European Journal of Medicinal Chemistry 82 (2014) 347–354

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and antimicrobial activity of amine linked bis- and tris-heterocycles

T. Bhanu Prakash, G. Dinneswara Reddy, A. Padmaja, V. Padmavathi*

Department of Chemistry, Sri Venkateswara University, Tirupati 517 502, Andhra Pradesh, India

ARTICLE INFO

Article history:
Received 29 January 2014
Received in revised form
16 May 2014
Accepted 1 June 2014
Available online 3 June 2014

Keywords:
Oxazole
Thiazole
Imidazole
Pyrazole
Antimicrobial activity

ABSTRACT

A series of amine linked bis- and tris-heterocycles were prepared from heteroaryl cinnamamides and tested for antimicrobial activity. The compounds **11c** and **12c** exhibited excellent antibacterial activity while **12a** and **12c** displayed excellent antifungal activity.

1. Introduction

Oxazoles are widely distributed in natural products including phenoxan [1], noricumazoles [2], hennoxazoles [3] and leiodolides A & B [4] and many of them possess significant biological activities such as antifungal, cytotoxic, anthelmintic etc., [5–7]. Thiazoles and their derivatives exhibit antibacterial [8], antifungal [9] and anti-inflammatory [10] activities. Multi-substituted imidazoles are significant core structures used in medicinal chemistry due to their remarkable activities such as antifungal, antibacterial, antiviral and anti-inflammatory [11–13]. Besides, cyclooxygenase-2 (COX-2) inhibitor Celecoxib [14], herbicide Fluazolate [15] and fungicide Pyraclostrobin [16] contains pyrazole moiety. The potent biological activity and the prevalence of azoles in natural products and as drug candidates stimulated intensive interest in the synthesis of hitherto unknown bis- and tris-heterocycles and to study their antimicrobial activity.

2. Chemistry

The synthetic intermediates (*E*)-*N*-(4-aryloxazol-2-yl)cinnamamide (**1**) [17], (*E*)-*N*-(4-arylthiazol-2-yl)cinnamamide (**2**) [17] and (*E*)-*N*-(4-aryl-1*H*-imidazol-2-yl)cinnamamide (**3**) [17] were prepared by the reaction of 4-aryloxazol-2-ylamine, 4-arylthiazol-2-ylamine and 4-aryl-1*H*-imidazol-2-ylamine with cinnamoyl chloride in the presence of toluene. The cyclocondensation reaction of **1**, **2** and **3** with thiosemicarbazide in the presence of sodium hydroxide in ethanol resulted in 3′-(4-aryloxazol-2-ylamino)-5′-aryl-4′,5′-dihydropyrazole-1′-carbothioamide (**4**), 3′-(4-arylthiazol-2-ylamino)-5′-aryl-4′,5′-dihydropyrazole-1′-carbothioamide (**5**) and 3′-(4-aryl-1*H*-imidazol-2-ylamino)-5′-aryl-4′,5′-dihydropyrazole-1′-carbothioamide (**6**), respectively. The ^{1}H NMR spectra of **4a**, **5a** and **6a** displayed an AMX splitting pattern for the methine and methylene protons of pyrazoline ring. The double doublets observed at δ 5.32, 3.85, 3.18 in **4a**, at 5.30, 3.79, 3.16 in **5a** and at 5.33, 3.86, 3.14 ppm in **6a** were attributed to H_A, H_M and H_x, respectively. In addition two broad singlets were observed at δ 5.54, 5.68 in **4a**, at 5.52, 5.64 in **5a** and at 5.62, 5.76 ppm in **6a** due to NH and NH_2. Besides, a broad singlet observed at δ 11.94 ppm in **6a** was due to NH of imidazole. The signals of highly acidic protons disappeared on deuteration. Oxidation of compounds **4**, **5** and **6** with chloranil afforded the aromatized products 3′-(4-aryloxazol-2-ylamino)-5′-aryl-1′*H*-pyrazole-1′-carbothioamide (**7**), 3′-(4-arylthiazol-2-ylamino)-5′-aryl-1′*H*-pyrazole-1′-carbothioamide (**8**) and 3′-(4-aryl-1*H*-imidazol-2-ylamino)-5′-aryl-1′*H*-pyrazole-1′-carbothioamide (**9**). The ^{1}H NMR spectra of **7a**, **8a** and **9a** showed a singlet at δ 7.04, 7.02 and 6.88 ppm due to $C_{4'}$–H in addition to the signals of other protons. The thioamide group in **7**, **8** and **9** was utilized to develop thiazole ring by cyclocondensation with phenacyl bromide. Thus the compounds 5′-aryl-*N*-(4-aryloxazol-2-yl)-1′-(4″-arylthiazol-2″-

* Corresponding author.
E-mail address: vkpuram2001@yahoo.com (V. Padmavathi).

http://dx.doi.org/10.1016/j.ejmech.2014.06.001
0223-5234/

yl)-1′*H*-pyrazol-3′-amine (**10**), 5′-aryl-*N*-(4-arylthiazol-2-yl)-1′-(4″-arylthiazol-2″-yl)-1′*H*-pyrazol-3′-amine (**11**) and 5′-aryl-*N*-(4-aryl-1*H*-imidazol-2-yl)-1′-(4″-arylthiazol-2″-yl)-1′*H*-pyrazol-3′-amine (**12**) were prepared. The ^{1}H NMR spectra of **10a**, **11a** and **12a** exhibited a singlet at δ 6.95, 6.88 and 6.93 ppm due to $C_{4'}$—H. However, the signals of C_5—H and $C_{5''}$—H appeared at downfield region and merged with aromatic protons. In addition a broad singlet at δ 5.47, 5.42 and 5.58 ppm was observed in these compounds due to NH. The compound **12a** displayed another broad singlet at δ 12.06 ppm due to NH of imidazole. The signals of NH disappeared on deuteration. The structures of all the new compounds were further ascertained by IR, ^{13}C NMR, mass and elemental analyses (see Scheme 1).

3. Biology

3.1. In vitro antimicrobial activity

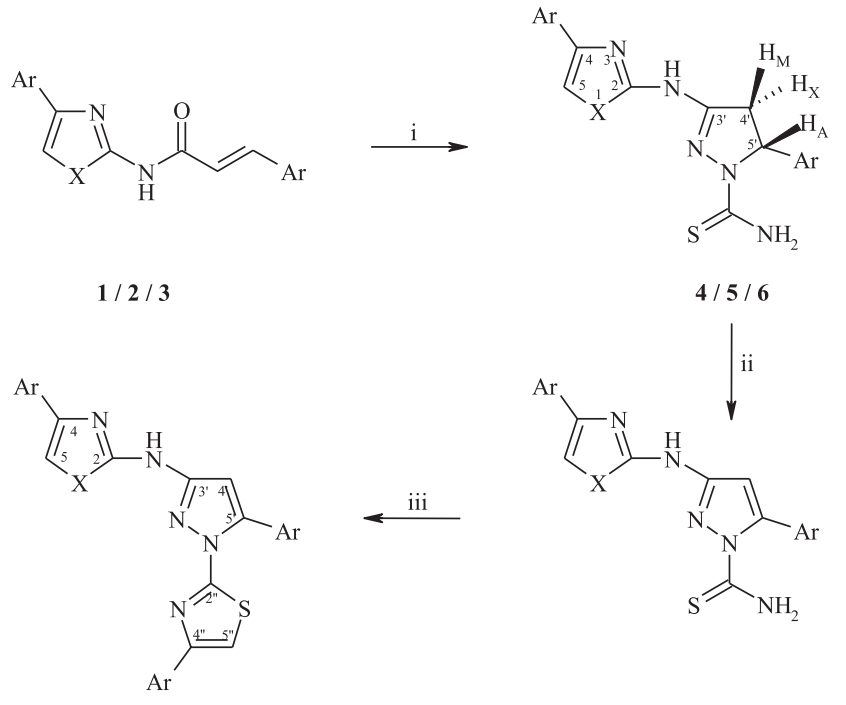
The compounds **4**–**12** were screened for antimicrobial activity at three different concentrations 25, 50 and 100 μg/well.

