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Synthesis and evaluation of novel thiazolidinedione derivatives for antibacterial activity

Shital L. Nawale* and Avinash S. Dhake

Department of Pharmaceutical Chemistry, S.M.B.T. College of Pharmacy, Nashik, Pune University, M.S., India.

ABSTRACT

A series of novel thiazolidinediones were prepared by incorporating pharmacologically significant moieties viz. ester, hydrazide and substituted amine groups linked to the central phenyl ring as well as replacement of phenyl by heterocycle like substituted furan ring by employing multistep synthetic protocols. The structures of the newly synthesized target molecules were established by spectral data. The synthesized compounds were tested for their in vitro antibacterial activity against the Gram-positive viz. Bacillus subtilis, Staphylococcus aureus and Gram-negative viz. Pseudomonas aeruginosa bacteria. The compounds A_2 *and* A_5 *containing thiosemicarbazide moiety showed good spectrum of activity with MIC values of 31.25µg/ml.*

Keywords: Thiazolidinediones, thiosemicarbazide, antibacterial activity.

INTRODUCTION

Wide spread resistance to many commercially available antibiotics is emerging and resistance to these agents will only increase day by day[1]. Bacterial infections can cause some of the most serious diseases and widespread epidemics in the world. With the increase in resistance of bacteria to antibiotic treatment, it is essential to develop novel approaches and new anti-bacterial agents as alternatives to various existing antimicrobial therapies[2].

The treatment of infectious diseases still remains an important and challenging issue because of a combination of factors including emerging infectious diseases and increasing number of multi-drug resistant microbial pathogens with particular relevance for Gram-positive bacteria. Although a large number of antibiotics and chemotherapeutics are available for medical use, the emergence of old and new antibiotic resistant bacterial strains in the last few decades constitutes a substantial need for the new classes of antibacterial agents[3].

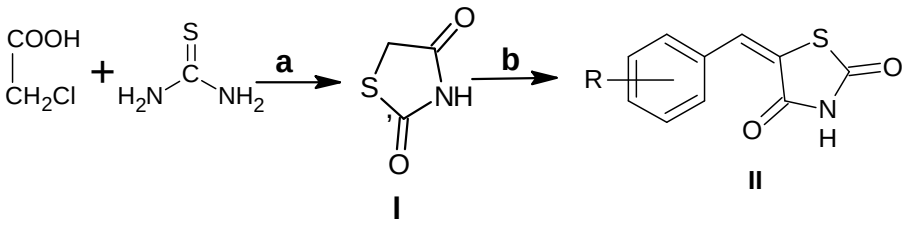
The 2,4-thiazolidinedione derivatives constitute an important class of heterocyclic compounds for which diverse biological properties such as antibacterial and antifungal, antiviral, antitumor and antidiabetic have been reported. Antibacterial activity is of particular importance given the dramatic rise of drug-resistant bacteria and the paucity of new agents currently in development[4].

Our aim was to study and develop a more chemically versatile and diverse thiazolidinediones incorporating the required elements of a suitable pharmacophore consisting of TZD-aromatic linker-ester/hydrazide/amine residues.

MATERIALS AND METHODS

1.1. Experimental

The melting points were determined by open cup capillary method and are uncorrected. TLC analyses were performed on glass plates using silica gel G_{60} and spots were visualized either by ultraviolet light or by iodine vapours. IR spectra were recorded as KBr pellets, using JASCO 4100 FTIR spectrophotometer. ^{1}H-NMR were obtained with BRUKER AVANCE II 400 NMR spectrometer and are reported as parts per million (ppm) downfield to TMS. A mass spectrum was recorded on PRA-O-336 wiff Turbo Spray mass spectrometer.

