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Short communication

Synthesis and biological evaluation of some 4-functionalized-pyrazoles as antimicrobial agents

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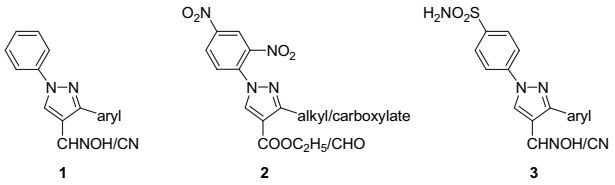
ABSTRACT

1,3-Diaryl-4-formylpyrazoles **8** bearing benzenesulfonamide moiety at position-1 were synthesized as important intermediates following Vilsmeier–Haack strategy. Aldehyde moiety of 4-formylpyrazole was then converted into carboxylic acid **9**, cyano **10** and carbothioamide **11** using established procedures. Out of these 4-functionalized pyrazoles, pyrazole-4-carboxylic acids **9** and carbothioamides **11** were evaluated for their *in vitro* antibacterial activity against four pathogenic bacterial strains namely, *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive), *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative), and *in vitro* antifungal activity against two pathogenic fungal strains namely, *Aspergillus niger* and *Aspergillus flavus*. Three tested compounds, **9e**, **11b** and **11f** exhibited moderate antibacterial activity against Gram-positive bacteria and **9g** showed moderate antifungal activity against the tested fungi. However, none of the compounds showed any activity against Gram-negative bacteria.

1. Introduction

Increasing resistance of microorganisms to currently available antimicrobial drugs is the major cause of morbidity and mortality throughout the world. Thus development of novel antimicrobial drugs is still in demand. Pyrazoles represent a key motif in heterocyclic chemistry and occupy a prime place in medicinal and pesticide chemistry due to their capability to exhibit a wide range of bioactivities such as antimicrobial [1–7], anticancer [8], anti-inflammatory [9–12], antidepressant [13,14], anticonvulsant [15,16], antipyretic [17], selective enzyme inhibitory activities [18], etc. Amongst a large array of medicinally important pyrazole derivatives, 4-functionalized pyrazoles occupy a unique position and their evaluation as antimicrobial agents has attracted much attention in the past [7,19–24]. Pyrazoles with various functional groups at position-4 such as cyano or oxime (**1**) [7], aldehyde or carboxylate (**2**) [19], etc., have been known to show good antimicrobial properties. Recently, Bekhit et al. [25] reported that 1,3-diaryl-4-functionalizedpyrazoles (**3**) bearing a benzenesulfonamide moiety at position-1 possess antimicrobial as well as anti-inflammatory activities. Thus the presence of an additional sulfonamide group at position-4 of the phenyl ring being the only difference between **1** and **3**, a direct comparison of the antimicrobial potential of **1** and **3** was possible which revealed that the presence of a sulfonamide group increases the activity against Gram-negative bacteria *Escherichia coli* especially in case of 4-oximepyrazole derivatives while retaining the activity against Gram-positive bacteria.



Presence of carboxylic acid [26–29] and carbothioamide [30–34] functionalities in a number of bioactive molecules has attracted the attention of chemists towards these moieties in the recent past. Motivated by these findings, we set out to synthesize some novel 4-functionalized pyrazoles bearing benzenesulfonamide moiety at position-1 and a carboxylic acid or carbothioamide moiety at position-4 for their evaluation as antibacterial and antifungal agents.

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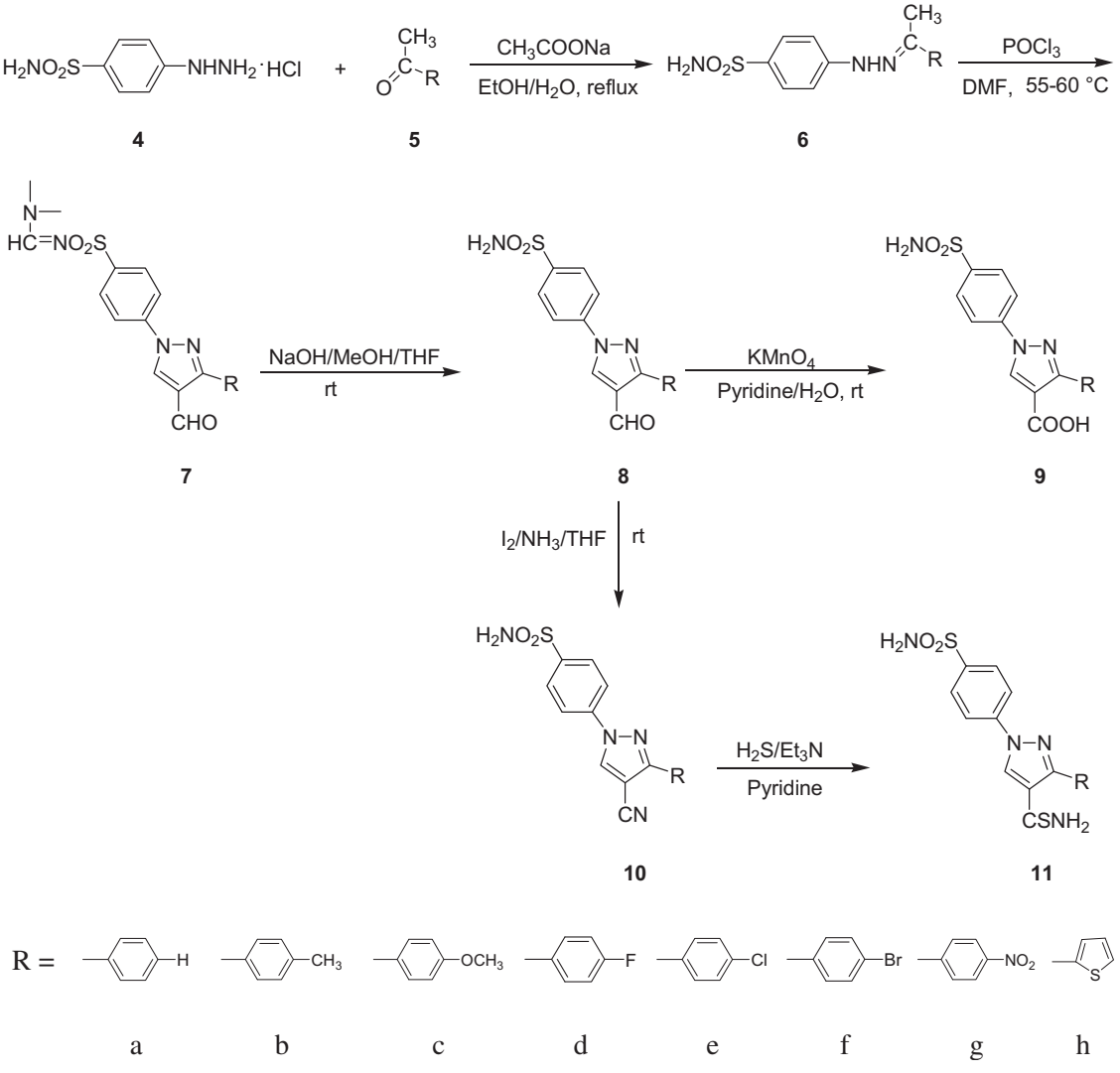
2. Results and discussion

