Research Article

Synthesis and Antimicrobial Studies of Pyrimidine Pyrazole Heterocycles

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Received 30 April 2014; Revised 19 July 2014; Accepted 29 July 2014; Published 25 August 2014

Academic Editor: Adriana I. Segall

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Prompted from the diversity of the wider use and being an integral part of genetic material, an effort was made to synthesize pyrimidine pyrazole derivatives of pharmaceutical interest by oxidative cyclization of chalcones with satisfactory yield and purity. A novel series of 1,3-dimethyl-6-hydroxy-2,4-dioxo-5-(1'-phenyl-3'-aryl-1*H*-pyrazol-5'-yl)-1,2,3,4-tetrahydropyrimidines (**5a-d**) and 1,3-diaryl-6-hydroxy-4-oxo-2-thioxo-5-(1'-phenyl-3'-aryl-1*H*-pyrazol-5'-yl)-1,2,3,4-tetrahydropyrimidines (**5e-l**) has been synthesized. The structures of these compounds were established on the basis of FT-IR, ¹H NMR, ¹³C NMR, and mass spectral analysis. All the synthesized compounds were screened for their antimicrobial activity against bacteria and fungi. Among all the compounds, **5g** was found to be the most active as its MIC was 31.25 µg/mL against *S. aureus* and *B. cereus*. The compounds **5h**, **5c**, and **5e** also possess antibacterial activity with MIC values as 62.50, 125.00, and 500.00 µg/mL, respectively. The compounds **5c** and **5j** were found to have antifungal activity against *Aspergillus* spp. As antifungal drugs lag behind the antibacterial drugs, therefore we tried *in vitro* combination of these two compounds with standard antifungal drugs (polyene and azole) against *Aspergillus* spp. The combination of ketoconazole with **5c** and **5j** showed synergy at 1 : 8 (6.25 : 50.00 µg/mL) and 1 : 4 (25 : 100 µg/mL) against *A. fumigatus* (ITCC 4517) and *A. fumigatus* (VPCI 190/96), respectively.

1. Introduction

Nitrogen heterocycles are of special interest as they constitute an important class of natural and nonnatural products, many of which exhibit useful biological activities. Pyrimidine, being an integral part of DNA and RNA, imparts diverse pharmacological properties, such as bactericide, fungicide, vermicide, insecticide, and anticancer and antiviral agents [1]. Certain pyrimidine derivatives are also known to display antimalarial, antifilarial, and antileishmanial activities [2].

The pyrazole derivatives are well known to have antimicrobial [3], antifungal [4], antitubercular [5], anticancer [6], analgesic [7], anti-inflammatory [8], antipyretic [9], anticonvulsant [10], antidepressant [11], muscle relaxing [12], antiulcer [13], antiarrhythmic [14], and antidiabetic [15] activities. With growing application of their synthesis and bioactivity, chemists and biologists in recent years have

directed considerable attention to the study of pyrazole derivatives. In view of the above mentioned importance of pyrimidines and pyrazoles, we tried to accommodate these moieties in a single molecular framework to synthesize the linked heterocycles for enhancing biological activity.

2. Results and Discussion

2.1. Chemistry. (*E*)-1-(1',3'-Dimethyl-6'-hydroxy-2',4'-dioxo-1',2',3',4'-tetrahydropyrimidin-5'-yl)-3-aryl-prop-2-ene-1-ones (**4a-d**) and (*E*)-1-(1',3'-diaryl-6'-hydroxy-4'-oxo-2'-thioxo-1',2',3',4'-tetrahydropyrimidin-5'-yl)-3-aryl-prop-2-ene-1-ones (**4e-l**) were synthesized by the Claisen condensation of 5-acetyl barbituric/thiobarbituric acid (**2a-c**) with aromatic aldehydes **3a-d** in methanol in the presence of NaOH as a base at 60°C [16]. Further, cyclocondensation of propenones **4a-l** with phenylhydrazine in acidic condition

in dioxane as solvent yielded 1,3-dimethyl-6-hydroxy-2,4-dioxo-5-(1'-phenyl-3'-aryl-1*H*-pyrazol-5'-yl)-1,2,3,4-tetrahydropyrimidines (**5a-d**) and 1,3-diaryl-6-hydroxy-4-oxo-2-thioxo-5-(1'-phenyl-3'-aryl-1*H*-pyrazol-5'-yl)-1,2,3,4-tetrahydropyrimidines (**5e-l**) in 46–81% yields [17]. Structure and yield of compounds, that is, **4a-l** and **5a-l**, are listed in Table 1. The 5-acetyl-1,3-diarylthiobarbituric acids (**2b-c**) in turn were synthesized by the following known method from 1,3-diarylthiobarbituric acids (**1b-c**) and acetic anhydride [18, 19] (Scheme 1).

All the compounds synthesized were characterized by IR, ¹H NMR, ¹³C NMR, and mass spectroscopy. Spectroscopic data was in complete agreement with the structures assigned for these compounds. IR spectrum of cyclized derivatives of barbituric acid (**5a-d**) showed band in the region of 1700–1740 cm⁻¹ for carbonyl group (at C2). The other carbonyl group at C4 showed band in the region of 1640–1699 cm⁻¹, whereas cyclized derivatives of thiobarbituric acid (**5e-l**) showed band in the region of 1050–1100 cm⁻¹, which indicates the presence of thiocarbonyl group (at C2) and other carbonyl groups at C4 showed band in the region of 1625–1680 cm⁻¹. Frequency band of OH group appears at 3200–3450 cm⁻¹ in the compounds **5a-l**. In ¹H NMR spectra, chemical shift values of all the compounds were in accordance with the expected values. Aromatic protons of compounds **4a-l** resonated in the region of δ 6.86–7.82. Two doublets of α -H (attached to C2) at δ 8.06 ($J = 16.11$ Hz) and β -H (attached to C3) at δ 8.41 ($J = 16.11$ Hz), respectively, of **4f** demonstrate the formation of α , β -unsaturated carbonyl moiety and $J = 16.11$ Hz indicates that the ethylene moiety in the enone linkage is in *trans* confirmation in the chalcone. Disappearance of these doublets in **5f** indicates the absence of chalcone moiety. All other phenyl protons in the compounds **5a-l** appeared in the aromatic region at δ 6.82–7.58. In ¹³C NMR of **4f**, all the characteristic peaks were in good agreement with the proposed structure. Carbonyl carbon at C-1 and C-4' appeared at δ 184.6 and 168.2, respectively. The characteristic peak of C=S appeared at δ 178.9. The C-2 and C-3 carbons appeared at δ 114.5 and 139.7, respectively. The OCH₃ carbon appeared at δ 55.4. The aromatic carbons attached to OCH₃ (i.e., C-4'') appeared at δ 162.9. The other aromatic carbons of **4f** resonated in the region of δ 127.3–131.7 and the aromatic carbon attached to nitrogen appeared at 148.6. In ¹³C NMR of **5f**, disappearance of peak at δ 184.6 indicates the cyclization of chalcone. Carbonyl carbon at C-4 appeared at δ 164.0. The characteristic peak of C=S appeared at δ 180.0. C-6 carbon appeared at δ 160.8. The pyrazole carbon at C-4' appeared at δ 88.3 [20]. The OCH₃ carbon appeared at δ 55.3. The aromatic carbon attached to OCH₃, that is, C-4'', appeared at δ 161.4. The other aromatic carbons resonated in the region of δ 127.4–129.5 and the aromatic carbon attached to nitrogen appeared at δ 147.13. Details of ¹H NMR and ¹³C NMR spectra of **5a-l** are given in experimental section.

