ± 5.43	121 ± 3.59*	-9.02
2	28e	18	119.8 ± 3.85	149.6 ± 6.76**	+24.87
3	33	18	123.2 ± 6.82	125.6 ± 6.23ns	+1.94

* P < 0.05, ns = non-significant
+ indicates increase in T.G. Levels
- indicates decrease in T.G. Levels

Antidiabetic Activity

Blood Glucose Lowering Activity in Rats

Animal : Albino wistar rats
Weight :150 – 220 mg
Vehicle :0.4 % Starch solution
Strength :25 mg/ ml

Table 9 Effect of fibrates analogues on blood glucose level in albino wistar rats

Animal group	Compoud	Dose (mg/kg)	Blood Glucose Level (mg/dl) ± SEM.		% Glucose Level changed
			Before drug treatment	4 hrs after drug treatment	
1	Finofibrate	18	128.7 ± 8.48	133.2 ± 9.95ns	+3.49
2	28e	18	133.4 ± 6.85	110.5 ± 6.11**	-17.16
3	33	18	129.6 ± 8.08	110.9 ± 4.60ns	-14.42

** P < 0.01, ns = non significant
+ indicates increase in glucose Levels
- indicates decrease in glucose Levels

The compounds **28e** and **33** were tested for lipid lowering and blood glucose lowering activity. No reduction was observed in blood triglyceride levels. However, **28e** and **33** were found to reduce blood glucose level by 17.15% and 14.42% but the value only for **28e** was found to be significant.

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