Synthesis and biological evaluation of novel 1,3,4-thiadiazole-linked indole derivatives as potential antimicrobial agents

Monika Barsagade[1*], Vijay Kumar Singh[1], Aarti Prajapati[2]

[1*]Shri Rawatpura Sarkar College of Pharmacy, Shri Rawatpura Sarkar University, NH-43, Dhamtari Road, Raipur, Chhattisgarh 492001

[2]Quantum School of Health Sciences, Quantum University Roorkee- Dehradun Highway (NH 73), Mandawar, Roorkee Uttarakhand 247167

Email: monikabarsagade627@gmail.com

ABSTRACT

The synthesis of some 2-phenyl-5-substituted-1, 3, 4-thiadiazol derivatives are synthesized and evaluated for antibacterial and antifungal activity. All the new compounds structures were elucidated by IR, ^{1}HNMR, and MASS spectral data. Antibacterial activity of all compounds was evaluated against *Bacillus subtilis, Bacillus megaterium, Escherichia coli* and *Pseudomonas Species* and antifungal activity was evaluated against *Candida albicans.* Study revealed that Compound **9f** showed potent activity against *Bacillus subtilis* and *Bacillus megaterium* with MIC 6.8 and 7.4µg/mL respectively. Compound **9e** showed potent activity against *Escherichia coli* with MIC 2.2 µg/mL. Compound **7i** showed potent activity against *Pseudomonas species* with 1.8µg/mL. Compound **9i** showed potent antifungal activity against *Candida albicans* with MIC 6.4µg/mL.

Keywords: Antibacterial, Antifungal, 1,3,4-Thiadiazole, *Bacillus subtilis*, *Bacillus megaterium*.

1. Introduction

An antimicrobial is an agent that either kills or inhibits the growth of microbes. Thus, this microbial agent may be either a chemical compound or any physical agents. These agents efficiently interfere with the growth and reproduction of causative microorganisms like bacteria, fungi, parasites, virus etc. The breakthrough obtained with the discovery of Penicillin in 1940 lead to development of an era which formed the basis of the prospering modern antimicrobial therapy. Diverse antibiotics and other agents have been discovered after the penicillin. The breakthrough of the antibiotic penicillin in the early 1940s became the vital mainstay for the era of [1]. The development of novel antimicrobial agents is an urgent and attractive task for medicinal chemists because of the increasing resistance to current antibiotic chemotherapy [2]. Drug resistant bacteria, such as vancomycin-resistant enterococci (VRE), methicillin resistant staphylococcus aureus (MRSA), multidrug resistant pseudomonas aeruginosa, and multi-drug resistant escherichia coli, cause lethal diseases and great difficulties in the treatment of community acquired and nosocomial infections, which severely threaten global public health and result in high economic costs. The main reason for this global problem is the widespread use of broad-spectrum antibiotics, anti-HIV and

anticancer drugs, which promote the development of resistance [3]. Thus; there is an extremely urgent need to develop new antimicrobial agents with a new mechanism of action and/or with the ability to overcome drug resistance. Heterocyclic chemistry is one of the most valuable sources of novel compounds with diverse biological activity. To medicinal chemists, the true utility of heterocyclic structures is the ability to synthesize one library based on one core scaffold and to evaluate its biological activity led to yield potential lead candidates for therapeutic use. Heterocycles form by far the largest of the classical divisions of organic chemistry and are of immense importance biologically, industrially, and indeed to understanding the life process and effort to improve the quality of life. In this regard, functionalized nitrogen-containing heterocycles are securing their place among the most highly recognized pharmacophore and therefore they have been intensively used as scaffolds for the drug development [4, 5]. Among them 1,3,4-thiadiazole scaffold have potent antimicrobial activity. Thiadiazoles are classified under azole compounds. These are five-membered heterocyclic compound containing a sulfur atom with two nitrogen atoms. Presence of two double bonds gave aromatic ring; with the name thiadiazole originating from the Hantzsch-Widman nomenclature [6]. Basically, there are four isomeric forms of thiadiazole shown in Figure 1.

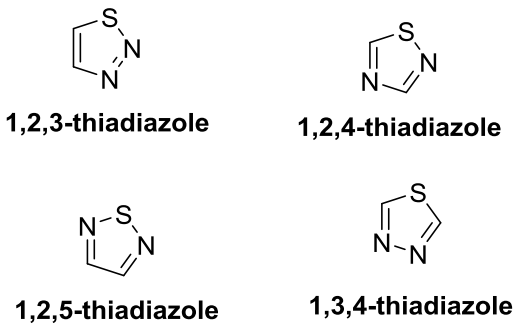


Figure 1. Isomers of thiadiazole

1,3,4-thiadiazole is amongst the highly useful isomeric form because of its diverse biological properties. Compounds bearing the 1,3,4-thiadiazole nucleus is known to have exclusive action against bacterial infection and anti-inflammatory activities in body. Structurally substituted derivatives of thiadiazole have also been found to bear diverse therapeutic activities such as analgesic, antimicrobial, antitubercular, anticonvulsant and anti-hepatitis B viral activities [7-9]. Based on these facts in this research work we synthesized derivatives of 1,3,4-thidiazole that have broad spectrum antimicrobial activity.

2. Designing of 1,3,4-thiadiazole derivatives

Molecular hybridization is an effective tool to design more active and novel chemical entities by covalently combining two or more drug pharmacophores into a single chemical entity. In view of interesting biological activities shown by 1,3,4-thiadiazole and indole analogues, we anticipated that the hybrid originating from the incorporation of the substituted 1,3,4-thiadiazole with indole scaffold may exhibit improved pharmacological profile and it may become lead candidates for the developments of drugs (Figure 2)[10].