4. Results and discussion

4.1. Antimicrobial activity

The results are presented in Table 1. The data revealed that Gram-positive bacteria were more susceptible towards the tested compounds than Gram-negative ones. It was observed that trisheterocyclic compounds, 5′-aryl-*N*-(4-aryloxazol-2-yl)-1′-(4″-arylthiazol-2″-yl)-1′*H*-pyrazol-3′-amine (**10**), 5′-aryl-*N*-(4-arylthiazol-2-yl)-1′-(4″-arylthiazol-2″-yl)-1′*H*-pyrazol-3′-amine (**11**) and 5′-aryl-*N*-(4-aryl-1*H*-imidazol-2-yl)-1′-(4″-arylthiazol-2″-yl)-1′*H*-pyrazol-3′-amine (**12**) displayed greater activity than the corresponding bisheterocycles, 3′-(4-aryloxazol-2-ylamino)-5′-aryl-1′*H*-pyrazole-1′-carbothioamide (**7**), 3′-(4-arylthiazol-2-ylamino)-5′-aryl-1′*H*-pyrazole-1′-carbothioamide (**8**) and 3′-(4-aryl-1*H*-imidazol-2-ylamino)-5′-aryl-1′*H*-pyrazole-1′-carbothioamide (**9**). In fact, 5′-(4-chlorophenyl)-*N*-(4-(4-chlorophenyl)thiazol-2-yl)-1′-(4″-(4-chlorophenyl)thiazol-2″-yl)-1′*H*-pyrazol-3′-amine (**11c**) and 5′-(4-chlorophenyl)-*N*-(4-(4-chlorophenyl)-1*H*-imidazol-2-yl)-1′-(4″-(4-chlorophenyl)thiazol-2″-yl)-1′*H*-pyrazol-3′-amine (**12c**) exhibited higher antibacterial activity greater than the standard drug Chloramphenicol particularly against *Staphylococcus aureus*. The compounds **8a**, **8c, 9c, 11a**, and **12a** exhibited good antibacterial activity whereas the compounds **7c**, **8b**, **9a**, **9b**, **10c**, **11b** and **12b** showed moderate activity. The presence of electron withdrawing chloro substituent on the aromatic ring increases the activity when compared with those having methyl and unsubstituted compounds.

All the tested compounds inhibited spore germination of the tested fungi except **4**, **5** and **6**. In general, most of the compounds showed slightly higher antifungal activity towards *Aspergillus niger* than *Penicillium chrysogenum*. The trisheterocyclic compounds having imidazole unit 5′-phenyl-*N*-(4-phenyl-1*H*-imidazol-2-yl)-1′-(4″-phenylthiazol-2″-yl)-1′*H*-pyrazol-3′-amine (**12a**) and 5′-(4-chlorophenyl)-*N*-(4-(4-chlorophenyl)-1*H*-imidazol-2-yl)-1′-(4″-(4-chlorophenyl)thiazol-2″-yl)-1′*H*-pyrazol-3′-amine (**12c**) exhibited excellent activity particularly against *A. niger* greater than the



i) $NH_2NHCSNH_2$ / NaOH / EtOH
ii) Chloranil / Xylene
iii) $ArCOCH_2Br$ / EtOH

1 / 4 / 7 / 10; X = O
2 / 5 / 8 / 11; X = S
3 / 6 / 9 / 12; X = NH

Ar = a) Ph
b) 4-Me. Ph
c) 4-Cl. Ph

Scheme 1. Synthesis of bis- and tris-heterocycles.

Table 1
The *in vitro* antibacterial activity of compounds **4**–**12**.

Compound	Zone of inhibition (mm)											
	Gram-positive bacteria						Gram-negative bacteria					
	Staphylococcus aureus			*Bacillus subtilis*			*Pseudomonas aeruginosa*			*Klebsiella pneumoniae*		
	25 μg/well	50 μg/well	100 μg/well	25 μg/well	50 μg/well	100 μg/well	25 μg/well	50 μg/well	100 μg/well	25 μg/well	50 μg/well	100 μg/well
4a	–	–	–	–	–	–	–	–	–	–	–	–
4b	–	–	–	–	–	–	–	–	–	–	–	–
4c	–	–	–	–	–	–	–	–	–	–	–	–
5a	–	–	–	–	–	–	–	–	–	–	–	–
5b	–	–	–	–	–	–	–	–	–	–	–	–
5c	–	–	–	–	–	–	–	–	–	–	–	–
6a	–	–	–	–	–	–	–	–	–	–	–	6 ± 2
6b	–	–	–	–	–	–	–	–	–	–	–	–
6c	–	–	–	–	–	6 ± 2	–	–	–	–	6 ± 2	7 ± 2
7a	6 ± 1	8 ± 2	10 ± 1	7 ± 1	9 ± 1	11 ± 1	–	–	–	7 ± 2	9 ± 2	12 ± 3
7b	–	–	6 ± 3	–	7 ± 2	8 ± 1	–	–	–	–	7 ± 2	8 ± 2
7c	15 ± 2	17 ± 1	19 ± 1	15 ± 2	16 ± 2	19 ± 2	8 ± 2	9 ± 2	12 ± 1	16 ± 2	17 ± 1	20 ± 2
8a	22 ± 2	25 ± 1	26 ± 3	21 ± 2	22 ± 2	24 ± 3	12 ± 2	15 ± 2	17 ± 1	23 ± 1	25 ± 1	28 ± 2
8b	11 ± 2	13 ± 3	15 ± 3	10 ± 1	12 ± 2	14 ± 3	–	6 ± 2	8 ± 2	11 ± 1	13 ± 1	16 ± 2
8c	24 ± 1	26 ± 2	29 ± 1	22 ± 1	25 ± 1	26 ± 2	14 ± 1	16 ± 1	18 ± 1	26 ± 2	28 ± 2	31 ± 1
9a	18 ± 3	20 ± 2	23 ± 2	18 ± 1	20 ± 2	22 ± 2	10 ± 1	12 ± 2	14 ± 1	19 ± 1	20 ± 1	24 ± 2
9b	10 ± 3	12 ± 2	14 ± 2	9 ± 2	10 ± 1	13 ± 2	–	–	7 ± 1	10 ± 2	12 ± 2	14 ± 1
9c	21 ± 1	23 ± 2	25 ± 2	19 ± 1	21 ± 1	23 ± 2	11 ± 2	13 ± 1	15 ± 2	20 ± 2	22 ± 2	26 ± 1
10a	8 ± 2	10 ± 2	13 ± 3	8 ± 2	10 ± 2	12 ± 2	–	–	6 ± 2	8 ± 3	10 ± 1	13 ± 2
10b	–	6 ± 3	8 ± 2	6 ± 2	8 ± 2	9 ± 2	–	–	–	6 ± 3	8 ± 1	10 ± 2
10c	17 ± 2	18 ± 3	21 ± 3	16 ± 2	18 ± 1	21 ± 1	9 ± 1	10 ± 1	13 ± 2	17 ± 1	19 ± 2	22 ± 1
11a	29 ± 3	31 ± 2	32 ± 2	25 ± 2	26 ± 3	28 ± 2	17 ± 1	18 ± 1	21 ± 3	30 ± 3	32 ± 2	35 ± 2
11b	14 ± 3	15 ± 2	18 ± 1	14 ± 1	15 ± 3	18 ± 1	7 ± 2	8 ± 1	11 ± 2	14 ± 2	15 ± 2	18 ± 1
11c	34 ± 2	36 ± 2	38 ± 3	29 ± 2	32 ± 1	35 ± 2	20 ± 2	22 ± 2	25 ± 2	35 ± 2	37 ± 3	41 ± 1
12a	27 ± 2	28 ± 1	30 ± 2	24 ± 2	26 ± 2	27 ± 1	15 ± 2	17 ± 2	20 ± 2	28 ± 1	29 ± 1	33 ± 2
12b	12 ± 2	14 ± 2	17 ± 2	12 ± 2	13 ± 2	16 ± 2	6 ± 1	7 ± 1	10 ± 1	13 ± 1	14 ± 2	17 ± 1
12c	32 ± 1	33 ± 3	35 ± 2	27 ± 1	29 ± 2	30 ± 3	18 ± 2	19 ± 2	23 ± 2	32 ± 2	34 ± 2	38 ± 1
Chloramphenicol	30 ± 3	33 ± 1	35 ± 2	32 ± 3	34 ± 3	38 ± 1	25 ± 2	27 ± 3	30 ± 1	38 ± 1	40 ± 2	42 ± 3
Control (DMSO)	–	–	–	–	–	–	–	–	–	–	–	–

(–) No activity. (±) Standard deviation.

standard drug Ketoconazole. The compounds **8c**, **9c** and **11a** displayed good activity. However, the compounds **7a**, **7c**, **8a**, **8b**, **9a**, **9b**, **10a**, **10b**, **10c**, **11b** and **12b**, showed moderate activity (Table 2).

The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) values of the tested compounds are listed in Table 3. MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism (But it is not sure that the microorganisms are completely killed). The MBC/MFC is the lowest concentration of antibiotic required to kill a particular bacterium/fungi. The MBC/MFC involves an additional set of steps performed once the minimum inhibitory concentration (MIC) is determined. The antimicrobials are usually regarded as bactericidal/fungicidal if the MBC/MFC is not greater than four times the MIC [18]. The compounds 5′-(4-chlorophenyl)-*N*-(4-(4-chlorophenyl)thiazol-2-yl)-1′-(4″-(4-chlorophenyl)-thiazol-2″-yl)-1′*H*-pyrazol-3′-amine (**11c**) and 5′-(4-chlorophenyl)-*N*-(4-(4-chlorophenyl)-1*H*-imidazol-2-yl)-1′-(4″-(4-chlorophenyl)thiazol-2″-yl)-1′*H*-pyrazol-3′-amine (**12c**) exhibited low MIC values when compared with 5′-phenyl-*N*-(4-phenylthiazol-2-yl)-1′-(4″-phenylthiazol-2″-yl)-1′*H*-pyrazol-3′-amine (**11a**) and 5′-phenyl-*N*-(4-phenyl-1*H*-imidazol-2-yl)-1′-(4″-phenylthiazol-2″-yl)-1′*H*-pyrazol-3′-amine (**12a**). It was observed that in compounds **11c** and **12c** the MBC value 2 × MIC in case of *S. aureus* whereas in **12c** the MFC value is 2 × MIC in case of *A. niger*.

