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ORIGINAL PAPER

One-pot synthesis of diphenyl pyrazolylmethylanilines via reductive amination using $NaBH_4/I_2$ and their antimicrobial screening

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Abstract Direct reductive amination of 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde and 3-(4-methylphenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde with various substituted aromatic amines using $NaBH_4$ in the presence of I_2 as reducing agent is described. The reaction has been carried out in anhydrous methanol under neutral conditions at room temperature. The structure of newly synthesized diphenyl pyrazolylmethylanilines was established on the basis of IR, ^1H, ^{13}C NMR, and mass spectral data. All diphenyl pyrazolylmethylaniline derivatives were tested in vitro for their antifungal and antibacterial activity against different strains of fungi and bacteria. Most of the compound exhibited considerable antifungal activity but poor antibacterial activity against the test strains.

Keywords Reductive amination · $NaBH_4/I_2$ · Diphenyl pyrazolylmethylaniline · NMR spectroscopy · Antimicrobial screening

Introduction

Amines are important organic compounds and their synthesis have been widely studied in organic chemistry. Moreover, amines bearing heteroaryl rings are a large class of organic compounds whose chemistry and biology have been exploited considerably for drug discovery [1–4]. Because of their interesting physiological properties, secondary amines in particular are extremely important and are consistently found in privileged structures (pharmacophores) in numerous biologically active compounds [5, 6]. Furthermore, secondary amines can be utilized as important scaffolds for structural manipulations. These amines have been demonstrated to be extremely useful intermediates for the synthesis of numerous natural products and bioactive molecules [7–9].

Reductive amination of aldehydes and ketones is an important direct transformation of carbonyl compounds into amines. In this important carbon–nitrogen bond-forming process, an aldehyde or ketone is reacted with ammonia, primary, or secondary amines in the presence of reducing agents to produce primary, secondary, and tertiary amines, respectively. A variety of reducing agents, such as hydrogen in the presence of metal catalyst, Zn–AcOH, $NaBH_4$–magnesium perchlorate, $ZnBH_4$–$ZnCl_2$, $NaBH_3CN$, $NaBH(OAc)_3$, borane–pyridine, $ZnCl_2$–$NaBH_4$, silica gel–$Zn(BH_4)_2$, and $NaBH_4$–wet clay/microwave, have been used for these transformations [10–19]. As part of our research for potential biologically active heterocycles containing a pyrazole ring and amines [20, 21], we report here an efficient method for the direct reductive amination of 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde (**3**) and 3-(4-methylphenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**4**) with various aromatic amines using $NaBH_4$ and a catalytic amount of iodine in absolute methanol in reasonably good yield. The newly synthesized diphenyl pyrazolylmethylanilines were also screened for possible antimicrobial activity against a panel of fungal and bacterial strains.

Results and discussion

Chemistry

The requisite key intermediates of pyrazolecarbaldehydes **3** and **4** were prepared by the reaction of the Vilsmeier–

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Haack reagent (*N*,*N*-dimethylformamide (DMF)/$POCl_3$) and phenyl hydrazones **1** and **2** [22]. The various new diphenyl pyrazolylmethylanilines **5a–5e** and **6a–6h** were synthesized by direct reductive amination using $NaBH_4$ in the presence of I_2 as reducing agent as outlined in Scheme 1.