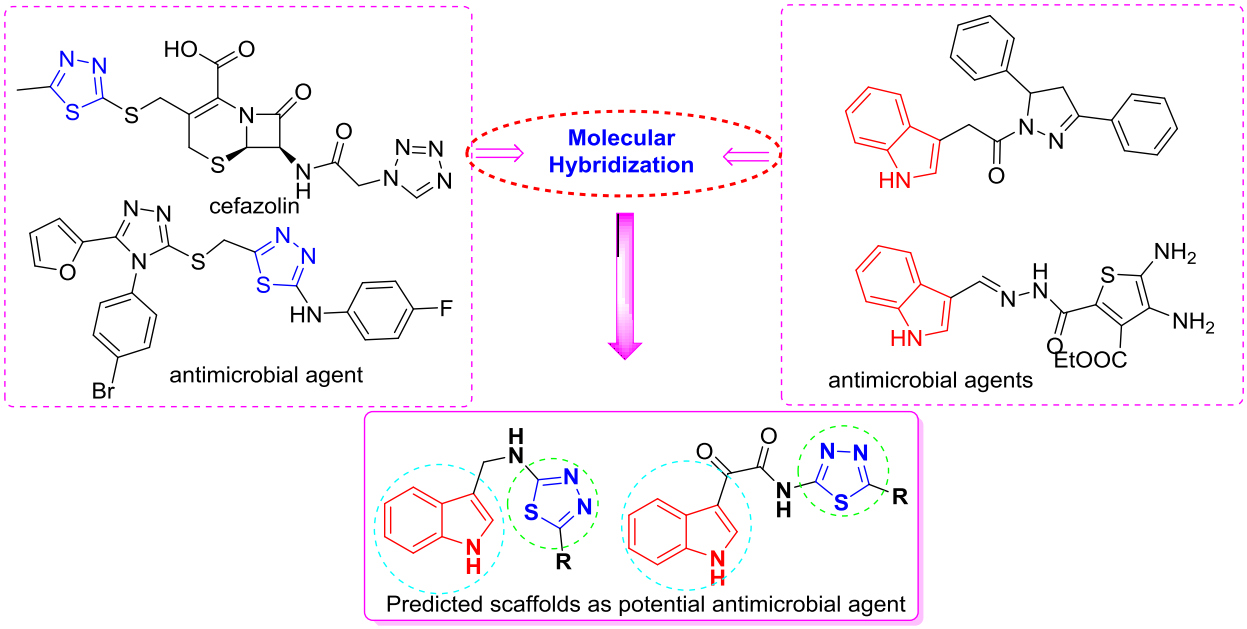


Figure 2. Design of 1,3,4-thiadiazole-linked indole scaffold as potential antimicrobial agent

3. EXPERIMENTAL WORK

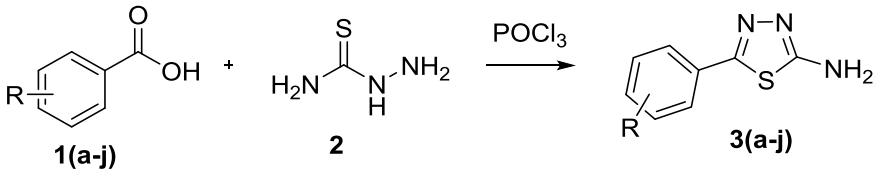
3.1. MATERIALS AND METHODS

All the reagents and solvents used in this research work were of analytical grade and obtained from Sigma- Aldrich, S.D. Fine chemicals and E. Merck India Ltd. All the reaction was carried out in anhydrous condition. Progress of the reactions was monitored with the help of thin layer chromatography, using ethyl acetate: chloroform as mobile phase. The results were visualized under UV light cabinet. Open glass capillaries melting point (MP) apparatus was used for the measurement of M.P of all synthesized compounds (˚C) and all the values were used uncorrected. Synthesized compounds were purified either by recrystallization in ethanol. Infrared spectra were recorded on Fourier Transform Infrared Spectroscopy in the range of 4000–400 cm^{-1} on Perkin Elmer RX1 Fourier transform spectrophotometer using KBr pellets.^{1}HNMR spectra were acquired on Bruker AVANCE III 400 MHz NMR Spectrometer using $CDCl_3$ as. Melting points were uncorrected and recorded on SMP30 melting point apparatus.

2.2. Chemistry

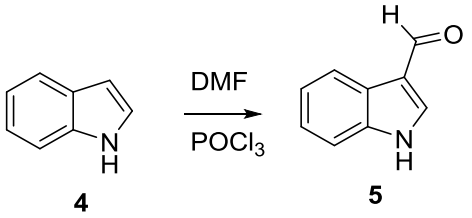
2.2.1. Procedure for synthesis of substituted 5-Phenyl-1,3,4-thiadiazol-2-amine (3a-j)

A mixture of the (different derivatives) benzoic acid **(1a-j)** (1.22 gm, 10 mmol), thio semicarbazide **(2)** (0.91 gm, 10mmol) and phosphorous oxychloride (5mL) was gently refluxed for 3 h. After cooling, water (25 mL) was added slowly and the reaction mixture was refluxed for 3 h and filtered. The solution was basified (pH 8–9) with concentrated potassium hydroxide solution and the precipitate was filtered and recrystallized from ethanol.



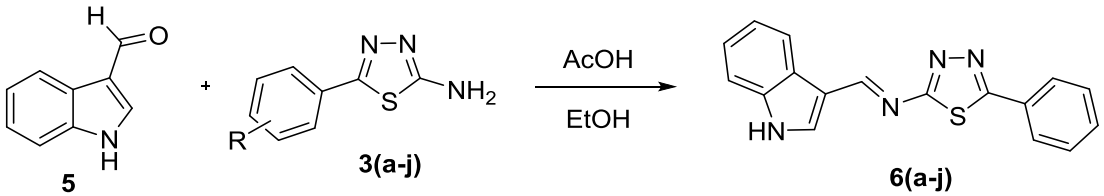
2.2.2. Procedure for 1*H*-Indole-3-carbaldehyde synthesis (5)

Phosphorus oxychloride (1.85 mL, 20 mmol) was added drop wise to DMF (5mL) at 4°C and stirred for 30 min under nitrogen. The solution of the indole (**4)** (1.17 gm, 10 mmol) in DMF (5 mL) was added drop by drop to this stirring solution and progressively warmed to room temperature. Upon completion, the reaction was poured into iced water, neutralized and kept at 4°C. The resulting precipitate was filtered off, dried then and recrystallized from ethanol [11].



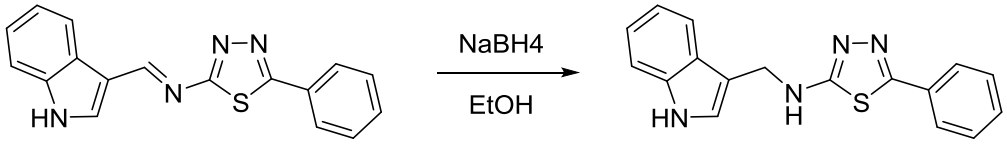
2.2.3. Procedure for the synthesis of substituted (*E*)-*N*-((1*H*-Indol-3-yl)methylene)-5-phenyl-1,3,4-thiadiazol-2-amine derivatives (6a-j)

The equimolar amount of different derivatives of 5-phenyl-1,3,4-thiadiazol-2-amine **3a-j** (0.61 gm, 3.44 mmol) and indole-3-carbaldehydes (**5)** (0.5 gm, 3.44mmol) in ethanol (20 mL) in presence of few drops of glacial acetic acid was refluxed for 6-8 h. The progress and completion of the reaction were checked by TLC. After completion of reaction, excess of solvent was distilled off and residues were poured on crushed ice. Solid precipitated was filtered, dried and recrystallized from ethanol.



