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Novel pyrazole-pyridine containing 4-thiazolidinone hybrids: Design, synthesis and antimicrobial activity

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The emergence of antimicrobial resistance (AMR) to currently available antimicrobial agents is a major source of concern for scientists. Antibiotic treatment against numerous bacteria and fungus is no longer effective due to antimicrobial resistance, as bacteria and fungi have developed to fight antibiotics and proven them ineffective or less efficient. Novel antimicrobial drugs that are effective against resistant strains are desperately needed. In continuation to this, we have designed and synthesized 4-thiazolidinone clubbed pyridine-pyrazole analogues **(5a-5o)** for the evaluation of antimicrobial activity. The structures of the newly prepared compounds were analyzed and confirmed by using IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. Synthesized compounds are tested against various bacterial and fungal strains. Among the tested compounds, compounds **5b, 5g** and **5m** (MIC value of 62.5 µg/mL) have exhibited potency against *E. coli* gram-negative strain while compounds **5a, 5c, 5g, 5i** and **5l** (MIC = 250 µg/mL) were active against *C. albicans* fungal strain.

Keywords: Pyrazole, Pyridine, 4-thiazolidinone, Antibacterial, Antifungal

The molecules which contain 4-thiazolidinone, pyridine, and pyrazole moiety have been synthesized to test their antimicrobial activity. Pyrazole moiety is active against anti-HIV, antiviral, anti-inflammatory and anti-proliferative[1-4]. Pyridine is also active as antidepressant, antitubercular and analgesic[5-9]. 4-Thiazolidinone is active as anticancer, antimicrobial, antioxidant and antiviral[10-15].

The concept of drug designing of reported molecules based on commercially available drug is given in Figure 1. We have taken furan and 4-thiazolidinone containing LJ-001 commercially available drug to design our targeted molecules (**5a-5o**). The various position of 4-thiazolidinone is modified as shown in Figure 1. Sulfur at second position and alkyl chain of third position is replaced by pyrazole and pyridine moiety respectively. Furan ring is replaced by various phenyl derivatives for the evaluation of antimicrobial activity of synthesized hybrids.

The hybrids of 4-thiazolidinone containing pyridine and quinazoline moiety were previously synthesized by our group for the development of antimicrobial agents[11]. The antimicrobial activity of the synthesized compounds was tested against various bacterial and fungal strains. Among the tested compounds, compound having methyl group on *meta* position of phenyl ring exhibited tremendous activity against gram-negative *E. coli* (12.5 µg/mL) and *P. aeruginosa* (62.5 µg/mL) strains. Inspired by these results, we have synthesized hybrids of 4-thiazolidinone bearing pyridine and pyrazole moiety (**5a-5o**). Pyridine of C-2 position of 4-thiazolidinone is replaced by pyrazole moiety while quinazoline of N-3 position is replaced by pyridine moiety. The results of antimicrobial activity revealed that synthesized hybrids exhibited excellent to moderate activity against tested bacterial and fungal strains. The rationale of our reported work is given in Figure 2.

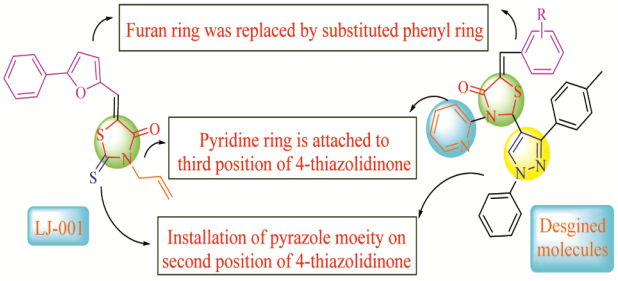


Figure 1 — The concept of drug designed of targeted molecules **(5a-5o)** based on commercially available drug

Results and Discussion

Chemistry

The synthetic pathway of reported compounds (**5a-5o**) is given in above Scheme I. Pyridine-2-amine **1** was dissolved in methanol and 1-phenyl-3-(*p*-tolyl)-1*H*-pyrazole-4-carbaldehyde **2** was added to it. After adding CH_3COOH in a catalytic amount, the reaction mixture was stirred at 80°C for 4 h to get compound **3**. *N*-((1-phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl) methylene)pyridin-2-amine **3** was dissolved in 1,4-dioxane and thioglycolic acid was added to it. The reaction mixture was stirred at 120°C for 8 h after the addition of $ZnCl_2$ as a catalyst to get compound **4**. Compound **4** was dissolved in ethanol and substituted benzaldehydes was added to it. After the addition of C_2H_5ONa as a catalyst, the reaction mixture was stirred at 60°C for 4 h to get compounds (**5a-o**).

