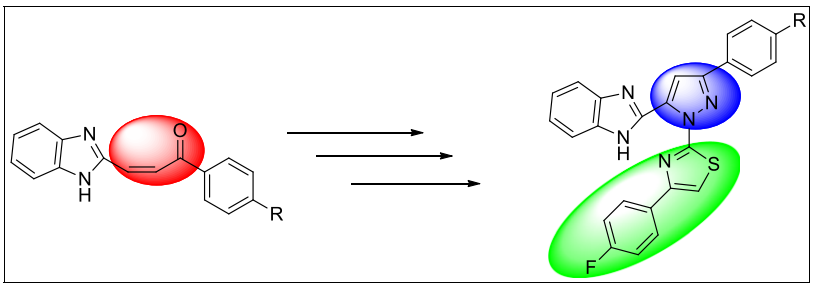
Design and Synthesis of Some New Benzimidazole Containing Pyrazoles and Pyrazolyl Thiazoles as Potential Antimicrobial Agents

Nemallapudi Bakthavatchala Reddy,[a] Grigory V. Zyryanov,[a,e] Guda Mallikarjuna Reddy,[a,b] Avula Balakrishna,[c] Adivireddy Padmaja,[d] Venkatapuram Padmavathi,[d] Cirandur Suresh Reddy,[d] Jarem Raul Garcia,[b] and Gundala Sravya[a]*

[a]Chemical Engineering Institute, Ural Federal University, Yekaterinburg 620002, Russia
[b]Department of Chemistry, State University of Ponta Grossa, Ponta Grossa 84030–900, Parana, Brazil
[c]Rajeev Gandhi Memorial College of Engineering and Technology (Autonomous), Nandyal 518501, Andhra Pradesh, India
[d]Department of Chemistry, Sri Venkateswara University, Tirupati 517 502, Andhra Pradesh, India
[e]Ural Division of the Russian Academy of Sciences, I. Ya. Postovskiy Institute of Organic Synthesis, 22 S. Kovalevskoy Street, Yekaterinburg 620219, Russia
*E-mail: sravyas72@yahoo.com
Received July 31, 2018
DOI 10.1002/jhet.3435
Published online 00 Month 2018 in Wiley Online Library (wileyonlinelibrary.com).



A new class of bis heterocycles-benzimidazolyl pyrazoles were prepared from the Michael acceptor (*E*)-3-(1*H*-benzimidazol-2-yl)-1-aryl-prop-2-en-1-one. The thiamide group was exploited to develop thiazole ring on treatment with *p*-fluorophenacyl bromide to get tris heterocycles. All the lead compounds were tested for antimicrobial activity. The compound **7d** having nitro substituent on the aromatic ring showed greater antimicrobial activity particularly against *Pseudomonas aeruginosa* and *Penicillium chrysogenum*.

J. Heterocyclic Chem., **00,** 00 (2018).

INTRODUCTION

Among heterocyclic pharmacophores, the benzimidazole ring system is quite common, which was first synthesized by Hoebrecker and subsequent exploration by Ladnberg and Wundt during late 1870s [1–3]. Such a heterocyclic scaffolds "benzimidazole" has been known as a "major scaffold" considering for their broad spectrum of biological profiles and empathies towards different targets [4,5]. The benzimidazole nucleus is considered as an important heterocyclic system due to its presence in a wide range of bioactive compounds [6]. Bendamustine [7] and Veliparib [8] are benzimidazole-based drugs approved for the cancers treatment (Fig. 1). The different substitutions on benzimidazole and its derivatives have been luring researchers throughout the world to investigate their therapeutic potential [9–12]. The compounds with pyrazole moieties are the most prominent class in active pharmaceutical drugs and agrochemicals in controlling infections, diseases, and pests [13]. In recent years, several drugs have been developed from pyrazole derivatives. For example, celecoxib demonstrates anti-inflammatory effects and inhibits COX-2; rimonabant functions as a cannabinoid receptor and is utilized to treat obesity; fomepizole inhibits alcohol dehydrogenase; and sildenafil inhibits phosphodiesterase [14] (Fig. 1).

Moreover, pyrazole derivatives have showed significant biological activities, such as antimicrobial [15], analgesic [16], anti-inflammatory [17], and anticancer [18] activities. The significance of thiazoles is emphasized by the fact that various drugs originate from them, such as antifungal agent (ravuconazole) [19] and antiulcer agent (nizatidine) [20] (Fig. 1). Besides, thiazolyl group is of great importance as it appears frequently in the structures of various natural products and biologically active compounds like thiamine (vitamin B) and also in some antibiotic drugs like penicillin, micrococcin [21], and many metabolic products of fungi and primitive marine animals. In addition, pyrazolyl thiazole scaffolds have been receiving several medical and pharmaceutical applications. They have potent antiviral [22], anti-inflammatory [23], and antimicrobial activities [24,25] as well as EP1 receptor antagonists [26,27]. Motivated by the aforesaid findings and pursuing our studies on different five-membered heterocycles [28], we were designed to synthesize a new series of benzimidazole linked pyrazolyl thiazoles and tested them as antimicrobial agents.

