PART-II

(Pharmacological Evaluation)
Recent advances on rhodanine and 2,4-thiazolidinedione: Synthetic and pharmacological developments
INTRODUCTION

Five membered heterocyclic molecules containing thiazole nucleus with carbonyl group on fourth carbon such as rhodanine and 2,4-thiazolidinedione derivatives have broad spectrum of pharmacological activities. In past two decades, rhodanines and 2,4-thiazolidinediones have emerged as potent antidiabetic agents and entered in clinical use such as ciglitazone, Enlitalzone, Pioglitazone, epalrestat and Troglitazone for the treatment of type 2 diabetes mellitus and diabetic complications. That is why investigation/molecular modification and pharmacological evaluation of these lead molecules have attracted special attention of synthetic chemists and pharmacologists respectively.

In recent years, a number of synthetic/pharmacological protocols to synthesize such type of molecules have appeared in the literature. These multifaceted molecules exhibit varied type of biological activities. Some recent developments in synthesis and pharmacological aspects of these molecules are discussed in this section.
Recent developments in Rhodanine Derivatives

D. B. Boyd, carried out a study based on rhodanine-containing molecules of pharmaceutical interest in 1997, he found out pharmacological importance of these molecules is limited because of poor solubility of rhodanine derivatives in water (except of rhodanine-3-acetic acids, as this problem can be overcome by modifying into suitable salts). Set aside this fault, these compounds exhibited a broad range of significant biological activities (Boyd et al., 1997). Rhodanine-3-acetic acid (RAA) 1 was prepared by Körner (Korner et al., 1908) in 1908, and its Knoevenagel condensation products with various aldehydes viz [(5Z)-(5-benzyldiene-4-oxo-2-thioxo-1,3-thiazolidin-3-yl)]acetic acids 2 were reported in the same year (Andreasch et al., 1908). From 1960 onwards studies revealed that such type of molecule have potential antimycobacterial (Taniyama et al., 1959; Singh et al., 2008), antifungal (Allan et al., 1960; Allan et al., 1961; Allan et al., 1961; Allan et al., 1963; Allan et al., 1962; Allan et al., 1964; Orchard et al., 2002; Orchard et al., 2002; Orchard et al., 2003; Orchard et al., 2004), pesticidal (Dovlatyan et al., 1973; Inamori et al., 1992; Muro et al., 1996), antihypertensive (Frankov et al., 1985), and antineoplastic (Friebe et al., 2001; Singh et al., 2004) activities. Their NMR characterization were performed in 1982 (Tanaouachi et al., 1982). In 2006, similar molecules were prepared under microwave irradiation (Zhou et al., 2006). The Knoevenagel products of rhodanine-3-acetic acid with pyridinecarbaldehydes were prepared in 1961 and shown to possess potential antibacterial and antifungal activities (Allan et al., 1961). {(5Z)-(4-Oxo-5-(pyridin-2-ylmethylidene)-2-thioxo-1,3-thiazolidin-3-yl)]acetic acid 3 was patented as a potential drug for the treatment of metabolic bone diseases (Esswein
et al., 2003; Esswein et al., 2004). It was later found out that they stimulate parathyroid hormone receptor-mediated cAMP formation and could be useful for the local and systemic treatment of rheumatoid arthritis, osteoarthritis and degenerative arthrosis (Esswein et al., 2003; Esswein et al., 2004).

Trypanocidal activity of substituted rhodanine-3-acetic acids has been reported recently (Smith et al., 2009). The only rhodanine acetic acid derivative that has been used clinically is the aldose reductase inhibitor epalrestat 4. It was marketed in Japan and was used to slow down eye damage associated with diabetes and to prevent diabetic peripheral neuropathy (Boyd et al., 1997, Tanaouchi et al., 1982, Ziegler et al., 2008, Ramirez et al., 2008, Tanaouchi et al., 1984). Aldose reductase is not the only enzyme inhibited by rhodanine carboxylic acids. It was found that many other enzymes are also inhibited by the derivatives of this structural class, and may be responsible for their various biological effects (Tomasic et al., 2009). Other rhodanine based molecules have also been popular as small molecule inhibitors of numerous targets such as HCV NS3 protease (Sing et al., 2001), anti-diabetic mechanism (Momose et al., 1991), aldose reductase (Fujishima et al., 2002), β-lactamase (Grant et al., 2000; Zervosen et al., 2004), histidine decarboxylase (Free et al., 1971), inhibitors of JSP-1 (Cutshall et al., 2005) etc. This part of dissertation deals with a brief account on synthesis and biological effects and subsequent recent developments of newly prepared potential
drugs based on nitrogen-sulphur containing heterocycles having rhodanine nucleus.

![](image)

**Rhodanine as anti-diabetic agent:**

Murugana *et al.* synthesized (Murugana *et al.*, 2009) a series of dispiropyrrolidines (16-compounds) by 1,3-dipolar cycloaddition reaction of azomethine ylides (*in situ* generated by the reaction of sarcosine with isatin) with 5-arylidene-1,3-thiazolidine-2,4-dione and 5-arylidene-4-thioxo-1,3-thiazolidine-2-one derivatives as dipolarophiles (Scheme 1). They performed molecular docking studies on 1FM9 protein and screened synthesized compounds for their anti-diabetic activity. The synthesized compounds exhibited attractive anti-diabetic properties and were found to be more effective than rosiglitazone in ameliorating stress condition.
Rhodanine as anti-apoptotic agent:

Wang and his co-worker synthesized, a series of BH3I-1 based dimeric modulators of 5. The over-expression of anti-apoptotic Bcl-2 proteins (which protects cells from apoptosis) is one mechanism for tumors to acquire drug resistance. In this study they found out dimeric modulators 6-7 have enhanced binding activity against anti-apoptotic Bcl-2 proteins and proved dimerization of monomeric modulators as one practical approach to enhance the bioactivity of Bcl-2 antagonists (Wang et al., 2008).
Moorthi and his group (Moorthy et al., 2010) designed and synthesized 5-isopropylidene derivatives of 5-benzilidene-3-ethyl rhodanine (BTR-1) 8, 3-dimethyl-2-thio-hydantoin (ITH-1) 9, and 3-ethyl-2-thio-2,4-oxazolidinedione (ITO-1) 10 and tested their chemotherapeutic properties. They found that all the compounds had induced cytotoxicity in a time- and concentration-dependent manner on leukemic cell line, CEM. Among these compound, BTR-1 8 found to be many fold potent in inducing cytotoxicity than ITH-1 9 and ITO-1 10 with an IC$_{50}$ value of $<10$ µM and affected cell division by inducing a block at S phase, which finally led to the activation of apoptosis.

Same research group reported (Ravi et al., 2010) the synthesis of 5-isopropylidene-3-ethyl rhodanine 11 by conventional and microwave assisted
method and they found that rhodanine ITR 11 treatment led to cytotoxicity in leukemic cell line, CEM by inducing apoptosis.

**Rhodanine as anti-microbial agent:**

Habib *et al.* reacted (Habib *et al.*, 1997) thiazolo[4,5]-dlpyrimidines with rhodanines and investigated the obtained products (7 compounds) 12 for antimicrobial screening and they found out that antifungal activity against *Aspergillus niger* and *Penicillium* sp with IZ 20-38 mm and MIC < 50 - < 25 µg/ml. They claimed compound 13 being the most active against *Aspergillus niger* while compound 14 found to be most active against *Penicillium* sp; and are 5-fold less active than the standard antibiotic clotrimazole. They concluded that the presence of an alkyl group at position 3 of the thiazolopyrimidine ring 13 is superior to that of other aromatic substituents; also the introduction of an arylideneamino group at position 6 of 14 enhanced the antifungal activity.
Opperman and his group disclosed (Opperman et al., 2009) that aryl rhodanines 15-18 did not exhibit antibacterial activity against any of the bacterial strains tested and were not cytotoxic against HeLa cells. Their study revealed that the aryl rhodanines 15-18 specifically inhibit the early stages of biofilm development by preventing attachment of the bacteria (specifically inhibit biofilm formation of S. aureus, S. epidermidis, Enterococcus faecalis, E. faecium, and E. gallinarum but not the gram-negative species Pseudomonas aeruginosa or Escherichia coli.) to surfaces.
Sim and his associates reported (Sim et al., 2002) benzylidene rhodanines 19-21 as novel inhibitors of uridine diphospho-N-acetylmuramate/L-alanine ligase. They observed that compounds 19-21 showed selective whole-cell activity against the Gram-positive methicillin resistant *Staphylococcus aureus* (MRSA) but not against the Gram-negative *Escherichia coli*. They also evaluated their cytotoxic effect on mammalian Chinese hamster ovary (CHO) cells.
Hardej et al. synthesized (Hardej et al., 2010) a series of glycine and phenylalanine-derived rhodanine analogs and evaluated their anti-MRSA activity. The antibacterial activity of compounds 22 and 23 against a panel of MRSA strains was significantly greater than that of the reference antibiotics penicillin G and ciprofloxacin. They claimed compound 23 exhibited only a 2-4-fold higher MIC value than that of vancomycin. They concluded from their study that the phenylalanine derived compounds 22 and 23 were promising templates for the development of new drugs to treat MRSA infections.

