

Original Research Article

Synthesis, Spectral Characterization and Anti Bacterial Activity of Novel Thiourea Analogues

Mahendra nath Mitta* and K. Madhava chetty

Department of Botany, S.V. University, Tirupati-517 502, Andhra Pradesh, India

*Corresponding author

ABSTRACT

Keywords

Urea,
Thiourea,
Antibacterial
activity,
Antifungal
activity

A series of novel urea/thiourea derivatives **3a-k** were synthesized by the reaction of equimolar quantities of 2-(benzo[d]thiazol-2-yl) aniline (**1**) in dry tetrahydrofuran (THF) and various isocyanates and thioisocyanates at room temperature in the presence of triethylamine (TEA) with high yields. All the title compounds were characterized by elemental; infrared (IR); ¹H and ¹³C NMR; and mass spectral data analyses. Synthesized compounds were screened for their antimicrobial activity. Among the all compounds 3a, d, fe and j exhibits potential antibacterial activity. Compounds 3d and 3i shows great antifungal activity at 100µg/mL concentration.

Introduction

The analyses of heterocycles are one of the major areas in medicinal chemistry and are privileged structures in drug discovery.[1, 2] Through high degree of binding affinity these privileged structures symbolize a class of molecules that act as ligands for various biological. Complications of multi-drug resistant microorganisms have reached on disturbing level in many countries around the world. In countries like United State and European a numbers of clinical reports explain the rising occurrence of meticillin-resistant S. aureus and other antibiotic-resistant human pathogenic microorganisms. There is a serious challenge to the medical community against the infections caused by those microorganisms and an effective

therapy is necessary which has led to a search for novel antimicrobial agents. Development of these molecules must permit us to promptly find novel biologically active compounds throughout a broad range of therapeutic areas in a smaller interval scale.

The initial organic compound synthesized in the laboratory is urea, which brought a green revolution throughout the world. Later on its similar structural compound thiourea had discovered which also had significant importance in agriculture for yield improvement [3-5]. Thiourea is important sulphur and nitrogen-containing compounds that have proved to be useful substances in

drug research in recent years [1–6]. Some urea derivatives possess valuable antituberculosis, antibacterial and anticonvulsant properties [7]. Most of these compounds include heterocyclic rings such as oxadiazoles, thiadiazoles, triazoles, and pyrazoles. It is well known that the 1, 2, 4-triazole-derived N-bridged heterocyclic find applications in the field of medicine, agriculture and industry [8-11]. The 1, 2, 4-triazole nucleus has also been incorporated into a wide variety of therapeutically important molecules to transform them into better drugs. Drugs such as fluconazole, itraconazole, and the new generation of triazoles posaconazole, voriconazole, and ravuconazole are the best examples of potent antifungal molecules possessing triazole nuclei [12-15]. Previous findings from our laboratory demonstrated that nucleoside and urea compounds with phosphorylation of drugs molecules shows various pharmacological properties including antimicrobial and antioxidant [16-21], anticancer [22,23], antidiabetics [24] and antialzheimers [25-31].

Urea was the initial organic compound synthesized in the laboratory, which brought a green revolution through out the world. Later on its similar structural compound thiourea had discovered which also had significant importance in agriculture for yield improvement. Later on urea and thiourea derivatives had been discovered which exhibited broad spectrum of biological activities such as herbicidal [4], inhibition of nitric oxide [5], anti-viral [6] and analgesic properties [7]. These potent biological activities of urea and thiourea derivatives have stimulated great interest in the synthesis of such compounds for extensive studies related to their biological activities. In view of these observations and applications of urea and Thiourea, we have focused on the synthesis of a series of novel

urea and thiourea derivatives by reacting 2-(benzo[d]thiazol-2-yl) aniline with various isocyanates and thioisocyanates in the presence of triethylamine.

Materials and Methods

All the chemicals were procured from Sigma-Aldrich, Merck and were used as such. Solvents used for spectroscopic and physical studies were reagent grade and were further purified by the literature methods. Melting points were determined in open capillary tubes by Guna digital melting point apparatus, expressed in (°C) and are uncorrected. Infrared spectra (ν_{\max} in cm^{-1}) were recorded as KBr pellets on a Perkin - Elmer, FT-IR 100 spectrophotometer. ^1H and ^{13}C spectra were recorded as solutions in DMSO-d_6 on a Bruker 400 MHz spectrometer operating at 400 MHz for ^1H , 100 MHz for ^{13}C . The ^1H and ^{13}C chemical shifts were expressed in parts per million (ppm) with reference to tetramethylsilane (TMS) and Mass spectra were recorded in E.S.I Mode on API-3000 mass spectrometer. Elemental analysis was performed on Thermo Finnigan Instrument at University of Hyderabad, Hyderabad.

