

Original Article

Synthesis and biological evaluation of some 5-substituted phenothiazine based thiazolidine-2,4-dione derivatives

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ABSTRACT

A new series of 1-(3-((phenylimino)methyl)-10H-phenothiazin-10-yl)ethanone (5a-5f) and 4-(10-acetyl-10H-phenothiazin-3-yl)-3-phenylthiazolidine-2,5-dione (6a-6f) have been prepared by the 5 step process. Structures are confirmed by spectral analysis. The biological evaluation for the anti diabetic activity has been performed. From the biological investigation, it is found that out of all the synthesized compounds two showed potent anti diabetic activity.

1. INTRODUCTION

Phenothiazine (Fig. 1) abbreviated PTZ is an organic compound that has the formula $S(C_6H_4)_2NH$ and is related to the thiazine-class of heterocyclic compounds. It contains two benzenes rings linked in a tricyclic system through nitrogen and sulfur atoms. Phenothiazine derivatives have amino alkyl side chain connected to the nitrogen atom of heterocyclic unit [1]. The compound known as phenothiazine was originally called “thiodiphenylamine” by Bernthsel the father of phenothiazine chemistry. Two other names are phenthiazine and 6-dibenzo-1, 4-thiazine. The chemical structure of phenothiazines provides an important molecular template for the development of the agent able to interact with variety of biological process and effective in the treatment as tranquilizer, anti-inflammatory, anti-psychotic, antimalarial, anti-tumour, anti-histaminic and analgesic activity.

Research into phenothiazine and its derivatives has remained unabated due to the wide range of application of this class of compounds as drugs, pesticides, dyes, industrial antioxidants, thermal stabilizers etc [2]. Phenothiazine the parent compound of the large number of medicinal compounds and thiazine dyes has been the subject of intensive study in industries and universities.

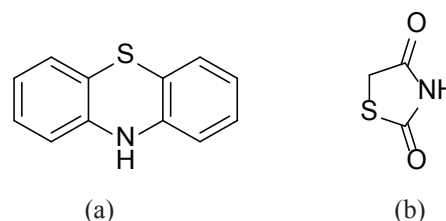


Fig. 1. Chemical structure of Phenothiazine (a) and Thiazolidine-2, 4-dione (b)

Thiazolidine-2, 4-dione (Fig 1) are derivatives of thiazolidine with two carbonyl groups at the 2 & 4-position. The first representative of group was ciglitazone followed by the synthesis of the other derivatives like Englitazone, Pioglitazone and Troglitazone. All share a common thiazolidine-2, 4-dione structure which is responsible for the majority of the pharmacological actions. Thiazolidine 2,4-dione also known as glitazones, are the class of compounds used in treatment of diabetes mellitus [3]. Thiazolidine-4-ones are usually solids, often melting with decomposition but the attachment of an alkyl group to the nitrogen lowers the melting point. Thiazolidine-4-ones are derivatives of thiazolidine with carbonyl group at the fourth position. The carbonyl group of thiazolidine-4-ones is highly un-reactive. Thiazolidine-4-ones are the derivatives,

which belongs to important groups of heterocyclic compounds containing sulfur and nitrogen in a five member ring.

The nucleus is also known as a wonder nucleus, because it shows different types of biological activities. Thiazolidine-4-one substituted moieties have received considerable attention during last two decades as they are gifted with variety of activities and have wide range of therapeutics properties. Thiazolidine-4-ones and its derivatives offer enormous scope in the field of medicinal chemistry [4]. Thiazolidine-4-ones are important compounds due to their broad range of biological activities and pharmacological properties i.e. antifungal [5], antioxidant [6], cytotoxic [7], anti-inflammatory, analgesic, anti YFV (yellow fever virus) activity [8], antitubercular, antimicrobial & antibacterial. Thiazolidine-4-one derivatives possess different pharmacological and biological activities. Antimicrobial activity is the most potent activity of thiazolidine-4-one. Antibacterial activity is strongly dependent on the nature of substituent at C-2 & N-3 position [9].