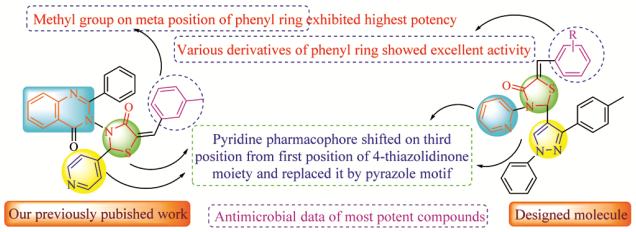
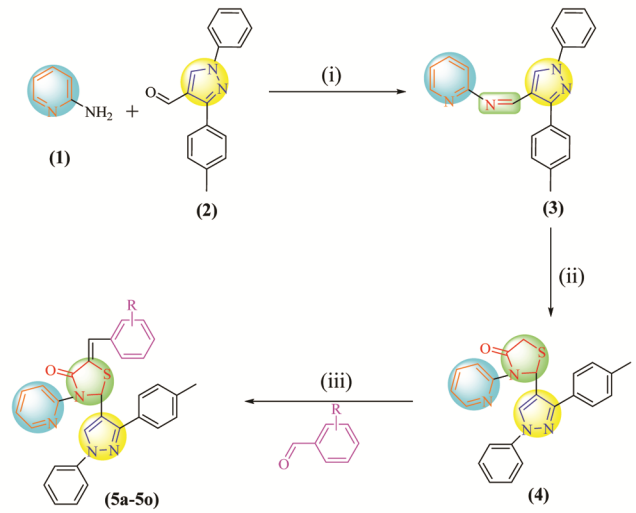


Figure 2 — The conceptualization of rationale for the designed molecules (**5a-5o**) in accordance to our previous work



Scheme I — Synthetic route for the preparation of 4-thiazolidinone hybrids (**5a-5o**)

The IR spectra of compound **5b** showed the peaks at 1727 cm^{-1} and 740 cm^{-1} which depicted the presence of >C=O group and C-S-C, respectively. Moreover, peaks observed at 1570 cm^{-1} and 663 cm^{-1} confirmed the presence of -C=N and -Br groups respectively. ^{1}H NMR of compound **5b** showed signals at 2.38 and 6.89-8.37 ppm corresponding to the $-CH_3$ group and aromatic protons of the proposed motif. Signals that appeared at 5.80 and 7.46 ppm are in accordance with methine proton of 4-thiazolidinone and proton of pyrazole ring respectively. ^{13}C NMR was also carried out for the reported molecule. Pyrazole ring affirmed by getting the peaks at 118.7, 122.2 and 152.9 ppm while peaks observed at 63.3 (methine carbon), 140.1 and 161.5 ppm are due to a 4-thiazolidinone moiety. Mass spectra at *m/z* 578 was in the agreement with the proposed compound.

Discussion on antimicrobial activity

4-Thiazolidinone hybrids (**5a-5o**) were synthesized for the development of novel antimicrobial agents. The results of the antimicrobial activity of synthesized compounds are given in Table I. Compounds having bromo on 2nd position, hydroxyl and nitro groups each on 4th position were most active against gram-negative strain *E. coli* having MIC value of 62.5 µg/mL while -4-F derivative was found to be most active against gram-positive strain *S. aureus* having MIC value of 62.5 µg/mL. Rest of the compounds were moderately active against tested bacterial strains. Results of antifungal activity of the synthesized compounds revealed that compounds containing -H, -2-Cl, -4-OH, $-4-OCH_3$ and $-2-NO_2$ derivatives showed tremendous activity having MIC value of 250 µg/mL against *C. albicans* compared to standard drug griseofulvin.

Structure-activity relationship

As shown in the Figure 3, an overview of the structure-activity relationship (SAR) of the titled compounds revealed that various electron-withdrawing and electron-donating groups present on phenyl ring may impact effectively on antimicrobial activity. The introduction of -Br group on second position while -F, $-OCH_3$ and $-NO_2$ groups on fourth position found to be the most active against *E. coli* and *S. aureus* strains having MIC value of 62.5 µg/mL. SAR study also revealed that both the electron-donating groups (-H, -4-OH, $-4-OCH_3$) and electron-withdrawing groups (-2-Cl, $-2-NO_2$) exhibited prominent potency against *E. coli* fungal strain showing MIC value of 250 µg/mL. Results revealed

Table I — Results of antimicrobial evaluation of 4-thiazolidinone based pyridine-pyrazole hybrids (**5a-o**)

Entry	-R	Minimum inhibitory concentration (MIC) in µg/mL				
		Bacteria µg/mL				Fungi µg/mL
		E. c.	*P. a.*	*S. a.*	*S. p.*	*C. a.*
5a	-H	200	200	250	100	250
5b	-2-Br	62.5	200	125	200	1000
5c	-2-Cl	200	200	500	250	250
5d	-3-Cl	100	100	250	125	1000
5e	-4-F	200	200	62.5	100	500
5f	-3-OH	250	100	125	250	500
5g	-4-OH	62.5	100	250	100	250
5h	-2-OCH_3	200	250	100	100	1000
5i	-4-OCH_3	250	250	250	200	250
5j	-3-CH_3	250	100	200	100	>1000
5k	-4-CH_3	500	500	100	125	500
5l	-2-NO_2	100	100	100	250	250
5m	-4-NO_2	62.5	100	100	250	500
5n	-2,3-$(OCH_3)_2$	125	100	500	500	500
5o	-2,4-$(CH_3)_2$	250	500	250	200	500
Chloramphenicol		50	50	50	50	—
Griseofulvin		—	—	—	—	500

Escherichia coli (E.c.) *MTCC*-442; *Pseudomonas aeruginosa* (P.a.) *MTCC*-441; *Staphylococcus aureus* (S.a.) *MTCC*-96; *Streptococcus pyogenes* (S.p.) *MTCC*-443; *Candida albicans* (C.a.) *MTCC*-227.