General procedure for the preparation of compounds 3a-k

1-(2-(Benzo [d]thiazol-2-yl) phenyl)-3-(4-nitrophenyl) urea (3a)

Yield: 74%, Pale yellow, mp 210-212 °C; IR (KBr) (ν_{\max} cm^{-1}): 1647(C=O),1074(C-O), 3428 (NH); $^1\text{H-NMR}$ (DMSO-d_6) δ ppm: 6.80-7.30 (m, 10H, Ar-H), 7.60 (d, 2H, Ar-H) , 8.75(s,1H,NH), 8.90(s, 1H, NH); $^{13}\text{C-NMR}$ (DMSO-d_6) δ (ppm): 116.3, 119.7, 121.4, 121.6, 125.1, 124.0, 124.3, 124.6, 127.3, 128.1, 128.7, 133.6, 136.1, 143.1, 145.1, 152.7, 154.1; Anal. Calcd. For $\text{C}_{20}\text{H}_{14}\text{N}_4\text{O}_3\text{S}$: C, 61.53; H, 3.61; N,

14.35; Found: C, 61.40, H, 3.56; N, 14.30. GC-MS m/z 390 (100, M^+), 269(40), 181(60), 130(70).

1-(2-(Benzo[d]thiazol-2-yl)phenyl)-3-(4-chlorophenyl)urea (3b)

Yield: 70%, Dark Brown solid, mp 180-182 °C; IR (KBr) (v_{max} cm^{-1}): 1640(C=O), 1080(C-O), 3400 (NH); 1H -NMR (DMSO- d_6) δ ppm: 6.82-7.40 (m, 12H, Ar-H), 8.68(s, 1H, NH), 8.78(s, 1H, NH); ^{13}C -NMR (DMSO- d_6) δ (ppm): 116.2, 120.6, 121.3, 121.5, 124.0, 124.1, 125.0, 127.5, 128.1, 128.6, 129.6, 133.2, 133.5, 136.2, 137.4, 152.6, 154.3, 166.3; Anal. Calcd. For $C_{20}H_{14}ClN_3OS$: C, 63.24; H, 3.71; N, 11.06. Found: C, 63.19, H, 3.68; N, 10.90; GC-MS m/z 379 (100, M^+), 381(33, M^++2), 269(55), 169, 143(65), 130(54).

1-(2-(Benzo[d]thiazol-2-yl) phenyl)-3-(4-bromophenyl) urea (3c)

Yield. 72%, Pale Brown solid, mp 167-169 °C; IR (KBr) (v_{max} cm^{-1}): 1633(C=O), 1083(C-O), 3430 (NH); 1H -NMR (DMSO- d_6) δ ppm: 6.82-7.40 (m, 12H, Ar-H), 8.70(s, 1H, NH), 8.80(s, 1H, NH); ^{13}C -NMR (DMSO- d_6) δ (ppm): 116.2, 121.5, 121.6, 121.7, 122.0, 124.3, 124.6, 125.2, 127.5, 128.7, 131.7, 133.6, 136.2, 138.1, 138.4, 152.7, 154.3, 166.4; Anal. Calcd. For $C_{20}H_{14}BrN_3OS$: C, 56.61; H, 3.33; N, 9.90; Found: C, 56.55, H, 3.28; N, 9.80. GC-MS m/z 424 (100, M^+), 426(100, M^++2), 269(28), 214(35).

1-(2-(Benzo[d]thiazol-2-yl)phenyl)-3-(4-fluorophenyl) urea (3d)

Yield. 69%, Pale Orange Solid, mp 152-154 °C; IR (KBr) (v_{max} cm^{-1}): 1643(C=O), 1080(C-O), 3450 (NH); 1H -NMR (DMSO- d_6) δ ppm: 6.81-7.50 (m, 12H, Ar-H), 8.70(s, 1H, NH), 8.92(s, 1H, NH); Anal.

Calcd. For $C_{20}H_{14}FN_3OS$ C, 66.10; H, 3.88; N, 11.56. Found: C, 66.19, H, 3.78; N, 11.46; GC-MS m/z 363 (100, M^+), 269(56), 154(64).

