Indian Journal of Chemistry
Vol. 54B, May 2015, pp. 643-649

Synthesis of novel pyrimidine and pyrazole hybrids as potential antimicrobial agents

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Received 17 July 2014; accepted (revised) 23 February 2015

As a part of the ongoing endeavours in the exploration of novel antimicrobial agents, herein is reported a novel synthesis of hybrid molecules 5-(4-arylidene-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(4-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-ones **4** by combining pyrimidine and pyrazole scaffolds having diverse pharmacological activities. The characterization of these newly synthesized compounds has been carried out by IR, ^{1}H and ^{13}C NMR and mass spectroscopy. All the synthesized compounds have been evaluated for their *in vitro* antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and fungi (*Candida albicans, Aspergillus niger* and *Aspergillus clavatus*) using serial broth dilution method. Among all these synthesized compounds **4l** and **4d** have emerged as highly potent molecules with 12.5 µg/mL potency against *Pseudomonas aeruginosa* and *Aspergillus niger* respectively.

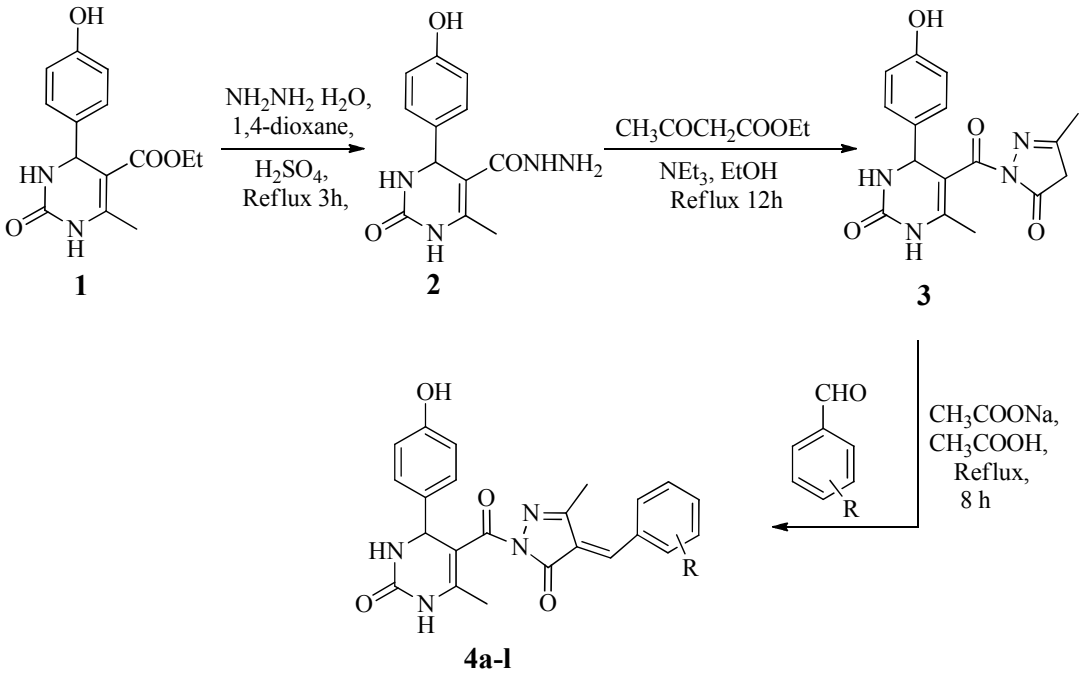
Keywords: Pyrimidine, pyrazole, hybrid molecules, antibacterial activity, antifungal activity, MIC

Pyrimidines represent one of the most active classes of compounds, possessing a wide spectrum of biological activity[1]. Pyrimidines and their fused ring derivatives have a broad spectrum of biological activity, best known as heterocyclic core of the nucleic acid bases. These ring systems are often incorporated into drugs designed for anticancer[2], antiviral[3], antihypertensive[4], analgesic and anti-inflammatory[1] activity. The 2,4,5,6-tetrasubstituted pyrimidines have been shown to be potent and selective cyclin-dependent kinase (CDK2) inhibitors and have inhibited *in-vitro* cellular proliferation in cultured human tumour cells[2]. Diaminopyrimidines halt the bacterial growth by inhibiting dihydrofolate reductase, the enzyme which is crucial in generating the active form of the co-enzyme tetrahydrofolic acid[5].