5. Conclusion

A series of amine linked bisheterocycles were prepared from heteroaryl cinnamamides by reaction with thiosemicarbazide followed by oxidation. The thioamide group was exploited to develop thiazole ring on treatment with phenacyl bromide to get trisheterocycles. All the compounds were tested for antimicrobial activity. The compounds thiazolyl/imidazolyl thiazolyl pyrazolyl amines **11c** and **12c** exhibited excellent antibacterial activity whereas imidazolyl thiazolyl pyrazolyl amines **12a** and **12c** displayed excellent antifungal activity. The presence of chloro substituent on the aromatic ring enhanced the activity.

6. Experimental

6.1. Chemistry

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The purity of the compounds was checked by TLC (silica gel H, BDH, ethyl acetate/hexane, 1:3). The IR spectra were recorded on a Thermo Nicolet IR 200 FT-IR spectrometer as KBr pellets and the wave numbers were given in cm^{-1}. The ^{1}H NMR spectra were recorded in DMSO-d_6 on a Bruker-400 spectrometer (400 MHz). The ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker spectrometer operating at 100 MHz. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. The mass spectra were recorded on Jeol JMS-D 300 and Finnigan Mat 1210 B at 70 eV with an emission current of 100 μA. The microanalyses were performed on a Perkin–Elmer 240C elemental analyzer.

6.1.1. General procedure for the synthesis of 3′-(4-aryloxazol-2-ylamino)-5′-aryl-4′,5′-dihydropyrazole-1′-carbothioamide (**4a**–**c**)/3′-(4-arylthiazol-2-ylamino)-5′-aryl-4′,5′-dihydropyrazole-1′-carbothioamide (**5a**–**c**)/3′-(4-aryl-1H-imidazol-2-ylamino)-5′-aryl-4′,5′-dihydropyrazole-1′-carbothioamide (**6a**–**c**)

To an equimolar (1 mmol) mixture of compound **1**/**2**/**3** and thiosemicarbazide in ethanol (2 ml), sodium hydroxide (1.5 mmol)

Table 2
The *in vitro* antifungal activity of compounds **4**–**12**.

Compound	Zone of inhibition (mm)					
	Aspergillus niger			*Penicillium chrysogenum*		
	25 μg/well	50 μg/well	100 μg/well	25 μg/well	50 μg/well	100 μg/well
4a	–	–	–	–	–	–
4b	–	–	–	–	–	–
4c	–	–	–	–	–	–
5a	–	–	–	–	–	–
5b	–	–	–	–	–	–
5c	–	–	–	–	–	–
6a	–	–	–	–	–	–
6b	–	–	–	–	–	–
6c	–	–	–	–	–	–
7a	8 ± 1	9 ± 1	11 ± 2	6 ± 1	8 ± 2	9 ± 3
7b	–	6 ± 2	7 ± 3	–	–	6 ± 3
7c	10 ± 2	11 ± 2	14 ± 2	8 ± 1	10 ± 2	13 ± 1
8a	17 ± 1	19 ± 1	22 ± 2	16 ± 2	20 ± 1	22 ± 3
8b	12 ± 3	13 ± 2	17 ± 1	12 ± 1	13 ± 2	16 ± 1
8c	20 ± 1	22 ± 1	25 ± 2	19 ± 3	21 ± 2	23 ± 1
9a	19 ± 1	20 ± 2	23 ± 1	17 ± 2	20 ± 3	22 ± 2
9b	14 ± 2	15 ± 2	18 ± 1	13 ± 2	15 ± 1	18 ± 3
9c	22 ± 1	24 ± 3	27 ± 1	21 ± 2	22 ± 1	25 ± 2
10a	9 ± 1	10 ± 1	12 ± 3	7 ± 2	9 ± 1	11 ± 2
10b	6 ± 2	8 ± 1	10 ± 1	–	7 ± 1	8 ± 2
10c	11 ± 2	12 ± 1	16 ± 1	10 ± 2	11 ± 1	15 ± 3
11a	25 ± 3	28 ± 2	30 ± 2	22 ± 1	24 ± 2	26 ± 1
11b	15 ± 1	16 ± 2	19 ± 3	14 ± 1	16 ± 3	19 ± 2
11c	29 ± 2	31 ± 1	33 ± 1	25 ± 2	27 ± 1	29 ± 2
12a	32 ± 1	34 ± 2	37 ± 2	27 ± 1	29 ± 2	31 ± 1
12b	16 ± 2	17 ± 3	21 ± 2	15 ± 3	18 ± 2	21 ± 1
12c	33 ± 2	36 ± 1	39 ± 3	29 ± 2	30 ± 1	32 ± 2
Ketoconazole	31 ± 3	33 ± 2	36 ± 2	35 ± 1	36 ± 2	38 ± 3
Control (DMSO)	–	–	–	–	–	–

(–) No activity. (±) Standard deviation.

was added and refluxed for 10–12 h. The contents were poured onto crushed ice. The solid separated was filtered and recrystallized from 2-propanol.

*6.1.1.1. 3′-(4-Phenyloxazol-2-ylamino)-5′-phenyl-4′,5′-dihydropyrazole-1′-carbothioamide (**4a**).* Light yellow solid (0.18 g, 74%); m.p. 206–208 °C; IR (KBr): 1342 (C═S), 1570 (C═N), 3248 (NH), 3341, 3450 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 3.18 (dd, 1H, H_x, $J_{Ax} = 6.7$ Hz, $J_{Mx} = 11.9$ Hz), 3.85 (dd, 1H, H_M, $J_{AM} = 14.8$ Hz, $J_{Mx} = 11.9$ Hz), 5.32 (dd, 1H, H_A, $J_{AM} = 14.8$ Hz, $J_{Ax} = 6.7$ Hz), 5.54 (bs, 1H, C_2–NH), 5.68 (bs, 2H, C–NH_2), 6.92–7.80 (m, 11H, Ar–H & C_5–H) ppm; ^{13}C NMR (DMSO-d_6): 43.6 (C-4′), 66.5 (C-5′), 138.8 (C-5), 141.2 (C-4), 151.6 (C-2), 158.3 (C-3′), 178.4 (C═S), 126.2, 127.6, 128.4, 130.1, 132.7, 133.5, 135.3, 140.2 (aromatic carbons) ppm; MS (*m/z*): 363.44 [$M^{+\bullet}$]; Anal. Calcd. for $C_{19}H_{17}N_5OS$: C, 62.79; H, 4.71; N, 19.27. Found: C, 62.90; H, 4.73; N, 19.43%.

*6.1.1.2. 3′-(4-(4-Methylphenyl)oxazol-2-ylamino)-5′-(4-methylphenyl)-4′,5′-dihydro-pyrazole-1′-carbothioamide (**4b**).* Light yellow solid (0.16 g, 69%); m.p. 218–220 °C; IR (KBr): 1350 (C═S), 1576 (C═N), 3243 (NH), 3336, 3444 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 2.28 & 2.31 (s, 6H, Ar–CH_3), 3.15 (dd, 1H, H_x, $J_{Ax} = 6.5$ Hz, $J_{Mx} = 11.6$ Hz), 3.78 (dd, 1H, H_M, $J_{AM} = 14.7$ Hz, $J_{Mx} = 11.6$ Hz), 5.36 (dd, 1H, H_A, $J_{AM} = 14.7$ Hz, $J_{Ax} = 6.5$ Hz), 5.51 (bs, 1H, C_2–NH), 5.69 (bs, 2H, C–NH_2), 7.02–7.75 (m, 9H, Ar–H & C_5–H) ppm; ^{13}C NMR (DMSO-d_6): 24.1 & 24.4 (Ar–CH_3), 43.2 (C-4′), 66.0 (C-5′), 137.5 (C-5), 140.8 (C-4), 151.9 (C-2), 158.9 (C-3′), 178.1 (C═S), 125.9, 127.2, 128.9, 131.2, 131.9, 133.2, 134.8, 139.6 (aromatic carbons) ppm; MS (*m/z*): 391.49 [$M^{+\bullet}$]; Anal. Calcd. for $C_{21}H_{21}N_5OS$: C, 64.43; H, 5.41; N, 17.89. Found: C, 64.51; H, 5.40; N, 18.01%.