1-(2-(Benzo[d]thiazol-2-yl)phenyl)-3-(3-chloro-4-(trifluoromethyl)phenyl)urea(3e)

Yield 74%, Pale Orange Solid, mp 163-165 °C; IR (KBr) (v_{max} cm^{-1}): 1630(C=O), 1085(C-O), 3300 (NH); 1H -NMR (DMSO- d_6) δ ppm: 6.90-7.60 (m, 12H, Ar-H), 8.50(s, 1H, NH), 8.80(s, 1H, NH); Anal. Calcd. For $C_{21}H_{13}ClF_3N_3OS$: C, 56.32 H, 2.93; N, 9.38. Found: C, 56.22, H, 2.79; N, 9.20.

1-(2-(Benzo[d]thiazol-2-yl)phenyl)-3-(4-nitrophenyl)thiourea (3f)

Yield. 72%, Pale Yellow Solid, mp 215-217 °C; IR (KBr) (v_{max} cm^{-1}): 1350(C=S), 3310 (NH); 1H -NMR (DMSO- d_6) δ ppm: 6.70-7.30 (m, 10H, Ar-H), 7.50(d, 2H, Ar-H), 8.50(s, 1H, NH), 8.80(s, 1H, NH); ^{13}C -NMR (DMSO- d_6) δ (ppm): 120.6, 120.7, 121.4, 121.6, 124.2, 124.3, 124.6, 125.2, 125.3, 127.4, 133.0, 133.6, 137.6, 143.7, 144.4, 154.2, 166.4, 178.8, ; Anal. Calcd. For $C_{20}H_{14}N_4O_2S_2$: C, 59.10; H, 3.47; N, 13.78. Found C, 58.94; H, 3.25; N, 13.55;. GC-MS m/z 406 (100, M^+), 284(47), 196(38).

1-(2-(Benzo[d]thiazol-2-yl)phenyl)-3-(4-chlorophenyl)thiourea (3g)

Yield. 68 %, White solid, mp 190-192 °C; IR (KBr) (v_{max} cm^{-1}): 1345(C=S), 3310 (NH); 1H -NMR (DMSO- d_6) δ ppm: 6.90-7.60 (m, 12H, Ar-H), 8.50(s, 1H, NH), 8.80(s, 1H, NH); ^{13}C -NMR (DMSO- d_6) δ (ppm): 120.9, 121.4, 121.6, 124.3, 124.9, 125.1, 127.6, 128.8, 129.6, 131.6, 133.0, 133.6, 136.4, 137.4, 154.2, 166.3, 179.7 ;Anal. Calcd. For $C_{20}H_{14}ClN_3S_2$: C, 60.67;

H, 3.56; N, 10.61. Found C, 60.67; H, 4.00; N, 10.60 ;. GC-MS m/z 395(100, M⁺), 397(33, M⁺+2), 284(54), 186(33).

1-(2-(Benzo[d]thiazol-2-yl)phenyl)-3-(4-bromophenyl)thiourea (3h)

Yield.78%, White Solid, mp 171-173 °C; IR (KBr) (ν_{\max} cm⁻¹): 1348(C=S), 3310 (NH); ¹H-NMR (DMSO-*d*₆) δ ppm: 6.90-7.60 (m, 12H, Ar-H), 8.50(s, 1H, NH), 8.80(s, 1H, NH); Anal. Calcd. For C₂₀H₁₄BrN₃S₂; C, 54.55; H, 3.20; N, 9.54. Found C, 54.45; H, 2.98; N, 9.44 ;. GC-MS m/z : 440 (100, M⁺), 442(100, M⁺+2), 284(49), 231(29).

1-(2-(Benzo[d]thiazol-2-yl)phenyl)-3-(4-fluorophenyl)thiourea (3i)

Yield. 76%, Dark Brown solid, mp 185-187 °C; IR (KBr) (ν_{\max} cm⁻¹): 1340(C=S), 3310 (NH); ¹H-NMR (DMSO-*d*₆) δ ppm: 6.94 - 7.80 (m, 12H, Ar-H), 8.50(s, 1H, NH), 8.80(s,1H,NH); Anal. Calcd. For C₂₀H₁₄FN₃S₂; C, 63.30; H, 3.72 N, 11.07. Found C, 63.25; H, 3.76; N, 11.18 ;. GC-MS m/z : 379 (100, M⁺), 284(53), 169(34).