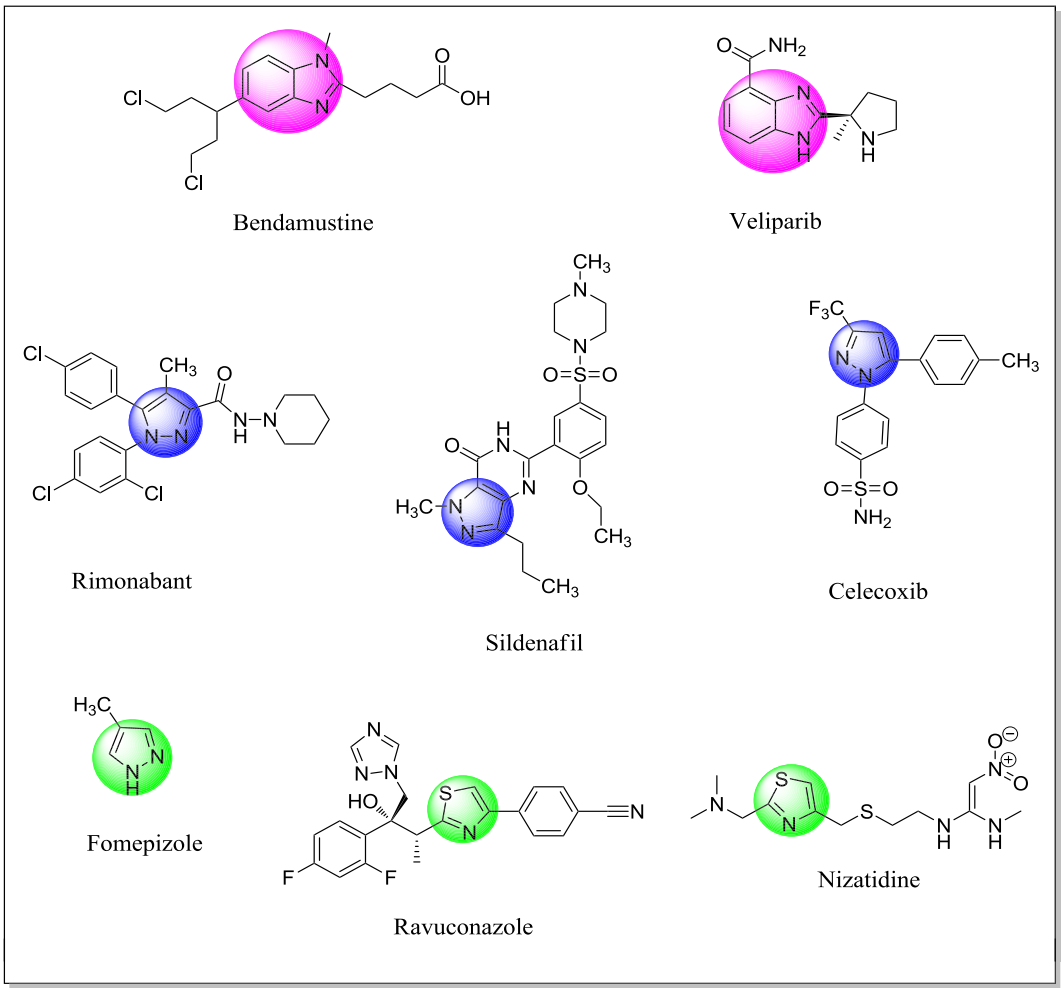


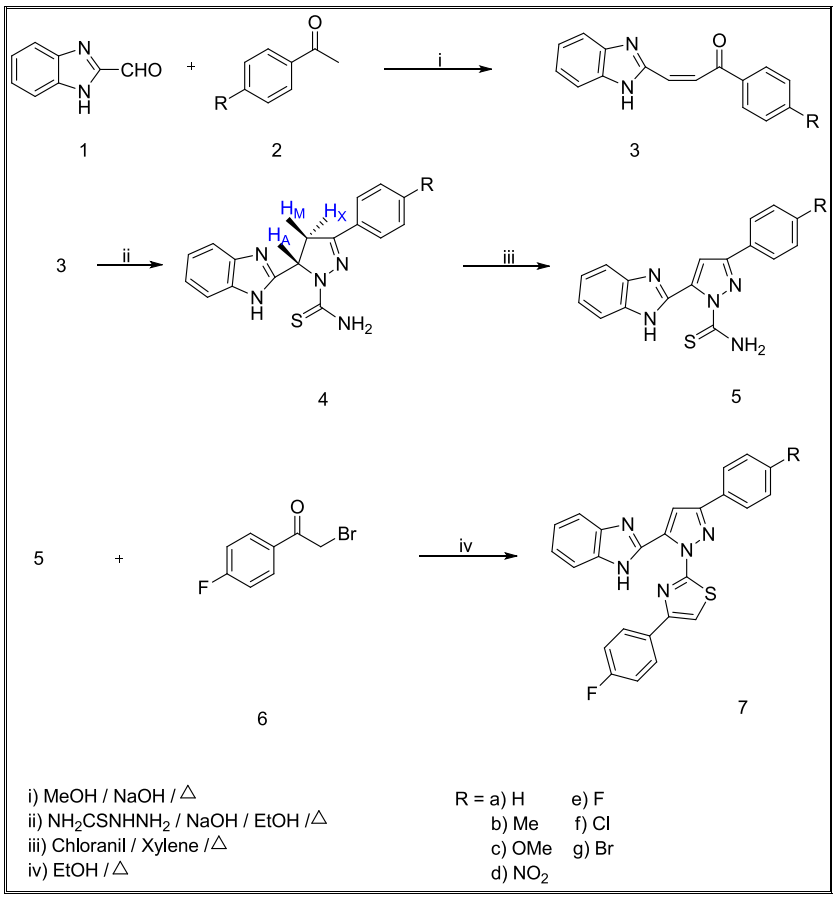
Figure 1. Drugs containing benzimidazole, pyrazole, and thiazole units. [Color figure can be viewed at wileyonlinelibrary.com]

RESULTS AND DISCUSSION

A new class of bis and tris heterocycles—4,5-dihydro-5-(1*H*-benzimidazol-2-yl)-3-aryl-pyrazole-1-carbothioamide (**4**), 5-(1*H*-benzimidazol-2-yl)-3-aryl-1*H*-pyrazole-1-carbothioamide (**5**), 2-(5-(1*H*-benzimidazol-2-yl)-3-aryl-1*H*-pyrazol-1-yl)-4-(4-fluorophenyl)thiazole (**7**)—were synthesized from (*E*)-3-(1*H*-benzimidazol-2-yl)-1-aryl-prop-2-en-1-one (**3**) (Scheme 1). In fact, the compound **3** was prepared by the Claisen–Schmidt reaction of benzimidazole-2-carboxaldehyde (**1**) and aryl ketones (**2**) in the presence of NaOH in methanol. The compound **4** was prepared by the reaction of **3** with thiosemicarbazide in the presence of NaOH in ethanol. The ^{1}H-NMR spectrum of **4a** exhibited an AMX splitting pattern for pyrazoline ring protons. The three doublets of doublets appeared at δ 4.14, 3.93, and 3.51 ppm were assigned to H_A, H_M, and H_X, respectively. The coupling constant values J_{AM} = 12.3, J_{MX} = 10.3, and J_{AX} = 6.3 Hz indicated that H_A and H_M are cis, H_A and H_x are trans, and H_M and H_X are geminal. In addition, two broad singlets were observed at δ 10.14 and 8.49 ppm due to NH and NH_2, respectively, which disappeared on deuteration.

