Synthesis, characterization and antimicrobial activity of novel thiazolidin-4-one derivatives

Subhas S. Karki[1]*, Mitul Prajapati[1]

[1]Department of Pharmaceutical Chemistry, College of Pharmacy, KLE Academy of Higher Education & Research (KLE University), Bengaluru-560010

Abstract

Purpose
To synthesize, characterize, and evaluate the antimicrobial activity of novel 4-thiazolidinone derivatives.

Methods
The intermediate compound, 4-substituted-2-(phenylimino)-thiazolidin-4-one, was synthesized by condensation of phenylthiourea, ethyl chloroacetate, and fused sodium acetate. It was further reacted with several aromatic aldehydes to yield thiazolidin-4-one derivatives.
The synthesized compounds were subsequently characterized by melting point determination, thin-layer chromatography (TLC), infrared (IR), proton nuclear magnetic resonance (1H NMR) spectroscopy, and mass spectroscopy. All compounds were screened for their antimicrobial activities by turbidometric and well diffusion method.

Results
Structures of the synthesized compounds were confirmed based on the elemental analysis. Additionally, the synthesized compounds displayed moderate activity against *Staphylococcus aureus, Bacillus subtils, Streptococcus mutans, Pseudomonas aeruginosa,* and *Escherichia coli*.

Conclusion
As the synthesized compounds showed antibacterial activity, further studies can be performed to evaluate the efficacy and safety of these derivatives.

Keywords: Thiazolidin-4-one derivatives, 4-substituted-2-(phenylimino)-thiazolidin-4-one, antimicrobial activity

Introduction

Medicines are the products of persistent effort made by human civilization from time to time. However, a large percentage of these medicines used for the treatment of infectious diseases are among the factors responsible for high mortality globally.[1] Discovery of novel and improved form of drugs has become necessary due to the inability of drugs prepared from natural sources (plants, animals, and minerals) to treat the newer form of diseases.[2]

Globally, a wide range of organic compounds is synthesized annually, which are being regularly screened for their biological activity. Moreover, production of established parent molecule derivatives is preferred over synthesizing new molecules as it minimizes toxicity and improves potency in relatively a shorter period of time. β-lactam antibiotics (penicillin G, penicillin V, and ampicillin), irreversible proton pump inhibitors (omeprazole, lansoprazole, rabeprazole), and benzodiazepine derivatives (diazepam and clonazepam) are few examples of such derivatives, which have helped in managing an extensive spectrum of diseases and disorders.[3] Even though few novel synthetic antimicrobial agents have been developed in the recent years, more stress has been put to explore the possibility of a new antibiotic-producing organism to develop semisynthetic derivatives of parent antibiotics with more anticipated properties.[4]

Alternating units of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) make up the glycan part of a peptidoglycan polymer, which is an essential component of a bacterial cell wall. It confers mechanical resistance to the higher internal osmotic pressure and helps in maintaining a defined cell shape.[5] During biosynthesis of peptidoglycan, the enzyme—Mur B aids in the reduction of enolpyruvyl uridine diphosphate NAG to uridine diphosphate NAM[6] which in turn is an intermediate in the formation of uridine NAM peptide

portion of the cell wall precursor. Thus, any molecule involved in competitively inhibiting Mur B may possess the potential to become an attractive antibacterial agent.

Compounds containing thiazolidin-4-one ring are known to have diverse biological activity. A thiazolidin-4-one ring has a carbonyl group, an atom of nitrogen, and sulfur and is known to be present in several pharmaceutical compounds such as rosiglitazone and pioglitazone.[7] The diversity in its biological response profile has engrossed the attention of many researchers to find the various uses of this compound. It has been reported that 4-thiazolidinones have the prospective to act as diphosphate surrogates and Mur B inhibitors.[8] On the other hand, various compounds having 4-thiazolidinone in core structure have also been found to have antidiabetic,[7] antiproliferative, tumor-inhibiting,[9] and anticonvulsive activities[10] along with the ability to inhibit the replication of human immunodeficiency virus (HIV).[11]

Thus, the following study had been conducted to synthesize and characterize thiazolidin-4-one derivatives. Additionally, the antimicrobial activity of these compounds against *Staphylococcus aureus*, *Bacillus subtils*, *Streptococcus mutans*, *Pseudomonas aeruginosa* and *Escherichia coli* was.