In the last 20 years pyrazole ring has attracted much attention as it has become fairly accessible and shows diverse properties. Pyrazoles and several *N*-substituted pyrazoles are known to possess numerous chemical, biological and medicinal applications because of their versatile biological activities such as antitumor[6], antileukemia[7], antidepressant[8,9], and antitubercular[10]. It is considered a typical model of the pyrazole containing diaryl-heterocyclic template that is known to selectively inhibit cyclooxygenase enzyme COX-2[11]. Celecoxib is a safe anti-inflammatory and analgesic agent. Considering these published data and on the basis of previous work[12-15] herein is reported the synthesis of novel potent antimicrobials. In the present paper, hybrid molecules containing pyrimidine and pyrazole scaffolds have been developed, with a dual mode of action with a view to kill multidrug resistant bacteria.

Results and Discussion

The synthetic strategies and methodologies to achieve intermediates and target compounds in this study are depicted in **Scheme I**. The compound ethyl 4-(4-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetra-hydropyrimidine-5-carboxylate **1** was obtained in excellent yield and purity by a single pot Biginelli condensation reaction of 4-hydroxy benzaldehyde, urea and ethyl acetoacetate in the presence of concentrated HCl. In the second step, compound **1** in 1,4-dioxane was condensed with hydrazine in the presence of concentrated H_2SO_4 by refluxing together for 3h to furnish product 4-(4-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide **2**. The key precursor 4-(4-hydroxyphenyl)-6-methyl-5-(3-methyl-5-oxo-4, 5-dihydro-1*H*-pyrazole-1-carbonyl)-3,4-dihydropyrimidin-2(1*H*)-one **3** to the target molecules was synthesized by condensing intermediate **2** with ethyl acetoacetate in the presence of a base



R = -H, -2-F, -4-F, -2-OH, -4-OH, -4-Cl, -2-NO_2, -3-NO_2, -4-NO_2, -2,6$(Cl)_2$, -2-OCH_3, -4-OCH_3

Scheme I — Synthetic pathway to newly synthesized compounds **4a-l**

triethyl amine and refluxing for 12 h in ethanol. The final step involves Knoevenegel condensation under completely anhydrous conditions, of compound **3** containing active methylene group with different aldehydes in the presence of sodium acetate to yield the desired products 5-(4-arylidene-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(4-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-ones **4**.

All the synthesized compounds were characterized by using IR, NMR and mass spectroscopy. Compound **2** showed characteristic peak in IR at 3417 cm^{-1} assignable to NH_2 group. The characteristic signals in ^1H NMR of compound **2** demonstrated singlets at δ 8.22 and 9.10 corresponding to NH_2 and NH groups respectively. The ^{13}C NMR spectra displayed a signal at δ 165.2 assignable to the carbohydrazide carbon (-$CONHNH_2$). The mass spectrum of compound **2** showed a molecular ion peak at *m/z* 262 (M^+) corresponding to the molecular formula $C_{12}H_{14}N_4O_3$. Compound **2** was cyclized by condensation with ethyl acetoacetate in the presence of a base to furnish the key intermediate **3**.

This compound represented strong absorption bands at 1756 and 3363 cm^{-1} corresponding to carbonyl and secondary amine group. In addition to this, ^1H NMR revealed the appearance of singlet peaks at δ 2.18 and 3.45 integrating three protons of the methyl group on pyrazole scaffold and two methylene protons respectively. ^{13}C NMR confirmed the proposed structure by the demonstration of signals at δ 16.4, 159.5 and 163.0 due to the carbon of methyl group, carbon attached to the methyl group and carbonyl carbon of the pyrazole scaffold respectively. Moreover, the mass spectrum of **3** showed a molecular ion peak at *m/z* 328 (M^+) corresponding to molecular formula $C_{16}H_{16}N_4O_4$. The aforementioned key intermediate **3** on Knoevenegel condensation led to our targeted molecule **4a-l** through their reaction with different aldehydes. Compound **4a** showed absorption bands in IR spectra at 3462 and 1714 cm^{-1} due to the secondary amine and carbonyl group respectively. In ^1H NMR, compound **4a** was confirmed by the appearance of the singlet along with other characteristic peaks, at δ 6.85 corresponding to the methine proton of Knovenegel adducts (thermally stable *Z*-isomer). The ^{13}C NMR further confirmed the formation of Knoevenegel adduct by the demonstration of peaks at δ 126.4 and 147.8 corresponding to the carbon attached to the methine carbon in the pyrazole scaffold and the double bonded carbon atom respectively. The mass spectrum authenticated the molecular formula $C_{23}H_{20}N_4O_4$ by showing a molecular ion peak at *m/z* 462 (M^+). Formation of products **4a-l** was explained based on

the literature precedence and the isomeric ratio of products was presumed to be mainly *Z* in all cases. It was reported that the thermodynamically stable *Z*-isomer pre-dominated with a ratio of *Z:E* isomers 10:1 after recrystallization for all arylidene **4a-l**. The ratio of the two geometrical stereo isomers was readily quantified by ^{1}H NMR as reported in the literature[15].