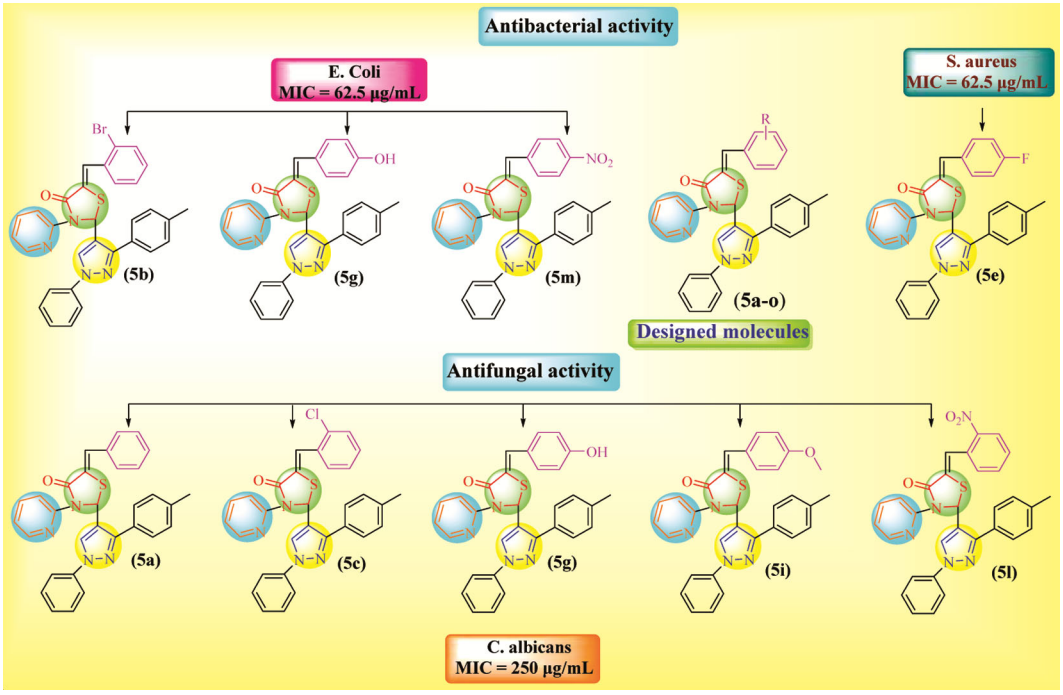


Figure 3 — Effects of various electron-withdrawing and electron-donating groups influencing antimicrobial activity

that second and fourth position of phenyl ring exhibited higher potency as antimicrobial agents.

Experimental Section

The completion of the reaction and the purity of all compounds was checked on aluminum-coated TLC plates 60, F_{254} (E. Merck) using n-hexane and ethyl acetate (7:3) as eluent and visualized under ultraviolet (UV) light, or iodine vapor. Elemental analysis was carried out by a Perkin-Elmer 2400 CHN analyzer. ^{1}H NMR spectra were recorded on a Bruker Avance II 400 MHz and ^{13}C NMR spectra on Varian Mercury-

400, 100 MHz in DMSO-d_6 as a solvent and tetramethylsilane (TMS) as an internal standard using 5mm tube. Chemical shifts were reported in parts per million (ppm). IR spectra were recorded on a Bruker FT-IR spectrophotometer while mass spectra were scanned on a Shimadzu LC-MS 2010 spectrometer[16].

Compound 3-(4-methyphenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde 2 was prepared as per the method given in the literature[17].

Preparation of N-((3-(4-methylphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)pyridin-2-amine(3)

2-Aminopyridine **1** (0.01 mol) was dissolved in methanol (20 mL) 3-(4-methyphenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde **2** (0.01 mol) was added to it. The reaction mixture was stirred at 80°C for 4 h after the addition of CH_3COOH as a catalyst. The reaction mixture was allowed to cool at RT and poured into crushed ice. The product obtained was filtered, dried and recrystallized from methanol to obtain compound **3**.

Yield: 82%. Solid. m.p.151-153°C; IR (KBr): 2852 (C–H, aromatic), 1626 (C=N), 1501 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.25 (s, 3H, Ar-CH_3), 7.11-7.55 (m, 13H, Ar-H), 7.43 (s, 1H, =CH-N) 9.01 (s, 1H, -C*H*=N-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 19.3 (Ar-CH_3), 118.3 (C_4 of pyrazole ring), 119.1 (Ar-C), 120.6 (Ar-C), 120.3 (C_5 of pyrazole ring), 125.1 (Ar-C), 127.4 (Ar-C), 129.2 (Ar-C), 129.4 (Ar-C), 134.2 (Ar-C), 138.2 (Ar-C), 138.4 (Ar-C), 139.6 (Ar-C), 148.3 (Ar-C), 149.2 (Ar-C), 149.5 (Ar-C), 153.3 (C_3 of pyrazole ring), 162.1 (-*C*H=N); MS: *m/z* 338 (M^+). Anal. Calcd for $C_{22}H_{18}N_4$: C, 78.08; H, 5.36; N, 16.56. Found: C, 78.16; H, 5.39; N, 16.52%.

Preparation of 2-(3-(4-methylphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-3-(pyridin-2-yl)thiazolidin-4-one (4)

Compound **3** (0.01 mol) was dissolved in 1,4-dioxane (20 mL) and thioglycolic acid (0.03 mol) was added to it. The reaction mixture was stirred at 120°C for 8 h after the addition of $ZnCl_2$ as a catalyst. The reaction mixture was allowed to cool at RT and poured into an ice-cold dilute $NaHCO_3$ solution. The product obtained was filtered, dried and recrystallized from methanol to obtain compound **4**.

