Synthesis and antifungal activity of novel (1-arylmethyl-3-aryl-1*H*-pyrazol-5-yl)(4-arylpiperazin-1-yl)methanone derivatives

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A series of novel (1-arylmethyl-3-aryl-1*H*-pyrazol-5-yl)(4-arylpiperazin-1-yl)methanone derivatives were synthesised. Preliminary study of the structure–activity relationship revealed that 4-chlorophenyl, 4-*tert*-butylphenyl, 4-fluorophenyl and 3-methoxyphenyl had a promising effect on the antifungal activity.

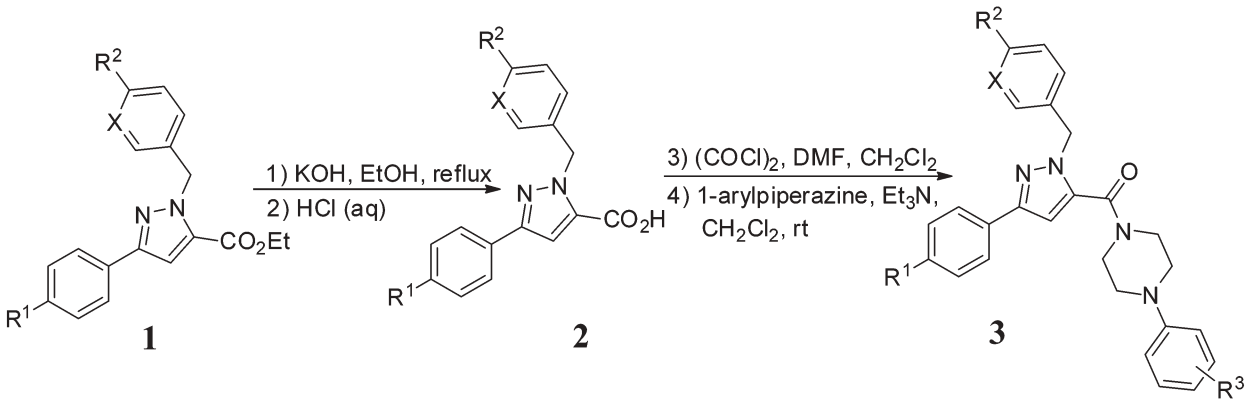
Keywords: synthesis, pyrazole carboxamide, piperazine, antifungal activity

Microbial resistance to known antibiotics is increasing and leads to an increasing incidence of infection.[1] Therefore, it is very necessary to develop new antimicrobial drugs. Pyrazoles are a class of important heterocyclic compounds that are widely used in the agrochemical and pharmaceutical industries. Many pyrazole derivatives are known to exhibit a wide range of bioactivities such as antimicrobial,[2] anti-inflammatory,[3] anticancer,[4] antidepressant,[5] anticonvulsant[6] and antiangiogenic[7] activities. Some pyrazole derivatives have been used clinically, such as Celecoxib, a well-known COX-2 inhibitor.[8] According to the literature, many piperazine derivatives exhibit antifungal activity.[9,10] In our previous work, we reported a series of novel pyrazole derivatives such as ethyl 1-(2-hydroxy-3-aroxypropyl)-3-aryl-1*H*-pyrazole-5-carboxylate derivatives, 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide derivatives, 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide hydrazone derivatives and 3-aryl-1-(2-(hydroxyimino)-2-arylethyl)-1*H*-pyrazole-5-carboxylate derivatives as potential anticancer agents.[11–14] We are also committed to the synthesis of pyrazole derivatives with antimicrobial activity. The combination of pyrazole and some heterocyclic compounds can form compounds with good antifungal activity.[15,16] Thus, we focused on the introduction of piperazine to enhance the antifungal activity of pyrazole derivatives. A series of novel pyrazole derivatives containing piperazine moiety was synthesised and evaluated for their antifungal activity *in vitro*.