The reaction was carried out by simply stirring 1.0 equivalent of aldehyde and 1.2 equivalents of aromatic amine in methanol in the presence of the $NaBH_4/I_2$ reagent. The iodine catalyzed the in situ formation of an imine at room temperature that was ultimately reduced in situ to the secondary amine by $NaBH_4$. The functional group transformation of –CHO in compounds **3** and **4** into $–CH_2NH–$ in compounds **5a–5e** and **6a–6h** was established on the basis of IR, ^1H, ^{13}C NMR including DEPT-135, and mass spectral data. In the ^1H NMR spectra of compound **3** and **4**, the aldehyde proton resonated as a singlet at $\delta = 10.06$ and 9.95 ppm, respectively, which disappeared in spectra of compounds **5a–5e** and **6a–6h**, and a new signal was observed at high upfield values at $\delta = 4.11–4.21$ ppm integrating for two protons. The signal due to the NH proton was observed at $\delta = 5.51–6.32$ ppm. The methylene group of $–CH_2NH–$ appeared as a singlet, broad singlet (bs), and in some compounds as a doublet ($J = 3.8–5.1$ Hz) due to coupling with the NH proton whereas the NH signal was observed as a broad singlet. Furthermore in the ^{13}C NMR spectra of the compounds **3** and **4**, the signal due to the carbonyl carbon was observed at $\delta = 178.9$ and 180.0 ppm, respectively. No carbonyl carbon signal was found in the spectra of compounds **5a–5e** and **6a–6h** and a new signal due to $–CH_2NH–$ appeared at 38.6–40.2 ppm. This assignment was also confirmed by DEPT-135 spectra of compounds **5a** and **5b** in which a negative peak was observed for $–CH_2–$ at 39.6 and 40.2 ppm, respectively. The above spectral analysis suggested the successful reductive amination of pyrazolecarbaldehydes **3** and **4**. This fact was further supported by FAB-MS spectra of compounds **5a–5e** and **6a–6h** (Table 1).

Preliminary antimicrobial activity

All the diphenyl pyrazolylmethylanilines were tested in vitro for antifungal activity against *Candida albicans* (ATCC 64550), *Aspergillus niger* (MTCC 281), *A. flavus* (MTCC 277), and *Penicillium citrinum* (NCIM 768) using the cup-plate agar diffusion method at concentrations of 6.25, 12.5, 25, 50, 100, and 200 μg/cm^3 [23, 24]. Fluconazole was used as a reference drug at a concentration of 6.25 μg/cm^3.

As shown in Table 2 among the series **5a–5e** and **6a–6h**, compounds **5a**, **5c**, **6b**, **6c**, and **6e** exhibited a minimum inhibitory concentration (MIC) in the range of 25–50 μg/cm^3 against *C. albicans*, whereas compounds **5a**, **5c**, **5d**, and **6a–6g** showed antifungal activity against *A. niger* and *A. flavus* with MIC values in the range of 25–50 μg/cm^3. The minimum concentration required to inhibit the growth of *P. citrinum* was found to be in the range of 50–100 μg/cm^3. The lipophilicity of the compounds is well known to play an important role in antifungal activity [25]. Our results demonstrate that increasing the lipophilic character of the compounds increased the antifungal activity of diphenyl pyrazolylmethylanilines having electron-withdrawing groups but not electron-releasing groups.

All compounds were also screened for their in vitro antibacterial activity against *Escherichia coli* (NCTC 10418), *Staphylococcus aureus* (NCTC 65710), and *Pseudomonas aeruginosa* (NCTC 10662). Ciprofloxacin was used as a reference drug. None of the test compounds showed good antibacterial activity, whereas compounds **5e** and **6h** exhibited moderate antibacterial activity against *E. coli* (NCTC 10418) and *S. aureus* (NCTC 65710). All test compounds were poorly active against *P. aeruginosa* (NCTC 10662).

Conclusion

On the basis of antifungal activity data, it can be concluded that the newly synthesized diphenyl pyrazolylmethylaniline

