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Synthesis and antibacterial activity of some new 1-heteroaryl-5-amino-3*H*/methyl-4-phenylpyrazoles

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Abstract—The regioselective synthesis of 1-heteroaryl-5-amino-4-phenylpyrazoles **3a–g** and 1-heteroaryl-5-amino-3-methyl-4-phenylpyrazoles **3h–n** was achieved by the treatment of heteroarylhydrazines **1a–g** with α-phenylformylacetonitrile **2a** and α-phenylacetylacetonitrile **2b**, respectively. The structures of the compounds **3** were established by the combined use of ^{1}H and ^{13}C NMR spectroscopy. All the fourteen compounds were tested for their in vitro antibacterial activity against three Gram-positive and two Gram-negative bacteria. Six compounds **3a**, **3d**, **3e**, **3g**, **3l**, and **3n** from this series were found to be equipotent or more potent than the commercial antibiotics (Linezolid and Cefroxime axetil).

1. Introduction

5-Aminopyrazoles are an important class of compounds because of physiological properties associated with them. They display many diverse types of biological properties such as antibacterial[1], anti-inflammatory[2], and herbicidal activities,[3] and have been extensively investigated over the past hundred years (e.g., antipyrine since 1884). They are also known to possess a good synthetic potential, especially in the preparation of condensed heterocycles such as pyrazolo[3,4-*d*] pyrimidines, imidazopyrazoles, etc., which are found to possess adenosine receptor affinity,[4] anti-tumor activity[5] besides being purine[6] and folate antagonists.[7] Urea derivatives of 5-aminopyrazoles have recently been reported as potent inhibitors of P^{38} kinase,[8] TNF-α production,[9] and cholesterol acyltransferase.[10] In view of these observations and in continuation of our work related to heteroarylpyrazoles,[11] it was envisaged in the present investigation to undertake the synthesis and evaluation of the antibacterial activity of some new 1-heteroaryl-5-amino-3*H*/methyl-4-phenylpyrazoles **3** with an expectation to find new and more potent antibacterial agents.

2. Chemistry

Synthesis of 1-heteroaryl-5-amino-4-phenylpyrazoles **3a–g** and 1-heteroaryl-5-amino-3-methyl-4-phenylpyrazoles **3h–n** is outlined in Scheme 1.

Starting compounds, α-phenylformylacetonitrile **2a** and α-phenylacetylacetonitrile **2b**, were readily prepared by the acylation of α-phenylacetonitrile with ethyl formate and ethyl acetate, respectively, in the presence of sodium ethoxide.[12] Refluxing **2** with heteroarylhydrazines **1** in ethanol provided 1-heteroaryl-5-aminopyrazoles in excellent yield. In principle, the reaction of heteroarylhydrazines and α-phenylacylacetonitrile may lead to the formation of isomeric pyrazoles **3** and **4** through the pathways detailed in Scheme 2.

However, in the reaction of **1** with **2**, a single isomer was obtained in each case. The product may be one of the two possible isomers having H/CH_3 located either at position 3 or 5 of the pyrazole ring. The isomer was identified as **3** a by combined use of ^{1}H and ^{13}C NMR spectroscopy.

Abbreviations: MTCC, microbial type culture collection; SCDA, soyabean casein digest agar; EtOH, ethanol; MHA, Muller–Hinton agar; MIC, minimum inhibitory concentration.

Keywords: 1-Heteroaryl-5-amino-4-phenylpyrazoles; 1-Heteroaryl-5-amino-3-methyl-4-phenylpyrazoles; NMR spectroscopy; Antibacterial activity.

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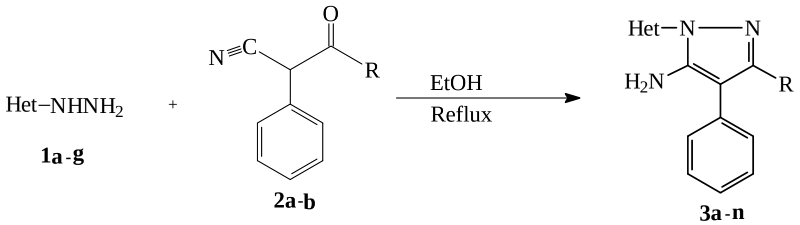


