

# Synthesis, Characterization and *In Vitro* Antimicrobial Evaluation of Novel Pyrazolothiazol-4(5*H*)-one Derivatives

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Gaikwad, *et al.*: Antimicrobial Evaluation of Pyrazolothiazol-4(5*H*)-one

Antimicrobial screening of several novel pyrazolothiazol-4(5*H*)-one derivatives (3a-3j) has been performed. Reaction of aromatic aldehydes with aromatic ketones yielded starting chalcones (1a-1j) which have been subsequently reacted with thiosemicarbazide for obtaining N-thiocarbamoylpyrazole derivatives (2a-2j). These were further cyclized to pyrazolothiazol-4(5*H*)-one derivatives (3a-3j) in the presence of ethylbromoacetate. The structures of newly synthesized compounds were confirmed by FTIR and <sup>1</sup>H NMR and/or MS. The *in vitro* antimicrobial activity of these compounds was evaluated against Gram positive bacteria, Gram negative bacteria and fungi. Their minimum inhibitory concentration was determined by tube dilution method. The results showed that most of the compounds have promising antimicrobial activity as compared to standard drugs. Among the test compounds, 2-[5(4-chlorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-thiazol-4(5*H*)-one (3e) was found to show the most potent antimicrobial activity.

**Key words:** Antimicrobial activity, chalcone, minimum inhibitory concentration, pyrazolothiazol-4(5*H*)-one, thiosemicarbazide

Antimicrobials are one of the most important weapons in fighting bacterial infections. However, over the past few decades, development of resistance to existing drugs is a constant growing phenomenon that has concerned researchers throughout the world. So there is still need for the development of new class of active antimicrobial agents<sup>[1-4]</sup>.

An extensive literature survey revealed that, thiazolone ring has broad spectrum of biological activities such as antimicrobial, antiproliferative, antiviral, antiinflammatory, and anticonvulsant<sup>[5,6]</sup>. Bondock *et al.* reported synthesis and antimicrobial activity of some new thiazole, thiophene and pyrazole derivatives containing benzothiazole moiety (fig. 1a)<sup>[7]</sup>. Abdelhamid reported a facile synthesis of 5-arylthiazoles and triazolino[4,3-*a*] pyrimidines as antimicrobial agents (fig. 1b)<sup>[8]</sup>.

On the other hand, pyrazole ring belongs to the privileged scaffolds in modern medicinal chemistry particularly in discovering new antimicrobial agents. The pyrazole motifs were found to possess an extensive spectrum of pharmacological activities like

anticancer, antibacterial, antiinflammatory, analgesic, antidepressant and antioxidant<sup>[9]</sup>. Ragavan *et al.* reported synthesis and antimicrobial activities of novel 1,5-diaryl pyrazoles (fig. 1c)<sup>[10]</sup>. Bekhit and his coworkers reported synthesis and biological evaluation of some thiazolylpyrazole derivatives as dual anti-inflammatory antimicrobial agents (fig. 1d)<sup>[11]</sup>. Shah *et al.* reported synthesis and antibacterial studies of some novel isoxazoline derivatives (fig. 1e)<sup>[12]</sup>.

All of these encouraged us to integrate these two heterocycles and screen new pyrazolothiazol-4(5*H*)-one derivatives, which might exhibit synergistic antimicrobial effect. Herein, we illustrate in the present work the designing, synthesis and biological evaluation of pyrazolothiazol-4(5*H*)-one derivatives as potential antimicrobial agents.

All reagents and solvents used were purchased from S. D. Fine-Chem. Ltd., Mumbai, India. Melting points were determined by open capillary method on a VMP-D apparatus (Veego Instruments Corporation, Mumbai, India). The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminum sheets (silica gel 60) obtained from Merck Ltd., Mumbai, India and visualized using UV lamp. The FTIR spectra were recorded in the