1-(2-(Benzo[d]thiazol-2-yl)phenyl)-3-(3-chloro-4-trifluoromethyl)phenyl)thiourea (3j)

Yield.70%, Pale Orange Solid, mp 185-187 °C; IR (KBr) (ν_{\max} cm⁻¹): 1350(C=S), 3310 (NH); ¹H-NMR (DMSO-*d*₆) δ ppm: 7.00-7.80 (m, 12H, Ar-H), 8.30(s, 1H, NH), 8.70(s, 1H, NH). Anal. Calcd. For C₂₁H₁₃ClF₃N₃S₂; C, 54.37; H, 2.82; N, 9.06. Found C, 54.28; H, 2.76; N, 8.95.

1 -Allyl-3- (2-(benzo [d] thiazol-2-yl) phenyl) thiourea (3k)

Yield 72%, Dark red solid, mp 175-177 °C; IR (KBr) (ν_{\max} cm⁻¹): 1347(C=S), 3400 (NH); ¹H-NMR (DMSO-*d*₆) δ ppm: 6.94 -

7.80 (m, 12H, Ar-H), 8.50(s, 1H, NH), 8.80(s, 1H, NH);Anal. Calcd. For : C₁₇H₁₅N₃S₂; C, 62.74; H, 4.65; N, 12.91. Found C, 62.70; H, 4.59; N, 12.81.

Biological activity

Antibacterial activity

All the newly synthesized compounds **3a-k** were screened for their antibacterial activity against gram positive bacteria such as *Staphylococcus aureus* (ATCC-29737) and *Bacillus subtilis* (ATCC-6633) and the gram negative bacteria such as *Escherichia coli* (ATCC-2343)and *Pseudomonas aeruginosa* (MTCC-1034) using disc diffusion method [32]. The cultures were diluted with sterilized saline to bring the final inoculum size of approximately 10⁵-10⁶ CFU/mL. These solutions containing 10⁶ cells/ mL were added to each Whatmann No.1 filter paper disc (6 mm diameter) and acetone and diethyl ether was used as the control.

Antifungal activity

The antifungal activity of newly synthesized compounds **3a-k** was tested against three pathogenic fungi including *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporium* by the poison plate technique. Test compounds were dissolved in acetone (10 mL) before mixing with Potato Dextrose Agar (PDA, 90 mL). The final concentration of the compounds in the medium was fixed at 100 μ g/ mL. Three kinds of fungi were incubated in PDA at 25±1 °C for 5 days to get new mycelium for antifungal assay, then a mycelia disc of approximately 0.45 cm diameter cut from the culture medium was picked up with a sterilized inoculation needle and inoculated in the center of PDA plate. The inoculated plates were incubated at 25±1°C for 5 days. Acetone in sterilized distilled water served as control, while

Amphotericin was used as positive control. For each treatment, three replicates were carried out. The radial growth of the fungal colonies was measured on the sixth day. The *in vitro* inhibiting effects of the test compounds on the fungi were calculated by the formula $CV = A-B/A$, where A represents the diameter of fungi growth on untreated PDA, B represents the diameter of fungi on treated PDA, and CV represents the rate of inhibition. The bacterial and fungal cultures containing discs were placed on the media and incubated at 37 °C for 24 h to 72 h for better observation. All the experiments were carried out in triplicates and the results were expressed as zone of inhibition in mm.

Results and Discussion

To a stirred solution of 2-(benzo[d]thiazol-2-yl) aniline (**1**) in dry tetrahydrofuran (THF) (15 mL) and various isocyanates/thioisocyanates were added at room temperature in the presence of triethylamine (TEA). After completion of the addition, the reaction mixture was stirred for 2h at 60 °C. The reaction progress was monitored by thin layer chromatography (TLC). After completion of the reaction, Et₃N.HCl was filtered off solvent was removed in a rota-evaporator to obtain crude product. It was purified by silica gel column chromatography eluting with ethylacetate: hexane (1:2) to afford the title compounds (3a-k).

The infrared spectral data of **3a-k** are given in experimental part. Characteristic IR stretching absorptions¹⁸ were observed in the regions 3228-3428, 1630-1647, 1341-1350 cm⁻¹ for N-H, C=O and C=S, respectively. Aromatic protons of all titled compounds appeared as complex multiplets in the region 6.80-7.80 ppm. The NH protons attached to -C=O / -C=S appeared as two distinct singlet in the region 8.50-

8.80 ppm. Aromatic carbons of all titled compound appeared in their expected region. The -C=O carbon of compounds **1**, **3a**, **3c** appeared as singlet in the region 152.6-152.7 ppm whereas the -C=S carbon of compound **3k** appeared as singlet in the region δ 178.8.