The outcome of antimicrobial studies of the newly synthesized compounds revealed that these compounds have significant antibacterial and antifungal activities. From antimicrobial activity data **(Table I)**, key precursor, pyrazole **3** showed poor antibacterial activity at MIC > 1,000. The pyrazole **3** afforded target compounds **4** by means of Knoevenegel condensation with aromatic aldehydes. Collectively, compounds **4** could be considered as significant potent, active, broad-spectrum antimicrobials. Preliminary microbiological screening results showed that compounds **4f** (-4-Cl-C_6H_4), **4h** (-3-NO_2-C_6H_4), **4i** (-4-NO_2-C_6H_4) possessed good activity against *E. coli*. On installation of -NO_2 at second position in phenyl group in **4g,** improved activity against *E. coli* was observed. Compound **4i** (-4-NO_2-C_6H_4) demonstrated good activity against *P. aeruginosa*, while compounds **4d** (-2-OH-C_6H_4), **4f (**-4-Cl-C_6H_4**)** and **4g** (2-NO_2-C_6H_4) showed very good activity against *P. aeruginosa*. When substituent-4-OCH_3-C_6H_4 was inserted in **4l,** increment in activity was enormous and it exhibited excellent activity against *P. aeruginosa*. When -4-OH-C_6H_4 was installed in **4e,** it possessed good activity against *S. aureus*. Compounds **4a** (-C_6H_5), **4b** (-2-F-C_6H_4), **4c** (-4-F-C_6H_4), **4e** (-4-OH-C_6H_4) and **4i** (-4-NO_2-C_6H_4) demonstrated good activity against *S. pyogenes.*

In case of antifungal activity, compound **4l** (-4-OCH_3-C_6H_4) exhibited good activity against *C. albicans.* When -2-OH-C_6H_4 was installed in **4d,** increment in activity was enormous and it demonstrated an excellent activity against *A. niger*. The antibacterial and antifungal activities have been compared based on standard drugs *Ciprofloxacin* and *Griseofulvin* respectively.

Structure activity relationship

Results of antimicrobial activity of final compounds and the precursor clearly suggested that the Knoevenegel adduct of pyrazole is decisive for a broad spectrum of antimicrobial activity. On considering of the relation-ships between the structure of the heterocyclic scaffolds **4a-l** and the antimicrobial property, the uniqueness of individual substitutions proved to be a vital parameter for influencing the activity of the synthesized compounds. For antimicrobial activity, it was observed that the introduction of electron-releasing groups on the phenyl ring resulted in a considerable increase in antibacterial potency of compounds. It was observed from the screening data that the compounds **4l** and **4d** containing methoxy and hydroxyl substituents respectively showed tremendous potency against bacteria *P. aeruginosa* and fungal strain *A. niger* at MIC 12.5 µg/mL. SAR studies discovered that the installation of an electron-releasing group on phenyl ring amplified the antimicrobial activity in comparison to the installation of electron-withdrawing atoms or groups.

Table I — Antimicrobial activity of synthesized compounds

Compd	-R	Minimum inhibitory concentration (MIC) for bacteria (µg/mL)				Minimum inhibitory concentration (MIC) for fungi (µg/mL)		
		E.c.	*P.a.*	*S.a.*	*S.p.*	*C.a.*	*A.n.*	*A.c.*
3	-	500	200	>1000	200	>1000	500	500
4a	-H	200	200	200	50	1000	500	1000
4b	-2-F	200	500	200	50	500	1000	1000
4c	-4-F	200	200	200	50	500	500	500
4d	-2-OH	200	25	200	100	100	12.5	100
4e	-4-OH	100	200	50	50	1000	1000	1000
4f	-4-Cl	50	25	200	200	500	500	500
4g	-2-NO_2	25	25	500	500	250	1000	400
4h	-3-NO_2	50	100	250	100	500	1000	1000
4i	-4-NO_2	50	50	200	50	500	500	500
4j	-2,6-$(Cl)_2$	200	100	100	200	1000	500	500
4k	-2-OCH_3	200	200	500	500	1000	1000	1000
4l	-4-OCH_3	100	12.5	200	100	50	100	100
Ciprofloxacin		25	25	50	50	-	-	-
Griseofulvin		-	-	-	-	500	100	100

*E.c.: Escherichia coli (*MTCC-443*), P.a.: Pseudomonas aeruginosa (*MTCC-1668*), S.a.: Staphylococcus aureus (*MTCC-96*), S.p.: Streptococcus pyogenes (*MTCC-442*); C.a.: Candida albicans (*MTCC-227*), A.n.: Aspergillus niger (*MTCC-282*), A.c.: Aspergillus clavatus (*MTCC-1323*)*