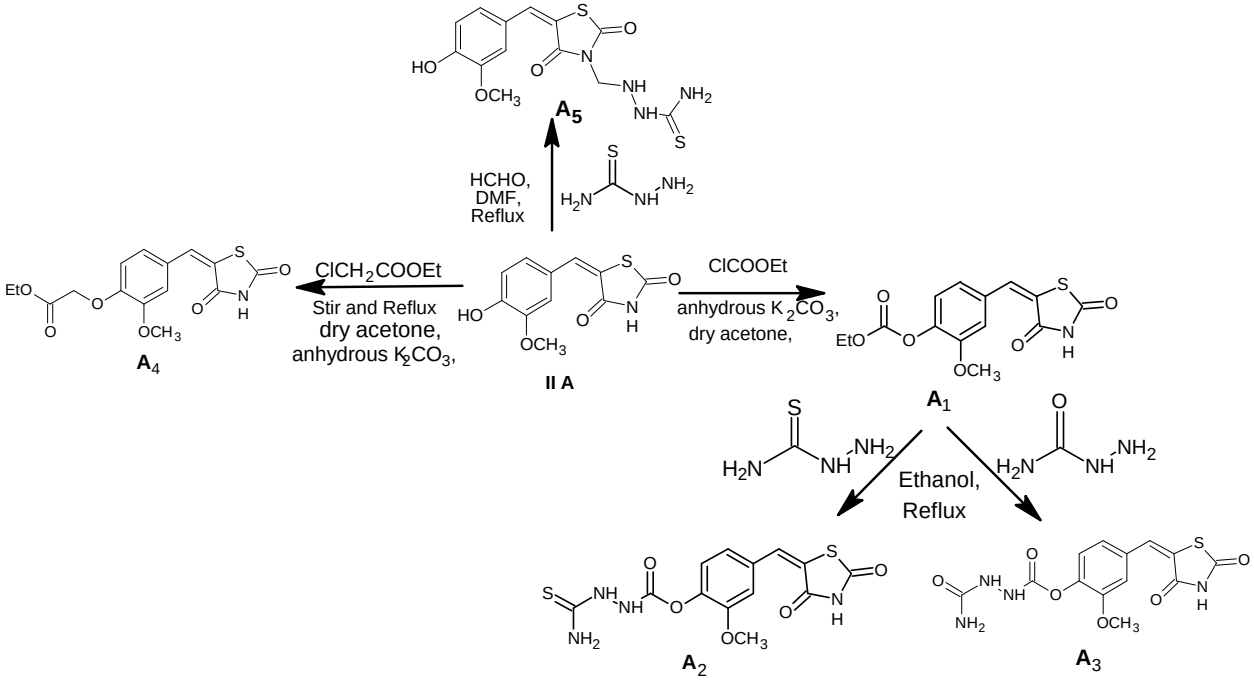


I **II**

***IIA**: R= 4-OH,3-OCH$_3$; **IIB**: R= 4-OH; **IIC**: R= 4-Cl.*

Scheme 1: Synthesis of 2,4-TZD(I) and substituted-benzylidene-2,4-TZDs(II)

***(a)**H$_2$O, conc. HCl, reflux for 10-15 h **(b)**substituted benzaldehyde, toluene, piperidine, reflux for 6-8 hrs.*



Scheme 2: Synthesis of A$_1$-A$_5$

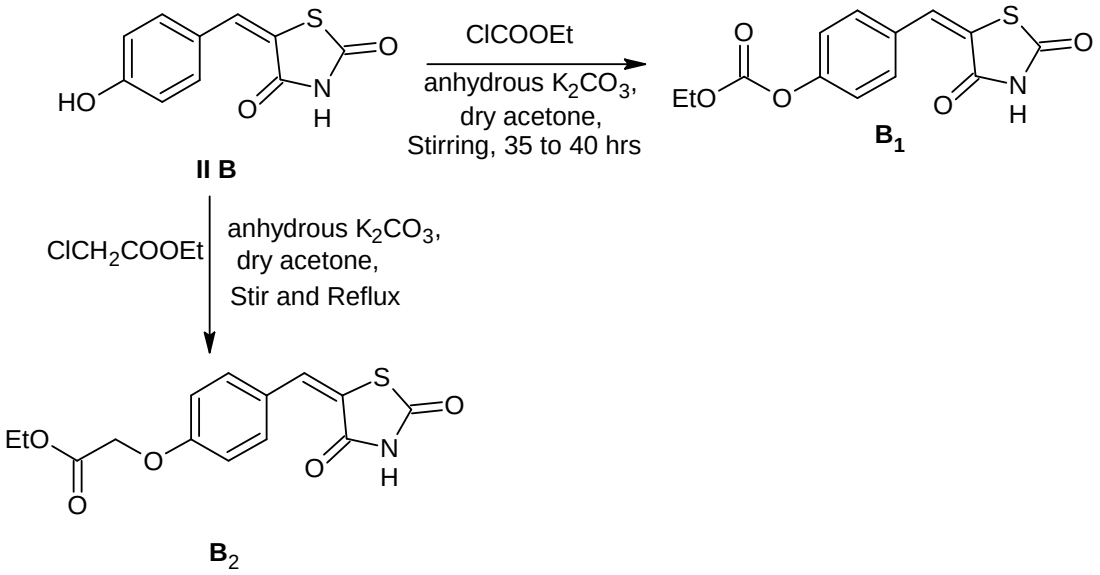
*1.1.1. Synthesis of 2,4-thiazolidinedione **(I)**[5,6,7] :*