In this study we have synthesized novel derivatives of urea/Thiourea compounds were tested for their antibacterial and antifungal activities at the concentration of 100 µg/mL. From the Table 1 it is demonstrated that, among the all compounds **3a**, **3d**, **3e** and **3j** were showed potential activity against *S. aureus*, *E.coli*, *B.substilis* and *P. auroginosa*. Remaining compounds were exhibits moderate antibacterial activity when compared to positive broad spectrum antibiotic Gatifloxacin.

In addition to the antibacterial activity, we have also carried out the antifungal activity against *Aspergillus niger*, *Candida albicans*, *Fusarium oxysporum*. The obtained results were suggested that compounds **3d** and **3i** shows potential antifungal activity and other compounds elicits moderate to good antifungal activity. The obtained results were measured in terms of zone of inhibition in milli meters (Table 2).

From the above results and aforementioned discussions it can be concluded that synthesized Thiourea derivatives (**3a**, **3d**, **3i** and **3j**) have shows potential antimicrobial activities against both gram positive and negative microbes at lower concentrations. The selected compounds were needed to be tested in animal models for their better pharmacotherapy.

Table.1 Antibacterial activity of synthesized Thiourea derivatives

Compounds	Zone of Inhibition (m)			
	<i>S.aureus</i>	<i>B. subtilis</i>	<i>E.coli</i>	<i>P. auroginosa</i>
3a	11.7±0.01	14.6±0.02	14.9±0.02	12.8±0.02
3b	09.7±0.02	10.4±0.03	09.1±0.03	09.9±0.04
3c	09.1±0.04	09.0±0.05	08.6±0.02	08.7±0.03
3d	08.5±0.02	11.0±0.03	11.4±0.04	10.6±0.01
3e	11.4±0.03	13.9±0.04	14.2±0.05	12.4±0.02
3f	11.2±0.02	14.8±0.06	13.4±0.05	12.0±0.06
3g	08.4±0.03	10.0±0.04	11.4±0.02	10.0±0.02
3h	08.0±0.02	09.8±0.02	09.5±0.01	10.5±0.03
3i	09.0±0.04	11.9±0.01	11.8±0.06	11.2±0.05
3j	10.4±0.01	13.9±0.03	14.0±0.04	13.3±0.02
3k	9.12±0.04	10.2±0.03	11.3±0.04	10.7±0.01
Gatifloxacin	07.1±0.01	08.8±0.02	07.2±0.01	08.0±0.08

Table.2 Antifungal activity of the title compounds measure in Zone of inhibition (mm)

Compound ^a	<i>A.niger</i>	<i>C.albicans</i>	<i>F.oxysporum</i>
3a	07.0±0.02	07.3±0.01	08.9±0.02
3b	08.0±0.02	09.0±0.02	09.3±0.03
3c	07.4±0.04	08.2±0.03	08.4±0.03
3d	11±0.04	10.57±0.02	11±0.02
3e	09.6±0.03	09.1±0.04	08.9±0.03
3f	07.8±0.02	08.6±0.03	08.4±0.02
3g	07.3±0.05	07.6±0.02	07.1±0.03
3h	08.7±0.03	07.0±0.04	09.0±0.02
3i	11.0±0.04	11.4±0.02	10.9±0.03
3j	09.5±0.04	07.4±0.02	09.8±0.05
3k	07.0±0.02	06.9±0.02	07.0±0.04
Amphotericin ^b	13.0±0.30	12.0±0.43	11.9±0.05