Scheme 1

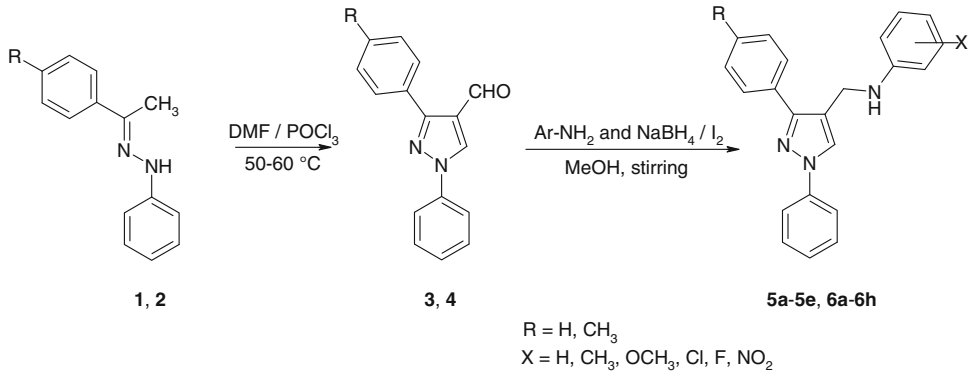

Table 1 Physicochemical data of diphenyl pyrazolylmethylaniline derivatives **5a–5e** and **6a–6h**

Compd	R	X	Yield[a] (%)	M.p. (°C)	Clog$P$[b]	R_f value[c]
5a	H	H	70	96	5.18 ± 1.02	0.72
5b	H	3-CH_3	68	109	5.64 ± 1.02	0.72
5c	H	4-F	65	88	5.63 ± 1.02	0.64
5d	H	2-Cl	71	117	5.69 ± 1.02	0.72
5e	H	4-NO_2	59	128	5.63 ± 1.02	0.80
6a	CH_3	H	67	64	5.64 ± 1.02	0.74
6b	CH_3	4-Cl	72	68	6.42 ± 1.02	0.72
6c	CH_3	4-F	52	75	6.09 ± 1.02	0.75
6d	CH_3	4-OCH_3	73	70	5.46 ± 1.02	0.69
6e	CH_3	3-Cl-4-F	63	64	7.33 ± 1.17	0.71
6f	CH_3	4-CH_3	68	68	6.42 ± 1.02	0.73
6g	CH_3	3-Cl	70	72	6.77 ± 1.17	0.80
6h	CH_3	4-NO_2	66	62	6.09 ± 1.02	0.73

[a] Recrystallization from ethanol/methanol/chloroform mixture

[b] ACD lab software version 12.0 (freeware)

[c] Benzene/acetone (9.5:0.5)

derivatives possess considerable antifungal activity. The antifungal activity was higher in compounds substituted with a chloro and fluoro group, whereas in the case of antibacterial activity the compound substituted with a nitro derivative exhibited slightly better activity compared to other derivatives of the **5a–5e** series. However the activity of the tested compounds is less than that of the reference drugs fluconazole and ciprofloxacin.

Experimental

All the chemicals were supplied by E. Merck (Germany) and S. D. Fine chemicals (India). Melting points were determined by the open tube capillary method. IR spectra were recorded as KBr pellets on a Bio Rad FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a 300-MHz Bruker DPX spectrophotometer using DMSO-d_6 or $CDCl_3$ as solvent. Mass spectra were recorded on a JEOL SX102/DA-6000 mass spectrometer, elemental analysis on a Vario EL III CHNOS-Elementar analyzer and results agreed favorably with calculated values. Thin-layer chromatography (TLC) was performed to monitor the progress of the reaction and purity of the compounds, the spots being visualized under iodine vapor or UV light. Solvents were purified using standard procedures. Compounds **3** and **4** were prepared according to a published method [22].

*General procedure for synthesis of compounds **5a–5e** and **6a–6h***

To a solution of appropriate 1*H*-pyrazole-4-carbaldehyde **3** or **4** (1.0 mmol) in 10 cm^3 of methanol, substituted aniline

Table 2 Antimicrobial activity data of diphenyl pyrazolylmethylaniline derivatives **5a–5e** and **6a–6h**