*6.1.1.3. 3′-(4-(4-Chlorophenyl)oxazol-2-ylamino)-5′-(4-chlorophenyl)-4′,5′-dihydro-pyrazole-1′-carbothioamide (**4c**).* Light yellow solid (0.18 g, 76%); m.p. 232–234 °C; IR (KBr): 1345 (C═S), 1566 (C═N), 3255 (NH), 3338, 3435 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 3.20 (dd, 1H, H_x, $J_{Ax} = 6.8$ Hz, $J_{Mx} = 12.0$ Hz), 3.84 (dd, 1H, H_M, $J_{AM} = 15.0$ Hz, $J_{Mx} = 12.0$ Hz), 5.35 (dd, 1H, H_A, $J_{AM} = 15.0$ Hz, $J_{Ax} = 6.8$ Hz), 5.58 (bs, 1H, C_2–NH), 5.72 (bs, 2H, C–NH_2), 7.05–7.68 (m, 9H, Ar–H & C_5–H) ppm; ^{13}C NMR (DMSO-d_6): 42.9 (C-4′), 66.7 (C-5′), 139.2 (C-5), 141.5 (C-4), 152.0 (C-2), 157.8 (C-3′), 178.7 (C═S),

Table 3
MIC, MBC and MFC of compounds **11a, 11c, 12a** and **12c**.

Compound	Minimum inhibitory concentration					
	MIC (MBC/MFC) μg/well					
	S. aureus	*B. subtilis*	*P. aeruginosa*	*K. pneumoniae*	*A. niger*	*P. chrysogenum*
11a	12.5 (50)	50 (200)	50 (>200)	50 (>200)	25 (100)	50 (200)
11c	6.25 (12.5)	25 (100)	50 (200)	25 (100)	12.5 (100)	50 (200)
12a	12.5 (50)	50 (200)	50 (>200)	50 (>200)	12.5 (100)	25 (100)
12c	6.25 (12.5)	50 (200)	50 (200)	50 (200)	6.25 (12.5)	25 (100)
Chloramphenicol	6.25	6.25	6.25	12.5	–	–
Ketoconazole	–	–	–	–	6.25	12.5

(–) No activity.

127.1, 128.3, 129.4, 131.6, 132.2, 134.3, 136.5, 140.8 (aromatic carbons) ppm; MS (*m/z*): 432.33 [$M^{+\bullet}$]; Anal. Calcd. for $C_{19}H_{15}Cl_2N_5OS$: C, 52.78; H, 3.50; N, 16.20. Found: C, 52.91; H, 3.53; N, 16.38%.

*6.1.1.4. 3′-(4-Phenylthiazol-2-ylamino)-5′-phenyl-4′,5′-dihydropyrazole-1′-carbothioamide (**5a**).* Light yellow solid (0.17 g, 71%); m.p. 214–216 °C; IR (KBr): 1336 (C═S), 1560 (C═N), 3246 (NH), 3345, 3446 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 3.16 (dd, 1H, H_x, $J_{Ax} = 6.6$ Hz, $J_{Mx} = 11.5$ Hz), 3.79 (dd, 1H, H_M, $J_{AM} = 14.5$ Hz, $J_{Mx} = 11.5$ Hz), 5.30 (dd, 1H, H_A, $J_{AM} = 14.5$ Hz, $J_{Ax} = 6.6$ Hz), 5.52 (bs, 1H, C_2–NH), 5.64 (bs, 2H, C–NH_2), 7.15–7.74 (m, 11H, Ar–H & C_5–H) ppm; ^{13}C NMR (DMSO-d_6): 43.3 (C-4′), 66.1 (C-5′), 103.2 (C-5), 149.2 (C-4), 158.0 (C-3′), 163.8 (C-2), 177.8 (C═S), 126.5, 127.8, 128.8, 129.6, 131.9, 133.2, 135.7, 141.4 (aromatic carbons) ppm; MS (*m/z*): 379.50 [$M^{+\bullet}$]; Anal. Calcd. for $C_{19}H_{17}N_5S_2$: C, 60.13; H, 4.52; N, 18.45. Found: C, 60.43; H, 4.54; N, 18.60%.

*6.1.1.5. 3′-(4-(4-Methylphenyl)thiazol-2-ylamino)-5′-(4-methylphenyl)-4′,5′-dihydro-pyrazole-1′-carbothioamide (**5b**).* Light yellow solid (0.19 g, 78%); m.p. 241–243 °C; IR (KBr): 1324 (C═S), 1552 (C═N), 3238 (NH), 3332, 3440 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 2.25 & 2.31 (s, 6H, Ar–CH_3), 3.19 (dd, 1H, H_x, $J_{Ax} = 6.5$ Hz, $J_{Mx} = 11.3$ Hz), 3.75 (dd, 1H, H_M, $J_{AM} = 14.3$ Hz, $J_{Mx} = 11.3$ Hz), 5.32 (dd, 1H, H_A, $J_{AM} = 14.3$ Hz, $J_{Ax} = 6.5$ Hz), 5.48 (bs, 1H, C_2–NH), 5.65 (bs, 2H, C–NH_2), 7.10–7.68 (m, 9H, Ar–H & C_5–H) ppm; ^{13}C NMR (DMSO-d_6): 24.0 & 24.2 (Ar–CH_3), 43.8 (C-4′), 65.7 (C-5′), 102.8 (C-5), 148.6 (C-4), 159.5 (C-3′), 163.2 (C-2), 176.3 (C═S), 126.1, 127.3, 128.2, 129.3, 130.9, 132.4, 134.5, 138.8 (aromatic carbons) ppm; MS (*m/z*): 407.55 [$M^{+\bullet}$]; Anal. Calcd. for $C_{21}H_{21}N_5S_2$: C, 61.89; H, 5.19; N, 17.18. Found: C, 61.83; H, 5.20; N, 17.31%.

*6.1.1.6. 3′-(4-(4-Chlorophenyl)thiazol-2-ylamino)-5′-(4-chlorophenyl)-4′,5′-dihydro-pyrazole-1′-carbothioamide (**5c**).* Light yellow solid (0.19 g, 81%); m.p. 248–250 °C; IR (KBr): 1328 (C═S), 1571 (C═N), 3250 (NH), 3339, 3455 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 3.12 (dd, 1H, H_x, $J_{Ax} = 6.8$ Hz, $J_{Mx} = 11.8$ Hz), 3.83 (dd, 1H, H_M, $J_{AM} = 14.9$ Hz, $J_{Mx} = 11.8$ Hz), 5.29 (dd, 1H, H_A, $J_{AM} = 14.9$ Hz, $J_{Ax} = 6.8$ Hz), 5.55 (bs, 1H, C_2–NH), 5.69 (bs, 2H, C–NH_2), 7.18–7.70 (m, 9H, Ar–H & C_5–H) ppm; ^{13}C NMR (DMSO-d_6): 42.4 (C-4′), 66.4 (C-5′), 103.5 (C-5), 149.7 (C-4), 158.6 (C-3′), 164.1 (C-2), 178.1 (C═S), 127.4, 128.6, 129.1, 131.6, 132.8, 133.9, 135.3, 139.6 (aromatic carbons) ppm; MS (*m/z*): 448.39 [$M^{+\bullet}$]; Anal. Calcd. for $C_{19}H_{15}Cl_2N_5S_2$: C, 50.89; H, 3.37; N, 15.62. Found: C, 50.97; H, 3.35; N, 16.79%.

*6.1.1.7. 3′-(4-Phenyl-1H-imidazol-2-ylamino)-5′-phenyl-4′,5′-dihydropyrazole-1′-carbo-thioamide (**6a**).* Light yellow solid (0.19 g, 77%); m.p. 226–228 °C; IR (KBr): 1346 (C═S), 1579 (C═N), 3240 (NH), 3352, 3451 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 3.14 (dd, 1H, H_x, $J_{Ax} = 6.9$ Hz, $J_{Mx} = 12.0$ Hz), 3.86 (dd, 1H, H_M, $J_{AM} = 14.4$ Hz, $J_{Mx} = 12.0$ Hz), 5.33 (dd, 1H, H_A, $J_{AM} = 14.4$ Hz, $J_{Ax} = 6.9$ Hz), 5.62 (bs, 1H, C_2–NH), 5.76 (bs, 2H, C–NH_2), 7.12–7.62 (m, 11H, Ar–H & C_5–H), 11.94 (bs, 1H, C_5–NH) ppm; ^{13}C NMR (DMSO-d_6): 43.9 (C-4′), 65.8 (C-5′), 120.8 (C-5), 137.4 (C-2), 142.0 (C-4), 157.4 (C-3′), 177.6 (C═S), 126.7, 127.1, 127.9, 128.5, 131.4, 134.6, 136.3, 139.6 (aromatic carbons) ppm; MS (*m/z*): 362.45 [$M^{+\bullet}$]; Anal. Calcd. for $C_{19}H_{18}N_6S$: C, 62.96; H, 5.01; N, 23.19. Found: C, 63.08; H, 5.04; N, 23.39%.