Oxidation of **4** with chloranil in xylene produced the aromatized product **5**. The ^{1}H-NMR spectrum of **5a** showed a singlet at δ 6.31 and two broad singlets at 8.57 and 10.08 ppm due to C_4–H, NH_2, and NH, respectively. The signals due to highly acidic protons disappeared when D_2O was added. Moreover, exploitation of compound **5** furnished compound **7**. Thus, the cyclocondensation reaction of **5** with *p*-fluorophenacyl bromide (**6**) resulted in 2-(5-(1*H*-benzimidazol-2-yl)-3-aryl-1*H*-pyrazol-1-yl)-4-(4-fluorophenyl)thiazole (**7**). The ^{1}H-NMR spectrum of **7a** displayed a singlet at δ 6.37 and a broad singlet at 10.10 ppm due to C_4–H and NH. Another singlet corresponding to C_5–H was observed at downfield region and merged with aromatic protons. The structures of all the compounds were further confirmed by IR, ^{13}C-NMR, mass spectra, and elemental analyses.

Scheme 1. [Color figure can be viewed at wileyonlinelibrary.com]



Antibacterial activity. The compounds **4**, **5**, and **7** were screened for antibacterial activity at four different concentrations 12.5, 25, 50, and 100 µg per well against *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive bacteria), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (Gram-negative bacteria), and Chloramphenicol, which was used as standard drug. The results of antibacterial activity shown in Table S1 specified that Gram-negative bacteria were more vulnerable towards the tested compounds than Gram-positive ones. It was observed that bis and tris heterocyclic compounds (**5** and **7**) showed slightly higher activity than the respective mono heterocyclic system (**4**). This may be due to the presence of electron withdrawing groups (NO_2, F, Cl, and Br) in bis heterocyclic compounds. The compounds having electron withdrawing groups (**5d**, **5e**, **5f**, **5g**, **7d**, **7e**, **7f**, and **7g**) were more active than those having donating groups (OCH_3 and CH_3). In fact, the compound **5d** showed excellent activity against *P. aeruginosa* when compared with the standard drug Chloramphenicol (Table S1 and Fig. S2). This may be due to the presence of more electronegative chlorine on the aromatic ring. Among bis heterocyclic compounds, aromatized derivatives (**5a–g**) were more effective. On the other hand, the non-aromatized (**4a–g**) compounds were inactive. This may be due to the greater electron withdrawing capacity of the aromatized compounds. Moreover, it was observed that pyrazolyl thiazole containing tris heterocycles displayed slightly higher activity than the respective pyrazole containing bis heterocycles.

Antifungal activity. All the tested compounds inhibited the spore germination against tested fungi. In general, most of the compounds showed slightly higher antifungal activity towards *Aspergillus niger* than *Penicillium chrysogenum*. Among all the compounds, **7d** displayed greater inhibitory activity particularly against *P. chrysogenum* when compared with the standard drug Ketoconazole (Table S2 and Fig. S3). Moreover, compounds **5d**, **5e**, **5f**, **5g**, **7e**, **7f**, and **7g** exhibited good activity. In fact, compounds having pyrazole and thiazole in combination with benzimidazole displayed high inhibitory activity than others.

Minimum inhibitory concentration, minimum bactericidal concentration, and minimum fungicidal concentration of the compounds 5d, **7d**, and **7e**. The minimum inhibitory concentration (MIC), minimum bactericidal concentration

(MBC), and minimum fungicidal concentration (MFC) values of the compounds tested are listed in Table S3. 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 MIC is determined. The antimicrobials are usually regarded as bactericidal/fungicidal if the MBC/MFC is not greater than four times the MIC [29]. The compound **7d** exhibited low MIC values when compared with **5d** and **7e**. In addition, MBC value in **7d** is 2 × MIC in case of *P. aeruginosa*, and MFC value is 2 × MIC in case of *P. chrysogenum*. However, the other compounds showed bactericidal and fungicidal effects greater than 2 × MIC.

The structure–antimicrobial activity relationship of the synthesized compounds revealed that tris heterocyclic compounds have greater activity than the corresponding bis heterocycles. Among tris heterocyclic systems, the nitro substituted **7d** displayed excellent antibacterial activity against *P. aeruginosa* with an inhibition zone of 34 mm at 100 μg per well and MIC and MBC of 6.25 and 12.5 μg/mL, respectively. The compound **5d** also displayed strong antifungal activity against *P. chrysogenum* with an inhibition zone of 41 mm at 100 μg per well and MIC and MFC of 12.5 and 25 μg/mL, respectively. Moreover, it was observed that the compounds having nitro substituent on aromatic ring enhanced the activity when compared with electron donating compounds.

Experimental procedure for antimicrobial activity. The *in vitro* antimicrobial studies were carried out by agar well diffusion method against test organisms [30,31]. 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. The compounds were dissolved in dimethyl sulfoxide (DMSO) of 5 mg/mL, and from this, 2.5, 5, 10, and 20 μL (12.5, 25, 50, and 100 μg per well) were added into the wells by using sterile pipettes. Simultaneously, the standard antibiotics, Chloramphenicol for antibacterial activity and Ketoconazole for antifungal activity (as positive control), were tested against the pathogens. The samples were dissolved in DMSO, which showed that no zone of inhibition acts as 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 antimicrobial activity.

Broth dilution test is used to determine MIC of the aforementioned samples [32,33]. Freshly prepared NB 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* was diluted 100 folds in NB (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, and 40 μL of stock solution contains 6.25, 12.5, 25, 50, 100, and 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.

To determine the MBC [34] and MFC [35] for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes that did not show any growth and inoculated on sterile NB (for bacteria) and Potato Dextrose Agar (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.

CONCLUSION

In conclusion, we have prepared a new class of bis heterocycles-benzimidazolyl pyrazoles from the Michael acceptor (*E*)-3-(1*H*-benzimidazol-2-yl)-1-aryl-prop-2-en-1-one. The thiamide group was exploited to develop thiazole ring on treatment with *p*-fluorophenacyl bromide to get tris heterocycles. All the lead compounds were tested for antimicrobial activity. The compound **7d** having nitro substituent on the aromatic ring showed greater antimicrobial activity particularly against *P. aeruginosa* and *P. chrysogenum*.

EXPERIMENTAL

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The ^{1}H-NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Jeol JNM λ-400 MHz spectrometer. The ^{13}C-NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Jeol JNM spectrometer operating at λ-100 MHz. High-resolution mass spectra were recorded on Micromass Q-TOF micromass spectrometer using electro spray ionization.