2.2. Biology

2.2.1. Antifungal Activity. The antifungal activity against *Aspergillus* spp. was evaluated by different methods [21–23],

that is, disc diffusion assay (DDA), microbroth dilution assay (MDA), and percent spore germination inhibition (PSGI). The Minimum Inhibitory Concentration (MIC) values of Amphotericin B (Amp B) and Nystatin (NYS) against all the three *Aspergillus* species were found to be 0.75 μ g/disc and 1.00 μ g/disc, respectively, by DDA and 1.95 μ g/mL and 3.90 μ g/mL, respectively, by MDA and PSGI. The MIC of **5c** compound was 46.75 μ g/disc against all the tested isolates of *Aspergillus* spp. by DDA, whereas **5j** compound possesses a slight higher MIC against *A. flavus* and *A. niger*, that is, 187.5 μ g/disc, but against *A. fumigatus* it possesses the same MIC, that is, 46.75 μ g/disc. The MIC of **5c** by MDA and PSGI was found to be 250.0 μ g/mL against *A. fumigatus* and 500.0 μ g/mL against *A. flavus* and *A. niger*. The MIC of **5j** was found to be 500.0 μ g/mL against *A. fumigatus* and *A. flavus* and 1000 μ g/mL against *A. niger*, respectively, by MDA and PSGI (Table 2).

Results revealed that the synthesized compounds **5c** and **5j** exhibited mild antifungal activity which is lower than the standard drugs. Some other substituted pyrimidines and pyrazoles have earlier been reported as potent antifungal agents against a number of pathogenic fungi alone and in combination [24, 25].

As these compounds showed promising activity, so we further tried these compounds in combination with standard antifungal drugs to evaluate their synergistic behaviour, if any. Therefore, the compounds **5c** and **5j** were tried for *in vitro* combination with polyenes and azoles.

(1) In Vitro Combination Study of Pyrimidine Pyrazole Analogues (5c** and **5j**) with Antifungal Drugs.** Among the human pathogenic species of *Aspergillus*, *A. fumigatus* is the primary causative agent of human infection followed by *A. flavus* and *A. niger* [23]. Therefore, *A. fumigatus* [ITCC 4517 (IARI, Indian Agricultural Research Institute, Delhi), ITCC 1634, clinical isolate VPCI 190/96 (VPCI, Vallabhbbhai Patel Chest Institute, Delhi)] was selected for *in vitro* combination study of pyrimidine pyrazole analogues with antifungal drugs. The data of *in vitro* combination was analysed by Fraction Inhibitory Concentration Index (FICI) model [24] and summarized in Tables 3–6.

(1.1) In Vitro FIC Index of **5c with Polyene (Amp B, NYS) and Azole (KTZ, FLZ).** In combination of Amp B and NYS with **5c**, the FICI values were found to be in the range of 0.8 to 1.03; indifference (IND) was declared against *A. fumigatus* strains (Table 3). The MIC end point value of **5c** reduced from 314.98 to 7.87/12.50 μ g/mL. But the MIC of Amp B and NYS almost remains the same, that is, 1.96 μ g/mL and 3.12 μ g/mL.

The combination of **5c** with KTZ and FLZ reduced the MIC end point value of KTZ and FLZ from 39.37 to 7.87 μ g/mL and from 314.98 to 62.90 μ g/mL, respectively, against *A. fumigatus* strains. The MIC of **5c** reduced from 314.98 to 62.90 μ g/mL in combination with KTZ and with FLZ it gets reduced to 251.98. Depending upon FICI model indifference (IND) and synergy (SYN) was observed. The combination of KTZ with **5c** showed SYN against only one strain of *A. fumigatus*, that is, ITCC 4517, at 1:8 (6.25:50.00 μ g/mL, FICI = 0.40) (Table 4). The FICI (GM)

TABLE 1: Structure, formula, and yields of compounds 5a-l.

Entry	R	X	Ar	Chalcone (4a-l)	Cyclised product (5a-l)	Yield (%)
2a, 3a, 4a, 5a	-CH ₃	O	<i>p</i> -CH ₃			66
2a, 3b, 4b, 5b	-CH ₃	O	<i>p</i> -OCH ₃			66
2a, 3c, 4c, 5c	-CH ₃	O	<i>p</i> -Br			80
2a, 3d, 4d, 5d	-CH ₃	O	<i>p</i> -Cl			66

TABLE 1: Continued.

Entry	R	X	Ar	Chalcone (4a-l)	Cyclised product (5a-l)	Yield (%)
2b, 3a, 4e, 5e	C ₆ H ₆	S	<i>p</i> -CH ₃			64
2b, 3b, 4f, 5f	C ₆ H ₆	S	<i>p</i> -OCH ₃			80
2b, 3c, 4g, 5g	C ₆ H ₆	S	<i>p</i> -Br			46
2b, 3d, 4h, 5h	C ₆ H ₆	S	<i>p</i> -Cl			78

TABLE I: Continued.