In a 250 ml three necked flask, to a solution of chloroacetic acid (0.6 mole) in 60 ml of water, was added a solution of thiourea (0.6 mole) dissolved in 60 ml of water. The mixture was stirred for 15 min to form a white precipitate, accompanied by considerable cooling. To the contents of the flask was then added slowly 60 ml of concentrated HCl dropwise, the flask was then connected with a reflux condenser and gentle heat applied to effect complete solution, after which the reaction mixture was stirred and refluxed for 10-15 hrs at 100-110°C. On cooling the contents of the flask solidified to a cluster of white needles. The product was filtered and washed with cold water to remove traces of hydrochloric acid and dried. It was purified by recrystallization from ethyl alcohol. White crystals yield 80%, m.p. 123-125°C.

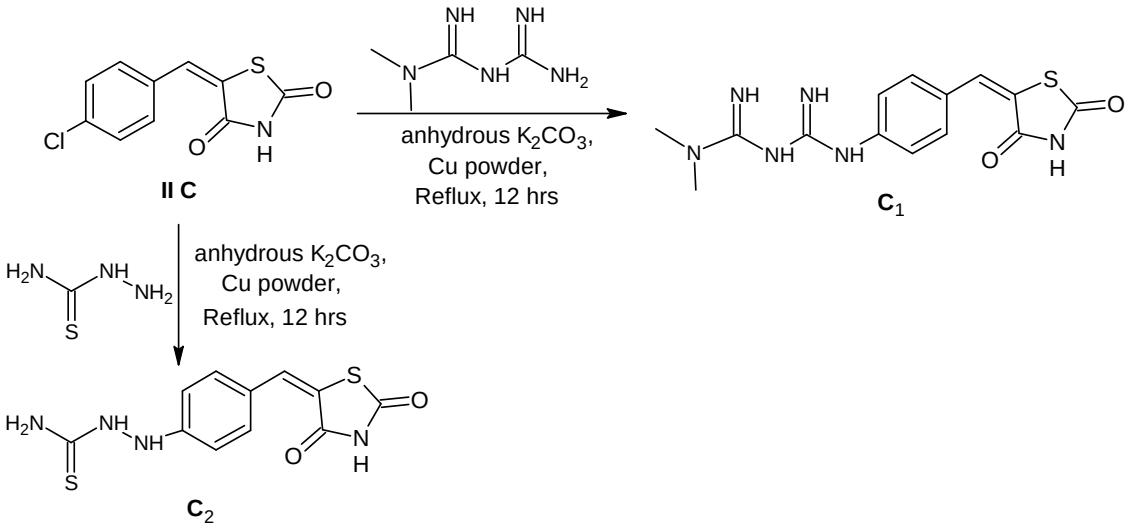
*1.1.2. Synthesis of 5-substituted-2,4-thiazolidinedione **(II)** [5,8,9] :*

In a 250 ml three-necked round-bottomed flask provided with a Dean-Stark apparatus, a substituted benzaldehyde i.e. vanillin or 4-hydroxy-benzaldehyde or 4-chlorobenzaldehyde (0.188 mole) and 2,4-thiazolidinedione (0.188 mole) were together suspended in toluene. To this a catalytic amount of piperidine (1ml) was added. The mixture was stirred and refluxed. After the complete removal of water and when the temperature reached above 110°C the

reaction mixture was stirred for further 6-7 hr. On cooling the product precipitated out from toluene. The product was filtered and washed with cold dry toluene and dry ethanol to give **IIA** or **IIB** or **IIC** respectively. TLC was performed in Benzene:Ethyl acetate (8:2). **IIA** [(5*E*)-5-(4-hydroxy-3-methoxybenzylidene)-1,3-thiazolidine-2,4-dione] : Yellow powder, yield 88%, m.p. 225-227°C, R_f value 0.52. **IIB [**(5*E*)-5-(4-hydroxybenzy-lidene)-1,3-thiazolidine-2,4-dione] : Yellow powder, yield 90%, m.p. 274-276°C, R_f value 0.47. **IIC [**(5*E*)-5-(4-chlorobenzylidene)-1,3-thiazolidine-2,4-dione] : pale yellow powder, yield 83%, m.p. 222-224°C, R_f value 0.86.



