A facile iodine(III)-mediated synthesis of 3-(3-aryl-1-phenyl-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyridines via oxidation of 2-((3-aryl-1-phenyl-1H-pyrazol-4-yl)methylene)-1-(pyridin-2-yl)hydrazines and their antimicrobial evaluations

Om Prakash1*, Khalid Hussain2, Deepak K Aneja2, Chetan Sharma3 and Kamal R Aneja3

Abstract

Background: Fused heterocyclic 1,2,4-triazoles have acquired much importance because of their interesting biological properties. Although a number of methods have been reported in the literature which includes oxidation with phosphorus oxychloride, lead tetraacetate, bromine, etc., hypervalent iodine reagents have emerged as reagents of choice for various synthetically useful transformations due to their low toxicity, ready availability and ease of handling.

Results: A series of new 3-(3-aryl-1-phenyl-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyridines 4 has been conveniently synthesized by oxidative cyclization of 2-(3-aryl-1-phenyl-1H-pyrazol-4-yl)methylene)-1-(pyridin-2-yl)hydrazines 3 promoted with iodobenzene diacetate under mild conditions (up to 90% isolated yields). All the new compounds were tested in vitro for their antimicrobial activity.

Conclusions: Iodine(III)-mediated oxidative approach has offered an easy access to new 3-(3-aryl-1-phenyl-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyridines 4. The antibacterial and antifungal activities of newly synthesized compounds have proved them potent antimicrobial agents.

Keywords: hypervalent iodine, antimicrobial activity, triazoles, pyrazole

Background

Fused heterocyclic 1,2,4-triazoles have acquired much importance because of their CNS depressant [1], antiallergy [2], antimicrobial [3] and anti-inflammatory [4] properties. Most methods for the preparation of fused 1,2,4-triazole derivatives are based on the oxidation of heterocyclic hydrazones or hydrazides with phosphorus oxychloride [5], lead tetraacetate [5,6], bromine [6,7], etc., which are associated with toxic properties. Therefore, alternative approach avoiding these reagents is always preferred.

Organohypervalent iodine reagents have emerged as reagents of choice for various synthetically useful transformations due to their low toxicity, ready availability and ease of handling [8-17]. We have recently reported the usefulness of iodobenzene diacetate (IBD) to effect oxidative cyclization of benzalhydrazones to 1,2,4-triazoles [18-22].

Pyrazoles form an integral part of many natural products of therapeutic importance and possess potentially reactive sites for a variety of chemical reactions to generate molecular diversity. (S)-3-Pyrazolylalanine [23], lonazolac [24], difenamizole [25], mepirizole [26], metamizol [27] and 4,5-dihydro-3-phenyl-6H-pyrrolo[1,2-b]pyrazole are some of the biologically active compounds endowed with antimicrobial [28], hypoglycaemic [29] and non-nucleoside HIV-1 reverse transcriptase inhibitor properties [30].

Our ongoing programme on the development of hypervalent iodine-mediated methodologies in heterocyclic...
synthesis coupled with the significant biological importance of fused 1,2,4-triazole derivatives and pyrazole derivatives, prompted us to undertake the synthesis of hitherto unknown fused 1,2,4-triazolopyridines. We report in this study on the synthesis of fused 3-(3-aryl-1-phenyl-1H-pyrazol-4-yl)etriazolo[4,3-a]pyridines 3 needed for their oxidative cyclization. These substrates were easily accessible in high yields (88-96%) and purity needed for their oxidative cyclization. These substrates

### Results and Discussion

#### Chemistry

First, we synthesized a series of 2-((3-aryl-1-phenyl-1H-pyrazol-4-yl)methylene)-1-(pyridin-2-yl)hydrazines 3 for their oxidative cyclization. These substrates were easily accessible in high yields (88-96%) and purity from the reaction of 2-pyridyllhydrazine 1 and 3-aryl-1-phenyl-1H-pyrazole-4-carbaldehydes 2 in ethanol (Scheme 1) [31]. Then, the reaction of 2-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)-1-(pyridin-2-yl)hydrazine (3a) (see Additional file 1) was carried out with 1.1 equivalents of IBD in dichloromethane by stirring at room temperature overnight. The usual work-up of the reaction afforded the expected product, 3-(1,3-diphenyl-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyridine (4a) (see Additional file 2) in 90% yield (Scheme 1). To study the scope of reaction, we carried out oxidation of a wide range of substituted 2-((3-aryl-1-phenyl-1H-pyrazol-4-yl)methylene)-1-(pyridin-2-yl)hydrazines 3b-g (see Additional files 3, 4, 5, 6, 7 and 8) under similar conditions. It was observed that IBD-mediated oxidative approach worked nicely to give the desired products 4b-g (see Additional files 9, 10, 11, 12, 13 and 14) in all cases in 82-90% yields.