2.2.4. Procedure for the synthesis of substituted *N*-((1*H*-indol-3-yl)methyl)-5-phenyl-1,3,4-thiadiazol-2-amine derivatives (7a-j)

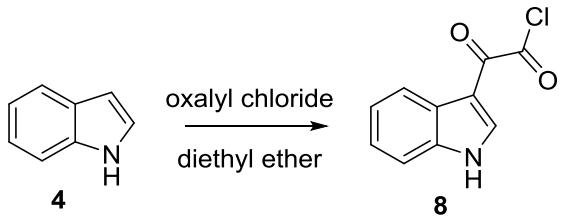
To a stirring suspension of 1-10 (0.5 gm, 1.64 mmol) in ethanol (20 mL), sodium borohydride (0.062 gm, 1.64 mmol) was added and the reaction mixture was stirred at 60^0C for 1-2 hrs. After completion of the reaction (monitored by TLC), the solvent was evaporated, cooled water (10 mL) was added to the residues. The solid obtained was filtered and dried. Finally, the product was purified by recrystallization by ethanol.



6(a-j) **7(a-j)**

2.2.5. Procedure for synthesis of 2-(1*H*-Indol-3-yl)-2-oxoacetyl chloride (8)

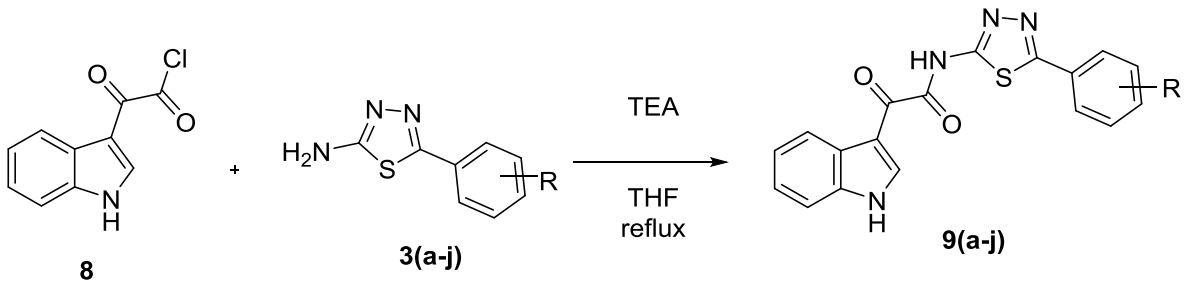
To a stirring solution of indole (**4)** (1.00 gm, 8.53 mmol) in diethyl ether (15 mL), a solution of oxalyl chloride (0.73 mL, 8.53 mmol) in diethyl ether (10 mL) was added drop wise at 0 °C. After complete addition, the reaction mixture was stirred at same for additional one hour and at room temperature for 30 minutes. After completion of reaction, as checked by TLC, the solid so formed was filtered, washed with chilled diethyl ether and dried. The product obtained was immediately used in the next-step without further purification.



4 **8**

2.2.6. Procedure for synthesis of 2-(1*H*-Indol-3-yl)-2-oxo-*N*-(5-phenyl-1,3,4-thiadiazol-2-yl)acetamide derivatives (9a-j)

To a stirring solution of 8 (0.85 gm, 4.81 mmol) in THF (15 mL), a solution of **3(a-j)** (1.0gm,4.81 mmol) in THF (10 mL) was added dropwise at 0 °C. After complete addition, the reaction mixture was stirred at room temperature for 10 minutes then refluxed for 4-5 hrs. After completion of reaction, as checked by TLC, the reaction mixture was evaporated. The residues were extracted DCM and organic layer was washed with water followed by saturated sodium bicarbonate solution. The collected organic layer was dried over anhydrous sodium sulphate, filtered and evaporated. The residue so obtained was recrystallized with ethanol to obtain the final compound.



8 **3(a-j)** **9(a-j)**

2.3. Structural analysis of synthesized compounds

2.3.1. Compound 7a

NMR:[1]H-NMR (DMSO-d_6): δ 11.011 (bs, 1H, -N*H*), 8.250 (t, 1H, -N*H*), 7.760-7.742 (d, 2H, Ar*H*), 7.644-7.624 (d, 1H, Ar*H*), 7.481-7.376 (m, 5H, Ar*H*), 7.123-7.086 (t, 1H, Ar*H*), 7.036-6.999 (t, 1H, Ar*H*), 4.676-4.663 (d, 2H, -NHCH_2). IR (KBr) ν (cm^{-1}): 3396, 3181 (N-H

stretch), 3020 (Aromatic C-H stretch), 2930 (Aliphatic C-H stretch), 1548 (C=C str., aromatic). ESI Mass (m/z): 307.2 $(M+1)^+$.

2.3.2. Compound 7b

NMR:^{1}H-NMR (DMSO-d_6): δ 11.012 (bs, 1H, -N*H*), 8.313 (t, 1H, -N*H*), 7.779-7.757 (d, 2H, Ar*H*), 7.634-7.614 (d, 1H, Ar*H*), 7.534-7.512 (d, 2H, Ar*H*), 7.403-7.373 (d, 2H, Ar*H*), 7.121-7.084 (t, 1H, Ar*H*), 7.033-6.996 (t, 1H, Ar*H*), 4.674-4.661 (d, 2H, -NHCH_2).IR (KBr) ν (cm^{-1}): 3269, 3212 (N-H stretch), 3016 (Aromatic C-H stretch), 2930 (Aliphatic C-H stretch), 1548 (C=C str., aromatic). ESI Mass (m/z): 341.1 $(M+1)^+$.