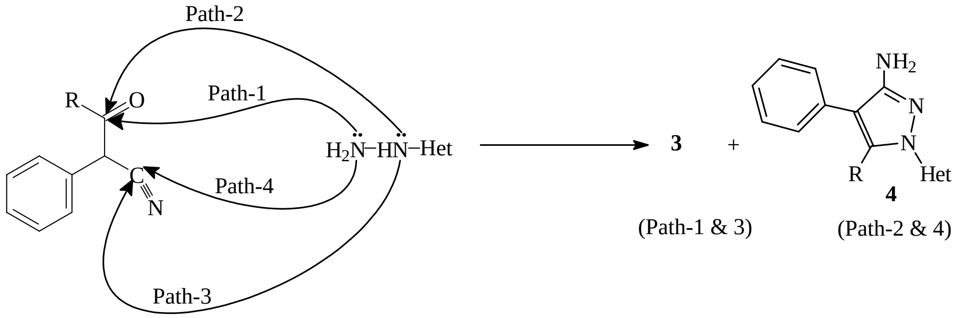
1	Het–
a	Benzothiazol-2-yl
b	6-Fluorobenzothiazol-2-yl
c	6-Chlorobenzothiazol-2-yl
d	6-Methylbenzothiazol-2-yl
e	4-Methylquinolin-2-yl
f	7-Chloroquinolin-4-yl
g	4,6-Dimethylpyrimidin-2-yl

2	R
a	H
b	CH_3

3	Het-	R
a	**1a**	H
b	**1b**	H
c	**1c**	H
d	**1d**	H
e	**1e**	H
f	**1f**	H
g	**1g**	H

3	Het-	R
h	**1a**	CH_3
i	**1b**	CH_3
j	**1c**	CH_3
k	**1d**	CH_3
l	**1e**	CH_3
m	**1f**	CH_3
n	**1g**	CH_3

Scheme 1.



Scheme 2.

The ^1H NMR spectra of **3a**–**g** displayed the pyrazole proton appearing as a sharp singlet at δ 7.7 ppm, while in **3h**–**n**, a singlet of three protons for methyl group appeared at δ 2.3 ppm. The chemical shifts of the pyrazole and methyl protons indicated that they are located at position 3 of the pyrazole ring. It has earlier been established in our laboratory that the H/methyl group located at position 3 of the pyrazole moiety resonates upfield as compared to position 5 (i.e., ~δ 8.7 for C_5-H/~δ 2.7 for C_5-CH_3).[13]

The presence of H/Me group at position 3 and consequent location of the amino group at position 5 finds further support by the study of ^{13}C NMR spectra of compounds **3a**–**n**. The compounds **3a**–**g** and **3h**–**n** displayed C-3 signal at δ 140–142 ppm and δ 149–152 ppm, respectively, and C-5 signal at δ 146–147 ppm which is a characteristic signal for C-5 bearing an amino group. These values are in complete agreement with earlier reports.[1b,14,15] Had the H/Me group been located at position 5 and amino group at position 3 of pyrazole ring, the C-5 would have resonated at δ 129–130 ppm/δ 140–142 ppm and C-3 at δ 156–157 ppm.[16,17]

The presence of H/Me substituent at position 3 suggests that the carbonyl carbon of α-phenylacylacetonitrile is more reactive toward nucleophiles than the cyano group and unsubstituted nitrogen of heteroarylhydrazine is involved in the initial reaction.

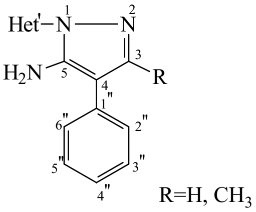
The complete assignment of the signals in ^{13}C NMR spectra of the compounds is given in Table 1.

IR spectra (in KBr) of all the compounds displayed the characteristic bands at 3280, 3380 cm^{-1} due to NH_2 str.