Yield: 85%. Solid. m.p.198-200°C. IR (KBr): 2851 (C–H, aromatic), 1744 (C=O), 1621 (C=N), 1508 (C=C), 684 cm^{-1} (C-S); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.21 (s, 3H, Ar-CH_3), 3.82 (s, 2H, -CH_2), 5.64 (s, 1H, -S-CH-N-), 7.01-7.55 (m, 13H), 7.48 (s, 1H, =CH-N-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 20.3 (Ar-CH_3), 33.4 (-CH_2-S-), 64.1 (-S-*C*H-N-), 116.4 (Ar-C), 118.4 (C_4 of pyrazole ring), 118.6 (Ar-C), 122.2 (C_5 of pyrazole ring), 125.1 (=*C*H-Ar), 127.3 (Ar-C), 128.5 (Ar-C), 128.7 (Ar-C), 129.3 (Ar-C), 137.1 (Ar-C), 138.7 (Ar-C), 139.3 (C_5 of thiazolidinone ring), 148.3 (Ar-C), 149.24 (Ar-C), 150.1 (Ar-C), 152.5 (C_3 of pyrazole ring), 172.5 (-C=O); MS: *m/z* 412 (M^+). Anal. Calcd for $C_{24}H_{20}N_4OS$: C, 69.88; H, 4.89; N, 13.58. Found: C, 69.83; H, 4.85; N, 13.62%.

General preparation of 5-arylidene-2-(3-(4-methylphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-3-(pyridin-2-yl)thiazolidin-4-ones (5a-o)

Compound **4** (0.01 mol) was dissolved in ethanol (20 mL) and substituted benzaldehydes (0.01 mol) was added to it. After the addition of C_2H_5ONa as a catalyst, the reaction mixture was stirred at 60°C for 4 h. The reaction mixture was allowed to cool at RT and poured into crushed ice. The product obtained was filtered, dried and recrystallized from methanol to obtain compound (**5a-o**).

Characterization of compound 5-(benzylidene)-2-(1-phenyl-3-(p-tolyl)-1*H*-pyrazol-4-yl)-3-(pyridin-2-yl)thiazolidin-4-one(5a)

Yield: 85%. Solid. m.p.234-236ºC. IR (KBr): 2921 (C–H, aromatic), 1725 (C=O), 1574 (C=N), 1505 (C=C), 744 cm^{-1} (C-S); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.32 (s, 3H, -CH_3), 5.16 (s, 1H, =CH-Ar), 5.83 (s, 1H, -S-CH-N-), 6.83-8.39 (m, 18H, Ar-H), 7.43 (s, 1H, =CH-N-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 17.8 (Ar-CH_3), 58.4 (-S-*C*H-N-), 116.9 (C_4 of pyrazole ring), 119.2, 124.8 (=*C*H-Ar), 126.1, 127.1, 128, 128.9, 129.2 (C_5 of thiazolidinone ring), 129.6, 129.7 (C_3 of pyrazole ring), 129.9, 130, 131.2, 132.4, 133.5, 134, 140.9, 141.6, 144.2, 144.7, 156.7 (C_5 of pyrazole ring), 170.2 (-C=O);MS: *m/z* 500 (M^+). Anal. Calcd for $C_{31}H_{24}N_4OS$: C, 74.38; H, 4.83; N, 11.19%. Found: C, 74.35; H, 4.86; N, 11.16%.

Characterization of compound 5-(2-bromobenzylidene)-2-(1-phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)-3-(pyridin-2-yl)thiazolidin-4-one(5b)

Yield: 60%. Solid. m.p.247-249ºC. IR (KBr): 2928 (C–H, aromatic), 1727 (C=O), 1570 (C=N), 1508 (C=C), 740 (C-S), 663 cm^{-1} (-Br); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.38 (s, 3H, -CH_3), 5.14 (s, 1H, =CH-Ar), 5.80 (s, 1H, -S-CH-N-), 6.91-8.37 (m, 17H, Ar-H), 7.46 (s, 1H, =CH-N-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 24.9 (Ar-CH_3), 57.4 (-S-*C*H-N-), 118.6 (C_4 of pyrazole ring), 119, 122.7 (=*C*H-Ar), 126.8,

127, 127.8, 128.2, 128.5, 128.6, 128.7, 129.1 (C_5 of thiazolidinone ring), 130.4, 130.6 (C_3 of pyrazole ring), 131.3, 132.4, 132.8, 134.4, 135.8, 141.4, 146.1, 148.9, 151.2, 160 (C_5 of pyrazole ring), 173.2 (-C=O); MS: *m/z* 578 (M^+). Anal. Calcd for $C_{31}H_{23}BrN_4OS$: C, 64.25; H, 4.00; N, 9.67%. Found: C, 64.21; H, 4.02; N, 9.63%.