2.3.3. Compound 7c

NMR:^{1}H-NMR (DMSO-d_6): δ 10.996 (bs, 1H, -N*H*), 8.121 (t, 1H, -N*H*), 7.688-7.666 (d, 2H, Ar*H*), 7.633-7.614 (d, 1H, Ar*H*), 7.389-7.368 (m, 2H, Ar*H*), 7.117-7.081 (m, 1H, Ar*H*), 7.028-6.993 (m, 3H, Ar*H*), 4.649-4.637 (d, 2H, -NHCH_2), 3.801 (s, 3H, -OCH_3).IR (KBr) ν (cm^{-1}): 3246, 3186 (N-H stretch), 3014 (Aromatic C-H stretch), 2930, 2841 (Aliphatic C-H stretch), 1551 (C=C str., aromatic). ESI Mass (m/z): 337.9 $(M+1)^+$.

2.3.4. Compound 7d

NMR:^{1}H-NMR (DMSO-d_6): δ 11.019 (bs, 1H, -N*H*), 8.207 (bs, 1H, -N*H*), 7.644-7.626 (d, 3H, Ar*H*), 7.396-7.373 (d, 2H, Ar*H*), 7.278-7.259 (d, 2H, Ar*H*), 7.103 (t, 1H, Ar*H*), 7.014 (t, 1H, Ar*H*), 4.660-4.649 (d, 2H, -NHCH_2), 3.801 (s, 3H, -CH_3).IR (KBr) ν (cm^{-1}): 3208, 3185 (N-H stretch), 3058 (Aromatic C-H stretch), 2930 (Aliphatic C-H stretch), 1548 (C=C str., aromatic). ESI Mass (m/z): 321.8 $(M+1)^+$.

2.3.5. Compound 7e

NMR:^{1}H-NMR (DMSO-d_6): δ 11.028 (bs, 1H, -N*H*), 8.267 (t, 1H, -N*H*), 7.821-7.786 (m, 2H, Ar*H*), 7.638-7.618 (d, 1H, Ar*H*), 7.407-7.376 (m, 2H, Ar*H*), 7.309 (t, 2H, Ar*H*), 7.106 (t, 1H, Ar*H*), 7.017 (t, 1H, Ar*H*), 4.670-4.657 (d, 2H, -NHCH_2).IR (KBr) ν (cm^{-1}): 3230, 3178 (N-H stretch), 3055 (Aromatic C-H stretch), 2980, 2876 (Aliphatic C-H stretch), 1548 (C=C str., aromatic). ESI Mass (m/z): 325.8 $(M+1)^+$.

2.3.6. Compound 7f

NMR:^{1}H-NMR (DMSO-d_6): δ 11.027 (bs, 1H, -N*H*), 8.299 (t, 1H, -N*H*),8.013-7.990 (m, 1H, Ar*H*), 7.644-7.594 (m, 2H, Ar*H*), 7.482-7.374 (m, 2H, Ar*H*), 7.106 (t, 1H, Ar*H*), 7.019 (t, 1H, Ar*H*), 4.684-4.672 (d, 2H, -NHCH_2).IR (KBr) ν (cm^{-1}): 3211, 3189 (N-H stretch), 3054 (Aromatic C-H stretch), 2934 (Aliphatic C-H stretch), 1557 (C=C str., aromatic). ESI Mass (m/z): 341.8 $(M+1)^+$.

2.3.7. Compound 7g

NMR:^{1}H-NMR (DMSO-d_6): δ 11.018 (bs, 1H, -N*H*), 8.195 (t, 1H, -N*H*), 7.649-7.629 (d, 1H, Ar*H*), 7.522-7.503 (d, 1H, Ar*H*), 7.409-7.261 (m, 5H, Ar*H*), 7.106 (t, 1H, Ar*H*), 7.020 (t, 1H, Ar*H*), 4.664-4.651 (d, 2H, -NHCH_2), 2.487 (s, 3H, -CH_3).IR (KBr) ν (cm^{-1}): 3242, 3180 (N-H stretch), 3014 (Aromatic C-H stretch), 2930, 2835 (Aliphatic C-H stretch), 1552 (C=C str., aromatic). ESI Mass (m/z): 321.8 $(M+1)^+$.

2.3.8. Compound 7h

NMR:^{1}H-NMR (DMSO-d_6): δ 10.982 (bs, 1H, -N*H*), 8.113-8.090 (dd, 1H, Ar*H*), 8.013 (t, 1H, -N*H*), 7.633-7.631 (d, 1H, Ar*H*), 7.446-7.365 (m, 3H, Ar*H*), 7.194-7.173 (d, 1H, Ar*H*), 7.114-7.051 (m, 2H, Ar*H*), 7.006 (t, 1H, Ar*H*), 4.662-4.650 (d, 2H, -NHCH_2), 3.909 (s, 3H, -OCH_3).IR (KBr) ν (cm^{-1}): 3251, 3211 (N-H stretch), 3014 (Aromatic C-H stretch), 2930 (Aliphatic C-H stretch), 1519 (C=C str., aromatic). ESI Mass (m/z): 337.9 $(M+1)^+$.

2.3.9. Compound 7i

NMR:^{1}H-NMR (DMSO-d_6): δ 11.026 (bs, 1H, -N*H*), 8.357 (t, 1H, -N*H*), 8.049-8.027 (d, 1H, -Ar*H*), 7.794-7.789 (d, 1H, Ar*H*), 7.641-7.622 (d, 1H, Ar*H*), 7.567-7.541 (dd, 1H, Ar*H*), 7.415-7.377 (m, 2H, Ar*H*), 7.126-7.089 (m, 1H, Ar*H*), 7.038-7.001 (m, 1H, Ar*H*), 4.693-4.680 (d, 2H, -NHCH_2).IR (KBr) ν (cm^{-1}): 3292, 3265 (N-H stretch), 3076, 3061 (Aromatic C-H stretch), 2968, 2904 (Aliphatic C-H stretch), 1548 (C=C str., aromatic). ESI Mass (m/z): 375.1 $(M)^+$, 377.1 $(M+2)^+$.

2.3.10. Compound 7j

NMR:^{1}H-NMR (DMSO-d_6): δ 11.011 (bs, 1H, -N*H*), 8.158 (t, 1H, -N*H*),7.641-7.622 (d, 1H, Ar*H*), 7.394-7.357 (m, 3H, Ar*H*),7.220-7.195 (d, 1H, Ar*H*), 7.122-7.085 (m, 1H, Ar*H*),7.033-6.998 (m, 2H, Ar*H*), 4.656-4.643 (d, 2H, -NHCH_2), 3.822 (s, 3H, -OCH_3), 3.796 (s, 3H, -OCH_3).IR (KBr) ν (cm^{-1}): 3250, 3215 (N-H stretch), 3004 (Aromatic C-H stretch), 2939 (Aliphatic C-H stretch), 1549 (C=C str., aromatic). ESI Mass (m/z): 367.2 $(M+1)^+$.