Values are mean ± S.D of three replicates (p< 0.05). a 100 µg/mL, b100 µg/mL

Scheme.1 Synthesis of urea and thiourea derivatives

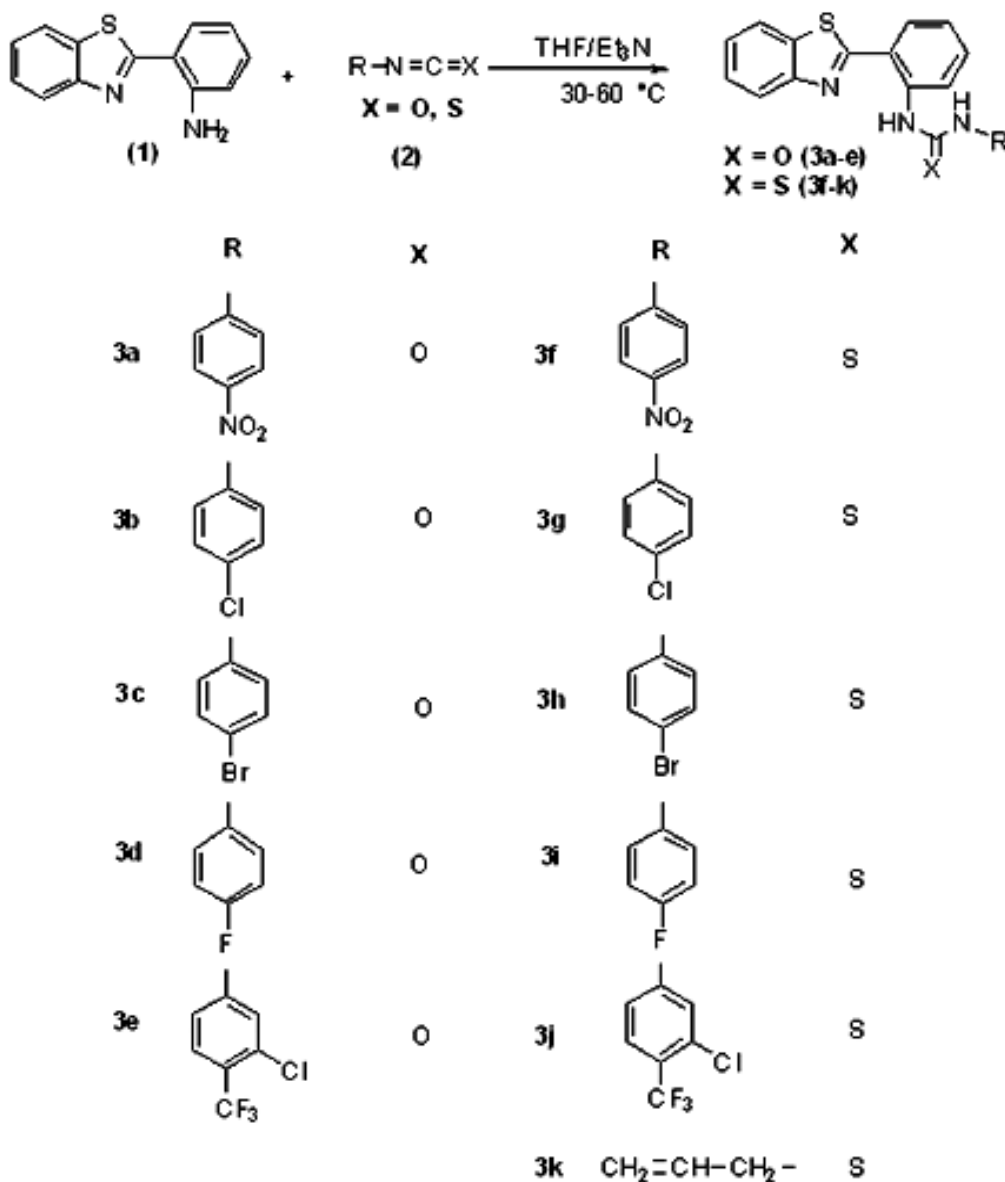
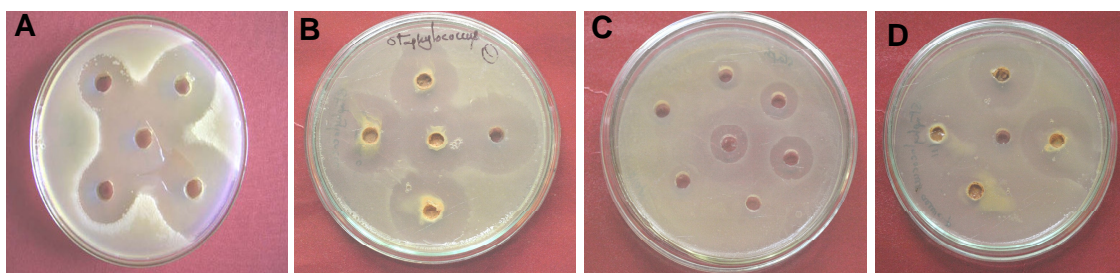


Figure.1 Schematic representation of Zone of inhibition showing anti bacterial activity of synthesized compound 3j tested against *Bacillus* (A), *Staphylococcus* (B), *E.coli* (C) and *Pseudomonas auroginosa* (D)



