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Design, Synthesis, Characterization and Biological Evaluation of Novel Thiazolidine-4-Ones

S. A. Gosavi[1]*, R.R. Kunkulol[2], S. S. Pawar[3], R.S. Shendge.[4]

[1]*Research Scholar, Pravara Institutes of Medical Sciences (DU), Loni-413 736, Ahmednagar, Maharashtra, India.

[2]Department of Pharmacology, Pravara Institutes of Medical Sciences (DU), Loni-413 736, Ahmednagar, Maharashtra, India

[3]Department of Pharmaceutical Chemistry, Sanjivani College of Pharmaceutical Education and Research, Kopargaon-423 603, Maharashtra, India

[4]Department of Pharmaceutics, Sanjivani College of Pharmaceutical Education and Research, Kopargaon-423 603, Maharashtra, India

*E-mail : seemagosavi@gmail.com

Mobile No.: 91- 9860077084, Fax: (02423) 222682,

Address for Postal Correspondance: Pravara Institutes of Medical Sciences (DU), Loni-413 736

ABSTRACT

A new series of substituted 3-(4-phenylpyrimidin-2-yl) thiazolidin-4-one (SG1-SG9) was synthesized in order to determine their antioxidant, antimicrobial and antitubercular activity and feasible structure–activity relationships. The structures of synthesized compounds were established on the basis of melting point, TLC, IR, ^{1}HNMR and HRMS. The derivatives were evaluated for the radical scavenging activity compared to the standard Ascorbic acid. The results of antioxidant study show that some of the derivatives possess mild to moderate activity as compared to standard. Further the derivatives were evaluated for the antibacterial activity against Gram positive bacteria *S.aureus* (ATCC 9144),*S.epidemidis* (ATCC12228) and Gram negative bacteria *E.coli* (ATCC 25922) , *Klebsiella* (ATCC 4352), while antifungal activity against *A.flavus* (ATCC 9643) *and A.niger* (ATCC 16404) by using Agar well diffusion method using Ciprofloxacin and Fluconazole as standard respectively. The results of antimicrobial studies show that some of the derivatives posses mild to moderate activity as compared to standard. The derivatives were evaluated for the antitubercular activity against Mycobacterium tuberculosis (Vaccine strain, H37 RV strain) ATCC27294 by Microplate Alamar Blue Assay (MABA) method using Pyrazinamide, Ciprofloxacin and Streptomycin as standard. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method.

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Key Words:thiazolidin-4-one, antioxidant, antimicrobial, antitubercular

INTRODUCTION

Thiazolidine-4-ones containing thiazole moiety, it had been synthesized by 6-amino coumarin, isatin, primary amines and aromatic aldehydes. Thiazolidine-4-ones has been considered as a magic moiety because it possess almost all types of biological activities such as antifungal, antitubercular, antimicrobial, antioxidant, antibacterial, cytotoxic, anti-inflammatory, analgesic, anti YFV (yellow fever virus) activities.

Heterocycles containing sulphur and nitrogen atoms in the core structure, it shows number of pharmacologically and biologically active compounds. Thiazolidine-4-ones are usually solids, often melting with decomposition but the attachment of an alkyl group to the nitrogen lowers the melting point. Thiazolidine-4-ones are derivatives of thiazolidine with carbonyl group at the fourth position. The carbonyl group of thiazolidine-4-ones is highly un-reactive. Thiazolidine-4-ones are the derivatives, which belongs to important groups of heterocyclic compounds containing sulfur and nitrogen in a five member ring.[1, 2]

Figure 1

The nucleus is also known as a wonder nucleus, because it shows different types of biological activities[2]. Thiazolidine-4-one substituted moieties have received considerable attention during last two decades as they are gifted with variety of activities and have wide range of therapeutics properties. Thiazolidine-4-ones and its derivatives offer enormous scope in the field of medicinal chemistry. Thiazolidine-4-ones are important compounds due to their broad range of biological activities and pharmacological properties i.e. antifungal[2,12], antioxidant[3], cytotoxic[3], anti-inflammatory[4], analgesic[4], anti YFV (yellow fever virus) activity[5], antitubercular[10,14], antimicrobial[2, 4, 10, 11, 15, 16, 17], antibacterial[9, 10, 12, 13, 17], thiazolidine-4-one derivatives possess different pharmacological and biological activities. Antimicrobial activity is the most potent activity of thiazolidine-4-one. Antibacterial activity is strongly dependent on the nature of substituent at C-2 & N-3 position.