Biological evaluation

Antibacterial assay

The newly synthesized compounds were screened for their antibacterial activity against Gram positive bacteria (*Staphylococcus aureus* (MTCC-96), *Streptococcus pyogenes* (MTCC-442)) and Gram negative (*Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa* (MTCC-1688)). Antibacterial activity was measured as per National Committee for Clinical Laboratory Standards (NCCLS) protocol by Mueller–Hinton Broth (Becton–Dickinson, USA)[16-18]. Standard strains were procured from CSIR-IMTECH, Chandigarh. Compounds were primarily screened for their antibacterial activity in six sets against *E. coli*, *S. aureus*, *P. aeruginosa* and *S. pyogenes* at different concentrations of 1000, 500 and 250 µg/mL as shown in **Table I**. The drugs found to be active in primary screening 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. The antibacterial activity is carried out as per the protocol[19]. The standard drug used in the present study was Ciprofloxacin for evaluating antibacterial activity which showed 25, 25, 50 and 50 µg/mL MIC against *E. coli, P. aeruginosa, S. aureus* and *S. pyogenes* respectively.

Antifungal assay

The same compounds were tested for antifungal activity as primary screening in six sets against *Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus* at various concentrations of 1000, 500, 200 and 100 µg/mL as shown in **Table I**. Results were recorded in the form of primary and secondary screening. The synthesized compounds were diluted to 1000 µg/mL concentration, as a stock solution. Those synthesized compounds which were found to be active in this primary screening were further tested in a second set of dilution against all microorganisms. *Griseofulvin* was used as a standard drug for antifungal activity, which showed 500, 100 and 100 µg /mL MIC against *C. albicans*, *A. Niger* and *A. Clavatus* respectively. 2% DMSO and sterilized distilled water were used as negative control, while *Griseofulvin* (1 U strength) was used as positive control. Results of antimicrobial evaluation of derivatives **4a-l** are shown in **Table I**. For fungal growth, in the present protocol, Sabourauds dextrose broth at 28°C was used under aerobic condition for 48 h.

Experimental Section

All reactions except those in aqueous media were carried out by standard techniques with the exclusion of moisture. Melting points were determined on an electro thermal melting point apparatus and are reported uncorrected. TLC on silica gel plates (Merck, 60, F_{254}) was used for checking homogeneity and reaction monitoring. Column chromatography over silica gel (Merck, 70-230 and 230-400 mesh ASTH for flash chromatography) was applied when necessary to isolate and purify the reaction products. Elemental analysis (% C, H, and N) was carried out using a Perkin-Elmer 2400 CHN analyser. IR spectra of all compounds have been recorded on a Perkin-Elmer FT-IR spectrophotometer in KBr. ^{1}H NMR spectra were recorded on Varian Gemini 300 MHz and ^{13}C NMR spectra on Varian Mercury-400, 100 MHz with DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on a Shimadzu LCMS 2010 spectrometer. Anhydrous reactions were carried out in oven dried glassware under nitrogen atmosphere.

Preparation of ethyl 4-(4-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate, 1

Compound **1** was prepared according to literature[20] method.

Preparation of 4-(4-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide, 2

Compound **1** ethyl 4-(4-hydroxyphenyl)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (0.01 mol) was dissolved in 1,4-dioxane (20 mL) and to this, hydrazine hydrate (99%) (0.01 mol) was added followed by the addition of a catalytic amount of conc. H_2SO_4 and the reaction mass allowed to stir for 3 h at 100°C. After completion of reaction, crude mass was allowed to cool to RT and poured over crushed ice. The crude product obtained as yellowish precipitate, was filtered and dried. Purification was carried out by recrystallization from ethanol (95%). Yield 69%; m.p. 195-98°C; IR (KBr): 3645 (OH), 3452, 3342 (NH), 3071 (C-H, aromatic), 1513 (C=C), 1685 cm^{-1} (C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.35 (s, 3H,-CH_3), 5.42 (s, 1H,-OH), 5.60 (s, 1H,-CH), 6.94 (d, 2H, Ar-H), 7.23 (d, 2H, Ar-H), 8.22 (s, 2H, NH_2, D_2O exch.), 9.10 (s, 1H,-N*H*NH_2), 10.05 (s, 1H,-N*H*CPh), 10.12 (s, 1H,-N*H*CCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.2, 156.5, 150.2, 146.1, 135.9, 126.1 (2), 115.7 (2), 108.6, 54.1, 18.1; LCMS: *m/z* 262 (M^+). Anal.

Calcd for $C_{12}H_{14}N_4O_3$: C, 54.96; H, 5.38; N, 21.36. Found: C, 54.84; H, 5.55; N, 21.12%.