Materials and Methods:

Microorganisms

Standard cultures of *Bacillus subtilus*, *Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, and *Escherichia coli* were obtained from Biotechnology Department, M. S. Ramaiah Institute of Technology, Bangalore.

Chemicals

Synthesis of parent compounds

Synthesis of 4-substituted-2-(phenylimino)-thiazolidin-4-one

Synthesis of 4-substituted phenylthiourea

Ammonium thiocyanate (0.11 M, 8.5 g) and acetone (50 mL) were added into a three-necked flask fitted with a reflux condenser, mechanical stirrer, and dropping funnel. The mixture was subsequently refluxed for 5 min after adding benzoyl chloride (0.1 M, 14.1 g). A solution of aromatic amine (0.1 M) was added in acetone with gentle refluxing. After refluxing, the mixture was added into cold water leading to the formation of a yellow precipitate (α-benzoyl-β-phenyl-thiourea). The precipitate, obtained after filtration of the mixture, was heated for 5 min in a boiling solution of sodium hydroxide (30 g) dissolved in water (270 ml). The resultant product was filtered to remove the insoluble components followed by acidification of the product with the addition of concentrated hydrochloric acid. Ammonium hydroxide was also added to make the product slightly basic in nature thereby aiding in the precipitation of 4-substituted phenylthiourea as a white crystalline product (Fig. 1). It was further processed by recrystallization from ethanol.[12, 13]

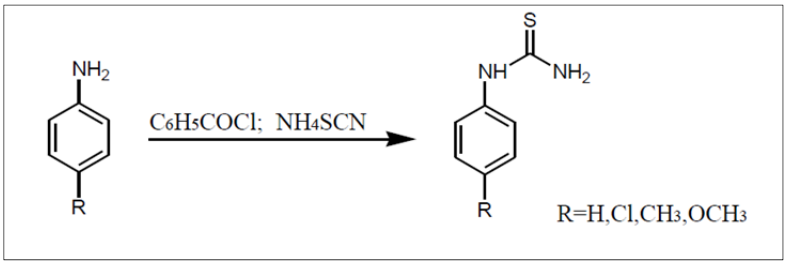

Fig.1. Synthesis of 4-substituted phenylthiourea from 4-substituted aromatic amine

Synthesis of 4-substituted-2-(phenylimino)-thiazolidin-4-one from 4-substituted phenylthiourea

A mixture of 4-substituted phenylthiourea (0.01 M), sodium acetate (0.01 M), ethylchloroacetate (0.01 M), and ethanol (15 mL) was refluxed for 3.5 h in a 100-mL round bottom flask fitted with a reflux condenser and a guard tube. Following this, the mixture was poured carefully into ice cold water. A white precipitate was formed, which was subsequently filtered, dried, and recrystallized from ethanol (Fig. 2).[14]

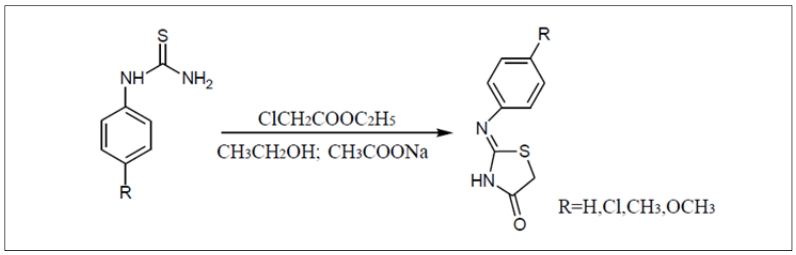

Fig.2. Synthesis of 4-substituted-2-(phenylimino)-thiazolidin-4-one from 4-Substituted phenylthiourea