2. EXPERIMENTAL

All the chemicals and solvents used were procured from Merck (India), S.D. Fine Chemicals (India) & Rankem (India). The present work reports the synthesis and anti-diabetic activity of a series of 4-(10-acetyl-10H-phenothiazin-3-yl)-3-phenylthiazolidine-2,5-dione derivatives (6a-6f) according to the synthetic schemes as shown in Figure 1. The homogeneity of the compounds was monitored by thin layer chromatography (TLC) using silica gel G as stationary phase and visualized by iodine vapors. The chemical structures of the synthesised compounds were characterized using IR, ¹H NMR and elemental analysis techniques. The physical constants of the synthesised compounds are presented in Table 1.

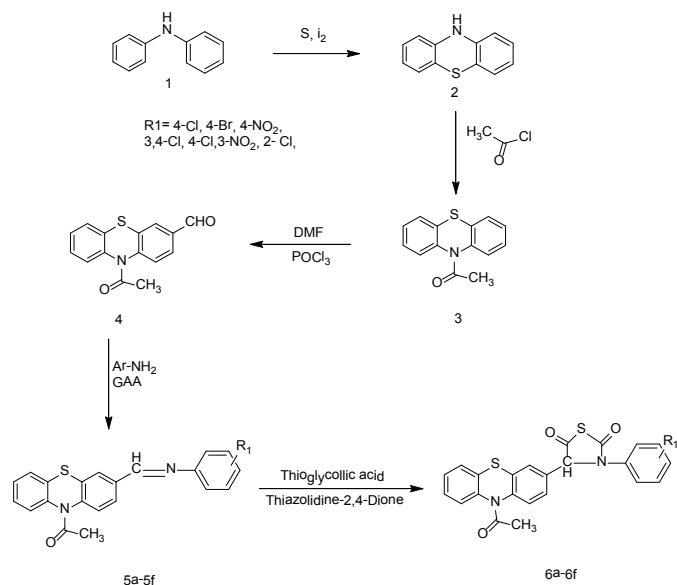


Fig. 2. Synthetic scheme for 4-(10-acetyl-10H-phenothiazin-3-yl)-3-phenylthiazolidine-2,5-dione derivatives 6(a-f)

2.1 Synthetic procedure

2.1.1 Synthesis of 10H-Phenothiazine (2): 1mmole of diphenyl amine, 4gm of sulphur and catalytic amount of iodine was taken in a porcelaine dish and triturated or uniform mixing. The solids were heated at 200°C for 1 hour. A brown color crystalline powder was formed on completion of reaction. Collect the solids and check melting point.

2.1.2 Synthesis of N-Acetyl Phenothiazine (3): 4gm of 10-H phenothiazine (2) was treated with 10ml of acetyl chloride and allowed to stir on magnetic stirrer for half hour. The resulting mixture is then allowed to reflux for 1 hour [10].

2.1.3 Synthesis of N-Acetyl Phenothiazinal (4): 1mmole of N-Acetyl phenothiazine was taken in round bottom flask. To that 1mmole of POCl₃ and 3 mmole of DMF equivalent was added and stirred for 30 mm. After that added POCl₃.

2.1.4 Synthesis of 1-(3-((phenylimino)methyl)-10H-phenothiazin-10-yl)ethanone 5(a-f): The resulting compound was treated with glacial acetic acid and substituted aniline and allowed to stir on magnetic stirrer for 15 minutes and then refluxed for 6 hour. The resulting compound is marked as compound 5(a-f) [11].

2.1.5 Synthesis of 4-(10-acetyl-10H-phenothiazin-3-yl)-3-phenylthiazolidine-2,5-dione 6(a-f): Compound 5(a-f) is then treated with thiazolidine-2,4-dione, thioglycolic acid and allowed to stir for 15 minutes and then refluxed for 6 hour. The resulting compound is marked as compound 6(a-f) [12].

