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Synthesis, Characterization and Evaluation of Antimicrobial profile of 4-Thiazolidinone derivatives

Asish Bhaumik*[1], M. Upender Kumar[1], M. Sucharitha[1], Dr. P. Panneerselvam[2], Sunil Kumar Sing[2]

[1]*Department of Pharmaceutical Chemistry, Teja College of Pharmacy, Kodad, Nalgonda-508206, Telangana, India*
[2]*Department of Pharmaceutical Chemistry, C.L. Baid Metha College of Pharmacy, Jyothinagar, Thoraipakkam, Chennai-600097, India.*

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Abstract

The objective of the present work was the synthesis of N-[2-(4-substituted phenyl)-4-oxo-1,3-thiazolidine-3-yl]-2-(naphthalene-2-yloxy)acetamide and evaluation of in-vitro antimicrobial activity. Based on this a new series of compound had been planned to synthesize by reacting β-naphthol, ethyl chloroacetate, hydrazine monohydrate, ethyl alcohol and various aromatic aldehydes in presence of anhydrous potassium carbonate The synthesized compounds were characterized by IR, NMR, and Mass spectroscopy. The in-vitro antimicrobial profile of newly synthesized compounds were carried out by using agar diffusion method using bacterial cultures of Staphylococcus aureus (ATCC 9144) and Escherichia coli (ATCC 25922) and fungal culture of Aspergillus niger (ATCC 9029). By observing it was found that most of the synthesized compounds executed moderate to good antimicrobial activity against the tested micro-organisms. The most of the synthesized compounds were active against all the tested micro-organisms for antimicrobial activity with an MIC range of 15-40µg/ml. The MIC values for the synthesized compounds were found to be A2 (MIC of 15 µg / ml), A10 (MIC of 16 µg / ml), and A5 (MIC of 17) against Staphylococcus aureus (ATCC 9144) and A2 (MIC of 16 µg / ml), A10 (MIC of 19 µg / ml), and A5 (MIC of 18) against Escherichia coli (ATCC 25922) and A1 (MIC of 16 µg / ml), A3 (MIC of 19 µg / ml), and A5 MIC of 18 µg / ml) against Aspergillus niger (ATCC9029).

Keywords: Thiazolidinone, Antimicrobial, IR, NMR, MIC

Contents

***Corresponding author**
Asish Bhaumik
Department of Pharmaceutical Chemistry,
Teja College of Pharmacy, Kodad,
Nalgonda-508206, Telangana, India
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1. Introduction

Antimicrobials (Disinfectants and Antiseptics) are the chemical agents which are used to destroy or inhibit the growth and development of pathogenic microorganisms [1]. To determine bacterial susceptibility, disc diffusion method is commonly used and provides the results within 24 hours. In view of the large number of antimicrobial agents available, it is not possible to test all the agents against the isolate and a particular compound can be used as a class representative e.g, one of the agents of first generation for that generation of cephalosporins. For organisms which have remained susceptible to the same drug since antibiotics were discovered e.g group a streptococci, most laboratories do not report susceptibility tests [2]. Several clinical pharmacokinetic indices are useful in adjusting the dosage of antimicrobial agents. The half-life of a drug and the volume of distribution, the MIC for the infecting organism, site of infection and host defence factors should be considered in determining dose frequency [3].

4-thiazolidinones are the derivatives of thiazolidine with a carbonyl group at the 4 position. Several methods for the synthesis are available. The synthesis of 2-amino 4-thiazolidinones-4-C has been reported by using thiourea and sodium salt of labeled monochloroacetic acid [4]. Another method of synthesis of 4-thiazolidinones is by using of thiocyanate, alkylisothio cyanate with hydrazide/acetamide followed by the treatment with ethylchloro or ethylbromo acetate and sodium acetate [5].