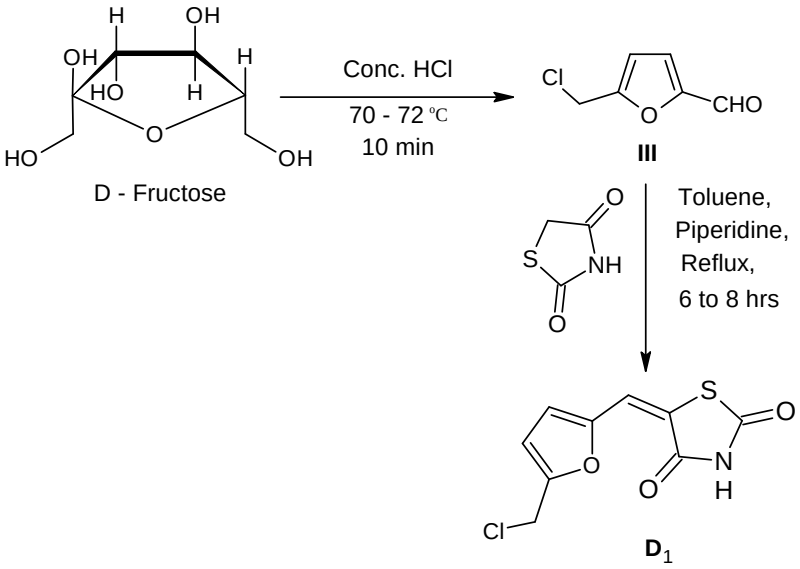
Scheme 3: Synthesis of B_1 and B_2



Scheme 4: Synthesis of C_1 and C_2

1.1.3. Synthesis of ***Thiosemicarbazide****[10] :*

To a 6.22 gm (6.4 ml) hydrazine hydrate (99 %) in 5 ml water was added 9.1 gm of solid ammonium-thiocyanate. The reaction mixture began to evolve ammonia immediately and additional quantity was removed on heating to boiling point. On reaching boiling point, heating was continued until the temperature reached 130 ºC following which the mixture was cooled to 15 ºC and 2 ml water was added. The product obtained was separated by filtration and washed by 6 ml of water which was combined with filtrate. A total of five boildowns were run as described above. The maximum temperatures reached in 2nd, 3rd, 4th and 5th boildowns were, respectively, 132,132,146 and 146 ºC while the quantity of fresh water added to the filtrates was 8,8,13.5 and 13.5 ml in the boildown procedure. All the residues were then combined and recrystallized from ethanol. Yield was 71 % and m.p. 176-178 ºC. (Ref. m.p.182-184 ºC[11]).



Scheme 5: Synthesis of D_1

1.1.4. General Procedure for synthesis of 4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene) methyl]-2-methoxyphenyl ethyl carbonate ***(A_1)*** *and 4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl ethyl carbonate* ***(B_1)*** *[12] :*
The compound **IIA** (for synthesis of ***A_1***) or **IIB** (for synthesis of ***B_1***) (1 mole) was suspended in dry acetone and to this anhydrous K_2CO_3 (1 mole) was added. To this mixture, ethylchloroformate (1mole) was then added dropwise. This was then stirred for 35- 40 hrs at room temperature and reaction was monitored by TLC. The product was then filtered and washed with water. It was purified by recrystallization from ethanol.

1.1.4.1. 4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]-2-methoxyphenylethyl- carbonate ***(A_1):*** $C_{14}H_{13}NO_6S$; Yield: 95 %; m.p.: 200-202 ºC; TLC- Benzene:Ethyl acetate (8:2), R_f value: 0.72. IR cm^{-1}: Imide C=O str.(1705,1756); ester C=O str.(1786); C-O-C (ether) str. (1262,1216); =C-H str.(2995); N-H str.(3223); C-N str.(1302); C-S str.(679); Aro. C=C str.(1547). ^{1}H NMR (δ ppm, DMSO): 1.27(t,3H,CH_3); 2.58(s,DMSO); 3.91(s,3H,OCH_3); 4.52(q,2H,CH_2); 7.11-7.75 (m,3H,Aro. CH); 7.93(s,H,CH-benzylidene); 11.76(s,H,N-H of TZD).

1.1.4.2. 4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl ethyl carbonate ***(B_1):*** $C_{13}H_{11}NO_5S$; Yield: 81 %; m.p.: 254-256ºC; TLC- Benzene:Ethyl acetate (8:2), R_f value: 0.58. IR cm^{-1} : Imide C=O str.(1690, 1746); ester C=O str.(1765); Aro. C=C str.(1501); C-S str. (687); alkyl C-H str.(2917); Aro. C-H str.(3015); N-H str. (3423).