*6.1.1.8. 3′-(4-(4-Methylphenyl)-1H-imidazol-2-ylamino)-5′-(4-methylphenyl)-4′,5′-dihydropyrazole-1′-carbothioamide (**6b**).* Light yellow solid (0.18 g, 73%); m.p. 244–246 °C; IR (KBr): 1338 (C═S), 1584 (C═N), 3236 (NH), 3346, 3447 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 2.27 & 2.30 (s, 6H, Ar–CH_3), 3.17 (dd, 1H, H_x, $J_{Ax} = 6.8$ Hz, $J_{Mx} = 11.7$ Hz), 3.77 (dd, 1H, H_M, $J_{AM} = 14.2$ Hz, $J_{Mx} = 11.7$ Hz), 5.27 (dd, 1H, H_A, $J_{AM} = 14.2$ Hz, $J_{Ax} = 6.8$ Hz), 5.60 (bs, 1H, C_2–NH), 5.78 (bs, 2H, C–NH_2), 7.09–7.68 (m, 9H, Ar–H & C_5–H), 11.98 (bs, 1H, C_5–NH) ppm; ^{13}C NMR (DMSO-d_6): 24.1 & 24.2 (Ar–CH_3), 43.2 (C-4′), 66.3 (C-5′), 120.5 (C-5), 137.2 (C-2), 141.7 (C-4), 158.6 (C-3′), 177.8 (C═S), 127.3, 128.0, 129.4, 131.7, 132.3, 133.8, 135.6, 140.6 (aromatic carbons) ppm; MS (*m/z*): 390.50 [$M^{+\bullet}$]; Anal. Calcd. for $C_{21}H_{22}N_6S$: C, 64.59; H, 5.68; N, 21.52. Found: C, 64.66; H, 5.67; N, 21.66%.

*6.1.1.9. 3′-(4-(4-Chlorophenyl)-1H-imidazol-2-ylamino)-5′-(4-chlorophenyl)-4′,5′-dihydropyrazole-1′-carbothioamide (**6c**).* Light yellow solid (0.18 g, 75%); m.p. 263–265 °C; IR (KBr): 1351(C═S), 1575 (C═N), 3249 (NH), 3338, 3458 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 3.15 (dd, 1H, H_x, $J_{Ax} = 7.0$ Hz, $J_{Mx} = 11.8$ Hz), 3.87 (dd, 1H, H_M, $J_{AM} = 14.6$ Hz, $J_{Mx} = 11.8$ Hz), 5.34 (dd, 1H, H_A, $J_{AM} = 14.6$ Hz, $J_{Ax} = 7.0$ Hz), 5.58 (bs, 1H, C_2–NH), 5.78 (bs, 2H, C–NH_2), 7.14–7.70 (m, 9H, Ar–H & C_5–H), 12.06 (bs, 1H, C_5–NH) ppm; ^{13}C NMR (DMSO-d_6): 43.5 (C-4′), 65.6 (C-5′), 121.3 (C-5), 137.8 (C-2), 142.6 (C-4), 157.9 (C-3′), 177.1 (C═S), 127.0, 128.5, 129.4, 130.7, 132.4, 133.5, 135.6, 139.3 (aromatic carbons) ppm; MS (*m/z*): 431.34 [$M^{+\bullet}$]; Anal. Calcd. for $C_{19}H_{16}Cl_2N_6S$: C, 52.91; H, 3.74; N, 19.48. Found: C, 53.00; H, 3.76; N, 19.60%.

*6.1.2. General procedure for the synthesis of 3′-(4-aryloxazol-2-ylamino)-5′-aryl-1′H-pyrazole-1′-carbothioamide (**7a**–**c**)/3′-(4-arylthiazol-2-ylamino)-5′-aryl-1′H-pyrazole-1′-carbothioamide (**8a**–**c**)/3′-(4-aryl-1H-imidazol-2-ylamino)-5′-aryl-1′H-pyrazole-1′-carbothioamide (**9a**–**c**)*

A solution of compound **4**/**5**/**6** (1 mmol) and chloranil (1.2 mmol) in xylene (10 ml) was refluxed for 23–25 h. Then it was treated with 5% NaOH solution. The organic layer was separated, repeatedly washed with water and dried over an. Na_2SO_4. The solvent was removed *in vacuo*. The solid obtained was purified by recrystallization from 2-propanol.

*6.1.2.1. 3′-(4-Phenyloxazol-2-ylamino)-5′-phenyl-1′H-pyrazole-1′-carbothioamide (**7a**).* Yellow solid (0.13 g, 66%); m.p. 228–230 °C; IR (KBr): 1337 (C═S), 1568 (C═N), 1637 (C═C), 3257 (NH), 3337, 3447 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 5.52 (bs, 1H, C_2–NH), 5.65 (bs, 2H, C–NH_2), 7.04 (s, 1H, $C_{4'}$–H), 7.14–7.68 (m, 11H, Ar–H & C_5–H) ppm; ^{13}C NMR (DMSO-d_6): 93.6 (C-4′), 137.8 (C-5), 139.3 (C-4), 144.2 (C-5′), 146.4 (C-3′), 147.1 (C-2), 178.2 (C═S), 127.2, 128.5, 129.6, 131.2, 132.5, 133.9, 135.3, 136.2 (aromatic carbons) ppm; MS (*m/z*): 361.42 [$M^{+\bullet}$]; Anal. Calcd. for $C_{19}H_{15}N_5OS$: C, 63.14; H, 4.18; N, 19.38. Found: C, 63.21; H, 4.22; N, 19.50%.

*6.1.2.2. 3′-(4-(4-Methylphenyl)oxazol-2-ylamino)-5′-(4-methylphenyl)-1′H-pyrazole-1′-carbothioamide (**7b**).* Yellow solid (0.13 g, 68%); m.p. 246–248 °C; IR (KBr): 1334 (C═S), 1573 (C═N), 1632 (C═C), 3260 (NH), 3332, 3439 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 2.26 & 2.28 (s, 6H, Ar–CH_3), 5.55 (bs, 1H, C_2–NH), 5.63 (bs, 2H, C–NH_2), 7.03 (s, 1H, $C_{4'}$–H), 7.12–7.63 (m, 9H, Ar–H & C_5–H) ppm; ^{13}C NMR (DMSO-d_6): 24.2 & 24.4 (Ar–CH_3), 94.3 (C-4′), 137.5 (C-5), 138.8 (C-4), 143.8 (C-5′), 145.2 (C-3′), 147.8 (C-2), 177.8 (C═S), 127.0, 128.7, 129.2, 130.7, 131.9, 133.5, 134.6, 135.8 (aromatic carbons) ppm; MS (*m/z*): 389.47 [$M^{+\bullet}$]; Anal. Calcd. for $C_{21}H_{19}N_5OS$: C, 64.76; H, 4.92; N, 17.98. Found: C, 64.85; H, 4.90; N, 18.13%.

*6.1.2.3. 3′-(4-(4-Chlorophenyl)oxazol-2-ylamino)-5′-(4-chlorophenyl)-1′H-pyrazole-1′-carbothioamide (**7c**).* Yellow solid (0.14 g, 72%); m.p. 258–260 °C; IR (KBr): 1345 (C═S), 1558 (C═N), 1625 (C═C), 3252 (NH), 3348, 3456 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 5.48 (bs, 1H, C_2–NH), 5.66 (bs, 2H, C–NH_2), 7.07 (s, 1H, $C_{4'}$–H), 7.18–7.60 (m, 9H, Ar–H & C_5–H) ppm; ^{13}C NMR (DMSO-d_6): 94.8 (C-4′), 138.1 (C-5), 139.7 (C-4), 144.7 (C-5′), 146.8 (C-3′), 148.1 (C-2), 178.4 (C═S), 127.5, 128.8, 129.4, 130.9, 132.4, 133.5, 134.2, 136.8 (aromatic carbons) ppm; MS (*m/z*): 430.31 [$M^{+\bullet}$]; Anal. Calcd. for

$C_{19}H_{13}Cl_2N_5OS$: C, 53.03; H, 3.05; N, 16.28. Found: C, 52.97; H, 3.09; N, 16.19%.

*6.1.2.4. 3′-(4-Phenylthiazol-2-ylamino)-5′-phenyl-1′H-pyrazole-1′-carbothioamide (**8a**).* Yellow solid (0.14 g, 70%); m.p. 237–239 °C; IR (KBr): 1340 (C=S), 1565 (C=N), 1640 (C=C), 3241 (NH), 3336, 3442 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 5.49 (bs, 1H, C_2–NH), 5.60 (bs, 2H, C–NH_2), 7.02 (s, 1H, $C_{4'}$–H), 7.08–7.65 (m, 11H, Ar–H & C_5–H) ppm; ^{13}C NMR (DMSO-d_6): 94.2 (C-4′), 102.8 (C-5), 143.8 (C-5′), 146.1 (C-3′), 147.3 (C-4), 162.7 (C-2), 177.5 (C=S), 126.4, 127.6, 128.2, 128.9, 130.6, 132.2, 133.9, 135.6 (aromatic carbons) ppm; MS (*m/z*): 377.49 [$M^{+\bullet}$]; Anal. Calcd. for $C_{19}H_{15}N_5S_2$: C, 60.45; H, 4.01; N, 18.55. Found: C, 60.53; H, 3.99; N, 18.69%.