The literature survey revealed that 4-thiazolidinone and their derivatives were possessed a wide range of pharmacological activities such as anti-inflammatory, analgesic, anticonvulsant, antimicrobial, local and spinal anesthetics, CNS stimulants, hypnotics, anti HIV, anti diabetic, anticancer, FSH receptor antagonist and CFTR inhibitor etc [6-7]. The objective of the present work is the synthesis of N-[2-(4-substituted phenyl)-4-oxo-1,3-thiazolidine-3-yl]-2-(naphthalene-2-yloxy) acetamide and evaluation of antimicrobial activity. Based on this a new series of compound have been planned to synthesize by reacting β-napthol, ethylchloro acetate, hydrazine monohydrate, ethyl alcohol and various aromatic aldehydes in the presence of anhydrous potassium carbonate.

2. Materials and Methods

The all chemicals used for the synthesis were of laboratory grade and analytical grade. The melting points of newly synthesized thiazolidinone compounds were determined by open capillary method. The IR spectra of synthesized compounds were recorded by ABB Bomen FT-IR spectrometer MB 104 IR spectra recorder with KBr pellets**.** The H^1-NMR spectra of synthesized compounds were recorded by BRUKER NMR spectrometer in DMSO. The Mass spectra of synthesized compounds were recorded by JEOL GCmate. The purification of newly synthesiszed compounds were done by TLC method.

TLC plates are pre-coated silica gel (HF254-200 mesh) aluminium plate using ethyl acetate and n-hexane as a solvent system and spots were visualized under U.V chamber. The IR, H^1-NMR and Mass spectra were assigned to elucidate the structure of synthesized compounds (A1-A10). The standard drugs Ciprofloxacin and Ketoconazole were purchased from the local retail pharmacy shop. Bacterial culctures of *Staphylococcus aureus (ATCC 9144)* and *Escherichia coli* (*ATCC 25922)* and fungal culture of *Aspergillus niger (ATCC 9029)* were obtained from the Biotechnology Lab of the Institute and maintained on Nutrient agar slant and fungus strain was maintained on Sabouraud dextrose broth at 4^0 C.

Steps involved in the synthesis of target compound [8]

Step-1: Preparation of ethyl-2-naphthalene -6-yloxy acetate:

2-napthol (1.44 gm, 10mmol), anhydrous potassium carbonate (1gm) and ethyl chloroactate (1.67gm, 10mmol) in 50ml of anhydrous acetone were refluxed on oil bath for 6 hours. The reaction mixure was filtered and the excess solvent was removed by distillation under pressure.

Step-2: Preparation of 2-(naphthalene-6-yloxy) acetohydrazide:

The residue and 1gm hydrazine monohydrate (20 mmol) were dissolved in 50 ml of absolute ethanol and refluxed on a steam bath for 1 hour. The solute must was filtered and dried and recrystalized from ethanol.

Step-3: Preparation of substituted benzaldehyde derivatives:

0.01mol of substituted benzaldehyde and 0.01mol of substance and 2-3 drops of glacial acetic acid and 20ml of ethanol were taken in round bottom flask and reflux for 6 hours on water bath. After cooling add ice cold water to the mixture to give solid white mass. Filtered and dried. Recrystallized from chloroform-methanol mixture.

Step-4: General method of synthesis of thiazolidinone derivatives:

A mixture of Schiff base (0.001mmol) and Thioglycolic acid (0.001mol) dissolved in 1,4-dioxane (20ml), anhydrous zinc chloride (0.5mg) was added and refluxed for 8 hours. The reaction was then cooled to 30°C and the resulting solid was washed with sodium bicarbonate solution. The final compound recrystallized from absolute ethanol.

Synthetic scheme:

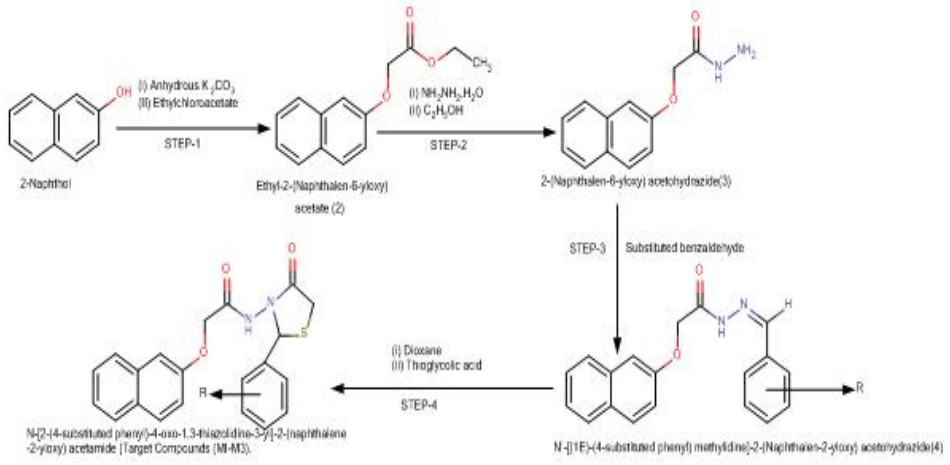


Figure 1

Spectral data:

Compound A1:

N-[2-(4-hydroxyphenyl]-4-oxo-1,3-thiazolidin-3-yl-2-(naphthalene-2-yloxylacetamide. M.F- $C_{21}H_{18}N_2O_4S$, M.W 394.44 ,M.P-180^{O}c, R_f-0.55, Yield-62.1%, IR (KBr) ν (cm^{-1}): 1624.11cm^{-1}(Ar-C=C),3177.12cm^{-1}(aliph-N-H),1026.57cm^{-1}(N-N),747.42cm^{-1}(C-S),3610.57cm^{-1}(O-H phe),1689.24cm^{-1}(C=O),1269.54cm^{-1}(C-N),1728.62cm^{-1}(C=O-thiazolidine), ^{1}H-NMR δ (ppm): 8.0(1H,-NH-),6.8-7.9(11H,Ar-H),5.92(1H,-N-CH-S-),5.21(1H,Ar-OH),5.0(2H,-O-CH_2-CO-),3.8(2H,-S-CH_2), Mass (m/e value): 394.5(30%)(M^+), 395.4(25%)(M+1), 377.1(50%), 301.0(70%), 274.0(38%), 228.1(58%), 200.7(67%), 185.1(24%), 157.1(48%), 127.1(73%), 102.0(44%), 100.4(100%)B

Copound A2:

N-[2(4-chlorophenyl-4-oxo-1,3-thiazolidin-3-yl]-2-(naphthalene-2-yloxy)acetamide.M.F: $C_{21}H_{17}ClN_2O_3S$, MW-412.89, M.P-172^{0}c, R_f- 0.46, Yield-65.2%, IR (KBr) ν (cm^{-1}): 1611.20cm^{-1} (Ar-C=C) ,3186.99cm^{-1} (Aliph-N-H), 1086.99cm^{-1}(N-N) ,695.56cm^{-1} (C-S) ,1668.87cm^{-1}(C=O), 1267.68cm^{-1} (C-N) ,750.35cm^{-1} (Ar-C-Cl) ,1716.32cm^{-1} (C=O- thiazolidine), ^{1}H-NMR δ (ppm): 8.3 (1H,-NH-) ,6.8-7.9 (11H,Ar-H) ,5.80 (1H,-N-CH-S-) ,5.0 (2H,-O-CH_2-CO-), 3.3(2H,-S-CH_2), Mass (m/e value): 412.9(24%)(M^+), 413.8(20%)(M+1), 377.1(50%), 301.0(70%), 274.0(38%), 228.1(58%), 200.7(67%), 185.1(24%), 157.1(48%), 127.1(73%), 102.0(44%),100.4(100%)B.

Copound A3:

N[2-(4-fluorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-(naphthalene)acetamide. M.F- $C_{21}H_{17}FN_2O_3S$, MW- 396.43, M.P-175^{0}c, R_f- 0.48, Yield- 55.7%, IR (KBr) ν (cm^{-1}): 1609.09cm^{-1}(Ar-C=C),3194.42cm^{-1}(Aliph-N-H),1026.76cm^{-1}(N-N) 1256.34cm^{-1}(C-N), 705.10cm^{-1}(C-S),1662.09cm^{-1}(C=O),1000.62cm^{-1} (Ar-C-F), 1721.94cm^{-1}(C=O-thiazolidin), ^{1}H-NMR δ (ppm): 8.20(1H,-NH-), 6.8-7.9(11H,Ar-H),6.0(1H,-N-CH-S-),4.90(2H,-O-CH_2-CO-), 3.5 (2H,-S-CH_2-), Mass (m/e value): 396.5(13%)(M^+), 397.4(11%)(M+1), 377.1(50%), 301.0(70%), 274.0(38%), 228.1(58%), 200.7(67%), 185.1(24%), 157.1(48%), 127.1(73%), 102.0(44%),100.4(100%)B.