Results and discussion

The synthesis of pyrazole carboxamide **3** was performed as outlined in Scheme 1. The starting material ethyl 3-aryl-1-arylmethyl-1*H*-pyrazole-5-carboxylate **1** was synthesised as described in a previous paper.[17] Pyrazolecarboxylic acids **2**[18,19] were obtained by the alkali hydrolysis of pyrazole esters **1**. Pyrazolecarboxylic acids first reacted with oxalyl chloride in CH_2Cl_2 under reflux to give pyrazole carbonyl chlorides. Then the target compound **3** was obtained by the reactions of pyrazole carbonyl chlorides with 1-arylpiperazines in the presence of triethylamine in CH_2Cl_2 at room temperature in yield 59.3–98.4%.

Structures of the compounds **3a–l** were determined by IR, ^{1}H NMR and HRMS spectroscopy. For example, IR spectrum of compound **3a** showed C=O stretching vibration at 1640 cm^{-1}. ^{1}H NMR spectral of compound **3a** showed four singles for



3a: R^1 = H, R^2 = *tert*-Bu, X = C, R^3 = H
3b: R^1 = H, R^2 = *tert*-Bu, X = C, R^3 = 2-F
3c: R^1 = H, R^2 = *tert*-Bu, X = C, R^3 = 4-F
3d: R^1 = H, R^2 = *tert*-Bu, X = C, R^3 = 3-MeO
3e: R^1 = Cl, R^2 = Cl, X = N, R^3 = H
3f: R^1 = Cl, R^2 = Cl, X = N, R^3 = 2-F
3g: R^1 = Cl, R^2 = Cl, X = N, R^3 = 4-F
3h: R^1 = Cl, R^2 = Cl, X = N, R^3 = 3-MeO
3i: R^1 = Cl, R^2 = *tert*-Bu, X = C, R^3 = H
3j: R^1 = Cl, R^2 = *tert*-Bu, X = C, R^3 = 2-F
3k: R^1 = Cl, R^2 = *tert*-Bu, X = C, R^3 = 4-F
3l: R^1 = Cl, R^2 = *tert*-Bu, X = C, R^3 = 3-MeO

Scheme 1 Synthesis of **3**.

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CH_2 of piperazine ring. A singlet at 6.60 was observed for pyrazole–H. The CH_2 connected with the pyrazole ring showed a singlet at δ 5.53. In the HRMS, compound **3a** gave a [M+H]-ion peak at *m/z* 479.2805 in accordance with the molecular structure of $C_{31}H_{35}N_4O$.

In vitro antifungal activity

Fluconazole was used as the reference drug for *C. albicans* (CMCC(B) 98001). The result of anti-*Candida* activity showed that all the synthesised compounds had no activity against the pathogenic strains of *C. albicans*.

Anti-*Aspergillus* activity of the synthesised compounds was tested against pathogenic fungal strain *Aspergillus flavus* (CGMCC 3.3950). The anti-*Aspergillus* activity of all the synthesised compounds was evaluated by the microbroth dilution (MDA) assays.[20] Amphotericin B was used as a standard drug. The minimum inhibitory concentrations (MICs) of the tested compounds against pathogenic fungi are shown in Table 1. Results of anti-*Aspergillus* activity demonstrated that **3c**, **3d**, **3e**, **3i** and **3j** showed mild to moderate anti-*Aspergillus* activity against the pathogenic strains used in the experiments. Compounds **3a**, **3b**, **3g**, **3h**, **3k** and **3l** were found to be potential inhibitor of *Aspergillus flavus*. They have significant MIC value at 64 μg mL^{-1} in MDA assay (Table 1). By comparing the anti-*Aspergillus* activity of **3e–h** and **3i–l**, it can be seen that 1-arylmethyl has no effect on the anti-*Aspergillus* activity, while 4-arylpiperazine shows significant impact. Compounds **3e**, **3i** and **3j** with phenyl and 2-fluorophenyl groups respectively show mild to moderate anti-*Aspergillus* activitiy, while compounds **3g**, **3h**, **3k** and **3l** with 4-fluorophenyl and 3-methoxyphenyl groups respectively are potential inhibitors of *Aspergillus flavus*.