Preparation of 4-(4-hydroxyphenyl)-6-methyl-5-(3-methyl-5-oxo-4, 5-dihydro-1*H*-pyrazole-1-carbonyl)-3, 4-dihydropyrimidin-2(1*H*)-one, 3

To a mixture of 4-(4-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide **2** (0.01 mol) and ethyl acetoacetate (0.01 mol) in absolute ethanol (99%, 20 mL), catalytic amount of triethylamine (1 mL) was added. The reaction mixture was refluxed for 12 h at 78°C using reflux condenser equipped with magnetic stirrer. After completion of reaction, the resultant heavy reddish syrup was allowed to cool to RT. It was washed thoroughly with ether to remove impurities. The crude solid thus separated out was filtered off under vacuum and purified by recrystallization from ethanol (95%) to give product **3**. Yield 65%; m.p. 202 °C; IR (KBr): 3578 (OH), 3432, 3363 (NH), 2973 (C-H, aromatic), 1520 (C=C), 1690 cm^{-1} (C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.18 (s, 3H, pyrazole-CH_3), 2.34 (s, 3H, pyrimidine –CH_3), 3.45 (s, 2H, pyrazole-CH_2), 5.39 (s, 1H,-OH), 5.62 (s, 1H, -CH), 6.85 (d, 2H, Ar-H), 7.18 (d, 2H, Ar-H), 10.04 (s, 1H,-N*H*CCH_3), 10.12 (s, 1H,-N*H*CPh); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.6, 163.0, 159.5, 156.5, 150.2, 146.1, 135.9, 126.1 (2), 115.7 (2), 108.6, 54.1, 42.4, 18.1, 16.4; LCMS: *m/z* 328 (M^+). Anal. Calcd for $C_{16}H_{16}N_4O_4$: C, 58.53; H, 4.91; N, 17.06. Found: C, 58.42; H, 4.25; N, 17.52%.

General procedure for the preparation of 5-(4-arylidene-3-methyl-5-oxo-4, 5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(4-hydroxyphenyl)-6-methyl-3, 4-dihydropyrimidin-2(1*H*)-one, 4

Compound **3** 4-(4-hydroxyphenyl)-6-methyl-5-(3-methyl-5-oxo-4, 5-dihydro-1*H*-pyrazole-1-carbonyl)-3,4-dihydropyrimidin-2(*1H*)-one (0.01 mol) and different aldehydes (0.01 mol) suspended in dry toluene (20 mL) were taken in a flask equipped with a Dean-Stark apparatus fitted with calcium chloride guard tube. Catalytic amount of piperidine (0.5 mL) was added and the mixture was refluxed with stirring for 8 h. On cooling, the crude product was precipitated, filtered under vacuum and washed with cold methanol to give compound **4**. Product was purified by recrystallization from ethanol/chloroform (1:1).

5-(4-Benzylidene-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(4-hydroxy-phenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one, 4a: Yield 63%; m.p.176-79°C; IR (KBr): 3567 (OH), 3462, 3536 (NH), 3045 (C-H, aromatic), 1578 (C=C), 1714 cm^{-1} (C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.10 (s, 3H, pyrazole-CH_3), 2.43 (s, 3H, pyrimidine-CH_3), 5.32 (s, 1H,-OH), 5.49 (s, 1H, –CH), 6.85 (s, 1H, =CH), 6.94-7.56 (m, 9H, Ar-H), 10.07 (s, 1H,-N*H*CPh), 10.25 (s, 1H,-N*H*CCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 165.6, 156.5, 150.2, 147.8, 146.1, 143.4, 135.9, 132.9, 128.9 (2), 128.4 (2), 127.9, 126.4, 126.1 (2), 115.7 (2), 108.6, 54.1, 18.1, 14.7; LCMS: *m/z* 416 (M^+). Anal. Calcd for $C_{23}H_{20}N_4O_4$: C, 66.34; H, 4.84; N, 13.45. Found: C, 66.07; H, 4.93; N, 13.65%.

5-(4-(2-Fluorobenzylidene)-3-methyl-5-oxo-4, 5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(4-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one, 4b: Yield 61%; m.p. 275-77°C; IR (KBr): 3667 (OH), 3378, 3456 (NH), 3075 (C-H, aromatic), 1567 (C=C), 1693 (C=O), 1102 cm^{-1} (C-F); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.16 (s, 3H, pyrazole-CH_3), 2.37 (s, 3H, pyrimidine-CH_3), 5.22 (s, 1H,-OH), 5.45 (s, 1H,-CH), 6.76 (s, 1H, =CH), 6.87-7.45 (m, 8H, Ar-H), 9.97 (s, 1H, -N*H*CPh), 10.03 (s, 1H,-N*H*CCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 165.6, 161.3, 156.5, 150.2, 147.8, 146.1, 143.4, 135.9, 129.5, 128.0, 126.6, 126.0 (2), 124.2, 123.1, 115.7 (2), 115.2, 108.6, 54.1, 18.1, 14.7; LCMS: *m/z* 434 (M^+). Anal. Calcd for $C_{23}H_{19}FN_4O_4$: C, 63.59; H, 4.41; N, 12.90. Found: C, 63.14; H, 4.50; N, 12.78%.