Characterization of compound 5-(2-chlorobenzylidene)-2-(1-phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)-3-(pyridin-2-yl)thiazolidin-4-one(5c)

Yield: 74%. Solid. m.p.221-223ºC. IR (KBr): 2924 (C–H, aromatic), 1726 (C=O), 1571 (C=N), 1509 (C=C), 745 (C-S), 713 cm^{-1} (-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.33 (s, 3H, -CH_3), 5.16 (s, 1H, =CH-Ar), 5.82 (s, 1H, -S-CH-N-), 6.86-8.38 (m, 17H, Ar-H), 7.45 (s, 1H, =CH-N-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 24.2 (Ar-CH_3), 59.4 (-S-*C*H-N-), 113.2, 117.3 (C_4 of pyrazole ring), 118.4, 125.3 (=*C*H-Ar), 126.3, 127.1, 127.4, 128.2, 128.4, 129.2 (C_5 of thiazolidinone ring), 129.5, 130.3 (C_3 of pyrazole ring), 130.5, 130.7, 131.2, 132.5, 133.2, 133.3, 133.6, 138.3, 138.8, 140.5, 143.4, 146.3, 156.5 (C_5 of pyrazole ring), 172.2 (-C=O); MS: *m/z* 534 (M^+). Anal. Calcd for $C_{31}H_{23}ClN_4OS$: C, 69.59; H, 4.33; N, 10.47%. Found: C, 69.55; H, 4.36; N, 10.44%.

Characterization of compound 5-(3-chlorobenzylidene)-2-(1-phenyl-3-(p-tolyl)-1*H*-pyrazol-4-yl)-3-(pyridin-2-yl)thiazolidin-4-one(5d)

Yield: 80%. Solid. m.p.249-251ºC. IR (KBr): 2925 (C–H, aromatic), 1724 (C=O), 1576 (C=N), 1503 (C=C), 743 (C-S), 718 cm^{-1} (-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.35 (s, 3H, -CH_3), 5.13 (s, 1H, =CH-Ar), 5.86 (s, 1H, -S-CH-N-), 6.85-8.39 (m, 17H, Ar-H), 7.47 (s, 1H, =CH-N-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 24.6 (Ar-CH_3), 59.5 (-S-*C*H-N-), 113.3, 117 (C_4 of pyrazole ring), 118.9, 125.1 (=*C*H-Ar), 126.5, 127.3, 127.8, 128.4, 128.7, 129 (C_5 of thiazolidinone ring), 129.1, 130 (C_3 of pyrazole ring), 130.2, 130.3, 131.1, 132.8, 133, 133.2, 133.4, 138.5, 138.7, 140.9, 143.9, 146.5, 156.7 (C_5 of pyrazole ring), 172.9 (-C=O);MS: *m/z* 534 (M^+). Anal. Calcd for $C_{31}H_{23}ClN_4OS$: C, 69.59; H, 4.33; N, 10.47%. Found: C, 69.55; H, 4.36; N, 10.44%.

Characterization of compound 5-(4-fluorobenzylidene)-2-(1-phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)-3-(pyridin-2-yl)thiazolidin-4-one(5e)

Yield: 60%. Solid. m.p.245-247ºC. IR (KBr): 2925 (C–H, aromatic), 1727 (C=O), 1573 (C=N), 1508 (C=C), 1390 (C-F), 744 cm^{-1} (C-S); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.33 (s, 3H, -CH_3), 5.11 (s, 1H, =CH-Ar), 5.87 (s, 1H, -S-CH-N-), 6.85-8.34 (m, 17H, Ar-H), 7.47 (s, 1H, =CH-N-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 21.1 (Ar-CH_3), 54.6 (-S-*C*H-N-), 111.1, 114.2, 114.5 (C_4 of pyrazole ring), 116.2, 119.3, 121.1, 125.5 (=*C*H-Ar), 126.2, 127.3, 128.1, 129.2 (C_5 of thiazolidinone ring), 129.4, 129.5 (C_3 of pyrazole ring), 130.3, 132.3, 132.6, 132.8, 134.3, 138.3, 141.3, 142.3, 144.2, 144.3, 156.4, 162.2 (C_5 of pyrazole ring), 170.3 (-C=O);MS: *m/z* 518 (M^+). Anal. Calcd for $C_{31}H_{23}FN_4OS$: C, 71.80; H, 4.47; N, 10.80%. Found: C, 71.82; H, 4.45; N, 10.83%.

Characterization of compound 5-(3-hydroxybenzylidene)- 2-(1-phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)-3-(pyridin-2-yl) thiazolidin-4-one(5f)

Yield: 69%. Solid. m.p.275-277ºC. IR (KBr): 3380 (-OH), 2923 (C–H, aromatic), 1724 (C=O), 1575 (C=N), 1506 (C=C), 748 cm^{-1} (C-S); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.36 (s, 3H, -CH_3), 5.13 (s, 1H, =CH-Ar), 5.29 (s, 1H, -OH), 5.86 (s, 1H, -S-CH-N-), 6.82-8.39 (m, 17H, Ar-H), 7.44 (s, 1H, =CH-N-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 21.3 (Ar-CH_3), 54.1 (-S-*C*H-N-), 111.3, 114.4, 114.6 (C_4 of pyrazole ring), 116.1, 119.4, 121.2, 125.4 (=*C*H-Ar), 126.3, 127.1, 128.3, 129.4 (C_5 of thiazolidinone ring), 129.5, 129.7 (C_3 of pyrazole ring), 130.4, 132.2, 132.5, 132.6, 134.5, 138.2, 141.4, 142.2, 144.4, 144.6, 156.3, 162.3 (C_5 of pyrazole ring), 170.1 (-C=O);MS: *m/z* 516 (M^+). Anal. Calcd for $C_{31}H_{24}N_4O_2S$: C, 72.07; H, 4.68; N, 10.85%. Found: C, 72.03; H, 4.65; N, 10.89%.