2.3.11. Compound 9a

NMR:^{1}H-NMR (DMSO-d_6): δ 13.308 (bs, 1H, -CON*H*), 12.462 (bs, 1H, -N*H*), 8.667-8.661 (m, 1H, Ar*H*), 8.265-8.245 (m, 1H, Ar*H*), 8.011-8.001 (m, 1H, Ar*H*), 7.567 (m, 5H, Ar*H*), 7.332-7.310 (m, 2H, Ar*H*). IR (KBr) ν (cm^{-1}): 3351 (amide -NH stretch),3149 (N-H stretch), 3020 (Aromatic C-H stretch), 1692, 1651 (C=O stretch, amide C=O stretch), 1537 (C=C str., aromatic). ESI Mass (m/z): 349.1 $(M+1)^+$.

2.3.12. Compound 9b

NMR:^{1}H-NMR (DMSO-d_6): δ 13.391 (bs, 1H, -CON*H*), 12.476 (bs, 1H, -N*H*), 8.662-8.654 (d, 1H, Ar*H*), 8.261-8.238 (m, 1H, Ar*H*), 8.040-8.026 (m, 2H, Ar*H*), 7.590-7.488 (m, 3H, Ar*H*), 7.332-7.310 (m, 2H, Ar*H*).IR (KBr) ν (cm^{-1}): 3289 (amide -NH stretch),3155 (N-H stretch), 3060 (Aromatic C-H stretch), 1701, 1629 (C=O stretch, amide C=O stretch), 1515 (C=C str., aromatic). ESI Mass (m/z): 383.9 $(M+1)^+$.

2.3.13. Compound 9c

NMR:^{1}H-NMR (DMSO-d_6): δ 13.214 (bs, 1H, -CON*H*), 12.457 (bs, 1H, -N*H*), 8.665-8.657 (m, 1H, Ar*H*), 8.266-8.244 (m, 1H, Ar*H*), 7.953-7.932 (d, 2H, Ar*H*), 7.592-7.570 (m, 1H, Ar*H*), 7.331-7.309 (m, 2H, Ar*H*), 7.124-7.102 (d, 1H, Ar*H*), 3.851 (s, 3H, -OCH_3).IR (KBr) ν (cm^{-1}): 3329 (amide -NH stretch),3181 (N-H stretch), 3076 (Aromatic C-H stretch), 1712, 1634 (C=O stretch, amide C=O stretch), 1537 (C=C str., aromatic). ESI Mass (m/z): 379.9 $(M+1)^+$.

2.3.14. Compound 9d

NMR:^{1}H-NMR (DMSO-d_6): δ 13.293 (bs, 1H, -CON*H*), 12.469 (bs, 1H, -N*H*), 8.658 (m, 1H, Ar*H*), 8.248 (m, 1H, Ar*H*), 7.902-7.833 (d, 2H, Ar*H*), 7.579-7.589 (m, 1H, Ar*H*), 7.384-7.319 (m, 4H, Ar*H*), 2.341 (s, 3H, -CH_3). IR (KBr) ν (cm^{-1}): 3321 (amide -NH stretch), 3151 (N-H stretch), 3074 (Aromatic C-H stretch), 1708, 1632 (C=O stretch, amide C=O stretch), 1524 (C=C str., aromatic). ESI Mass (m/z): 363.9 $(M+1)^+$.

2.3.15. Compound 9e

NMR:^{1}H-NMR (DMSO-d_6): δ 13.345 (bs, 1H, -CON*H*), 12.473 (bs, 1H, -N*H*), 8.740-8.580 (m, 1H, Ar*H*), 8.360-8.168 (m, 1H, Ar*H*), 8.167-7.980 (m, 2H, Ar*H*), 7.700-7.515 (m, 1H, Ar*H*), 7.515-7.365 (m, 2H, Ar*H*),7.365-7.245 (m, 2H, Ar*H*). IR (KBr) ν (cm^{-1}): 3316 (amide -NH stretch),3148 (N-H stretch), 3075 (Aromatic C-H stretch), 1704, 1632 (C=O stretch, amide C=O stretch), 1524 (C=C str., aromatic). ESI Mass (m/z): 367.2 $(M+1)^+$.

2.3.16. Compound 9f

NMR:^{1}H-NMR (DMSO-d_6): δ 13.396 (bs, 1H, -CON*H*), 12.482 (bs, 1H, -N*H*), 8.700-8.694 (d, 1H, Ar*H*), 8.267-8.247 (m, 1H, Ar*H*), 8.194-8.178 (m, 1H, Ar*H*), 7.735-7.716 (m, 1H, Ar*H*), 7.620-7.565 (m, 3H, Ar*H*), 7.331-7.310 (m, 2H, Ar*H*). IR (KBr) ν (cm^{-1}): 3351 (amide -NH stretch),3152 (N-H stretch), 3075 (Aromatic C-H stretch), 1698, 1637 (C=O stretch, amide C=O stretch), 1509 (C=C str., aromatic). ESI Mass (m/z): 405.3 $(M+1)^+$.

2.3.17. Compound 9g

NMR:^{1}H-NMR (DMSO-d_6): δ 13.308 (bs, 1H, -CON*H*), 12.470 (bs, 1H, -N*H*), 8.677-8.668 (d, 1H, Ar*H*), 8.269-8.246 (m, 1H, Ar*H*), 7.746-7.727 (d, 1H, Ar*H*), 7.598-7.566 (m, 1H, Ar*H*), 7.481-7.432 (m, 2H, Ar*H*), 7.406-7.365 (m, 1H, Ar*H*), 7.333-7.310 (m, 2H, Ar*H*). IR (KBr) ν (cm^{-1}): 3349 (amide -NH stretch),3175 (N-H stretch), 3020 (Aromatic C-H stretch), 1694, 1651 (C=O stretch, amide C=O stretch), 1509 (C=C str., aromatic). ESI Mass (m/z): 363.1 $(M+1)^+$.

2.3.18. Compound 9h

NMR:^{1}H-NMR (DMSO-d_6): δ 13.104 (bs, 1H, -CON*H*), 12.474 (bs, 1H, -N*H*), 8.707-8.699 (d, 1H, Ar*H*), 8.344-8.321 (dd, 1H, Ar*H*), 8.269-8.246 (m, 1H, Ar*H*), 7.588-7.538 (m, 2H, Ar*H*), 7.343-7.297 (m, 3H, Ar*H*), 7.188-7.151 (t, 1H, Ar*H*), 4.063 (s, 3H, -CH_3).IR (KBr) ν (cm^{-1}): 3364 (amide -NH stretch),3151 (N-H stretch), 3020 (Aromatic C-H stretch), 1677, 1631 (C=O stretch, amide C=O stretch), 1581 (C=C str., aromatic). ESI Mass (m/z): 379.2 $(M+1)^+$.