Piao and his group synthesized (Chen et al., 2010) several hybrid compounds (19 compounds) having chalcone and rhodanine-3-acetic acid moieties (Scheme 2) and tested for their anti-bacterial activity. Some compounds exhibited good anti-microbial activities against Gram-positive bacteria (including the multidrug-resistant clinical isolates) equivalent to that of standard drug (norfloxacin) but less active than oxacillin.

Scheme 2
Tomasic et al. reported (Tomasic et al., 2010) the synthesis and antibacterial activity for a series of rhodanine-, rhodanine-N-acetic acid-, thiazolidine-2,4-dione-, barbituric- and thiobarbituric acid-based compounds bearing an ylidene substituent at position 5. The most potent compound of the series, \((Z)-5-(2,3,4\text{-trifluorobenzylidene})\text{rhodanine 24, inhibited the growth of S. aureus at 0.5 µg/ml and MRSA at 32 µg/ml.}\)

Orchard and his group (Orchard et al., 2004) synthesized rhodanine-3-acetic acid based compounds 25-26 and described as inhibitors of fungal protein: mannosyl transferase 1 (PMT1). They observed 5-[[3-(1-phenylethoxy)-4-(2-phenylethoxy)phenyl]methylene]-4-oxo-2-thioxo-3-thiazolidineacetic acid 27, inhibit *Candida albicans* PMT1 with IC50s in the range 0.2–0.5 µM. Members of the series were found to be effective in inducing changes in morphology of *C. albicans* in vitro that have previously been associated with loss of the transferase activity. According to them, these compounds 25-26 could serve as useful tools for studying the effects of protein O-mannosylation and its relevance in the search for novel antifungal agents.
Sortino et al. reported (Sortino et al., 2007) benzyliden-rhodanines 28 which acted as antifungal agents. They evaluated the compounds 29 and 30 that showed fungicidal activity and were most active against *Candida genus* and *C. neoformans* including clinical isolates. Other compounds of this series showed a very good activity against dermatophytes.
Rhodanine as Anti-Hepatitis C virus (HCV) agent:

Sing et al. disclosed (Sing et al., 2001) arylalkylidene rhodanines 31-32 inhibit HCV NS3 protease at moderate concentrations. They claimed that these rodanine derivatives were better inhibitors of serine proteases such as chymotrypsin and plasmin. They concluded that selectivity of arylmethylidene rhodanines 31-32 with bulkier and more hydrophobic functional groups showed increased activity towards HCV NS3 protease respectively by 13- and 25-folds.

\[
\text{31}
\]

\[
\text{32}
\]

Rhodanine as HIV-1 integrase inhibitors:

Rajamaki and his associate synthesized (Rajamaki et al., 2009) and biologically evaluated rhodanine based compounds 33 and identified these
exhibiting anti HIV-1 integrase activity and moderate inhibition of HIV-1 cell replication.

![Chemical structure of compound 33](image)

**Rhodanine as anti-inflammatory agent:**

Cutshall *et al.* reported (Cutshall *et al.*, 2005) synthesis and evaluation of rhodanine-based compounds 34 as inhibitors of JSP-1. On SAR studies they demonstrated that stronger electron-withdrawing functional groups appended to the aryl-benzylidene position provided analogs with the greatest potencies as illustrated by compound 35. Compound 35 had showed reversible and competitive bind with substrate with a high degree of enzyme selectivity against other phosphatases.

![Chemical structure of compound 34 and 35](image)
Irvine et al. identified (Irvine et al., 2008) a series of rhodanine derivatives as novel inhibitors of PDE4. Compounds 37 and 38 displayed the most significant activity of the compounds synthesized, being some 20- and 24-fold more potent than lead compound 36.

![Chemical structures of compounds 36, 37, and 38]

**Rhodanines for Sleeping sickness:**

Smith et al. developed (Smith et al., 2009) the first small molecular inhibitors of dolicholphosphate mannose synthase (DPMS), a mannosyltransferase critically involved in glycoconjugate biosynthesis in *T. brucei*. Thiazolidinones 39, 40 and 41 in particular were promising candidates for further development because of their respective activities against trypanosomal DPMS and GPI anchor biosynthesis. They reported that these DPMS inhibitors prevent the biosynthesis of glycosylphosphatidylinositol (GPI) anchors, and possess trypanocidal activity against live trypanosomes. Drug-like molecules 39-41 with activity against
*Trypanosoma brucei* are urgently required as potential therapeutics for the treatment of African sleeping sickness.

![Chemical structures](image)

Rhodanines as tyrosinase inhibitors:

Liu *et al.* synthesized (Liu *et al.*, 2011) a series of dihydropyrimidin-(2H)-one analogues and rhodanine derivatives and evaluated for their inhibitory effects on the diphenolase activity of mushroom tyrosinase. They found that some of the synthesized compounds exhibited significant inhibitory activities. Especially, compound 42 bearing a hydroxyethoxyl group at position-4 of phenyl ring exhibited most potent tyrosinase inhibitory activity with IC₅₀ value of 0.56 mM. The inhibition mechanism analysis of compound 42 demonstrated that the inhibitory effect of the compound on the tyrosinase was irreversible. These results suggested that such compounds might be served as lead compounds for further designing new potential tyrosinase inhibitors.
Rhodanines as PRL-3 inhibitors

Ahn et al. synthesized (Ahn et al., 2006) and evaluated a series of rhodanine derivatives 43 for their ability to inhibit PRL-3. Benzylidene rhodanine derivative 43 showed good biological activity, while compound 44 was found to be the most active in this series exhibiting IC$_{50}$ value of 0.9 Lm in vitro and showed a reduced invasion in cell-based assay.

Pharmacological developments in 2,4-Thiazolidinedione

The most commonly used antidiabetic agents have been sulfonylureas, metformin, and certain alphaglucosidase inhibitors and meglitinides. These agents increase insulin secretion from pancreatic β-cells, but sometimes induce severe hypoglycemia and weight gain (Holman et al., 1991) and hyperinsulinemia is
known to be a risk factor for ischemic heart disease (Depres et al., 1996). In addition, high rates of both primary and secondary failure were observed with these drugs (The Diabetes Control and Complications Trials, 1993; American Diabetic Association, 1993; Harrower et al., 1994; U.K. Prospective Diabetes Study Group, 1995). Therefore, drugs that ameliorate the insulin resistance without stimulating insulin release from β-cells have been developed for the treatment of type 2 diabetes. Type 2 diabetes is a multifactorial disease defined by a high plasma glucose level, and is characterized by both insulin resistance and impaired insulin secretion by pancreatic β-cells (DeFronzo et al., 1988). The prototypical 2,4-thiazolidinedione, ciglitazone 45 was discovered (Sohda et al., 1982) by Takeda Chemical Industries, Ltd., Japan and has antihyperglycemic activity in insulin-resistant animal models, KKAy mice (Iwatsuka et al., 1970) and Wistar fatty rats (Ikeda et al., 1981), but no effect in insulin-deficient animal models of diabetes (Fujita et al., 1983; Chang et al., 1983). During structure–activity relationship studies on 2,4-thiazolidinediones and related compounds, they discovered highly potent compounds, such as pioglitazone 46 (Sohda et al., 1990), and AD-5061 47 (Sohda et al., 1992). Since the discovery of ciglitazone 45, a number of pharmaceutical companies have been evaluating new 2,4-thiazolidinedione analogs as agents for improving insulin resistance. Troglitazone 48 (Yoshioka et al., 1989) was launched first in the market, but had been withdrawn because of liver toxicity and related deaths associated with the drug. Nowadays, two 2,4-thiazolidinedione class agents, pioglitazone 46 and rosiglitazone 49 (Cantello et al., 1994) are in clinically use. Many companies are still endeavouring to find a new glucose lowering agent. (Rami et al., 2000; Lohray et al., 1998; Lohray et al., 2001; Oguchi et al., 2000; Nomura et al., 1999;
Although the precise mechanism of action of these drugs remains unknown, a recent study suggested that antidiabetic thiazolidinediones interact with a family of nuclear receptors known as peroxisome proliferator-activated receptor (PPAR)-\(\gamma\) (Lehmann et al., 1995). PPAR\(\gamma\) is one of a subfamily of PPARs encoded by independent genes. Three human PPARs, designated PPAR\(\alpha\), PPAR\(\gamma\), and PPAR\(\delta\), have been identified till date (Isseman et al., 1990; Schmidt et al., 1992; Kliewer et al., 1994). It was also observed that the potency for activation of PPAR\(\gamma\) in vitro mirrored the in vivo glucose lowering activity in diabetic ob/ob mice (Willson et al., 1996). This would indicate that the major mechanism of
action of 2,4-thiazolidinediones involve PPARγ. As far as 2,4-thiazolidinediones already in the market are concerned, several side effects, such as anemia, edema, and body weight gain, have been reported (Iwamoto et al., 1996). Therefore, search for a new compounds with fewer side effects and a more advanced profile than existing drug molecules is the main focus of attention for chemists as well as for pharmacologists. Recent developments in the synthesis and evaluations of thiazolidinedione based compounds for a variety of biological activities along with anti-diabetic activity are discussed here in the forthcoming pages.