Compd	MIC (zone of inhibition)						
	Antifungal activity				Antibacterial activity		
	C. albicans	*A. flavus*	*A. niger*	*P. citrinum*	*E. coli*	*S. aureus*	*P. aeruginosa*
5a	50 (6.5)	50 (5.5)	50 (5.5)	100 (7.5)	200 (6.0)	200 (6.5)	–
5b	100 (6.5)	100 (8.0)	100 (7.5)	100 (6.5)	200 (6.5)	200 (7.0)	200 (5.5)
5c	50 (7.5)	50 (7.0)	50 (8.0)	100 (7.0)	200 (7.0)	200 (7.0)	–
5d	100 (8.5)	50 (6.0)	50 (5.5)	100 (6.5)	200 (7.5)	200 (7.0)	–
5e	100 (6.5)	100 (7.5)	100 (7.0)	–	100 (6.5)	100 (7.5)	200 (7.5)
6a	100 (8.0)	50 (5.5)	50 (6.0)	100 (8.5)	100 (5.5)	200 (8.0)	–
6b	50 (6.0)	25 (5.0)	25 (5.5)	50 (6.5)	100 (6.0)	100 (7.0)	200 (7.0)
6c	25 (6.0)	25 (6.5)	25 (5.5)	50 (7.0)	100 (5.5)	100 (6.0)	–
6d	100 (7.5)	50 (5.0)	50 (5.5)	100 (7.5)	200 (7.0)	200 (7.0)	–
6e	25 (6.5)	25 (6.5)	25 (7.0)	50 (7.5)	100 (7.0)	200 (5.5)	200 (6.0)
6f	100 (8.5)	50 (6.5)	50 (6.5)	100 (6.5)	200 (6.5)	200 (7.0)	–
6g	100 (7.0)	50 (8.5)	50 (7.5)	100 (7.5)	200 (6.0)	200 (6.5)	–
6h	100 (6.5)	100 (6.0)	–	–	200 (8.5)	100 (6.5)	200 (7.5)
Fluconazole	6.25 (9.5)	6.25 (9.0)	6.25 (8.5)	6.25 (10.0)	NT	NT	NT
Ciprofloxacin	NT	NT	NT	NT	6.25 (10.0)	6.25 (9.5)	6.25 (8.5)

MIC data are presented in μg/cm^3; zone of inhibition data are in presented in mm

NT not tested; – absence of activity

(1.2 mmol) was added and then 50 mg iodine (0.4 mmol) was added with stirring at room temperature. To the stirred solution 55 mg of sodium borohydride (1.4 mmol) was added slowly, stirring further for 3–6 h. A precipitate was formed which was filtered, washed with water, dried, and recrystallized from ethanol to give crystalline products **5a**–**5e** and **6a**–**6h**. The progress of the reaction and purity of the compound were checked by TLC, using benzene/acetone (9:1) as mobile phase. Physicochemical data of the compounds are presented in Table 1.

N-(1,3-Diphenyl-1H-pyrazol-4-ylmethyl)aniline (**5a**, $C_{22}H_{19}N_3$)
IR (KBr): $\bar{\nu}$ = 3,211 (N–H), 1,620 (C=N), 1,548 (C=C), 1,029 (C–N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 4.20 (bs, 2H, CH_2), 5.91 (bs, 1H, NH), 6.52–6.66 (m, 3H, Ar–H), 7.04–7.09 (m, 2H, Ar–H), 7.22–7.30 (m, 3H, Ar–H), 7.46–7.51 (m, 2H, Ar–H), 7.67–7.69 (m, 2H, Ar–H), 7.83–7.86 (m, 2H, Ar–H), 8.53 (s, 1H, pyrazole-H-5) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 39.6 (CH_2), 113.6, 117.0, 126.1, 127.4, 127.8, 128.6, 129.3, 131.3, 135.2, 139.9, 145.5, 151.6 ppm; FAB-MS: m/z = 325 (M^+).