In conclusion, a series of novel pyrazole carboxamide derivatives containing a piperazine moiety **3a–l** were synthesised and characterised by IR, ^{1}H NMR and HRMS spectroscopy. Preliminary evaluation for antifungal activity *in vitro* showed that the compounds **3c**, **3d**, **3e**, **3i** and **3j** had moderate anti-*Aspergillus* activity against the pathogenic strains used in the experiments. While **3a**, **3b**, **3g**, **3h**, **3k** and **3l** were found to be potential inhibitors of the growth of *Aspergillus flavus* with significant MIC value at 64 μg mL^{-1} in MDA assay. Structure–activity relationships analysis showed that the 4-fluorophenyl and 3-methoxyphenyl groups significantly enhanced the anti-*Aspergillus* activity.

Experimental

All reagents were of analytical grade or chemically pure. TLC was performed on silica gel $60F_{254}$ plates (Merck KGaA). Melting point was determined on an *XD-4* digital micro melting point apparatus. ^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer, using $CDCl_3$ as solvent and TMS as internal standard. IR spectra were recorded with an IR spectrophotometer Avtar 370 FT-IR (Termo Nicolet). HRMS spectra were recorded on a LTQ Orbitrap Hybrid mass spectrometer.

General procedure

KOH (30 mmol) was added to a solution of **1** (10 mmol) in ethanol (50 mL). The mixture was refluxed for 2 h. After cooling, ethanol was removed under reduced pressure. The residue was dissolved in 50 mL water. The solution was added to hydrochloric acid (3 M) until pH = 4–5. The precipitate was filtered and dried to give **2** as pure product. A solution of **2** (1 mmol), oxalyl chloride (0.5 mL) and two drops of DMF in CH_2Cl_2 (10 mL) was refluxed for 4 h. After cooling, the solvent was removed. The residue was dissolved in CH_2Cl_2 (5 mL) and added dropwise to a solution of 1-arylpiperazine in CH_2Cl_2 (10 mL) with Et_3N (0.5 mL) at room temperature. The mixture was stirred for 2–4 h. The end of reaction was detected by TLC. After which the solution was washed by water (20 mL × 2), the water phase was extracted with CH_2Cl_2 (15 mL × 1). The combined organic phase was dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using PE/EtOAc (2:1, v/v) as an eluent to afford title compound **3** in 44.8–82.9%.

[1-(4-tert-Butylbenzyl)-3-phenyl-1H-pyrazol-5-yl](4-phenylpiperazin-1-yl)methanone (**3a**): White solid, yield 59.3%; m.p. 178–181 °C; IR (ν_{max} cm^{-1}): 1640 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz): 1.14 (s, 9H, $C(CH_2)_3$), 2.57 (s, 2H, CH_2), 3.07 (s, 2H, CH_2), 3.44 (s, 2H, CH_2), 3.82 (s, 2H, CH_2), 5.53 (s, 2H, CH_2), 6.60 (s, 1H, pyrazole–H), 6.83 (d, 2H, *J* = 7.8 Hz, ArH), 6.89 (t, 1H, *J* = 7.2 Hz, ArH), 7.17–7.29 (m, 6H, ArH), 7.31–7.36 (m, 1H, ArH), 7.42 (t, 2H, *J* = 7.6 Hz, ArH), 7.83 (d, 2H, *J* = 7.0 Hz, ArH). HRMS calcd for $[M+H]^+$ $C_{31}H_{35}N_4O$: 479.2811, found 479.2805.

[1-(4-tert-Butylbenzyl)-3-phenyl-1H-pyrazol-5-yl][4-(2-fluorophenyl)piperazin-1-yl]methanone (**3b**): White solid, yield 65.0%; m.p. 155–158 °C; IR (ν_{max} cm^{-1}): 1627 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz): 1.16 (s, 9H, $C(CH_2)_3$), 2.46 (s, 2H, CH_2), 2.98 (s, 2H, CH_2), 3.43 (s, 2H, CH_2), 3.84 (s, 2H, CH_2), 5.53 (s, 2H, CH_2), 6.60 (s, 1H, pyrazole–H), 6.81 (t, 1H, *J* = 8.0 Hz, ArH), 6.92–7.07 (m, 3H, ArH), 7.18–7.30 (m, 4H, ArH), 7.30–7.36 (m, 1H, ArH), 7.42 (t, 2H, *J* = 7.6 Hz, ArH), 7.82 (d, 2H, *J* = 7.4 Hz, ArH). HRMS calcd for $[M+H]^+$ $C_{31}H_{34}FN_4O$: 497.2717, found 497.2704.