5-(4-(4-Fluorobenzylidene)-3-methyl-5-oxo-4, 5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(4-hydroxy-phenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one, 4c: Yield 67%; m.p. 247-50°C; IR (KBr): 3520 (OH), 3456, 3534 (NH), 2978 (C-H, aromatic), 1547 (C=C), 1722 (C=O), 1145 cm^{-1} (C-F); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.20 (s, 3H, pyrazole-CH_3), 2.45 (s, 3H, pyrimidine-CH_3), 5.34 (s, 1H,-OH), 5.56 (s, 1H,-CH), 6.87 (s, 1H, =CH), 7.12-7.55 (m, 8H, Ar-H), 10.08 (s, 1H,-N*H*CCH_3), 10.17 (s, 1H,-*NHCPh*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 165.6, 162.1, 156.5, 150.2, 147.8, 146.1, 143.4, 135.9, 129.5, 128.0, 126.6, 126.0 (2), 124.2, 123.1, 115.7 (2), 115.2, 108.6, 54.1, 18.1, 14.7; LCMS: *m/z* 434 (M^+). Anal. Calcd for $C_{23}H_{19}FN_4O_4$: C, 63.59; H, 4.41; N, 12.90. Found: C, 63.17; H, 4.27; N, 12.68%.

5-(4-(2-Hydroxybenzylidene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(4-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one, 4d: Yield 76%; m.p. 228-30°C; IR (KBr): 3533 (OH), 3389, 3454 (NH), 3057 (C-H, aromatic), 1576 (C=C), 1714 cm^{-1} (C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.98 (s, 3H, pyrazole-CH_3), 2.33 (s, 3H, pyrimidine -CH_3), 5.40 (s, 1H,-OH), 5.45 (s, 1H,-OH), 5.63 (s, 1H,

-CH), 6.95 (s, 1H, =CH), 6.75-7.29 (m, 8H, Ar-H), 9.98 (s, 1H,-N*H*CCH$_3$), 10.18 (s, 1H,-N*H*CPh); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 165.6, 157.1, 156.5, 150.2, 147.8, 146.1, 143.4, 135.9, 129.5, 128.0, 126.6, 126.0 (2), 124.2, 123.1, 115.7 (2), 115.2, 108.6, 54.1, 18.1, 14.7; LCMS: *m/z* 432 (M^+). Anal. Calcd for $C_{23}H_{20}N_4O_5$: C, 63.88; H, 4.66; N, 12.96. Found: C, 63.62; H, 4.84; N, 12.16%.

5-(4-(4-Hydroxybenzylidene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(4-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one, 4e: Yield 56%; m.p. 258-60°C; IR (KBr): 3639 (OH), 3345, 3456 (NH), 2982 (C-H, aromatic), 1563 (C=C), 1692 cm^{-1} (C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.08 (s, 3H, pyrazole-CH$_3$), 2.25 (s, 3H, pyrimidine -CH$_3$), 5.35 (s, 1H,-OH), 5.49 (s, 1H,-OH), 5.76 (s, 1H, -CH), 6.87-7.39 (m, 8H, Ar-H), 7.06 (s, 1H, =CH), 10.09 (s, 1H,-N*H*CPh), 10.14 (s, 1H,-N*H*CCH$_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 165.6, 157.7, 156.5, 150.2, 147.8, 146.1, 143.4, 135.9, 129.5, 128.0, 126.6, 126.0 (2), 124.2, 123.1, 115.7 (2), 115.2, 108.6, 54.1, 18.1, 14.7; LCMS: *m/z* 432 (M^+). Anal. Calcd for $C_{23}H_{20}N_4O_5$: C, 63.88; H, 4.66; N, 12.96. Found: C, 63.78; H, 4.74; N, 12.64%.

5-(4-(4-Chlorobenzylidene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(4-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one, 4f: Yield 71%; m.p. 254-56°C; IR (KBr): 3540 (OH), 3351, 3469 (NH), 2934 (C-H, aromatic), 1536 (C=C), 1699 (C=O), 756 cm^{-1} (C-Cl); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.13 (s, 3H, pyrazole-CH$_3$), 2.38 (s, 3H, pyrimidine-CH$_3$), 5.55 (s, 1H,-OH), 5.65 (s, 1H,-CH), 6.78 (s, 1H, =CH), 6.94-7.56 (m, 8H, Ar-H), 9.94 (s, 1H,-N*H*CCH$_3$), 10.14 (s, 1H,-N*H*CPh); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 165.6, 156.5, 150.2, 147.8, 146.1, 143.4, 135.9, 134.9 (2), 133.5, 131.0, 128.7 (2), 126.5, 126.1 (2), 115.7 (2), 108.6, 54.1, 18.1, 14.7; LCMS: *m/z* 450 (M^+). Anal. Calcd for $C_{23}H_{19}ClN_4O_4$: C, 61.27; H, 4.25; N, 12.43. Found: C, 61.62; H, 4.55; N, 12.34%.