References

1. Cragg GM, Newman DJ. Biodiversity: A continuing source of novel drug leads. *Pure Appl Chem*, 2005; 77:7-24.
2. Sahu MP, Singh D. Role of thiourea in improving productivity of wheat (*Triticum aestivum* L.). *Journal of Plant Growth Regulation*, 1995; 4: 169-173
3. Sahu M P, Solanki NS, Dashora LN. Effects of Thiourea, Thimine and ascorbic acid on growth and yield of maize. *Journal of Agronomy and Crop Science*, 1993; 171: 65-69.
4. Sahu MP, Solanki NS. Role of sulphydral compounds in improving dry matter partitioning and grain production of maize. *Journal of Agronomy Crop Science*, 1991; 167: 356-359.
5. Yon ova PA, Stoilkova GM. Synthesis and biological activity of urea and Thiourea derivatives from 2-aminoheterocyclic compounds. *Journal of Plant Growth Regulation*, 2004; 23: 280-291.
6. Kim YJ, Ryu JH, Cheon YJ, Lim HJ, Jeon R. Design and synthesis of urea and thiourea derivatives and their inhibitory activities on lipopolysaccharide-induced NO production. *Bioorg Med Chem Lett*, 2007; 17:3317-3321.
7. Rojas J, Paya M, Domínguez JN, Ferrandiz L, Bioorg K. The synthesis and effect of fluorinated chalcone derivatives on nitric oxide production. *Medicinal Chemistry Letters*, 2002; 12: 1951-1954.
8. Saeed S, Rashid N, Jones PG, Ali M, Hussain R. N-Cyclohexyl-N'-(4-nitrobenzoyl) Thiourea. *European Journal of Medicinal Chemistry*, 2010; 45: 1323-1328.
9. Azam F, Alkskas IA, Ahmed MA. Synthesis of some urea and thiourea derivatives of 3-phenyl/ethyl-2-thioxo-2,3-dihydrothiazolo[4,5-d] pyrimidine and their antagonistic effects on haloperidol-induced catalepsy and oxidative stress in mice. *European Journal of Medicinal Chemistry*. 2009; 44(10): 3889-97
10. Babu KR, Rao VK, Kumar YN, Polireddy K, Subbaiah KV, C.Nagaraju. Identification of substituted [3, 2-a] pyrimidines as selective antiviral agents: 3 Molecular modeling studies. *Antiviral Research*, 2012; 95:118-127.
11. Babu KR, Koteswara Rao Valasani, Nanda Kumar Yellapu, Hari Prasad Osuru, Chandra Sekhar Kuruva, Bhaskar Matcha, and Naga Raju Chamarthi. Design, synthesis, in silico and in vitro studies of novel 4-methylthiazole-5-carboxylic acid derivatives as potent anti-cancer agents. *Bioorganic and medicinal chemistry letters*, 2014; 24 (18):4580-4585.
12. C. Limban, Chifiriuc, A. V. Missir, I. C. Chirița , C. Bleotu. Antimicrobial Activity of Some New Thioureides Derived from 2-(4-Chlorophenoxymethyl) benzoic Acid. *Molecules*, 2008; 13: 567-580.
13. Bhandari K, Srinivas N, Keshava GBS and Shukla PK. Tetrahydronaphthylazole oxime ethers: the conformationally rigid analogues of oxiconazole as antibacterials. *European Journal of Medicinal Chemistry*. 2009; 44(1): 437- 447.
14. Liav A, Angala SK, Brennan PJ, Jackson M. N-D-aldopentofuranosyl-N'-[p-(isoamyloxy)phenyl]-thiourea derivatives: potential anti-TB therapeutic agents *Bioorg Med Chem Lett*, 2008; 18(8):2649-51.