[1-(4-tert-Butylbenzyl)-3-phenyl-1H-pyrazol-5-yl][4-(4-fluorophenyl)piperazin-1-yl]methanone (**3c**): White solid, yield 89.9%; m.p. 190–192 °C; IR (ν_{max} cm^{-1}): 1640 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz): 1.13 (s, 9H, $C(CH_2)_3$), 2.47 (s, 2H, CH_2), 2.98 (s, 2H, CH_2), 3.45 (s, 2H, CH_2), 3.82 (s, 2H, CH_2), 5.53 (s, 2H, CH_2), 6.60 (s, 1H, pyrazole–H), 6.77 (dd, 2H, J_1 = 8.4 Hz, J_2 = 4.4 Hz, ArH), 6.95 (t, 2H, *J* = 8.4 Hz, ArH), 7.19 (d, 2H, *J* = 8.1 Hz, ArH), 7.26 (d, 2H, *J* = 8.1 Hz, ArH), 7.34 (t, 1H, *J* = 7.4 Hz, ArH), 7.42 (t, 2H, *J* = 7.6 Hz, ArH), 7.82 (d, 2H, *J* = 7.4 Hz, ArH). HRMS calcd for $[M+H]^+$ $C_{31}H_{34}FN_4O$: 497.2717, found 497.2710.

[1-(4-tert-Butylbenzyl)-3-phenyl-1H-pyrazol-5-yl][4-(3-methoxyphenyl)piperazin-1-yl]methanone (**3d**): White solid, yield 98.4%; m.p. 151–154 °C. IR (ν_{max} cm^{-1}): 1640 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz): 1.14 (s, 9H, $C(CH_2)_3$), 2.58 (s, 2H, CH_2), 3.07 (s, 2H, CH_2), 3.43 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3), 3.81 (s, 2H, CH_2), 5.53 (s, 2H, CH_2), 6.35 (s, 1H, ArH), 6.40–6.47 (m, 2H, ArH), 6.59 (s, 1H, pyrazole–H), 7.15 (t, 1H, *J* = 8.2 Hz, ArH), 7.20 (d, 2H, *J* = 8.2 Hz, ArH), 7.27 (d, 2H, *J* = 8.2 Hz, ArH), 7.33 (t, 1H, *J* = 7.6 Hz, ArH), 7.42 (t, 2H, *J* = 7.6 Hz, ArH), 7.82 (d, 2H, *J* = 7.6 Hz, ArH). HRMS calcd for $[M+H]^+$ $C_{32}H_{37}N_4O_2$: 509.2917, found 509.2922.

{3-(4-Chlorophenyl)-1-[(6-chloropyridin-3-yl)methyl]-1H-pyrazol-5-yl}(4-phenylpiperazin-1-yl)methanone (**3e**): White solid, yield 79.1%; m.p. 168–170 °C. IR (ν_{max} cm^{-1}): 1745 (C=N), 1630 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz): 2.93 (s, 2H, CH_2), 3.18 (s, 2H, CH_2), 3.72 (s, 2H, CH_2), 3.89 (s, 2H, CH_2), 5.54 (s, 2H, CH_2), 6.63 (s, 1H, pyrazole–H), 6.88–6.98 (m, 3H, ArH), 7.27–7.34 (m, 3H, ArH), 7.39 (d, 2H, *J* = 8.6 Hz, ArH), 7.68 (dd, 1H, J_1 = 8.3 Hz, J_2 = 2.4 Hz, ArH), 7.72 (d, 2H, *J* = 8.6 Hz, ArH), 8.39 (d, 1H, *J* = 2.4 Hz, ArH). HRMS calcd for $[M+H]^+$ $C_{26}H_{24}Cl_2N_5O$: 492.1358, found 492.1362.