The structures of all the compounds 3 and 4 were confirmed by their spectral (IR, $^1$H NMR, Mass) and elemental analytical data. For example, the IR spectrum of the compound 3a exhibited characteristic absorption band at 3190 cm$^{-1}$ due to NH functional group. The $^1$H NMR spectrum of the product 3a showed two singlets due to C(5)-H of pyrazole ring and N=CH at $\delta$ 8.93 and $\delta$ 8.17, respectively, and also a broad singlet due to NH at $\delta$ 10.66 which disappeared on the addition of D$_2$O. Other protons appeared as multiplet in the aromatic regions. Mass spectrum of the compound 3a exhibited molecular ion peak at m/z 340.06 [M + 1]$^+$. The characterization of products 4 was based upon a careful comparison of their IR and $^1$H NMR spectra with those of 3. IR spectra of 4 were found to be transparent in the region of NH stretch, thus confirming the oxidation of 3 into 4. An important characteristic feature in the $^1$H NMR spectra of 4 was the disappearance of the singlet due to N=CH around $\delta$ 8.12-8.93, which was present in the spectra of 3. The plausible mechanism for the oxidation of 3 to 4 is analogous to our earlier reports [18,19] and given in Scheme 2.

#### Pharmacology

**In vitro antibacterial activity**

All the synthesized compounds, 3a-g and 4a-g were evaluated in vitro for their antibacterial activity against two Gram-positive bacterial strains, *Staphylococcus aureus* and *Bacillus subtilis* and two Gram-negative bacteria namely, *Escherichia coli* and *Pseudomonas aeruginosa*, and their activity was compared to a well-known commercial antibiotic, ciprofloxacin. All the compounds possessed variable antibacterial activity against Gram-positive bacteria, *S. aureus* and *B. subtilis*. Results of antibacterial evaluation are summarized in Table 1 and Figure 1. Compounds 3a-g and 4a-g showed zone of inhibition ranging between 12.8 and 24.6 mm. On the basis of zone of the inhibition produced against the test bacteria, compound 3d was found to be the most effective against *S. aureus* and *B. subtilis* showing the maximum zone of inhibition of 18.6 and 19.3 mm, respectively, when compared with commercial antibiotic.
ciprofloxacin, which showed maximum zone of inhibition of 27.6 and 26.3 mm against Gram-positive bacteria, *S. aureus* and *B. subtilis*, respectively. Compounds 4a, 4b, 4c and 4f were found to be the most effective against both Gram-positive bacteria showing maximum zone of inhibition ranging between 20.3 and 22.6 mm. Rest of compounds showed fair activity against Gram-positive bacterial strains (Table 1, Figure 1). All the synthesized compounds showed fair activity against Gram-negative bacterial strains. In the whole series, the MIC value of various synthesized compounds (3 and 4) ranges between 16 and 256 μg/mL against Gram-positive and Gram-negative bacteria (Table 2, Figure 2). Out of compounds 3a-3g, compound 3d was found to be the most effective against both Gram-positive bacteria having the lowest MIC value 64 μg/mL when compared with commercial antibiotic ciprofloxacin, which showed MIC value 5 μg/mL for both Gram-positive bacteria. Out of compounds 4a-4g, compounds 4a, 4b, 4c and 4f possessed good antibacterial activity against *B. subtilis* with MIC of 16, 16, 32 and 32 μg/mL, respectively (Table 2, Figure 2).

### In vitro antifungal activity

All the newly synthesized compounds (3 and 4) were also tested in vitro for their antifungal activity against two fungi, namely *Aspergillus niger* and *Aspergillus flavus*. Standard antibiotic fluconazole was used for comparison with antifungal activity shown by compounds 3a-g and 4a-g. A careful analysis of percentage mycelial growth inhibition revealed that compounds 3a, 3b, 3c and 3d exhibit good antifungal activity against both *A. flavus* and *A. niger*. Out of compounds 4a-g, with commercial antibiotic ciprofloxacin, which showed MIC value 5 μg/mL for both Gram-positive bacteria.