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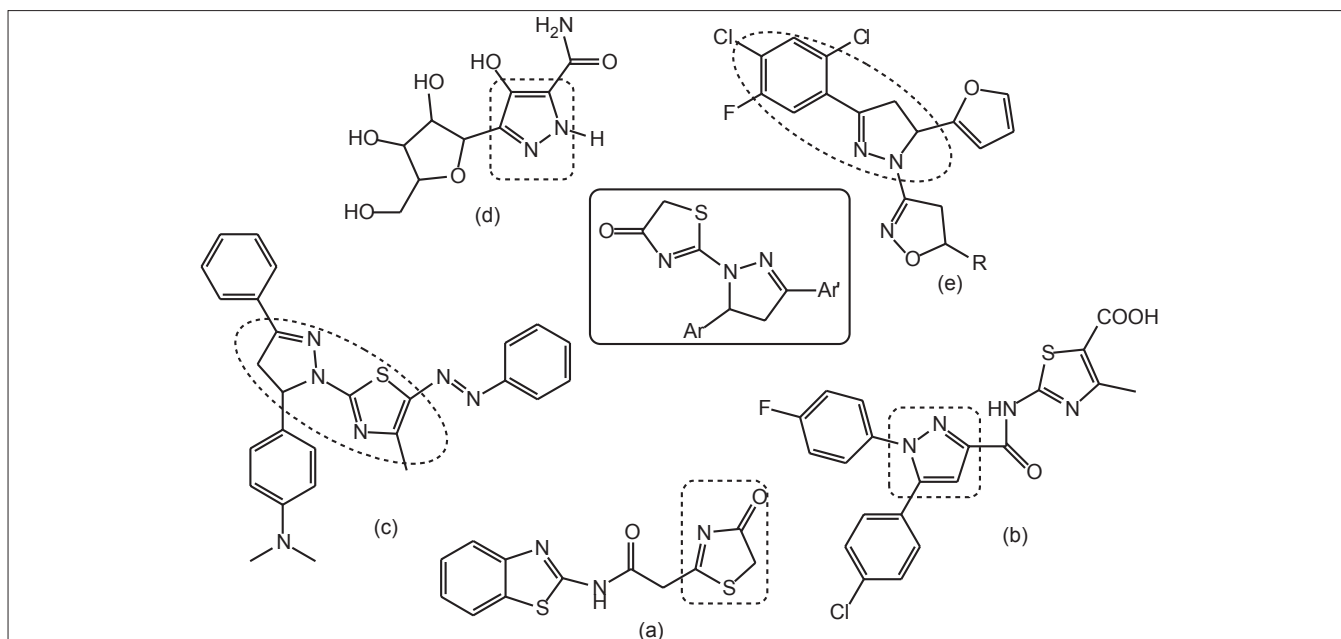


Fig. 1: Examples of lead compounds containing pyrazole and thiazolone rings with antimicrobial activity and designing of the pyrazolothiazol-4(5H)-one derivatives.

range of 4000-400  $\text{cm}^{-1}$  by KBr disk method using FTIR 8400S Shimadzu spectrometer. Spectral analyses were recorded at Sophisticated Analytical Instrumental Facility (SAIF), IIT, Mumbai, India.  $^1\text{H}$  NMR spectra were recorded on Varian Mercury Plus (300 MHz) spectrometer in  $\text{DMSO-}d_6$  or  $\text{CDCl}_3$  as solvent using tetramethylsilane (TMS) as an internal reference standard and values are expressed in  $\delta$  ppm. Mass spectra were recorded on Varian Inc. mass spectrometer.

General pathway for synthesis of compounds (3a-3j) was outlined in Scheme 1 and the physicochemical characterization is given in Table 1. For synthesis of chalcones (1a-1j) a suspension of aromatic ketone (40 mmol) and aromatic aldehyde (40 mmol) in ethanolic NaOH was stirred for appropriate period of time. The reaction mixture was poured into cold water to produce solid compound which was recrystallized with suitable solvent in order to obtain pure chalcone.

General method for synthesis of N-thiocarbamoylpyrazole derivatives (2a-2j) described as mixture of chalcone (10 mmol) and thiosemicarbazide (20 mmol) was refluxed in ethanolic KOH. Precipitate formed was filtered under vacuum. Recrystallization was carried out with acetone or ethanol.