3. Biological results and discussion

All the 14 compounds were tested in vitro for their antibacterial activity against three Gram-positive bacteria namely, *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermidis* (MTCC 2639), and *Bacillus pumilus* (MTCC 1456), and two Gram-negative bacteria namely, *Salmonella typhi* (MTCC 733) and *Pseudomonas aeruginosa* (MTCC 3541) (Table 2). Six of these compounds **3a**, **3d**, **3e**, **3g**, **3l**, and **3n** exhibited good antibacterial activity against both Gram-positive and Gram-negative bacteria. The compound **3n** showed maximum antibacterial activity against *S. aureus* (MIC 0.5 μg/ml) and *S. epidermidis* (MIC 1 μg/mL). It also displayed inhibitory activity against *B. pumilus* (MIC 2 μg/mL) and *S. typhi* (MIC 4 μg/mL).

Table 1. ^{13}C NMR data for some compounds **(3)**



Carbons[*]	**3a**	**3b**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**	**3j**	**3k**	**3l**	**3n**
C-3	142.78	141.96	142.49	140.50	140.32	141.76	151.07	150.93	152.12	150.55	148.72	150.28
C-4	103.15	103.50	104.04	104.80	104.84	104.44	102.81	102.12	103.94	102.58	103.55	103.75
C-5	144.80	144.90	144.69	145.31	142.98	145.44	144.86	144.90	144.91	144.82	146.06	146.01
C-2′	161.93	160.50	161.04	153.40	151.05	168.42	161.25	160.55	162.11	160.33	153.01	168.28
C-3′	—	—	—	113.61	117.55	—	—	—	—	—	113.27	—
C-3′a	150.81	148.31	148.72	—	—	—	150.52	147.03	149.71	148.39	—	—
C-4′	121.79	121.71	121.30	145.51	149.71	157.66	121.07	121.87	122.36	120.51	145.25	157.46
C-4′a	—	—	—	125.44	122.28	—	—	—	—	—	125.67	—
C-5′	125.86	114.42 (d, $^2J_{C\text{-}F}$ = 24.25 Hz)	125.77	123.82	128.61	116.58	127.95	113.84 (d, $^2J_{C\text{-}F}$ = 24.15 Hz)	126.60	125.53	123.58	116.04
C-6′	124.57	159.12 (d, $^1J_{C\text{-}F}$ = 268.5 Hz)	134.56	125.66	127.90	157.66	123.72	159.07 (d, $^1J_{C\text{-}F}$ = 268.5 Hz)	132.13	133.54	125.81	157.46
C-7′	121.31	108.72 (d, $^2J_{C\text{-}F}$ = 27.51 Hz)	121.15	126.55	135.61	—	120.87	107.44 (d, $^2J_{C\text{-}F}$ = 27.51 Hz)	121.09	120.51	125.60	—
C-7′a	131.54	132.55	132.19	—	—	—	131.87	132.75	132.99	131.83	—	—
C-8′	—	—	—	129.82	125.63	—	—	—	—	—	129.49	—
C-8′a	—	—	—	147.42	142.07	—	—	—	—	—	146.95	—
C-1″	132.19	131.05	131.56	133.13	132.32	133.05	131.03	131.25	129.84	131.07	133.05	133.02
C-2″, 6″	129.02	128.80	128.75	128.73	128.61	129.04	128.95	128.25	128.48	128.22	128.79	128.82
C-3″, 5″	126.02	125.76	127.79	128.26	125.68	126.16	127.95	127.95	129.12	127.89	128.30	128.53
C-4″	126.44	125.51	125.93	126.10	125.41	125.71	125.80	125.58	127.00	127.06	127.94	126.03
3-CH_3	—	—	—	—	—	—	12.81	12.68	13.26	12.58	13.26	13.53
CH_3	—	—	21.35	18.72	—	24.12	—	—	—	—	18.73	24.10

[*] Carbons having single prime and double prime represent heteroaryl and phenyl carbons, respectively.