*6.1.2.5. 3′-(4-(4-Methylphenyl)thiazol-2-ylamino)-5′-(4-methylphenyl)-1′H-pyrazole-1′-carbothioamide (**8b**).* Yellow solid (0.13 g, 67%); m.p. 261–263 °C; IR (KBr): 1326 (C=S), 1561 (C=N), 1628 (C=C), 3247 (NH), 3327, 3436 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 2.25 & 2.27 (s, 6H, Ar–CH_3), 5.46 (bs, 1H, C_2–NH), 5.58 (bs, 2H, C–NH_2), 6.98 (s, 1H, $C_{4'}$–H), 7.06–7.66 (m, 9H, Ar–H & C_5–H) ppm; ^{13}C NMR (DMSO-d_6): 24.0 & 24.3 (Ar–CH_3), 94.9 (C-4′), 102.4 (C-5), 142.5 (C-5′), 144.7 (C-3′), 146.0 (C-4), 162.3 (C-2), 176.2 (C=S), 126.6, 127.3, 128.7, 130.3, 131.8, 132.7, 134.3, 135.1 (aromatic carbons) ppm; MS (*m/z*): 405.54 [$M^{+\bullet}$]; Anal. Calcd. for $C_{21}H_{19}N_5S_2$: C, 62.19; H, 4.72; N, 17.27. Found: C, 62.29; H, 4.77; N, 17.40%.

*6.1.2.6. 3′-(4-(4-Chlorophenyl)thiazol-2-ylamino)-5′-(4-chlorophenyl)-1′H-pyrazole-1′-carbothioamide (**8c**).* Yellow solid (0.14 g, 73%); m.p. 269–271 °C; IR (KBr): 1348 (C=S), 1573 (C=N), 1632 (C=C), 3244 (NH), 3342, 3452 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 5.50 (bs, 1H, C_2–NH), 5.59 (bs, 2H, C–NH_2), 7.05 (s, 1H, $C_{4'}$–H), 7.14–7.60 (m, 9H, Ar–H & C_5–H) ppm; ^{13}C NMR (DMSO-d_6): 94.7 (C-4′), 103.1 (C-5), 142.2 (C-5′), 146.9 (C-3′), 147.7 (C-4), 163.0 (C-2), 176.6 (C=S), 126.9, 127.7, 128.4, 130.1, 132.5, 133.3, 134.6, 135.8 (aromatic carbons) ppm; MS (*m/z*): 446.38 [$M^{+\bullet}$]; Anal. Calcd. for $C_{19}H_{13}Cl_2N_5S_2$: C, 51.12; H, 2.94; N, 15.69. Found: C, 51.19; H, 2.97; N, 15.80%.

*6.1.2.7. 3′-(4-Phenyl-1H-imidazol-2-ylamino)-5′-phenyl-1′H-pyrazole-1′-carbothioamide (**9a**).* Yellow solid (0.12 g, 62%); m.p. 250–252 °C; IR (KBr): 1336 (C=S), 1577 (C=N), 1622 (C=C), 3254 (NH), 3340, 3458 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 5.48 (bs, 1H, C_2–NH), 5.74 (bs, 2H, C–NH_2), 6.88 (s, 1H, $C_{4'}$–H), 7.10–7.68 (m, 11H, Ar–H & C_5–H), 11.90 (bs, 1H, C_5–NH) ppm; ^{13}C NMR (DMSO-d_6): 94.3 (C-4′), 121.6 (C-5), 137.4 (C-2), 138.8 (C-4), 146.7 (C-5′), 148.2 (C-3′), 177.6 (C=S), 125.6, 127.1, 129.7, 131.3, 132.5, 133.6, 135.2, 136.3 (aromatic carbons) ppm; MS (*m/z*): 360.44 [$M^{+\bullet}$]; Anal. Calcd. for $C_{19}H_{16}N_6S$: C, 63.31; H, 4.47; N, 23.32. Found: C, 63.36; H, 4.56; N, 23.40%.

*6.1.2.8. 3′-(4-(4-Methylphenyl)-1H-imidazol-2-ylamino)-5′-(4-methylphenyl)-1′H-pyrazole-1′-carbothioamide (**9b**).* Yellow solid (0.13 g, 65%); m.p. 276–278 °C; IR (KBr): 1331 (C=S), 1565 (C=N), 1636 (C=C), 3243 (NH), 3347, 3452 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 2.26 & 2.29 (s, 6H, Ar–CH_3), 5.54 (bs, 1H, C_2–NH), 5.72 (bs, 2H, C–NH_2), 6.86 (s, 1H, $C_{4'}$–H), 7.08–7.62 (m, 9H, Ar–H & C_5–H), 11.93 (bs, 1H, C_5–NH) ppm; ^{13}C NMR (DMSO-d_6): 24.1 & 24.3 (Ar–CH_3), 94.1 (C-4′), 121.3 (C-5), 137.8 (C-2), 138.2 (C-4), 146.4 (C-5′), 147.7 (C-3′), 178.2 (C=S), 125.2, 126.8, 128.4, 129.1, 131.6, 133.2, 134.8, 135.3 (aromatic carbons) ppm; MS (*m/z*): 388.49 [$M^{+\bullet}$]; Anal. Calcd. for $C_{21}H_{20}N_6S$: C, 64.92; H, 5.19; N, 21.63. Found: C, 65.01; H, 5.25; N, 21.79%.

*6.1.2.9. 3′-(4-(4-Chlorophenyl)-1H-imidazol-2-ylamino)-5′-(4-chlorophenyl)-1′H-pyrazole-1′-carbothioamide (**9c**).* Yellow solid (0.13 g, 68%); m.p. 285–287 °C; IR (KBr): 1347 (C=S), 1556 (C=N), 1630 (C=C), 3258 (NH), 3351, 3466 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): 5.56 (bs, 1H, C_2–NH), 5.76 (bs, 2H, C–NH_2), 6.94 (s, 1H, $C_{4'}$–H), 7.12–7.74 (m, 9H, Ar–H & C_5–H), 11.95 (bs, 1H, C_5–NH) ppm; ^{13}C NMR (DMSO-d_6): 94.8 (C-4′), 121.1 (C-5), 138.2 (C-2), 139.5 (C-4), 146.9 (C-5′), 148.7 (C-3′), 177.3 (C=S), 126.3, 127.5, 128.7, 129.3, 130.6, 131.1, 133.8, 135.5 (aromatic carbons) ppm; MS (*m/z*): 429.33 [$M^{+\bullet}$]; Anal. Calcd. for $C_{19}H_{14}Cl_2N_6S$: C, 53.15; H, 3.29; N, 19.57. Found: C, 53.26; H, 3.33; N, 19.74%.

*6.1.3. General procedure for the synthesis of 5′-aryl-N-(4-aryloxazol-2-yl)-1′-(4″-arylthiazol-2″-yl)-1′H-pyrazol-3′-amine (**10a**–**c**)/5′-aryl-N-(4-arylthiazol-2-yl)-1′-(4″-arylthiazol-2″-yl)-1′H-pyrazol-3′-amine (**11a**–**c**)/5′-aryl-N-(4-aryl-1H-imidazol-2-yl)-1′-(4″-arylthiazol-2″-yl)-1′H-pyrazol-3′-amine (**12a**–**c**)*

To a solution of compound **7**/**8**/**9** (1 mmol) in ethanol (10 ml), phenacyl bromide (1 mmol) was added and refluxed for 4–6 h. The solid separated on cooling was filtered and purified by column chromatography using ethyl acetate–hexane (1:1) as eluent.

*6.1.3.1. 5′-Phenyl-N-(4-phenyloxazol-2-yl)-1′-(4″-phenylthiazol-2″-yl)-1′H-pyrazol-3′-amine (**10a**).* Yellow solid (0.16 g, 63%); m.p. 236–238 °C; IR (KBr): 1574 (C=N), 1634 (C=C), 3252 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): 5.47 (bs, 1H, C_2–NH), 6.95 (s, 1H, $C_{4'}$–H), 7.12–7.66 (m, 17H, Ar–H, C_5–H & $C_{5''}$–H) ppm; ^{13}C NMR (DMSO-d_6): 94.4 (C-4′), 112.8 (C-5″), 137.5 (C-5), 138.6 (C-4), 146.0 (C-3′), 150.2 (C-5′), 153.5 (C-4″), 160.8 (C-2″), 162.8 (C-2), 126.5, 127.2, 127.9, 128.9, 129.6, 130.8, 131.5, 132.4, 133.1, 134.7, 135.5, 136.7 (aromatic carbons) ppm; MS (*m/z*): 461.54 [$M^{+\bullet}$]; Anal. Calcd. for $C_{27}H_{19}N_5OS$: C, 70.26; H, 4.15; N, 15.17. Found: C, 70.33; H, 4.13; N, 15.29%.