2.2 Spectral data of 4-(10-acetyl-10H-phenothiazin-3-yl)-3-phenylthiazolidine-2,5-dione derivatives (6a-6f)

2.2.1 4-(10-acetyl-10H-phenothiazin-3-yl)-3-(4-chlorophenyl)thiazolidine-2,5-dione (6a): FTIR (KBr, cm⁻¹): 1311.23(aromatic, 3-amine, C-N), 1124.45(aliphatic, C-N), 1702.28(saturated, acyclic, C=O), 1771.24(4-membered, C=O), 707.23(str, equatorial, C-Cl). ¹H-NMR (DMSO-d₆, 500 MHz, δ/ppm) 7.74 (1H, s, Ar-H); 7.51 (1H, d, Ar-H), 7.52 (1H, d, Ar-H), 7.48 (1H, s, Ar-H), 7.44 (1H, s, Ar-H), 7.29 (1H, d, Ar-H), 7.21 (2H, tri, Ar-H), 7.18 (2H, tri, Ar-H), 6.91(1H, s, Ar-H), 6.79(1H, s, Ar-H), 6.74(1H, s, Ar-H), 4.82(1H, s, CH), 4.30(1H s, CH₂), 3.55(1H, s, CH)

2.2.2 (S)-4-(10-acetyl-10H-phenothiazin-3-yl)-3-(4-bromophenyl)thiazolidine-2,5-dione (6b): FTIR (KBr, cm⁻¹): 1311.25(aromatic, 3-amine, C-N), 1138.43(aliphatic, C-N), 1731.29(saturated, acyclic, C=O), 1771.24(4-membered, C=O), 711.23(str, equatorial, C-Br). ¹H-NMR (DMSO-d₆, 500 MHz, δ/ppm) 8.39 (1H, d, CH); 7.90 (1H, d, Ar-H), 7.74 (1H, d, Ar-H), 7.68 (1H, d, Ar-H), 7.60 (1H, d, Ar-H), 7.52 (1H, s, Ar-H), 7.50 (1H, d, Ar-H), 7.42 (1H, d, Ar-H), 7.40 (1H s, Ar-H), 7.18(1H, s, Ar-H), 6.49(1H, s, Ar-H), 4.28(1H, s, CH₂)

2.2.3 4-(10-acetyl-10H-phenothiazin-3-yl)-3-(4-nitrophenyl)thiazolidine-2,5-dione (6c): FTIR (KBr, cm⁻¹): 1313.25(aromatic, 3-amine, C-N), 1124.43(aliphatic, C-N), 1722.29(saturated, acyclic, C=O), 1774.24(4-membered, C=O), 1541.23(str, aromatic, C-NO₂) ¹H-NMR (DMSO-d₆, 500 MHz, δ/ppm) 8.39 (1H, d, CH); 7.90 (1H, d, Ar-H), 7.74 (1H, d, Ar-H), 7.68 (1H, d, Ar-H), 7.60 (1H, d, Ar-H), 7.52 (1H, s, Ar-H), 7.50 (1H, d, Ar-H), 7.42 (1H, d, Ar-H), 7.40 (1H, s, Ar-H), 7.18 (1H, s, Ar-H), 6.49 (1H, s, Ar-H), 4.28 (1H, s, CH₂);

2.2.4 4-(10-acetyl-10H-phenothiazin-3-yl)-3-(3,4-dichlorophenyl)thiazolidine-2,5-dione (6d): FTIR (KBr, cm⁻¹): 1318.25(aromatic, 3-amine, C-N), 1124.43(aliphatic, C-N), 1720.29(saturated, acyclic, C=O), 1773.24(4-membered, C=O), 782.23(str, equatorial, C-Cl). ¹H-NMR (DMSO-d₆, 500 MHz, δ/ppm) 10.601 (s, 1H, -NH-of 2° amide); 7.964 (s, 1H, -SO₂NH); 7.855 (trip, 1H, 2° amine of urea); 7.706 (d, 2H, Ar-H); 7.614 (d, 2H, Ar-H); 7.511 (s, 1H, Ar-H); 7.310 (d, 1H, Ar-H); 7.221 (d, 1H, Ar-H); 4.275 (d, 2H, -CH₂-); 2.238 (s, 3H, Ar-CH₃);