2.1. Chemistry

The synthetic route used to synthesize the target pyrazole-4-carboxylic acids **9** and carbothioamides **11** is outlined in Scheme 1. The treatment of aqueous solution of 4-hydrazinobenzenesulfonamide hydrochloride **4** with sodium acetate generated free hydrazine which on condensation with various substituted methylketones **5** in aqueous ethanol afforded corresponding hydrazones **6**. Subsequent reactions of hydrazones **6** under Vilsmeier–Haack [35] conditions afforded 4-formylpyrazoles **7** with protected sulfonamide group. The deprotection of sulfonamide group to afford aldehyde **8** with free SO_2NH_2 group was accomplished under basic conditions using methanolic solution of NaOH with THF as co-solvent. It is pertinent to mention here that just after we finished the synthesis of 4-formylpyrazoles **8** in our lab in the beginning of 2009, an article by Bekhit et al. [25] appeared revealing the synthesis and characterization of some of the 4-formylpyrazoles (**8a**, **8b**, **8e**, **8f** and **8g**) synthesized by us. However, in our group, deprotection of sulfonamide group was achieved under basic conditions while their group reported acidic conditions for the same. Oxidation of aldehyde **8** underwent smoothly using $KMnO_4$ in aqueous pyridine [36,37] to afford carboxylic acids **9**. On the other hand, oxidation of **8** using ammoniacal solution of iodine [38] in THF afforded 4-cyanopyrazoles **10**. This transformation presumably proceeded with an intermediate *N*-iodoaldimine, which eliminated an HI molecule in ammonia to afford 4-cyanopyrazoles **10**. While we achieved the synthesis of eight 4-cyanopyrazoles **10** from 4-formylpyrazoles **8** using a single step protocol, a recent report described the synthesis of five of these 4-cyanopyrazoles (**10a**, **10b**, **10e**, **10f** and **10g**) by a two step protocol involving the conversion of aldehyde to the oxime followed by dehydration of oxime using $POCl_3$ [25]. The single step protocol adopted by us seems to be advantageous over two step protocol in terms of using milder and greener materials (I_2, NH_3 as compared to $POCl_3$) as well as in terms of yields which are generally higher. 4-Cyanopyrazoles **10** were converted into the corresponding carbothioamides **11** by passing H_2S gas through their solution in pyridine in the presence of triethylamine [39,40]. Spectral data (^{1}H NMR, ^{13}C NMR, IR and mass) of the newly synthesized compounds were in full agreement with the proposed structures. In general, pyrazole-4-carboxylic acids **9a**–**h** were characterized by a broad exchangeable singlet in the range of δ 12.62–12.93 due to COOH group in ^{1}H NMR spectra and a broad absorption band from 2500 to 3150 cm^{-1} due to O–H stretching of COOH along with a sharp band at



Scheme 1. Synthesis of 1,3-diaryl-4-functionalized pyrazoles.

Table 1
In vitro antibacterial activity and MIC of compounds **9** and **11**.

Compound[a]	Diameter of growth of inhibition zone[b] (mm)				Minimum inhibitory concentration (MIC) (μg/mL)	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
9a	15.3	16.6	–	–	128	128
9b	14.6	17.3	–	–	>128	64
9c	15.6	17.0	–	–	128	64
9d	15.0	18.6	–	–	128	64
9e	18.6	21.6	–	–	64	32
9f	15.6	14.3	–	–	128	>128
9g	14.6	15.0	–	–	>128	128
9h	18.6	15.6	–	–	64	128
11a	15.3	15.0	–	–	128	128
11b	21.6	16.3	–	–	32	128
11c	16.6	15.6	–	–	128	128
11d	17.6	15.3	–	–	64	128
11e	18.3	14.6	–	–	64	>128
11f	21.6	18.3	–	–	32	64
11h	20.3	17.6	–	–	64	64
Ciprofloxacin	27.6	26.3	25.3	25.0	5	5

– No activity.
[a] Concentration 4.0 mg/mL.
[b] Values, including diameter of the well (8 mm), are means of three replicates.

Table 2
In vitro antifungal activity of compounds **9** and **11** through poisoned food method.

Compound[a]	Mycelial growth inhibition (%)	
	A. niger	*A. flavus*
9a	50.0	55.5
9b	46.6	51.1
9c	44.4	44.4
9d	52.5	48.8
9e	45.5	44.4
9f	51.1	45.5
9g	61.1	57.7
9h	57.7	55.5
11a	50.0	53.3
11b	44.4	51.1
11c	55.5	57.7
11d	44.4	61.1
11e	50.0	52.5
11f	44.4	51.1
11h	61.1	55.5
Fluconazole	75.3	74.6

[a] Concentration 4.0 mg/mL.

1690–1705 cm^{-1} corresponding to C=O stretch in FT-IR. 4-Cyanopyrazoles **10a–h** were characterized by disappearance of a singlet due to CHO group in ^{1}H NMR spectra and a sharp band resonating at 2230–2236 cm^{-1} due to C≡N stretch in FT-IR. Pyrazole-4-carbothiamides **11a–f** and **11h** were characterized by two exchangeable singlets in the range of δ 9.84–10.03 and δ 9.25–9.56 corresponding to NH and SH protons in ^{1}H NMR. 1-[4-(Aminosulfonyl)phenyl]-3-(4-nitrophenyl)-1*H*-pyrazole-4-carbothioamide **11g** could not be synthesized by the same method inspite of repeated attempts. We are at loss to explain this exceptional behavior in case of nitro substituent.

2.2. Biological evaluation

2.2.1. In vitro antibacterial activity

All the newly synthesized carboxylic acids (**9a–h**) and carbothioamides (**11a–f** and **11h**) were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 121) representing Gram-positive bacteria, and *E. coli* (MTCC 1652) and *Pseudomonas aeruginosa* (MTCC 741) representing Gram-negative bacteria (Table 1) by agar well diffusion method [41] using ciprofloxacin as the reference drug. The results were recorded for each tested compound as the average diameter of inhibition zones of bacterial growth surrounding the well in millimetres. The Minimum Inhibitory Concentration (MIC) measurements were performed using a macrodilution tube method [42,43] (Table 1).

Results revealed that in general, all the tested compounds possessed moderate to good antibacterial activity against Gram-positive bacteria (*S. aureus*, *B. subtilis*). However, none of them was found to be effective against any of the Gram-negative bacteria (*E. coli*, *P. aeruginosa*). On the basis of zone of inhibition against the test bacterium, compounds **11b** and **11f** were found to be the most effective against *S. aureus* showing the maximum zone of inhibition of 21.6 mm and compound **9e** against *B. subtilis* producing 21.6 mm zone of inhibition (Table 1) as compared with the standard drug ciprofloxacin which showed the zone of inhibition 27.6 mm against

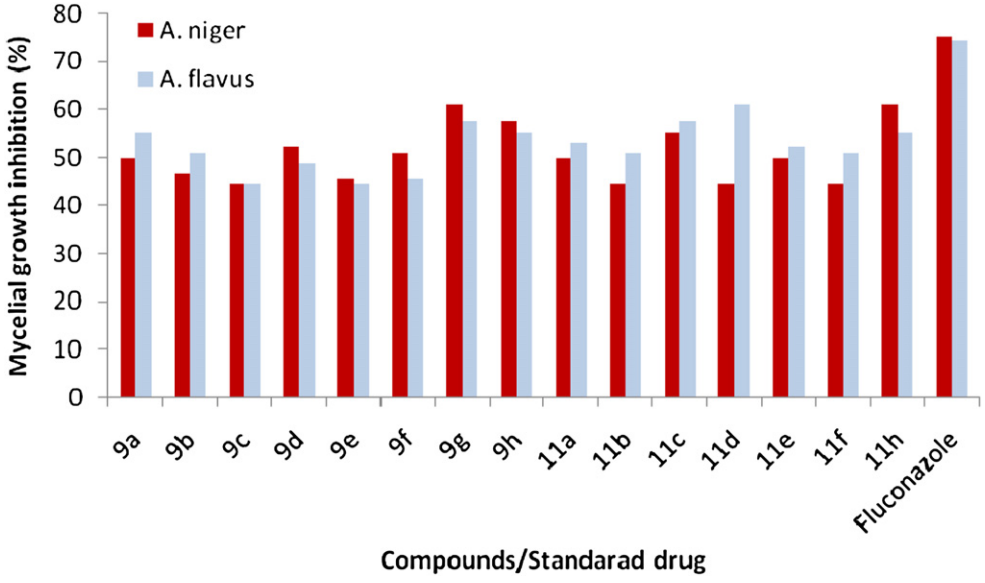


Fig. 1. Comparison of antifungal activity of newly synthesized compounds and standard antifungal drug against test microorganisms.