2.3.19. Compound 9i

NMR:^{1}H-NMR (DMSO-d_6): δ 13.429 (bs, 1H, -CON*H*), 12.483 (bs, 1H, -N*H*), 8.694 (s, 1H, Ar*H*), 8.254-8.130 (m, 2H, Ar*H*), 8.000-7.870 (m, 1H, Ar*H*), 7.719-7.520 (m, 2H, Ar*H*), 7.420-7.220 (m, 2H, Ar*H*).IR (KBr) ν (cm^{-1}): 3316 (amide -NH stretch),3159 (N-H stretch), 3080 (Aromatic C-H stretch), 1712, 1627 (C=O stretch, amide C=O stretch), 1515 (C=C str., aromatic). ESI Mass (m/z): 417.1 $(M)^+$, 419.2 $(M+1)^+$.

2.3.20. Compound 9j

NMR:^{1}H-NMR (DMSO-d_6): δ 13.246 (bs, 1H, -CON*H*), 12.471 (bs, 1H, -N*H*), 8.657 (s, 1H, Ar*H*), 8.355-8.165 (m, 1H, Ar*H*), 7.680-7.435 (m, 3H, Ar*H*), 7.435-7.205 (m, 2H, Ar*H*), 7.205-7.005 (m, 1H, Ar*H*), 3.887 (s, 3H, -OCH_3), 3.849 (s, 3H, -OCH_3).IR (KBr) ν (cm^{-1}): 3289 (amide -NH stretch),3148 (N-H stretch), 3005 (Aromatic C-H stretch), 2905 (Aliphatic C-H), 1687, 1629 (C=O stretch, amide C=O stretch), 1509 (C=C str., aromatic). ESI Mass (m/z): 409.3 $(M+1)^+$.

2.4. Biological evaluation

2.4.1. Antimicrobial activity

All the newly synthesized compounds were evaluated by the agar cup diffusion technique [12, 13] using 1 mg/mL solution of synthesized compound in DMSO. The test organisms utilized were *Bacillus subtilis(MTCC-441)* and *Bacillus megaterium(MTCC-428)*as examples of Gram positive bacteria and *Escherichia coli(MTCC-443)* and *Pseudomonas spp. (2496)* as examples of Gram negative bacteria. Antifungal activity was screened against fungal strain, *Candida albicans (MTCC-227)*. Inocculatedmüller Hinton agar for bacteria and yeast peptone dextrose agar for fungi was poured onto the sterilized petri dishes (25-30mL: each petri dish). The poured material was allowed to set (30 min) and thereafter the cups (8 mm diameter) was made by punching into the agar surface with a sterile cork borer and scooping out the punched part of the agar. The test solution (200 µL) was added into the cups with the help of a micro pipette. The plates were incubated at 37°C and the results were recorded for antibacterial activity after 24 h and for antifungal activity after 72 h. A control using DMSO without the test compound was included for each organism. Ampicillin and Gentamicin were used as standard antibacterial agents, whereas Amphotericin B was used as antifungal reference drugs. Each experiment was carried out in triplicate and the results were recorded as the average diameter of inhibition zones of bacterial or fungal growth in mm. The minimal inhibitory concentration (MIC, µg/mL) determination method of the biologically active compounds (Table 1) was applied using different series of dilutions against Gram-positive, Gram-negative bacteria and fungi.

Table 1: *In vitro* antimicrobial activity of compounds 7(a-j) and 9(a-j)

S. No.	Minimum inhibitory concentration MIC (µg/mL)[a]						
	Comp. Code	R	Gram positive		Gram negative		Fungi
			Bs	Bm	Ec	Ps	Ca
1.	**7a**	Ph	32.8	38.2	6.8	12.6	61.6
2.	**7b**	4Cl	24.6	1 8.8	2.86	9.8	12.6
3.	**7c**	4-OMe	34.2	-	0.62	10.6	19.8
4.	**7d**	4-Me	68.6	72.4	34.7	-	-
5.	**7e**	4-F	44.2	38.6	3.3	3.3	7.6
6.	**7f**	2-Cl	24.4	26.7	12.8	18.2	14.2
7.	**7g**	2Me	38.6	48.6	58.2	-	-
8.	**7h**	2-OMe	36.4	42.2	22.6	44.2	-

9.	**7i**	2,4-dichloro	18.6	15.8	0.86	1.8	4.6
10.	**7j**	3,4-dimethoxy	22.8	21.6	-	-	-
11.	**9a**	Ph	18.7	16.4	8.4	14.6	28.7
12.	**9b**	4Cl	8.6	12.2	2.4	4.8	12.2
13.	**9c**	4-OMe	22.6	-	7.8	14.6	-
14.	**9d**	4-Me	32.8	14.6	-	58.2	8.4
15.	**9e**	4-F	24.2	28.8	2.2	10.6	21.6
16.	**9f**	2-Cl	6.8	7.4	-	5.2	-
17.	**9g**	2Me	34.8	44.6	18.6	26.2	58.1
18.	**9h**	2-OMe	22.2	28.6	-	-	-
19.	**9i**	2,4-dichloro	24.8	21.7	4.6	4.8	6.4
20.	**9j**	3,4-dimethoxy	38.4	44.6	14.6	8.2	9.6
21.	Ampicillin	--	21	21	NT	NT	NT
22.	Gentamycin	--	NT	NT	3	6	NT
23.	Amphotericin B	--	NT	NT	NT	NT	8

Bs: *Bacillus subtilis*, Bm: *Bacillus megaterium*, Ec: *Escherichia coli*, Ps: *Pseudomonas spp.*, Ca: *Candida albicans.*