Copound A4:

N-[2-(4-bromophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-(naphthalene-2-yloxy)acetamide. M.F- $C_{21}H_{17}BrN_2O_3S$, M.W-457.34, M.P- 178^{0}c, R_f- 0.51, Yield- 64.96%, IR (KBr) ν (cm^{-1}): 1621.73cm^{-1}(Ar-C=C), 3198.97 cm^{-1}(Aliph-N-H), 1031.38 cm^{-1}(N-N), 758.36 cm^{-1}(C-S), 1681.77 cm^{-1}(C=O), 1530.18 cm^{-1}(Ar-C-Br), 1721.46 cm^{-1}(C=O-thiazolidine), ^{1}H-NMR δ (ppm): 8.0(1H,-NH-), 6.8-7.9(11H,Ar-H), 5.9(1H,-N-CH-S-), 5.2(2H,-O-CH_2-CO-), & 3.3(2H,-S-CH_2-), Mass (m/e value): 457.4(10%)(M^+), 458.3(9%)(M+1), 377.1(50%), 301.0(70%), 274.0(38%), 228.1(58%), 200.7(67%), 185.1(24%), 157.1(48%), 127.1(73%), 102.0(44%),100.4(100%)B.

Copound A5:

2-(naphthalene-2-yloxy)-N-[2-(4-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]acetamide. M.F- $C_{21}H_{17}N_3O_5S$, M.W-423.44, M.P- 160^{0}c, R_f- 0.71,Yield- 68.2%, IR (KBr) ν (cm^{-1}): 1605.0cm^{-1}(Ar-C=C), 3181.81 cm^{-1},(Aliph-N-H), 1050.57 cm^{-1}(N-N), 1248.07 cm^{-1}(C-N), 752.45 cm^{-1}(C-S), 1685.27 cm^{-1}(C=O), 1521.57 cm^{-1}(Ar-NO_2), 1721.09 cm^{-1}(C=O-thiazolidine), ^{1}H-NMR δ (ppm): 8.2(1H,-NH-), 6.8-7.9(11H, Ar-H), 5.8(1H,-N-CH-S-), 5.1(2H, -O=CH_2-CO-), & 3.4(2H, -S-CH_2-), Mass (m/e value) **:** 423.5(11%)(M^+), 424.4(9%)(M+1), 377.1(50%), 301.0(70%), 274.0(38%), 228.1(58%), 200.7(67%), 185.1(24%), 157.1(48%), 127.1(73%), 102.0(44%),100.4(100%)B.

Copound A6:

2(naphthalene-2-yloxy)-N-[2-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]acetamide. M.F- $C_{21}H_{17}N_3O_5S$, M.W- 423.44, M.P- 165^{0}c, R_{f-} 0.69, Yield- 68.2%, IR (KBr) ν (cm^{-1}): 1613.0cm-1(Ar-C=C), 3211.27 cm-1(Aliph-N-H), 1061.45

cm-1(N-N), 1248.01 cm-1(C-N), 774.86 cm-1(C-S),1681.31 cm-1(C=O),1516.23 cm-1(NO_2), 1717.68 cm-1(C=O-thiazolidine), ^{1}H-NMR δ (ppm): 8.2(1H, -NH-), 6.8-7.9(11-H, Ar-H), 5.8(1H,-N-CH-S-), 5.2(2H,-O-CH_2-CO-),3.4(2H, -S-CH_2-), Mass (m/e value): 423.5(9%)(M^+), 424.4(8%)(M+1), 377.1(50%), 301.0(70%), 274.0(38%), 228.1(58%), 200.7(67%), 185.1(24%), 157.1(48%), 127.1(73%), 102.0(44%),100.4(100%)B.