Characterization of compound 5-(4-hydroxybenzylidene)-2-(1-phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)-3-(pyridin-2-yl)thiazolidin-4-one(5g)

Yield: 67%. Solid. m.p.261-263ºC. IR (KBr): 3384 (-OH), 2926 (C–H, aromatic), 1727 (C=O), 1573 (C=N), 1507 (C=C), 745 cm^{-1} (C-S); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.32 (s, 3H, -CH_3), 5.16 (s, 1H, =CH-Ar), 5.25 (s, 1H, -OH), 5.82 (s, 1H, -S-CH-N-), 6.85-8.36 (m, 17H, Ar-H), 7.42 (s, 1H, =CH-N-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 21.2 (Ar-CH_3), 54.3 (-S-*C*H-N-), 111.3, 114.1, 114.4 (C_4 of pyrazole ring), 116.1, 119.2, 121.2, 125.4 (=*C*H-Ar), 126.3, 127.4, 128.2, 129.1 (C_5 of thiazolidinone ring), 129.3, 129.6 (C_3 of pyrazole ring), 130.2, 132.1, 132.2, 132.5, 134.2, 138.5, 141.1, 142.5, 144.1, 144.4, 156.5, 162.4 (C_5 of pyrazole ring), 170.5 (-C=O);MS: *m/z* 516 (M^+). Anal. Calcd for $C_{31}H_{24}N_4O_2S$: C, 72.07; H, 4.68; N, 10.85%. Found: C, 72.03; H, 4.65; N, 10.89%.

Characterization of compound 5-(2-methoxybenzylidene)-2-(1-phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)-3-(pyridin-2-yl)thiazolidin-4-one(5h)

Yield: 84%. Solid. m.p.272-274ºC. IR (KBr): 2923 (C–H, aromatic), 1729 (C=O), 1571 (C=N), 1505 (C=C), 744 cm^{-1} (C-S); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.31 (s, 3H, -CH_3), 3.85 (s, 3H, -OCH_3), 5.19 (s, 1H, =CH-Ar), 5.86 (s, 1H, -S-CH-N-), 6.88-8.38 (m, 17H, Ar-H), 7.41 (s, 1H, =CH-N-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 21.1 (Ar-CH_3), 54.1 (-S-*C*H-N-), 58.4 (Ar-OCH_3), 111.2, 114.5, 114.8 (C_4 of pyrazole ring), 116.5, 119.2, 121.4, 125.1 (=*C*H-Ar), 126.4, 127.3, 128.2, 129.7 (C_5 of thiazolidinone ring), 129.8, 129.9 (C_3 of pyrazole ring), 130.2, 132.2, 132.4, 132.5, 134.53 138.1, 141.2, 142.3, 144.2, 144.6, 156.5, 162 .3(C_5 of pyrazole ring), 170.3 (-C=O);MS: *m/z* 530 (M^+). Anal. Calcd for $C_{32}H_{26}N_4O_2S$: C, 72.43; H, 4.94; N, 10.56%. Found: C, 72.41; H, 4.98; N, 10.59%.

Characterization of compound 5-(4-methoxybenzylidene)-2-(1-phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)-3-(pyridin-2-yl)thiazolidin-4-one(5i)

Yield: 88%. Solid. m.p.279-281ºC. IR (KBr): 2925 (C–H, aromatic), 1725 (C=O), 1574 (C=N), 1508 (C=C), 743 cm^{-1} (C-S); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.34 (s, 3H, -CH_3), 3.82 (s, 3H, -OCH_3), 5.14 (s, 1H, =CH-Ar), 5.89 (s, 1H, -S-CH-N-), 6.82-8.34 (m, 17H, Ar-H), 7.46 (s, 1H, =CH-N-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 21.6 (Ar-CH_3), 54 (-S-*C*H-N-), 58.8 (Ar-OCH_3), 111.3, 114.3, 114.7 (C_4 of pyrazole ring), 116.7, 119.4, 121.2, 125.9 (=*C*H-Ar), 126.5, 127.7, 128.3, 129 (C_5 of thiazolidinone ring), 129.3, 129.8 (C_3 of pyrazole ring), 130.8, 132.6, 132.8, 132.9, 134.5, 138.4, 141.1, 142.4, 144.1, 144.5, 156, 162 (C_5 of pyrazole ring), 170.2 (-C=O); MS: *m/z* 530 (M^+). Anal. Calcd for $C_{32}H_{26}N_4O_2S$: C, 72.43; H, 4.94; N, 10.56%. Found: C, 72.41; H, 4.98; N, 10.59%.