1.1.5. General Procedure for synthesis of 4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene) methyl]-2-methoxyphenyl 2-carbamothioylhydrazinecarboxylate ***(A_2)*** *and 4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]-2-methoxyphenyl 2-carbamoylhydrazinecarboxylate* ***(A_3)*** *[12]:*

To a suspention of A1 (0.01 mole) in 40 ml ethanol, thiosemicarbazide (for synthesis of ***A_2***) or semicarbazide (for synthesis of ***A_3***) (0.015 mole) was added and the reaction mixture was refluxed for 7-8 hrs. The resulting mixture was allowed to cool in ice bath and filtered. The product obtained was then dried and recrystallized with water.

4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)-methyl]-2-methoxyphenyl-2-carbamo-thioylhydrazine-carboxylate ***(A_2):*** $C_{13}H_{12}N_4O_5S_2$; Yield: 65%; m.p.: 218-220ºC; TLC- Benzene:Ethyl acetate (8:2), R_f value: 0.57. IR cm^{-1}: Imide C=O str.(1608,1705); Ar-O-C=O str.(1746); C-O-C (ether) str.(1266, 1300); =C-H str.(2998); NH_2 str.(3459); N-H str.(3219, 3423); NH_2-CS-NH str.(1169); C=S str.(1369); C-S str. (680); C=C str. (1518). ^{1}H NMR (δ ppm, DMSO): 3.90(s,3H,OCH_3); 2.59 (s,DMSO); 7.10-7.66(m,3H,Aro. CH); 7.73(s,H,CH-benzylidene); 8.64(s,2H,NH_2); 10.05-10.38 (m,2H,NH of TSC group); 12.31(s,H,NH of TZD).

4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]-2-methoxyphenyl-2-carbamoyl-hydrazinecarboxylate ***(A_3):***
$C_{13}H_{12}N_4O_6S_2$; Yield: 60%; m.p.: 174-176ºC; TLC- Benzene:Ethyl acetate (8:2), R_f value: 0.34. IR cm^{-1}: Imide C=O str.(1608, 1704); Ar-O-C=O str.(1745); NH_2-C=O (1517); C-O-C(ether) str.(1262, 1302); =C-H str.(3072); NH_2 str.(3463); N-H str.(3226, 3419); NH_2-CS-NH str.(1172); C=S str.(1369); C-S str. (680), Aro. C-H str. (2997).

*1.1.6. General Procedure for synthesis of ethyl {4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene) methyl]-2-methoxyphenoxy}acetate **(A_4)** and ethyl {4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}acetate **(B_2)**[13] :*

A mixture of **IIA** (for synthesis of ***A_4***) or **IIB** (for synthesis of ***B_2***) (0.1 mole) and anhydrous K_2CO_3 (0.15 mole) in an excess of dry acetone was stirred at reflux temperature for 3 h. To the stirred suspension, ethylchloroacetate (0.1 mole) in dry acetone (30 ml) was added dropwise over a period of 1 h at reflux temperature, and the refluxing continued for 10-12 hrs. After keeping the reaction mixture overnight, the excess of solvent was removed, and the residue was poured into crushed ice. The solid was then filtered, washed with water and recrystallized from ethanol.

*Ethyl{4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]-2-methoxyphenoxy}-acetate**(A_4):*** $C_{15}H1_5NO_6S$; Yield: 82%; m.p.: 114-116ºC; TLC- Benzene:Ethyl acetate (8:2), R_f value: 0.89. IR cm^{-1}: Imide C=O str.(1730, 1689); ester C=O str.(1766), C-O-C(ether) str.(1226, 1261); =C-H str.(2990); N-H str.(3445); C-S str. (670).

*1.1.6.1. Ethyl-{4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}acetate**(B_2):*** $C_{14}H_{13}NO_5S$; Yield: 79%; m.p.: 148-150ºC; TLC- Benzene:Ethyl acetate (8:2), R_f value: 0.8. IR cm^{-1}: Imide C=O str.(1670, 1721); ester C=O (1751); Aro. C=C str.(1509); C-S str. (686); alkyl C-H str.(2948); Aro. C-H str.(2995); N-H str. (3454).

*1.1.7. Synthesis of 2-{[(5E)-5-(4-hydroxy-3-methoxybenzylidene)-2,4-dioxo-1,3-thiazolidin-3-yl]methyl}hydrazine carbothioamide**(A_5)**[14] :*

The compound **IIA** (0.004 mole) was dissolved in N,N-dimethyl formamide (15 ml). To this, thiosemicarbazide (0.004 mole) was added followed by addition of formalin solution (38%) (0.004 mole) and was refluxed for 20 hrs. The refluxed solution was kept in the refrigerator for 48 hrs. The product obtained was filtered, dried and recrystallized using ethyl acetate or ethanol.