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EXPERIMENTAL

Material and Methods

All the chemicals in the synthesis were purchased from S.D. Fine Chemicals LTD., Mumbai. Melting points were determined by open capillary method on Veego (model: VMP-D) electronic apparatus and are uncorrected. The IR spectra of the synthesized compounds were recorded on Shimadzu 8400-S FT-IR Spectrophotometer using ATR sampling technique.^{1}H NMR spectra was obtained on Bruker AV III 500 MHz spectrometer spectra in $CDCl_3$ and chemical shifts are given in parts per million, downfield from Tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained from Bruker Impact HD 3050 system instrument at the SPPU, Pune. To monitor the reactions, as well as, to establish the identity and purity of reactants and products, thin layer chromatography was performed on microscopic slides (2 x 7.5 cm) coated with silica gel G F_{254}, using Benzene: Methanol (7:3) solvent systems and the spots were visualized under ultra-violet light (254nm) or by exposure to iodine vapours.

General method for preparation of chalcones (Step I):

To a solution of 5.5 g of sodium hydroxide in 50 ml of distilled water, 30 ml of ethanol was added in the flask .The flask was immersed in a bath of crushed ice. After adding 0.1 mol of acetophenone (substituted), then the mixture was stirred. Then 0.1 mol of aromatic aldehyde was added. This mixture was vigorously stirred for 2-3 h maintained at 25^0C. After stirring the reaction mixture was kept in the ice chest for 24 h & the solid obtained were filtered & washed using cold water until neutral to the litmus. Progress of reaction was monitored by TLC. On completion, the reaction mixture was neutralised by gradual addition of dilute hydrochloric acid with stirring until neutral to litmus. Crude product recrystallized using rectified spirit.

General procedure for the preparation of pyrimidines (Step II)

A mixture of appropriate chalcones and guanidine hydrochloride in absolute ethanol (10 ml) were refluxed on a water bath. The progress of reaction was monitored by TLC. On completion the solvent was completely evaporated and the residue was poured into ice cold water. The precipitated solid was collected by filtration.

Synthesis of schiff's base of n-(4-methoxy benzylidine)-4, 6-diphenyl pyrimidine 2-amine (Step III)

A mixture of 0.01 mol of pyrimidine and aldehyde (0.01 mol) and 2–3 drops of glacial acetic acid in ethanol was refluxed. The progress of reaction was monitored by TLC. The solvent was removed under reduced pressure to afford product Schiff base. Recrystallized from of rectified spirit.

Synthesis of - (4, 6-disubstituted phenyl) pyrimidin-2yl-2-(4methoxyphenyl) thiazolidine-4-one (Step IV)

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A mixture of appropriate (0.01 mol)*N*-(4-methoxy benzylidine)-4, 6-diphenyl pyrimidine 2-amine, thioglycolic acid (0.015 mol) and a pinch of anhydrous $ZnCl_2$ in dry 1, 4-dioxane was refluxed for 12–14 h. The reaction mixture was cooled and neutralized with 10% sodium bicarbonate solution. The separated solid was filtered, washed with water and recrystallized from ethanol.

Synthesis of final derivatives substituted 4-oxothiazolidin-5-yl (Step V)

An equimolar quantity (0.004 mol) of substituted amine in 10 ml of ethanol was added to slurry containing product IV and aq. formaldehyde solution dissolve in 10 ml of ethanol. The reaction mixture was stirred for 1h at room temperature and refrigerated for 48 h the product was separated by suction filtration and recrystallize from ethanol.