Copound A7:

N-[2-(3,4-dimethoxyphenyl)-4-oxo-thiazolidin-3-yl]-2-(naphthalene-2-yloxy)acetamide.M.F- $C_{23}H_{22}N_2O_5S$,M.W-438.12, M.P-185^{0}c, R_f-0.66, Yield-58.6%, IR (KBr) ν (cm^{-1}): 1619.0cm-1(Ar-C=C), 3202.17cm-1(Aliph-N-H), 1026.57cm-1(N-N), 1265.59cm-1(C-N), 747.42cm-1(C-S), 1663.99cm-1(C=O), 1126.82cm-1(-C-O-C-), 1723.15cm-1(C=O-thiazolidine), ^{1}H-NMR δ (ppm): 8.2(1H, -NH-), 6.8-7.9(11H, Ar-H), 6.1(1H, -N-CH-S-), 5.3(2H, -O-CH_2-CO-), 3.8(6H, -O-CH_3), 3.4(2H, -S-CH_2-), Mass (m/e value): 438.1(6%)(M^+), 439.1(5%)(M+1), 377.1(50%), 301.0(70%), 274.0(38%), 228.1(58%), 200.7(67%), 185.1(24%), 157.1(48%), 127.1(73%), 102.0(44%),100.4(100%)B.

Copound A8:

N-[2-(2-chlorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-(naphthalene-2-yloxy)acetamide. M.F- $C_{21}H_{17}ClN_2O_3S$, M.W-412.89, M.P- 198^{0}C, R_f – 0.44, Yield-71.2%, IR (KBr) ν (cm^{-1}): 1614.07cm-1(Ar-C=C), 3188.27cm-1(Aliph-N-H), 1048.26cm-1(N-N), 1267.13cm-1(C-N), 774.55cm-1(C-S), 1685.07cm-1(C=O), 700.46cm-1(Ar-C-Cl), 1721.07cm-1(C=O-thiazolidine), ^{1}H-NMR δ (ppm): 8.4(1H, -NH-), 6.8-7.9(11H, Ar-H), 6.2(1H, -N-CH-S-), 5.2(2H, -O-CH_2-CO-), 3.7(2H, -S-CH_2-), Mass (m/e value): 412.9(14%)(M^+), 413.8(13%)(M+1), 377.1(50%), 301.0(70%), 274.0(38%), 228.1(58%), 200.7(67%), 185.1(24%), 157.1(48%), 127.1(73%), 102.0(44%),100.4(100%)B.

Copound A9:

2-(naphthalene-2-yloxy)-N-[2-(3-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]acetamide. M.F- $C_{21}H_{17}N_3O_5S$, M.W-423.44, M.P-166^{0}c, R_f - 0.68, Yield-71.5%, IR (KBr) ν (cm^{-1}): 1612.32cm-1(Ar-C=C), 3217.42cm-1(Aliph-N-H), 1050.57cm-1(N-N), 1237.20cm-1(C-N), 703.59cm-1(C-S), 1682.57cm-1(C=O), 1507.14cm-1(Ar-NO_2), 1721.38cm-1(C=O-thiazolidine), ^{1}H-NMR δ (ppm): 8.2(1H, -NH-), 6.8-7.9(11H, Ar-H), 6.1(1H, -N-CH-S-), 5.1(2H, -O-CH_2-CO-), 3.6(2H, -S-CH_2-), Mass (m/e value): 423.5(9%)(M^+), 424.4(M+1), 377.1(50%), 301.0(70%), 274.0(38%), 228.1(58%), 200.7(67%), 185.1(24%), 157.1(48%), 127.1(73%), 102.0(44%),100.4(100%)B.