Synthesis of 2-chloro-3-formylquinoline derivatives from 4-substituted acetanilide

Phosphoryl chloride (0.35 M) was added dropwise, with continuous stirring, into precooled (at 0°C) N, N-dimethylformamide (0.125 M, 9.1 g). The temperature of the mixture was increased to 80°C for 18 h after the addition of acetanilide (0.05 M). The mixture was consequently poured into ice water (300 mL) and stirred for 1 h at 0–10°C leading to the formation of 2-chloro-3-formylquinoline precipitate (Fig.3). The precipitate was filtered, washed, and dried followed by recrystallization from ethyl acetate.[15]

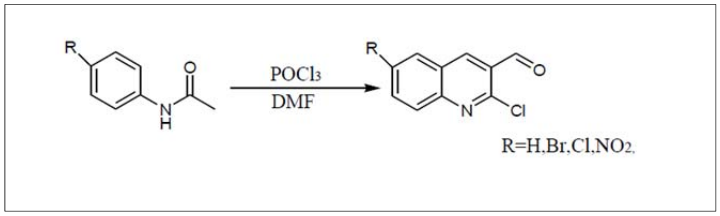

Fig.3. Synthesis of 2-chloro-3-formylquinoline derivatives from 4-substituted Acetanilide

Synthesis of intermediates

Synthesis of the intermediates was done in a round bottom flask fitted with a reflux condenser and a guard tube.

General procedure for the synthesis of 2(arylimino)-5-[(substituted halo quinolin-3-yl) methylene] thiazolidin-4-one.

Halo-3-formyl quinolone (0.02 M)/formyl quinolone, (4-substituted phenylimino)-thiazolidin-4-one (0.01 M), fused sodium acetate (0.02 M), and glacial acetic acid (30 mL) were added into a flask. The reaction mixture was refluxed for 5 h at 120℃. Resultant precipitate ((4-substituted phenylimino)-5-[(2-chloro-2, 3-dihydroquinoline-3yl) methylene] thiazolidin-4-one from 2-chloro-3-formylquinoline; (4-substitutedphenylimino)-5-(2, 6-dichloro-2, 3- dihydroquinoline-3yl) methylene) thiazolidin-4-one) was dried and recrystallized with ethanol after filtration (Figs.4 and 5).

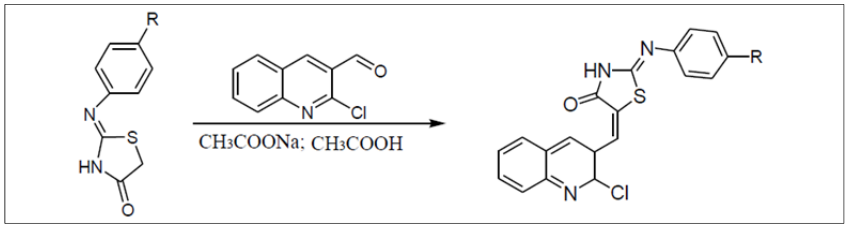

Fig.4. Synthesis of (4-substituted phenylimino)-5-[(2-chloro-2, 3-dihydroquinoline-3yl) methylene] thiazolidin-4-one from 2-chloro-3-formylquinoline

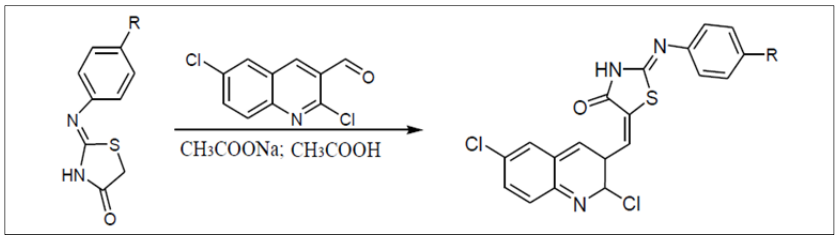

Fig.5. Synthesis of (4-substituted phenylimino)-5-(2, 6-dichloro-2,3- dihydroquinoline-3yl) methylene) thiazolidin-4-one from 2,6-dichloro-3-formylquinoline