Table 2. In vitro antibacterial activity of **3a**–**n** by using agar diffusion assay technique

Compound	Diameter of zone of growth inhibition (mm)[a]				
	Sa	Se	Bp	St	Pa
3a	20 ± .81	24 ± .57	—	15.16 ± .37	15.83 ± .37
3b	—	—	—	—	—
3c	22.16 ± .37	15 ± .81	—	—	—
3d	20.83 ± .37	22 ± .81	—	18.16 ± .37	15 ± .81
3e	15.16 ± .37	20 ± .81	14.83 ± .37	15 ± .81	20.16 ± .37
3f	—	—	—	—	—
3g	18 ± .81	15.16 ± .37	—	14.83 ± .37	15.16 ± .37
3h	20.16 ± .37	15 ± .81	—	—	—
3i	—	—	—	—	—
3j	20 ± .57	20.16 ± .37	—	—	—
3k	—	—	—	—	—
3l	22.16 ± .37	15.83 ± .37	22.16 ± .37	15.83 ± .37	18 ± .57
3m	—	—	—	—	—
3n	40 ± .81	30 ± .57	25.16 ± .37	20.83 ± .37	15 ± .81
Ethanol	6.83 ± .37	6.83 ± .37	6.16 ± .37	6.83 ± .37	6.16 ± .37
Linezolid	22.83 ± .37	20 ± .81	15.16 ± .37	10.83 ± .37	12.83 ± .37
Cefroxime axetil	10.83 ± .37	12.16 ± .37	10 ± .81	12.16 ± .37	10 ± .57

—, no activity.

Sa, *S. aureus* (MTCC 3160); Se, *S. epidermidis* (MTCC 2639); Bp, *B. pumilus* (MTCC 1456); St, *S. typhi* (MTCC 733); and Pa, *P. aeruginosa* (MTCC 3541).

[a] Mean of six replicate ± standard deviation.

Compound **3l** was found to be effective against *S.aureus* and *B. pumilus* showing a MIC of 2 μg/mL. It also inhibited the growth *P. aeruginosa* at 4 μg/mL.

Compounds **3a** and **3d** displayed the same inhibitory activity against all the test organisms. They possess good activity against *S. epidermidis* (MIC 2 μg/mL) and *S. aureus* (MIC 4 μg/mL). Compound **3e** inhibited the growth of *S. epidermidis* and *P. aeruginosa* at 4 μg/mL; while *S.aureus*, *B. pumilus*, and *S. typhi* were inhibited at 4 μg/mL. The last compound of this series, that is, **3g** inhibited the growth of all the organisms, except for *B. pumilus* at MICs 4 and 8 μg/mL.

A careful analysis of MIC data shows some interesting trends. Comparison of the MIC data of 1-(benzothiazol-2′-yl)-5-amino-3*H*/methyl-4-phenylpyrazoles **3a–d** and **3h–k** revealed that replacement of pyrazole proton with a methyl group, in general, reduces the antibacterial activity. The most striking example in this series is that of **3d** and **3k**. While the compound **3d** exhibits significant level of antibacterial activity against Sa, Se, St, and Pa, compound **3k** is completely inactive toward all the microorganisms.

Moreover, incorporation of fluorine at position 6 of benzothiazolyl also results in the complete loss of antibacterial activity as in **3b** and **3i**. On the other hand, in 1-(quinolin-2′-yl)-5-amino-3*H*/methyl-4-phenylpyrazoles **3e** and **3l** and 1-(4′,6′-dimethylpyrimidin-2′-yl)-5-amino-3*H*/methyl-4-phenylpyrazoles **3g** and **3n** the antibacterial activity increases to a significant extent when the pyrazole proton is replaced by the methyl group toward certain microorganisms. It was also observed that when 2-quinoline nucleus is replaced by 4-quinoline at position 1 of the pyrazole, there is complete loss of antibacterial activity (**3e** vs **3f** and **3l** vs **3m**).

4. Conclusion

Using easily obtainable compounds **2** and **1** we have prepared a new series of 1-heteroaryl-5-amino-3*H*/methylpyrazoles regioselectively. Many of these compounds **3a**, **3d**, **3e**, **3g**, **3l**, and **3n** showed excellent activity as displayed in Table 2 and Fig. 1. The antibacterial activity of these compounds was also compared with those of two commercial antibiotics namely Linezolid and Cefroxime axetil.

5. Experimental

Melting points were determined in open capillaries with an electrical apparatus and are uncorrected. IR spectra were recorded on a Buck Scientific IR M500 instrument. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker instrument at 300 MHz and 75 MHz, respectively. High resolution mass spectra (HRMS) were measured in EI mode on a Kratos MS-50 spectrometer.

Heteroarylhydrazines[18] **1a–g** were synthesized according to the literature procedures.