All chemical shifts were reported in δ (ppm) using TMS as an internal standard. The microanalyses were performed on a Perkin-Elmer 240C elemental analyzer. The temperature was measured by flexible probe throughout the reaction.

General procedure for the synthesis of 4,5-dihydro-5-(1H-benzimidazol-2-yl)-3-aryl-pyrazole-1-carbothioamide (4a–g). An equimolar (1 mmol) mixture of compound **3** and thiosemicarbazide, ethanol (3 mL), and sodium hydroxide (1.5 mmol) was added. It was refluxed for 7–8 h. After completion of the reaction (monitored by TLC), the contents of the flask were poured onto crushed ice. The separated solid was collected by filtration and purified by recrystallization from isopropyl alcohol.

4,5-Dihydro-5-(1H-benzimidazol-2-yl)-3-phenylpyrazole-1-carbothioamide (4a). Yellow solid; yield 76%; m.p. 146–148°C; IR (KBr) (cm^{-1}): 3445, 3336 (NH_2), 3229 (NH), 1584 (C=N), 1365 (C=S); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.51 (dd, 1H, H_X, J_{AX} = 6.3 Hz, J_{MX} = 10.3 Hz), 3.93 (dd, 1H, H_M, J_{AM} = 12.3 Hz, J_{MX} = 10.3 Hz), 4.14 (dd, 1H, H_A, J_{AM} = 12.3 Hz, J_{AX} = 6.3 Hz), 8.49 (bs, 2H, NH_2), 7.21–7.71 (m, 9H, Ar–H), 10.14 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 43.6 (C-4), 57.2 (C-5), 118.5, 126.6, 131.1, 131.9, 133.7, 137.8, 140.8, 143.5, 154.4 (aromatic carbons), 174.3 (C=S) ppm; MS (*m/z*): 344.3888 [M + Na]; *Anal*. Calcd for $C_{17}H_{15}N_5S$: C, 63.53; H, 4.70; N, 21.79%; found: C, 63.62; H, 4.71; N, 21.92%.

4,5-Dihydro-5-(1H-benzimidazol-2-yl)-3-p-tolylpyrazole-1-carbothioamide (4b). Yellow solid; yield 72%; m.p. 137–139°C; IR (KBr) (cm^{-1}): 3438, 3332 (NH_2), 3236 (NH), 1575 (C=N), 1333 (C=S); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.36 (s, 3H, Ar–CH_3), 3.49 (dd, 1H, H_X, J_{AX} = 6.1 Hz, J_{MX} = 10.2 Hz), 4.07 (dd, 1H, H_M, J_{AM} = 12.1 Hz, J_{MX} = 10.2 Hz), 4.87 (dd, 1H, H_A, J_{AM} = 12.1 Hz, J_{AX} = 6.1 Hz), 8.32 (bs, 2H, NH_2), 7.22–7.76 (m, 8H, Ar–H), 10.08 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 21.8 (Ar–CH3), 43.1 (C-4), 56.5 (C-5), 118.3, 126.2, 130.4, 131.2, 133.5, 137.4, 140.5, 143.2, 154.1 (aromatic carbons), 173.9 (C=S) ppm; MS (*m/z*): 358.4147 [M + Na]; *Anal*. Calcd for $C_{18}H_{17}N_5S$: C, 64.45; H, 5.11; N, 20.88%; found: C, 64.55; H, 5.12; N, 21.06%.

4,5-Dihydro-5-(1H-benzimidazol-2-yl)-3-(p-methoxyphenyl) pyrazole-1-carbothioamide (4c). Yellow solid; yield 70%; m.p. 140–142°C; IR (KBr) (cm^{-1}): 3440, 3331 (NH_2), 3239 (NH), 1578 (C=N), 1339 (C=S); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.45 (dd, 1H, H_X, J_{AX} = 6.1 Hz, J_{MX} = 10.2 Hz), 3.88 (dd, 1H, H_M, J_{AM} = 12.1 Hz, J_{MX} = 10.2 Hz), 3.81 (s, 3H, Ar–OCH_3), 4.01 (dd, 1H, H_A, J_{AM} = 12.1 Hz, J_{AX} = 6.1 Hz), 8.27 (bs, 2H, NH_2), 7.06–7.95 (m, 8H, Ar–H), 10.02 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 42.9 (C-4), 56.7 (Ar–OCH_3), 56.1 (C-5), 118.1, 126.1, 130.0, 131.1, 133.2, 137.3, 140.1, 143.0, 153.9 (aromatic carbons), 173.5 (C=S) ppm; MS (*m/z*): 374.4163 [M + Na]; *Anal*. Calcd for $C_{18}H_{17}N_5OS$: C, 61.52; H, 4.88; N, 19.93%; found: C, 61.63; H, 4.90; N, 20.15%.

4,5-Dihydro-5-(1H-benzimidazol-2-yl)-3-(p-nitrophenyl) pyrazole-1-carbothioamide (4d). Yellow solid; yield 84%; m.p. 172–174°C; IR (KBr) (cm^{-1}): 3426, 3333 (NH_2), 3231 (NH), 1570 (C=N), 1338 (C=S); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.65 (dd, 1H, H_X, J_{AX} = 6.8 Hz, J_{MX} = 10.8 Hz), 4.12 (dd, 1H, H_M, J_{AM} = 12.8 Hz, J_{MX} = 10.8 Hz), 4.52 (dd, 1H, H_A, J_{AM} = 12.8 Hz, J_{AX} = 6.8 Hz), 8.65 (bs, 2H, NH_2), 7.26–8.37 (m, 8H, Ar–H), 10.48 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 45.2 (C-4), 58.8 (C-5), 119.4, 127.6, 132.3, 132.9, 135.0, 139.3, 142.3, 144.8, 155.7 (aromatic carbons), 175.3 (C=S) ppm; MS (*m/z*): 389.3875 [M + Na]; *Anal*. Calcd for $C_{17}H_{14}N_6O_2S$: C, 55.73; H, 3.85; N, 22.94%; found: C, 55.81; H, 3.87; N, 23.18%.