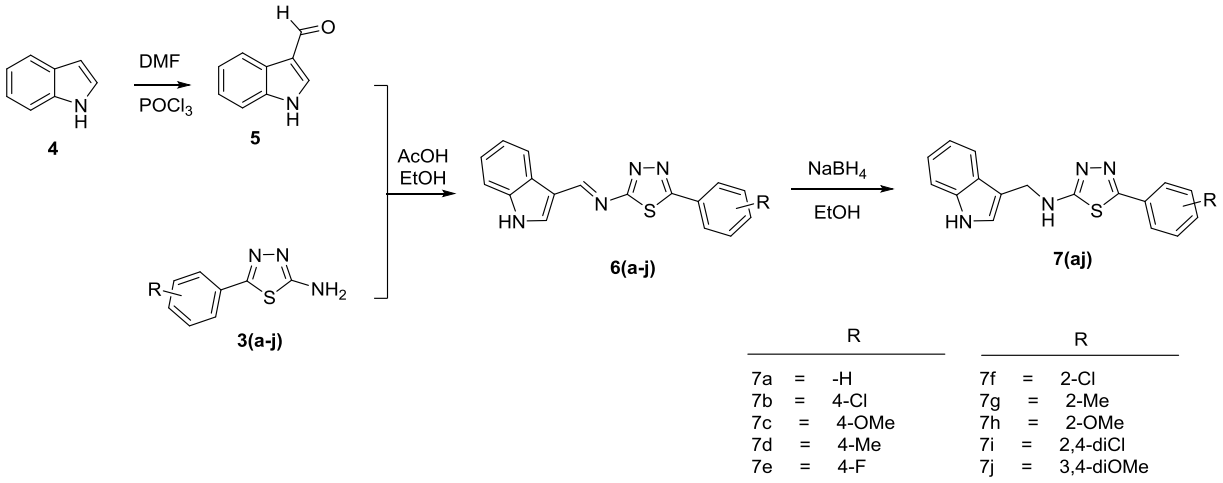
[a](-): Inactive (MIC > 100 µg/mL).

NT: Not tested.

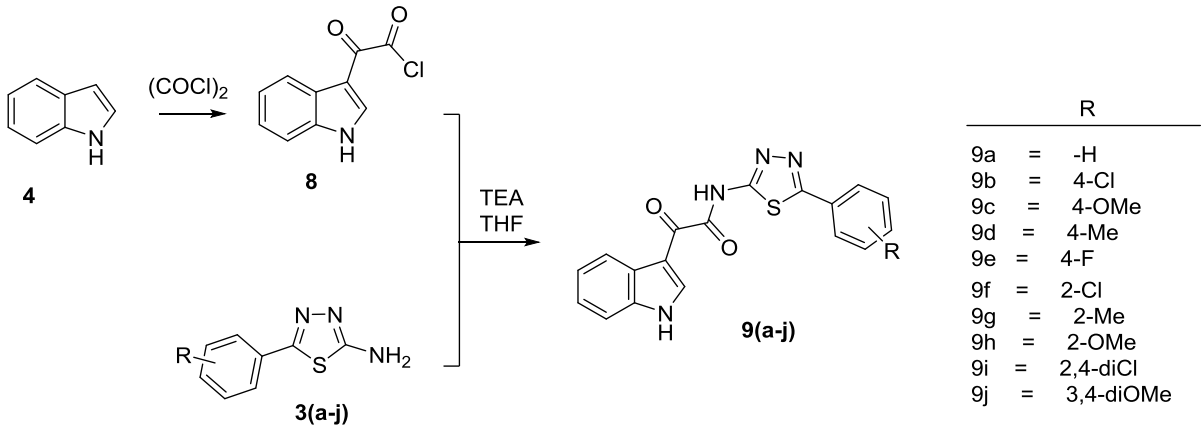
3. Results and discussion

3.1. Chemistry

In the present study, 20 derivatives of 1,3,4-thiadiazole have been synthesized as highlighted in Scheme 1 and Scheme 2. For Scheme 1 two intermediate are prepared, the first intermediate 5-Phenyl-1,3,4-thiadiazol-2-amine (3a-j) was prepared by the refluxing different derivatives of benzoic acid 1(a-j), thio semicarbazide (2) and phosphorous oxychloride. The second intermediate 1*H*-Indole-3-carbaldehyde (5) was prepared by the reaction of phosphorus oxychloride with indole (4) solution in DMF under continuous stirring solution at room temperature. For Scheme 2 2-(1*H*-Indol-3-yl)-2-oxoacetyl chloride (8) was prepared by stirring solution of indole (4) in diethyl ether and oxalyl chloride at 0°C. The final compounds 7(a-j) were prepared by the reaction of 5 with different derivatives 3(a-j) (Scheme 1). Compounds 9(a-j) was prepared by the reaction of 8 with different 3(a-j) (Scheme 2). The depicted structures of all the synthesized derivatives were strengthened by the different spectroscopy analysis such as IR, ^{1}HNMR, and mass spectral data. IR spectral data of synthesized compounds 7(a-j) and 9(a-j) showed characteristic peaks at υ cm^{-} 3212, 3016, and 1548 due to presence of N-H, C-H, and C=C. The mass spectrum of compound 1A displayed a molecular ion peak at m/z 307.2 $(M+1)^{+}$, compound 7c 337.9 $(M+1)^{+}$ and compound 9a showed molecular ion peak at m/z 349.1 (M+1)+.



Scheme 1. Synthesis of 1,3,4-thiadiazole derivatives (7a-j)



Scheme 2. Synthesis of 1,3,4-thiadiazole derivatives (9a-j)

3.2. Antimicrobial Activity

All the synthesized compounds of series 1 (7a-j) and series 2 (9a-j) were evaluated for antibacterial activity against were *Bacillus subtilis(MTCC-441),Bacillus megaterium(MTCC-428)*, *Escherichia coli(MTCC-443)* and *Pseudomonas spp.* Antifungal activity of all synthesized compound also evaluated against *Candida albicans (MTCC-227)*. The results of antibacterial and antifungal activity were expressed as MIC (minimum inhibitory concentration) values and are presented in Table 1. Among 1 series compound 7i showed most potent antibacterial activity against *Bacillus subtilis, Bacillus megaterium*, *Escherichia coli* and *Pseudomonas* with MIC value18.6 and 15.8, 0.86 and 1.8µg/mL respectively. 7i compound also showed potent antifungal activity against *Candida albicans* with MIC 4.6 µg/mL. Compound 7c have no antibacterial activity against *Bacillus megaterium.* 7d showed no antibacterial activity against *Pseudomonas* and no antifungal activity against *Candida albicans*. In second series compound 9f showed potent activity against gram positive bacteria *Bacillus subtilis* and *Bacillus megaterium* with MIC 6.8 and 7.4 (µg/mL) respectively. Compound 9f have no antibacterial activity against *Escherichia coli* and no antifungal