For synthesis of pyrazolothiazol-4(5H)-one derivatives (3a-3j), N-thiocarbamoylpyrazole derivative (10 mmol) was refluxed with ethylbromoacetate (33 mmol) for

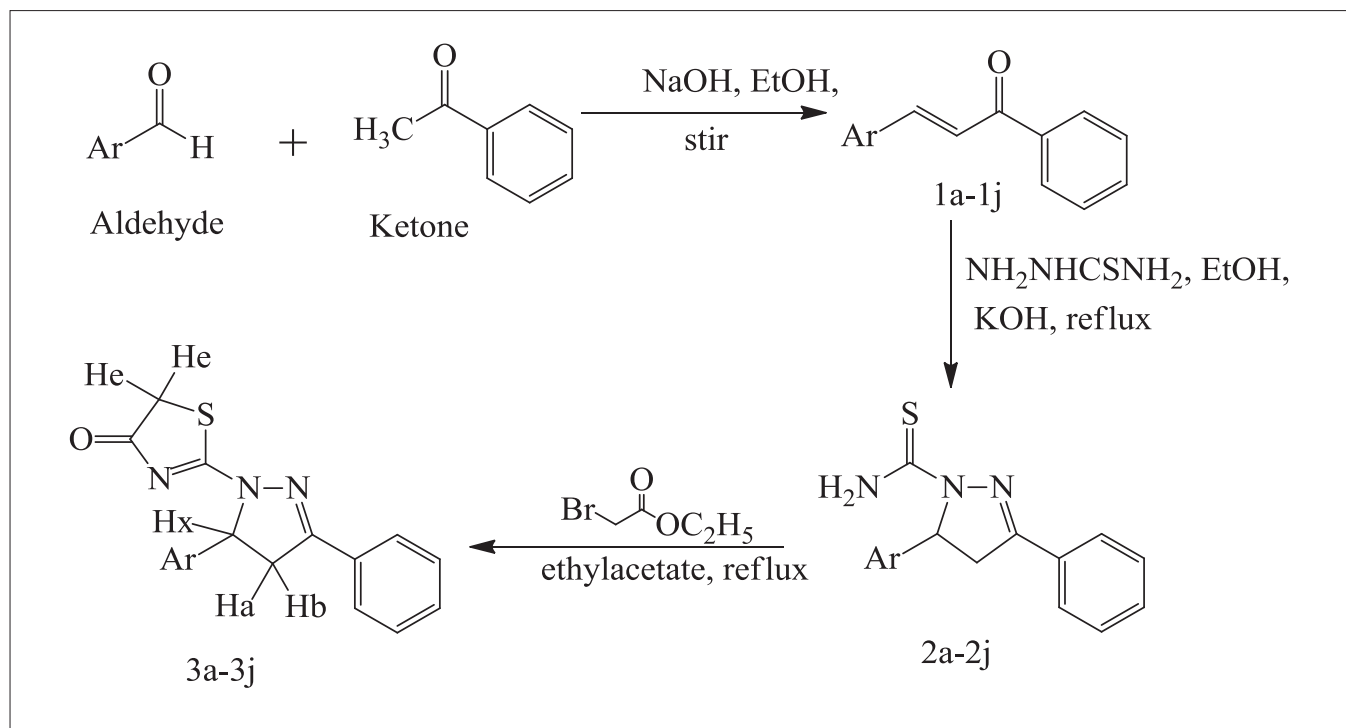
TABLE 1: PHYSICOCHEMICAL CHARACTERIZATION OF NEWLY SYNTHESIZED COMPOUNDS (3a-3j)

Compound	Ar	Molecular formula	Molecular weight	Mp ( $^{\circ}$ )	% yield	$R_f$ value*
3a	$\text{C}_6\text{H}_5$	$\text{C}_{18}\text{H}_{15}\text{N}_3\text{OS}$	321.40	222	72	0.32
3b	$\text{C}_4\text{H}_4\text{O}$	$\text{C}_{16}\text{H}_{13}\text{N}_3\text{O}_2\text{S}$	311.36	230	68	0.35
3c	$\text{C}_4\text{H}_4\text{S}$	$\text{C}_{16}\text{H}_{13}\text{N}_3\text{OS}_2$	327.42	233	80	0.77
3d	2- $\text{ClC}_6\text{H}_4$	$\text{C}_{18}\text{H}_{14}\text{ClN}_3\text{OS}$	355.84	272	74	0.82
3e	4- $\text{ClC}_6\text{H}_4$	$\text{C}_{18}\text{H}_{14}\text{ClN}_3\text{OS}$	355.84	249	77	0.77
3f	2-Cl, 4- $\text{ClC}_6\text{H}_3$	$\text{C}_{18}\text{H}_{13}\text{Cl}_2\text{N}_3\text{OS}$	390.29	287	65	0.59
3g	2-Cl, 6- $\text{FC}_6\text{H}_3$	$\text{C}_{18}\text{H}_{13}\text{ClFN}_3\text{OS}$	373.83	251	81	0.85
3h	4- $\text{FC}_6\text{H}_4$	$\text{C}_{18}\text{H}_{14}\text{FN}_3\text{OS}$	339.39	210	73	0.74
3i	3- $\text{NO}_2\text{C}_6\text{H}_4$	$\text{C}_{18}\text{H}_{14}\text{N}_4\text{O}_3\text{S}$	366.39	299	66	0.31
3j	4- $\text{OHC}_6\text{H}_4$	$\text{C}_{18}\text{H}_{15}\text{N}_3\text{O}_2\text{S}$	337.40	300	62	0.35