5.1. 1-Heteroaryl-5-amino-3*H*/methyl-4-phenylpyrazoles (3a–n)

General procedure: α-phenylacylacetonitrile (**2a–b**, 0.01 mol) was dissolved in ethanol (25 mL) and an equimolar amount of appropriate heteroarylhydrazine (**1a–h**, 0.01 mol) was added to it. The reaction mixture was refluxed for 1 h. Excess of the solvent was removed by distillation. The crude products so obtained on cooling were recrystallized from ethanol.

The physical, analytical, and spectral data of synthesized compounds are given below:

5.1.1. 1-(Benzothiazol-2′-yl)-5-amino-4-phenylpyrazole (3a). Yield 78%; mp 220–22 °C; IR (KBr, cm^{-1}) 3298, 3410 (NH_2 str.); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ: 6.18 (br s, 2H, NH_2), 7.24–7.49 (m, 7H, 5′, 6′ Bz-H, Ph-H), 7.70 (s, 1H, 3-H), 7.83–7.87 (m, 2H, 4′, 7′ Bz-H); MS: *m/z* M$^+$ 292. Anal. Calcd for $C_{16}H_{12}N_4S$: N, 19.18%. Found: 18.84%.

5.1.2. 1-(6′-Fluorobenzothiazol-2′-yl)-5-amino-4-phenylpyrazole (3b). Yield 80%; mp 265 °C; IR (KBr, cm^{-1}) 3300, 3412 (NH_2 str.); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ: 6.70 (br s, 2H, NH_2), 7.17–7.52 (m, 6H, 5′-H, Ph-H), 7.60 (dd, 1H, 7′-H, $J_{o(H-F)}$ = 8.1 Hz, J_m = 2.4 Hz), 7.69 (s, 1H, 3-H), 7.82 (dd, 1H, 4′-H, J_o = 9.0 Hz, $J_{m(H-F)}$ = 4.7 Hz); MS: *m/z* M$^+$ 310. Anal. Calcd for $C_{16}H_{11}FN_4S$: N, 18.06%. Found: 17.92%.

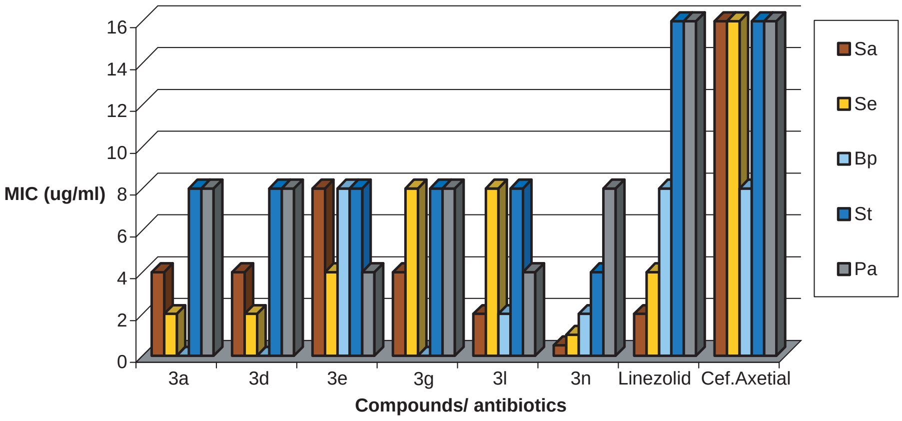


Figure 1. Comparison of minimum inhibitory concentration (MIC) of some compounds and standard antibiotics against test microorganisms.

5.1.3. 1-(6′-Chlorobenzothiazol-2′-yl)-5-amino-4-phenylpyrazole (3c). Yield 77%; mp 270–271 °C; IR (KBr, cm^{-1}) 3298, 3408 (NH_2 str.); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ: 6.54 (br s, 2H, NH_2), 7.45–7.56 (m, 6H, 5′-H, Ph-H), 7.92 (d, 1H, 4′-H, J_o = 7.8 Hz), 7.94 (s, 1H, 3-H), 8.08 (s, 1H, 7′-H); MS: *m/z* 326 (M^+) and 328 (M^++2) in the ratio of 3:1 showing the typical isotopic profile. The exact mass measurement of the two isotopes was $C_{16}H_{11}ClN_4S$ requiring 326.0393 (Found: 326.0406) and 328.0381 (Found: 328.0366), respectively, in the ratio 3:1. Anal. Calcd for $C_{16}H_{11}ClN_4S$: N, 17.18%. Found: 16.87%.