Entry	R	X	Ar	Chalcone (4a-l)	Cyclised product (5a-l)	Yield (%)
2c, 3a, 4i, 5i	<i>o</i> -OCH ₃ C ₆ H ₅	S	<i>p</i> -CH ₃			68
2c, 3b, 4j, 5j	<i>o</i> -OCH ₃ C ₆ H ₅	S	<i>p</i> -OCH ₃			68
2c, 3c, 4k, 5k	<i>o</i> -OCH ₃ C ₆ H ₅	S	<i>p</i> -Br			80
2c, 3d, 4l, 5l	<i>o</i> -OCH ₃ C ₆ H ₅	S	<i>p</i> -Cl			81

TABLE 2: Antifungal activity of pyrimidine pyrazole analogues.

Compound	DDA ($\mu\text{g}/\text{disc}$)			MIC			PSGI ($\mu\text{g}/\text{mL}$)		
	<i>A. flavus</i>	<i>A. niger</i>	<i>A. fumigatus</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>A. fumigatus</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>A. fumigatus</i>
5c	46.75	46.75	46.75	500	500	250	500	500	250
5e	—	—	—	—	—	—	—	—	—
5f	—	—	—	—	—	—	—	—	—
5g	—	—	—	—	—	—	—	—	—
5h	—	—	—	—	—	—	—	—	—
5i	—	—	—	—	—	—	—	—	—
5j	187.5	187.5	46.75	500	1000	500	500	1000	500
5k	—	—	—	—	—	—	—	—	—
5l	—	—	—	—	—	—	—	—	—

—: no significant inhibition.

TABLE 3: *In vitro* combination of compound 5c with polyene (AmpB, NYS) against *A. fumigatus*.

Strain	MIC ($\mu\text{g}/\text{mL}$)				FIC index		Interpretation		
	5c	AmpB	NYS	AmpB + 5c	NYS + 5c	AmpB + 5c	NYS + 5c	AmpB + 5c	NYS + 5c
ITCC 4517	250	1.95	3.9	1.56/6.25	3.12/12.50	0.83 (0.8 + 0.03)	0.85 (0.8 + 0.05)	IND	IND
ITCC 1634	500	1.95	3.9	3.12/12.50	3.12/12.50	1.62 (1.6 + 0.02)	0.83 (0.8 + 0.03)	IND	IND
VPCI 190/96	250	1.95	3.9	1.56/6.25	3.12/12.50	0.83 (0.8 + 0.03)	0.85 (0.8 + 0.05)	IND	IND
Geometric mean	314.98	1.95	3.9	1.96/7.87	3.12/12.50	1.03 (1.00 + 0.3)	0.83 (0.8 + 0.03)	IND	IND

IND: indifference.

Compounds 1-5	R	X
1a, 2a, 4a-d, 5a-d	-CH ₃	O
1b, 2b, 4e-h, 5e-h	-C ₆ H ₅	S
1c, 2c, 4i-l, 5i-l	<i>o</i> -OCH ₃ C ₆ H ₄	S

Compounds 3-5	Y
3a, 4a, 4e, 4i, 5a, 5e, 5i	CH ₃
3b, 4b, 4f, 4j, 5b, 5f, 5j	OCH ₃
3c, 4c, 4g, 4k, 5c, 5g, 5k	Br
3d, 4d, 4h, 4l, 5d, 5h, 5l	Cl

SCHEME 1: Synthesis of pyrimidine pyrazole derivatives.

TABLE 4: *In vitro* combination of compound **5c** with azole (KTZ, FLZ) against *A. fumigatus*.

Strain	MIC ($\mu\text{g/mL}$)					FIC index		Interpretation	
	5c	KTZ	FLZ	KTZ + 5c	FLZ + 5c	KTZ + 5c	FLZ + 5c	KTZ + 5c	FLZ + 5c
ITCC 4517	250	31.25	500	6.25/50.0	50.0/200.0	0.40 (0.2 + 0.2)	0.9 (0.1 + 0.8)	SYN	IND
ITCC 1634	500	62.50	250	25.0/100.0	100.0/400.0	0.60 (0.4 + 0.2)	1.2 (0.4 + 0.8)	IND	IND
VPCI 190/96	250	31.25	250	12.50/50.0	50.0/200.0	0.60 (0.4 + 0.2)	1.0 (0.2 + 0.8)	IND	IND
Geometric mean	314.98	39.37	314.98	7.87/62.9	62.9/251.98	0.52 (0.32 + 0.20)	1.0 (0.2 + 0.8)	IND	IND

IND: indifference; SYN: synergy.

TABLE 5: *In vitro* combination of compound **5j** with polyene (AmpB, NYS) against *A. fumigatus*.

Strain	MIC ($\mu\text{g/mL}$)					FIC index		Interpretation	
	5j	AmpB	NYS	AmpB + 5j	NYS + 5j	AmpB + 5j	NYS + 5j	AmpB + 5j	NYS + 5j
ITCC 4517	250.00	1.95	3.90	1.56/6.25	3.12/12.50	0.83 (0.8 + 0.03)	0.85 (0.8 + 0.05)	IND	IND
ITCC 1634	500.00	1.95	3.90	3.12/12.50	6.25/25.00	1.62 (1.6 + 0.02)	1.65 (1.6 + 0.05)	IND	IND
VPCI 190/96	500.00	1.95	3.90	1.56/3.12	3.12/12.50	0.81 (0.8 + 0.01)	0.83 (0.8 + 0.03)	IND	IND
Geometric mean	396.85	1.95	3.90	1.96/6.24	3.93/15.70	1.03 (1.00 + 0.3)	1.05 (1.00 + 0.5)	IND	IND

IND: indifference.

values for the rest of combination were 0.52 and 1.02; IND occurred.

Since the MIC value of KTZ is significantly reduced in combination with **5c**, this compound may be a potential candidate for further research and may be developed as a potential candidate to be used in combination therapy against fungal infections.

(1.2) *In Vitro* FIC Index of **5j** with Polyene (Amp B, NYS) and Azole (KTZ, FLZ). The MIC (GM) end point value of Amp B and NYS in combination with **5j** remains almost the same, that is, 1.96 and 3.93 $\mu\text{g/mL}$, respectively. But the MIC (GM) end point value of **5j** in combination with Amp B and NYS reduced from 396.84 to 6.24 and 15.70 $\mu\text{g/mL}$, respectively. The FICI (GM) values were found to be 1.03 and 1.05 with Amp B and NYS combination with **5j**; showed IND against the tested strain (Table 5).