2.2.5 4-(10-acetyl-10H-phenothiazin-3-yl)-3-(4-chloro-3-

nitrophenyl)thiazolidine-2,5-dione (6e): FTIR (KBr, cm⁻¹): 1311.25(aromatic, 3-amine, C-N), 1122.53(aliphatic, C-N), 1728.19(saturated, acyclic, C=O), 1772.54(4-membered, C=O), 751.25(str, equatorial, C-Cl), 1490.23(str, aromatic, C-NO₂) ¹H-NMR (DMSO-d₆, 500 MHz, δ/ppm) 10.54 (s, 1H, -NH-of 2° amide); 7.56 (s, 1H, -SO₂NH); 7.53-7.51 (trip, 1H, 2° amine); 7.49-7.48 (d, 2H, Ar-H); 7.37-7.36 (d, 2H, Ar-H); 7.205 (s, 1H, Ar-H); 7.14-7.13 (d, 1H, Ar-H); 6.92-6.91 (d, 1H, Ar-H); 4.34 (d, 2H, -CH₂-); 2.29 (s, 3H, Ar-CH₃)

2.2.6 4-(10-acetyl-10H-phenothiazin-3-yl)-3-(2-chlorophenyl)thiazolidine-2,5-dione (6f): FTIR (KBr, cm⁻¹): 1311.23(aromatic, 3-amine, C-N), 1124.45(aliphatic, C-N), 1702.28(saturated, acyclic, C=O), 1771.24(4-membered, C=O), 707.23(str, equatorial, C-Cl). ¹H-NMR (DMSO-d₆, 500 MHz, δ/ppm) 7.74 (1H, s, Ar-H); 7.51 (1H, d, Ar-H), 7.52 (1H, d, Ar-H), 7.48 (1H, s, Ar-H), 7.44 (1H, s, Ar-H), 7.29 (1H, d, Ar-H), 7.21 (2H, tri, Ar-H), 7.18 (2H, tri, Ar-H), 6.91 (1H, s, Ar-H), 6.79 (1H, s, Ar-H), 6.74 (1H, s, Ar-H), 4.82 (1H, s, CH), 4.30 (1H, s, CH₂), 3.55 (1H, s, CH)

Table. 1 Physicochemical data of 4-(10-acetyl-10H-phenothiazin-3-yl)-3-phenylthiazolidine-2,5-dione (6a-6f)

S. no.	Molecular formula	Molecular weight	Solubility	Melting point (°C)	Color	Rf value
6a	C ₂₃ H ₁₅ ClN ₂ O ₃ S ₂	466.96	Benzene, DMSO	170	White	0.81
6b	C ₂₃ H ₁₅ BrN ₂ O ₃ S ₂	511.41	Benzene, DMSO	175	Yellowish Grey	0.87
6c	C ₂₃ H ₁₅ N ₃ O ₃ S ₂	477.51	Chloroform, DMSO	168	White	0.84
6d	C ₂₃ H ₁₄ Cl ₂ N ₂ O ₃ S ₂	501.40	Benzene, DMSO	178	Grey	0.87
6e	C ₂₃ H ₁₄ ClN ₃ O ₃ S ₂	511.96	Chloroform, DMSO	180	White	0.89
6f	C ₂₃ H ₁₅ ClN ₂ O ₃ S ₂	466.96	Benzene, DMSO	180°	White	0.82

2.3 Anti-diabetic evaluation

2.3.1 Selection of drugs and chemicals: For the purpose of this work Rosiglitazones (Standard drug), test drugs (self made), thioglycollic acid, dimehthyl formamide, acetyl chloride and glacial acetic acid was selected.

2.3.2 Preparation of drugs and chemical solutions: The solutions prepared for administration were normal saline 10ml/kg by oral route, STZ dissolved in 0.1 M sodium citrate buffer pH 4.5 at a dose of 60 mg/kg body weight, Rosiglitazone 8mg/kg by oral route, test compound 1 (5mg/kg) by oral route; test compound 2 (10mg/kg) by oral route.

2.3.3 Selection of experimental animals: Wister rats weighing between 150-250gm were taken for anti diabetic activity. Animals were acclimatized to laboratory conditions for 7 days prior to

taking them for experimentation. Food was withdrawn 12 hours prior to drug administration till completion of experiment. The animals were weighed and numbered appropriately. The test and standard drugs were given orally. Each animal, at the commencement of its dosing was between 8 and 12 weeks old. Proper housing and feeding conditions were provided to the animals that is the temperature in the experimental animal room should be 22°C (± 3°C), the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. All the animals were obtained from Animal house of the IIMT College of Medical Sciences, Meerut, Uttar Pradesh. All the experiment was done in CPCSEA approved lab and registration no. is 1297/Po/Re/S/2009/CPCSEA.