N-(1,3-Diphenyl-1H-pyrazol-4-ylmethyl)-3-methylaniline (**5b**, $C_{23}H_{21}N_3$)
IR (KBr): $\bar{\nu}$ = 3,264 (N–H), 1,599 (C=N), 1,515 (C=C), 1,067 (C–N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.13 (s, 3H, CH_3), 4.20 (d, 2H, CH_2, J = 4.6 Hz), 5.69 (bs, 1H, NH), 6.57 (d, 2H, Ar–H, J = 8.1 Hz), 6.89 (d, 2H, Ar–H, J = 8.1 Hz), 7.31–7.49 (m, 6H, Ar–H), 7.80 (d, 2H, Ar–H, J = 6.9 Hz), 7.85 (d, 2H, Ar–H, J = 7.5 Hz), 8.54 (s, 1H, pyrazole-H-5) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 20.6 (CH_3), 40.1 (CH_2), 113.2, 115.2, 118.3, 126.9, 127.3, 128.8, 129.6, 131.1, 136.2, 139.5, 145.1, 151.6 ppm; FAB-MS: m/z = 339 (M^+).

N-(1,3-Diphenyl-1H-pyrazol-4-ylmethyl)-4-fluoroaniline (**5c**, $C_{22}H_{18}FN_3$)
IR (KBr): $\bar{\nu}$ = 3,309 (N–H), 1,599 (C=N), 1,506 (C=C), 1,025 (C–N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 4.21 (s, 2H, CH_2), 5.83 (bs, 1H, NH), 6.62–6.67 (m, 2H, Ar–H), 6.88–6.94 (m, 2H, Ar–H), 7.30–7.52 (m, 6H, ArH), 7.79 (d, 2H, Ar–H, J = 7.2 Hz), 7.85 (d, 2H, Ar–H, J = 8.1 Hz), 8.53 (s, 1H, pyrazole-H-5) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 39.8 (CH_2), 112.9, 116.2, 119.3, 125.9, 127.3, 127.8, 128.4, 129.3, 131.3, 135.2, 139.9, 145.5, 151.6 ppm; FAB-MS: m/z = 343 (M^+).

2-Chloro-N-(1,3-diphenyl-1H-pyrazol-4-ylmethyl)aniline (**5d**, $C_{22}H_{18}ClN_3$)
IR (KBr): $\bar{\nu}$ = 3,241 (N–H), 1,596 (C=N), 1,504 (C=C), 1,059 (C–N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 4.22 (d, 2H, CH_2, J = 4.5 Hz), 6.32 (bs, 1H, NH), 6.54–6.67 (m, 3H, Ar–H), 7.07 (t, 1H, Ar–H, J = 7.8 Hz), 7.30–7.53 (m, 6H, Ar–H), 7.78 (d, 2H, Ar–H, J = 7.8 Hz), 7.86 (d, 2H, Ar–H, J = 8.1 Hz), 8.57 (s, 1H, pyrazole-H-5) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 39.4 (CH_2), 112.7, 119.0, 120.2, 121.3, 126.4, 127.1, 127.8, 128.5, 130.3, 132.9, 136.1, 138.4, 141.8, 150.7 ppm.

N-(1,3-Diphenyl-1H-pyrazol-4-ylmethyl)-4-nitroaniline (**5e**, $C_{22}H_{18}N_4O_2$)
IR (KBr): $\bar{\nu}$ = 3,218 (N–H), 1,603 (C=N), 1,497 (C=C), 1,067 (C–N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 4.20 (d, 2H, CH_2, J = 5.1 Hz), 5.81 (bs, 1H, NH), 6.61 (d, 2H, Ar–H, J = 8.7 Hz), 7.02 (d, 2H, Ar–H, J = 8.7 Hz), 7.24–7.48 (m, 6H, Ar–H), 7.71 (d, 2H, Ar–H, J = 7.2 Hz), 7.77 (d, 2H, Ar–H, J = 7.8 Hz), 8.48 (s, 1H, pyrazole-H-5) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 39.8 (CH_2), 39.4, 117.8, 119.5, 121.9, 123.1, 126.3, 127.1, 127.8, 128.6, 130.3, 133.1, 139.5, 141.2, 148.7, 152.6 ppm.