S. aureus and 26.3 mm against *B. subtilis*. Besides **11b** and **11f**, compounds **9e**, **9h**, **11e** and **11h** showed moderate antibacterial activity against *S. aureus* with zone of inhibition >18.0 mm while compounds **9d** and **11f** showed moderate antibacterial activity against *B. subtilis* with zone of inhibition >18.0 mm. However, in terms of MIC, none of the compounds was found to possess appreciable antibacterial activity. Amongst all the compounds, the MIC ranged between 32 (**11b**, **11f**, **9e**) and 128 μg/mL against Gram-positive bacteria (Table 1). A comparison between the two series of compounds (**9** and **11**) indicates that in general, 4-carbothioamide derivatives (**11**) exhibit better activity against *S. aureus* while a reverse trend is observed against *B. subtilis*. Within the individual series, no correlation between the antibacterial activity with respect to the substituent on phenyl ring is observed. However, in general, compounds containing a halogen substituent showed better antibacterial activity than the compounds with other substituents. A comparative study with corresponding 4-cyano derivatives (**3**) [25] revealed that replacement of cyano group with carboxylic acid moiety (**9**) or carbothioamide moiety (**11**) resulted in complete loss of activity against Gram-negative bacteria and a decreased activity against Gram-positive bacteria. It is an established fact that Gram-negative bacteria are hard to target owing to their cell wall structure, however, it seems from the comparison that increased hydrophilicity due to the replacement of a cyano group with carboxylic acid moiety or carbothioamide moiety is detrimental to activity against Gram-negative bacteria.

2.2.2. In vitro antifungal activity

Carboxylic acids **9** and carbothioamides **11** were also evaluated for their *in vitro* antifungal activity against two fungal strains, *Aspergillus niger* (MTCC 282) and *Aspergillus flavus* (MTCC 871) by poisoned food method [44]. Fluconazole was used as the reference drug and the results were recorded as the percentage inhibition of mycelial growth. When compared with reference drug (75.3% and 74.6% inhibition against *A. niger* and *A. flavus* respectively), it was noted that compounds **9g**, **9h**, **11c** and **11h** showed moderate antifungal activity with ≥55% inhibition of mycelial growth against *A. niger* and compounds **9a**, **9g**, **9h**, **11c**, **11d** and **11h** showed ≥55% inhibition of mycelial growth against *A. flavus* (Table 2). Comparison of antifungal activity of newly synthesized compounds with standard drug fluconazole against both fungal strains in terms of % mycelial growth inhibition is shown in Fig. 1.

3. Conclusions

In the present study, some novel 1,3-diaryl-4-functionalizedpyrazoles bearing benzenesulfonamide moiety at position-1 and an aldehyde, carboxylic acid, cyano and carbothioamide functionality at position-4 were synthesized. Newly synthesized pyrazole-4-carboxylic acids **9** and carbothioamides **11** were screened for their *in vitro* antibacterial activity and antifungal activity. Out of the tested compounds, **9e**, **11b** and **11f** exhibited moderate antibacterial activity against Gram-positive bacteria and **9g** showed moderate antifungal activity against the tested fungi. However, none of the newly synthesized compounds was found to be superior over the reference drugs.

4. Experimental protocols

Melting points were determined in open glass capillaries in an electrical melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu-21 FT-IR or Perkin–Elmer IR Spectrophotometer using the KBr pellet technique. ^{1}H NMR and ^{13}C NMR spectra were recorded either in pure DMSO-d_6 or in $CDCl_3$/DMSO-d_6 mixture on Bruker NMR spectrometers at 300/400 MHz and 75.5/100 MHz respectively using tetramethylsilane (TMS) as internal standard. Chemical shifts are expressed in δ, ppm. Mass spectra (*DART-MS*) were recorded on a JEOL-AccuTOF JMS-T100LC Mass spectrometer having a DART (*Direct Analysis in Real Time*) source in ES^+ mode. The purity of the compounds was checked by ^{1}H NMR and thin layer chromatography (TLC) on silica gel plates using a mixture of petroleum ether and ethyl acetate as eluent. Iodine or UV lamp was used as a visualizing agent. Abbreviations 's' for singlet, 'm' for multiplet, 'ex' for exchangeable proton are used for NMR assignments; 's' for strong, 'm' for medium for IR and 'br' for broad in NMR as well as IR assignments. 'd' stands for decomposition in melting point data.

4.1. General procedure for the synthesis of hydrazones, *6a–h*

To a solution of 4-hydrazinobenzenesulfonamide hydrochloride (**4**, 5.3 mmol) in water (15 mL) was added anhydrous sodium acetate (5.3 mmol) whereupon a solid separated out which was dissolved by the addition of ethanol (20 mL). Subsequently, an appropriate methylketone (**5**, 5.3 mmol) was added and the resulting reaction mixture was refluxed for 1 h. On cooling, a solid separated out which was filtered, dried and crystallized from ethanol to afford hydrazones **6**.

Hydrazones **6a**, **6b**, **6e–6g** were identified by the comparison of their melting point and spectral data with those reported in literature [25].

4.1.1. 4-{2-[1-(4-Methoxyphenyl)ethylidene]hydrazino} benzenesulfonamide (*6c*)

Yield 86%; m.p. 230–232 °C; IR (KBr, cm^{-1}): 3386, 3267 and 3078 (m, N–H stretch), 1605 (s, C=N stretch), 1512 (s, N–H bend), 1311 and 1142 (s, SO_2 stretch); ^{1}H NMR ($CDCl_3$/DMSO-d_6, 400 MHz): δ 8.99 (s, ex, 1H, NH), 7.75 (d, 2H, J = 8.8 Hz, Ar), 7.71 (d, 2H, J = 8.8 Hz, Ar), 7.28 (d, 2H, J = 8.8 Hz, Ar), 6.90 (d, 2H, J = 8.8 Hz, Ar), 6.53 (s, ex, 2H, SO_2NH_2), 3.83 (s, 3H, OCH_3), 2.27 (s, 3H, CH_3).

4.1.2. 4-{2-[1-(4-Fluorophenyl)ethylidene]hydrazino} benzenesulfonamide (*6d*)

Yield 89%; m.p. 210–212 °C; IR (KBr, cm^{-1}): 3357, 3251 and 3110 (m, N–H stretch), 1604 (s, C=N stretch), 1512 (s, N–H bend), 1319 and 1149 (s, SO_2 stretch); ^{1}H NMR ($CDCl_3$/DMSO-d_6, 300 MHz): δ 9.70 (s, ex, 1H, NH), 7.86–7.90 (m, 2H, Ar), 7.65 (d, 2H, J = 8.7 Hz, Ar), 7.31 (d, 2H, J = 8.7 Hz, Ar), 7.23 (t, 2H, J = 8.7 Hz, Ar), 7.08 (s, ex, 2H, SO_2NH_2), 2.28 (s, 3H, CH_3).