5.1.4. 1-(6′-Methylbenzothiazol-2′-yl)-5-amino-4-phenylpyrazole (3d). Yield 81%; mp 238–239 °C; IR (KBr, cm^{-1}) 3291, 3405 (NH_2 str.); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ: 2.48 (s, 3H, 6′-CH_3), 6.45 (br s, 2H, NH_2), 7.25 (d, 1H, 5′-H, J_o = 8.7 Hz), 7.43–7.47 (m, 5H, Ph-H), 7.64 (s, 1H, 7′-H), 7.68 (s, 1H, 3-H), 7.73 (d, 1H, 4′-H, J_o = 8.6 Hz); MS: *m/z* M^+ 306. Anal. Calcd for $C_{17}H_{14}N_4S$: N, 18.30%. Found: 18.22%.

5.1.5. 1-(4′-Methylquinolin-2′-yl)-5-amino-4-phenylpyrazole (3e). Yield 78%; mp 148 °C; IR (KBr, cm^{-1}) 3276, 3311 (NH_2 str.); ^{1}H NMR ($CDCl_3$) δ: 2.76 (s, 3H, 4′-CH_3), 6.62 (br s, 2H, NH_2), 7.22–7.55 (m, 5H, Ph-H), 7.67–7.72 (m, 2H, 6′, 7′-H), 7.70 (s, 1H, 3-H), 7.92 (dd, 1H, 5′-H, J_o = 8.4 Hz, J_m = 1.2 Hz), 7.99 (dd, 1H, 8′-H, J_o = 8.4 Hz, J_m = 1.2 Hz), 8.11 (s, 1H, 3′-H); MS: *m/z* M^+ $C_{19}H_{16}N_4$ requires 300.1372 (Found: 300.1374). Anal. Calcd for $C_{19}H_{16}N_4$: N, 18.67%. Found: 18.61%.

5.1.6. 1-(7′-Chloroquinolin-4′-yl)-5-amino-4-phenylpyrazole (3f). Yield 76%; mp 182–183 °C; IR (KBr, cm^{-1}) 3287, 3405 (NH_2 str.); ^{1}H NMR ($CDCl_3$) δ: 4.60 (br s, 2H, NH_2), 7.23–7.56 (m, 7H, 3′, 6′, Ph-H), 7.78 (s, 1H, 3-H), 8.02 (d, 1H, 2′-H, J_o = 8.7 Hz), 8.10 (d, 1H, 8′-H, J_m = 2.1 Hz), 8.96 (d, 1H, 5′-H, J = 4.9 Hz); MS: *m/z* M^+ 320. Anal. Calcd for $C_{18}H_{13}ClN_4$: N, 17.50%. Found: 17.12%.

5.1.7. 1-(4′,6′-Dimethylpyrimidin-2′-yl)-5-amino-4-phenylpyrazole (3g). Yield 74%; mp 182 °C; IR (KBr, cm^{-1}) 3288, 3468 (NH_2 str.); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ: 2.48 (s, 6H, 4′, 6′-CH_3), 6.18 (br s, 2H, NH_2), 6.81 (s, 1H, 5′-H), 7.31–7.43 (m, 5H, Ph-H), 7.66 (s, 1H, 3-H); MS: *m/z* M^+ $C_{15}H_{15}N_5$ requires 265.1327 (Found: 265.1330). Anal. Calcd for $C_{15}H_{15}N_5$: N, 26.41%. Found: 26.04%.

5.1.8. 1-(Benzothiazol-2′-yl)-5-amino-3-methyl-4-phenylpyrazole (3h). Yield 81%; mp 182–183 °C; IR (KBr, cm^{-1}) 3283, 3410 (NH_2 str.); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ: 2.26 (s, 3H, 3-CH_3), 6.28 (br s, 2H, NH_2), 7.26–7.29 (m, 9H, Ph-H, Bz-H); MS: *m/z* M^+ 306. Anal. Calcd for $C_{17}H_{14}N_4S$: N, 18.30%. Found: 17.82%.