Scheme I:

Figure 2. General structure of substituted thiazolidine-4-one

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Table 1: Physical characteristics of synthesized derivatives (SG1-SG9):

Sr. No	R	R'	R"	R"'	Comp Code	Mol. formula	Mol. Wt.	M.P (^{0}C)	R_f Value	% Yield
1	-H				SG1	$C_{32}H_{24}N_6O_4SCl$	623.45	101-103	0.56	74.84
2	-H				SG2	$C_{30}H_{23}N_7O_4SCl$	612.45	102-105	0.47	78.51
3	-H				SG3	$C_{32}H_{23}N_5O_5S_2Cl$	670.44	108-110	0.53	68.52
4					SG4	$C_{34}H_{28}N_6O_3SCl$	635.45	109-111	0.50	62.54
5					SG5	$C_{32}H_{27}N_6O_3SCl$	610.45	115-117	0.58	78.24
6					SG6	$C_{33}H_{29}N_6O_4S_2Cl$	672.45	119-121	0.54	64.36
7					SG7	$C_{31}H_{23}N_6O_3SCl_2$	629.9	120-122	0.60	68.57
8					SG8	$C_{30}H_{22}N_6O_3SCl_2$	616.9	125-127	0.53	69.43
9					SG9	$C_{32}H_{23}N_5O_4S_2Cl_2$	675.9	124-126	0.51	61.98

*All melting points are uncorrected.
**Mobile phase- Ethyl acetate: Hexane (2:1)
Recrystallization solvent: Ethanol (80%).

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Biological Evaluation

Free Radical Scavenging Activity (DPPH Assay)

The radical scavenging activity of the synthesized compounds against stable free radical 2.2-diphenyl-2- picrylhydrazyl hydrate (DPPH, Sigma-Aldrich Chemie, Steinheim, Germany) was determined spectrophotometric ally. When DPPH reacts with antioxidant compounds, which can donate hydrogen, it is reduced. Following the reduction, its deep violet colour in methanol bleached to yellow, showing a significant absorption decrease at 517 nm. Then 3ml of various concentrations (2, 4,8,16 and 32 μg/ml) of the compounds (3a-3j) dissolved in ethanol were added to 1ml of ethanol solution of DPPH. (Accurately 4.079 mg of DPPH was weighed and added to 100 ml ethanol). After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm (Shimadzu UV-Vis spectrophotometer) Ascorbic acid was used as the reference compound. All tests and analyses were done in three replicates and the results were averaged. Free radical DPPH inhibition in percentage (AA %) was calculated as follows:

$$\text{Scavenging Effect (\%)} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{Control}}} \text{ X } 100$$

Statistical analysis

Results were analyzed using one-way analysis of variance (ANOVA) followed by the Tukey's test using statistical software package, GraphPad Prism; version 5.03. Values were expressed as a mean ± standard deviation of the mean and (***) $P < 0.001$ was considered as statistically highly significant. Values of $P \leq 0.05$ (*) and $P \leq 0.01$ (**) were considered statistically significant. To calculate the IC50 values using Microsoft Excel. IC50 was calculated from linear equation relationship, i.e., y = mx + c, (IC_{50}: Half maximal inhibitory concentration)

Antibacterial Activity

All the synthesized compounds have been screened in-vitro for the antibacterial activity by the agar well diffusion method using DMSO as solvent, against two strains of gram positive and gram negative bacteria at five concentrations in a Brain Heart Infusion agar medium. Antibacterial activity of test compound was evaluated against gram positive bacteria

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S.aureus(ATCC 9144) and *S.epidermis*(ATCC12228) and gram negative bacteria *E. coli* (ATCC 25922) and *Klebsiella* (ATCC 9027), using Ciprofloxacin as standard. Plates were read only if the lawn of growth is confluent or nearly confluent and diameter of inhibition zone was measured to nearest whole millimeter by holding the measuring device.

Antifungal Activity

Fungicidal activity of the test compound was evaluated against *A. Niger* (ATCC 16404) and*A.flavas*(ATCC 9643) by Agar well diffusion method, Sabouraud agar medium is used instead of Brain heart infusion agar. The method of testing the antifungalactivity is same as that adopted for evaluating antibacterial activity. Fluconazole was used as the standard; DMSO was used as solvent.

Antitubercular activity

Anti mycobacterial activity of compounds were assessed against M. Tuberculosis (Vaccine strain, H37 RV strain) ATCC27294 using microplate Alamar Blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth. The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink.