Synthesis of 5-(2-chloroquinoline-3-yl-methylene)-2-thioxothiazolidin-4-one

5-(2-chloroquinoline-3-yl-methylene)-2-thioxothiazolidin-4-one was synthesized by the addition of 2-chloro-3-formylquinoline (0.01 M), 2-thioxothiazolidine-4-one (0.01 M), piperidine (2–3 drops), and methanol (15 mL) in a flask. The reaction mixture was refluxed for 1 h resulting in the formation of a precipitate. The precipitate was filtered and dried followed by recrystallization with ethanol (Fig. 6).

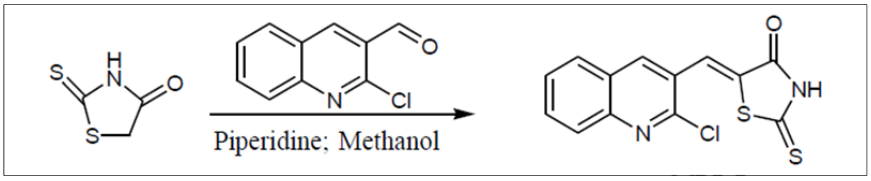

Fig.6. Synthesis of 5-((2-chloroquinoline-3-yl) methylene)-2-thioxothiazolidin-4-one

General procedure for synthesis of 5-(4/2-hydroxysubstituted-5/3-nitrosubstitutedbenzylidene)-2-(4-substituted phenylimino) thiazolidin-4-one.

Fused sodium acetate (0.02 M) and glacial acetic acid (30 mL) were added into a flask containing 4-hydroxy-3-nitrobenzaldehyde/2-hydroxy-5-nitrobenzaldehyde (0.01 M), (4-substituted phenylimino)-thiazolidin-4-one (0.01 M). The reaction mixture was refluxed for a period of 5 h at 120℃. The precipitate obtained was filtered, dried, and recrystallized with ethanol to obtain the final product (Figs.7 and 8).

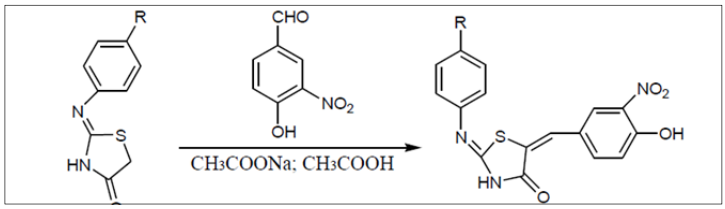

Fig.7. Synthesis of 2-(4-substituted phenylimino)-5-(4-hydroxy-3-nitrobenzylidene) thiazolidin-4-one

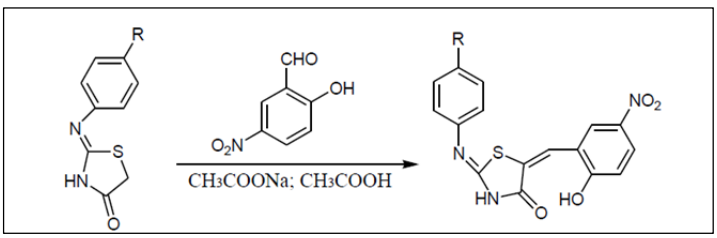

Fig.8. Synthesis of 5-(2-hydroxy-5-nitrobenzylidene)-2-(4-substituted phenylimino) thiazolidin-4-one

Synthesis of 4-thiazolinone derivatives

The 4-thiazolinone derivatives (labelled as MBP) were synthesized by the substitution of R-groups (H, Cl, and CH_3). Hydrogen was substituted in MBP-1, MBP-4, MBP-8, and MBP-11 derivatives. Chlorine was the

functional R-group in MBP-2, MBP-5, MBP-9, and MBP-12 derivatives. MBP-3, MBP-6, MBP-10, and MBP-13 had methyl as the functional R-group.