Copound A10:

N-[2-(3-hydroxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-(naphthalene-2-yloxy)- acetamide. M.F- $C_{21}H_{18}N_2O_4S$, M.W-394.44, M.P- 187^{0}c, R_f.0.58, Yield- 62.3%, IR (KBr) ν (cm^{-1}): 1603.86cm-1(Ar- C=C), 3210.68cm-1(Aliph-N-H), 1048.26cm-1(N-N), 1258.95cm-1(C-N), 703.47cm-1(C-S), 1686.85cm-1(C=O),3610.93cm-1(O-H-Ph), 1721.63cm-1(C=O-thiazolidine), ^{1}H-NMR δ (ppm): 8.3(1H, -NH-), 6.8-7.9(11H, Ar-H), 6.1(1H, -N-CH-S-), 5.2(2H, -O-CH_2-CO-), 4.9(1H, Ar-OH), 3.3(2H, -S-CH_2-), Mass (m/e value): 394.5(26%)(M^+), 395.4(25%)(M+1), 377.1(50%), 301.0(70%), 274.0(38%), 228.1(58%), 200.7(67%), 185.1(24%), 157.1(48%), 127.1(73%), 102.0(44%),100.4(100%)B.

Table 2: for Minimum Inhibitory Concentration (MIC) of the Synthesized Compounds

Compounds	**Bacteria**						**Fungi**		
	S.aureus			***E.coli***			**A.niger**		
	Concentration (µgm/ml)								
	50	100	150	50	100	150	50	100	150
A1	18	21	25	19	21	25	19	21	24
A2	22	24	29	22	25	29	20	22	23
A3	23	27	31	18	21	24	23	25	28
A4	17	19	23	15	18	20	19	21	28
A5	25	29	32	23	27	29	22	25	31
A6	20	24	27	19	21	24	18	21	24
A7	23	25	27	24	28	31	23	26	31
A8	20	24	28	18	21	25	20	24	27
A9	17	19	22	19	22	24	22	25	29
A10	24	28	32	23	27	31	20	21	24
Ciprofloxacin (100 µgm/ml)	38			38			–		
Ketoconazole (100 µgm/ml)	–			–			38		

Evaluation of Antimicrobial Activity by paper disc diffusion method [9 10,11]

The sterilized (autoclaved at 120^{o}C for 30 min) medium was inoculated (1mL/100mL of medium) with the suspension [10^5 cfu m/l (colony forming unit per milliliter)] of the microorganism (matched to McFarland barium

sulphate standard) and poured in Petridish to give a depth of 3-4mm. The paper impregnated with the test compounds (50, 100,150 µg/ml in dimethyl formamide) was placed on the solidified medium. The plates were pre-incubated for 1hr at RT and incubated at 37 ^{o}C for 24 hr for anti-bacterial and antifungal activities respectively. Ciprofloxacin (100 µg/disc) and Ketoconazoloe (100 µg/disc) was used as a standard.

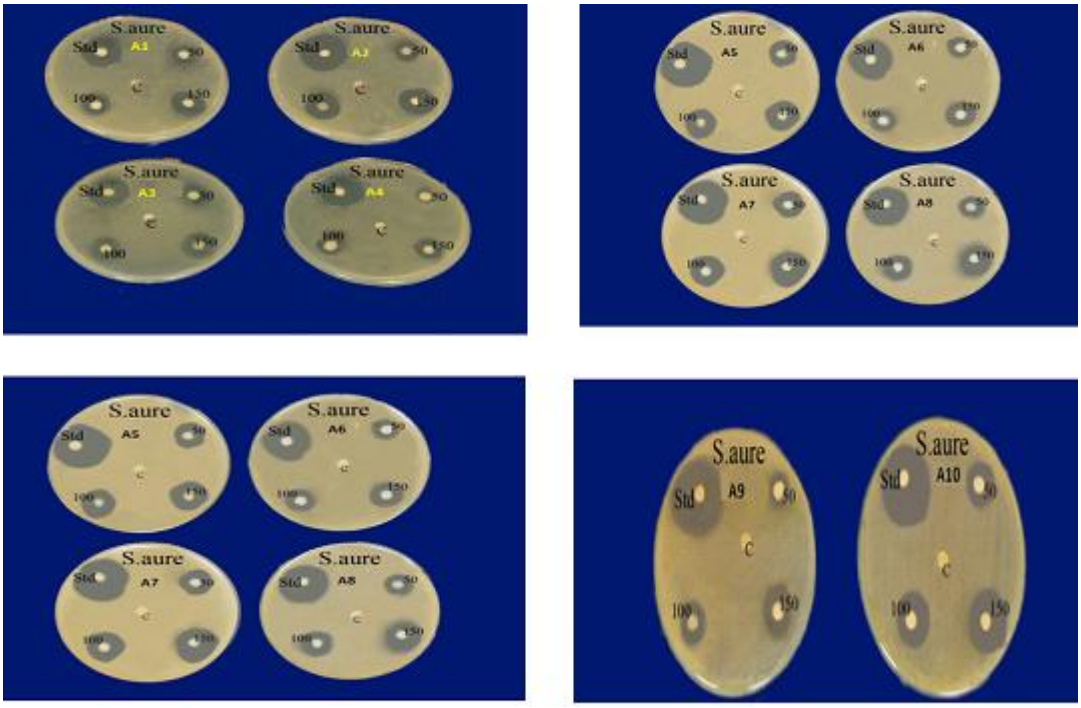
Determination of MIC by agar streak dilution method [12]