4.1.3. 4-{2-[1-(2-Thienyl)ethylidene]hydrazino} benzenesulfonamide (*6h*)

Yield 82%; m.p. 230–232 °C; IR (KBr, cm^{-1}): 3325, 3240 and 3109 (m, N–H stretch), 1597 (s, C=N stretch), 1512 (s, N–H bend), 1319 and 1149 (s, SO_2 stretch); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.73 (s, ex, 1H, NH), 7.66 (d, 2H, J = 8.7 Hz, Ar), 7.47 (d, 1H, J = 4.8 Hz, thienyl), 7.32 (d, 1H, J = 3.0 Hz, thienyl), 7.23 (d, 2H, J = 8.7 Hz, Ar), 7.04–7.06 (m, 3H, SO_2NH_2, thienyl), 2.31 (s, 3H, CH_3).

4.2. General procedure for the synthesis of sulfonamide protected-4-formylpyrazoles, *7a–h*

To a cold, stirred solution of dimethylformamide (30 mL) and phosphorous oxychloride (83.58 mmol) was added hydrazone (**6**, 20.24 mmol) following the literature procedure [35]. The reaction mixture was stirred at 55–60 °C for 5 h, cooled to room temperature, poured into ice cold water and neutralized with saturated aqueous sodium bicarbonate solution whereupon a solid separated out that was filtered, washed with excess of cold water, dried and crystallized from acetic acid to afford aldehydes **7**.

Aldehydes **7a**, **7b**, **7e**–**g** were identified by the comparison of their melting point and spectral data with those reported in literature [25].

*4.2.1. N-[(Dimethylamino)methylidene]-4-[4-formyl-3-(4-methoxyphenyl)-1H-pyrazol-1-yl]-benzenesulfonamide (**7c**)*

Yield 88%; m.p. 162–163 °C; IR (KBr, cm^{-1}): 2930 (m, aldehyde, C–H stretch), 1684 (s, C=O stretch), 1630 (s, C=N stretch), 1339 and 1147 (s, SO_2 stretch); ^1H NMR (DMSO-d_6, 300 MHz): δ 9.86 (s, 1H, CHO), 8.80 (s, 1H, pyrazole C_5–H), 8.10 (s, 1H, CH=N), 7.92–7.96 (m, 4H, Ar), 7.75 (d, 2H, $J = 8.7$ Hz, Ar), 6.94 (d, 2H, $J = 8.7$ Hz, Ar), 3.80 (s, 3H, OCH_3), 3.18 (s, 3H, N–CH_3), 3.02 (s, 3H, N–CH_3); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 184.6 (CHO), 160.1, 159.9, 152.8, 141.6, 140.6, 135.6, 130.0, 127.6, 123.3, 122.4, 119.3, 113.9, 55.2 (OCH_3), 40.9 (NCH_3), 35.1 (NCH_3).

*4.2.2. N-[(Dimethylamino)methylidene]-4-[3-(4-fluorophenyl)-4-formyl-1H-pyrazol-1-yl]-benzenesulfonamide (**7d**)*

Yield 87%; m.p. 188–189 °C; IR (KBr, cm^{-1}): 2928 (m, aldehyde, C–H stretch), 1684 (s, C=O stretch), 1623 (s, C=N stretch), 1340 and 1143 (s, SO_2 stretch); ^1H NMR (DMSO-d_6, 300 MHz): δ 10.06 (s, 1H, CHO), 8.61 (s, 1H, pyrazole C_5–H), 8.19 (s, 1H, CH=N), 8.06 (d, 2H, $J = 8.7$ Hz, Ar), 7.91 (d, 2H, $J = 8.7$ Hz, Ar), 7.85–7.88 (m, 2H, Ar), 7.22 (t, 2H, $J = 8.7$ Hz, Ar), 3.18 (s, 3H, N–CH_3), 3.07 (s, 3H, N–CH_3); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 184.5 (CHO), 162.8 (d, $^1J_{CF} = 241.6$ Hz), 159.1, 151.9, 141.5, 136.1, 130.9 (d, $^3J_{CF} = 8.3$ Hz), 127.7, 127.5, 122.5, 119.4, 115.5 (d, $^2J_{CF} = 21.8$ Hz), 41.0 (NCH_3), 35.1 (NCH_3).

*4.2.3. N-[(Dimethylamino)methylidene]-4-[4-formyl-3-(2-thienyl)-1H-pyrazol-1-yl]benzene-sulfonamide (**7h**)*

Yield 84%; m.p. 143–144 °C; IR (KBr, cm^{-1}): 2932 (m, aldehyde, C–H stretch), 1682 (s, C=O stretch), 1628 (s, C=N stretch), 1335 and 1142 (s, SO_2 stretch); ^1H NMR (DMSO-d_6, 300 MHz): δ 10.06 (s, 1H, CHO), 9.43 (s, 1H, pyrazole C_5–H), 8.24 (s, 1H, CH=N), 8.07–8.13(m, 3H, Ar, thienyl), 7.94 (d, 2H, $J = 8.7$ Hz, Ar), 7.60 (d, 1H, $J = 4.8$ Hz, thienyl), 7.14–7.17 (m, 1H, thienyl), 3.17 (s, 3H, N–CH_3), 2.94 (s, 3H, N–CH_3); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 184.6 (CHO), 160.3, 147.4 142.3, 140.7, 137.5, 133.5, 129.8, 128.6, 128.4, 128.2, 122.3, 119.8, 41.4 (NCH_3), 35.5 (NCH_3).

*4.3. General procedure for the synthesis of 4-formylpyrazoles, **8a**–**h***

To a stirred solution of NaOH (23.56 mmol) in methanol (20 mL) was added *N*-protected formylpyrazole (**7**, 7.85 mmol) followed by THF (60 mL) to get a clear homogeneous solution. Subsequently reaction mixture was stirred for 14 h, whereupon it was poured into ice cold water and neutralized with dilute hydrochloric acid. Solid separated out which was filtered, washed with excess of cold water, dried and crystallized from ethanol to afford aldehydes **8**.

Aldehydes **8a**, **8b**, **8e**–**g** were identified by the comparison of their melting point and spectral data with those reported in literature [25].

*4.3.1. 4-[4-Formyl-3-(4-methoxyphenyl)-1H-pyrazol-1-yl] benzenesulfonamide (**8c**)*

Yield 86%; m.p. 213–214 °C; IR (KBr, cm^{-1}): 3325 and 3240 (m, N–H stretch), 2939 (m, aldehyde, C–H stretch), 1666 (s, C=O stretch), 1597 (s, C=N stretch), 1327 and 1165 (s, SO_2 stretch); ^1H NMR (DMSO-d_6, 300 MHz): δ 10.02 (s, 1H, CHO), 9.05 (s, 1H, pyrazole C_5-H), 8.09 (d, 2H, $J = 8.7$ Hz, Ar), 8.03 (d, 2H, $J = 8.7$ Hz, Ar), 7.87 (d, 2H, $J = 8.7$ Hz, Ar), 7.29 (s, ex, 2H, SO_2NH_2), 7.01 (d, 2H, $J = 8.7$ Hz, Ar), 3.85 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 185.1 (CHO), 160.6, 153.3, 143.1, 141.1, 136.0, 130.5, 127.8, 122.9, 119.7, 114.4, 55.7 (OCH_3).