**Thiazolidinedione as anti-diabetic agent:**

Rakowitz et al. synthesised (Rakowitz et al., 2006) and tested several 5-benzyl-2,4-thiazolidinediones 50-51 as *in vitro* aldose reductase inhibitors (ARIs). Their evaluation showed N-unsubstituted 5-benzyl-2,4-thiazolidinediones 50 and (5-benzyl-2,4-dioxothiazolidin-3-yl)acetic acids 51, displayed moderate to high inhibitory activities. The insertion of an acetic acid chain on N-3 significantly enhanced the AR inhibitory potency, leading to compound 51 which proved to be the most effective among the tested compounds. In N-unsubstituted derivatives 50 the presence of an additional aromatic ring on the 5-benzyl moiety was generally beneficial.
Madhavan et al. synthesized (Madhavan et al., 2006) and evaluated 2,4-Thiazolidinedione derivatives of 1,3-benzoxazinone for their PPAR-\(\alpha\) and \(\gamma\) dual activation. A compound DRF-2519 (52), through SAR of TZD derivatives of benzoxazinone, has shown potent dual PPAR activation. In \(ob/ob\) mice, it showed better efficacy than the other similar molecules. In fat fed rat model, it showed significant improvement in lipid parameters, which was found to be better than fibrates.

Dundar et al. prepared (Dundar et al., 2008) new series of chromonyl-2,4-thiazolidinediones 53 aiming to reduce diabetic complications especially which have effect on the cataract formation. The synthesized compounds were tested for their ability to inhibit rat kidney AR by an \textit{in vitro} spectrophotometric assay. Compound 54 showed the highest inhibitory activity. They concluded that the increasing inhibitory effect of compounds 53 might be due to the acetic acid side chain of 2,4-TZD and such compounds especially 54 could display therapeutic potential in the prevention and the treatment of diabetic complications as promising ARIs.
Maccari et al. reported (Maccari et al., 2010) new ARIs through *in vitro* evaluation of a series of 5-arylidene-3-(3,3,3-trifluoro-2-oxopropyl)-2,4-thiazolidinediones, the compound 56 as promising ARIs. This led to the identification of two new non-carboxylic acid containing 5-arylidene-2,4-thiazolidinedione derivatives (57 and 58) that are active at low micromolar doses.

**Thiazolidinedione as Anti-cancer agent:**

Patil et al. synthesized (Patil et al., 2010) and evaluated ten derivatives of 5-benzylidene-2,4-thiazolidinediones 59 for their antiproliferative activity in a panel of 7 cancer cell lines using four concentrations at 10-fold dilutions. Sulforhodamine B (SRB) protein assay was used to estimate cell stability or
These compounds showed varying degrees of cytotoxicity in the tested cell lines, most marked effect was shown by compound 60 in MCF7 (breast cancer), K562 (leukemia) and GURAV (nasopharyngeal cancer) cell lines with log₁₀ GI₅₀ values of -6.7, -6.72 and -6.73 respectively.

**Thiazolidinedione as anti-inflammatory agent:**

Barros et al. synthesized (Barros et al., 2010) 5-arylidene-3-benzyl-thiazolidine-2,4-diones 61 with halide groups on their benzyl rings (8 compounds) and assayed in vivo to investigate their anti-inflammatory activities and 3-(2-bromo-benzyl)-5-(4-methanesulfonyl-benzylidene)-thiazolidine-2,4-dione, compound 62, showed higher anti-inflammatory activity than the rosiglitazone reference drug as it bound PPARγ with 200-fold lower affinity than the reference ligand.
Alagawadi et al. described (Alagawadi et al., 2011) the synthesis and antimicrobial activity of 5-substituted-2,4-thiazolidinedione derivatives 63 and 66. They evaluated all compounds for their preliminary *in vitro* antibacterial and antifungal activity. The investigation of antimicrobial screening revealed that some of the tested compounds showed moderate to good bacterial and fungal inhibition. Particularly compounds 64, 65, 67 and 68 have shown good activity against *S. aureus* and *E. faecalis* with minimum inhibitory concentrations (MIC) values between 4 and 32 µg/ml. All compounds were active against tested fungal strains at 1–64 µg/ml concentration. Compounds 64, 65, 67 and 68 also showed good antifungal activity against *C. albicans* at 1–4 µg/ml and *C. neoformans*, *A. flavus*, *A. niger* at 2–8 µg/ml concentration.
Liu et al. synthesized (Liu et al., 2011) a series of chalcone derivatives bearing the 2,4-thiazolidinedione and benzoic acid moieties 69 and evaluated for their anti-bacterial activity. Tested compounds, were the most effective with MIC value in the range of 0.5-4 \( \mu \text{g/ml} \) against six Gram-positive bacteria (including multidrug-resistant clinical isolates).
Thiazolidinedione as antioxidant:

Jeong et al. synthesized (Jeong et al., 2004) multi-substituted benzyldenethiazolidine-2,4-diones 70 by Knoevenagel condensation of di- or trisubstituted 4-hydroxybenzaldehydes with thiazolidine-2,4-dione and evaluated for antioxidant activities against Cu$^{2+}$-induced oxidation of human low-density lipoproteins (LDL). Among compounds, 71 was found to be superior to probucol in LDL-antioxidant activities and found to be 9-fold more active than probucol.

Hossain et al. synthesized (Hossain et al., 2007) a series of 5-arylidene-2,4-thiazolidinediones and its geranyloxy or prenyloxy derivatives were studied for their radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Their scavenging activities were expressed as IC$_{50}$ value. Compounds 72-74 showed appreciable radical scavenging activities. The vanillin based thiazolidinedione compound 72 displayed highest activity comparable to that of α-tocopherol. But in vivo, compound 74 showed better results in inducing phase II detoxifying/antioxidative enzyme. The compounds 72-74 were found to be
effective in enhancing the host antioxidant defense system such as superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), and reduced Glutathione (GSH), and at the same time lowering the serum ALT and AST level at the preliminary screening dose of 3 mg/kg in normal Swiss albino mice given orally for 20 days as compared to the control animals. The hepatic lipid-peroxidation level (LPO) remained unchanged.

Ottana et al. explored (Ottana et al., 2011) 5-arylidene-4-thiazolidinones as antioxidant agents and aldose reductase inhibitors. They found compound 75 and 76 proved to be interesting inhibitors of the enzyme as well as excellent antioxidant agents that are potentially able to counteract the oxidative stress associated with both diabetic complications as well as other pathologies.
Thiazolidinedione as anti-obesity:

Baihua Hu et al. disclosed (Hu et al., 2001) synthesis of methylsulfonamide substituted 2,4-thiazolidinedione (6-compounds) 77 and found 78 a potent (EC$_{50}$=0.01 mM, IA=1.19) and selective (more than 110-fold over $\beta_1$ and $\beta_2$ agonist activity) $\beta_3$ agonist. This compound has also been proven to be active and selective in an in vivo mode.
Bhattarai et al. synthesized (Bhattarai et al., 2009) benzylidene-2,4-thiazolidinedione derivatives (9-compounds) with substitutions on the phenyl ring at the ortho or para positions of the thiazolidinedione group 79 as PTP1B inhibitors with IC\textsubscript{50} values in a low micromolar range. Compound 80, the lowest, bore an IC\textsubscript{50} of 5.0 \(\mu\)M. In vivo efficacy of 80 as an antiobesity and hypoglycemic agent was evaluated in a mouse model system. This compound also significantly suppressed weight gain and significantly improved blood parameters such as TG, total cholesterol and NEFA. Compound 80 was also found to activate peroxisome proliferator-activated receptors (PPARs) indicating multiple mechanisms of action.

![Chemical structures of 79 and 80](image)

Same research group (Bhattarai et al., 2010) synthesized benzylidene-2,4-thiazolidinedione derivatives (12 compounds) 81 as PTP1B inhibitors with IC\textsubscript{50} values in a low micromolar range. Compound 82, the lowest, bore an IC\textsubscript{50} of 1.3 \(\mu\)M. In a peroxisome proliferator-activated receptor-\(\gamma\) (PPAR-\(\gamma\)) promoter reporter gene assay, compound 82 found to activate the transcription of the reporter gene with potencies comparable to those of troglitazone, rosiglitazone, and pioglitazone. In vivo efficacy of 82 as an anti-obesity and hypoglycemic agent was
evaluated in a mouse model system. Compound 82 significantly suppressed weight gain and significantly improved blood parameters such as TG, total cholesterol and NEFA without overt toxic effects.