### Table 1 In vitro antibacterial activity of compounds 3 and 4

| Compounds | Diameter of growth of inhibition zone (mm)* | *Staphylococcus aureus* | *Bacillus subtilis* | *Escherichia coli* | *Pseudomonas aeruginosa* |
|-----------|------------------------------------------|------------------------|--------------------|--------------------|-------------------------|
| 3a        | 17.3                                     | 19.3                   | 13.3               | 15.6               |
| 3b        | 16.6                                     | 17.3                   | -                  | 13.2               |
| 3c        | 17.3                                     | 18.6                   | 13.0               | 12.8               |
| 3d        | 18.6                                     | 19.3                   | 14.3               | 15.0               |
| 3e        | 16.3                                     | 17.6                   | 14.2               | -                  |
| 3f        | 16.3                                     | 17.6                   | -                  | 13.5               |
| 3g        | 17.0                                     | 18.3                   | 13.8               | 14.3               |
| 4a        | 22.6                                     | 24.6                   | 19.6               | 17.3               |
| 4b        | 21.3                                     | 24.6                   | 18.0               | 16.6               |
| 4c        | 20.3                                     | 21.6                   | 16.3               | 15.3               |
| 4d        | 17.0                                     | 18.6                   | 15.6               | 13.6               |
| 4e        | 15.5                                     | 16.0                   | -                  | 16.2               |
| 4f        | 21.6                                     | 22.6                   | 17.3               | 15.6               |
| 4g        | 18.6                                     | 19.3                   | 14.6               | 15.3               |
| Ciprofloxacin | 27.6                                    | 26.3                   | 25.0               | 25.3               |

* no activity

*Values including diameter of the well (8 mm), are means of three replicates

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**Figure 1** Comparison of diameter of growth of inhibition of compounds (3 and 4) and standard drug

**Figure 2** Comparison of diameter of growth of inhibition of compounds 3 and 4 with commercial antibiotic ciprofloxacin.
compounds 4a, 4b, 4c and 4f showed excellent activity against both antifungal strains as shown in Table 3 and Figure 3. It indicates that fused triazoles 4a-g containing electron-releasing substituents (4a, 4b and 4c) at para position of aryl ring of pyrazole moiety are more antifungal than triazoles having electron-withdrawing groups (4d, 4e, 4f and 4g) at the same position.

A careful analysis of MIC data revealed some interesting results (Table 2) which are as follows

(i) Compounds 3a-g have shown marginal activity, but after oxidative cyclization of these compounds, it was found that antibacterial activity has been increased.

(ii) Inhibitory data of compounds 4a-g suggested that the replacement of para proton of aryl ring of pyrazole moiety (at 3-position) in 3-(3-aryl-1-phenyl-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyridines (4a-g) with electron-releasing groups the antibacterial activity increases while replacing the same aryl proton with electron-withdrawing group the antibacterial activity decreases. In compound 4b, the proton at para position of aryl ring of pyrazole moiety is replaced with methyl group, and in compound 4c, the proton at para position of aryl ring of pyrazole moiety is replaced with methoxy group, both of these compounds exhibited significant level of antibacterial activity. Compounds 4d, 4e, 4f (containing halogen at para position of aryl ring of pyrazole

| Compounds | Minimum inhibitory concentration (μg/mL) |
|-----------|------------------------------------------|
|           | Staphylococcus aureus | Bacillus subtilis | Escherichia coli | Pseudomonas aeruginosa |
| 3a        | 128                       | 64               | 256             | 128                   |
| 3b        | 128                       | 128              | NT              | 256                   |
| 3c        | 128                       | 64               | 256             | 256                   |
| 3d        | 64                        | 64               | 256             | 256                   |
| 3e        | 128                       | 128              | 256             | NT                    |
| 3f        | 128                       | 128              | NT              | 256                   |
| 3g        | 128                       | 64               | 256             | 256                   |
| 4a        | 32                        | 16               | 64              | 64                    |
| 4b        | 32                        | 16               | 64              | 128                   |
| 4c        | 64                        | 32               | 128             | 128                   |
| 4d        | 128                       | 64               | 128             | 256                   |
| 4e        | 128                       | 128              | 256             | 128                   |
| 4f        | 32                        | 32               | 64              | 128                   |
| 4g        | 64                        | 64               | 256             | 256                   |

Ciprofloxacin 5 5 5 5

NT, not tested.