The intermediates synthesized for the production of derivatives are as follows:

1. MBP-1, MBP-2, and MBP-3: 4-substituted phenylimino-5-[(2-chloro-2, 3-dihydroquinoline-3yl) methylene] thiazolidin-4-one
2. MBP-4, MBP-5, and MBP-6: 4-substituted phenylimino-5-[(2,6-dichloro-2,3-dihydroquinoline-3yl)methylene] thiazolidin-4-one
3. MBP-7: 5-(2-chloroquinoline-3-yl-methylene)-2-thioxothiazolidin-4-one
4. MBP-8, MBP-9, and MBP-10: 2-(4-substituted phenylimino)-5-(4-hydroxy-3-nitrobenzylidene) thiazolidin-4-one
5. MBP-11, MBP-12, and MBP-13: 5-(2-hydroxy-5-nitrobenzylidene)-2-(4-substituted phenylimino) thiazolidin-4-one

Identification and characterization of synthesized derivatives

After synthesis, the derivatives were identified and characterized by determining melting point and performing thin layer chromatography (TLC), and infrared (IR), proton-nuclear magnetic resonance (1H-NMR) and mass spectroscopy.

Melting points were determined by capillary tube method (using Thiel's melting point tube) whereas TLC was performed with chloroform:methanol as mobile phase. Polar solvent—dimethyl sulfoxide (DMSO) was used for solubilizing the synthesized compounds. On the other hand, Fourier transform infrared spectroscopy (FTIR) spectra were recorded with potassium bromide powder on a JASCO V410 FTIR whereas ^{1}H-NMR spectra was measured in Bruker Ultraspec 500 MHz/AMX400 MHz spectrometer using $CDCl_3$ and d6-DMSO. Triple quadrupole LC/MS-6410 from Agilent technologies was used for mass spectroscopy.

Antimicrobial activity

Preliminary pharmacological screening was performed for the synthetic compounds by subjecting them to antimicrobial evaluation.[16] The compounds were tested against *Staphylococcus aureus*, *Bacillus subtils*, *Streptococcus mutans*, *Pseudomonas aeruginosa,* and *Escherichia coli*.

Turbidometric method

The 4-thiazolidinone derivatives (in graded concentrations) were added into a sterile nutrient medium followed by the inoculation of microorganisms. Medium was thereafter incubated at 37°C until the development of turbidity. Blank was maintained along with the positive and negative controls. Activity of the derivatives was evaluated by measuring the turbidity of the medium at 530 nm after incubation.

Agar diffusion method

The test compounds were added into the wells present in the Petri plates containing solidified agar inoculated with the respective microorganisms. Ciprofloxacin was used as the standard (40 µg/100 µL of DMSO) concomitantly with the test samples. The plates were subsequently incubated for 18–24 h at 37°C. Potency of each of the chemical was analyzed on the basis of the zone of inhibition (measured in terms of mm) developed in each plate.[17]

Results and discussion

A series of 13 derivatives of (4-substituted phenylimino)-5-[(2-chloro-2,3-di hydro quinolin-3-yl)methylene]thiazolidin-4-one, (4-substituted phenylimino)-5-(2,6-di chloro-2,3-dihydroquinolin-3-yl)methylene)thiazolidin-4-one, 5-((2-chloroquinolin-3-yl methylene)-2-thiaoxothiazolidin-4-one and 5-(4/2-hydroxy substituted-5/3-nitro substituted benzylidene)-2-(4-substituted phenylimino)thiazolidin-4-one were synthesized by reacting 4-substituted-2-(phenylimino)-thiazolidin-4-one with different aromatic aldehyde. However, the key intermediate, 4-substituted-2-(phenylimino)-thiazolidin-4-one, required for the synthesis of the title compounds was prepared by reacting 4-substituted/unsubstituted phenylthiourea with ethylchloroacetate, ethanol, and fused sodium acetate. Moreover, phenylthiourea acted as an adaptable starting material for the synthesis of MBP 1–6.