Spectral Analysis

***N*'-((2-(4-hydroxyphenyl)-3-(4-(4-hydroxyphenyl)-6-phenylpyrimidin-2-yl)-4-oxothiazolidin-5-yl) methyl) isonicotinohydrazide**: **SG1**

IR (ATR, cm^{-1}): CH-Ar stretch (3055.35), C=C (Ar) (1595.18), C=O of Ketone (1670.41), Broad O-H of Ar (3500-3000), N-H (Secondary Stretch) (3250.16), Ar, tertiary C-N (Amines)

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(1357.93), N-N str. of hydrazide (1066.67), -NH str. Of hydrazide (3495.13), ^{1}*H NMR (δ ppm):* 7.842 (1H, s, -NH amine), 7.260 (1H, s, -NH), 7.654 (2H, d, Aromatic hydrogen), 8.006 (2H, d, Aromatic hydrogen), 1.254 (2H, -CH_2), 0.869 (1H, t, thiazolidine-4-one ring proton-CH), 2.249 (2H, thiazolidine-4-one ring proton-CH), 6.990 (2H, d, Aromatic hydrogen), 7.441 (2H, d, Aromatic hydrogen), 5.310 (1H, s, hydroxyl –OH), 7.561 (1H, s, aromatic hydrogen), 7.457 (1H, s, aromatic hydrogen), 7.532 (1H, s, aromatic hydrogen), 7.107 (1H, s, aromatic hydrogen), 7.430 (1H, s, aromatic hydrogen), 4.811 (1H, s, hydroxyl -OH), 8.194 (1H, t, aromatic hydrogen) HRMS m/z (%):590.65, 498.1705, 377.1300, 348.148, 257.0912 and 215.027

4-(((2-(4-hydroxyphenyl)-3-(4-(4-hydroxyphenyl)-6-phenylpyrimidin-2-yl)-4-xothiazolidin-5-yl)methyl) amino)benzenesulfonamide : SG2

IR (ATR, cm^{-1}): Merged with broad –OH (3150-3050), C=C of Ar (1575.89), C=O of ketone (1646.30), Broad O-H of Ar (3500-3000), N-H of Secondary Stretch (3261.74), C-N of Amines (1342.50), -S=O asymmetric stretch (1325.14), -S=O symmetric stretch (1168.90), aromatic tertiary –C-N (1291.39), HRMS m/z (%):625.1023, 533.0969, 516.1394, 377.1471, 257.3156 & 215.0736

***N*'-((2-(4-hydroxyphenyl)-3-(4-(4-hydroxyphenyl)-6-phenylpyrimidin-2-yl)-4-oxothiazolidin-5-yl)methyl)pyrazine-2-carbohydrazide: SG3**

IR (ATR, cm^{-1}): CH of aromatic stretch (3000.37), C=C of aromatic (1588.43), C=O of ketone (1666.55), broad O-H of aromatic (3500-3000) N-H of Secondary Stretch (3200.01), aromatic tertiary C-N (Amines) (1277.88), N-N stretch of hydrazide (1090.78), -NH stretch of hydrazide (3399.65)

***N*'-((2-(3-chlorophenyl)-3-(4-(4-hydroxyphenyl)-6-(4-methoxy phenyl) pyrimidin-2-yl)-4-oxothiazolidin-5-yl) methyl)isonicotinohydrazide : SG4**

IR (ATR, cm^{-1}): CH aromatic stretch (3063.06), C=C of aromatic (1597.11), C=O of ketone (1643.41), broad O-H of aromatic (3500-3000), N-H of Secondary stretch (3209.66, 3147.93) Ar, tertiary C-N (Amines) (1365.65), N-N stretch of hydrazide (1091.75), -NH stretch of

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hydrazide (3531.78), HRMS m/z (%):639.1858, 528.1324, 498.1049, 377.1471, 257.3156 & 215.0394

SG5, 4-(((2-(3-chlorophenyl)-3-(4-(4-hydroxyphenyl)-6-(4-methoxy phenyl) pyrimidin-2-yl)-4-oxothiazolidin-5-yl) methyl) amino) benzenesulfonamide, IR (ATR, cm^{-1}): CH of aromatic stretch (3000), C=C of aromatic (1593.31), C=O of Ketone (1683.94), broad O-H of aromatic (3500-3000), N-H of Primary Stretch (3211.59, 3279.32), C-N (Amines) (1360.68), -S=O asymmetric stretch (1317.23), -S=O symmetric stretch 1148.41

***N*'-((2-(3-chlorophenyl)-3-(4-(4-hydroxyphenyl)-6-(4-methoxy phenyl) pyrimidin-24-oxothiazolidin-5-yl)methyl) pyrazine-2-carbohydrazide : SG6**