N-[3-(4-Methylphenyl)-1-phenyl-1H-pyrazol-4-ylmethyl]aniline (**6a**, $C_{23}H_{21}N_3$)
IR (KBr): $\bar{\nu}$ = 3,268 (N–H), 1,625 (C=N), 1,530 (C=C), 1,020 (C–N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.31 (s, 3H, CH_3), 4.20 (bs, 2H, CH_2), 5.91 (bs, 1H, NH), 6.52–6.66 (m, 3H, Ar–H), 7.04–7.09 (m, 2H, Ar–H), 7.22–7.30 (m, 3H, Ar–H), 7.46–7.51 (m, 2H, Ar–H), 7.67–7.69 (m, 2H, Ar–H), 7.83–7.86 (m, 2H, Ar–H), 8.53 (s, 1H, pyrazole-H-5) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 21.3 (CH_3), 40.2 (CH_2), 112.4, 115.9, 119.0, 126.8, 127.5, 127.9, 128.4, 129.7, 130.3, 133.8, 138.0, 140.1, 144.8, 151.1 ppm; FAB-MS: m/z = 339 (M^+).

4-Chloro-N-[3-(4-methylphenyl)-1-phenyl-1H-pyrazol-4-ylmethyl]aniline (**6b**, $C_{23}H_{20}ClN_3$)
IR (KBr): $\bar{\nu}$ = 3,274 (N–H), 1,684 (C=N), 1,515 (C=C), 1,011 (C–N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.31 (s, 3H, CH_3), 4.19 (s, 2H, CH_2), 6.16 (bs, 1H, NH), 6.65 (d, 2H, Ar–H, J = 7.2 Hz), 7.09 (d, 2H, Ar–H, J = 7.2 Hz), 7.25–7.50 (m, 5H, Ar–H), 7.66 (d, 2H, Ar–H, J = 6.9 Hz), 7.85 (d, 2H, Ar–H, J = 6.9 Hz), 8.52 (s, 1H, pyrazole-H-5) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 21.5 (CH_3), 39.9 (CH_2), 113.2, 118.8, 119.8, 120.9, 126.5, 127.2, 127.9, 128.0, 129.7, 130.4, 133.5, 137.8, 141.3, 144.5, 152.0 ppm; FAB-MS: m/z = 374 (M^+), 376 [$(M + 2)^+$].

4-Fluoro-N-[3-(4-methylphenyl)-1-phenyl-1H-pyrazol-4-ylmethyl]aniline (**6c**, $C_{23}H_{20}FN_3$)
IR (KBr): $\bar{\nu}$ = 3,305 (N–H), 1,590 (C=N), 1,520 (C=C), 1,017 (C–N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.32 (s, 3H, CH_3), 4.17 (d, 2H, CH_2, J = 5.1 Hz), 5.87 (bs, 1H, NH), 6.61–6.66 (m, 2H, Ar–H), 6.92 (t, 2H, Ar–H, J = 9.0 Hz), 7.23–7.31 (m, 3H, Ar–H), 7.49 (t, 2H, Ar–H, J = 7.9 Hz), 7.68 (d, 2H, Ar–H, J = 8.1 Hz), 7.85 (d, 2H, Ar–H, J = 8.4 Hz), 8.53 (s, 1H, pyrazole-H-5)

ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 21.4$ (CH_3), 39.8 (CH_2), 112.6, 116.7, 118.3, 119.7, 126.8, 127.2, 128.1, 128.7, 129.9, 131.3, 135.8, 138.9, 140.5, 151.1, 156.5 ppm; FAB-MS: $m/z = 357$ (M^+).