4,5-Dihydro-5-(1H-benzimidazol-2-yl)-3-(p-fluorophenyl) pyrazole-1-carbothioamide (4e). Yellow solid; yield 81%; m.p. 166–168°C; IR (KBr) (cm^{-1}): 3437, 3329 (NH_2), 3237 (NH), 1572 (C=N), 1333 (C=S); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.02 (dd, 1H, H_X, J_{AX} = 6.7 Hz, J_{MX} = 10.7 Hz), 4.03 (dd, 1H, H_M, J_{AM} = 12.6 Hz, J_{MX} = 10.7 Hz), 4.46 (dd, 1H, H_A, J_{AM} = 12.6 Hz, J_{AX} = 6.7 Hz), 8.60 (bs, 2H, NH_2), 7.25–8.08 (m, 8H, Ar–H), 10.39 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 44.8 (C-4), 58.2 (C-5), 119.3, 127.4, 132.1, 132.5, 134.8, 139.0, 142.1, 144.5, 155.4 (aromatic carbons), 175.0 (C=S) ppm; MS (*m/z*): 362.3789 [M + Na]; *Anal*. Calcd for $C_{17}H_{14}FN_5S$: C, 60.16; H, 4.16; 5.88; N, 20.64%; found: C, 60.24; H, 4.18; N, 20.81%.

4,5-Dihydro-5-(1H-benzimidazol-2-yl)-3-(p-chlorophenyl) pyrazole-1-carbothioamide (4f). Yellow solid; yield 79%; m.p. 158–160°C; IR (KBr) (cm^{-1}): 3427, 3319 (NH_2), 3228 (NH), 1561 (C=N), 1322 (C=S); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.58 (dd, 1H, H_X, J_{AX} = 6.5 Hz, J_{MX} = 10.5 Hz), 3.98 (dd, 1H, H_M, J_{AM} = 12.5 Hz, J_{MX} = 10.5 Hz), 4.32 (dd, 1H, H_A, J_{AM} = 12.5 Hz, J_{AX} = 6.5 Hz), 8.58 (bs, 2H, NH_2), 7.24–7.87 (m, 8H, Ar–H), 10.26 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 44.1 (C-4), 57.9 (C-5), 119.1, 127.2, 131.6, 132.3, 134.5, 138.7, 141.7, 144.1, 155.2 (aromatic carbons), 174.8 (C=S) ppm; MS (*m/z*): 378.8351 [M + Na]; *Anal*. Calcd for $C_{17}H_{14}ClN_5S$: C, 57.38; H, 3.97; N, 19.68%; found: C, 57.49; H, 4.00; N, 19.86%.

4,5-Dihydro-5-(1H-benzimidazol-2-yl)-3-(p-bromophenyl) pyrazole-1-carbothioamide (4g). Yellow solid; yield 78%; m.p. 152–154°C; IR (KBr) (cm^{-1}): 3447, 3339 (NH_2), 3248 (NH), 1582 (C=N), 1343 (C=S); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.54 (dd, 1H, H_X, J_{AX} = 6.3 Hz, J_{MX} = 10.4 Hz), 3.96 (dd, 1H, H_M, J_{AM} = 12.4 Hz, J_{MX} = 10.4 Hz), 4.20 (dd, 1H, H_A, J_{AM} = 12.4 Hz, J_{AX} = 6.3 Hz), 8.54 (bs, 2H, NH_2), 7.23–

7.79 (m, 8H, Ar–H), 10.19 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 43.8 (C-4), 57.5 (C-5), 118.7, 127.0, 131.2, 132.0, 134.1, 138.2, 141.1, 143.8, 154.9 (aromatic carbons), 174.6 (C=S) ppm; MS (*m/z*): 423.2864 [M + Na]; *Anal*. Calcd for $C_{17}H_{14}BrN_5S$: C, 51.01; H, 3.53; N, 17.50%; found: C, 51.14; H, 3.54; N, 17.69%.

General procedure for the synthesis of 5-(1H-benzimidazol-2-yl)-3-aryl-1H-pyrazole-1-carbothioamide (5a–g). A solution of compound **4** (1 mmol) and chloranil (1.2 mmol) in xylene (10 mL) was refluxed for 24 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 isopropyl alcohol.

5-(1H-benzimidazol-2-yl)-3-phenyl-1H-pyrazole-1-carbothioamide (5a). Yellow solid; yield 78%; m.p. 141–143°C; IR (KBr) (cm^{-1}): 3437, 3329 (NH_2), 3237 (NH), 1571 (C=N), 1332 (C=S); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.31 (s, 1H, C_4–H), 7.28–7.99 (m, 9H, Ar–H), 8.57 (bs, 2H, NH_2), 10.08 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 103.6 (C-4), 115.5, 123.7, 127.4, 128.4, 129.1, 129.8, 133.1, 140.9, 141.7, 150.5, 152.3 (aromatic carbons), 176.7 (C=S) ppm; MS (*m/z*): 342.3745 [M + Na]; *Anal*. Calcd for $C_{17}H_{13}N_5S$: C, 63.93; H, 4.10; N, 21.93%; found: C, 64.09; H, 4.12; N, 22.20%.

5-(1H-benzimidazol-2-yl)-3-p-tolyl-1H-pyrazole-1-carbothioamide (5b). Yellow solid; yield 74%; m.p. 149–152°C; IR (KBr) (cm^{-1}): 3438, 3330 (NH_2), 3239 (NH), 1574 (C=N), 1333 (C=S); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.36 (s, 3H, Ar–CH_3), 6.70 (s, 1H, C_4–H), 7.23–8.06 (m, 8H, Ar–H), 8.51 (bs, 2H, NH_2), 10.05 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 21.5 (Ar–CH3), 103.5 (C-4), 115.3, 123.1, 127.3, 127.1, 129.0, 128.6, 132.7, 140.6, 141.5, 150.1, 151.9 (aromatic carbons), 176.4 (C=S) ppm; MS (*m/z*): 356.4011 [M + Na]; *Anal*. Calcd for $C_{18}H_{15}N_5S$: C, 64.84; H, 4.53; N, 21.01%; found: C, 64.95; H, 4.55; N, 21.22%.