4-(4-Hydroxyphenyl)-6-methyl-5-(3-methyl-4-(2-nitrobenzylidene)-5-oxo-4, 5-dihydro-1*H*-pyrazole-1-carbonyl)-3,4-dihydropyrimidin-2(1*H*)-one, 4g: Yield 69%; m.p. 220-22°C; IR (KBr): 3667 (OH), 3345, 3472 (NH), 3081 (C-H, aromatic), 1536 (C=C), 1556 (NO$_2$), 1723 cm^{-1} (C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.19 (s, 3H, pyrazole-CH$_3$), 2.42 (s, 3H, pyrimidine-CH$_3$), 5.39 (s, 1H,-OH), 5.59 (s, 1H,-CH), 7.04 (s, 1H, =CH), 7.13-8.28 (m, 8H, Ar-H), 10.08 (s, 1H,-*NHCPh*), 10.15 (s, 1H,-N*H*CCH$_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.7, 165.6, 156.5, 150.2, 147.9, 147.6, 146.1, 143.4, 135.9, 134.7, 130.4, 130.0, 128.8, 126.4, 126.0 (2), 123.8, 115.7 (2), 108.6, 54.1, 18.1, 14.7; LCMS: *m/z* 461 (M^+). Anal. Calcd for $C_{23}H_{19}N_5O_6$: C, 59.87; H, 4.15; N, 15.18. Found: C, 59.49; H, 4.45; N, 15.36%.

4-(4-Hydroxyphenyl)-6-methyl-5-(3-methyl-4-(3-nitrobenzylidene)-5-oxo-4, 5-dihydro-1*H*-pyrazole-1-carbonyl)-3,4-dihydropyrimidin-2(1*H*)-one, 4h: Yield 72%; m.p. 282-84°C; IR (KBr): 3678 (OH), 3371, 3450 (NH), 2954 (C-H, aromatic), 1568 (C=C), 1567 (NO$_2$), 1725 cm^{-1} (C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.24 (s, 3H, pyrazole-CH$_3$), 2.37 (s, 3H, pyrimidine-CH$_3$), 5.47 (s, 1H,-OH), 5.64 (s, 1H,-CH), 7.15 (s, 1H, =CH), 7.25-8.16 (m, 8H, Ar-H), 9.97 (s, 1H,-N*H*CCH$_3$), 10.02 (s, 1H,-N*H*CPh); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 165.6, 156.5, 150.2, 147.8 (2), 146.1, 143.4, 135.9, 134.3, 129.5, 126.5, 126.1, 125.3, 123.6, 123.1, 115.7 (2), 108.6, 54.1, 18.1, 14.7; LCMS: *m/z* 461 (M^+). Anal. Calcd for $C_{23}H_{19}N_5O_6$: C, 59.87; H, 4.15; N, 15.18. Found: C, 59.56; H, 4.67; N, 15.32%.

4-(4-Hydroxyphenyl)-6-methyl-5-(3-methyl-4-(4-nitrobenzylidene)-5-oxo-4, 5-dihydro-1*H*-pyrazole-1-carbonyl)-3,4-dihydropyrimidin-2(1*H*)-one, 4i: Yield 67%; m.p. 240-44°C; IR (KBr): 3619 (OH), 3341, 3425 (NH), 3031 (C-H, aromatic), 1559 (C=C), 1529 (NO$_2$), 1718 cm^{-1} (C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.12 (s, 3H, pyrazole-CH$_3$), 2.56 (s, 3H, pyrimidine -CH$_3$), 5.23 (s, 1H,-OH), 5.70 (s, 1H, –CH), 6.84 (s, 1H, =CH), 7.34-8.39 (m, 8H, Ar-H), 9.93 (s, 1H,-N*H*CPh), 9.98 (s, 1H,-N*H*CCH$_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 165.6, 156.5, 150.2, 147.8, 147.1, 146.1, 143.4, 139.0, 135.9, 132.2 (2), 126.2, 126.0 (2), 123.8 (2), 115.7 (2), 108.6, 54.1, 18.1, 14.7; LCMS: *m/z* 461 (M^+). Anal. Calcd for $C_{23}H_{19}N_5O_6$: C, 59.87; H, 4.15; N, 15.18. Found: C, 59.34; H, 4.26; N, 15.47%.