*4.3.2. 4-[3-(4-Fluorophenyl)-4-formyl-1H-pyrazol-1-yl] benzenesulfonamide (**8d**)*

Yield 78%; m.p. 192–193 °C; IR (KBr, cm^{-1}): 3435 and 3327 (m, N–H stretch), 2930 (m, aldehyde, C–H stretch), 1671 (s, C=O stretch), 1597 (s, C=N stretch), 1327 and 1165 (s, SO_2 stretch); ^1H NMR (DMSO-d_6, 300 MHz): δ 10.03 (s, 1H, CHO), 9.10 (s, 1H, pyrazole C_5–H), 8.08 (d, 2H, $J = 8.7$ Hz, Ar), 8.03 (d, 2H, $J = 8.7$ Hz, Ar), 7.94–7.98 (m, 2H, Ar), 7.22 (t, 2H, $J = 8.7$ Hz, Ar), 7.17 (s, ex, 2H, SO_2NH_2); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 184.9 (CHO), 163.2 (d, $^1J_{CF} = 249.1$ Hz), 152.3, 143.3, 141.0, 136.5, 131.4, 131.3 (d, $^3J_{CF} = 8.3$ Hz), 127.96, 122.91, 127.8, 122.9, 119.8, 115.9 (d, $^2J_{CF} = 22.6$ Hz).

*4.3.3. 4-[4-Formyl-3-(2-thienyl)-1H-pyrazol-1-yl] benzenesulfonamide (**8h**)*

Yield 80%, m.p. 187–188 °C; IR (KBr, cm^{-1}): 3302 and 3124 (m, N–H stretch), 2929 (m, aldehyde, C–H stretch), 1674 (s, C=O stretch), 1597 (s, C=N stretch), 1335 and 1165 (s, SO_2 stretch); ^1H NMR (DMSO-d_6, 300 MHz): δ 10.06 (s, 1H, CHO), 9.47 (s, 1H, pyrazole C_5-H), 7.99–8.15 (m, 5H, Ar, thienyl), 7.77 (d, 1H, $J = 4.8$ Hz, thienyl), 7.51 (s, ex, 2H, SO_2NH_2), 7.20–7.23 (m, 1H, thienyl); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 184.6 (CHO), 147.4, 143.3, 140.8, 137.4, 133.4, 129.8, 128.6, 128.4, 127.9, 122.3, 119.7.

*4.4. General procedure for conversion of 4-formylpyrazoles into respective pyrazole-4-carboxylic acids, **9a**–**h***

To a cold, stirred solution of appropriate 4-formylpyrazole (**8**, 2 mmol) in aqueous pyridine (20 mL, 1:1 v/v) was added potassium permanganate (2 mmol) in small portions maintaining the temperature below 20 °C. The mixture was stirred till the violet color disappeared whereupon aqueous NaOH solution (30 mL, 5% w/v) was added and reaction mixture was further stirred for half an hour. Solid thus formed was filtered and to the filtrate was added cold dilute hydrochloric acid with vigorous stirring till precipitates were obtained. The precipitates were filtered, washed with excess of cold water, dried and crystallized from ethanol to afford acids **9**.

*4.4.1. 1-[4-(Aminosulfonyl)phenyl]-3-phenyl-1H-pyrazole-4-carboxylic acid (**9a**)*

Yield 65%; m.p. 262 °C (d); IR (KBr, cm^{-1}): 3325 and 3232 (m, N–H stretch), 2550–3100 (br m, O–H stretch), 1674 (s, C=O stretch), 1597 (s, C=N stretch), 1535 (s, N–H bend), 1342 and 1157 (s, SO_2 stretch); ^1H NMR (DMSO-d_6, 400 MHz): δ 12.68 (br s, ex, 1H, COOH), 9.20 (s, 1H, pyrazole C_5–H), 8.20 (d, 2H, $J = 8.4$ Hz, Ar), 7.97 (d, 2H, $J = 8.4$ Hz, Ar), 7.84 (d, 2H, $J = 8.4$ Hz, Ar), 7.46 (m, 5H, SO_2NH_2, Ar); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.5, 153.3, 142.3, 140.8, 134.2, 131.7, 129.1, 129.0, 128.6, 127.8, 127.2, 119.0, 114.6; DART MS *m/z* 344.11 $(M+H)^+$, $C_{16}H_{13}N_3O_4SH^+$ calcd. 344.07.

*4.4.2. 1-[4-(Aminosulfonyl)phenyl]-3-(4-methylphenyl)-1H-pyrazole-4-carboxylic acid (**9b**)*

Yield 75%; m.p. 258 °C (d); IR (KBr, cm^{-1}): 3356 and 3232 (m, N–H stretch), 2650–3150 (br m, O–H stretch), 1705 (s, C=O stretch), 1597 (s, C=N stretch), 1528 (s, N–H bend), 1342 and 1157 (s, SO_2 stretch); ^1H NMR (DMSO-d_6, 400 MHz): δ 12.65 (br s, ex, 1H, COOH), 9.17 (s, 1H, pyrazole C_5–H), 8.18 (d, 2H, $J = 8.4$ Hz, Ar), 7.98 (d, 2H, $J = 8.4$ Hz, Ar), 7.74 (d, 2H, $J = 8.0$ Hz, Ar), 7.47 (s, ex, 2H, SO_2NH_2), 7.26 (d, 2H, $J = 8.0$ Hz, Ar), 2.36 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.6, 153.4, 142.3, 140.8, 138.1, 134.1, 129.0, 128.9, 128.4, 127.3, 119.0, 114.5, 20.9 (CH_3); DART MS *m/z* 358.11 $(M+H)^+$, $C_{17}H_{15}N_3O_4SH^+$ calcd. 358.09.

4.4.3. 1-[4-(Aminosulfonyl)phenyl]-3-(4-methoxyphenyl)-1H-pyrazole-4-carboxylic acid (**9c**)

Yield 80%; m.p. 265 °C (d); IR (KBr, cm^{-1}): 3256 (m, N–H stretch), 2500–3150 (br m, O–H stretch), 1697 (s, C=O stretch), 1597 (s, C=N stretch), 1528 (s, N–H bend), 1342 and 1157 (s, SO_2 stretch); ^1H NMR (DMSO-d_6, 400 MHz): δ 12.63 (br s, ex, 1H, COOH), 9.16 (s, 1H, pyrazole C_5–H), 8.18 (d, 2H, $J = 8.4$ Hz, Ar), 7.96 (d, 2H, $J = 8.4$ Hz, Ar), 7.82 (d, 2H, $J = 8.0$ Hz, Ar), 7.46 (s, ex, 2H, SO_2NH_2), 7.01 (d, 2H, $J = 8.0$ Hz, Ar), 3.81 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.7, 159.6, 153.1, 142.2, 140.8, 134.1, 130.4, 127.2, 124.1, 118.9, 114.3, 113.2, 55.1 (OCH_3); DART MS m/z 374.12 $(M + H)^+$, $C_{17}H_{15}N_3O_5SH^+$ calcd. 374.08.