The properties like substituents present, molecular formula, molecular weight, Mp=Melting point, percentage yield and  $R_f$  value. \*Mobile phase for TLC (hexane:ethyl acetate, 1:1)

appropriate time. After completion of reaction, residue obtained was recrystallized with suitable solvent.

2-(3,5-Diphenyl-4,5-dihydropyrazol-1-yl)-thiazol-4(5H)-one (3a). Yield: 72%; mp 222–223 $^{\circ}$ ; FTIR ( $\nu$ ,  $\text{cm}^{-1}$ )=2970 (C-H, aromatic), 1697 (C=O), 1543 (C=N), 1226 (C-N), 700 (C-S);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm=3.34–3.42 (dd, 1H, Ha), 3.85 (s, 2H, He), 3.90-4.00 (dd, 1H, Hb), 5.76–5.81 (dd, 1H, Hx), 7.21-7.36 (m, 5H, Ar-H), 7.43–7.54 (m, 3H, Ar-H), 7.78-7.85 (dd, 2H, Ar-H). MS:  $m/z$  =322 ( $\text{M}^+$  +1, 100%), 323 ( $\text{M}^+$  +2, 18.56%).



Scheme I: Synthesis of the pyrazolothiazol-4(5H)-one derivatives (3a-3j).

2-(5-Furan-2-yl-3-phenyl-4,5-dihydropyrazol-1-yl)-thiazol-4(5H)-one (3b). Yield: 68%; mp 230-232°; FTIR ( $\nu$ ,  $\text{cm}^{-1}$ )=2970 (C-H, aromatic), 1695 (C=O), 1543 (C=N), 1222 (C-N), 690 (C-S);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm=3.63-3.71 (dd, 1H, Ha), 3.74-3.84 (dd, 1H, Hb), 3.86 (s, 2H, He), 5.84-5.90 (dd, 1H, Hx), 6.31-6.33 (dd, 1H, Ar-H), 6.54-6.55 (d, 1H, Ar-H), 7.30-7.31 (d, 1H, Ar-H), 7.44-7.54 (m, 3H, Ar-H), 7.80-7.84 (dd, 2H, Ar-H).

2-(3-Phenyl-5-thiophen-2-yl-4,5-dihydropyrazol-1-yl)-thiazol-4(5H)-one (3c). Yield: 80%; mp 233-235°; FTIR ( $\nu$ ,  $\text{cm}^{-1}$ )=2966 (C-H, aromatic), 1701 (C=O), 1543 (C=N), 1211 (C-N), 692 (C-S);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm=3.53-3.60 (dd, 1H, Ha), 3.87 (s, 2H, He), 3.88-3.97 (dd, 1H, Hb), 6.08-6.13 (dd, 1H, Hx), 6.91-6.94 (dd, 1H, Ar-H), 7.15-7.20 (d, 1H, Ar-H), 7.21-7.23 (dd, 1H, Ar-H), 7.44-7.55 (m, 3H, Ar-H), 7.80-7.84 (dd, 2H, Ar-H).

2-[5-(2-Chlorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-thiazol-4(5H)-one (3d). Yield: 74%; mp 272-274°; FTIR ( $\nu$ ,  $\text{cm}^{-1}$ )=2924 (C-H, aromatic), 1697 (C=O), 1541 (C=N), 1222 (C-N), 756 (Aromatic C-Cl), 688 (C-S); MS:  $m/z$  =356.1( $\text{M}^+$ , 100%), 358 ( $\text{M}^+$  +2, 38.51%), 357 ( $\text{M}^+$  +1, 24.68%).

