Synthesis and Evaluation of Antimicrobial Activity of 4 - Thiazolidinones of Ketoanils

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Abstract **- A new series of 4-Thiazolidinones with heterocyclic nucleus have been synthesised by cyclocondensation of ketoanils with thioglycolic acid under reflux. The ketoanils obtained by condensation of methyl glyoxal, methyl–bis-glyoxal and glyoxoacetanilide with various amines under reflux.The structure of synthesised 4-Thiazolidinoes confirmed by elemental analysis and spectral (IR, H^1NMR) data. Some of these compounds were evaluated for *in- vitro* growth inhibitory activities against *Staphylococcus aureus, Stalophylococcus epidermidis, Micrococcus leuteus* bacteria and C*andida ablicanes* fungi.**

Keywords- **Glyoxals, Ketoanils, Amines, Thioglycolic acid, Thiazolidinones, Antibacterial and Antifungal activities.**

I. INTRODUCTION

High versatility in properties of Thiazolidinones, leading to manifold applications in several field of development, viz. medical sciences[1-7,11,15,18,23], industries [22],analytical[8,9], coordination[24] and synthetic chemistry[10,11,15].etc. high stability, easy synthesis[10] and tendency to participate in substitution and condensation reactions have been temptation in frequent synthesis and characterization of Thiazolidinones. Novel structural features of Thiazolidinones leading to occurrence of keto enolic forms in equilibrium on account of adjacent position of active methylene and ketonic groups in ring make these compounds preponderant over their heterocyclics. Scare knowledge in the chromatographic[8,19] and coordination chemistry of Thiazolidinones in spite of colours and presence of three potent donors, nitrogen, oxygen and sulphur, in heterocyclic ring, attach, enhanced interest with these organics.

Some important methods of synthesis of Thiazolidinones involving cyclocondensation or cyclisation open chain compound are also reported. Distinguished structural[11,15], biological[15,16, 23], analytical and and coordination features of Thiazolidinones in general, and scare knowledge in cyclocondensation products of Ketoanils with Thiocarboxylic acids and nonavailability of any reference on Thiazolidinones obtained by cyclocondensation of Ketoazomethinines of Methyl glyoxal, Methyl-bis-glyoxal and Glyoxoacetanilide with amines in particular aroused our interest to synthesise to some new compounds by the method of cyclocondensation of these glyoxals. The choice of method of cyclocondensation for synthesising Thiazolidinones lies in its easy and convenient handling and high yield.

II. MATERIALS AND METHODS

Reagents and Conditions:

1. Acetone, Acetyl acetone, Acetoacetanilide, Selenium dioxide, Ether, reflux for 6 h.
2. Methyl glyoxal, Methyl-bis-glyoxal and Glyoxoacetanilide, Amines, Ether,
3. Ether- Benzene (1:1v/v) , reflux for 6-8 h.
4. Ketoanil, Thioglycolic acid(98%),Absolute Alcohol, Dry Benzene , reflux for 15-25 h.

Synthesis of 4-Thiazolidinones takes place in three steps;

Step-1: Preparation of Glyoxals

For the precipitation of methyl glyoxal 1 mole of Selenium dioxide was mixed with 1mole of acetone containing 200 ml of ether and refluxed for 6 hours. Mustered yellow reaction mixture was decanted and solvent was driven off on water bath. Glyoxal was passed through the column of silica gel using ether as developer to ensure compete removal of selenium metal.

Methyl-bis-glyoxal and glyoxoacetanilide were prepared by mixing 0.5 mole of acetylacetone or 1 mole of acetoacetanilide with 1 mole of selenium dioxide in alcohol(95%) and refluxing the reaction mixture for 6-8 hours. Brown yellow reaction mixture of methyl-bis-glyoxal and brown coloured mixture of glyoxoacetanilide were decanted and alcohol was evaporated on water bath. Viscous semisolid glyoxals were purified by distillation under reduced pressure.