5.1.9. 1-(6′-Fluorobenzothiazol-2′-yl)-5-amino-3-methyl-4-phenylpyrazole (3i). Yield 78%; mp 195–197 °C; IR (KBr, cm^{-1}) 3283, 3399 (NH_2 str.); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ: 2.22 (s, 3H, 3-CH_3), 6.41 (br s, 2H, NH_2), 7.18–7.45 (m, 6H, 5′, Ph-H), 7.59 (dd, 1H, 7′-H, $J_{o(H\text{-}F)}$ = 8.2 Hz, $J_{m(H\text{-}H)}$ = 2.5 Hz), 7.74 (dd, 1H, 4′-H, J_o = 8.8 Hz, $J_{m(H\text{-}F)}$ = 4.5 Hz); MS: *m/z* M^+ 324. Anal. Calcd for $C_{17}H_{13}FN_4S$: N, 17.28%. Found: 16.94%.

5.1.10. 1-(6′-Chlorobenzothiazol-2′-yl)-5-amino-3-methyl-4-phenylpyrazole (3j). Yield 76%; mp 206 °C; IR (KBr, cm^{-1}) 3330, 3471 (NH_2 str.); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ: 2.22 (s, 3H, 3-CH_3), 5.83 (br s, 2H, NH_2), 7.18–7.40 (m, 6H, 5′, Ph-H), 7.65 (d, 1H, 4′-H, J_o = 8.7 Hz), 7.71 (d, 1H, 7′-H, J_m = 2.1 Hz); MS: *m/z* M^+ 340. Anal. Calcd for $C_{17}H_{13}ClN_4S$: N, 16.47%. Found: 16.06%.

5.1.11. 1-(6′-Methylbenzothiazol-2′-yl)-5-amino-3-methyl-4-phenylpyrazole (3k). Yield 72%; mp 178–179 °C; IR (KBr, cm^{-1}) 3223, 3334 (NH_2 str.); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ: 2.25 (s, 3H, 3-CH_3), 2.45 (s, 3H, 6′-CH_3), 6.45 (br s, 2H, NH_2), 7.23–7.46 (m, 6H, 5′, Ph-H), 7.63 (s, 1H, 7′-H), 7.67 (d, 1H, 4′-H, J_o = 8.4 Hz); MS: *m/z* M^+ 320. Anal. Calcd for $C_{18}H_{16}N_4S$: N, 17.50%. Found: 17.11%.

5.1.12. 1-(4′-Methylquinolin-2′-yl)-5-amino-3-methyl-4-phenylpyrazole (3l). Yield 82%; mp 100 °C; IR (KBr, cm^{-1}) 3280, 3376 (NH_2 str.); ^{1}H NMR ($CDCl_3$) δ: 2.34 (s, 3H, 3-CH_3), 2.74 (s, 3H, 4′-CH_3), 6.42 (br s, 2H, NH_2), 7.25–7.50 (m, 6H, 6′, Ph-H), 7.66–7.71 (m, 1H, 7′-H), 7.88 (dd, 1H, 5′-H, J_o = 8.4 Hz, J_m = 1.1 Hz), 7.94 (dd, 1H, 8′-H, J_o = 8.4 Hz, J_m = 1.2 Hz), 8.075 (s, 1H, 3′-H); MS: *m/z* M^+ 314. Anal. Calcd for $C_{20}H_{18}N_4$: N, 17.83%. Found: 17.65%.

5.1.13. 1-(7′-Chloroquinolin-4′-yl)-5-amino-3-methyl-4-phenylpyrazole (3m). Yield 76%; mp 156–157 °C; IR (KBr, cm^{-1}) 3281, 3403 (NH_2 str.); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ: 2.36 (s, 3H, 3-CH_3), 3.91 (br s, 2H, NH_2), 7.29–7.58 (m, 7H, 3′, 6′, Ph-H), 8.07 (d, 1H, 2′-H, J_o = 8.7 Hz), 8.14 (d, 1H, 8′-H, J_m = 2.1 Hz), 8.96 (d, 1H, 5′-H, J_o = 4.5 Hz); MS: *m/z* M^+ 334. Anal. Calcd for $C_{19}H_{15}ClN_4$: N, 16.77%. Found: 16.45%.