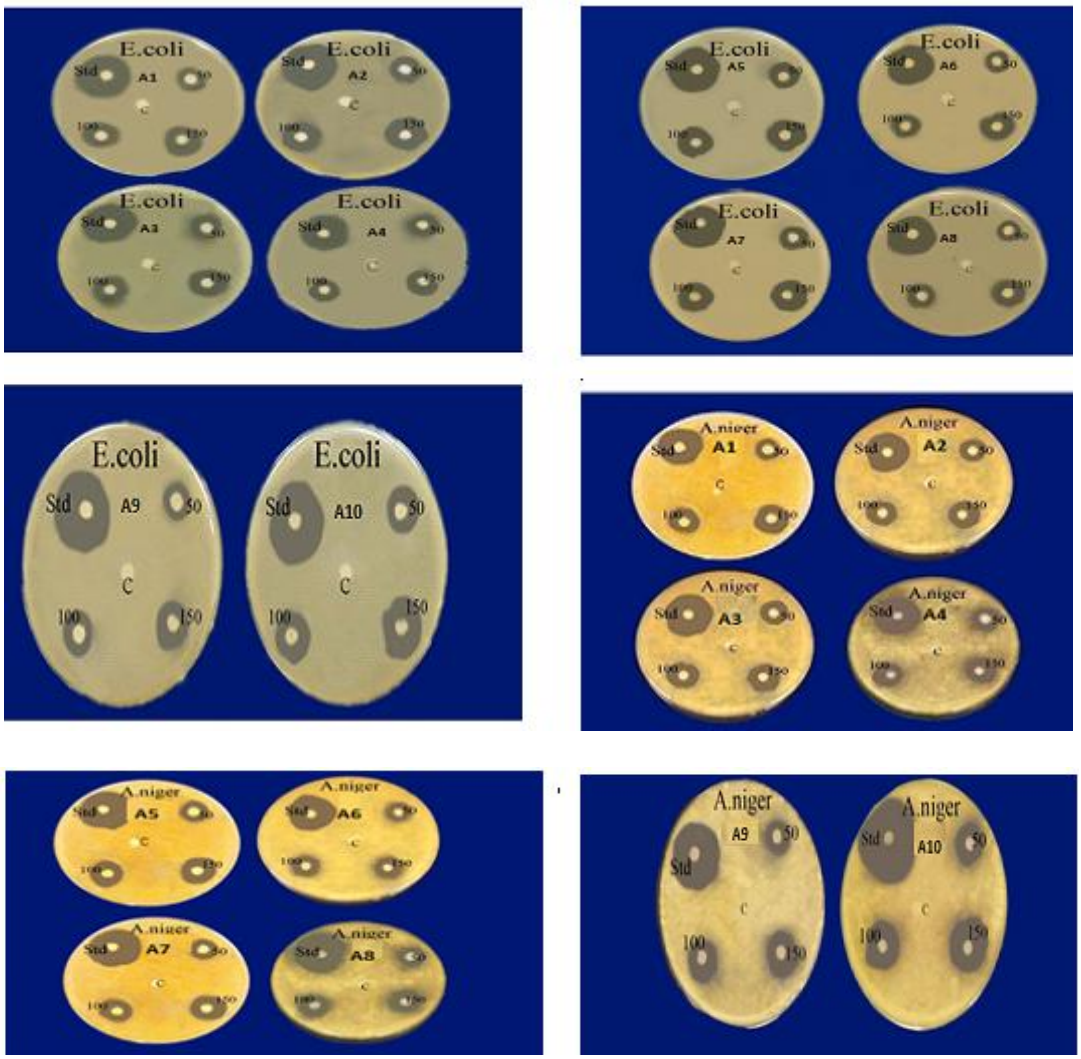
MIC of the synthesized compounds was determined by agar streak dilution method. A stock solution of the synthesized compounds (100µg/ml) in Dimethyl formamide was prepared and graded quantities of the test compounds were incorporated in specified quantities of molten nutrient agar medium. A specified quantity of the medium containing the compounds was poured into a Petri dish to give a depth of 3-4mm and allowed to solidify. Suspension of the micro-organism were prepared to contain approximately10^5 cfu m/l and applied to plates with serially diluted compounds in Dimethyl formamide to be tested and incubated at 37^{o}C for 24hr. for bacteria and fungi. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria on the plate.