2-[5-(4-Chlorophenyl)-3-phenyl-4,5-dihydropyrazol-1-

yl]-thiazol-4(5H)-one (3e). Yield: 77%; mp 249-251°; FTIR ( $\nu$ ,  $\text{cm}^{-1}$ )=2929 (C-H, aromatic), 1703 (C=O), 1533 (C=N), 1226 (C-N), 827 (Aromatic C-Cl), 688 (C-S); MS:  $m/z$ =356 ( $\text{M}^+$ , 100%), 358 ( $\text{M}^+$  +2, 39.55%), 357( $\text{M}^+$  +1, 19.54%), 255 ( $\text{M}^+$  -100, 11.82%).

2-[5-(2,4-Dichlorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-thiazol-4(5H)-one (3f). Yield: 65%; mp 287-289°; FTIR ( $\nu$ ,  $\text{cm}^{-1}$ ) =3074 (C-H, aromatic), 1689 (C=O), 1533 (C=N), 1226 (C-N), 763 (Aromatic C-Cl), 692 (C-S);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm=3.22-3.29 (dd, 1H, Ha), 3.92 (s, 2H, He), 3.99-4.09 (dd, 1H, Hb), 6.01-6.06 (dd, 1H, Hx), 6.94-6.97 (d, 1H, Ar-H), 7.18-7.19 (dd, 1H, Ar-H), 7.42-7.53 (m, 4H, Ar-H), 7.76-7.80 (dd, 2H, Ar-H).

2-[5-(2-Chloro-6-fluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-thiazol-4(5H)-one (3g). Yield: 81%; mp 251-253°; FTIR ( $\nu$ ,  $\text{cm}^{-1}$ )=2966 (C-H, aromatic), 1701 (C=O), 1537 (C=N), 1224 (C-N), 1116 (Aromatic C-F), 790 (Aromatic C-Cl), 694 (C-S); MS:  $m/z$ =374 ( $\text{M}^+$  +1, 100%), 340 ( $\text{M}^+$  -34, 32.16%), 273 ( $\text{M}^+$  - 100, 45.84%), 239 ( $\text{M}^+$  - 135, 31.23%).

2-[5-(4-Fluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-thiazol-4(5H)-one (3h). Yield: 73%; mp 210-212°; FTIR ( $\nu$ ,  $\text{cm}^{-1}$ )=2926 (C-H, aromatic), 1708 (C=O),

1568 (C=N), 1161 (C-N), 1124 (Aromatic C-F), 688 (C-S); MS:  $m/z=340$  ( $M^+ +1$ , 100%),  $341(M^+ +2$ , 20.39%).

2-[5-(3-Nitrophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-thiazol-4(5H)-one (3i). Yield: 66%; mp 298-299°C; FTIR ( $\nu$ ,  $\text{cm}^{-1}$ )=3061 (CH, aromatic), 1697 (C=O), 1539 (C=N), 1222 (C-N), 692 (C-S); MS:  $m/z=367$  ( $M^+ +1$ , 100%), 368 ( $M^+ +2$ , 20.54%), 232 ( $M^+ -135$ , 7.36%).

2-[5-(4-Hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-thiazol-4(5H)-one (3j). Yield: 62%; mp 298-300°C; FTIR ( $\nu$ ,  $\text{cm}^{-1}$ )=3460 (C-OH), 2970 (C-H, aromatic), 1688 (C=O), 1579 (C=N), 1230 (C-N), 693 (C-S);  $^1\text{H NMR}$  (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  ppm=3.35-3.42 (dd, 1H, Ha), 3.91 (s, 2H, He), 4.01-4.11 (dd, 1H, Hb), 5.65-5.70 (dd, 1H, Hx), 6.70-6.73 (d, 2H, Ar-H), 7.01-7.04 (d, 2H, Ar-H), 7.49-7.58 (m, 3H, ArH), 7.83-7.86 (dd, 2H, ArH), 9.48 (s, 1H, OH).