The synthesized compounds were recrystallized from ethanol and characterized by determining melting points and performing TLC, FTIR, ^{1}H NMR, and mass spectroscopy.

4-substituted phenylimino-5-[(2-chloro-2, 3-dihydroquinoline-3yl) methylene] thiazolidin-4-one

MBP -1 (R = H): Dark yellow colored powder; molecular weight 367; yield 37%; melting point 249–251℃; Rf 0.85; IR (KBr, cm^{-1}): 3150 (N–H), 3095 (aromatic C–H), 1677 (C=O), 1491 (C=C), 1104 (C–N).

MBP-2 (R=Cl): Light yellow powder; molecular weight 401; yield 42%; melting point 267–269; Rf 0.83; IR (KBr, cm^{-1}): 3237 (N–H), 3150 (aromatic C-H), 2170 C–S–C, 1651 (C=O), 1488 (C=C), 1019 (C-N); ^{1}H NMR (DMSO/$CDCl_3$): 7.07 (1H, d, aliphatic C-H), 7.16 (1H, t, aromatic H), 7.21 (1H, t, aromatic C–H), 7.32-7.54 (4H, m, aromatic H), 7.79 (2H, d, aromatic H), 7.98 (1H, s, aromatic H), 12.12 (1H, d, N–H).

MBP-3 (R=CH_3): Light green powder; molecular weight 381; yield 48%; melting point 320–322 ℃; Rf 0.70; IR (KBr, cm^{-1}): 3262 (N–H), 3196 (aromatic C–H), 2852 (Aromatic CH_3), 1667 (C=O), 1439 (C=C), 1113 (C–N); ^{1}H NMR (DMSO/$CDCl_3$): 6.96 (1H, s, aliphatic C–H), 7.21-7.54 (5H, m, aromatic H), 7.67–7.78 (2H, d, aromatic H), 7.96-8.41 (2H, s, aromatic H), 12.11 (1H, s, N-H); LC-MS (m/z): 382.1 (M+1).

4-substituted phenylimino-5-[(2,6-dichloro-2,3-dihydroquinoline-3yl)methylene] thiazolidin-4-one

MBP-4 (R=H): Yellow compound; molecular weight 402; yield 35%; melting point 258–260℃; Rf 0.71; IR (KBr, cm^{-1}): 3257 (N–H), 3141 (aromatic C–H), 1652 (C=O), 1514 (C=C), 1088 (C–N).

MBP-5 (R=Cl): Greenish yellow powder; molecular weight 436; yield 35%; melting point 270–271℃; Rf 0.72; IR (KBr, cm^{-1}): 3276 (N–H), 3040 (aromatic C-H), 1647 (C=O), 1556 (C=C), 1319 (C–N).

MBP-6 (R = CH_3): Light yellow powder; molecular weight 416; yield 40%; melting point 298-300℃; Rf 0.78; LC-MS (m/z): 416.

5-(2-chloroquinoline-3-yl-methylene)-2-thioxothiazolidin-4-one

MBP-7 : Yellow powder; molecular weight 306; yield 60%; melting point 170–172℃; Rf 0.64; IR (KBr, cm^{-1}): 3413 (N–H), 3049 (aromatic C–H), 2109 (C–S–C), 1723 (C=O), 1573 (C–N), 1484 (C=C); LC-MS (m/z): 305 (M−1).