IR (ATR, cm^{-1}): CH of aromatic stretch (3091.99), C=C of aromatic (1550.82), C=O of ketone (1681.98), broad O-H of aromatic (3541.42), aromatic, tertiary C-N (Amines) 1313.57, N-N str. of hydrazide1037.74, -NH stretch of hydrazide (3400.62), C-Cl (775.41)

***N*'-((3-(4-(4-chlorophenyl)-6-(4-hydroxyphenyl)pyrimidin-2-yl)-2-(4-(dimethylamino)phenyl)-4-oxothiazolidin-5-yl)methyl)isonicotinohydrazide : SG7**

IR (ATR, cm^{-1}): CH of aromatic stretch (3015.80), C=C of aromatic (1580.72), C=O of ketone (1646.30), broad O-H of aromatic (3500-3000), N-H of secondary stretch) 3229.91, Ar, tertiary C-N (Amines) 1344.43, N-N stretch of hydrazide (1090.78), C-Cl (747.44), ^{1}H *NMR (δ ppm):* 6.0 & 5.4 (1H, s, -NH), 7.755 & 7.957 (2H, d, Aromatic hydrogen), 0.869 (2H, d, -CH2), 1.240 (1H, t, thiazolidine-4-one ring proton-CH), 2.61 (1H, s, thiazolidine-4-one ring proton-CH), 6.883, 7.521, 7.385, 7.888, 6.923, 6.773, 7.478 (1H, s, aromatic hydrogen), 5.10 (1H, s, hydroxyl –OH), HRMS m/z (%):652.1956, 617.1470, 533.2063, 498.2928, 377.1023 & 285.1333

4-(((3-(4-(4-chlorophenyl)-6-(4-hydroxyphenyl)pyrimidin-2-yl)-2-(4(dimethylamino)phenyl)-4-oxothiazolidin-5-yl)methyl)amino)benzenesulfonamide : SG8

IR (ATR, cm^{-1}): CH of aromatic stretch (3191.33), C=C of aromatic (1588.43), C=O of ketone (1644.37), broad O-H of aromatic (3500-3000), N-H of secondary stretch (3257.88), C-N (Amines) (1342.50), -S=O asymmetric stretch (1322.25), -S=O symmetric stretch (1169.87), aromatic, tertiary –C-N (1293.31), C-Cl (813.99), ^{1}H *NMR (δ ppm):* 9.5-9.7 (2H, s, $-SO_2NH_2$), 7.961 & 7.891 (2H, d, aromatic hydrogen), 3.085 (1H, s, -NH), 0.869 (2H, d, $-CH_2$), 1.299 (1H, t, Thiazolidine-4-one ring proton-CH), 2.598 (1H, s, Thiazolidine-4-one ring proton-CH), 6.884 (2H, d, aromatic hydrogen), 2.985 (6H, s, Aliphatic-CH_3), 5.431 (1H, s, hydroxyl –OH), 6.580,

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7.791, 6.771, 7.565 & 7.36 (1H, s, aromatic hydrogen), 4.77 (1H, s, Hydroxyl –OH), HRMS m/z (%):687.1958, 652.1858, 516.1049, 377.1471 & 257.0570

***N*'-((3-(4-(4-chlorophenyl)-6-(4-hydroxyphenyl)pyrimidin-2-yl)-2-(4-dimethylamino)phenyl)-4-oxothiazolidin-5-yl)methyl)pyrazine-2-carbohydrazide : SG9**

IR (ATR, cm^{-1}): CH of aromatic stretch (3022.55), C=C of aromatic (1589.40), C=O of ketone (1644.37), Broad O-H of aromatic (3500-3000), N-H of secondary stretch (3235.70), aromatic, tertiary C-N (Amines) (1343.46), N-N stretch of hydrazide (1090.78), -NH stretch of hydrazide (3400.62), C-Cl (775.41)