{3-(4-Chlorophenyl)-1-[(6-chloropyridin-3-yl)methyl]-1H-pyrazol-5-yl}[4-(2-fluorophenyl) piperazin-1-yl]methanone (**3f**): White solid, yield 72.2%; m.p. 130–132 °C; IR (ν_{max} cm^{-1}): 1744 (C=N), 1629 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz): 2.77 (s, 2H, CH_2), 3.05 (s, 2H, CH_2), 3.71 (s, 2H, CH_2), 3.90 (s, 2H, CH_2), 5.55 (s, 2H, CH_2), 6.62 (s, 1H, pyrazole–H), 6.90 (t, 1H, *J* = 8.0 Hz, ArH), 6.96–7.12 (m, 3H, ArH), 7.28 (d, 1H, *J* = 8.4 Hz, ArH), 7.38 (d, 2H, *J* = 8.5 Hz, ArH), 7.68 (dd, 1H, J_1 = 8.2 Hz, J_2 = 2.3 Hz, ArH), 7.72 (d, 2H, *J* = 8.5 Hz, ArH), 8.40 (d, 1H, *J* = 2.3 Hz, ArH). HRMS calcd for $[M+H]^+$ $C_{26}H_{23}Cl_2FN_5O$: 510.1264, found 510.1271.

Table 1 Antimicrobial activity of pyrazole carboxamide derivatives **3a–l** expressed as MIC (μg mL^{-1})

Compound	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**	**3j**	**3k**	**3l**	Amphotericin B
Aspergillus	64	64	128	128	128	–	64	64	128	128	64	64	1

– No activity.

{3-(4-Chlorophenyl)-1-[(6-chloropyridin-3-yl)methyl]-1H-pyrazol-5-yl}[4-(4-fluorophenyl) piperazin-1-yl]methanone (**3g**): White solid, yield 88.2%; m.p. 199–202 °C; IR (ν_{max} cm^{-1}): 1745 (C=N), 1623 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz): 2.79 (s, 2H, CH_2), 3.06 (s, 2H, CH_2), 3.69 (s, 2H, CH_2), 3.88 (s, 2H, CH_2), 5.55 (s, 2H, CH_2), 6.62 (s, 1H, pyrazole–H), 6.87 (dd, 2H, J_1 = 8.4 Hz, J_2 = 4.5 Hz, ArH), 7.00 (t, 2H, J = 8.4 Hz, ArH), 7.27 (d, 1H, J = 7.0 Hz, ArH), 7.39 (d, 2H, J = 8.3 Hz, ArH), 7.68 (dd, 1H, J_1 = 8.2 Hz, J_2 = 2.3 Hz, ArH), 7.72 (d, 2H, J = 8.3 Hz, ArH), 8.39 (d, 1H, J = 2.3 Hz, ArH). HRMS calcd for $[M+H]^+$ $C_{26}H_{23}Cl_2FN_5O$: 510.1264, found 510.1259.

{3-(4-Chlorophenyl)-1-[(6-chloropyridin-3-yl)methyl]-1H-pyrazol-5-yl}[4-(3-methoxyphenyl) piperazin-1-yl]methanone (**3h**): White solid, yield 80.1%; m.p. 154–157 °C; IR (ν_{max} cm^{-1}): 1745 (C=N), 1627 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz): 2.95 (s, 2H, CH_2), 3.18 (s, 2H, CH_2), 3.70 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2), 5.54 (s, 2H, CH_2), 6.44 (s, 1H, ArH), 6.48–6.53 (m, 2H, ArH), 6.62 (s, 1H, pyrazole–H), 7.20 (t, 1H, J = 8.2 Hz, ArH), 7.27 (d, 1H, J = 6.8 Hz, ArH), 7.39 (d, 2H, J = 8.5 Hz, ArH), 7.68 (dd, 1H, J_1 = 8.2 Hz, J_2 = 2.5 Hz, ArH), 7.72 (d, 2H, J = 8.5 Hz, ArH), 8.39 (d, 1H, J = 2.5 Hz, ArH). HRMS calcd for $[M+H]^+$ $C_{27}H_{26}Cl_2N_5O_2$: 522.1464, found 522.1468.