4-Methoxy-N-[3-(4-methylphenyl)-1-phenyl-1H-pyrazol-4-ylmethyl]aniline (**6d**, $C_{24}H_{23}N_3O$)
IR (KBr): $\bar{\nu} = 3{,}228$ (N–H), 1,600 (C=N), 1,510 (C=C), 1,030 (C–N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.31$ (s, 3H, CH_3), 3.61 (s, 3H, OCH_3), 4.14 (d, 2H, CH_2, $J = 5.1$ Hz), 5.51 (bs, 1H, NH), 6.59–6.72 (m, 4H, Ar–H), 7.22–7.28 (m, 3H, Ar–H), 7.48 (t, 2H, Ar–H, $J = 7.95$ Hz), 7.68 (d, 2H, Ar–H, $J = 8.1$ Hz), 7.84 (d, 2H, Ar–H, $J = 8.7$ Hz), 8.51 (s, 1H, pyrazole-H-5) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 21.3$ (CH_3), 39.9 (CH_2), 56.8 (OCH_3), 112.9, 115.3, 117.7, 119.1, 126.2, 126.6, 128.7, 129.6, 129.9, 132.2, 135.5, 140.5, 149.6, 152.3 ppm; FAB-MS: $m/z = 369$ (M^+).

3-Chloro-4-fluoro-N-[3-(4-methylphenyl)-1-phenyl-1H-pyrazol-4-ylmethyl]aniline (**6e**, $C_{23}H_{19}ClFN_3$)
IR (KBr): $\bar{\nu} = 3{,}291$ (N–H), 1,603 (C=N), 1,503 (C=C), 1,058 (C–N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.32$ (s, 3H, CH_3), 4.18 (d, 2H, CH_2, $J = 3.8$ Hz), 6.17 (s, 1H, NH), 6.59–6.62 (m, 2H, N-phenyl-H), 6.74–6.77 (m, 1H, N-phenyl-H), 7.24–7.32 (m, 3H, Ar–H), 7.67 (d, 2H, Ar–H, $J = 7.8$ Hz), 7.86 (d, 2H, Ar–H, $J = 8.1$ Hz), 7.12 (t, 2H, Ar–H, $J = 8.2$ Hz), 7.50 (t, 1H, Ar–H, $J = 7.2$ Hz), 8.55 (s, 1H, pyrazole-H-5) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 21.3$ (CH_3), 38.9 (CH_2), 115.6, 116.4, 118.1, 118.7, 119.6, 121.5, 127.2, 127.8, 128.1, 129.9, 130.6, 133.5, 138.9, 140.1, 148.8, 150.2, 156.3 ppm; FAB-MS: $m/z = 392$ (M^+), 394 [$(M + 2)^+$].

4-Methyl-N-[3-(4-methylphenyl)-1-phenyl-1H-pyrazol-4-ylmethyl]aniline (**6f**, $C_{24}H_{23}N_3$)
IR (KBr): $\bar{\nu} = 3{,}210$ (N–H), 1,582 (C=N), 1,530 (C=C), 989 (C–N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.07$ (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 4.11 (d, 2H, CH_2, $J = 4.2$ Hz), 5.62 (bs, 1H, NH), 6.51 (d, 2H, N-phenyl-H-2/6, $J = 7.8$ Hz), 6.83 (d, 2H, Ar–H, $J = 7.8$ Hz), 7.02–7.24 (m, 3H, Ar–H), 7.42 (t, 2H, Ar–H, $J = 7.5$ Hz), 7.62 (d, 2H, Ar–H, $J = 7.8$ Hz), 7.78 (d, 2H, Ar–H, $J = 7.8$ Hz), 8.44 (s, 1H, pyrazole-H-5) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 20.5$ (CH_3), 21.6 (CH_3), 39.1 (CH_2), 113.8, 118.3, 119.1, 126.5, 127.2, 127.9, 128.5, 130.7, 132.8, 137.9, 141.2, 149.6, 151.2 ppm; FAB-MS: $m/z = 353$ (M^+).