![Chemical structures of compounds](image)

**Thiazolidinedione as anti-prostaglandins:**

Wu *et al.* synthesized (Wu *et al.*, 2010) a range of benzylidene thiazolidinedione derivatives (27 compounds with 75-88% yields) with different substituents on the phenyl ring 83 and evaluated their inhibitory 15-hydroxyprostaglandin dehydrogenase (15-PGDH) activity. Based on the structures of the thiazolidinediones analogues and inhibitory activity, replacement of the cyclohexylethyl group of 84 with the hetero five-member ring increased the inhibitory potency. However, replacement of the cyclohexylethyl group with a hetero six-member ring decreased the inhibitory potency significantly. It was
found that compound 85 (5-(4-(2-(thiophen-2-yl)ethoxy)benzylidene)thiazolidine-2,4-dione) was the most potent inhibitor and was effective in the nanomolar range.

**Thiazolidinedione as thyroid hormone receptor antagonists:**

Komatsu et al. designed and synthesized (Komatsu et al., 2007) diphenylamine derivatives 86 with a thiazolidinedione moiety as the terminal polar group and evaluated as thyroid hormone receptor (TR) antagonists. Thiazolidinedione derivatives 86 with N-alkyl group showed antagonistic activities towards both the hTRα1 and hTRβ1 subtypes.
Looking over the abovementioned trends in these two classes of lead compounds (Rhodanines and 2,4-Thiazolidinediones), it was worthwhile to pharmacologically evaluate the newly synthesized novel nitrogen sulphur based heterocyclic compounds viz. rhodanine and 2,4-thiazolidinedione derivatives, for their antibacterial and antihyperglycemic activities. Their pharmacological evaluations and results have been discussed exhaustively in upcoming section-2 & section-3 of the chapter-IV.
Pharmacological evaluation of novel chromonyl-rhodanine and chromonyl–thiazolidinedione derivatives: As Antibacterials
INTRODUCTION

The use of mixtures with antimicrobial properties to treat infections were described over 2000 years ago (Lindblad et al., 2008; Forrest et al., 1982; Wainwright et al., 1989). The observations made in mid of 20th century in the laboratory regarding antibiosis among micro-organisms led to the discovery of natural antibacterials produced by microorganisms (Kingston et al., 2008). These natural antibacterials were given the name antibiotics. The term antibiosis, means "against life," was introduced by the Vuillemin as a descriptive name of the phenomenon exhibited by these early antibacterial drugs (Calderon et al., 2007; Foster et al., 1974). Antibiosis was first described in 1877 in bacteria when Louis Pasteur and Robert Koch observed that an airborne bacillus could inhibit the growth of Bacillus anthracis (Landsberg et al., 1949). These drugs were later renamed antibiotics by S. Waksman, in 1942 (Calderon et al., 2007; Waksman et al., 1947). John Tyndall was the first who described antagonistic activities by fungi against bacteria in England in 1875 (Kingston et al., 2008). Synthetic antibiotic chemotherapy as a science and further development of antibacterials on these lines began in Germany with Paul Ehrlich in the late 1880s and he discovered a medicinally useful drug, the synthetic antibacterial Salvarsan (Calderon et al., 2007; Limbird et al., 2004; Bosch et al., 2008) now called Arsphenamine. In 1928, Alexander Fleming observed antibiosis against bacteria by a fungus of the genus Penicillium. Fleming postulated that the effect was mediated by an antibacterial compound named penicillin, and its antibacterial properties could be exploited for chemotherapy (Fleming et al., 1980; Sykes et al., 2001). First commercially available antibacterial and the first sulphonamide
‘Prontosil’ was developed by a research team led by Gerhard Domagk in 1932 at the Bayer Laboratories of the IG Farben conglomerate in Germany (Bosch et al., 2008). Domagk received the 1939 Nobel Prize for Medicine for his efforts. Prontosil had a relatively broad spectrum activity against Gram-positive cocci, but not against enterobacteria. Research was stimulated apace by its success. The discovery and development of this sulfonamide drug opened the era of antibacterials/antibiotics (Figure 1).

Figure 1 Discovery of new classes of antibiotics.

In 1939, coinciding with the start of World War II, Rene Dubos reported the discovery of the first naturally derived antibiotic, gramicidin from B. brevis. It was one of the first commercially manufactured antibiotics universally and was effectively used to treat wounds and ulcers of the wounded soldiers during World War II (Van Epps et al., 2006). Florey and Chain succeeded in purifying the first
penicillin, penicillin G procaine in 1942, which displayed potent antibacterial activity against a wide range of bacteria and had low toxicity in humans. Furthermore, its activity was not inhibited by biological constituents such as pus, unlike the synthetic sulfonamides (Florey et al., 1945). For the discovery and development of penicillin as a therapeutic drug, Ernst Chain, Howard Florey, and A. Fleming shared the 1945 Nobel Prize in Medicine. Florey credited Dubos for pioneering approach of deliberate and systematic search for antibacterial compounds, which led to the discovery of gramicidin and revived Florey's research in penicillin (Van Epps et al., 2006). A chronological order of antibacterials/antibiotics coming in the market in the modern era is given in the following table (Table 1).
Antibacterial/antibiotics are commonly classified depending upon their mechanism of action, chemical structure, spectrum of activity, targetive bacterial functions or growth processes (Calderon et al., 2007). Anti-bacterials that target the bacterial cell wall (penicillins and cephalosporins) or the cell membrane
(polymixins), or interfere with essential bacterial enzymes (quinolones and sulfonamides) have bactericidal activities and those target protein synthesis (aminoglycosides, macrolides, and tetracyclines) are usually bacteriostatic (Finberg et al., 2004). Further categorization is based on their target specificity. "Narrow-spectrum" antibacterial antibiotics target specific types of bacteria, such as Gram-negative or Gram-positive bacteria, whereas broad-spectrum antibiotics affect a wide range of bacteria. After a 40-year gap in discovering new classes of antibacterial compounds, three new classes of antibacterial antibiotics have been brought into clinical use: cyclic lipopeptides (such as daptomycin), glycylicyclines (such as tigecycline), and oxazolidinones (such as linezolid) (Cunha et al., 2009).

**Why New Antibacterial Agents is needed?**

The drug resistant bacterial strains are growing at an alarming rate in both developing and developed countries (Projan et al., 2007). From this statement alone, it should be clear that the need for the development of novel antibacterial agents is of utmost importance. In the current antibacterial drug pipeline, there is only a miniscule glimmer of hope (Projan et al., 2007; Theuretzbacher et al., 2009; Fischbach et al., 2009; Gill et al., 2010; http://www.sciencedaily.com). This rapid increase in resistant bacteria coupled with the slow emergence of novel agents has led some experts to call this time the “dawn of the post-antibiotic era (Colson et al., 2008; Alanis et al., 2005; Walsh et al., 2003).

There exists a perpetual need for new antibiotics. Most of the drugs will remain just as effective in the future as they are today, but that is not the case with
antibiotics/antibacterials. Eventually, the inevitable rise of resistance will erode the utility of today’s antibiotics (Fischbach et al., 2009; Falagas et al., 2007). There are three factors that will intensify this supply problem of discouraging antibiotic development (Nathan et al., 2005). First, antibiotics are used in smaller quantities than other drugs. The standard antibiotic course lasts only few days or week compared to drugs used in for chronic illness which can even last a lifetime. Therefore, antibiotics yield lower revenues than most of the drugs. Second, the use of newly approved antibiotics is often limited to serious bacterial infections and scope of success is very limited. The third reason is an increase in regulatory requirements to get a drug licensed, which involves lengthy clinical trials as cost prohibitive. However, most of the newly approved drugs can be prescribed to all who may be benefited from their use. These factors ultimately result in this quandary: Resistance is on the rise while antibiotic discovery and development are on the decline (Fischbach et al., 2009; Nathan et al., 2004; Nussbaum et al., 2006).

Three classes of antibiotic-resistant pathogens are emerging as major threats to public health. The first pathogen of concern is MRSA. It is estimated that MRSA is responsible for approximately 19,000 deaths per year in the United States alone. The rising prevalence of MRSA increases the likelihood of emerging vancomycin-resistant Staphylococcus aureus (VRSA), which is just as deadly as MRSA but more challenging to treat, will become a major concern in hospitals (Klevenets et al., 2007; Weigel et al., 2003). The second class of pathogens are multidrug-resistant (MDR) and pandrug-resistant (PDR) gram-negative bacteria. These bacteria may be less prevalent than MRSA, but they pose a severe risk of
infections that are truly untreatable. These strains of *Acinetobacter baumannii*, *Esherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* are becoming resistant to antibacterials/antibiotics that are used in some (MDR) or all (PDR) gram-negative bacteria: penicillins, cephalosporins, carbapenems, monobactams, quinolones, aminoglycosides, tetracyclines, and polymixins (Falagas *et al.*, 2005). The third class of pathogens are MDR and extensively drug-resistant (XDR) strains of Mycobacterium tuberculosis. These strains are an ever increasing threat in developing nations (Dorman *et al.*, 2007). MDR-TB treatments requires a 2-year course of antibiotics accompanied with serious side effects; XDR-TB is even more difficult to treat and often fatal (Kim *et al.*, 2008).