The *in vitro* antimicrobial activity of newly synthesized compounds (3a-3j) was determined by tube dilution method. In this work, two Gram positive organisms, *i.e.* *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538P and two Gram negative organisms, *i.e.* *Escherichia coli* ATCC 9002(NCTC) and *Pseudomonas aeruginosa* ATCC 25619 were used to investigate the antibacterial activity. They were also evaluated for their *in vitro* antifungal activity against *Aspergillus niger* ATCC 9029 and *Candida albicans* ATCC 2091. The bacterial and the fungal strains were procured from National Chemical Laboratory, Pune, India. Streptomycin and fluconazole (Sigma

Aldrich, Bangalore, India) were used as standard for antibacterial and antifungal studies, respectively. Two-fold dilutions of the compounds and standard drugs were prepared in dimethylsulfoxide (DMSO) ranging from 0.8 to 400  $\mu\text{g/ml}$ . Seeded broth was prepared in nutrient broth from 24 h old bacterial cultures on nutrient agar at  $37\pm 1^\circ$  while fungal spores from 24 h old Sabourauds agar slant cultures were suspended in Sabourauds dextrose broth. The colony forming units of the seeded broth were adjusted in the range of Mcfarland standard number one. Testing was performed at  $\text{pH } 7.4\pm 0.2$ . A set of assay tubes containing only inoculated broth was kept as control and likewise solvent controls were also run simultaneously. The tubes were incubated in incubators at  $37^\circ$  for bacteria and at  $28^\circ$  for fungi. The minimum inhibitory concentrations were recorded by visual observations after 24 h (bacteria) and 72-96 h (fungi) of incubation. Results of antimicrobial studies have been presented in Table 2.

The synthesis of compounds (3a-3j) was followed by the general pathway outlined in Scheme 1. In the first step, chalcones (1a-1j) were obtained by direct condensation of the aromatic aldehydes and the aromatic ketones, using sodium hydroxide as catalyst in ethanol. In the second step, cyclization of different chalcones with thiosemicarbazide under basic condition leads to the formation of N-thiocarbamoylpyrazole derivatives (2a-2j). In the last step, pyrazolothiazol-4(5H)-one derivatives (3a-3j) were obtained by refluxing compounds (2a-2j) with ethylbromoacetate in ethylacetate. All of the synthesized compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted

**TABLE 2: IN VITRO ANTIMICROBIAL ACTIVITY OF COMPOUNDS (3a-3j)**

Compound	Minimum inhibitory concentration ( $\mu\text{g/ml}$ )					
	Gram positive bacteria		Gram negative bacteria		Fungi	
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. niger</i>	<i>C. albicans</i>
3a	200	100	1.55	50	12.5	25
3b	400	50	400	200	400	400
3c	25	6.25	100	400	100	100
3d	3.12	12.5	6.25	25	6.25	50
3e	1.55	12.5	1.55	3.12	6.25	12.5
3f	3.12	25	12.5	6.25	12.5	6.25
3g	6.25	3.12	6.25	12.5	3.12	12.5
3h	6.25	3.12	25	50	25	200
3i	1.55	100	12.5	25	50	100
3j	25	200	200	100	50	400
Std a	3.12	6.25	6.25	6.25	-	-
Std b	-	-	-	-	8	16

Std=standard, a=streptomycin and Std, b=fluconazole are standards for antibacterial and antifungal activity, respectively

structures. The physicochemical characteristics of newly synthesized compounds are given in Table 1.

These synthesized pyrazolothiazol-4(5*H*)-one derivatives were evaluated for their *in vitro* antimicrobial activity (Table 2). The study revealed that some of the tested compounds showed moderate to excellent antimicrobial activity against pathogenic strains. Interestingly, among the synthesized compounds, 3e showed excellent antibacterial activity against *B. subtilis*, *E. coli*, and *P. aeruginosa* as compared to standard drug streptomycin. Also 3a, 3e, 3g, 3h and 3i exhibited moderate antibacterial activity. 3c, 3d, 3f and 3g showed similar antibacterial activity as that of standard. Similarly, 3e and 3g showed excellent activity against both *A. niger* and *C. albicans* as compared to standard drug fluconazole. Also 3d and 3f showed good activity against *A. niger* and *C. albicans*, respectively.

From the above results it was observed that, compounds bearing electronegative substitutions such as Cl or F showed pronounced activity against most of the bacterial and fungal strains. While the compounds bearing aromatic heterocyclic rings without substitutions like furan or thiophene were found to be inactive. Therefore, in future pyrazolothiazol-4(5*H*)-one derivatives would represent a novel lead for the development of a new class of antimicrobial agents. These compounds are recommended for further *in vivo* screening.

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