4.4.4. 1-[4-(Aminosulfonyl)phenyl]-3-(4-fluorophenyl)-1H-pyrazole-4-carboxylic acid (**9d**)

Yield 85%; m.p. 266 °C (d); IR (KBr, cm^{-1}): 3371 and 3240 (m, N–H stretch), 2500–3150 (br m, O–H stretch), 1697 (s, C=O stretch), 1597 (s, C=N stretch), 1535 (s, N–H bend), 1342 and 1157 (s, SO_2 stretch); ^1H NMR (DMSO-d_6, 400 MHz): δ 12.74 (br s, ex, 1H, COOH), 9.20 (s, 1H, pyrazole C_5–H), 8.19 (d, 2H, $J = 8.0$ Hz, Ar), 7.90–7.98 (m, 4H, Ar), 7.47 (s, ex, 2H, SO_2NH_2), 7.27–7.31 (m, 2H, Ar); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.5, 162.4 (d, $^1J_{CF} = 244.3$ Hz), 152.4, 142.4, 140.7, 134.3, 131.3 (d, $^3J_{CF} = 8.7$ Hz), 128.24, 128.21, 127.2, 119.1, 114.7 (d, $^2J_{CF} = 21.1$ Hz); DART MS m/z 362.10 $(M + H)^+$, $C_{16}H_{13}FN_3O_4SH^+$ calcd. 362.06.

4.4.5. 1-[4-(Aminosulfonyl)phenyl]-3-(4-chlorophenyl)-1H-pyrazole-4-carboxylic acid (**9e**)

Yield 78%; m.p. 261 °C (d); IR (KBr, cm^{-1}): 3364 and 3263 (m, N–H stretch), 2500–3150 (br m, O–H stretch), 1697 (s, C=O stretch), 1597 (s, C=N stretch), 1535 (s, N–H bend), 1335 and 1157 (s, SO_2 stretch); ^1H NMR (DMSO-d_6, 400 MHz): δ 12.77 (br s, ex, 1H, COOH), 9.21 (s, 1H, pyrazole C_5–H), 8.19 (d, 2H, $J = 7.2$ Hz, Ar), 7.97 (d, 2H, $J = 7.2$ Hz, Ar), 7.89 (d, 2H, $J = 7.6$ Hz, Ar), 7.52 (d, 2H, $J = 7.6$ Hz, Ar), 7.47 (s, ex, 2H, SO_2NH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.5, 152.1, 142.4, 140.7, 134.4, 133.5, 130.9, 130.8, 130.6, 127.9, 127.29, 127.25, 119.1, 119.0, 114.6; DART MS m/z 378.06 $(M + H)^+$, $C_{16}H_{13}ClN_3O_4SH^+$ calcd. 378.03.

4.4.6. 1-[4-(Aminosulfonyl)phenyl]-3-(4-bromophenyl)-1H-pyrazole-4-carboxylic acid (**9f**)

Yield 63%; m.p. 258 °C (d); IR (KBr, cm^{-1}): 3364 and 3263 (m, N–H stretch), 2500–3150 (br m, O-H stretch), 1690 (s, C=O stretch), 1597 (s, C=N stretch), 1535 (s, N–H bend), 1335 and 1157 (s, SO_2 stretch); ^1H NMR (DMSO-d_6, 400 MHz): δ 12.73 (br s, ex, 1H, COOH), 9.20 (s, 1H, pyrazole C_5-H), 8.19 (d, 2H, $J = 8.4$ Hz, Ar), 7.98 (d, 2H, $J = 8.4$ Hz, Ar), 7.83 (d, 2H, $J = 8.0$ Hz, Ar), 7.66 (d, 2H, $J = 8.0$ Hz, Ar), 7.47 (s, ex, 2H, SO_2NH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.6, 152.2, 142.4, 140.75, 140.71, 134.3, 131.1, 131.0, 130.8, 130.6, 127.3, 127.2, 122.2, 119.1, 114.8; DART MS m/z 422.03 $(M + H)^+$, $C_{16}H_{13}BrN_3O_4SH^+$ calcd. 421.98.

4.4.7. 1-[4-(Aminosulfonyl)phenyl]-3-(4-nitrophenyl)-1H-pyrazole-4-carboxylic acid (**9g**)

Yield 64%; m.p. 263 °C (d); IR (KBr, cm^{-1}): 3356 and 3263 (m, N–H stretch), 2500–3150 (br m, O–H stretch), 1705 (s, C=O stretch), 1597 (s, C=N stretch), 1535 (s, N–H bend), 1342 and 1157 (s, SO_2 stretch); ^1H NMR (DMSO-d_6, 400 MHz): δ 12.93 (br s, ex, 1H, COOH), 9.27 (s, 1H, pyrazole C_5–H), 8.31 (d, 2H, $J = 8.4$ Hz, Ar), 8.15 (d, 2H, $J = 8.0$ Hz, Ar), 7.99 (d, 2H, $J = 8.4$ Hz, Ar), 7.91 (d, 2H, $J = 8.0$ Hz, Ar), 7.49 (s, ex, 2H, SO_2NH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.3, 151.2, 147.4, 142.7, 140.6, 138.2, 134.7, 130.3, 127.3, 123.0, 119.3, 115.2; DART MS m/z 389.09 $(M + H)^+$, $C_{16}H_{12}N_4O_6SH^+$ calcd. 389.06.

4.4.8. 1-[4-(Aminosulfonyl)phenyl]-3-(2-thienyl)-1H-pyrazole-4-carboxylic acid (**9h**)

Yield 70%; m.p. 240 °C (d); IR (KBr, cm^{-1}): 3248 (m, N–H stretch), 2550–3150 (br m, O–H stretch), 1705 (s, C=O stretch), 1597 (s, C=N stretch), 1528 (s, N–H bend), 1335 and 1165 (s, SO_2 stretch); ^1H NMR (DMSO-d_6, 400 MHz): δ 12.88 (br s, ex, 1H, COOH), 9.20 (s, 1H, pyrazole C_5-H), 8.14–8.21 (m, 3H, Ar, thienyl), 7.98 (d, 2H, $J = 8.4$ Hz, Ar), 7.62 (d, 1H, $J = 5.2$ Hz, thienyl), 7.47 (s, ex, 2H, SO_2NH_2), 7.16–7.18 (m, 1H, thienyl); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.5, 147.4, 142.4, 140.5, 134.7, 133.4, 129.4, 127.6, 127.4, 127.3, 119.0, 113.6; DART MS m/z 350.05 $(M + H)^+$, $C_{14}H_{11}N_3O_4S_2H^+$ calcd. 350.03.

4.5. General procedure for conversion of 4-formylpyrazoles into respective 4-cyanopyrazoles, **10a–h**

To a stirred solution of appropriate 4-formylpyrazole (**8**, 1 mmol) in THF (6 mL) was added aqueous NH_3 (30%, 8 mL) followed by I_2 (1.2 mmol). Reaction mixture was stirred at room temperature until dark violet/brown solution became colorless whereupon it was poured into aqueous $Na_2S_2O_3$ solution (25 mL, 5% w/v). The precipitates so obtained were filtered, washed with excess of cold water, dried and crystallized from ethanol to afford cyanopyrazoles **10**.

4-Cyanopyrazoles **10a**, **10b**, **10e–g** were identified by the comparison of their melting point and spectral data with those reported in literature [25].

4.5.1. 4-[4-Cyano-3-(4-methoxyphenyl)-1H-pyrazol-1-yl] benzenesulfonamide (**10c**)

Yield 96%; m.p. 222–224 °C; IR (KBr, cm^{-1}): 3364 and 3271 (m, N–H stretch), 2230 (s, C≡N stretch), 1605 (s, C=N stretch), 1535 (s, N–H bend), 1336 and 1163 (s, SO_2 stretch); ^1H NMR (DMSO-d_6, 300 MHz): δ 9.51 (s, 1H, pyrazole C_5-H), 8.14 (d, 2H, $J = 8.7$ Hz, Ar), 8.01 (d, 2H, $J = 8.7$ Hz, Ar), 7.94 (d, 2H, $J = 8.7$ Hz, Ar), 7.51 (s, ex, 2H, SO_2NH_2), 7.14 (d, 2H, $J = 8.7$ Hz, Ar), 3.84 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 161.0, 153.4, 143.4, 140.8, 137.2, 128.4, 127.8, 122.7, 119.8, 115.0, 114.7, 91.3 (C≡N), 55.8 (OCH_3).