activity against *Candida albicans*.Compound 9e showed potent activity against *Escherichia coli* with MIC 2.2 (µg/mL). Compound 9i showed potent antifungal activity against *Candida albicans* with MIC 6.4(µg/mL). The structure activity relationships (SARs) indicate that the 1,3,4-thiadiazole and indole scaffold is important for antibacterial and antifungal activity. Substitution of electronegative group at phenyl ring enhanced both antibacterial and antifungal activity. In Compound 7i substitution of 2,4-di-chloro atphenyl ring showed more potent activity compared to standard drug Ampicillin and other synthesized compounds. Substitution of methyl group at 2nd position of phenyl ring reduced antimicrobial activity in 7d compound. Unsubstituted phenyl ring have less activity compared to standard drug. Substitution of methoxy group at 4th position or *meta* position of phenyl ring (7c) showed more potent antimicrobial activity compared to 2nd position methoxy group in phenyl ring (2H). Substitution of methyl ring at *ortho* position of phenyl showed potent activity compared to *para* position methyl group in phenyl ring. Study revealed that in series 1 electronegative group at phenyl ring are play an important role for antibacterial and antifungal activity. In series 2, substitution of chloro group at *ortho* position of at phenyl ring showed more potent antibacterial activity compared to standard drug but does not showed antifungal activity. Substitution of chloro group at *meta* position showed potent antibacterial and antifungal activity. Substitution of chloro group at 2nd and 4th position showed more potent antifungal activity compared to standard drug and other synthesized compounds of series 2. Substitution of methoxy group at 3rd and 4th position of phenyl ring reduced antibacterial and antifungal activity.

4. Conclusion

In the present study design, synthesis, characterization and evaluation of antibacterial and antifungal activity have been performed on a series of 1,3,4-thiadiazole derivatives against *Bacillus subtilis, Bacillus megaterium*, *Escherichia coli*, *Pseudomonas* and *Candida albicans* species. All compounds of series 1 (7a-7j) was synthesized by the refluxing the mixture of 5-Phenyl-1,3,4-thiadiazol-2-amine (3a-j) and 1*H*-Indole-3-carbaldehyde (5). Series 2 compounds (9a-9j) was synthesized by refluxing the 5-Phenyl-1,3,4-thiadiazol-2-amine (3a-j) with 2-(1*H*-Indol-3-yl)-2-oxoacetyl chloride. The structure activity relationships (SARs) revealed that the 1,3,4-thiadiazole and indole scaffold is essential for anti-microbial activity. Substituted phenyl ring with electronegative group at showed more potent antibacterial and antifungal activity. Compound 7i with 2,4-di-chloro at phenyl ring showed highest activity compared to standard drug Ampicillin and other synthesized compound. Phenyl ring electropositive group showed less activity compared to electronegative group. In series 2, chloro substituted phenyl ring showed more potent antibacterial activity compared to standard drug Ampicillin but does not showed antifungal activity. 2,4-di chloro substituted phenyl ring at 2nd position of 1,3,4,-thidiazole ring showed highest antifungal activity compared to standard drug and other synthesized compounds of series 2. Therefore, we conclude that electronegative group at phenyl ring and 1,3,4-thiadiazole ring are essential for antibacterial and antifungal activity.

Reference

1. Levinreisman, I.; Ronin, I.; Gefen, O.; Braniss, I.; Shoresh, N.; Balaban, N.Q. Antibiotic tolerance facilitates the evolution of resistance. Science 2017, 355, 826.
2. GomesM.N.,MuratovE.N.,PereiraM.Peixoto,RossetoJ.C.,CravoL.P.,P.V.L,Andrade,C. H.,Neves, Chalcone B.J. Byproduct: rising opening mark for drug layout. Molecules 2017, 22,1210.
3. Kar Mahapatra, KumarBhart D, Asati S.V. Anticancer falcones: Structure and atomic target angle Eur. J. Medicinal Chemistry. 2015, 98, 69–114.
4. Rybka M., Mercader A.G.,Castro, E.A. Predictive QSAR research of falconer biproduct cytotoxicity action opposing HT-29 hominid clone adenocarcinoma unit boundary. ChemomIntell. Lab. Syst. 2014, 132,18–29.
5. ChenJ.J., ChengM.J., Shu, C.W.,Sung, P.J., Lim, Y.P., Cheng, L.Y., Wang, S.L., Chen, L.C. A Narrative falcon and Anti-oxidant essential of Glycyrrhiza glabra. Chemistry Nat. Compound. 2017, 53, 632–634.
6. Bush, K.; Courvalin, P.; Dantas, G.; Davies, J.; Eisenstein, B.; Huovinen, P.; Jacoby, G.A.; Kishony, R.; Kreiswirth, B.N.; Kutter, E.; Lerner, S.A.; Levy, S.; Lewis, K.; Lomovskaya, O.; Miller, J.H.; Mobashery, S.; Piddock, L.J.; Projan, S.; Thomas, C.M.;Tomasz, A.; Tulkens, P.M.; Walsh, T.R.; Watson, J.D.; Witkowski, J.; Witte, W.; Wright, G.; Yeh, P.; Zgurskaya, H. I. Tackling antibiotic resistance. Nat. Rev. Microbiol. 2011, 9, 894-896\
7. Kaushal M, Kaur A. A review on some 2,5-disubstituted [1,3,4] thiadiazole substituted thiazolidinone derivatives as a potent antimicrobial agent. World J Pharm Res. 2016;5(6):1966–77.
8. Mehta D, Taya P Neetu, author. A review on the various biological activities of thiadiazole. Int J Pharm Pharm Sci. 2015;7(4):39–47.
9. Rao MRK, Shil S. Some observations on thin layer chromatography technique. Int J Recent Technol Eng. 2019;8(2):1700–2
10. Salimon, J.; Salih, N.; Hameed, A.; Ibraheem, H.; Yousif, E. Synthesis and antibacterial activity of some new 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives. J. Appl. Sci. Res. 2010, 6, 866–870.
11. Yousif, E.; Majeed, A.; Al-Sammarrae, K.; Salih, N.; Salimon, J.; Abdullah, B. Metal complexes of Schiff base: preparation, characterization and antibacterial activity. Arabian J Chem. 2017, 10, S1639-S1644.
12. A.L. Barry, The Antimicrobic Susceptibility Test: Principles and Practices; Ilus Lea &Febiger: Philadelphia, 1976. p 180.
13. P. M. Reddy, Y.P. Ho, K. Shanker, R. Rohini, V. Ravinder, Eur. J. Med. Chem. 44 (2009) 2621.