*6.1.3.2. 5′-(4-Methylphenyl)-N-(4-(4-methylphenyl)oxazol-2-yl)-1′-(4″-(4-methylphenyl)-thiazol-2″-yl)-1′H-pyrazol-3′-amine (**10b**).* Yellow solid (0.16 g, 61%); m.p. 251–252 °C; IR (KBr): 1586 (C=N), 1639 (C=C), 3240 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): 2.26, 2.28 & 2.29 (s, 9H, Ar–CH_3), 5.43 (bs, 1H, C_2–NH), 6.92 (s, 1H, $C_{4'}$–H), 7.06–7.63 (m, 14H, Ar–H, C_5–H & $C_{5''}$–H) ppm; ^{13}C NMR (DMSO-d_6): 24.1, 24.2 & 24.3 (Ar–CH_3), 93.2 (C-4′), 112.5 (C-5″), 137.1 (C-5), 138.9 (C-4), 145.7 (C-3′), 151.9 (C-5′), 153.3 (C-4″), 160.5 (C-2″), 162.4 (C-2), 126.3, 126.9, 127.6, 128.1, 128.5, 130.4, 131.1, 132.6, 133.2, 133.8, 134.7, 136.2 (aromatic carbons) ppm; MS (*m/z*): 503.62 [$M^{+\bullet}$]; Anal. Calcd. for $C_{30}H_{25}N_5OS$: C, 71.55; H, 5.00; N, 13.91. Found: C, 71.61; H, 5.03; N, 14.05%.

*6.1.3.3. 5′-(4-Chlorophenyl)-N-(4-(4-chlorophenyl)oxazol-2-yl)-1′-(4″-(4-chlorophenyl)-thiazol-2″-yl)-1′H-pyrazol-3′-amine (**10c**).* Yellow solid (0.18 g, 69%); m.p. 267–269 °C; IR (KBr): 1578 (C=N), 1636 (C=C), 3234 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): 5.49 (bs, 1H, C_2–NH), 7.00 (s, 1H, $C_{4'}$–H), 7.18–7.72 (m, 14H, Ar–H, C_5–H & $C_{5''}$–H) ppm; ^{13}C NMR (DMSO-d_6): 93.7 (C-4′), 113.2 (C-5″), 137.8 (C-5), 138.4 (C-4), 146.4 (C-3′), 151.4 (C-5′), 153.7 (C-4″), 161.2 (C-2″), 163.1 (C-2), 127.1, 127.8, 128.7, 129.4, 130.6, 131.7, 132.4, 132.9, 133.5, 134.2, 134.8, 136.2 (aromatic carbons) ppm; MS (*m/z*): 564.87 [$M^{+\bullet}$]; Anal. Calcd. for $C_{27}H_{16}Cl_3N_5OS$: C, 57.41; H, 2.85; N, 12.40. Found: C, 57.51; H, 2.87; N, 12.56%.

*6.1.3.4. 5′-Phenyl-N-(4-phenylthiazol-2-yl)-1′-(4″-phenylthiazol-2″-yl)-1′H-pyrazol-3′-amine (**11a**).* Yellow solid (0.17 g, 70%); m.p. 239–241 °C; IR (KBr): 1563 (C=N), 1628 (C=C), 3230 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): 5.42 (bs, 1H, C_2–NH), 6.88 (s, 1H, $C_{4'}$–H), 7.08–7.62 (m, 17H, Ar–H, C_5–H & $C_{5''}$–H) ppm; ^{13}C NMR (DMSO-d_6): 93.8 (C-4′), 102.5 (C-5), 112.4 (C-5″), 145.7 (C-3′), 147.0 (C-4), 149.8 (C-5′), 153.2 (C-4″), 159.4 (C-2″), 162.4 (C-2), 126.2, 126.9, 127.5, 128.6, 129.2, 130.5, 131.2, 131.8, 132.6, 133.3, 134.0, 135.8

(aromatic carbons) ppm; MS (*m/z*): 477.60 [$M^{+\bullet}$]; Anal. Calcd. for $C_{27}H_{19}N_5S_2$: C, 67.90; H, 4.01; N, 14.66. Found: C, 67.99; H, 4.05; N, 14.79%.

*6.1.3.5. 5′-(4-Methylphenyl)-N-(4-(4-methylphenyl)thiazol-2-yl)-1′-(4″-(4-methylphenyl)-thiazol-2″-yl)-1′H-pyrazol-3′-amine (**11b**).* Yellow solid (0.16 g, 65%); m.p. 280–282 °C; IR (KBr): 1570 (C=N), 1620 (C=C), 3226 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): 2.24, 2.26 & 2.28 (s, 9H, Ar–CH_3), 5.43 (bs, 1H, C_2–NH), 6.84 (s, 1H, $C_{4'}$–H), 7.02–7.60 (m, 14H, Ar–H, C_5–H & $C_{5''}$–H) ppm; ^{13}C NMR (DMSO-d_6): 24.0, 24.2 & 24.3 (Ar–CH_3), 92.4 (C-4′), 101.3 (C-5), 112.0 (C-5″), 144.2 (C-3′), 146.6 (C-4), 149.5 (C-5′), 152.8 (C-4″), 160.1 (C-2″), 162.7 (C-2), 126.7, 127.1, 128.2, 128.9, 130.1, 130.7, 131.4, 132.2, 132.9, 133.5, 134.6, 135.2 (aromatic carbons) ppm; MS (*m/z*): 519.68 [$M^{+\bullet}$]; Anal. Calcd. for $C_{30}H_{25}N_5S_2$: C, 69.33; H, 4.85; N, 13.48. Found: C, 69.25; H, 4.83; N, 13.57%.

*6.1.3.6. 5′-(4-Chlorophenyl)-N-(4-(4-chlorophenyl)thiazol-2-yl)-1′-(4″-(4-chlorophenyl)-thiazol-2″-yl)-1′H-pyrazol-3′-amine (**11c**).* Yellow solid (0.17 g, 67%); m.p. 288–290 °C; IR (KBr): 1565 (C=N), 1632 (C=C), 3237 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): 5.45 (bs, 1H, C_2–NH), 6.87 (s, 1H, $C_{4'}$–H), 7.14–7.68 (m, 14H, Ar–H, C_5–H & $C_{5''}$–H) ppm; ^{13}C NMR (DMSO-d_6): 94.1 (C-4′), 101.9 (C-5), 112.7 (C-5″), 146.1 (C-3′), 147.8 (C-4), 150.3 (C-5′), 153.5 (C-4″), 160.7 (C-2″), 163.7 (C-2), 126.5, 127.3, 128.3, 129.4, 130.9, 131.7, 132.1, 132.3, 132.9, 133.7, 135.2, 136.2 (aromatic carbons) ppm; MS (*m/z*): 580.94 [$M^{+\bullet}$]; Anal. Calcd. for $C_{27}H_{16}Cl_3N_5S_2$: C, 55.82; H, 2.78; N, 12.06. Found: C, 55.91; H, 2.82; N, 12.19%.

*6.1.3.7. 5′-Phenyl-N-(4-phenyl-1H-imidazol-2-yl)-1′-(4″-phenyl-thiazol-2″-yl)-1′H-pyrazol-3′-amine (**12a**).* Yellow solid (0.17 g, 68%); m.p. 272–274 °C; IR (KBr): 1580 (C=N), 1637 (C=C), 3240 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): 5.58 (bs, 1H, C_2–NH), 6.93 (s, 1H, $C_{4'}$–H), 7.12–7.74 (m, 17H, Ar–H, C_5–H & $C_{5''}$–H), 12.06 (bs, 1H, C_5–NH) ppm; ^{13}C NMR (DMSO-d_6): 94.2 (C-4′), 113.0 (C-5″), 121.6 (C-5), 137.4 (C-2), 138.9 (C-4), 146.5 (C-3′), 151.7 (C-5′), 154.2 (C-4″), 161.6 (C-2″), 127.6, 128.6, 129.4, 130.8, 131.3, 132.5, 133.1, 134.6, 135.1, 135.7, 136.3, 137.6 (aromatic carbons) ppm; MS (*m/z*): 460.55 [$M^{+\bullet}$]; Anal. Calcd. for $C_{27}H_{20}N_6S$: C, 70.41; H, 4.38; N, 18.25. Found: C, 70.48; H, 4.41; N, 18.19%.

*6.1.3.8. 5′-(4-Methylphenyl)-N-(4-(4-methylphenyl)-1H-imidazol-2-yl)-1′-(4″-(4-methyl-phenyl)thiazol-2″-yl)-1′H-pyrazol-3′-amine (**12b**).* Yellow solid (0.18 g, 71%); m.p.: 263–265 °C; IR (KBr): 1588 (C=N), 1633 (C=C), 3248 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): 2.23, 2.25 & 2.26 (s, 9H, Ar–CH_3), 5.63 (bs, 1H, C_2–NH), 6.90 (s, 1H, $C_{4'}$–H), 7.08–7.66 (m, 14H, Ar–H, C_5–H & $C_{5''}$–H), 12.11 (bs, 1H, C_5–NH) ppm; ^{13}C NMR (DMSO-d_6): 24.1, 24.3 & 24.4 (Ar–CH_3), 93.8 (C-4′), 112.4 (C-5″), 121.2 (C-5), 137.2 (C-2), 139.1 (C-4), 146.8 (C-3′), 152.3 (C-5′), 153.9 (C-4″), 160.5 (C-2″), 127.3, 128.1, 128.9, 129.7, 130.3, 131.3, 132.5, 132.9, 134.2, 135.7, 136.1, 137.3 (aromatic carbons) ppm; MS (*m/z*): 502.63 [$M^{+\bullet}$]; Anal. Calcd. for $C_{30}H_{26}N_6S$: C, 71.69; H, 5.21; N, 16.72. Found: C, 71.64; H, 5.22; N, 16.82%.