The combination of azole (KTZ and FLZ) with **5j** reduced the MIC (GM) end point value of KTZ from 39.33 to 25.00 and from 314.98 to 62.99 $\mu\text{g/mL}$ of FLZ. The MIC (GM) end point value of **5j** reduced from 396.85 to 100 $\mu\text{g/mL}$ with KTZ and 251.98 $\mu\text{g/mL}$ with FLZ. But this reduction is not as much significant as the combination of KTZ with **5j**, which showed synergy against only one *A. fumigatus* VPCI 190/96, that is, 1:4 (25:100 $\mu\text{g/mL}$). The FICI (GM) values for the other combinations were 0.70 and 0.83; indifference was declared (Table 6).

2.2.2. *Antibacterial Activity*. Among all the analogues the most active compound was **5g** whose MIC was 31.25 $\mu\text{g/mL}$ against *S. aureus* and *B. cereus* and the second and third most active compounds were **5h** and **5c** which showed MIC at 62.50 $\mu\text{g/mL}$ against *B. cereus* and *S. aureus* and 125 $\mu\text{g/mL}$ against *S. aureus*, respectively. The other two compounds **5e** and **5j** showed activity at 500 $\mu\text{g/mL}$ against *S. aureus* and *E. coli*, respectively. Erythromycin was used as a standard drug (Table 7).

It has already been reported that the pyrimidine pyrazole analogues have strong antibacterial activity against a number of pathogenic bacteria [26]. Therefore, we have tried to evaluate their *in vitro* antibacterial potential against gram positive as well as gram negative bacteria.

The compound **5g** showed potent antibacterial activity against gram positive bacteria *S. aureus* and *B. cereus*. These results suggest that there may be a useful practical application from the chemistry of pyrimidine pyrazole analogues.

3. Experimental

3.1. *General*. All reagents were of commercial grade and were used as received. Solvents were dried and purified using standard techniques. $^1\text{H-NMR}$ (400 MHz) and $^{13}\text{C-NMR}$ (100.5 MHz) were recorded on JNM ECX-400P (Jeol, USA) spectrometer using TMS as an internal standard. Chemical shifts are reported in parts per million (ppm). Mass spectra were recorded on API-2000 mass spectrometer. IR absorption spectra were recorded in the 400–4000 cm^{-1} range on a Perkin-Elmer FT-IR spectrometer model 2000 using KBr pellets. Melting points were determined using Buchi M-560 and are uncorrected. These reactions were monitored by thin layer chromatography (TLC), on aluminium plates coated with silica gel 60 F₂₅₄ (Merck). UV radiation and iodine were used as the visualizing agents. Column chromatography was performed on silica gel (100–200 mesh).

3.2. *General Procedure for the Synthesis of Chalcone Analogues (4a–l)*. A solution of **2a–c** (1 mmol) and corresponding aryl aldehydes **3a–d** (1 mmol) in 20 mL of methanol was treated with sodium hydroxide as base at 60°C. The reaction mixture was refluxed for 50 h. After completion of reaction, it was concentrated and extracted with chloroform (3 × 20 mL). The combined organic extract was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was purified by column chromatography.

TABLE 6: *In vitro* combination of compound **5j** with azole (KTZ and FLZ) against *A. fumigatus*.

Strain	MIC ($\mu\text{g/mL}$)					FIC index		Interpretation	
	5j	KTZ	FLZ	KTZ + 5j	FLZ + 5j	KTZ + 5j	FLZ + 5j	KTZ + 5j	FLZ + 5j
ITCC 4517	250.00	31.25	500.00	25.00/100.00	50.00/200.00	1.20 (0.8 + 0.4)	0.90 (0.1 + 0.8)	IND	IND
ITCC 1634	500.00	62.50	250.00	25.00/100.00	100.00/400.00	0.60 (0.4 + 0.2)	1.20 (0.4 + 0.8)	IND	IND
VPCI 190/96	500.00	31.25	250.00	25.0/100.00	50.00/200.00	0.50 (0.4 + 0.1)	0.60 (0.2 + 0.4)	SYN	IND
Geometric mean	396.85	39.37	314.90	25.00/100.00	62.99/251.98	0.7 (0.5 + 0.2)	0.83 (0.2 + 0.63)	IND	IND

IND: indifference.

TABLE 7: Antibacterial activity of pyrimidine pyrazole analogues.

Compound	Bacterial pathogens (MIC $\mu\text{g/mL}$)						
	<i>S. aureus</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>M. luteus</i>	<i>B. pumilus</i>	<i>B. subtilis</i>
5c	125	—	—	—	—	—	—
5e	500	—	—	—	—	—	—
5f	—	—	—	—	—	—	—
5g	31.25	31.25	—	—	—	—	—
5h	62.50	62.50	—	—	—	—	—
5i	—	—	—	—	—	—	—
5j	—	—	—	—	—	—	—
5k	—	—	—	—	—	—	—
5l	—	—	—	—	—	—	—
ERY	15.62	15.62	7.81	31.25	15.62	15.62	15.62

ERY: erythromycin (standard drug).

—: no significant inhibition.

3.2.1. (*E*)-1-(1',3'-Dimethyl-6'-hydroxy-2',4'-dioxo-1',2',3',4'-tetrahydropyrimidin-5'-yl)-3-(*p*-tolyl)-prop-2-ene-1-one, (**4a**). The product was obtained as mentioned in general procedure from **2a** and **3a** as yellow solid in 72% yield; M.p. 187.0°C; IR ν_{max} (cm^{-1}) = 1662, 1718 (C=O), 2925 (C-H), 3432 (OH); ^1H NMR (400 MHz, CDCl_3): δ (ppm) 2.34 (3H, s, $-\text{CH}_3$), 3.20 (6H, s, $\text{N}-\text{CH}_3$), 7.29 (2H, d, J = 7.32 Hz, ArH), 7.62 (2H, d, J = 8.04 Hz, ArH), 7.95 (1H, d, J = 16.11 Hz, α -H) 8.45 (1H, d, J = 16.11 Hz, β -H), 16.94 (1H, s, $-\text{OH}$); ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 21.05, 27.67, 27.84, 119.10, 126.37, 128.43, 129.00, 129.98, 131.85, 139.38, 141.81, 145.65, 149.84, 154.69, 163.22, 165.83, 182.02.