5.1.14. 1-(4′,6′-Dimethylpyrimidin-2′-yl)-5-amino-3-methyl-4-phenylpyrazole (3n). Yield 75%; mp 146–148 °C; IR (KBr, cm^{-1}) 3308, 3474 (NH_2 str.); ^{1}H NMR ($CDCl_3$) δ: 2.33 (s, 3H, 3-CH_3), 2.59 (s, 6H, 4′, 6′-CH_3), 6.08 (br s, 2H, NH_2), 6.83 (s, 1H, 5′-H), 7.25–7.45 (m, 5H, Ph-H); MS: *m/z* M^+ 279. Anal. Calcd for $C_{16}H_{17}N_5$: N, 25.09%. Found: 24.92%.

6. In vitro biological assay

6.1. Medium

Two solid media, namely Muller–Hinton Agar (MHA; Beef infusion 300 g/L, casein acid hydrolysate 17.5 g/L, starch 1.5 g/L, agar-agar 17 g/L, and distilled water 1000 mL, adjusted to pH 7.4) and soyabean casein digest agar (SCDA; casein enzymatic hydrolysate 17.0 g/L, papain digest of soyabean 3.0 g/L, NaCl 5.0 g/L, dipotassium phosphate 2.5 g/L, dextrose 2.5 g/L, and distilled water 1000 mL, adjusted to pH 7.3), were used for the biological assays.

6.2. Test microorganisms

Three Gram-positive bacteria *S. aureus* (MTCC 3160), *S. epidermidis* (MTCC 2639), and *B. pumilus* (MTCC 1456), and two Gram-negative bacteria *S. typhi* (MTCC 733) and *P. aeruginosa* (MTCC 3541) were used for the biological assays.

6.3. Primary screening

Primary screening of fourteen compounds **3a**–**n** was done by the agar diffusion assay technique by following the method of Kumar et al.[1b] 24-h-old bacterial cultures of all test microorganisms were used as inoculum, which was adjusted to 0.5 McFarland Standard, that is, 1.5×10^8 CFU/mL.[19] The stock solutions of all the test compounds (1 mg/mL) were prepared by dissolving 1 mg of the test compound in ethanol (1 mL). Linezolid, Cefroxime axetil, and ethanol were used as positive and negative controls, respectively.

20 mL of molten and cooled MHA and 500 µl of each test bacterial culture were mixed (separate flasks were used for each bacterial culture) and poured in sterilized and labelled petri plates. The wells of 6 mm were punched in the solidified petri plates, aseptically. Fifty microliters from stock solutions of all the compounds as well as controls was added to each well of labelled petri plates and incubated at 35 °C for 24 h. The diameter of the zone of growth inhibition around each well was measured after incubation using a Vernier Caliper.

6.4. Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) is the lowest concentration of the antimicrobial agent that prevents the development of visible growth after overnight incubation.[20] MIC of compounds against Gram-positive and Gram-negative test bacteria was determined by the method of NCCLS.[21] All the test cultures were streaked on SCDA and incubated overnight at 37 °C. Turbidity of all the bacterial cultures was adjusted to 0.5 McFarland Standard by preparing bacterial suspension of 3–5-well isolated colonies of the same morphological type selected from an agar plate culture. The cultures were further diluted 10-fold to get an inoculum size of 1.2×10^7 CFU/mL. Stock solution of 4 mg/mL of each compound was prepared in ethanol and was appropriately diluted to get a final concentration of 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, and 0.12 mg/mL.

Standard antibiotics (Linezolid, Manufacturer-Unichem Lab. Ltd., Batch No. N1953005; Cefroxime axetil, Manufacturer-Glaxo, Batch No. HD-313) were also diluted in the same manner. Three hundred and twenty microliters of each dilution was added to 20 mL molten and cooled MHA (separate flasks were taken for each dilution). After thorough mixing, the medium was poured in sterilized petri plates. The test bacterial cultures were spotted in a predefined pattern by ascetically transferring 5 ml of each bacterial culture on the surface of solidified agar plates and incubated at 35 °C for 24 h.

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