3-Chloro-N-[3-(4-methylphenyl)-1-phenyl-1H-pyrazol-4-ylmethyl]aniline (**6g**, $C_{23}H_{20}ClN_3$)
IR (KBr): $\bar{\nu} = 3{,}254$ (N–H), 1,664 (C=N), 1,520 (C=C), 1,020 (C–N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.32$ (s, 3H, CH_3), 4.20 (d, 2H, CH_2, $J = 3.9$ Hz), 6.30 (bs, 1H, NH), 6.54–6.66 (m, 3H, Ar–H), 7.07 (t, 1H, Ar–H, $J = 8.1$ Hz), 7.23–7.31 (m, 3H, Ar–H), 7.49 (t, 2H, Ar–H, $J = 7.2$ Hz), 7.66 (d, 2H, Ar–H, $J = 7.8$ Hz), 7.85 (d, 2H, Ar–H, $J = 8.1$ Hz), 8.54 (s, 1H, pyrazole-H-5) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 20.9$ (CH_3), 38.6 (CH_2), 119.0, 119.8, 121.3, 126.3, 127.2, 127.9, 128.7, 130.6, 131.9, 137.7, 139.4, 141.3, 148.7, 150.8 ppm; FAB-MS: $m/z = 374$ (M^+), 376 [$(M + 2)^+$].

N-[3-(4-Methylphenyl)-1-phenyl-1H-pyrazol-4-ylmethyl]-4-nitroaniline (**6h**, $C_{23}H_{20}N_4O_2$)
IR (KBr): $\bar{\nu} = 3{,}307$ (N–H), 1,593 (C=N), 1,573 (C=C), 1,028 (C–N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.32$ (s, 3H, CH_3), 4.16 (d, 2H, CH_2, $J = 5.1$ Hz), 5.87 (bs, 1H, NH), 6.61–6.66 (m, 2H, Ar–H), 6.92 (t, 2H, Ar–H, $J = 9.0$ Hz), 7.23–7.31 (m, 3H, Ar–H), 7.49 (t, 2H, Ar–H, $J = 7.9$ Hz), 7.67 (d, 2H, Ar–H, $J = 8.1$ Hz), 7.84 (d, 2H, Ar–H, $J = 8.4$ Hz), 8.53 (s, 1H, pyrazole-H-5) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 21.5$ (CH_3), 39.4 (CH_2), 118.0, 119.6, 121.1, 122.9, 126.0, 126.8, 127.7, 129.5, 131.2, 133.6, 139.4, 141.7, 149.7, 151.2 ppm; FAB-MS: $m/z = 384$ (M^+).

Antimicrobial screening

Compounds **5a–5e** and **6a–6h** were tested against a panel of bacterial strains such as *E. coli* (NCTC 10418), *S. aureus* (NCTC 65710), *P. aeruginosa* (NCTC 10662) and fungal strains, viz. *A. niger* (MTCC 281), *A. flavus* (MTCC 277), *Monascus purpureus* (MTCC 369), and *P. citrinum* (NCIM 768), by the cup-plate method [23, 24]. Potato dextrose agar (PDA) and nutrient agar were used as culture medium for antifungal and antibacterial activity, respectively. Normal saline with Tween 80 (0.01%) was used to make a suspension of fungal and bacterial spores for lawning. PDA medium (50 cm^3) was poured into each petri dish (15 cm diameter). Five cubic centimeter of the spore suspension was spread over the solid agar medium and plates were dried in an incubator at 37 °C for 1 h. An agar punch was used to make wells on the seeded agar plates, and solutions of test compounds in dimethyl sulfoxide (DMSO) in concentrations of 6.25, 12.5, 25.0, 50, 100, and 200 μg/cm^3 were added into each well, labeled previously. A control was also prepared using DMSO as solvent. The petri plates were prepared in duplicate and incubated at 30 °C for 72 h for fungi and 37 °C for 24 h for bacteria. Antifungal and antibacterial activity was determined by measuring the zone of inhibition and the minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the test drug at which there was no visible growth. Activity of each compound **5a–5e** and **6a–6h** was compared with the standards fluconazole and ciprofloxacin and results are summarized as MIC (average zone of inhibition of two readings in millimeter) in Table 1.

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