2.3.4 Evaluation of anti-diabetic activity

Streptozotocin induced diabetes: Streptozotocin (STZ) is synthesized by *Streptomyces achromogenes* and is used to induce insulin-dependent diabetes mellitus in experimental animals. Streptozotocin is a nitrosourea analogue in which the *N*-methyl-*N*-nitrosourea (MNU) moiety is linked to the carbon-2 of a hexose. STZ is taken up by pancreatic β -cells via the GLUT 2 transporter where it causes β -cell death by DNA fragmentation due to the nitrosourea moiety. Three major pathways associated with cell death are: (i) DNA methylation through the formation of carbonium ion (CH_3^+) resulting in the activation of the nuclear enzyme poly ADP-ribose synthetase as part of the cell repair mechanism and consequently, NAD^+ depletion; (ii) Nitric oxide production (iii) Free radical generation as hydrogen peroxide [13].

After overnight fasting (deprived of food for 16 hours, had been allowed free access to water), diabetes was induced in male Wister albino rats by intraperitoneal injection of freshly prepared STZ dissolved in 0.1 M sodium citrate buffer pH 4.5 at a dose of 60 mg/kg body weight. After the injection they had free access to food and water. The animals were allowed to drink 5 % glucose solution overnight to overcome the hypoglycaemic shock. The development of diabetes was confirmed after 48 h of the Streptozotocin injection by collecting blood from tail vein. The animals having fasting blood glucose level in range of 275-300 mg/dL were considered as diabetic rats and used for experimentation [14].

Table 2. Anti-diabetic activity of synthesized compounds by streptozotocin induced method

Group (mg/kg body wt)	OGTT blood glucose level (mg/dl) \pm SEM (Oral Glucose Tolerance Test) (mg/dl)			
	0 min	60 min	120 min	180 min
Control	91.5 \pm 0.13	96.2 \pm 0.02	93.12 \pm 0.17	93.67 \pm 0.01
Roziglitazone	91.5 \pm 0.23	110.0 \pm 0.94	108 \pm 0.89	97.83 \pm 0.47
Test compound 1 (6a)	95 \pm 0.25	125.3 \pm 0.22	110. \pm 0.28	99 \pm 0.96
Test compound 2 (6d)	95 \pm 0.22	128 \pm 0.53	114 \pm 0.60	97.8 \pm 0.27

Table 3. Fasting blood glucose level (mg/dl) by synthesized compounds

Group (mg/kg body wt)	0 days	7 days	14 days	21 days
Control	91 \pm 1.00	115.7 \pm 0.9	114 \pm 0.8	100 \pm 0.47
Diabetic control	244.7 \pm 0.96	281.3 \pm 0.51	311 \pm 0.99	336 \pm 0.12
Roziglitazone	311.3 \pm 0.99	247.8 \pm 0.4	126.7 \pm 0.8	106.5 \pm 0.9

Test compound 1 (6a)	285.3 \pm 0.28	195 \pm 0.58	152 \pm 0.66	102.17 \pm 0.35
Test compound 2 (6d)	311.7 \pm 0.07	120.1 \pm 0.84	116.5 \pm 0.04	97.12 \pm 0.26

3. RESULTS AND DISCUSSION

It is the well established fact that phenothiazine based thiazolidine-2,4dione posses anti diabetic property. They stimulate insulin secretion from pancreatic β -cells by inhibiting of KATP channels that cause depolarization of the β cell membrane; in turn, this triggers the opening of voltage-gated Ca^{2+} channels, eliciting Ca^{2+} influx and a rise in intracellular Ca^{2+} which stimulates the exocytosis of insulin-containing secretory granules. Based on these facts, the present study investigated the antidiabetic activity. The anti diabetic activity was determined by streptozotocin induced method and it was found that compounds **6a** and **6d** were potent.

4. CONCLUSION

The main objective of this research work was to combine two moieties phenothiazine & thiazolidine-2,4-dione to prepare more potent anti-diabetic derivatives. From the above study it was clear that test compounds **6a** and **6d** were found to possess anti-diabetic activity.

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