2-(4-substituted phenylimino)-5-(4-hydroxy-3-nitrobenzylidene) thiazolidin-4-one

MBP-8 (3-NO_2-4-OH): Yellow powder; molecular weight 341; yield 62%; melting point 192–194℃; Rf 0.60; IR (KBr, cm^{-1}): 3481 (N–H), 3334 (OH), 3140 (aromatic C–H), 1673 (C=O), 1019 (C–N), 1497 (C=C), 1331 (aromatic NO_2); LC-MS (m/z): 342 (M+1).

MBP-9 (3-NO_2-4-OH): Dark yellow powder; molecular weight 375; yield 70%; melting point 212-214℃; Rf 0.72; IR (KBr, cm^{-1}): 3267 (N–H), 3348 (OH), 3041 (aromatic C–H), 1019 (C–N), 1451 (C=C), 1316 (aromatic NO_2).

MBP-10 (3-NO_2-4-OH): Light yellow powder; molecular weight 355; yield 62%; melting point 197-200℃; Rf 0.74; IR (KBr, cm^{-1}): 3258 (N–H), 3438 (OH), 3199 (aromatic C–H), 3126 (aromatic CH_3), 1019 (C–N), 1329 (C=C), 1496 (aromatic NO_2).

5-(2-hydroxy-5-nitrobenzylidene)-2-(4-substituted phenylimino) thiazolidin-4-one

MBP-11 (2-NO_2-5-OH): Light yellow powder; molecular weight 341; yield 55%; melting point 212-214℃; Rf 0.73.

MBP-12 (2-NO_2-5-OH): Light yellow powder; molecular weight 376; yield 50%; melting point 197–199℃; Rf 0.72; LC-MS (m/z): 376.

MBP-13 (2-NO_2-5-OH): Reddish brown powder; molecular weight 355; yield 58%; melting point 184–186℃; Rf 0.74; IR (KBr, cm^{-1}): 3486 (N–H), 3345 (OH), 3288 (aromatic C–H), 1666 (C=O), 1335.46 (C–N), 1614 (C=C), 1441 (aromatic NO_2).

Structures of various novel 4-thiazolidinone derivatives were confirmed based on the above spectral studies. Further, the resulting compounds were evaluated for their antimicrobial activity. Literature review reveals that 4-thiazolidinone derivatives exhibit diverse activities (antimicrobial, antifungal, and antiviral).

Antimicrobial activities of the synthesized 4-thiazolidinone derivatives were carried out by agar well diffusion method and the average diameter of zone of inhibition was recorded. In comparison with standard ciprofloxacin, MBP-1 and MBP-7 displayed moderate antimicrobial activity against both gram-positive organisms and gram-negative organisms including *Staphylococcus aureus*, *Bacillus subtils*, *Streptococcus mutans*, and *Escherichia*

coli; however, MBP-4, MBP-5, and MBP-10 showed moderate activity only against *Escherichia coli*. Also, *P. aeruginosa* was inhibited to some extent by MBP-1 and MBP-4; however, none of the compounds displayed higher activity than the standard drug **(Table 1)**.