The number of new antibiotic agents approved by the Food and Drug Administration (FDA) has fallen steadily since 1980 (Colson *et al.*, 2008). It is also pertinent to note that during that time 75% of the approved drugs were in two classes, beta-lactams and quinolones. Between 1935 and 1968 there were 14 classes of antibiotics introduced for human used; since then, only five have been introduced. Those five classes are; the oxazolidinones, lipopeptides, glycyclcyclins, pleuromutilins, and mupirocin (Colson *et al.*, 2008). While most of the new antibiotics are coming from existing classes, yet more of the diversity is being seen within these classes. These new agents are more effective and safer than earlier drugs in their class (Table 1) (Outterson *et al.*, 2007).

The FDA is the approving body for new antibiotics in the United States. Once a new drug has the approval of the FDA, it can be sold for use in the United States. Since 1998, the FDA has approved a number of new antibiotics. However,
only a limited number of these agents possess a novel mechanism of action. Having new antibiotics approved for use is a remarkable achievement, but those antibiotics which utilize the same mechanism of action as previously approved drugs always run the risk of increasing the rate of resistance. Anti-bacterials possessing a novel mechanism of action are greatly needed to alleviate this burden. FDA approved and clinically used antibiotics with their trade names are given below from 2000 to onwards (Colson et al., 2008).
In addition, some anti-bacterials/antibiotics are in pipeline that can emerge on the horizon in near future. The majority of these agents are aimed at treating infections caused by gram-positive organisms.
Over the past several years, there has been a drastic increase in the number of multi-drug resistant isolates of gram-negative bacteria (O'Fallon et al., 2009). The outlook for antibiotics that treat gram-negative infections is not as positive as in the gram-positive arena. This is causing major concern within the scientific community. There are currently no such antibacterials/antibiotics being reviewed for final approval by the FDA. There is not even single agent currently in Phase 3 clinical trials. This is a major cause of concern because without any agents that have advanced past Phase 2 clinical trials, it will be minimum of 5 years before the first such agent becomes routinely available in the clinic. While most of the agents in the pipeline are modifications of existing classes of antibiotics, there are a few novel classes arising through the pipeline as well. Given the widespread resistance problem and the propensity to intensify the effect of class-specific resistance, most scientists would prefer to develop more novel classes with no pre-existing potential cross-resistance (Wang et al., 2006). There are currently three agents that are in Phase 2 clinical trials for the treatment of gram-negative bacteria. The first agent is combination agent. It combines ceftazidime and NXL 104 (Figure 1.35). Ceftazidime is a cephalosporin, and NXL 104 is a new β-lactamase inhibitor.

![Diagram of NXL 104](image)

The second agent in Phase 2 clinical trials is IC43. This is a recombinant subunit vaccine consisting of two outer membrane proteins of *Pseudomonas aeruginosa*. It is being tested for the treatment of ventilator-associated pneumonia.
The final agent that is currently undergoing Phase 2 trials is KBPA101. This agent is a monoclonal antibody that targets Pseudomonas aeruginosa serotype O11. It is also associated with the co-development of a multivalent diagnostic test for rapid serotyping.

It is quite evident from exhaustive survey that the need to develop novel antibacterials is still strong. In previous section-1 (Chapter-III), it was discussed that the rhodanine and 2,4-thiazolidines derivatives have potent antimicrobial agent along with other significant pharmacological properties. Herein, we report the pharmacological evaluation of novel chromonyl-rhodanine and chromonyl-thiazolidinedione compounds against gram-positive (*Staphlococcus aureus*) and gram-negative bacteria (*Klebsiella aerogens*).

**Materials and Methods**

**General Procedure**

**Cup and plate method**

Modified cup and plate method was used for the evaluation of bacterial susceptibility of the synthesized compounds. With the help of a sterile borer, wells of 8mm. diameter were made in the solidified agar plate that was previously inoculated with the test Bacterium. Specified amount of test compounds were added to the wells and petri dishes were then incubated for 24 h at 37°C. After incubation the petri plates were observed for Inhibition Zones (IZ). The diameter
of inhibition zone is directly proportional to the antibacterial activity (Sadashiva et al., 2004).

**Bacterial strains**

The following Bacterial strains were used to study the antibacterial activity

- **Gram positive Staphlococcus aureus**
- **Gram negative Klebsilla aerogens**

**Preparations of test solutions**

All samples were tested at 2µg/ml, 4 µg/ml, 8 µg/ml, 16 µg/ml, 32 µg/ml and 64 µg/ml dosing pattern. Samples were made in DMSO. All the dosing solutions were prepared under aseptic conditions.

**Preparation of Muller Hinton Agar media**

**Composition of Muller Hinton Agar media is**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef Infusion</td>
<td>300ml</td>
</tr>
<tr>
<td>Casein Hydrosylate</td>
<td>17.5gm</td>
</tr>
<tr>
<td>Starch</td>
<td>1.5gm</td>
</tr>
<tr>
<td>Agar</td>
<td>10.0gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
</tbody>
</table>
The media was prepared by dissolving the suitable quantities of dehydrated of dehydrated medium in purified water in a conical flask by heating it on a water bath. Conical flask was closed with cotton plug and sterilized by autoclaving at 121°C for 15 min.

RESULTS AND DISCUSSION

Preparation of target compounds (Scheme 1, Table 1) and their characterization through $^1$H-NMR and $^{13}$C-NMR and spectral techniques has already been discussed in Chapter: I-III.

![Scheme 1](image)

**Table 1** Chromonyl-rhodanines and Chromonyl-thiazolidinediones.  
(CTZ-1 to CTZ-6) (CTZ-11 to CTZ-16)

<table>
<thead>
<tr>
<th>Target Molecules</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Table 1](image)
All the synthesized reduced Knoevenagel products CTZ 1-6 and CTZ 11-16 were subjected to *in vitro* screening against gram positive and negative bacteria using the agar well diffusion method. Two bacterial strains, *Staphylococcus aureus* (Gram positive) and *Klebesilla aerogens* (Gram negative), were used in the present study.

The antibacterial activity of newly synthesized novel compounds CTZ 1-6 *(Rhodanine based)* and CTZ 11-16 *(2,4-Thiazolidinedione based)* were evaluated by the agar well diffusion method (Sadashiva *et al.*, 2004). About 25ml of nutrient agar medium was poured into each petri plate and the agar plates were swabbed with 100 μl inocula of each test bacterium and kept for 15 min for adsorption. Using sterile cork borer of 8mm diameter, wells were bored into the
seeded agar plates and these were loaded with a 50 μl volume of test solutions. All the plates were incubated at 37 °C for 24 h. Antibacterial activity of each synthesized compound was evaluated by measuring the zone of growth inhibition & MIC against the test organisms with zone reader (Hi Antibiotic zone scale). MIC was determined as the lowest concentration of the compound tested that was able to inhibit visible growth of bacteria. Dimethylsulphoxide (DMSO) was used as a negative control whereas Cefixime was used as a reference drug. The experiments were performed in triplicates.

After checking the inhibitory zone diameters, it had been found out that some of the synthesized compounds (Table 1) have shown considerable antibacterial activity against the bacteria *Staphylococcus aureus* (Gram positive) and *Klebsilla aerogens* (Gram negative). Results of the study indicate that antibacterial activity of various synthesized compounds varied significantly depending upon the type of substituent attached to the benzene rings. The susceptibilities of gram-positive bacteria & gram negative bacteria were checked against newly synthesized 5-[(4-Oxo-4H-chromen-3-yl)methyl]-2-thioxo-1,3-thiazolidine-4-ones (*CTZ-1 to 6*) and 5-[(4-Oxo-4H-chromen-3-yl)methyl]thiazolidine-2,4-diones (*CTZ-11 to 16*) using Cefixime as reference antibacterial.
Table 2  *In vitro* antibacterial activity of chemical compounds through agar well diffusion method.