[1-(4-tert-Butylbenzyl)-3-(4-chlorophenyl)-1H-pyrazol-5-yl](4-phenylpiperazin-1-yl)methanone (**3i**): White solid, yield 79.9%; m.p. 205–208 °C; IR (ν_{max} cm^{-1}): 1637 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz): 1.14 (s, 9H, $C(CH_2)_3$), 2.58 (s, 2H, CH_2), 3.08 (s, 2H, CH_2), 3.43 (s, 2H, CH_2), 3.82 (s, 2H, CH_2), 5.52 (s, 2H, CH_2), 6.57 (s, 1H, pyrazole–H), 6.82 (d, 2H, J = 8.2 Hz, ArH), 6.90 (t, 1H, J = 7.2 Hz, ArH), 7.17–7.24 (m, 3H, ArH), 7.24–7.28 (m, 3H, ArH), 7.38 (d, 2H, J = 8.4 Hz, ArH), 7.76 (d, 2H, J = 8.4 Hz, ArH). HRMS calcd for $[M+H]^+$ $C_{31}H_{34}ClN_4O$: 513.2421, found 513.2419.

[1-(4-tert-Butylbenzyl)-3-(4-chlorophenyl)-1H-pyrazol-5-yl][4-(2-fluorophenyl)piperazin-1-yl]methanone (**3j**): White solid, yield 64.0%; m.p. 167–169 °C; IR (ν_{max} cm^{-1}): 1639 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz): 1.16 (s, 9H, $C(CH_2)_3$), 2.46 (s, 2H, CH_2), 2.98 (s, 2H, CH_2), 3.42 (s, 2H, CH_2), 3.84 (s, 2H, CH_2), 5.52 (s, 2H, CH_2), 6.57 (s, 1H, pyrazole–H), 6.81 (t, 1H, J = 8.1 Hz, ArH), 6.92–7.18 (m, 3H, ArH), 7.20 (d, 2H, J = 8.3 Hz, ArH), 7.29 (d, 2H, J = 8.3 Hz, ArH), 7.38 (d, 2H, J = 8.5 Hz, ArH), 7.75 (d, 2H, J = 8.5 Hz, ArH). HRMS calcd for $[M+H]^+$ $C_{31}H_{33}ClFN_4O$: 531.2327, found 531.2327.

[1-(4-tert-Butylbenzyl)-3-(4-chlorophenyl)-1H-pyrazol-5-yl][4-(4-fluorophenyl)piperazin-1-yl]methanone (**3k**): White solid, yield 67.9%; m.p. 220–222 °C; IR (ν_{max} cm^{-1}): 1638 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz): 1.14 (s, 9H, $C(CH_2)_3$), 2.47 (s, 2H, CH_2), 2.97 (s, 2H, CH_2), 3.42 (s, 2H, CH_2), 3.81 (s, 2H, CH_2), 5.51 (s, 2H, CH_2), 6.56 (s, 1H, pyrazole–H), 6.77 (dd, 2H, J_1 = 8.8 Hz, J_2 = 4.5 Hz, ArH), 6.95 (t, 2H, J = 8.8 Hz, ArH), 7.19 (d, 2H, J = 8.2 Hz, ArH), 7.27 (d, 2H, J = 8.2 Hz, ArH), 7.38 (d, 2H, J = 8.5 Hz, ArH), 7.76 (d, 2H, J = 8.5 Hz, ArH). HRMS calcd for $[M+H]^+$ $C_{31}H_{33}ClFN_4O$: 531.2327, found 531.2324.

[1-(4-tert-Butylbenzyl)-3-(4-chlorophenyl)-1H-pyrazol-5-yl][4-(3-methoxyphenyl)piperazin-1-yl]methanone (**3l**): White solid, yield 60.0%; m.p. 179–182 °C; IR (ν_{max} cm^{-1}): 1638 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz): 1.14 (s, 9H, $C(CH_2)_3$), 2.58 (s, 2H, CH_2), 3.07 (s, 2H, CH_2), 3.41 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3), 3.79 (s, 2H, CH_2), 5.51 (s, 2H, CH_2), 6.35 (s, 1H, ArH), 6.39–6.47 (m, 2H, ArH), 6.56 (s, 1H, pyrazole–H), 7.15 (t, 1H, J = 8.2 Hz, ArH), 7.19 (d, 2H, J = 8.5 Hz, ArH), 7.27 (d, 2H, J = 8.5 Hz, ArH), 7.38 (d, 2H, J = 8.4 Hz, ArH), 7.75 (d, 2H, J = 8.4 Hz, ArH). HRMS calcd for $[M+H]^+$ $C_{32}H_{36}ClN_4O_2$: 543.2527, found 543.2530.