Step-2: Synthesis of Ketoanils

Solution of amine (0.10 mole) and methyl glyoxal (0.10 mole), methyl-bis-glyoxal (0.05 mole) and glyoxoacetanilide (0.10 mole) in ether or ether-benzene(1:1,v/v) were mixed together with vigorous stirring at room temperature. Product

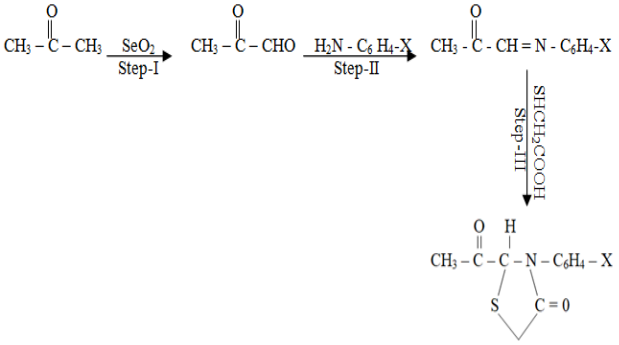
ketoanil precipitated either immediately or obtained as residue after evaporation of solvent on water bath was purified by recrystalization from acetone, chloroform or alcohol.

Step-3: Synthesis of Thiazolidinones:

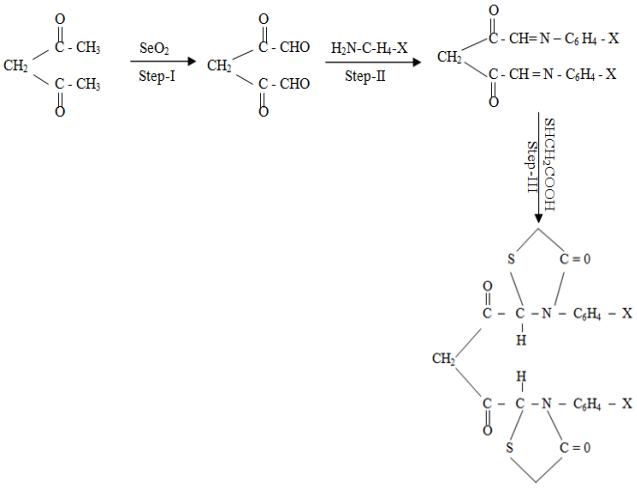
For the synthesis of Thiazolidinones equimolar (0.05 mol) solution of thioglycollic acid and ketoanil(0.05mole of methyl ketoanil) and (0.025mole of dimethyl-bis-ketoanil) and (0.05 mole of ketoanil of glyoxoacetanilide) in dry alcohol or benzene were mixed and refluxed for 20-25h. Reaction mixtures concentrated by evaporaration were neutralized with aqueous solution of sodium bicarbonate to remove excess of acid. Solid mass thus obtained were filtered out and washed with water and dried in hot air oven at about 50^0C. The chemical used in this work were Merck, Qualigens, CDH laboratory reagents.

Chemical reactions :

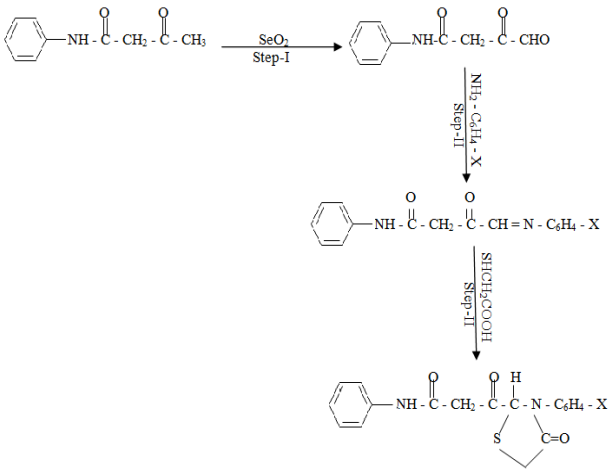
(1) Synthesis of 4-Thiazolidinones from Methyl glyoxal



(2) Synthesis of 4-Thiazolidinones from Methyl-bis-glyoxal



(3) Synthesis of 4-Thiazolidinones from Glyoxoacetanilide



Where, X= o-, m-, p-NO2/ Cl/ Br,/I

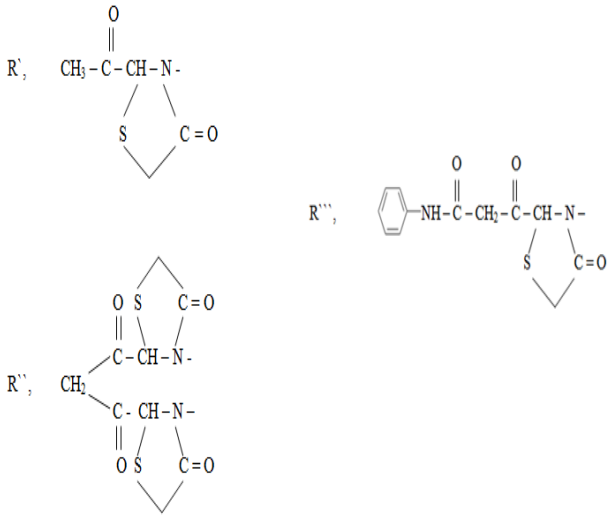
Homogeneity Test and Purification of Products:

For testing homogeniety and/or purity of Thiazolidinones their thin-layer chromatographic analysis was carried out along with corresponding Ketoanils. Sample solution in acetone/DMSO were loaded on starch bound silica gel layer and developed in different solvents. Resolving solvents of each mixture of Ketoanils and Thiazolidinones have been noted in Table -1.