5-(1H-benzimidazol-2-yl)-3-(p-methoxyphenyl)-1H-pyrazole-1-carbothioamide (5c). Yellow solid; yield 72%; m.p. 126–128°C; IR (KBr) (cm^{-1}): 3443, 3335 (NH_2), 3244 (NH), 1577 (C=N), 1338 (C=S); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.81 (s, 3H, Ar–OCH_3), 6.63 (s, 1H, C_4–H), 7.21–8.01 (m, 8H, Ar–H), 8.49 (bs, 2H, NH_2), 10.02 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 103.2 (C-4), 57.2 (Ar–OCH_3), 115.0, 122.7, 127.0, 126.8, 128.4, 128.3, 132.5, 140.4, 141.1, 149.3, 151.6 (aromatic carbons), 176.1 (C=S) ppm; MS (*m/z*): 372.3981 [M + Na]; *Anal*. Calcd for $C_{18}H_{15}N_5OS$: C, 61.87; H, 4.33; N, 20.04%; found: C, 61.99; H, 4.34; N, 20.21%.

5-(1H-benzimidazol-2-yl)-3-(p-nitrophenyl)-1H-pyrazole-1-carbothioamide (5d). Yellow solid; yield 86%; m.p. 167–169°C; IR (KBr) (cm^{-1}): 3426, 3315 (NH_2), 3222 (NH), 1560 (C=N), 1321 (C=S); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.92 (s, 1H, C_4–H), 7.32–8.15 (m, 8H, Ar–H), 8.74 (bs, 2H, NH_2), 10.19 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 104.8 (C-4), 116.8, 124.6, 128.9, 129.7, 130.6, 131.1, 134.7, 142.8, 151.6, 153.3 (aromatic carbons), 177.6 (C=S) ppm; MS (*m/z*): 387.3721 [M + Na]; *Anal*. Calcd for $C_{17}H_{12}N_6O_2S$: 56.04; H, 3.32; N, 23.06%; found: C, 56.15; H, 3.35; N, 23.39%.

5-(1H-benzimidazol-2-yl)-3-(p-fluorophenyl)-1H-pyrazole-1-carbothioamide (5e). Yellow solid; yield 83%; m.p. 162–164°C; IR (KBr) (cm^{-1}): 3422, 3314 (NH_2), 3223 (NH), 1566 (C=N), 1321 (C=S); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.90 (s, 1H, C_4–H), 7.30–8.14 (m, 8H, Ar–H), 8.71 (bs, 2H, NH_2), 10.17 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 104.5 (C-4), 116.5, 124.3, 128.6, 129.4, 130.3, 130.8, 134.0, 141.9, 142.5, 151.3, 153.0 (aromatic carbons), 177.4 (C=S) ppm; MS (*m/z*): 360.3633 [M + Na]; *Anal*. Calcd for $C_{17}H_{12}FN_5S$: C, 60.52; H, 3.59; N, 20.76%; found: C, 63.62; H, 3.61; N, 20.89%.

5-(1H-benzimidazol-2-yl)-3-(p-chlorophenyl)-1H-pyrazole-1-carbothioamide (5f). Yellow solid; yield 81%; m.p. 158–160°C; IR (KBr) (cm^{-1}): 3417, 3309 (NH_2), 3207 (NH), 1551 (C=N), 1312 (C=S); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.87 (s, 1H, C_4–H), 7.29–8.12 (m, 8H, Ar–H), 8.69 (bs, 2H, NH_2), 10.14 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 104.1 (C-4), 116.2, 124.0, 128.3, 129.1, 130.0, 129.6, 133.7, 141.6, 142.2, 151.0, 152.7 (aromatic carbons), 177.1 (C=S) ppm; MS (*m/z*): 376.8192 [M + Na]; *Anal*. Calcd for $C_{17}H_{12}ClN_5S$: C, 60.45; H, 3.58; N, 20.73%; found: C, 60.60; H, 3.59; N, 20.90%.

5-(1H-benzimidazol-2-yl)-3-(p-bromophenyl)-1H-pyrazole-1-carbothioamide (5g). Yellow solid; yield 78%; m.p. 153–155°C; IR (KBr) (cm^{-1}): 3459, 3352 (NH_2), 3258 (NH), 1592 (C=N), 1353 (C=S); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.78 (s, 1H, C_4–H), 7.27–8.08 (m, 8H, Ar–H), 8.62 (bs, 2H, NH_2), 10.10 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 103.9 (C-4), 115.9, 123.7, 128.0, 127.6, 129.7, 129.3, 133.4, 141.3, 141.9, 150.7, 152.4 (aromatic carbons), 176.9 (C=S) ppm; MS (*m/z*): 421.2699 [M + Na]; *Anal*. Calcd for $C_{17}H_{12}BrN_5S$: C, 53.42; H, 3.16; N, 18.32%; found: C, 53.51; H, 3.17; N, 18.52%.

General procedure for the synthesis of 2-(5-(1H-benzimidazol-2-yl)-3-aryl-1H-pyrazol-1-yl)-4-(4-fluorophenyl) thiazole (7a–g). A solution of compound **5** (1 mmol) in ethanol (10 mL) and *p*-fluorophenacyl bromide (**8**) (1 mmol) was added and refluxed for 5 h. The solid separated on cooling was filtered and purified by column chromatography using ethyl acetate/hexane (1:1) as eluent.

2-(5-(1H-benzimidazol-2-yl)-3-phenyl-1H-pyrazol-1-yl)-4-(4-fluorophenyl)thiazole (7a). Yellow solid (73%); m.p. 170–172°C; IR (KBr) (cm^{-1}): 3243 (NH), 1638 (C=C),

1579 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.37 (s, 1H, C_4–H), 7.36–8.00 (m, 14H, Ar–H, C_5'–H), 10.10 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 104.8 (C-4), 108.6 (C-5′), 115.8, 116.7, 123.7, 127.4, 128.7, 129.4, 129.7, 130.3, 131.0, 133.1, 141.6, 142.5, 149.5, 154.8, 160.8, 163.7 (aromatic carbons) ppm; MS (*m/z*): 460.4816 [M + Na]. *Anal*. Calcd for $C_{25}H_{16}FN_5S$: C, 68.63; H, 3.69; N, 16.01%; found: C, 63.75; H, 3.70; N, 16.22%.