*2-{[(5E)-5-(4-hydroxy-3-methoxybenzylidene)-2,4-dioxo-1,3-thiazolidin-3-yl]methyl}hydrazine-carbothioamide**(A_5):*** $C_{13}H_{14}N_4O_4S_2$; Yield: 76%; m.p.: 202-204ºC; TLC- Benzene:Ethyl acetate (8:2), R_f value: 0.64. IR cm^{-1}: Imide C=O str.(1690,1734); C-O-C (ether) str.(1281); =C-H str.(3056); Aro. C=C str.(1514); NH_2 str.(3451); NH_2 ben.(1591); NH ben.(1514); NH-CS-NH_2 (1149); C=S str.(1213); Ali.C-N str.(1033), C-S str. (686); alkyl C-H str.(2776); Aro. C-H str.(2929). ^{1}H NMR (δ ppm, DMSO): 3.87(s,3H,OCH_3); 2.55(s,DMSO); 4.21(d,2H,CH_2); 6.91-7.05(m,3H, Aro. CH); 7.67(s,H,CH- benzylidene); 8.03-8.29(m,4H,NH and NH_2 of TSC gr.); 9.80 (s,H,OH).

*1.1.8. General Procedure for synthesis of N'-{4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl}-N,N-dimethylimidodicarbonimidic diamide**(C_1)** and 2-{4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl] phenyl} hydrazinecarbothioamide **(C_2)**[15] :*

A mixture of 4-chlorobenzylidene 2,4-thiazolidinedione (**IIC**) (0.003 mole) and metformin (for synthesis of ***C_1***) or thiosemicarbazide (for synthesis of ***C_2***) (0.003 mole) dissolved in ethanol was refluxed in DMF with anhydrous K_2CO_3 (0.003 mole) and catalytic amount of copper for 12 hrs. The resulting mixture was then allowed to cool in ice bath and filtered. The product was washed with water and dried. It was then recrystallized from ethanol.

*1.1.8.1.N'-{4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl}-N,N-dimethylimido-dicarbnimidic diamide**(C_1):*** $C_{14}H_{16}N_6O_2S$; Yield: 67%; m.p.: 250-252ºC; TLC- Benzene:Ethyl acetate (8:2), R_f value: 0.19. IR cm^-: Imide C=O str.(1670,1617); =C-H str.(2954); Aro.C=C str.(1547); =NH str.(3352); NH str. (3174); NH ben.(1489); Ali.C-N str.(1227); Aro.C-N(1310); C-S str. (676); alkyl C-H str.(2847); Aro.C-H str.(2923). ^{1}H NMR (δ ppm, DMSO): 2.54 (s,6H,2CH_3); 3.38-4.09(m,4H,NH and =NH); 7.39-7.51(m,4H, Aro.CH); 8.15(s,H,CH-benzylidene); 11.46 (s,H,N-H of TZD); MS: m/z : $(M-2)^+$ = 330.3.

*1.1.8.1. 2-{4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl}hydrazinecarbothioamide**(C_2):***
$C_{11}H_{10}N_4O_2S_2$; Yield: 61%; m.p.: 242-244ºC; TLC- Benzene:Ethyl acetate (8:2), R_f value: 0.68. IR cm^{-1}: Imide C=O str.(1690,1735); =C-H str.(2918); Aro. C=C str.(1516); NH_2 str. (3444); NH str.(3306); Ali.C-N str.(1286); Aro. C-N(1337); C-S str. (655); C=S str. (1404).

*1.1.9. Synthesis of (5E)-5-{[5-(chloromethyl)furan-2-yl]methylidene}-1,3-thiazolidine-2,4-dione **(D_1)** :*

In a conical flask, was placed 6-7 ml of 32 % HCl and was heated to 50 ºC. To this, powdered D-fructose (0.005 mole) was added and dissolved with shaking. The dark coloured solution was heated rapidly to 70-72 ºC, kept at this temperature for 10 min (lengthening the time of heating or raising the temperature above 72 ºC is harmful) and poured at once onto crushed ice in large bath (preferably in hood). The product **III** was then filtered, washed with water and dried[16]. The yield was 28 % and m.p. > 250 ºC.

The compound **III** and 2,4-thiazolidinedione (**I**) (0.188 mole) were together suspended in toluene in a 250 ml three-necked round-bottomed flask provided with a Dean-Stark apparatus. To this, a small amount of DMSO (5 ml) and

catalytic amount of piperidine (1ml) was added. The mixture was stirred and refluxed. After the complete removal of water and when the temperature reached above 110°C the reaction mixture was stirred for further 6-7 hr. On cooling the product precipitated out from toluene. The product was filtered and washed with cold dry toluene and dry ethanol[5,8,9].