Figure 2 Comparison of MIC of compounds (3 and 4) and standard drug.
moiety) and 4g (containing nitro group at para position of aryl ring of pyrazole moiety) have shown marginal activities against all four bacteria.

Conclusions

We have described in this study an efficient and convenient synthesis of some new 3-(3-aryl-1-phenyl-1H-pyrazol-4-yl)-1,2,4]triazolo[4,3-a]pyridines (4a-g) with electron-releasing groups the antibacterial activity increased while as the para proton of aryl ring of pyrazole moiety is replaced with electron-withdrawing group, the antibacterial activity decreases.

Triazoles 4 having electron-releasing substituents at para position of aryl ring of pyrazole moiety (4a, 4b and 4c) are more antifungal than triazoles containing electron-withdrawing groups (4a, 4e, 4f and 4g) at the same position.

Experimental

Melting points were taken on slides in an electrical apparatus Labindia visual melting range apparatus and are uncorrected. The IR spectra were obtained with a Buck Scientific IR M-500 spectrophotometer. The 1H NMR spectra were recorded on a Bruker (300 MHz) spectrometer using tetramethylsilane as an internal standard. All the compounds gave satisfactory analytical results (within 0.4% of the theoretical values). The starting material 4-formylpyrazoles 2 were prepared by the literature method [32].

| Compounds/Antifungal drug | Mycelial growth of inhibition (%) |
|---------------------------|---------------------------------|
| Aspergillus niger | Aspergillus flavus |
| 3a | 52.5 | 51.1 |
| 3b | 51.1 | 50.6 |
| 3c | 52.5 | 51.1 |
| 3d | 50.0 | 49.8 |
| 3e | 44.4 | 45.5 |
| 3f | 45.5 | 44.4 |
| 3g | 47.7 | 45.5 |
| 4a | 66.5 | 58.8 |
| 4b | 62.3 | 62.5 |
| 4c | 60.5 | 60.6 |
| 4d | 52.5 | 51.1 |
| 4e | 48.8 | 50.0 |
| 4f | 69.5 | 68.5 |
| 4g | 51.1 | 50.0 |
| Fluconazole | 81.1 | 77.7 |

Table 3 In vitro antifungal activity of compounds 3 and 4

Figure 3 Comparison of antifungal activity of compounds (3 and 4) and standard drug.
2-((1-Phenyl-3-p-tolyl-1H-pyrazol-4-yl)methylene)-1-(pyridin-2-yl)hydrazine (3b)

Yield 94%, Mp 206°C, IR (νmax, KBr): 3194 cm⁻¹ (-NH str.); ¹H NMR (DMSO-d₆, 300 MHz): δ 6.87-6.93 (m, 1H), 7.20-7.23 (d, 1H, J = 9 Hz), 7.32-7.38 (m, 3H), 7.51-7.59 (d, 2H, J = 9 Hz), 7.62-7.65 (m, 3H), 7.98-8.09 (m, 3H), 8.15 (s, 1H), 8.93 (s, 1H, N=CH), 8.97 (s, 1H), 10.67 (bs, 1H, exchangeable with D₂O); Anal. Calculated for C₂₁H₁₆BrN₅: C 60.30, H 3.83, N 16.74; Found: C 60.33, H 3.86, N 16.77; ESI-MS m/z: 385.12 ([M + 1]⁺).

2-((4-Nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-1-(pyridin-2-yl)hydrazine (3g)

Yield 92%, Mp 234°C, IR (νmax, KBr): 3195 cm⁻¹ (-NH str.); ¹H NMR (DMSO-d₆, 300 MHz): δ 6.69-6.73 (m, 1H), 7.12-7.14 (m, 1H), 7.35-7.38 (m, 1H), 7.50-7.59 (m, 3H), 7.71 (m, 4H), 7.95-7.98 (d, 2H, J = 8.1 Hz), 8.06-8.07 (d, 1H, J = 4.5 Hz), 8.12 (s, 1H, N=CH), 8.93(s, 1H), 10.67 (bs, 1H, exchangeable with D₂O); Anal. Calculated for C₂₁H₁₄BrN₅: C 60.30, H 3.86, N 16.74; Found: C 60.30, H 3.83, N 16.77; ESI-MS m/z: 385.12 ([M + 1]⁺), 387.02 [M + 3]⁺.