References

[1] Microbial Evolution and Co-Adaptation: A Tribute to the Life and Scientific Legacies of Joshua Lederberg: Workshop Summary. The National Academies Collection: Reports funded by National Institutes of Health. Washington (DC)2009.
[2] Ji HF, Li XJ, Zhang HY. Natural products and drug discovery. Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia? EMBO reports. 2009,10(3):194-200.
[3] Randa Hilal-Dandan, Chapter name: Renin and Angiotensin, Book name: Goodman & Gilman's: The Pharmacological Basis of Therapeutics,12th edition; Chapter 26, Editors: Laurence L. Brunton, Bruce A. Chabner, Björn C. Knollmann..
[4] KD. T. Essential of Medicinal Pharmacology. 5th ed. Tripathi M, editor. New Delhi: Jaypee Brothers; 2008.
[5] Silhavy TJ, Kahne D, Walker S. The bacterial cell envelope. Cold Spring Harbor perspectives in biology. 2010,2(5):a000414.
[6] Chen MW. Structural stuides of enzymes regulating bacterial life style and cell wall biogenesis. Stockholm, Sweden: Karolinska Institutet; 2013.
[7] Singh T, Dudhe R, Kumar N, Sharma P. Absence of antidiabetic activity in some novel thiazolidinone derivatives. Journal of Pharmaceutical Negative Results. 2013,4(1):39.
[8] Andres CJ, Bronson JJ, D'Andrea SV, Deshpande MS, Falk PJ, Grant-Young KA, et al. 4-Thiazolidinones: novel inhibitors of the bacterial enzyme MurB. Bioorganic & medicinal chemistry letters. 2000,10(8):715-7.
[9] Sharath Kumar KS, Hanumappa A, Vetrivel M, Hegde M, Girish YR, Byregowda TR, et al. Antiproliferative and tumor inhibitory studies of 2,3 disubstituted 4-thiazolidinone derivatives. Bioorganic & medicinal chemistry letters. 2015,25(17):3616-20.
[10] Capan G, Ulusoy N, Ergenc N, Cevdet Ekinci A, Vidin A. Synthesis and anticonvulsant activity of new 3-[(2-furyl)carbonyl]amino-4-thiazolidinone and 2-[(2-furyl)carbonyl]hydrazono-4-thiazoline derivatives. Farmaco. 1996,51(11):729-32.
[11] Barreca ML, Chimirri A, De Luca L, Monforte AM, Monforte P, Rao A, et al. Discovery of 2,3-diaryl-1,3-thiazolidin-4-ones as potent anti-HIV-1 agents. Bioorganic & medicinal chemistry letters. 2001,11(13):1793-6.
[12] Wang AB. Synthesis of phenyl thioureas containing para-substituted tertiary amino-groups. Science China Mathematics. 1954,3(3):3.
[13] Robert L. Frank and Paul V. Smith RLFaPV. a-Phenylthiourea. Organic Syntheses. 1948,28:89.
[14] Bhandari SV, Bothara KG, Patil AA, Chitre TS, Sarkate AP, Gore ST, et al. Design, Synthesis and Pharmacological Screening of Novel Antihypertensive Agents Using Hybrid Approach. Bioorganic & Medicinal Chemistry. 2009,17(1):390-400.
[15] Tóth J, Blaskó G, Dancsó A, Tőke L, Nyerges M. Synthesis of New Quinoline Derivatives. Synthetic Communications. 2006,36(23):3581-9.
[16] Ghatage SL, Navale, S.S., Mujawar, N.K., Patil, S., Patil, V. Antimicrobial Screening. Indian Journal of Drugs. 2014,2(3):4.
[17] Kramer J, Kirshbaum A. Effect of paper on the performance assay in the control of antibiotic sensitivity discs. Applied microbiology. 1961,9:334-6.

Table 1. Antimicrobial activity of synthesized compounds

Code	**Concentration (µg/mL)**	**Zone of inhibition (mm)**				
		Staphylococcus aureus (+ve)	***Streptococcus mutans (+ve)***	***Bacillus subtils (+ve)***	***Escherichia coli (-ve)***	***Pseudomonas aeruginosa (-ve)***
Standard (ciprofloxacin)	40	26	33	26	29	27
MBP-1		18.5	–	14	17	17.5
MBP-2		10	–	–	–	–
MBP-3		–	–	–	–	–
MBP-4		–	–	10	12.5	16
MBP-5		–	–	–	11	–
MBP-6		–	–	–	–	–
MBP-7		10	13.5	14	19	–
MBP-8		–	–	–	–	–
MBP-9		–	–	11.5	–	–
MBP-10		–	–	–	–	–
MBP-11		–	–	–	–	–
MBP-12		–	–	10	10	–
MBP-13		–	–	–	–	–