RESULT AND DISCUSSION

Several approaches for the synthesis of chalcones have been presented in research papers, the most convenient of which is one involving the Claisen-Schmidt condensation of equimolar quantities of a substituted acetophenone and substituted aldehydes in the presence of aqueous alcoholic alkali. Synthesis started with condensation of unsubstituted/substituted aromatic aldehyde and ketone in presence of sodium hydroxide via well-known Claisen–Schmidt condensation reaction to yield chalcones. The chalcones formed were cyclized with guanidine hydrochloride to form corresponding pyrimidine analogues. Substituted thiazolidine-4-ones were formed by condensing pyrimidines with appropriately substituted aldehyde to form Schiff base, followed by cyclization with thioglycolic acid. Finally 5-substituted thiazolidine-4-ones were obtained by condensation with primary amine (isonicotinic acid hydrazide, pyrazinoic acid hydrazide and sulphanilamide) in presence of formaldehyde via well-known mannich reaction.Structures of all derivatives have been elucidated by ^{1}H-NMR, HRMS and IR spectral measurements. The results obtained from this study confirmed that the product has formed. The solid state IR (ATR,cm^{-1}) spectra of these compounds reveal a characteristic N-H (secondary stretch) 3200-3250.16 of hydrazide and aromatic Stretch between 3150-3050 cm^{-1}. The amine group of thiazolidine ring (C-N) group present in the thiazolidine ring reveal peaks at 1350-1000 cm^{-1} .The C=C group of Aromatic ring showed stretching vibrations at around 1600 and 1475 cm^{-1}. C=O (ketone) group reveal peaks at 1725-1705 cm-1. The ^{1}H NMR spectra of all target derivatives (SG1-SG9)were recorded in $CDCl_3$. ^{1}H NMR has revealed signal around at δ 3.50-3.64 accounting for thiazolidine nucleus. Signal for the aromatic protons were present in between δ 8 and 7. Thus, all

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the protons were accounted for the respective structures. Mass spectra were also in accordance with the proposed structures.

Radical scavenging activities are of great significance due to the deleterious role of free radicals in biological systems. The *in vitro* antioxidant properties of the newly synthesized compounds at different concentrations were examined by a well-documented assay like DPPH free radical scavenging assay. The effect of antioxidants on DPPH radicals is considered due to their hydrogen donating ability. Antioxidant molecule can quench DPPH free radicals and convert them to a colourless/bleached product ultimately resulting in a decrease in the absorbance. The *in vitro* antioxidant activity of the synthesized compounds SG1-SG9 compared to ascorbic acid as standard are shown in **Table 2**. Our results indicate that newly synthesized compounds showed moderate to good antioxidant activity at low concentrations as compared to ascorbic acid. In an attempt to establish some structure activity relationship based on the position and presence of different substituents and to understand as to how different functionalities have an effect on the antioxidant properties, a series of new Thiazolidine-4-ones were synthesized. The DPPH radical scavenging efficacy of SG2-SG9 did not show a regular trend. The scavenging of DPPH radicals by most of these compounds occurred in a concentration-dependent manner from 2 to 32 µg/ml with SG1 analogue showing maximum effect of 83.54 %, respectively. Whereas for its unsubstituted counterpart and chloro analogue, the moderate free radical scavenging activity was SG5 (20.22±0.134) and 58.63 % at a concentration of 32 µg/ml.

Antibacterial study of titled derivatives revealed that, amongst the nine synthesized derivatives, SG3, SG6 andSG9exhibited promising activity SG1, SG2, SG5, SG7, SG8, exhibit moderate, activity against *S Aureus*. SG3, SG4, SG5, SG6, SG7 exhibit potent activity against *S. Epidermis* .SG2, SG3, SG4, SG5, SG6, SG7, SG8and SG9 exhibited promising activity against *Klebsiell*and SG2, SG3, SG8 and SG9against *E. coli*, gram negative bacteria. The compounds SG1, SG2, SG3, SG4, SG5, SG6, SG7SG8, and SG9 exhibited promising activity against fungi *A. niger*and *A. flavus*respectively.

From the structure of potent antimicrobial compounds amongst the synthesized series it has been observed that groups like –Cl, -OH and OCH_3 at substituent on phenyl ring as well as isonicotinic acid hydrazide/sulphanilamide on thiazolidine-4-one positively contributes for antimicrobial potential. The results were depicted in **Table 3 and 4.**

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All the synthesized derivatives were tested for antimycobacterial activity against M. Tuberculosis using microplate Alamar Blue assay (MABA). Compound SG3 and SG9 showed equivalent antitubercular activity as standard. Hence in the present study, the aromatic substituted ketone, aromatic substituted aldehydes and isonicotinic acid hydrazide/sulphanilamide when linked with isoxazole moiety showed good potential for further development as antimycobacterial and antimicrobial agents. Further study is required to predict mechanism of action. The information revealed in this article may be helpful lead for the medicinal chemist who is working in this area.The results were depicted in **Table 5**