3.2.2. (*E*)-1-(1',3'-Diphenyl-6'-hydroxy-4'-oxo-2'-thiooxo-1',2',3',4'-tetrahydropyrimidin-5'-yl)-3-(*p*-tolyl)-prop-2-ene-1-one, (**4e**). The product was obtained as mentioned in general procedure from **2b** and **3a** as yellow solid in 67% yield; M.p. 284.6°C; IR ν_{max} (cm^{-1}) = 1039 (C=S), 1690 (C=O), 2924 (C-H), 3433 (OH); ^1H NMR (400 MHz, CDCl_3): δ (ppm) 2.38 (3H, s, $-\text{CH}_3$), 7.18 (2H, d, J = 8.05 Hz, ArH), 7.28–7.31 (2H, m, ArH), 7.45–7.58 (10H, m, ArH), 8.09 (1H, d, J = 15.38 Hz, α -H), 8.51 (1H, d, J = 16.84 Hz, β -H), 16.79 (1H, s, $-\text{OH}$); ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 21.68 ($-\text{CH}_3$), 119.15, 128.55, 128.63, 128.77, 129.11, 129.55, 129.61, 129.67, 129.81, 131.82, 139.72, 142.96, 148.62, 159.94, 168.63, 178.74 (C=S), 185.18.

3.2.3. (*E*)-1-(1',3'-Bis(2''-methoxyphenyl)-6'-hydroxy-4'-oxo-2'-thiooxo-1',2',3',4'-tetrahydropyrimidin-5'-yl)-3-(*p*-tolyl)-prop-2-ene-1-one, (**4i**). The product was obtained as mentioned in general procedure from **2c** and **3a** as yellow solid in 68% yield; M.p. 220.5°C; IR ν_{max} (cm^{-1}) = 1025 (C=S), 1663 (C=O), 2926 (C-H), 3434 (OH); ^1H NMR (400 MHz, CDCl_3): δ (ppm) 2.35 (3H, s, $-\text{CH}_3$), 3.84 (6H, s, $-\text{OCH}_3$), 7.03–7.10 (4H, m, ArH), 7.16 (2H, d, J = 7.32 Hz, ArH), 7.21–7.26 (2H, m, ArH), 7.44 (2H, d, J = 8.05 Hz, ArH), 7.55 (2H, d, J = 8.05 Hz, ArH), 8.03 (1H, d, J = 16.11 Hz, α -H), 8.51 (1H, d, J = 16.11 Hz, β -H), 16.84 (1H, s, $-\text{OH}$); ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 21.63 ($-\text{CH}_3$), 56.14 ($-\text{OCH}_3$), 119.57, 121.06, 121.19, 128.57, 129.53, 129.72, 129.82, 130.22, 130.59, 131.96, 142.46, 147.83, 159.53, 168.40, 178.54 (C=S), 184.94.

3.3. General Procedure for the Synthesis of Pyrimidine Pyrazole Heterocycles (**5a-l**). To the mixture of corresponding chalcone **4a-l** (1 mmol) and phenylhydrazine (1.5 mmol) in 20 mL of 1,4-dioxane, 2 drops of acetic acid were added. The reaction mixture was refluxed at 110°C overnight. After completion of reaction as monitored by TLC, reaction mixture was concentrated and extracted with chloroform (3 $\times$ 20 mL). The combined organic extract was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was purified by column chromatography (40% Ethyl acetate: pet ether).

3.3.1. 1,3-Dimethyl-6-hydroxy-2,4-dioxo-5-(1'-phenyl-3'-(*p*-tolyl)-1*H*-pyrazol-5'-yl)-1,2,3,4-tetrahydropyrimidine, (**5a**). The product was obtained as mentioned in general procedure from **4a** as white solid; M.p. 150–152°C; IR $\nu_{\max}$ (cm⁻¹) 1646, 1702, (2 × C=O), 2924 (C–H), 3210 (–OH); ¹H NMR (400 MHz, CDCl₃): δ (ppm) 2.34 (3H, s, –CH₃), 3.28 (3H, s, N–CH₃), 3.36 (3H, s, N–CH₃), 6.93 (2H, d, *J* = 8.08 Hz, ArH), 7.06 (1H, t, ArH), 7.18 (2H, d, *J* = 8.08 Hz, ArH), 7.25–7.30 (4H, m, Pyrazole H, ArH), 12.85 (1H, s, –OH); ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 21.04 (–CH₃), 27.51 (N–CH₃), 107.42, 115.89, 126.00, 129.46, 129.57, 129.79, 139.19, 147.81, 153.50, 159.21, 165.28; ESI-MS *m/z*: [M + H]⁺ = 388.1.

3.3.2. 1,3-Dimethyl-6-hydroxy-2,4-dioxo-5-(1'-phenyl-3'-(*p*-methoxyphenyl)-1*H*-pyrazol-5'-yl)-1,2,3,4-tetrahydropyrimidine, (**5b**). The product was obtained as mentioned in general procedure from **4b** as light brown solid, M.p. 170–171°C; IR $\nu_{\max}$ (cm⁻¹) 1647, 1716 (2 × C=O), 2924 (C–H), 3245 (–OH); ¹H NMR (400 MHz, CDCl₃): δ (ppm) 3.35 (3H, s, N–CH₃), 3.39 (3H, s, N–CH₃), 3.80 (3H, s, –OCH₃), 6.89–6.94 (4H, m, ArH), 7.07 (1H, t, ArH), 7.25–7.33 (5H, m, pyrazole H, ArH), 12.83 (1H, s, –OH); ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 27.65 (N–CH₃), 29.67 (N–CH₃), 55.33 (–OCH₃), 86.08, 114.45, 116.27, 124.13, 127.47, 129.54, 132.39, 148.08, 151.88, 159.61, 161.54, 162.08, 165.49; ESI-MS *m/z*: [M + H]⁺ = 404.1.