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Diameter of growth of inhibition zone IZ (mm) &amp; MIC in µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>CTZ-1</td>
<td>4.5 (&gt;16)</td>
</tr>
<tr>
<td>CTZ-2</td>
<td>03 (&gt;64)</td>
</tr>
<tr>
<td>CTZ-3</td>
<td>-</td>
</tr>
<tr>
<td>CTZ-4</td>
<td>02 (&gt;64)</td>
</tr>
<tr>
<td>CTZ-5</td>
<td>05 (&gt;16)</td>
</tr>
<tr>
<td>CTZ-6</td>
<td>-</td>
</tr>
<tr>
<td>CTZ-11</td>
<td>03 (&gt;64)</td>
</tr>
<tr>
<td>CTZ-12</td>
<td>-</td>
</tr>
<tr>
<td>CTZ-13</td>
<td>-</td>
</tr>
<tr>
<td>CTZ-14</td>
<td>-</td>
</tr>
<tr>
<td>CTZ-15</td>
<td>-</td>
</tr>
<tr>
<td>CTZ-16</td>
<td>02 (&gt;64)</td>
</tr>
<tr>
<td>Cefixime</td>
<td>03 (2)</td>
</tr>
</tbody>
</table>

- No activity

Among the newly synthesized compounds (CTZ-1 to CTZ-6 and CTZ-11 to CTZ-16), it was found out that rhodanine based compounds **CTZ-1** and **CTZ-5** showed the promising antibacterial activity against *Staphlococcus aureus* (Gram positive) [IZ (MIC) = 4.5 (>16) and 5 (>16)]. While **CTZ-1** showed a comprehensive strong antibacterial activity against *Klebesilla aerogens* (Gram negative) [IZ (MIC) = 10 (>8)]. The thiazolidinedione based compounds did not show any appreciable activity against both strains i.e. *Staphlococcus aureus* (Gram positive) & *Klebesilla aerogens* (Gram negative). This particular observation demonstrates the importance of >C=S functional group of rhodanine nucleus towards antibacterial activity. Simultaneously the –NO₂ at 6-position of chromonyl part of rhodanine series has also shown potential against
Staphlococcus aureus (Gram positive) also indicates that electron withdrawing effect on this part of the series might have some influence on the overall bacterial activity.

On comparison of inter species antibacterial activity of the most effective rhodanine compound CTZ-1, it was seen that it showed much promise against Gram negative sp. Klebesilla aerogens. It might be inferred that a slight structural resemblance of rhodanine nucleus with the β-lactamase antibiotics might be the reason for its appreciable antibacterial activity against Gram negative sp. Klebesilla aerogens.

Although the antibacterial profile of synthesized novel compounds is somewhat less than the reference drug Cefixime, yet rhodanine based compounds have shown potential to give more antibacterial agents with further modifications. The basic structure of the rhodanine based compounds may thus serve as a template for the future building of more potent antibacterial agents with less toxic and resistance aspects.

![Figure 1](image1.png)  ![Figure 2](image2.png)

Agar plates showing antibacterial activity of CTZ-1 against Gram positive Staphlococcus aureus (Photo 1) and Gram negative Klebesilla aerogens (Photo 2)
Pharmacological evaluation of novel chromonyl-rhodanine and chromonyl–thiazolidinedione derivatives: As Antihyperglycemics
INTRODUCTION (Diabetes - an overview)

Diabetes mellitus is a heterogeneous group of metabolic conditions caused by either a lack of insulin, resistance to its effects, or both (Daneman et al., 2006). Diabetic patients universally experience hyperglycaemia as a result of the body’s inability to maintain normal blood glucose levels through homeostatic mechanisms. Diabetes has been recognised for millennia and was, until the development of insulin therapy, a fatal disease (Banting et al., 1922). Now all types of diabetes mellitus are treatable with insulin or anti-diabetic drugs although long term complications remain high.

Diabetes mellitus is the fifth most common cause of death in the world and it is estimated that one in eight deaths (12.2%) among 20 to 79-year-olds were attributable to this malady in 2010 (International Diabetes Federation). Diabetes mellitus is a chronic condition according to International Diabetes Federation (IDF), the number of diabetes patients has risen sharply in recent years (International Diabetes Federation (IDF), 2009; 2011). In 1985, 30 million people had diabetes worldwide; the number rose to 150 million in 2000, 285 million in 2010 and is estimated to be 435 million - 7.8% of the adult world population by 2030.

India has the highest number of diabetics in the world. By next year, the country will be home to 50.8 million diabetics, making it the world's unchallenged diabetes capital. And the number is expected to go up to 87 million - 8.4% of the country's adult population by 2030.
Diabetes mellitus is classified by four distinct categories based on aetiopathogenesis although two main categories of diabetes make up the bulk of cases. Type 1 diabetes mellitus (T1DM) (previously known as insulin dependent diabetes mellitus (IDDM)) and Type 2 diabetes mellitus (previously known as non-insulin dependent diabetes mellitus (NIDDM)) are the predominant in all areas of the world (International Diabetes Federation (IDF), 1998; 2009; 2011). Other categories include gestational diabetes and other specific types of diabetes. The latter are those associated with gene defects of pancreatic β-cell function and insulin resistance; other syndromes associated with diabetes; diseases of the exocrine pancreas; and endocrinopathies and diabetes induced by drugs, chemicals or infective agents, Detailed classification is given below (American Diabetes Association, 2003; 2009; 2011).

Classification of Diabetes Mellitus (American Diabetes Association)

I. Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency)
   A. Immune mediated
   B. Idiopathic

II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)

III. Other specific types
   A. Genetic defects of β-cell function
      1. Chromosome 12, HNF-1 (MODY*3)
2. Chromosome 7, glucokinase (MODY2)

3. Chromosome 20, HNF-4 (MODY1)

4. Mitochondrial DNA

**B. Genetic defects in insulin action**

1. Type A insulin resistance

2. Leprechaunism

3. Rabson-Mendenhall syndrome

4. Lipoatrophic diabetes

**C. Diseases of the exocrine pancreas**

1. Pancreatitis

2. Trauma/pancreatectomy

**D. Endocrinopathies**

1. Acromegaly

2. Cushing’s syndrome

3. Glucagonoma

4. Pheochromocytoma

5. Hyperthyroidism

6. Somatostatinoma

7. Aldosteronoma

**E. Drug- or chemical-induced**

**F. Infections**

**G. Uncommon forms of immune-mediated diabetes**

**H. Other genetic syndromes sometimes associated with diabetes**

**IV. Gestational diabetes mellitus (GDM)**

* Maturity onset diabetes of the young
The above classification includes changes to reflect the aetiopathogenesis rather than the therapeutic implications of the groups. It also reflects the fact that there are a range of presentations, as well as therapeutic treatments, all of which can change with time, meaning that patients should not be classified according to these overlapping criteria. The terms insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus and their acronyms, IDDM and NIDDM, were therefore removed from the classification as a result of the confusion that their use had generated. The terms type 1 and type 2 diabetes mellitus were retained, with Arabic numerals being used (American Diabetes Association 2011).

Type 2 diabetes mellitus includes the most prevalent form of diabetes, which results from insulin resistance, with or without a secretory defect. It primarily occurs with increasing age and is associated with genetic and environmental risk factors. Type 2 diabetes is commonly preceded by a long period of abnormal glycaemic control and is part of the metabolic syndrome associated with hypertension, dyslipidaemia and hyperglycaemia. The condition has a stronger genetic aetiology than T1DM although environmental factors such as diet, exercise, obesity and smoking will impact on the development of type 2 diabetes (Stumvoll et al., 2005).

About half of all diabetic patients have complications (Poortvliet et al., 2007). There are two types of complications, acute and chronic. Acute complications are hyper or hypoglycemia, with good blood-glucose control, this complication can be resolved. An acute hyperglycemia results in fatigue, a feeling of malaise, and thirst. Hypoglycemia results in sweating, trembling, and dizziness.
These symptoms are resolved when the glucose levels return to normal levels. Severe disturbance of glucose levels can lead to coma.

Chronic hyperglycemia is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body’s systems, especially the nerves and blood vessels. It can lead to micro- and macrovascular complications such as retinopathy, neuropathy, nephropathy, foot problems and cardiovascular diseases. Diabetes type 2 imparts a 2-fold to 4-fold risk of cardiovascular disease (Skyler et al., 2009), is also the most common cause of new blindness in the adults (Schellhase et al., 2003), and imparts an increased risk of amputations (Lavery et al., 2005). Treatment in Controlling hyperglycemia can be difficult and can require, in addition to lifestyle changes, oral antidiabetics and in addition insulin. Risk of complications can be reduced by reducing total cardiovascular risk. Not only a reduction of Hba1c but also tight control of blood pressure and lipids, along with lifestyle changes (weight, smoking behavior and physical activity) can reduce the risk of complications (Stratton et al., 2000; Manley et al., 2003; Liebl et al., 2002; Adler et al., 2008; UKPDS 38, 1998). Frequent patient education and checks (feet, weight, eyes, blood pressure and lipids) are needed to prevent and control for diabetes complications. Maintaining near to normal glucose levels and reducing cardiovascular risk factors lead to a reduction in mortality and morbidity rates in diabetes type 2 patients (Stratton et al., 2000; UKPDS 33, 1998). To overcome/control diabetes and induced complications a variety of molecules are available in the market which are prescribed to the patient with/without combination. Type of drugs with their brand names (in parenthesis) is listed below:
**Sulfonylureas** (Amaryl®, Diabeta®, Glynase Glucotrol®, Glucotrol XL®)

**Biguanides** (Glucophage®, Glucophage XR®)

**Thiazolidinediones** (Actos®, Avandia®)

**Alpha-glucosidase inhibitors** (Precose®, Glyset®)

**Meglitinides** (Prandin®, Starlix®)

**Dipeptidyl peptidase 4 inhibitors** (Januvia®, Onglyza®)

**Combinations of sulfonylureas plus metformin** (Glucovance®)

**Other Combinations** (Actosplus Met®, Avandaryl®, Avandamet®, Duetact®, Janumet®, Kombiglyze XR®)

Many companies are still endeavouring to find a new glucose lowering agent because of existing molecules have several disadvantages along with advantages (Table 1) (Rami *et al*., 2000; Lohray *et al*., 1998; Lohray *et al*., 2001; Oguchi *et al*., 2000; Nomura *et al*., 1999; Henke *et al*., 1998; Collins *et al*., 1998; Cobb *et al*., 1998; Shinkai *et al*., 1998; Reginato *et al*., 1998; Hulin *et al*., 1996; Clark *et al*., 1991; Momose *et al*., 1991).