Antifungal activity of compounds **3a–l**

Amphotericin B was purchased from North China Pharmaceutical Company Ltd, China. Pathogenic strain of *Candida albicans* (CMCC(B) 98001) was obtained from American Type Culture Collection. *Aspergillus flavus* (CGMCC 3.3950) was obtained from China General Microbiological Culture Collection Center.

Candida albicans was grown on Modified Martin Broth at 28 °C and *Aspergillus flavus* was grown on Czapek's medium at 28 °C.

Antifungal activity assay

All the compounds were assayed by microbroth dilution.

Microbroth dilution

Test compounds were dissolved in distilled dimethylsulfoxide and then were plated in 96-well microplates. Each tested compound (256 μg mL^{-1} in DMSO) was transferred to each microplate well, to be diluted in 100 μL of broth and 100 μL of inocula to obtain final concentrations at 128, 64, 32, 16, 8, 4, 2, 1, 0.5 and 0.25 μg mL^{-1}, respectively. Dimethylsulfoxide never exceeded 1% v/v. After incubation at 28 °C for 48 h, the last tube remaining clear with no growth of microorganism was recorded to represent MIC. The MICs were read after incubation at 28 °C for 48 h. Growth controls consisting of media and media with 1% v/v dimethylsulfoxide were employed.

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References

1 P.K. Sharma, N. Chandak, P. Kumar *et al. Eur. J. Med. Chem.*, 2011, **46**, 1425.
2 M. Amir, S.A. Javed and M. Zaheen Hassan, *Med. Chem. Res.*, 2012, **21**, 1261.
3 N.M. Gawad, H.H. Georgey, N.A. Ibrahim, *et al. Arch. Pharm. Res.*, 2012, **35**, 807.
4 S. David, R.S. Perkins, F.R. Fronczek, *et al. J. Inorg. Biochem.*, 2012, **111**, 33.
5 D. Secci, A. Bolasco, P. Chimenti and S. Carradori, *Curr. Med. Chem.*, 2011, **18**, 5114.
6 A.H. Mandour, E.R. El-Sawy, M.S. Ebaid and S.M. Hassan, *Acta Pharm.*, 2012, **62**, 15.
7 A.H. Abadi, A.A. Eissa and G.S. Hassan, *Chem. Pharm. Bull.*, 2003, **51**, 838.
8 T.D. Penning, J.J. Talley, S.R. Bertenshaw, *et al. J. Med. Chem.*, 1997, **40**, 1347.
9 M. Rani, P. Parthiban, R. Ramachandran and S. Kabilan, *Med. Chem. Res.*, 2012, **21**, 653.
10 J. Xu, Y. Cao, J. Zhang, S. Yu, *et al. Eur. J. Med. Chem.*, 2011, **46**, 3142.
11 F. Wei, B.X. Zhao, B. Huang, *et al. Bioorg. Med. Chem. Lett.*, 2006, **16**, 6342.
12 Y. Xia, Z.W. Dong, B.X. Zhao, *et al. Bioorg. Med. Chem.*, 2007, **15**, 6893.
13 Y. Xia, C.D. Fan, B.X. Zhao, J. Zhao *et al. Eur. J. Med. Chem.*, 2008, **43**, 2347.
14 L.W. Zheng, Y. Li, D. Ge, *et al. Bioorg. Med. Chem. Lett.*, 2010, **20**, 4766.
15 N.J. Thumar and M.P. Patel, *Med. Chem. Res.*, 2011, **21**, 1751.
16 K.A. Ali, H.M. Hosni, E.A. Ragab and S.I. El-Moez, *Arch. Pharm.*, 2012, **345**, 231.
17 X.L. Ding, N. Meng, Y. Xia, *et al. Chin. J. Org. Chem.*, 2007, **27**, 1542.
18 Z. Tang, X.L. Ding, Y.S. Xie and B.X. Zhao, *Acta Crystallog. E*, 2009, **65**, o1273.
19 I. Vujasinovic, A. Paravic-Radicevic, K. Mlinaric-Majerski, *et al. Bioorg. Med. Chem.*, 2012, **20**, 2101.
20 V. Yadav, R. Mandhan, R. Dabur, *et al. J. Med. Microbiol.*, 2005, **54**, 375.