4.5.2. 4-[4-Cyano-3-(4-fluorophenyl)-1H-pyrazol-1-yl] benzenesulfonamide (**10d**)

Yield 96%; m.p. 232–234 °C; IR (KBr, cm^{-1}): 3356 and 3262 (m, N–H stretch), 2235 (s, C≡N stretch), 1597 (s, C=N stretch), 1528 (s, N–H bend), 1339 and 1161 (s, SO_2 stretch); ^1H NMR (DMSO-d_6, 300 MHz): δ 9.54 (s, 1H, pyrazole C_5-H), 8.14 (d, 2H, $J = 7.8$ Hz, Ar), 8.01–8.03 (m, 4H, Ar), 7.52 (s, ex, 2H, SO_2NH_2), 7.43 (t, 2H, $J = 8.1$ Hz, Ar); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 163.4 (d, $^1J_{CF} = 248.3$ Hz), 152.7, 143.3, 140.7, 137.0, 129.2 (d, $^3J_{CF} = 9.0$ Hz), 127.8, 126.6, 119.9, 116.6 (d, $^2J_{CF} = 21.9$ Hz), 114.3, 91.7 (C≡N).

4.5.3. 4-[4-Cyano-3-(2-thienyl)-1H-pyrazol-1-yl] benzenesulfonamide (**10h**)

Yield 98%; m.p. 216–220 °C; IR (KBr, cm^{-1}): 3296 and 3126 (m, N–H stretch), 2236 (s, C≡N stretch), 1596 (s, C=N stretch), 1531 (s, N–H bend), 1335 and 1164 (s, SO_2 stretch); ^1H NMR (DMSO-d_6, 300 MHz): δ 9.52 (s, 1H, pyrazole C_5–H), 8.09 (d, 2H, $J = 8.7$ Hz, Ar), 8.01 (d, 2H, $J = 8.7$ Hz, Ar), 7.76–7.78 (m, 2H, thienyl), 7.52 (s, ex, 2H, SO_2NH_2), 7.24–7.27 (m, 1H, thienyl); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 148.9, 143.6, 140.5, 137.2, 132.11, 129.0, 128.8, 127.9, 127.4, 119.9, 114.1, 91.1 (C≡N).

4.6. General procedure for conversion of 4-cyanopyrazoles into respective pyrazole-4-carbothioamides, **11a–f** and **g**

To an ice cold solution of appropriate 4-cyanopyrazole (**10**, 2 mmol) in pyridine (20 mL) was added triethylamine (5 mL)

whereupon H_2S gas was passed through the solution for 10 h with occasional stirring. Color of the solution changed first from yellow to green and finally to dark brown indicating the reaction progression. Reaction mixture was kept as such for overnight whereupon it was poured into ice cold water (150 mL) and neutralized with cold dilute hydrochloric acid with vigorous stirring till precipitates were obtained. The resulting precipitates were filtered, washed with excess of cold water, dried and crystallized from ethanol to afford carbothioamides **11**.

4.6.1. 1-[4-(Aminosulfonyl)phenyl]-3-phenyl-1H-pyrazole-4-carbothioamide (**11a**)

Yield 68%; m.p. 210–212 °C; IR (KBr, cm^{-1}): 3441, 3302, 3225 and 3101 (m, N–H stretch), 1651 (s, C=N stretch), 1597 (s, C=N stretch), 1551 (s, N–H bend), 1319 and 1157 (s, SO_2 stretch); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.85 (s, ex, 1H, NH/SH), 9.29 (s, ex, 1H, NH/SH), 8.89 (s, 1H, pyrazole C_5–H), 8.11 (d, 2H, J = 8.4 Hz, Ar), 7.96 (d, 2H, J = 8.4 Hz, Ar), 7.45–7.72 (m, 7H, SO_2NH_2, Ar); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 194.2, 149.7, 141.4, 132.2, 129.8, 128.9, 128.7, 128.4, 127.8, 126.4, 118.9; DART MS m/z 359.08 $(M+H)^+$, $C_{16}H_{14}N_4O_2S_2H^+$ calcd. 359.07.

4.6.2. 1-[4-(Aminosulfonyl)phenyl]-3-(4-methylphenyl)-1H-pyrazole-4-carbothioamide (**11b**)

Yield 80%; m.p. 222–225 °C; IR (KBr, cm^{-1}): 3310, 3232 and 3101 (m, N–H stretch), 1659 (s, C=N stretch), 1597 (s, C=N stretch), 1551 (s, N–H bend), 1327 and 1157 (s, SO_2 stretch); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.84 (s, ex, 1H, NH/SH), 9.25 (s, ex, 1H, NH/SH), 8.87 (s, 1H, pyrazole C_5–H), 8.12 (d, 2H, J = 8.4 Hz, Ar), 7.95 (d, 2H, J = 8.4 Hz, Ar), 7.62 (d, 2H, J = 7.8 Hz, Ar), 7.45 (s, ex, 2H, SO_2NH_2), 7.26 (d, 2H, J = 7.8 Hz, Ar), 2.35 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 193.4, 149.6, 142.4, 138.3, 129.8, 129.3, 128.3, 127.8, 126.3, 118.8, 21.3 (CH_3); DART MS m/z 373.11 $(M+H)^+$, $C_{17}H_{16}N_4O_2S_2H^+$ calcd. 373.08.

4.6.3. 1-[4-(Aminosulfonyl)phenyl]-3-(4-methoxyphenyl)-1H-pyrazole-4-carbothioamide (**11c**)

Yield 93%; m.p. 203–205 °C; IR (KBr, cm^{-1}): 3325, 3248 and 3109 (m, N–H stretch), 1651 (s, C=N stretch), 1597 (s, C=N stretch), 1551 (s, N–H bend), 1342 and 1165 (s, SO_2 stretch); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.85 (s, ex, 1H, NH/SH), 9.26 (s, ex, 1H, NH/SH), 8.85 (s, 1H, pyrazole C_5–H), 8.11 (d, 2H, J = 9.0 Hz, Ar), 7.95 (d, 2H, J = 9.0 Hz, Ar), 7.66 (d, 2H, J = 8.7 Hz, Ar), 7.46 (s, ex, 2H, SO_2NH_2), 7.02 (d, 2H, J = 8.7 Hz, Ar), 3.79 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 194.3, 159.9, 149.7, 142.1, 141.4, 129.8, 127.8, 125.9, 124.4, 118.8, 114.2, 55.6 (OCH_3); DART MS m/z 389.10 $(M+H)^+$, $C_{17}H_{16}N_4O_3S_2H^+$ calcd. 389.08.