Table-1: Formule, Colour, M.P. and Nitrogen (%) for Thiazolidinones:

Comp. code	Thiazolidinones	Colour	Melting Point(^{O}C)	Nitrogen (%) (Calculated)(Found)
1a	R'-C_6H_4-NO_2-o	Yellow brown	63	10.50 (10.85
1b	R'-C_6H_4-NO_2-m	Brown	>200	10.50 (10.24)
1c	R'-C_6H_4-NO_2-p	Brown	82	10.50 (9.98)
1d	R'-C_6H_4-Cl-p	Dark brown	114	5.48 (6.00)
1e	R'-C_6H_4-Br-p	Dark brown	90	4.66 (5.00)
1f	R'-C_6H_4-I –p	Black	69	4.03 (4.05)
2a	R"= (C_6H_4-NO_2-o) $_2$	Orange brown	60	10.85 (10.45)
2b	R"= (C_6H_4-NO_2-m) $_2$	Light brown	158	10.85 (10.86)
2c	R"= (C_6H_4-NO_2-p) $_2$	Brown yellow	118	10.85 (10.95)
2d	R"= (C_6H_4-Cl-p) $_2$	Brown	120	5.66 (6.06)
2e	R"= (C_6H_4-Br-p) $_2$	Brown	102	4.79 (5.00)
2f	R"= (C_6H_4-I-p) $_2$	Dark brown	95	4.13 (4.15)
3a	R''' = (C_6H_4-NO_2-o	Yellow brown	-	10.90 (11.39)
3b	R'''- C_6H_4-NO_2-m	Dark brown	122	10.90 (11.39)
3c	R''' - C_6H_4-NO_2-p	Brown	74	10.90 (10.74)

3d	R‴- C_6H_4-.Cl-p	Brown	61	7.47 (7.75)
3e	R‴- C_6H_4-Br-p	Brown	119	6.68 (6.75)
3f	R‴- C_6H_4-I-p	Dark brown	124	6.00 (5.86)



After knowing the resolving solvents by TLC test, products were purified by column chromatography conducted in columns of different sizes as per requirement. Each column is provided stop-cock at lower end and mounted vertically was packed by pouring silica gel (60-120 mesh CDH) suspension in mobile phase. The solutions of of impure products(containing unreacted ketoanil) in their resolving solvents were loaded in column and developed with their resolving solvents. Fast moving fraction with high R_F, Ketoanil , was collected in beaker and low R_F fraction which retained in column was eluted by acetone.Elutes of Thiazolidinones were treated with sodium bicarbonate solution and precipited solids were washed with 1% HCl and water successively and dried in hot air oven at about 60^0C.

Analysis and Physical Measurements:

Melting point determined in open glass capillary were uncorrected, Nitrogen analysis of all samples was done at CDRI, Lucknow. Infra red spectra of samples were recorded on Perkin Elmer spectrophotometer in 200-4000 cm^{-1} rang in Nujol medium whereas NMR spectra were recorded in DMSO medium on Perkin Elmer EM-360 NMR spectrometer.

III. RESULTS AND DISCUSSION

Preliminary TLC analysis of cyclocondensation products of ketoanils has received that all the product were contaiminated with ketoanils and none of them was multicomponent system. Physico-chemical data on pure mono-component solids have been noted in table-1.along with the formulae as derived from their nitrogen analysis.

Frequencies of band occurring at ca.1648cm^{-1} ,ca.1683 cm^{-1},ca.1585cm^{-1} ca683 cm^{-1},and ca.647 cm^{-1},in the infra red spectra corresponding to the stretching vibrations of characteristic groups C=O(ring),C=O (acyclic), C-N and C-S-C respectively of Thiazolidinones reveal the cyclocondensation at azomethine group of ketoanils. Other peaks appearing at ca.1098 cm^{-1},ca .1165 cm^{-1},ca.1210 cm^{-1},ca.1241 cm^{-1},ca.1303 cm^{-1},and ca.1374 cm^{-1}, and ca.2917 cm^{-1},which could only be attributed to δO-H(enolic) and νC=O (enolic) and νC=O(intra molecularly bonded) vibrations respectively indicate the presence of enolic structure alongwith ketonic form in the isolated solids. frequencies of other important groups, viz. νC=C(aromatic), ring breating and disubstitution have also been calculated.