**Table 1** Advantages and Disadvantages of Diabetes Drugs (Bennett *et al*., 2011; Bennett *et al*., 2011)

<table>
<thead>
<tr>
<th>Advantages:</th>
<th>Disadvantages:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>The sulfonylureas (glyburide, glimepiride, glipizide)</strong></td>
<td></td>
</tr>
<tr>
<td>-Fast onset of action</td>
<td>-Weight gain (5 to 10 pounds on average)</td>
</tr>
<tr>
<td>-No affect on blood pressure</td>
<td>-Heightened risk of hypoglycemia</td>
</tr>
<tr>
<td>-No affect on LDL cholesterol</td>
<td>-Glyburide has slightly higher risk of hypoglycaemia compared with glimepiride and glipizide</td>
</tr>
<tr>
<td>-Convenient dosing</td>
<td></td>
</tr>
<tr>
<td>-Low cost</td>
<td></td>
</tr>
</tbody>
</table>
- Lower risk of GI side effects than metformin

### Metformin

<table>
<thead>
<tr>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Low risk of hypoglycaemia</td>
<td>-Higher risk of GI side effects (nausea and diarrhea)</td>
</tr>
<tr>
<td>-Not linked to weight gain</td>
<td>-Cannot be taken by people with diabetes who have moderate or severe kidney disease or heart failure because of risk of lactic acid build-up</td>
</tr>
<tr>
<td>-Good effect on LDL cholesterol</td>
<td>-Less convenient dosing</td>
</tr>
<tr>
<td>-Good effect on triglycerides</td>
<td></td>
</tr>
<tr>
<td>-No effect on blood pressure</td>
<td></td>
</tr>
<tr>
<td>-Low cost</td>
<td></td>
</tr>
</tbody>
</table>

### The alpha-glucosidase inhibitors (acarbose, miglitol)

<table>
<thead>
<tr>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Slightly lower risk of hypoglycaemia compared to sulfonylureas</td>
<td>-Less effective than most other diabetes pills in lowering HbA1c.</td>
</tr>
<tr>
<td>-Not associated with weight gain</td>
<td>-Higher risk of GI side effects than other diabetes pills except metformin</td>
</tr>
<tr>
<td>-Decreases triglycerides</td>
<td>-Inconvenient dosing</td>
</tr>
<tr>
<td>-No effect on cholesterol</td>
<td>-High cost</td>
</tr>
</tbody>
</table>

### The thiazolidinediones (Actos, Avandia)

<table>
<thead>
<tr>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Low risk of hypoglycaemia</td>
<td>-Higher risk of heart failure</td>
</tr>
<tr>
<td>-Slight increase in “good” (HDL) cholesterol</td>
<td>-Weight gain (5 to 10 pounds)</td>
</tr>
<tr>
<td>-Actos linked to decreased triglycerides</td>
<td>-Linked to higher risk of edema (fluid build-up)</td>
</tr>
<tr>
<td>-Convenient dosing</td>
<td>-Linked to higher risk of anemia</td>
</tr>
<tr>
<td></td>
<td>-Increase in “bad” (LDL) cholesterol</td>
</tr>
<tr>
<td></td>
<td>-Avandia linked to increased triglycerides and higher risk of heart attack</td>
</tr>
<tr>
<td></td>
<td>-Actos linked to increased risk of bladder cancer</td>
</tr>
<tr>
<td></td>
<td>-Slower onset of action</td>
</tr>
<tr>
<td></td>
<td>-Rare risk of liver problems; requires monitoring</td>
</tr>
<tr>
<td></td>
<td>-Linked to increased risk of upper and lower limb fractures</td>
</tr>
<tr>
<td>The meglitinides (nateglinide, repaglinide)</td>
<td></td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>-No bad effect on cholesterol</td>
<td>-Repaglinide associated with risk of hypoglycemia and weight gain similar to sulfonylureas</td>
</tr>
<tr>
<td>-Rapid onset of action</td>
<td>-Nateglinide has less effect on HbA1c</td>
</tr>
<tr>
<td></td>
<td>-Inconvenient dosing</td>
</tr>
<tr>
<td></td>
<td>-High cost</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The DPP-inhibitors (Januvia, Onglyza)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>-When added to metformin, lower risk of hypoglycaemia compared with a sulfonylurea drugs</td>
<td>-Reduce HbA1c less than several other diabetes drugs</td>
</tr>
<tr>
<td>Few known side effects (but they are new drugs)</td>
<td>-May only be valuable as second drugs added to another medication</td>
</tr>
<tr>
<td>-Lower risk of GI side effects than metformin</td>
<td>-Less data on potential side effects compared to older drugs</td>
</tr>
<tr>
<td>-Convenient dosing</td>
<td>-High cost</td>
</tr>
</tbody>
</table>

In the case of those 2,4-thiazolidinediones already in the market have several side effects, such as anemia, edema, and body weight gain, have been reported (Iwamoto et al., 1996). Therefore, search for a new compounds with fewer side effects and a more advanced profile than existing drug molecules is the main focus of attention for chemists as well as for pharmacologists.
Materials and Methods

Animals:

White albino mice, aged 3 weeks (24-36g), were obtained from Sanjay Biologicals, Amritsar. The mice were housed in individual cages in an animal room (Animal Holding Unit, Punjabi University, Patiala) and put on standard pelleted diet, with water. Mice 5 weeks of age, were used for studies. They were housed about 5 mice per cage in a room with a 12h light and 12h dark regular alternate exposure and an ambient temperature of 22-25ºC.

Preparation of chromonyl-thiazolidines CTZ (1-6 & 11-16) solutions:

Stock solutions of Chromonyl-thiazolidinediones and Chromonyl-rhodanines were prepared by suspending in 200mg in 20 ml 0.25% CMC solution, 150mg in 25ml 0.25% CMC solution, and 150mg in 50ml 0.25% CMC solution for different doses. Freshly prepared doses were used for injection (intraperitoneally) in mice.

Streptozotocin (STZ)-induced diabetic mice:

The mice were intraperitoneally injected with a single dose of 100mg/kg STZ (Ito et al., 1999), freshly dissolved in citrate buffer (0.01 M, pH 4.5). Animals had free access to food and water after STZ injection. Diabetes in the mice was identified by polydipsia, polyuria and by measuring non-fasting serum
glucose concentration 48-h after injection of STZ. Mice with a serum glucose level above 200 mg/dl were selected for experiments.

**Determination of blood glucose by the glucose assay kit**

Glucose is first oxidized to gluconic acid and hydrogen peroxide. This reaction is catalyzed by glucose oxidase. The hydrogen peroxide formed reacts in the presence of peroxidase with 4-aminoantipyrine and $p$-hydroxybenzene sulfonate to form quinoneimine dye, with an absorbance maximum at 505 nm. The absorbance measured from the Auto analyser at Dept. Of Pharmaceutical science and Drug Research, Punjabi University, Patiala. The intensity of the color produced is directly proportional to the glucose concentration in the sample. The serum glucose concentration was expressed as mg/dl.

**Statistical analysis**

The results were expressed as means ±standard error of the mean (SEM). The data obtained from various groups were statistically analysed using one-way analysis of variance (ANOVA) followed by Tukey's multiple range test. The p value <0.05 was considered statistically significant.
RESULTS AND DISCUSSION

Preparation of target compounds (Scheme 1, Table 1) and their characterization through $^1$H-NMR and $^{13}$C-NMR and spectral techniques has already been discussed in Chapter: I-III.

Scheme 1

Table 1 Chromonyl-rhodanines and Chromonyl-thiazolidinediones.

<table>
<thead>
<tr>
<th>Target Molecules</th>
<th>(CTZ-1 to CTZ-6)</th>
<th>(CTZ-11 to CTZ-16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
</tr>
<tr>
<td>2</td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
</tr>
<tr>
<td>3</td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
</tr>
<tr>
<td>4</td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
</tr>
</tbody>
</table>
With a thought for a quest for new antihyperglycemic agents in mind, thiazolidinedione and rhodanine derivatives have been synthesized. This section deals with pharmacological evaluation of newly synthesized novel chromonyl rhodanine and chromonyl thiazolidinediones for antihyperglycemic activity in streptozotocin (STZ) induced diabetic mice (Ito et al., 1999).