3. Result and Discussion

Table 2: For Minimum Inhibitory Concentration (Mic) of the Synthesized Compounds

Compounds	Minimum inhibitory concentration (µg/ml)		
	Bacteria		Fungi
	S.aureus	E.coli	A.niger
A1	36	31	38
A2	15	16	16
A3	33	35	19
A4	38	40	33
A5	17	18	18
A6	34	37	25
A7	20	21	17
A8	35	35	40
A9	31	33	23
A10	16	19	25
Ciprofloxacin	0.2	0.3	-
Ketoconazole	-	-	6.1





Chemistry:

The synthesis of target compound-N-[2-(4-substituted phenyl)-4-oxo-1,3-thiazolidine-3-yl]-2-(naphthalene-2-yloxy)acetamide was carried out by reacting β-napthol, ethyl chloroacetate, hydrazine monohydrate, ethyl alcohol and various aromatic aldehydes in the presence of anhydrous potassiumcarbonate. The synthesized compounds were characterized by IR, NMR, and Mass spectroscopy and proposed the structure by spectral data The purity of the synthesized compounds were ascertained by TLC and spectral analysis.

Antimicrobial screening:

The synthesized compounds were (50, 100 and 150 µg/ml) screened for antimicrobial activity by paper disc diffusion method. The experimental data had shown that most of the synthesized compounds executed moderate to good antimicrobial activity against the tested micro-organisms. When compared to standard drugs (Ciprofloxacin) compounds A5, A2, A7 and A10 were found to exhibit good Anti-bacterial activity. When compared to standard drugs (Ketoconazole) compounds A5, A7, A9 and A3 were found to exhibit good Anti-fungal activity. The MIC of synthesized compounds were screened by agar streak dilution method. The experimental data had shown that most of the synthesized compounds executed moderate to good antibacterial and antifungal activity with an MIC range of 15-40µg/ml. The MIC values for the synthesized compounds was found to be A2 (MIC of 15 µg / ml), A10 (MIC of 16 µg / ml), and A5 (MIC of 17) against *Staphylococcus aureus (ATCC 9144)* and A2 (MIC of 16 µg / ml), A10 (MIC of 19 µg / ml), and A5 (MIC of 18) against Escherichia coli *(ATCC 25922)* and A1 (MIC of 16 µg / ml), A3 (MIC of 19 µg / ml), and A5 MIC of 18 µg / ml) against *Aspergillus niger(ATCC9029).*

4. Conclusion

By observing it was found that most of the newly synthesized compounds exhibited moderate to good antimicrobial activity against the tested micro-organisms. The synthesized compounds were active against all the tested micro-organism for antibacterial activity with an MIC range of 15-40µg/ml against *Staphylococcus aureus (ATCC 9144)*

and *Escherichia coli (ATCC 25922)* and for antifungal activity with an MIC range of 15-40µg/ml against *Aspergillus niger(ATCC9029).*

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