In order to seek confirmation i.r results,n.m.r. spectra of few Thiazolidinones, as typical examples, were also examined, peaks corresponding to common characteristic groups.CH-N / CH-S, cyclic CH_2 and Ar-H of Thiazolidinone occur at ca.2.44τ, ca.6.39 τ and ca.3.35 τ respectively. An additional band at ca.5.89 τ, attributable to Ar-H nonbenzenoid protons, appeared invariably in the n.m.r. spectra of products. revealing presence of enolic structure, supports the infrared results. Besides the common peaks of Thiazolidinones comprised of enolic and ketonic tautomeric forms of equilibrium typical peaks of their differenciating groups, HN-C=O , CH_3C=O and CH2 saturated non cyclic have also been observed at .ca. 4.80τ ,ca.7.30τ and 8.67τ respectively.

Based on above studies, the structures of Thiazolidinones reveals that active methylene group adjacent to carbonyl group(s) of chain as well as of Thiazolidinones ring might have led to enolisation of ketonic group.

Antibacterial and Antifungal activities

Preparation of solutions and plates for testing:

Standard solutions of concentrations 500μg/ml, 100 μg/ml, and 50μg/ml,were prepared by dissolving known quantity of Thiazolidinones in known volume of dimethylformamide.

On the blank plates , sterilized at 121^0C in an autoclave for 15 minutes, sterilized nutrient agar (Himedia Laboratories, Bombay) mixed with cultures (*Staphylococcus aureus, Stalophylococcus epidermidis, Micrococcus leuteus* bacteria and *Candida ablicanes* fungi separately) was spread at about 50^0C and cooled to room temperature. Holes of media plates were loaded with 0.1 ml of each sample solution and plates were left for 30 minutes for diffusion; loaded plates were kept at 37^0C for 24 h for antibacterial activity and 30^0C for 72 h. for antifungal activity testing respectively and Zone inhibitions are noted inTable-2.

Table-2: Antibacterial and Antifungal activities of Thiazolidinones

Comp. code	Thiazolidinones	Zone inhibition in mm										
		Staphylococcus aureus			*Stalophylococcus epidermidis,*			*Micrococcus leuteus*			*Candida ablicanes*	
		Stock soln.			Stock soln.			Stock soln.			Stock soln.	
		500 µg	100 µg	50 µg	500 µg	100 µg	50 µg	500 µg	100 µg	50 µg	500 µg	100 µg
1d	R`-C_6H_4-Cl-p	15	13	13	8	*	*	12	11	11	16	14
2c	R"= (C_6H_4-NO_2-p) $_2$	13	13	12	10	*	*	11	10	8	17	15
2d	R"= (C_6H_4-Cl-p) $_2$	16	12	12	9	*	*	11	9	6	15	12
2e	R"= (C_6H_4-Br-p) $_2$	16	14	13	10	*	*	13	14	9	14	12
2f	R"=, (C_6H_4-I-p) $_2$	15	14	12	12	*	*	11	10	9	16	12
3b	R"' - C_6H_4-NO_2-m	12	10	7	7	5	*	10	7	5	15	9
3c	R"' - C_6H_4-NO_2-p	13	12	9	9	6	*	10	8	6	15	9
3d	R"' - C_6H_4-.Cl-p	11	10	9	8	5	*	9	8	7	8	7
3e	R"' - C_6H_4-.Br-p	10	9	9	10	7	*	9	7	6	8	9
3f	R"'- C_6H_4-I-p	10	9	8	11	7	*	10	9	*	9	7

* Does not show zone inhibition

Perusal of Table-2 reveals that most of the compounds are active against two of three bacteria used in different concentrations. Among the three effective bacteria *Stalophylococcus epidermidis* however, has been found little affected by sample of Thiazolidinones. Antifungal activities of all typical Thiazolidinones is of worth interest.

p-Chlorothiazolidinone of Methyl glyoxal, and p-nitro, p-Chloro, p-Bromo, p-Iodothiazolidinones of Methyl-bis-glyoxal exhibiting highest antibacterial and antifungal activity.

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