4.6.4. 1-[4-(Aminosulfonyl)phenyl]-3-(4-fluorophenyl)-1H-pyrazole-4-carbothioamide (**11d**)

Yield 91%; m.p. 230–232 °C; IR (KBr, cm^{-1}): 3433, 3286 and 3148 (m, N–H stretch), 1636 (s, C=N stretch), 1597 (s, C=N stretch), 1543 (s, N–H bend), 1342 and 1157 (s, SO_2 stretch); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.87 (s, ex, 1H, NH/SH), 9.34 (s, ex, 1H, NH/SH), 8.91 (s, 1H, pyrazole C_5–H), 8.13 (d, 2H, J = 8.7 Hz, Ar), 7.97 (d, 2H, J = 8.7 Hz, Ar), 7.75 (dd, 2H, $^4J_{HF}$ = 5.7 Hz, $^3J_{HH}$ = 8.7 Hz, Ar), 7.46 (s, ex, 2H, SO_2NH_2), 7.31 (t, 2H, J = 8.7 Hz, Ar); ^{13}C NMR (DMSO-d_6, 75.5 MHz): 194.2, 162.5 (d, $^1J_{CF}$ = 248.2 Hz), 149.7, 141.9, 141.4, 130.8 (d, $^3J_{CF}$ = 8.3 Hz), 129.6, 128.3, 127.9, 125.6, 119.2, 115.6 (d, $^2J_{CF}$ = 21.9 Hz); DART MS m/z 377.09 $(M+H)^+$, $C_{16}H_{13}FN_4O_2S_2H^+$ calcd. 377.06.

4.6.5. 1-[4-(Aminosulfonyl)phenyl]-3-(4-chlorophenyl)-1H-pyrazole-4-carbothioamide (**11e**)

Yield 94%; m.p. 216–219 °C; IR (KBr, cm^{-1}): 3572, 3441, 3340 and 3148 (m, N–H stretch), 1643 (s, C=N stretch), 1597 (s, C=N stretch), 1551 (s, N–H bend), 1342 and 1157 (s, SO_2 stretch); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.88 (s, ex, 1H, NH/SH), 9.37 (s, ex, 1H, NH/SH), 8.91 (s, 1H, pyrazole C_5–H), 8.13 (d, 2H, J = 8.4 Hz, Ar), 7.96 (d, 2H, J = 8.4 Hz, Ar), 7.73 (d, 2H, J = 7.8 Hz, Ar), 7.53 (d, 2H, J = 7.8 Hz, Ar), 7.46 (s, ex, 2H, SO_2NH_2); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 194.1, 149.3, 142.5, 141.3, 133.6, 130.2, 129.9, 128.8, 127.8, 126.3, 119.0; DART MS m/z 393.06 $(M+H)^+$, $C_{16}H_{13}ClN_4O_2S_2H^+$ calcd. 393.03.

4.6.6. 1-[4-(Aminosulfonyl)phenyl]-3-(4-bromophenyl)-1H-pyrazole-4-carbothioamide (**11f**)

Yield 85%; m.p. 211–213 °C; IR (KBr, cm^{-1}): 3564, 3441, 3348 and 3171 (m, N–H stretch), 1643 (s, C=N stretch), 1597 (s, C=N stretch), 1551 (s, N–H bend), 1335 and 1157 (s, SO_2 stretch); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.87 (s, ex, 1H, NH/SH), 9.36 (s, ex, 1H, NH/SH), 8.91 (s, 1H, pyrazole C_5–H), 8.13 (d, 2H, J = 8.7 Hz, Ar), 7.97 (d, 2H, J = 8.7 Hz, Ar), 7.66–7.68 (m, 4H, Ar), 7.46 (s, ex, 2H, SO_2NH_2); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 193.8, 148.9, 142.5, 141.3, 131.7, 131.4, 130.5, 129.9, 127.8, 126.3, 122.3, 119.0; DART MS m/z 437.01 $(M+H)^+$, $C_{16}H_{13}BrN_4O_2S_2H^+$ calcd. 436.98.

4.6.7. 1-[4-(Aminosulfonyl)phenyl]-3-(2-thienyl)-1H-pyrazole-4-carbothioamide (**11h**)

Yield 79%; m.p. 207–209 °C; IR (KBr, cm^{-1}): 3441, 3340, 3256 and 3124 (m, N–H stretch), 1628 (s, C=N stretch), 1597 (s, C=N stretch), 1535 (s, N–H bend), 1350 and 1165 (s, SO_2 stretch); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.03 (s, ex, 1H, NH/SH), 9.56 (s, ex, 1H, NH/SH), 8.89 (s, 1H, pyrazole C_5–H), 8.09 (d, 2H, J = 8.7 Hz, Ar), 7.96 (d, 2H, J = 8.7 Hz, Ar), 7.60–7.62 (m, 2H, thienyl), 7.47 (s, ex, 2H, SO_2NH_2), 7.13–7.16 (m, 1H, thienyl); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 194.0, 144.0, 142.4, 141.1, 133.8, 129.7, 128.0, 127.9, 127.5, 127.3, 125.9, 118.8; DART MS m/z 365.05 $(M+H)^+$, $C_{14}H_{12}N_4O_2S_3H^+$ calcd. 365.02.

4.7. Antimicrobial assay

4.7.1. In vitro antibacterial assay

The antibacterial activity of newly synthesized compounds was evaluated by agar well diffusion method [41]. All the microbial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5×10^8 cfu/mL [42,45]. 20 mL of Mueller Hinton agar medium was poured into each Petri plate and the agar plates were swabbed with 100 μL inocula of each test bacterium and kept for 15 min for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into seeded agar plates and these were loaded with a 100 μL volume with concentration of 4.0 mg/mL of each compound reconstituted in dimethylsulphoxide (DMSO). All the plates were incubated at 37 °C for 24 h. Antibacterial activity of 15 newly synthesized compounds was evaluated by measuring the zone of growth inhibition against the test bacteria with zone reader (Hiantibiotic zone scale). DMSO was used as a negative control whereas ciprofloxacin was used as a positive control. The experiments were performed in triplicates. The antibacterial activity of the compounds was compared with ciprofloxacin as standard. Minimum Inhibitory Concentration (MIC) of newly synthesized compounds against tested bacteria was determined using macrodilution tube method as recommended by NCCLS [42,43]. MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. In this method, various test concentrations of newly synthesized compounds were prepared from 128 to 0.25 μg/mL in sterile tubes No. 1–10. 100 μL sterile Mueller Hinton Broth (MHB) was poured in each sterile tube followed by addition of 200 μL test compound in tube 1. Two fold serial dilutions were carried out from

tube 1 to tube 10 and excess broth (100 μL) was discarded from the last tube No. 10. To each tube, 100 μL of standard inoculum (1.5×10^8 cfu/mL) was added. Ciprofloxacin was used as control. Turbidity was observed after incubating the inoculated tubes at 37 °C for 24 h.

4.7.2. In vitro antifungal assay

The antifungal activity of newly synthesized compounds was evaluated by poisoned food method [44]. The molds were grown on Saburaud Dextrose Agar (SDA) at 25 °C for 7 days and used as inocula. 15 mL of molten SDA (45 °C) was poisoned by the addition of 100 μL volume of each compound having concentration of 4.0 mg/mL, reconstituted in DMSO, poured into a sterile Petri plate and allowed to solidify at room temperature. The solidified poisoned agar plates were inoculated at the centre with fungal plugs (8 mm diameter), obtained from the actively growing colony and incubated at 25 °C for 7 days. DMSO was used as a negative control whereas fluconazole was used as a positive control. The experiments were performed in triplicates. Diameter of the fungal colonies was measured and expressed as percent mycelial inhibition determined by applying the following formula:

$$\text{Inhibition of mycelial growth } \% = (dc - dt)/dc \times 100$$

where dc = average diameter of fungal colony in negative control plates; dt = average diameter of fungal colony in experimental plates.

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