3.3.3. 1,3-Dimethyl-6-hydroxy-2,4-dioxo-5-(1'-phenyl-3'-(*p*-bromo)-1*H*-pyrazol-5'-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine, (**5c**). The product was obtained as mentioned in general procedure from **4c** as white solid, M.p. 197–198°C; IR $\nu_{\max}$ (cm⁻¹) 1699, 1734 (2 × C=O), 2925 (C–H), 3417 (–OH); ¹H NMR (400 MHz, CDCl₃): δ (ppm) 3.28 (3H, s, N–CH₃), 3.35 (3H, s, N–CH₃), 6.92 (2H, d, *J* = 8.05 Hz, ArH), 7.09 (1H, t, ArH), 7.27–7.31 (5H, m, Pyrazole H, ArH), 7.52 (2H, d, *J* = 8.05 Hz, ArH), 12.82 (1H, s, –OH); ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 27.84 (N–CH₃), 86.14, 116.18, 122.26, 124.36, 127.87, 129.66, 132.29, 139.77, 147.76, 151.45, 161.45, 161.95, 164.99; ESI-MS *m/z*: [M + H]⁺ = 453.3.

3.3.4. 1,3-Dimethyl-6-hydroxy-2,4-dioxo-5-(1'-phenyl-3'-(*p*-chloro)-1*H*-pyrazol-5'-yl)-1,2,3,4-tetrahydropyrimidine, (**5d**). The product was obtained as mentioned in general procedure from **4d** as light brown solid, M.p. 121–123°C; IR $\nu_{\max}$ (cm⁻¹) 1642, 1702 (2 × C=O), 2923 (C–H), 3319 (–OH); ¹H NMR (400 MHz, CDCl₃): δ (ppm) 3.28 (3H, s, N–CH₃), 3.36 (3H, s, N–CH₃), 6.92 (2H, d, *J* = 8.05 Hz, ArH), 7.07–7.11 (2H, m, ArH), 7.24–7.48 (6H, m, ArH, pyrazole H), 12.83 (1H, s, –OH); ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 27.71 (N–CH₃), 87.69, 116.27, 119.88, 121.09, 124.36, 125.04, 127.56, 129.00, 129.33, 129.49, 130.04, 134.16, 139.09, 147.62, 151.71, 161.40; ESI-MS *m/z*: [M + H]⁺ = 408.1.

3.3.5. 1,3-Diphenyl-6-hydroxy-4-oxo-2-thiooxo-5-(1'-phenyl-3'-(*p*-tolyl)-1*H*-pyrazol-5'-yl)-1,2,3,4-tetrahydropyrimidine, (**5e**). The product was obtained as mentioned in general procedure from **4e** as light brown solid, M.p. 131–133°C; IR $\nu_{\max}$ (cm⁻¹) 1071 (C=S), 1676 (C=O), 2924 (C–H), 3245 (–OH); ¹H NMR

(400 MHz, CDCl₃): δ (ppm) 2.35 (3H, s, –CH₃), 6.90 (2H, d, *J* = 8.08 Hz, ArH), 7.06 (1H, t, ArH), 7.17–7.30 (9H, m, ArH), 7.32–7.38 (3H, m, ArH), 7.40–7.53 (5H, m, Pyrazole H, ArH), 12.82 (1H, s, –OH); ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 21.24 (N–CH₃), 88.32, 124.27, 125.55, 128.32, 128.70, 128.86, 129.38, 129.55, 129.82, 137.48, 138.25, 139.53, 140.35, 146.93, 160.75, 161.33, 163.97, 180.24 (C=S); ESI-MS *m/z*: [M + H]⁺ = 528.1.

3.3.6. 1,3-Diphenyl-6-hydroxy-4-oxo-2-thiooxo-5-(1'-phenyl-3'-(*p*-methoxyphenyl)-1*H*-pyrazol-5'-yl)-1,2,3,4-tetrahydropyrimidine, (**5f**). The product was obtained as mentioned in general procedure from **4f** as green solid, M.p. 120–121°C; IR $\nu_{\max}$ (cm⁻¹) 1030 (C=S), 1676 (C=O), 2924 (C–H), 3214 (–OH); ¹H NMR (400 MHz, CDCl₃): δ (ppm) 3.82 (3H, s, –OCH₃), 6.90–6.93 (4H, m, ArH), 7.08 (1H, t, ArH), 7.21–7.40 (10H, m, ArH), 7.43–7.58 (5H, m, Pyrazole H, ArH), 12.83 (1H, s, –OH); ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 55.32 (–OCH₃), 88.35, 114.49, 116.41, 127.45, 128.68, 129.37, 129.44, 129.47, 129.53, 131.2, 139.76, 140.32, 147.13, 159.80, 160.86, 161.49, 164.09, 180.03 (C=S); ESI-MS *m/z*: [M + H]⁺ = 545.4.

3.3.7. 1,3-Diphenyl-6-hydroxy-4-oxo-2-thiooxo-5-(1'-phenyl-3'-(*p*-bromo)-1*H*-pyrazol-5'-yl)-1,2,3,4-tetrahydropyrimidine, (**5g**). The product was obtained as mentioned in general procedure from **4g** as light green solid, M.p. 209–210°C; IR $\nu_{\max}$ (cm⁻¹) 1071 (C=S), 1675 (C=O), 2925 (C–H), 3182 (–OH); ¹H NMR (400 MHz, CDCl₃): δ (ppm) 6.89 (2H, d, *J* = 8.05 Hz, ArH), 7.08 (1H, t, ArH), 7.26–7.28 (4H, m, ArH), 7.33 (2H, d, *J* = 7.32 Hz, ArH), 7.40 (2H, d, *J* = 8.05 Hz, ArH), 7.44–7.57 (9H, m, Pyrazole H, ArH), 12.80 (1H, s, –OH); ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 88.36, 106.31, 116.31, 122.11, 124.93, 125.37, 127.83, 128.65, 129.39, 129.46, 129.66, 132.33, 136.11, 139.09, 139.89, 146.74, 160.55, 161.35, 164.18, 179.92 (C=S); ESI-MS *m/z*: [M + H]⁺ = 592.0.

3.3.8. 1,3-Diphenyl-6-hydroxy-4-oxo-2-thiooxo-5-(1'-phenyl-3'-(*p*-chloro)-1*H*-pyrazol-5'-yl)-1,2,3,4-tetrahydropyrimidine, (**5h**). The product was obtained as mentioned in general procedure from **4h** as dark green solid, M.p. 207–208°C; IR $\nu_{\max}$ (cm⁻¹) 1089 (C=S), 1675 (C=O), 2924 (C–H), 3198 (–OH); ¹H NMR (400 MHz, CDCl₃): δ (ppm) 6.89 (2H, d, *J* = 8.05 Hz, ArH), 7.08 (1H, t, ArH), 7.18–7.29 (5H, m, ArH), 7.32–7.40 (6H, m, ArH), 7.44–7.57 (6H, m, Pyrazole H, ArH), 12.80 (1H, s, –OH); ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 88.50, 105.95, 116.30, 127.51, 128.64, 129.39, 129.47, 129.65, 134.23, 138.66, 139.48, 139.89, 146.59, 160.96, 161.35, 164.21, 179.94 (C=S); ESI-MS *m/z*: [M + H]⁺ = 548.1.