Table 2: Data of IC_{50} of antioxidant activity for selected thiazolidine-4-one:

Sample	IC_{50}µg/ml (n=6)						Mean ± SD
AA	14.3	14.35	14.22	14.27	14.02	14.23	14.23±0.11
SG1	15.9511	15.8072	15.9321	17.7092	16.18	16.003	16.1±0.71
SG2	21.28	21.23	21.684	20.001	20.89	21.07	21.00±0.56
SG3	21.28	21.23	20.684	20.88	20.29	21.07	20.90±0.37
SG4	20.26	20.19	21.48	21.51	21.55	21.38	21.06±0.65
SG5	20.45	20.38	19.66	20.45	20.44	19.99	20.22±0.33
SG6	20.17	20.81	20.21	20.81	22.75	21.52	21.04±0.97
SG7	21.77	21.85	20.87	21.96	22.75	21.52	21.78±0.61
SG8	25.82	26.72	25.86	25.87	24.92	25.97	25.86±0.57
SG9	26.41	26.22	26.72	27.07	26.31	26.3	26.50±0.32

Data were expressed as mean ± SD (n = 6)

By applying Tukey-Kramer Multiple Comparisons Test there is a highly significant difference between mean values % Scavenging Activity of (SG1-SG9)

(i. e. ***P<0.001vs Ascorbic acid with the same concentration)

Compounds SG1 (IC_{50}=16.1±0.71) has been shown promising antioxidant activity while SG3, SG5, SG6, SG4, SG8 and SG9 (20.90±0.37, 20.22±0.33, 20.22±0.33, 21.06±0.65, 25.86±0.57 and 26.50±0.32 respectively) showed less antioxidant activity when compared with standard drug ascorbic acid (14.23±0.11).

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Table 3: Results of zone of inhibition of bacteria of selected thiazolidine-4-ones

Compound Code	Mean zone of inhibition (in mm)																			
	Gram +ve bacteria										Gram –ve bacteria									
	S. Aures					*S. Epidermis*					*Klebsiella*					*E.coli*				
Conc. In μg/ml	75	50	25	10	5	75	50	25	10	5	75	50	25	10	5	75	50	25	10	5
SG1	17	15	13	10	8	17	16	14	12	9	29	26	22	18	13	15	10	-	-	-
SG2	21	19	17	9	-	21	20	16	10	-	43	39	35	29	24	23	21	10	-	-
SG3	23	20	18	15	10	23	20	18	15	12	40	38	35	30	24	25	22	20	10	-
SG4	20	18	14	13	11	20	17	13	11	10	40	38	35	30	24	13	10	-	-	-
SG5	24	21	18	10	7	24	20	16	14	8	40	38	35	30	24	12	-	-	-	-
SG6	25	22	20	18	13	25	23	20	17	12	38	33	29	25	20	10	-	-	-	-
SG7	17	14	13	10	5	18	15	12	8	5	40	37	33	29	25	12	-	-	-	-
SG8	15	13	10	8	-	15	13	10	8	-	45	40	38	30	26	25	22	20	10	-
SG9	24	22	20	17	15	26	24	21	18	15	42	39	35	30	28	28	23	19	14	-
Ciprofloxacin (10μg)	26					26					30					32				

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Table 4: Results of zone of inhibition of fungi of selected thiazolidine-4-ones:

Compound Code	Mean zone of inhibition (in mm)									
	A. niger					*A. flavus*				
Conc. In µg/ml	75	50	25	10	5	75	50	25	10	5
SG1	33	23	21	23	23	42	32	22	18	22
SG2	34	24	19	14	14	42	32	22	12	12
SG3	32	22	20	22	22	43	33	23	13	23
SG4	38	28	22	18	18	47	37	27	17	17
SG5	34	24	20	24	24	43	33	23	13	23
SG6	33	23	21	23	23	41	31	30	28	15
SG7	32	29	23	20	17	45	35	25	15	10
SG8	34	24	22	14	14	46	36	26	16	10
SG9	37	27	21	17	12	48	38	28	18	13
Fluconazole (30 µg)	26					26				