(5E)-5-{[5-(chloromethyl)furan-2-yl]methylidene}-1,3-thiazolidine-2,4-dione ***(D_1)***: $C_9H_6ClNO_3S$; Yield: 85%; m.p.: >250ºC; TLC- Benzene:Ethyl acetate (8:2), R_f value: 0.48. IR cm^{-1}: Imide C=O str.(1741,1705); =C-H str.(2923); C-S str. (665); alkyl C-H str. (2853); Furan ring skeleton (1595); C-Cl str. (777); C-H of Furan (1011), N-H str.(3447). ^{1}H NMR (δ ppm, DMSO): 3.37(s,2H,CH_2); 2.66(s,DMSO); 7.23 (d,H,CH); 7.66(d,H,CH); 8.10(s,H,CH Benzylidene); 10.28(s,H,NH of TZD).

1.2 Antibacterial screening [17,18]:

For the antibacterial activity, the compounds were dissolved in dimethylsulfoxide (DMSO). To each tube containing sterilized Nutrient broth medium (5 ml), 5 ml of drug solution was added. The serial dilutions were made to obtain concentrations (in µg/ml) such as 250, 125, 62.5, 31.25, 15.62. The minimum inhibitory concentrations (MIC) were determined using the serial dilution technique. Each tube was inoculated with particular microorganism i.e. *Bacillus subtilis*, *Pseudomonas aerugenosa* and *Staphylococcus aureus* and then all the tubes were incubated at 35-37°C for 24 hrs. Positive control tubes (microorganism + broth + Streptomycin) and negative control tubes (microorganism + broth + DMSO) were also prepared.

RESULTS AND DISCUSSION

The desired compounds have been synthesized by convenient synthetic route as shown in Schemes 1-5. In particular, the recognized pharmacophores like thiosemicarbazide, semicarbazide, guanidine and ester units were tagged on to the TZDs. First, the basic nucleus 2,4-thiazolidinedione (**I)** was obtained by reacting equimolar amounts of thiourea and chloroacetic acid[5,6,7]. The substituted benzylidene TZDs (**IIA-C**) were obtained via Knoevenagel condensation of 2,4-thiazolidinedione and substituted aldehydes in toluene at reflux in the presence of piperidine (scheme 1)[8,9]. The compound **IIA** and **IIB** were then treated with ethyl chloroacetate and ethyl chloroformate to give $\mathbf{A_1}$, $\mathbf{A_4}$, $\mathbf{B_1}$ and $\mathbf{B_2}$ by stirring at room temperature in acetone (schemes 2,3) [12,13]. The ethoxy group in $\mathbf{A_1}$ was replaced with thiosemicarbazide and semicarbazide by refluxing in ethanol to give $\mathbf{A_2}$ and $\mathbf{A_3}$ (scheme 2)[12]. The 3-N substitution in TZD ring was carried out in **IIA** by reaction with thiosemicarbazide and formaldehyde in DMF to give $\mathbf{A_5}$ (scheme 5) [14]. The compound $\mathbf{C_1}$ and $\mathbf{C_2}$ were obtained from **IIC** by refluxing with metformin and thiosemicarbazide in presence of anhydrous K_2CO_3 and Cu powder to replace -Cl group (scheme 4) [15]. The levulose was used to obtain substituted furfural (**III**)[16] which on Knoevenagel condensation with **I** gave $\mathbf{D_1}$ (scheme 6)[8,9].