2-(5-(1H-benzimidazol-2-yl)-3-p-tolyl-1H-pyrazol-1-yl)-4-(4-fluorophenyl)thiazole (7b). Yellow solid; yield 75%; m.p. 155–176°C; IR (KBr) (cm^{-1}): 3246 (NH), 1641 (C=C), 1581 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.75 (s, 1H, C_4–H), 7.24–8.11 (m, 14H, Ar–H, C_5'–H), 10.07 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 104.5 (C-4), 108.3 (C-5′), 115.7, 116.5, 123.2, 127.1, 128.2, 128.8, 129.0, 129.7, 130.7, 131.8, 141.4, 142.3, 149.3, 154.4, 160.5, 163.0 (aromatic carbons) ppm; MS (*m/z*): 474.5086 [M + Na]. *Anal*. Calcd for $C_{26}H_{18}FN_5S$: C, 69.16; H, 4.02; N, 15.51%; found: C, 69.26; H, 4.05; N, 15.72%.

2-(5-(1H-benzimidazol-2-yl)-3-(p-methoxyphenyl)-1H-pyrazol-1-yl)-4-(4-fluorophenyl)thiazole (7c). Yellow solid; yield 72%; m.p. 166–168°C; IR (KBr) (cm^{-1}): 3252 (NH), 1645 (C=C), 1585 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.72 (s, 1H, C_4–H), 7.20–8.07 (m, 14H, Ar–H, C_5'–H), 10.05 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 104.1 (C-4), 108.0 (C-5′), 115.4, 116.2, 122.4, 126.9, 127.6, 128.3, 128.8, 129.4, 130.4, 131.4, 141.0, 142.0, 149.0, 154.0, 160.1, 162.7 (aromatic carbons) ppm; MS (*m/z*): 490.5072 [M + Na]. *Anal*. Calcd for $C_{26}H_{18}FN_5OS$: C, 66.79; H, 3.88; N, 14.98%; found: C, 66.91; H, 3.89; N, 15.19%.

2-(5-(1H-benzimidazol-2-yl)-3-(p-nitrophenyl)-1H-pyrazol-1-yl)-4-(4-fluorophenyl)thiazole (7d). Yellow solid; yield 77%; m.p. 215–217°C; IR (KBr) (cm^{-1}): 3242 (NH), 1636 (C=C), 1577 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.89 (s, 1H, C_4–H), 7.35–8.24 (m, 14H, Ar–H, C_5'–H), 10.18 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 105.6 (C-4), 109.9 (C-5′), 116.7, 117.8, 124.6, 128.5, 129.6, 129.9, 130.4, 130.9, 131.5, 134.4, 142.7, 143.7, 150.4, 155.6, 161.7, 163.7 (aromatic carbons) ppm; MS (*m/z*): 505.4792 [M + Na]. *Anal*. Calcd for $C_{25}H_{15}FN_6O_2S$: C, 62.23; H, 3.13; N, 17.42%; found: C, 62.33; H, 3.15; N, 17.63%.

2-(5-(1H-benzimidazol-2-yl)-3-(p-fluorophenyl)-1H-pyrazol-1-yl)-4-(4-fluorophenyl)thiazole (7e). Yellow solid; yield 71%; m.p. 208–210°C; IR (KBr) (cm^{-1}): 3240 (NH), 1634 (C=C), 1576 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.87 (s, 1H, C_4–H), 7.31–8.20 (m, 14H, Ar–H, C_5'–H), 10.16 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 105.2 (C-4), 109.6 (C-5′), 116.5, 117.4, 124.3, 128.2, 129.3, 129.7, 130.1, 130.6, 131.3, 134.2, 142.5, 143.4, 150.2, 155.3, 161.4, 163.5 (aromatic carbons) ppm; MS (*m/z*): 478.4722 [M + Na]. *Anal*. Calcd for $C_{25}H_{15}F_2N_5S$: C, 65.92; H, 3.32; N, 15.38%; found: C, 66.06; H, 3.33; N, 15.56%.

2-(5-(1H-benzimidazol-2-yl)-3-(p-chlorophenyl)-1H-pyrazol-1-yl)-4-(4-fluorophenyl)thiazole (7f). Yellow solid; yield 78%; m.p. 199–201°C; IR (KBr) (cm^{-1}): 3233 (NH), 1632 (C=C), 1571 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.83 (s, 1H, C_4–H), 7.29–8.18 (m, 14H, Ar–H, C_5'–H), 10.14 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 105.0 (C-4), 109.3 (C-5′), 116.2, 117.1, 124.0, 128.0, 129.1, 129.5, 129.8, 130.3, 131.2, 133.9, 142.2, 143.1, 150.0, 155.1, 161.2, 163.2 (aromatic carbons) ppm; MS (*m/z*): 494.9273 [M + Na]. *Anal*. Calcd for $C_{25}H_{15}ClFN_5S$: C, 63.62; H, 3.20; N, 14.84%; found: C, 63.73; H, 3.21; N, 15.06%.

2-(5-(1H-benzimidazol-2-yl)-3-(p-bromophenyl)-1H-pyrazol-1-yl)-4-(4-fluorophenyl)thiazole (7g). Yellow solid; yield 76%; m.p. 188–190°C; IR (KBr) (cm^{-1}): 3256 (NH), 1647 (C=C), 1588 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.81 (s, 1H, C_4–H), 7.27–8.15 (m, 14H, Ar–H, C_5'–H), 10.11 (bs, 1H, NH) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ 108.9 (C-4), 112.5 (C-5′), 116.0, 116.9, 123.8, 127.6, 128.9, 129.3, 129.6, 130.0, 131.1, 133.4, 142.0, 142.9, 149.8, 154.8, 161.0, 163.6 (aromatic carbons) ppm; MS (*m/z*): 539.3773 [M + Na]. *Anal*. Calcd for $C_{25}H_{15}BrFN_5S$: C, 58.15; H, 2.93; N, 13.56%; found: C, 58.27; H, 2.94; N, 13.78%.

Acknowledgments. The authors G. Sravya and N. Bakthavatchala Reddy are thankful to Sri Padmavathi Mahila Viswavidyalayam, Tirupati, for evaluating the antimicrobial activity and also thankful to Ural Federal University, Yekaterinburg, Russia, for providing lab facilities.

REFERENCES AND NOTES

[1] Sharma, P.; Senwar, K. R.; Jeengar, M. K.; Reddy, T. S.; Naidu, V. G. M.; Kamal, A.; Shankaraiah, N. Eur J Med Chem 2015, 104, 11.

[2] Senwar, K. R.; Reddy, T. S.; Thummuri, D.; Sharma, P.; Bharghava, S. K.; Naidu, V. G. M.; Shankaraiah, N. Bioorg Med Chem Lett 2016, 26, 4061.