3.3.9. 1,3-Bis(2''-methoxyphenyl)-6-hydroxy-4-oxo-2-thiooxo-5-(1'-phenyl-3'-(*p*-tolyl)-1*H*-pyrazol-5'-yl)-1,2,3,4-tetrahydropyrimidine, (**5i**). The product was obtained as mentioned in general procedure from **4i** as light brown solid, M.p. 107–109°C; IR $\nu_{\max}$ (cm⁻¹) 1075 (C=S), 1677 (C=O), 2925 (C–H), 3302 (–OH); ¹H NMR (400 MHz, CDCl₃): δ (ppm) 2.36 (3H, s, –CH₃), 3.85 (6H, s, –OCH₃), 6.91 (2H, d, *J* = 7.32 Hz, ArH), 7.00–7.12 (6H, m, ArH), 7.18–7.30 (5H, m, ArH),

7.30–7.43 (5H, m, Pyrazole H, ArH), 12.89 (1H, s, –OH); ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 29.61 (– CH_3), 56.01 (– OCH_3), 85.53, 105.37, 112.20, 112.72, 115.94, 121.02, 125.57, 128.51, 129.56, 129.67, 129.79, 129.99, 140.16, 148.36, 154.66, 178.37 (C=S); ESI-MS m/z : $[\text{M} + \text{H}]^+ = 588.1$.

3.3.10. 1, 3-Bis(2''-methoxyphenyl)-6-hydroxy-4-oxo-2-thio-oxo-5-(1'-phenyl-3'-(p-methoxyphenyl)-1H-pyrazol-5'-yl)-1,2,3,4-tetrahydropyrimidine, (**5j**). The product was obtained as mentioned in general procedure from **4j** as light green solid, M.p. 127–129°C; IR ν_{max} (cm^{-1}) 1074 (C=S), 1627 (C=O), 2926 (C–H), 3422 (–OH); ^1H NMR (400 MHz, CDCl_3): δ (ppm) 3.83 (9H, s, – OCH_3), 6.83–6.92 (7H, m, ArH), 7.01–7.11 (7H, m, ArH), 7.20–7.32 (4H, m, pyrazole H, ArH), 12.80 (1H, s, –OH); ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 55.32 (– OCH_3), 88.15, 112.78, 113.53, 114.45, 116.33, 121.01, 121.53, 121.81, 124.32, 127.53, 129.16, 129.46, 129.84, 130.18, 146.75, 154.93, 159.55, 167.76, 185.32 (C=S); ESI-MS m/z : $[\text{M} + \text{H}]^+ = 604.1$.

3.3.11. 1, 3-Bis(2''-methoxyphenyl)-6-hydroxy-4-oxo-2-thio-oxo-5-(1'-phenyl-3'-(p-bromo)-1H-pyrazol-5'-yl)-1,2,3,4-tetrahydropyrimidine, (**5k**). The product was obtained as mentioned in general procedure from **4k** as light yellow solid, M.p. 112–113°C; IR ν_{max} (cm^{-1}) 1044 (C=S), 1674 (C=O), 2925 (C–H), 3287 (–OH); ^1H NMR (400 MHz, CDCl_3): δ (ppm) 3.78 (6H, s, – OCH_3), 6.82–6.84 (2H, m, ArH), 6.93–7.04 (5H, m, ArH), 7.12–7.25 (6H, m, ArH), 7.32–7.39 (3H, m, ArH), 7.45–7.47 (2H, m, pyrazole H, ArH), 12.77 (1H, s, –OH); ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 56.36 (– OCH_3), 88.36, 116.26, 121.03, 121.13, 127.90, 128.00, 129.60, 129.70, 129.89, 130.25, 132.30, 139.30, 139.36, 146.77, 154.51, 159.70, 160.94, 163.93, 179.53 (C=S); ESI-MS m/z : $[\text{M} + \text{H}]^+ = 652.0$.

3.3.12. 1, 3-Bis(2''-methoxyphenyl)-4-oxo-2-thiooxo-6-hydroxy-5-(1'-phenyl-3'-(p-chloro)-1H-pyrazol-5'-yl)-1,2,3,4-tetrahydropyrimidine, (**5l**). The product was obtained as mentioned in general procedure from **4l** as light yellow solid, M.p. 232–233°C; IR ν_{max} (cm^{-1}) 1043 (C=S), 1654 (C=O), 2927 (C–H), 3437 (–OH); ^1H NMR (400 MHz, CDCl_3): δ (ppm) 3.85 (6H, s, – OCH_3), 6.89–6.90 (2H, m, ArH), 6.98–7.09 (5H, m, ArH), 7.23–7.28 (5H, m, ArH), 7.33–7.45 (6H, m, pyrazole H, ArH), 12.83 (1H, s, –OH); ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 56.19 (– OCH_3), 88.18, 112.23, 116.06, 120.91, 124.52, 127.60, 129.34, 129.59, 129.88, 134.26, 138.88, 154.89, 160.60, 163.97, 185.19 (C=S); ESI-MS m/z : $[\text{M} + \text{H}]^+ = 609.3$.

3.4. Antifungal Susceptibility Test. The pathogenic isolates of *Aspergillus fumigatus* (ITCC 4517 (IARI, Indian Agricultural Research Institute Delhi), ITCC 1634 (IARI, Delhi), clinical isolate 190/96 (VPCI, Vallabhbhai Patel Chest Institute Delhi)), *Aspergillus flavus* (clinical isolate 223/96 (VPCI, Delhi)), and *Aspergillus niger* (clinical isolate 56/96 (VPCI, Delhi)) were employed in the current study. These pathogenic species of *Aspergillus*, namely, *A. fumigatus*, *A. flavus*, and

A. niger, were cultured in laboratory on Sabouraud dextrose (SD) agar plates. The plates were inoculated with stock cultures of *A. fumigatus*, *A. flavus*, and *A. niger* and incubated in a BOD incubator at 37°C. The spores were harvested from 96 h cultures and suspended homogeneously in phosphate buffer saline (PBS). The spores in the suspension were counted and their number was adjusted to 10^8 spores/mL before performing the experiments. The antifungal activity of compounds was analysed by MDA, DDA, and PSGI. Each assay was repeated at least three times on different days. Amp B was used as a standard drug in antifungal susceptibility test.