Table 5: Results of antitubercular activity:

Sr. No.	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	**SG1**	S	R	R	R	R	R	R	R
2	**SG2**	S	R	R	R	R	R	R	R
3	**SG3**	S	S	R	R	R	R	R	R
4	**SG4**	S	R	R	R	R	R	R	R
5	**SG5**	S	R	R	R	R	R	R	R
6	**SG6**	S	R	R	R	R	R	R	R
7	**SG7**	S	R	R	R	R	R	R	R
8	**SG8**	S	R	R	R	R	R	R	R
9	**SG9**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**R**

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10	**Pyrazinamide**	S	S	S	S	S	S	R	R
11	**Ciprofloxacin**	S	S	S	S	S	S	R	R
12	**Streptomycin**	S	S	S	S	S	R	R	R

NOTE: S - Sensitive R- Resistant

CONCLUSION

The present study reveals synthesis and antimicrobial evaluation of some novel thiazolidine-4-one derivatives were synthesized (as per **Scheme I**) by chalcone preparation through condensation of unsubstituted / substituted aromatic aldehyde and ketone in presence of sodium hydroxide via well-known Claisen–Schmidt condensation reaction. The chalcones formed were cyclized with guanidine hydrochloride to form corresponding pyrimidine analogues. Finally 5-substituted thiazolidine-4-ones were obtained by condensation with primary amine (isonicotinic acid hydrazide, pyrazinoic acid hydrazide and sulphanilamide) in presence of formaldehyde via well-known mannich reaction. The solid state IR (ATR, cm^{-1}) spectra of these compounds reveal a characteristic N-H (secondary stretch) 3200-3250.16 of hydrazide and aromatic Stretch between 3150-3050 cm^{-1}. The amine group of thiazolidine ring (C-N) group present in the thiazolidine ring reveal peaks at 1350-1000 cm^{-1} .The C=C group of Aromatic ring showed stretching vibrations at around 1600 and 1475 cm^{-1}. C=O (ketone) group reveal peaks at 1725-1705 cm-1. The ^1H NMR spectra of all target derivatives (SG1-SG9)were recorded in $CDCl_3$. ^1H NMR has revealed signal around at δ 3.50-3.64 accounting for thiazolidine nucleus. Signal for the aromatic protons were present in between δ 8 and 7. Thus, all the protons were accounted for the respective structures. Mass spectra were also in accordance with the proposed structures. We have achieved a convenient protocol for the synthesis thiazolidine-4-ones moiety in good yield and evaluated their*in vitro* antioxidant activity by using DPPH radical scavenger assay. By applying Tukey-Kramer Multiple Comparisons Test there is a highly significant difference between mean values % Scavenging Activity of (SG1-SG9)(i. e. ***P<0.001 vs Ascorbic acid with the same concentration) Compounds SG1(IC_{50}=16.1±0.71)has been shown promising antioxidant activity while SG3, SG5, SG6, SG4, SG8 and SG9 (20.90±0.37, 20.22±0.33, 20.22±0.33, 21.06±0.65, 25.86±0.57 and 26.50±0.32 respectively) showed less antioxidant activity when compared with standard drug ascorbic acid (14.23±0.11) Our antioxidant screening

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results indicate that exciting DPPH radical scavenging activity was observed in compounds in comparison with standard ascorbic acid. Antibacterial study of titled derivatives revealed that, amongst the nine synthesized derivatives, SG3, SG6 and SG9 exhibited promising activity SG1, SG2, SG5, SG7, SG8, exhibit moderate, activity against *S Aures*. SG3, SG4, SG17, SG18, SG19 exhibit potent activity against *S. Epidermis*. SG2, SG3, SG4, SG17, SG18, SG19, SG8 and SG21 exhibited promising activity against *Klebsiella* and SG2, SG3, SG8 and SG21 against *E. coli*, gram negative bacteria. The compounds SG1, SG2, SG3, SG4, SG17, SG18, SG19 SG8, and SG21 exhibited promising activity against fungi *A. niger*and *A. flavus* respectively. From the structure of potent antimicrobial compounds amongst the synthesized series it has been observed that groups like –Cl, -OH and OCH_3 at substituent on phenyl ring as well as isonicotinic acid hydrazide/sulphanilamide on thiazolidine-4-one positively contributes for antimicrobial potential.

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