[3] Senwar, K. R.; Sharma, P.; Reddy, T. S.; Jeengar, M. K.; Nayak, V. L.; Naidu, V. G. M.; Kamal, A.; Shankaraiah, N. Eur J Med Chem 2015, 102, 413.

[4] Yadav, G.; Ganguly, S. Eur J Med Chem 2015, 97, 419.

[5] (a) Akhtar, W.; Khan, M. F.; Verma, G.; Shaquiquzzaman, M.; Rizvi, M. A.; Mehdi, S. H.; Akhter, M.; Alam, M. M. Eur J Med Chem 2017, 126, 705; (b) Future of Drug Discovery; Kamal, A.; Nekkanti, S.; Shankaraiah, N.; Manda, S. Drug Resistance in Bacteria, Fungi, Malaria, and Cancer; Switzerland: Springer International Publishing, 2017, p. 609.

[6] Keri, R. S.; Hiremathad, A.; Budagumpi, S.; Nagaraja, B. M. Chem Biol Drug Des 2015, 86, 19.

[7] Kath, R.; Blumenstengel, K.; Fricke, H. J.; Hoffken, K. J Cancer Res Clin Oncol 2001, 127, 48.

[8] Wagner, L. M. Onco Targets Ther 1931, 2015, 8.

[9] (a) Chen, G.; Liu, Z.; Zhang, Y.; Shan, X.; Jiang, L.; Zhao, Y.; He, W.; Feng, Z.; Yang, S.; Liang, G. ACS Med Chem Lett 2013, 4, 69; (b) Kamal, A.; Suresh, P.; Ramaiah, M. J. P.; Reddy, T. S.; Kapavarapu,

R. K.; Imthiajali, S.; Reddy, T. L. N.; Pushpavalli, S. N. C. V. L.; Shankaraiah, N.; Bhadra, M. P. Bioorg Med Chem 2013, 21, 5198; (c) Kamal, A.; Reddy, T. S.; Vishnuvardhan, M. V. P. S.; Nimbarte, V. K.; Rao, A. V. S.; Srinivasulu, V.; Shankaraiah, N. Bioorg Med Chem 2015, 23, 4608.

[10] Khan, I.; Tantray, M. A.; Hamid, H.; Alam, M. S.; Kalam, A.; Dhulap, A. Bioorg Med Chem Lett 2016, 26, 4020.

[11] Reddy, T. S.; Kulhari, H.; Reddy, V. G.; Bansal, V.; Kamal, A.; Shukla, R. Eur J Med Chem 2015, 101, 790.

[12] Sharma, P.; Thummuri, D.; Reddy, T. S.; Senwar, K. R.; Naidu, V. G. M.; Srinivasulu, G.; Bharghava, S. K.; Shankaraiah, N. Eur J Med Chem 2016, 122, 584.

[13] Kumar, V.; Kaur, K.; Gupta, G. K.; Sharma, A. K. Eur J Med Chem 2013, 69, 735.

[14] Ansari, A.; Ali, A.; Asif, M. ShamsuzzamanNew J Chem 2017, 41, 16.

[15] Isloor, A. M.; Kalluraya, B.; Shetty, P. Eur J Med Chem 2009, 44, 3784.

[16] Isloor, A. M.; Kalluraya, B.; Rao, M. J Saudi Chem Soc 2000, 4, 265.

[17] Kalluraya, B.; Isloor, A. M.; Frank, P. V.; Jagadesha, R. L.; Shenoy, S. Indian J Heterocycl Chem 2001, 11, 159.

[18] Sunil, D.; Isloor, A. M.; Shetty, P. Der Pharma Chemica 2009, 1, 19.

[19] Cuenca-Estrella, M.; Gomez-Lopez, A.; Mellado, E.; GarciaEffron, G.; RodriguezTudela, J. L. Antimicrob Agents Chemother 2004, 48, 3107.

[20] Mohareb, R. M.; Zaki, M. Y.; Abbas, N. S. Steroids 2015, 98, 80.

[21] Rogers, M. J.; Cundliffe, E.; Mccutchan, T. F. Antimicrob Agents Chemother 1966, 42, 715.

[22] El-Sabbagh, O. I.; Baraka, M. M.; Ibrahim, S. M.; Pannecouque, C.; Andrei, G.; Snoeck, R.; Balzarini, J.; Rashad, A. A. Eur J Med Chem 2009, 44, 3746.

[23] Kumar, P.; Chandak, N.; Kaushik, P.; Sharma, C.; Kaushik, D.; Aneja, K. R.; Sharma, P. K. Med Chem Res 2012, 21, 3396.

[24] Patel, H.; Ugale, V.; Ingale, A.; Bari, S. Lett Drug Des Discov 2012, 9, 840.

[25] Padmavathi, V.; Kumari, C. P.; Venkatesh, B. C.; Padmaja, A. Eur J Med Chem 2011, 46, 5317.

[26] Atobe, M.; Naganuma, K.; Kawanishi, M.; Morimoto, A.; Kasahara, K. I.; Ohashi, S.; Suzuki, H.; Hayashi, T.; Miyoshi, S. Bioorg Med Chem Lett 2013, 23, 6064.

[27] Atobe, M.; Naganuma, K.; Kawanishi, M.; Morimoto, A.; Kasahara, K. I.; Ohashi, S.; Suzuki, H.; Hayashi, T.; Miyoshi, S. Bioorg Med Chem Lett 2013, 23, 6569.

[28] Padmavathi, V.; Jagan Mohan Reddy, B.; Mahesh, K.; Triveni, P.; Padmaja, A. J Heterocyclic Chem 2010, 47, 825.

[29] French, G. L. J Antimicrob Chemother 2006, 58, 1107.

[30] Chung, K. T.; Thomasson, W. R.; Wu-Yuan, C. D. J Appl Bacteriol 1990, 69, 498.

[31] Azoro, C. World J Biotechnol 2002, 3, 347.

[32] Janovska, D.; Kubikova, K.; Kokoska, L. J Food Sci 2003, 21, 107.

[33] Bishnu, J.; Sunil, L.; Anuja, S. J Sci Eng Technol 2009, 5, 143.

[34] National Committee for Clinical Laboratory Standards, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, third ed., Approved Standard, NCCLS Publication M7-A3, Villanova, PA, 1993.

[35] National Committee for Clinical Laboratory Standards, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Proposed Standard, NCCLS Document M27-P, Villanova, PA, 1992.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.