Characterization of compound 5-(3-methylbenzylidene)-2-(1-phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)-3-(pyridin-2-yl)thiazolidin-4-one(5j)

Yield: 71%. Solid. m.p.267-269ºC. IR (KBr): 2921 (C–H, aromatic), 1726 (C=O), 1574 (C=N), 1501 (C=C), 749 cm^{-1} (C-S); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.36 (s, 6H, -CH_3), 5.15 (s, 1H, =CH-Ar), 5.88 (s, 1H, -S-CH-N-), 6.86-8.38 (m, 17H, Ar-H), 7.44 (s, 1H, =CH-N-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.2 (Ar-CH_3), 55.3 (-S-*C*H-N-), 111.5, 115.3 (C_4 of pyrazole ring), 119.2, 125.3 (=*C*H-Ar), 126.3, 127.1, 127.4, 128.1, 128.4, 128.6, 129.3 (C_5 of thiazolidinone ring), 129.5, 130.4 (C_3 of pyrazole ring), 130.5, 133.2, 137.4, 139.5, 142.3, 144.4, 145.2, 147.5, 149.5, 154.2 (C_5 of pyrazole ring), 169.7 (-C=O);MS: *m/z* 514 (M^+). Anal. Calcd for $C_{32}H_{26}N_4OS$: C, 74.68; H, 5.09; N, 10.89%. Found: C, 74.65; H, 5.12; N, 10.92%.

Characterization of compound 5-(4-methylbenzylidene)-2-(1-phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)-3-(pyridin-2-yl)thiazolidin-4-one(5k)

Yield: 76%. Solid. m.p.247-249ºC. IR (KBr): 2926 (C–H, aromatic), 1724 (C=O), 1578 (C=N), 1503 (C=C), 745 cm^{-1} (C-S); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.36 (s, 6H, -CH_3), 5.14 (s, 1H, =CH-Ar), 5.82 (s, 1H, -S-CH-N-), 6.82-8.37 (m, 17H, Ar-H), 7.49 (s, 1H, =CH-N-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.6 (Ar-CH_3), 55.7 (-S-*C*H-N-), 111.6, 115 (C_4 of pyrazole ring), 119, 125.1 (=*C*H-Ar), 126.7, 127.3, 127.9, 128.2, 128.7, 128.8, 129.1 (C_5 of thiazolidinone ring), 129.2, 130 (C_3 of pyrazole ring), 130.8, 133.3, 137.7, 139.7, 142.8, 144.5, 145.5, 147.8, 149.9, 154.3 (C_5 of pyrazole ring), 169.2 (-C=O);MS: *m/z* 514 (M^+). Anal. Calcd for $C_{32}H_{26}N_4OS$: C, 74.68; H, 5.09; N, 10.89%. Found: C, 74.65; H, 5.12; N, 10.92%.

Characterization of compound 5-(2-nitrobenzylidene)-2-(1-phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)-3-(pyridin-2-yl)thiazolidin-4-one(5l)

Yield: 62%. Solid. m.p.258-260ºC. IR (KBr): 2921 (C–H, aromatic), 1726 (C=O), 1574 (C=N), 1526 (N-O), 1501 (C=C), 749 cm^{-1} (C-S); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.36 (s, 6H, -CH_3), 5.15 (s, 1H, =CH-Ar), 5.88 (s, 1H, -S-CH-N-), 6.86-8.38 (m, 17H, Ar-H), 7.44 (s, 1H, =CH-N-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 18.1 (Ar-CH_3), 60.5 (-S-*C*H-N-), 113.2, 115.5 (C_4 of pyrazole ring), 118.3, 125.5 (=*C*H-Ar), 126.2, 126.6, 127.3, 127.5, 128.3, 129.4 (C_5 of thiazolidinone ring), 130.3, 130.5 (C_3 of pyrazole ring), 130.7, 131.4, 131.6, 133.3, 133.6, 137.4, 139.3, 145.2, 147.4, 148.6, 157.5 (C_5 of pyrazole ring), 168.6 (-C=O);MS: *m/z* 545 (M^+). Anal. Calcd for $C_{31}H_{23}N_5O_3SC$, 68.24; H, 4.25; N, 12.84%. Found: C, 68.26; H, 4.22; N, 12.87%.

Characterization of compound 5-(4-nitrobenzylidene)-2-(1-phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)-3-(pyridin-2-yl)thiazolidin-4-one(5m)

Yield: 69%. Solid. m.p.264-266ºC. IR (KBr): 2925 (C–H, aromatic), 1726 (C=O), 1574 (C=N), 1530 (N-

O), 1501 (C=C), 749 cm^{-1} (C-S); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.36 (s, 6H, -CH_3), 5.15 (s, 1H, =CH-Ar), 5.88 (s, 1H, -S-CH-N-), 6.88-8.38 (m, 17H, Ar-H), 7.44 (s, 1H, =CH-N-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 18.8 (Ar-CH_3), 60.8 (-S-*C*H-N-), 113.3, 115 (C_4 of pyrazole ring), 118.1, 125.2 (=*C*H-Ar), 126.1, 126.4, 127.1, 127.9, 128.7, 129.3 (C_5 of thiazolidinone ring), 130, 130.1 (C_3 of pyrazole ring), 130.8, 131.5, 131.6, 133.1, 133.7, 137.7, 139.4, 145.8, 147.5, 148.5, 157.7 (C_5 of pyrazole ring), 168.5 (-C=O);MS: *m/z* 545 (M^+). Anal. Calcd for $C_{31}H_{23}N_5O_3SC$, 68.24; H, 4.25; N, 12.84%. Found: C, 68.26; H, 4.22; N, 12.87%.