Synthesis of 3-(3-aryl-1-phenyl-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyridines (4a-g)

General procedure: To a suspension/solution of 3 (0.010 mol) in dichloromethane (25 mL), IBD (0.011 mol) was added in small portions, and the reaction mixture was stirred overnight. Then, the solvent was evaporated on water bath. To the resulting residue was added ethanol (5-10 mL), and the mixture was warmed to obtain a clear solution. On cooling at room temperature, solid separated out was filtered and washed with cold alcohol to give pure fused 1,2,4-triazole derivatives 4a-g.

(1,3-Diphenyl-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyridine (4a)

Yield 90%, Mp 162°C, IR (νmax, KBr): transparent in the region of -NH str.; ¹H NMR (DMSO-d₆, 300 MHz): δ 6.89-6.94 (m, 1H), 7.33-7.35 (m, 3H), 7.38-7.45 (m, 2H), 7.54-7.63 (m, 4H), 7.84-7.87 (s, 1H), 7.99-8.05 (m, 2H), 8.15-8.17 (d, 1H, J = 6 Hz), 9.16 (s, 1H); Anal. Calculated for C₂₁H₁₄FN₅: C 75.18, H 4.45, N 20.73; ESI-MS m/z: 338.09 [M + 1]⁺.

3-(1-Phenyl-3-p-tolyl-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyridine (4b)

Yield 88%, Mp 210°C, IR (νmax, KBr): transparent in the region of -NH str.; ¹H NMR (DMSO-d₆, 300 MHz): δ 2.28 (s, 3H), 6.89-6.93 (m, 1H), 7.13-7.16 (m, 2H), 7.38-7.44 (m, 4H), 7.57-7.62 (m, 2H), 7.80-7.84 (m, 1H), 8.01-8.03 (d, 2H, J = 6 Hz), 8.12-8.14 (d, 1H, J = 6 Hz), 9.13 (s, 1H); Anal. Calculated for C₂₂H₁₅N₅: C 75.19, H 4.88, N 19.93; Found: C 75.18, H 4.86, N 19.96; ESI-MS m/z: 352.11 [M + 1]⁺.
3-(3-(3-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyridine (4c)

Yield 82%,Mp 164°C, IR (νmax KBr): transparent in the region of -NH str.; 1H NMR (DMSO-d6, 300 MHz): δ 3.73 (s, 3H), 6.89-6.94 (m, 3H), 7.36-7.40 (m, 2H), 7.47-7.49 (m, 2H), 7.56-7.61 (m, 2H), 7.80-7.87 (m, 1H), 7.95-8.02 (m, 2H), 8.14-8.16 (m, 1H), 9.12 (s, 1H); Anal. Calculated for C21H14F3N5: C 70.98, H 3.99, N 19.69; ESI-MS m/z: 368.06 [M + 3]+.

3-(3-(3-(4-Methylphenyl)-1-phenyl-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyridine (4d)

Yield 85%, Mp 193°C, IR (νmax KBr): transparent in the region of -NH str.; 1H NMR (DMSO-d6, 300 MHz): δ 6.92-6.97 (m, 1H), 7.17-7.23 (m, 2H), 7.39-7.44 (m, 2H), 7.57-7.68 (m, 4H), 7.85-7.88 (m, 1H), 8.02-8.05 (d, 2H, J = 9 Hz), 8.24-8.26 (d, 1H, J = 6 Hz), 9.19 (s, 1H); Anal. Calculated for C21H14ClN5: C 67.83, H 3.80, N 18.84; Found: C 67.81, H 3.73, N 18.80; ESI-MS m/z: 372.01 [M + 1]+, 374.06 [M + 3]+.

3-(3-(3-(4-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyridine (4e)

Yield 89%, Mp 140°C, IR (νmax KBr): transparent in the region of -NH str.; 1H NMR (DMSO-d6, 300 MHz): δ 6.92-6.96 (m, 1H), 7.38-7.43 (m, 2H), 7.54-7.60 (m, 6H), 7.83-7.86 (m, 1H), 7.99-8.02 (d, 2H, J = 8.1 Hz), 8.23-8.26 (d, 1H, J = 6.9 Hz), 9.17 (s, 1H); Anal. Calculated for C21H14BrN5: C 60.59, H 3.39, N 16.82; Found: C 60.56, H 3.37, N 16.81; ESI-MS m/z: 416.04 [M + 1]+, 418.06 [M + 3]+.