5-(4-(2,6-Dichlorobenzylidene)-3-methyl-5-oxo-4, 5-dihydro-1*H*-pyrazole-1-carbonyl)-4-(4-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one, 4j: Yield 77%; m.p. 219-22°C; IR (KBr): 3672 (OH), 3367, 3418 (NH), 3063 (C-H, aromatic), 1578 (C=C), 1707 (C=O), 768 cm^{-1} (C-Cl); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.33 (s, 3H, pyrazole-CH$_3$), 2.63 (s, 3H, pyrimidine-CH$_3$), 5.58 (s, 1H,-OH), 5.83 (s, 1H,-CH), 7.24 (s, 1H, =CH), 7.29-7.87 (m, 7H, Ar-H), 10.05 (s, 1H,-N*H*CPh), 10.11 (s, 1H,-N*H*CCH$_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 165.6, 156.5, 150.2, 147.8, 146.1, 143.4, 135.9, 135.7, 132.5 (2), 128.0 (2), 126.6, 126.2 (2), 125.9, 115.7 (2), 108.6, 54.1, 18.1,

14.7; LCMS: *m/z* 485 (M^+). Anal. Calcd for $C_{23}H_{19}N_5O_6$: C, 56.92; H, 3.74; N, 11.54. Found: C, 56.43; H, 3.37; N, 11.67%.

4-(4-Hydroxyphenyl)-5-(4-(2-methoxybenzylidene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one, 4k: Yield 58%; m.p. 235-39°C; IR (KBr): 3656 (OH), 3372, 3423 (NH), 3043 (C-H, aromatic), 1542 (C=C), 1709 cm^{-1} (C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.15 (s, 3H, pyrazole-CH_3), 2.45 (s, 3H, pyrimidine -CH_3), 3.13 (s, 3H,-OCH_3), 5.47 (s, 1H,-OH), 5.64 (s, 1H, -CH), 6.93 (s, 1H, =CH), 6.99-7.45 (m, 8H, Ar-H), 9.97 (s, 1H,-N*H*CPh), 10.04 (s, 1H,-N*H*CCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 165.6, 159.2, 156.5, 150.2, 147.8, 146.1, 143.4, 135.9, 134.0, 128.9, 126.5, 126.1 (2), 122.1, 120.9, 115.7 (2), 114.2, 108.6, 56.2, 54.1, 18.1, 14.7; LCMS: *m/z* 446 (M^+). Anal. Calcd for $C_{24}H_{22}N_4O_5$: C, 64.57; H, 4.97; N, 12.55. Found: C, 64.27; H, 4.89; N, 12.45%.

4-(4-Hydroxyphenyl)-5-(4-(4-methoxybenzylidene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbonyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one, 4l: Yield 64%; m.p. 178-80°C; IR (KBr): 3648 (OH), 3352, 3482 (NH), 3078 (C-H, aromatic), 1567 (C=C), 1724 cm^{-1} (C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.11 (s, 3H, pyrazole-CH_3), 2.39 (s, 3H, pyrimidine -CH_3), 2.86 (s, 3H,-OCH_3), 5.38 (s, 1H,-OH), 5.52 (s, 1H, -CH), 6.78 (s, 1H, =CH), 7.06-7.38 (m, 8H, Ar-H), 10.08 (s, 1H,-N*H*CPh), 10.12 (s, 1H,-N*H*CCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 165.6, 159.8, 156.5, 150.2, 147.8, 146.1, 143.4, 135.9, 130.2 (2), 126.4, 126.1 (2), 125.2, 115.7 (2), 114.2 (2), 108.6, 55.8, 54.1, 18.1, 14.7; LCMS: *m/z* 446 (M^+). Anal. Calcd for $C_{24}H_{22}N_4O_5$: C, 64.57; H, 4.97; N, 12.55. Found: C, 64.85; H, 4.54; N, 12.77%.

Conclusion

As stated earlier, the goal of the current study was to synthesize and explore antimicrobial activity of some new structural hybrids of pyrimidine and pyrazole frameworks with anticipation of generating new leads having potent activity against various Gram-positive, Gram-negative bacterial and fungal strains. The presence of electron releasing groups like hydroxyl and methoxy substituents in the Knoevenegel adduct increased the activity of compounds compared to those with other substituents. The electronic diversity also played a crucial role in activity. In regards to the relationships between the structure of the heterocyclic scaffold and the detected antimicrobial properties, the compounds showed diverse pharmacological activity. Thus, for a compound, optimum electron density is inevitable so as to gain significant antimicrobial activity. Thus, in future, this new class of pyrimidine based pyrazolinones may be used as templates for generating better lead molecules to fight against bacterial and fungal infections.

Acknowledgements

Authors are thankful to the University Grants Commission, New Delhi and Department of Science and Technology, New Delhi for financial support under the NON-SAP and DST-FIST programs respectively.

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