Preference of use of Streptozotocin over Alloxan in Diabetic animal models

Chemically induced type I & II diabetes is the most commonly used animal model of diabetes. Alloxan (2, 4, 5, 6-tetraoxo hexahydro pyrimidine) was the first agent that was reported to produce permanent diabetes in laboratory animals (Dunn et al., 1943). Streptozotocin (STZ) has replaced alloxan as the principal agent used to produce experimental diabetes. This is due to the greater selectivity of β-cells for STZ (Junod et al., 1969) and lower mortality rate seen in STZ-diabetic animals (effective diabetogenic dose of STZ is four or five times less than its lethal dose) (Hoftiezer et al., 1973).
The chemical structure of STZ (Figure 1) comprises a glucose molecule with a highly reactive nitrosourea side chain that is thought to initiate its cytotoxic action. As previously reported, diabetes was consistently produced at doses of 50-70 mg/kg of STZ (ArRajab et al., 1993). The absence of ketosis in animals having received intravenous STZ at doses of 65 mg/kg or less is adequately explained by incomplete, although marked, insulin depletion (Junod et al., 1969).

The glucose moiety directs this agent to the pancreatic β-cells, where it binds to a membrane receptor to cause structural damage (Johansson et al., 1978). The deleterious effect of STZ results from the generation of highly reactive carbonium ions (CH$_3^+$) that cause DNA breaks by alkylating DNA bases at various positions, resulting in activation of the nuclear enzyme, poly(ADP-ribose) synthetase, thereby depleting the cellular enzyme substrate (NAD$^+$), leading to cessation of NAD$^+$-dependent energy and protein metabolism.

This in turn leads to reduced insulin secretion (Yamamoto et al., 1981). It has been suggested that free radical stress occurred during β-cell destruction mediated by mononuclear phagocytes and cytokines (Pitkanen et al., 1992; Nagy et al., 1989). Since free radical scavengers have been demonstrated to protect
against the diabetogenic properties of STZ (Robbins et al., 1980), it is likely that oxidative stress may play a role in determining STZ toxicity.

In the present study, the antihyperglycemic effects of chromonyl-rhodanines and thiazolidinediones (CTZ 1–6 and CTZ 11-16) on STZ-induced diabetic mice model, were evaluated taking Rosiglitazone as a reference drug. Firstly, two of the prototype compounds CTZ 1 and CTZ 11 of each class i.e. chromonyl- rhodanines and chromonyl-thiazolidinediones, were investigated for hypoglycaemic effect using OGTT (Trinder et al., 1969). Animals were grouped into five groups of five mice (weight 24-36g) for each compound. Animals fasted overnight. Retaining one group as control other three groups were administered CTZ-1/CTZ-11 in dose of 50mg/kg, 100mg/kg, 200mg/kg of the bodyweight intraperitonealy. Last group was injected Rosiglitazone intraperitonealy. Glucose (1mg/gm) was administered to the mice and blood samples were withdrawn at 0, 30, 60, 90 and 120 min. Test compounds significantly decreased the glucose level of the animals as compared to the control animals.
It is apparently clear from figure 7 that CTZ-1 with a dose of 100mg/kg and 200mg/kg has significantly reduced the glucose level in comparison to dose 50mg/kg. It is further concluded that dose response of CTZ-1 with 100mg/kg and 200mg/kg was near to that obtained from standard drug rosiglitazone 4 mg/kg.

Similarly (OGTT) was done for 2,4-thiazolidinedione based compound CTZ-11 and better results were obtained (Figure 8). CTZ-11 in a dose of 200mg/kg decreased glucose level as effectively as standard drug rosiglitazone 4 mg/kg. Similarly, dose response of CTZ-11 with 50mg/kg and 100mg/kg was also found good.

**Figure 7** Hypoglycemic effect of CTZ-1 in OGTT
Based on the results, 50mg/kg, 100mg/kg and 200mg/kg dose pattern was selected for CTZ-1 and CTZ-11 for evaluation of anti hyperglycaemic effects on streptozotocin induced diabetic mice model. Their respective doses were prepared in 0.25% CMC solution for intraperitoneal administration.

To induce diabetes streptozotocin was injected in white albino mice (100 mg/kg, i.p.). After 48 h, glucose level was estimated. After the confirmation of diabetes (glucose level>200 mg/dl), drug treatment was started and continued for 28 days. Test compounds were found to significantly attenuate the increased glucose level as compared to control animals. Rosiglitazone was used as standard drug. It is quite evident from graphical illustrations that CTZ-1 in 200mg/kg showed very good diabetic control in 28 days (figure 9,10 and 11) bringing uniform decrease in glucose level, which was not experienced in standard drug rosilitazone (irregular decrease in glucose level) (figure 9 and 10).
Figure 9 Effect of CTZ-1 (50mg, 100mg, 200mg) treatment on increased glucose level (n=5). The values are expressed as means ±SEM. a p<0.05 vs control 0 week, b p<0.05 vs 1st week, c p<0.05 vs 2nd week, and d p<0.05 vs 3rd week.

Figure 10 Effect of CTZ-1 (50mg, 100mg, 200mg) treatment on increased glucose level (n=5). The values are expressed as means ±SEM. a p<0.05 vs control 0 week, b p<0.05 vs 1st week, c p<0.05 vs 2nd week, and d p<0.05 vs 3rd week.
Other doses of CTZ-1 have also shown good antihyperglycemic activity (figure 9 and 10). On comparing the antihyperglycemic activity of CTZ-1 (200 mg/kg) with other rhodanine based compounds CTZ-2 to CTZ-6 (200 mg/kg), only CTZ-1 was found to have shown the best results (Figure 11).

Figure 11 Effect of CTZ-1-6 (200mg) treatment on increased glucose level (n=5). The values are expressed as means ±SEM. a$p<0.05$ vs control 0 week, b$p<0.05$ vs 1st week, c$p<0.05$ vs 2nd week, and d$p<0.05$ vs 3rd week.

Similarly, daily administration of CTZ-11 (50mg/kg, 100mg/kg and 200mg/kg) 28 days in streptozotocin induced diabetic mice, caused a significant reduction in blood glucose level when compared with the vehicle-treated control mice and day zero value (Figure 12-14). Similarly administration of rosiglitazone (4 mg/kg) 28 days caused a significant reduction in the blood glucose level (P<0.001) in streptozotocin induced diabetic mice when compared to vehicle and day zero values. There was a significant decrease in serum glucose level in the CTZ-11 treated mice when compared to the vehicle-treated control mice. CTZ-11
in doses 100mg/kg and 200mg/kg showed glucose-lowering activity profile comparable to standard drug rosiglitazone (figure 12-13).

**Figure 12** Effect of CTZ-11 (50mg, 100mg, 200mg) treatment on increased glucose level (n=5). The values are expressed as means ±SEM. a<p<0.05 vs control 0 week, b<p<0.05 vs 1st week, c<p<0.05 vs 2nd week, and d<p<0.05 vs 3rd week.

**Figure 13** Effect of CTZ-11 (50mg, 100mg, 200mg) treatment on increased glucose level (n=5). The values are expressed as means ±SEM. a<p<0.05 vs control 0 week, b<p<0.05 vs 1st week, c<p<0.05 vs 2nd week, and d<p<0.05 vs 3rd week.
Other doses of CTZ-11 have also shown good antihyperglycemic activity (figure 9 and 10). On comparison, antihyperglycemic activity of CTZ-11 (200 mg/kg) with CTZ-12 to CTZ-16 (200 mg/kg), only CTZ-11 have shown the best results, yet CTZ-15 has also shown good effect (P<0.05) up to third week (Figure 14).

Figure 14 Effect of CTZ-11-16 (200mg) treatment on increased glucose level (n=5). The values are expressed as means ±SEM. \(^{a}p<0.05\) vs control 0 week, \(^{b}p<0.05\) vs 1\(^{st}\) week, \(^{c}p<0.05\) vs 2\(^{nd}\) week, and \(^{d}p<0.05\) vs 3\(^{rd}\) week.

The antihyperglycemic activity of the screened compounds revealed that unsubstituted compound CTZ-1 and CTZ-11 have reduced elevated blood sugar levels significantly (P<0.05). Compound CTZ-11 (dose 200mg/kg), being most active derivative, has shown glucose-lowering activity profile comparable to standard drug rosiglitazone. Compound CTZ-1 (dose 200mg/kg) has produced moderate decrease in glucose levels on comparison with control (Figure 15). The high bioactivity of compounds CTZ-1 and CTZ-11 makes them a suitable lead to
develop new chemical entities for potential use in the treatment of Type-2 diabetes.

**Figure 15** Effect of CTZ-1 & 11 (200mg) treatment on increased glucose level (n=5). The values are expressed as means ±SEM. a\(p<0.05\) vs control 0 week, b\(p<0.05\) vs 1\(^{st}\) week, c\(p<0.05\) vs 2\(^{nd}\) week, and d\(p<0.05\) vs 3\(^{rd}\) week.