15. L. D. Santos, L. A Lima, V.C. Filho, R. Corrêa, F. D C. Buzzi, and R. J. Nunes, *Bioorg. Medicinal Chem*, 2008; 16, 8526-8534.
16. Koteswara Rao V, Boppudi Hari Babu, Kilaru Raveendra Babu, Doddaga Srinivasulu, Chamarthi Naga Raju. Ecofriendly synthesis of tetrahydropyrimidine derivatives in aqueous medium under ultrasonic irradiation." *Synthetic Communications*, 2012; 42 (22): 3368-3376.
17. Koteswara Rao V, Sanapalli Subba Reddy, Echchukattula Dada Peer, Alahari Janardhan Rao, and Chamarthi Naga Raju. Synthesis and Antimicrobial Activity of Novel Iminophosphocin Derivatives. *Chem Inform*, 2010; 41(17)
18. Alahari Janardhan, Valasani Koteswara Rao, Pasupuleti Visweswara Rao, Chamarthi Naga Raju, and Sunil Kumar Ghosh. Synthesis and bioactivity of phosphorylated derivatives of stavudine. *European Journal of Chemistry*, 2010; 1 (4): 297-301.
19. Reddy, S. Subba, V. Koteswara Rao, B. Satheesh Krishna, C. Suresh Reddy, P. Visweswara Rao, and C. Naga Raju. Synthesis, antimicrobial, and antioxidant activity of new α -aminophosphonates. *Phosphorus, Sulfur, and Silicon and the Related Elements*, 2011; 186 (7): 1411-1421.
20. Sanapalli S Reddy, AU Ravi Sankar, C Naga Raju, V Koteswara Rao. Synthesis and antimicrobial activity of new α -aminophosphonic acid esters. *South African Journal of Chemistry*, 2008; 61:97-101.
21. Satheesh Krishna, A Janardhan Rao, K Reddi Mohan Naidu, V Koteswara Rao, C Naga Raju. Synthesis and antibacterial activity studies of novel 2-substituted-1, 3, 2-oxazaphosphole-2-oxide derivatives of (S)-(+)-prolinol. *J Organic communications*, 2010; 3: 98-105.
22. Rao VK, Reddy SS, Krishna BS, Naidu KRM, Raju CN. Synthesis of Schiff's bases in aqueous medium: a green alternative approach with effective mass yield and high reaction rates. *Green Chemistry Letters and Reviews*, 2010; 3(3):217-223.
23. Koteswara Rao, Valasani, Sanapalli S Reddy, Balam S Krishna, Cirandur S Reddy, Nimmanapalli P Reddy, Tamatam CM Reddy, Chamarthi N Raju, and S. K Ghosh. "Design, synthesis and anti colon cancer activity evaluation of phosphorylated derivatives of lamivudine (3TC)." *Letters in Drug Design & Discovery*, 2011; 8 (1): 59-64.
24. Rao VK, Rao AJ, Reddy SS, Raju CN, Rao PV. Synthesis, spectral characterization and biological evaluation of phosphorylated derivatives of galanthamine. *European journal of medicinal chemistry*, 2010; 45(1):203-209.
25. Valasani KR, Chaney MO, Day VW, Yan SS. Acetyl cholinesterase inhibitors: structure based design, synthesis, pharmacophore modeling, and virtual screening. *Journal of chemical information and modeling*, 2013; 53(8):2033-2046.
26. Jhansi V, Rani, Koteswara Rao V, Xueqi Gan, and Shirley ShiDu Yan. Identification of human presequence protease (hPreP) agonists for the treatment of Alzheimer's disease. *European journal of medicinal chemistry*, 2014;76: 506-516.
27. Jhansi Rani V, Koteswara Rao Valasani, Du Fang, Todd D Williams, Shirley ShiDu Yan. Determination of small molecule ABAD inhibitors

- crossing blood-brain barrier and pharmacokinetics. *J Alzheimer's Disease*, 2014;42(1):333-344
28. Valasani, Koteswara R., Gang Hu, Michael O. Chaney, and Shirley S. Yan. "Structure - Based Design and Synthesis of Benzothiazole Phosphonate Analogues with Inhibitors of Human ABAD - A β for Treatment of Alzheimer's Disease." *Chemical biology and drug design*, 2013; 81 (2): 238-249.
 29. Valaasani KR, Sun Q, Hu G, Li J, Du F. Identification of Human ABAD Inhibitors for Rescuing A β -Mediated Mitochondrial Dysfunction. *Current Alzheimer research*, 2014; 11(2):128-136.
 30. Valasani KR, Vangavaragu JR, Day VW, Yan SS. Structure Based Design, Synthesis, Pharmacophore Modeling, Virtual Screening, and Molecular Docking Studies for Identification of Novel Cyclophilin D Inhibitors. *Journal of chemical information and modeling*, 2014; 54(3):902-912.
 31. Rao VK, Carlson EA, Yan SS. Mitochondrial permeability transition pore is a potential drug target for neurodegeneration. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 2014; 1842(8):1267-1272
 32. Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing; Sixteen International Supplements. CLSI document M100 S16. vol. 26-3; M7-A7, vol. 26-2; M2-A9, vol. 26-1. Wayne, PA, USA, 2006.