Structures of all the synthesized compounds were confirmed by IR, ^{1}H NMR, and Mass spectral analyses. IR spectra of all the final compounds, showed characteristic peaks for N-H stretching in TZD ring in the range of 3174-3454cm^{-1} and C=O stretching in the range of 1608-1756 cm^{-1} to confirm the presence of thiazolidinedione ring system. ^{1}H NMR showed a characteristic broad singlet peak in the range of 10.28-12.31 δ ppm to confirm the presence of NH proton of thiazolidinedione scaffolds in final compounds. This large deshielding effect on NH proton is attributed to the presence of electron withdrawing carbonyl groups. The NMR data of compound $\mathbf{A_1}$ showed presence of CH_3 (1.27 ppm), CH_2 (4.5 ppm), N-H (11.76 ppm) and OCH_3 (3.9 ppm). While in case of $\mathbf{A_2}$, the peaks between 8.6-10.7 showed presence of 4 hydrogens. For compounds $\mathbf{B_1}$ and $\mathbf{B_2}$, the IR spectra reveals the presence of ester C=O group at 1765 and 1750 cm^{-1} respectively. The IR spectrum of $\mathbf{A_5}$ showed the peak of 1° amine function at 3451 cm^{-1} and C=S str. at 1213 cm^{-1} which are absent in its starting material **IIA** while the NMR spectrum showed absence of imide N-H which normally appears at 10.28 – 12.31 ppm in thiazolidinedione ring. The presence of 4 hydrogens of amine function in thiosemicarbazide moiety was confirmed by appearance of peak at 8.02-8.26 ppm. The attachment of metformin moiety directly to the phenyl ring at p-position was achived by replacing p-Cl group of 5-(4-chlorobenzylidene)-1,3-thiazolidine-2,4-dione (IIC). The IR spectrum of $\mathbf{C_1}$ reveals presence of =N-H (imine) at 3352 cm^{-1} and broad peak of N-H (amino) at 3174 cm^{-1}. The NMR spectrum showed presence of guanidine N-H between 3.38-4.09 ppm, six hydrogens of $2CH_3$ at 2.54 ppm. Also, the IR spectrum showed presence of NH_2 at 3444 cm^{-1}, C=S str. at 1404 cm^{-1} and aromatic C-N str. at 1337 cm^{-1}. The mass spectrum showed molecular ion peak at m/z=330.3 i.e. $(M-2)^+$. In case of compound $\mathbf{D_1}$, IR spectrum showed N-H str. at 3447 cm^{-1}, furan ring skeleton str. at 1595 cm-1 and CH_2Cl str. at 777 cm^{-1} and NMR spectrum reveals the peak of N-H at 10.28 ppm, arylidine =C-H at 8.10 ppm, CH_2 hydrogens at 3.37 ppm and C-H of furan ring at 7.1 and 7.5 ppm. This data conforms the structure of $\mathbf{D_1}$.

The synthesized compounds were tested for their *in vitro* antibacterial activity against the Gram-positive bacteria *viz. Bacillus subtilis, Staphylococcus aureus* and Gram-negative bacteria viz. *Pseudomonas aeruginosa*. The MIC

values were determined by using broth dilution method in nutrient broth media. Streptomycin was used as standard. The MIC was determined on the basis of presence or absence of turbidity. **Table no. 1** indicates the results of MIC for screened compounds in µg/ml.

Table No. 1: MIC (µg/ml) values for the screened TZD compounds

Comp. code	Microorganism		
	Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
A_1	62.5	31.25	62.5
A_2	31.25	31.25	31.25
A_3	62.5	31.25	31.25
A_4	62.5	31.25	31.25
A_5	31.25	31.25	31.25
B_1	62.5	62.5	125
B_2	62.5	31.25	125
C_1	62.5	125	62.5
C_2	31.25	62.5	125
D_1	125	125	62.5
Streptomycin	3.90	3.90	3.90

The synthesized compounds showed MIC values between 31.25–125 µg/ml. The investigation of antimicrobial screening revealed that some of the tested compounds exhibited moderate to good antibacterial activity. Particularly compounds **A_2**, **A_5** and **C_2** have shown good activity against *B. subtilis* with MIC value 31.25µg/ml while compound **D_1** showed less activity. All the compounds showed good activity (31.25µg/ml) against *S. aureus* except **B_1** , **C_2** (62.5µg/ml) and **D_1** (125µg/ml). In case of Gram-negative bacteria, the compounds **A_2 - A_5** have shown good activity (31.25µg/ml).

In conclusion, all of the compounds tested have shown moderate antibacterial activity and we noticed that the compounds with thiosemicarbazide group (**A_2** and **A_5**) were found to be most potent followed by compounds with semicarbazide and ester residue (**A_3** and **A_4**) as lipophilic chain. However, the antibacterial potency of all compounds was less than that of the standard used, streptomycin.

CONCLUSION

All the new synthesized compounds were tested for their *in vitro* antibacterial activity against the Gram-positive *viz. Bacillus subtilis*, *Staphylococcus aureus* and Gram-negative viz. *Pseudomonas aeruginosa* bacteria using broth dilution method. The MIC was determined and streptomycin was used as a standard drug.

The screened compounds showed MIC values between 31.25–125µg/ml against all the bacteria used for testing. The compounds **A_2** and **A_5** containing thiosemicarbazide moiety showed good spectrum of activity at MIC values of 31.25 µg/ml and compounds **A_3** and **A_4** containing semicarbazide moiety showed moderate activity. All compounds were less active than the standard drug streptomycin.

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