3.4.1. Disc Diffusion Assay (DDA). The disc diffusion assay was performed in radiation sterilized petri plates (10.0 cm diameter, Tarsons). The SD agar plates were prepared and plated with a standardized suspension of 1×10^8 spore/mL of *Aspergillus* spp. Then, plates were allowed to dry and discs (5.0 mm in diameter) of Whatman filter paper number 1 were placed on the surface of the agar. The different concentrations of compounds in the range of 750–1.0046 μg were impregnated on the discs. An additional disc for solvent (DMSO) was also placed on agar plate. The plates were incubated at 37°C and examined at 24 h, 48 h for zone of inhibition, if any, around the discs. The concentration, which developed the zone of inhibition of at least 6.0 mm diameter, was taken as end point (Minimum Inhibitory Concentration, MIC).

3.4.2. Percent Spore Germination Inhibition Assay (PSGI). Different concentrations of the test compounds in 90.0 μL of culture medium were prepared in 96-well flat-bottomed microculture plates (Tarson) by double dilution method. Each well was then inoculated with 10.0 μL of spore suspension (100 ± 5 spores). The plates were incubated at 37°C for 16 h and then examined for spore germination under inverted microscope (Nikon, diphot). The number of germinated and nongerminated spores was counted. The lowest concentration of the compound, which resulted in >90% inhibition of germination of spores in the wells, was considered as MIC₉₀.

3.4.3. Microbroth Dilution Assay (MDA). The test was performed in 96-well culture plates (Tarson). Various concentrations of compounds in the range of 1250–4.3 $\mu\text{g}/\text{mL}$ were prepared in 90.0 μL of culture medium by double dilution method. Each well was inoculated with 10 μL of spore suspension (1×10^8 spore/mL) and incubated for 48 h at 37°C. After 48 h, the plates were assessed visually. The optically clear well was taken as end point, MIC.

3.5. Antifungal Drugs and Pyrimidine Pyrazole Analogues Checkerboard Testing. *In vitro* combination of pyrimidine pyrazole analogues was studied with antifungal drug AmpB (Himedia) and NYS (Himedia). The starting range of final concentration was taken as approximate one fold higher than individual MIC to compute all *in vitro* interactions (Antagonistic; Synergy, SYN; and Indifference, IND). The final concentrations of antifungal agents which ranged

from 3.125 to 0.02 $\mu\text{g/mL}$ for Amp B, 6.25 to 0.09 for NYS, and 400 to 3.125 $\mu\text{g/mL}$ for **5c**, **5j** were taken. Aliquots of 45 μL of each drug at a concentration four times the targeted final were dispensed in the wells in order to obtain a two-dimensional checkerboard (8×8 combination) [27]. Each well then was inoculated with 10 μL of spore suspension (1×10^8 spore/mL). The plates were incubated at 37°C for 48 h. The plates were then assessed visually. The optically clear well was taken as end point, MIC.

3.6. Drug Interaction Modelling. The drug interaction was determined by the most popular FICI model. The FICI represents the sum of the FICs (Fraction Inhibitory Concentration) of each drug tested. The FIC of a drug was defined as MIC of a drug in combination divided by MIC of the same drug alone (MIC of drug in combination/MIC of drug alone), $\text{FICI} = 1$ (revealed indifference), $\text{FICI} \leq 0.5$ (revealed synergy), and $\text{FICI} > 4$ (revealed antagonism) [28].

3.7. Antibacterial Susceptibility Test. The antibacterial activity of compound was analysed by microbroth dilution Resazurin based assay [29]. Each assay was repeated at least three times on different days. The different pathogenic species of bacteria, *Staphylococcus aureus* (MTCC number 3160), *Bacillus cereus* (MTCC number 10085), *Escherichia coli* (MTCC number 433), *Salmonella typhi* (MTCC number 733), *Micrococcus luteus* (MTCC number 8132), *Bacillus pumilis* (MTCC number 2299), and *Bacillus subtilis* (MTCC number 8142), were cultured in Luria broth. Using aseptic techniques, a single colony was transferred into a 100 mL Luria broth and placed in incubator at 35°C . After 12–18 h of incubation, the culture was centrifuged at 4000 rpm for 5 minutes. The supernatant was discarded and pellet was resuspended in 20 mL PBS and centrifuged again at 4000 rpm for 5 min. This step was repeated until the supernatant was clear. The pellet was then suspended in 20 mL PBS. The optical density of the bacteria was recorded at 500 nm and serial dilutions were carried out with appropriate aseptic techniques until the optical density was in the range of 0.5–1.0, representing 5×10^6 CFU/mL.

3.7.1. Resazurin Based Microtitre Dilution Assay. Resazurin based MDA was performed in 96-well plates under aseptic conditions. The concentrations of compounds in the range of 2000–7.8 $\mu\text{g/mL}$ were prepared in 100 μL of culture medium by serial dilution method. 10 μL of Resazurin indicator solution (5X) was added in each well. Finally, 10 μL of bacterial suspension was added (5×10^6 CFU/mL) to each well to achieve a concentration of 5×10^5 CFU/mL. Each plate had a set of controls: a column with erythromycin as positive control. The plates were prepared in triplicate and incubated at 37°C for 24 h. The colour change was then assessed visually. The lowest concentration at which colour change occurred was taken as the MIC value.

4. Conclusion

In search of novel antimicrobial molecules, we came across that pyrimidine pyrazole heterocycles can be of interest as **5g** showed significant antibacterial activity. The compounds **5e** and **5h** also showed moderate antibacterial activity. **5j** showed moderate antifungal activity. Out of all heterocycles, **5c** possesses both antifungal and antibacterial activity. Our studies showed that these novel heterocycles can supplement the existing antifungal therapy. Monotherapy can be replaced by combination therapy. Therefore **5c**, **5g**, and **5j** might be of great interest for the development of novel antimicrobial molecule.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors would like to thank Council of Scientific and Industrial Research (CSIR), New Delhi, and Defence Research and Development Organisation (DRDO) for the financial support.

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