*6.1.3.9. 5′-(4-Chlorophenyl)-N-(4-(4-chlorophenyl)-1H-imidazol-2-yl)-1′-(4″-(4-chloro-phenyl)thiazol-2″-yl)-1′H-pyrazol-3′-amine (**12c**).* Yellow solid (0.17 g, 66%); m.p. 277–279 °C; IR (KBr): 1576 (C=N), 1635 (C=C), 3243 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): 5.65 (bs, 1H, C_2–NH), 6.96 (s, 1H, $C_{4'}$–H), 7.18–7.70 (m, 14H, Ar–H, C_5–H & $C_{5''}$–H), 12.08 (bs, 1H, C_5–NH) ppm; ^{13}C NMR (DMSO-d_6): 94.7 (C-4′), 113.8 (C-5″), 120.8 (C-5), 136.7 (C-2), 139.7 (C-4), 147.2 (C-3′), 152.8 (C-5′), 155.2 (C-4″), 162.3 (C-2″), 126.8, 127.4, 128.8, 129.1, 130.6, 131.5, 132.8, 133.1, 133.9, 134.5, 136.1, 137.8 (aromatic carbons) ppm; MS (*m/z*): 563.89 [$M^{+\bullet}$]; Anal. Calcd. for $C_{27}H_{17}Cl_3N_6S$: C, 57.51; H, 3.04; N, 14.90. Found: C, 57.61; H, 2.98; N, 15.02%.

6.2. Biological assays

6.2.1. Cells

The compounds **4**–**12** were dissolved in DMSO at different concentrations of 25, 50 and 100 μg/well. Bacterial strains *S. aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and fungi *A. niger*, *P. chrysogenum* were obtained from the Department of Microbiology, S.V. University, Tirupati.

6.2.2. Antibacterial and antifungal assays

The *in vitro* antimicrobial studies were carried out by agar well diffusion method against test organisms [19,20]. Nutrient broth (NB) plates were swabbed with 24 h old broth culture (100 μl) of test bacteria. Using the sterile cork borer, wells (6 mm) were made into each petriplate. Various concentrations of DMSO dissolved compounds (25, 50 100 μg/well) were added into the wells by using sterile pipettes. The standard antibiotics, Chloramphenicol, for antibacterial activity and Ketoconazole, for antifungal activity (as positive control) were simultaneously tested against the pathogens. The samples were dissolved in DMSO which showed no zone of inhibition acts as a negative control. The plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. After appropriate incubation, the diameter of zone of inhibition of each well was measured. Duplicates were maintained and the average values were calculated for eventual antibacterial activity.

6.2.3. Minimum inhibitory concentration assay

Broth dilution test was used to determine Minimum Inhibitory Concentration (MIC) of the above mentioned samples [21,22]. Freshly prepared nutrient broth was used as diluents. The 24 h old culture of the test bacteria *S. aureus*, *B. subtilis*, *P. aeruginosa* and *K. pneumoniae* and the test fungi *A. niger* and *P. chrysogenum* were diluted 100 fold in nutrient broth (100 μl bacterial cultures in 10 ml NB). The stock solution of the synthesized compounds was prepared in DMSO by dissolving 5 mg of the compound in 1 ml of DMSO. Increasing concentrations of the test samples (1.25, 2.5, 5, 10, 20, 40 μl of stock solution contains 6.25, 12.5, 25, 50, 100, 200 μg of the compounds) were added to the test tubes containing the bacterial and fungal cultures. All the tubes were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. The tubes were examined for visible turbidity and using NB as control. Control without test samples and with solvent was assayed simultaneously. The lowest concentration that inhibited visible growth of the tested organisms was recorded as MIC.

6.2.4. Minimum bactericidal/fungicidal concentration

To determine the Minimum Bactericidal Concentration (MBC) [23] and Minimum Fungicidal Concentration (MFC) [24] for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient broth (for bacteria) and PDA (for fungi) by streaking. Plates inoculated with bacteria and fungi were incubated at 37 °C for 24 h and at 28 °C for 48 h, respectively. After incubation, the lowest concentration was noted as MBC (for bacteria) or MFC (for fungi) at which no visible growth was observed.

Acknowledgments

The authors are grateful to Council of Scientific and Industrial Research (CSIR), New Delhi for financial assistance under major research project. One of the authors, T. Bhanu Prakash is thankful to University Grants Commission (UGC), New Delhi for the sanction of UGC-BSR fellowship.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.06.001.

References

[1] R. Jansen, B. Kunze, V. Wray, H. Reichenbach, E. Jurkiewicz, G. Hunsmann, G. Hofle, Liebigs Ann. 12 (1991) 707–708.
[2] J. Barbier, R. Jansen, H. Irschik, S. Benson, K. Gerth, B. Böhlendorf, G. Höfle, H. Reichenbach, J. Wegner, C. Zeilinger, A. Kirschning, R. Müller, Angew. Chem. Int. Ed. 51 (2012) 1256–1260.
[3] T. Ichiba, W.Y. Yoshida, R.J. Scheuer, T. Higa, D.G. Gravalos, J. Am. Chem. Soc. 113 (1991) 3173–3174.
[4] J.S. Sandler, P.L. Colin, M. Kelly, W. Fenical, J. Org. Chem. 71 (2006) 7245–7251.
[5] R. Raju, O. Gromyko, V. Fedorenko, A. Luzhetskyy, R. Müller, Tetrahedron Lett. 53 (2012) 3009–3011.
[6] Z. Jin, Nat. Prod. Rep. 26 (2009) 382–455.
[7] Z. Jin, Nat. Prod. Rep. 22 (2005) 196–229.
[8] P. Vicini, A. Geronikaki, K. Anastasia, M. Incerti, F. Zani, Bioorg. Med. Chem. 14 (2006) 3859–3864.
[9] S. Bae, H.G. Hahn, K.D. Nam, H. Mah, J. Comb. Chem. 7 (2005) 7–9.
[10] S.M. Sondhi, J. Singh, A. Kumar, H. Jamal, P.P. Gupta, Eur. J. Med. Chem. 44 (2009) 1010–1015.
[11] M. Hranjec, I. Piantanida, M. Kralj, L. Suman, K. Pavelıc, G. Karminski-Zamola, J. Med. Chem. 51 (2008) 4899–4910.
[12] M. Lhassani, O. Chavignon, J.M. Chezal, J.C. Teulade, J.P. Chapat, R. Snoeck, G. Andrei, J. Balzarini, E. De Clercq, A. Gueiffier, Eur. J. Med. Chem. 34 (1999) 271–274.
[13] H. Nakano, T. Inoue, N. Kawasaki, H. Miyataka, H. Matsumoto, T. Taguchi, N. Inagaki, H. Nagai, T. Satoh, Bioorg. Med. Chem. 8 (2000) 373–380.
[14] T.D. Penning, J.J. Talley, S.R. Bertenshaw, J.S. Carter, P.W. Collins, S. Docter, M.J. Graneto, L.F. Lee, J.W. Malecha, J.M. Miyashiro, R.S. Rogers, D.J. Rogier, S.S. Yu, G.D. Anderson, E.G. Burton, J.N. Cogburn, S.A. Gregory, J. Med. Chem. 40 (1997) 1347–1365.
[15] K. Hirai, A. Uchida, R. Ohno, in: P. Boger, K. Hirai, K. Wakabyashi (Eds.), Herbicide Classes in Development, Springer, Heidelberg, Germany, 2002, pp. 179–289.
[16] J.V. Mercader, C.S. Pantaleon, C. Agullo, A. Abad-Somovilla, A. Abad-Fuentes, J. Agric. Food Chem. 56 (2008) 7682–7690.
[17] V. Padmavathi, C. Prema Kumari, B.C. Venkatesh, A. Padmaja, Eur. J. Med. Chem. 46 (2011) 5317–5326.
[18] G.L. French, J. Antimicrob. Chemother. 58 (2006) 1107–1117.
[19] K.T. Chung, W.R. Thomasson, C.D. Wu-Yuan, J. Appl. Bacteriol. 69 (1990) 498–503.
[20] C. Azoro, World J. Biotechnol. 3 (2002) 347–357.
[21] D. Janovska, K. Kubikova, L. Kokoska, Czech J. Food Sci. 21 (2003) 107–110.
[22] J. Bishnu, L. Sunil, S. Anuja, J. Sci. Eng. Technol. 5 (2009) 143–150.
[23] Clinical and Laboratory Standards Institute, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, seventh ed., 2006. Approved standard, CLSI Document M7-A7, Ways, Pennsylvania.
[24] Clinical and Laboratory Standards Institute, Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, 2002. Approved Standard NCCLS Document M27-A2, Ways, Pennsylvania.