Characterization of compound 5-(2,3-dimethoxybenzylidene)-2-(1-phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)-3-(pyridin-2-yl)thiazolidin-4-one(5n)

Yield: 80%. Solid. m.p.261-263ºC. IR (KBr): 2924 (C–H, aromatic), 1726 (C=O), 1575 (C=N), 1504 (C=C), 748 cm^{-1} (C-S); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.36 (s, 3H, -CH_3), 3.86 (s, 6H, -OCH_3), 5.14 (s, 1H, =CH-Ar), 5.87 (s, 1H, -S-CH-N-), 6.83-8.37 (m, 16H, Ar-H), 7.46 (s, 1H, =CH-N-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 21.1 (Ar-CH_3), 54.2 (-S-*C*H-N-), 58.2 (Ar-OCH_3), 111.2, 114.1, 114.3 (C_4 of pyrazole ring), 116.5, 119.2, 121.4, 125.3 (=*C*H-Ar), 126.2, 127.4, 128.1, 129.2 (C_5 of thiazolidinone ring), 129.4, 129.6 (C_3 of pyrazole ring), 130.6, 132.4, 132.5, 132.6, 134.3, 138.2, 141.2, 142.3, 144.3, 144.4, 156.8, 162.5 (C_5 of pyrazole ring), 170.4 (-C=O);MS: *m/z* 560 (M^+). Anal. Calcd for $C_{33}H_{28}N_4O_3S$: C, 70.69; H, 5.03; N, 9.99%. Found: C, 70.67; H, 5.01; N, 9.95%.

Characterization of compound 5-(2,4-dimethylbenzylidene)-2-(1-phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)-3-(pyridin-2-yl)thiazolidin-4-one(5o)

Yield: 88%. Solid. m.p.241-243ºC. IR (KBr): 2922 (C–H, aromatic), 1727 (C=O), 1574 (C=N), 1501 (C=C), 748 (C-S); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.39 (s, 9H, -CH_3), 5.16 (s, 1H, =CH-Ar), 5.87 (s, 1H, -S-CH-N-), 6.80-8.33 (m, 16H, Ar-H), 7.46 (s, 1H, =CH-N-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 18.1 (Ar-CH_3), 60.6 (-S-*C*H-N-), 113.2, 115.5 (C_4 of pyrazole ring), 118.3, 125.3 (=*C*H-Ar), 126.2, 126.5, 127.2, 127.6, 128.5, 129.4 (C_5 of thiazolidinone ring), 130.2, 130.4 (C_3 of pyrazole ring), 130.5, 131.2, 131.4, 133.2, 133.4, 137.3, 139.2, 145.4, 147.6, 148.2, 157.6 (C_5 of pyrazole ring), 168.3 (-C=O);MS: *m/z* 528 (M^+). Anal. Calcd for $C_{33}H_{28}N_4OS$: C, 74.97; H, 5.34; N, 10.60%. Found: C, 74.93; H, 5.37; N, 10.64%.

Antimicrobial assay

Antibacterial assay

The compounds (**5a-5o**) were screened for their antibacterial activity in triplicate sets against these bacteria at different concentrations of 1000, 500, 250, and 200 μg/mL. The drugs which were found to be active in primary analysis were further diluted and evaluated. 10 μg/mL suspensions were further inoculated on appropriate media and the growth was noted after one or two days. Minimum inhibitory concentration is the lowest concentration, which shows no growth of microbes after spot subculture for each drug. The test mixture should contain 10^8 cells/mL. In this study, DMSO and sterilized distilled water used as negative controls, while chloramphenicol was used as a positive control for evaluating the antibacterial activity[18].

Antifungal assay

The same newly synthesized compounds **(5a-5o)** were screened for their antifungal activity in six sets against *Candida albicans* (MTCC-227) at various primary concentrations of 1000, 500 and 250 μg/mL. The primary screen active compounds were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25, and 12.5 μg/mL concentrations for secondary screening to test in a second set of dilution against all microorganisms. 'Griseofulvin' was used as a standard drug for antifungal activity, which showed 500 μg/mL MIC against *C. albicans*. For growth of fungi, in the present procedure, we have used Sabourauds dextrose broth at 28°C in aerobic condition for 48 h. DMSO and sterilized distilled water used as negative controls while 'Griseofulvin' (1 U strength) was used as a positive control[18].

Conclusion

We have designed and synthesized 4-thiazolidinone bearing pyridine-pyrazole hybrids (**5a-5o**) in our attempt to develop effective antimicrobial agents. Pyridine-2-amine and substituted pyrazole carbaldehydes were taken as substrates. Schiff base was formed when primary amine reacted with aldehyde in the presence of acetic acid followed by cyclization of 4-thiazolidinone by thioglycolic acid. In the final step of the reaction, Michael condensation was performed, where active methylene group of 4-thiazolidinone reacted with substituted benzaldehydes to furnish desire products. The antimicrobial screening of the synthesized hybrids was tested

against bacterial and fungal species. The outcome of the antimicrobial activity revealed that electron-withdrawing bromo and nitro derivatives were most active against *E. coli* strain showing MIC value of 62.5 µg/mL while electron-donating hydroxyl group also exhibited excellent activity against *E. coli* strain with same MIC value. Results of antifungal activity showed that compounds having electron-donating groups (-H, -4-OH, -4-OCH_3) and electron-withdrawing groups (-2-Cl, -2-NO_2) found to be most active against *C. albicans* having MIC value of 250 µg/mL, which is two-fold of standard drug griseofulvin.

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