Biological assay

Test microorganisms

Total six microbial strains were selected on the basis of their clinical importance in causing diseases in humans. Two Gram-positive bacteria (Staphylococcus aureus MTCC 96 and Bacillus subtilis MTCC 121); two Gram-negative bacteria (Escherichia coli MTCC 1652 and Pseudomonas aeruginosa MTCC 741) and two fungi, Aspergillus niger and A. flavus, the ear pathogens isolated from the patients of Kurukshetra, were used in the present study for the evaluation of antimicrobial activity of the compounds [33]. All the cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. The bacteria were subcultured on nutrient agar, whereas fungi on Sabouraud dextrose.

In vitro antibacterial activity

The antibacterial activity of chemical compounds was evaluated by the agar well diffusion method. All the cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately 1.5 × 108 cfu/mL. 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 the seeded agar plates, and these were loaded with a 100 μL volume with concentration of 2.0 mg/mL of each compound reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 37°C for 24 h. Antibacterial activity of each compound was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi Antibiotic Zone Scale). The DMSO was used as a negative control, whereas ciprofloxacin was used as a positive control. This procedure was performed in three replicate plates for each organism [34].

Determination of minimum inhibitory concentration (MIC)

The MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. The MIC of all the synthesized compounds (3 and 4) against bacterial strains was tested through a modified agar well-diffusion method [35]. In this method, a twofold serial dilution of each compound was prepared by first reconstituting the compound in DMSO followed by dilution in sterile, distilled water to achieve a decreasing concentration range of 256-0.5 μg/mL. A 100 μL volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100 μL of standardized inoculum (105 cfu/mL) of the test microbial strain. All test plates were incubated aerobically at 37°C for 24
In vitro antifungal activity

The antifungal activity of the newly synthesized compounds was evaluated by poison food technique. The moulds were grown on Sabouraud dextrose agar (SDA) at 25°C for 7 days and used as inocula. 15 mL of molten volume of each compound having concentration of 4.0 mg/mL, reconstituted in the 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. The DMSO was used as the negative control, whereas fluconazole was used as the positive control. The experiments were performed in triplicates. Diameter of the fungal colonies was measured and observed for the inhibition zones. The MIC, taken at the centre with fungal plugs (8 mm diameter), that completely inhibited the growth of the microbe, was recorded for each test organism. Ciprofloxacin was used as positive control while DMSO as negative control.

Additional material

**Additional file 1:** 1H NMR spectra (3a). 1H NMR of 2-(1,3-Diphenyl-1H-pyrazol-4-yl)-methylene)-1-(pyridin-2-yl)hydrazine.

**Additional file 2:** 1H NMR spectra (4a). 1H NMR of (1,3-Diphenyl-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyridine.

**Additional file 3:** 1H NMR spectra (3b). 1H NMR of 2-((1-Phenyl-3-p-toly1-1H-pyrazol-4-yl)methylene)-1-(pyridin-2-yl)hydrazine.

**Additional file 4:** 1H NMR spectra (3c). 1H NMR of 2-((3-(3-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-1-(pyridin-2-yl)hydrazine.

**Additional file 5:** 1H NMR spectra (3d). 1H NMR of 2-((3-(4-Fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-1-(pyridin-2-yl)hydrazine.

**Additional file 6:** 1H NMR spectra (3e). 1H NMR of 2-((3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-1-(pyridin-2-yl)hydrazine.

**Additional file 7:** 1H NMR spectra (3f). 1H NMR of 2-((3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-1-(pyridin-2-yl)hydrazine.

**Additional file 8:** 1H NMR spectra (3g). 1H NMR of 2-((3-(4-Nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-1-(pyridin-2-yl)hydrazine.

**Additional file 9:** 1H NMR spectra (4b). 1H NMR of 3-((1-Phenyl-3-p-toly1-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyridine.

**Additional file 10:** 1H NMR spectra (4c). 1H NMR of 3-((3-(4-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyridine.

**Additional file 11:** 1H NMR spectra (4d). 1H NMR of 3-((3-(4-Fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyridine.

Inhibition of mycelial growth % = (dc – dt)/dc × 100

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

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Abbreviations

DMSO: dimethylsulphoxide; IBD: iodobenzene diacetate; MIC: minimum inhibitory concentration; MTCC: microbial type culture collection; SDA: sabouraud dextrose agar.

Acknowledgements

The authors are thankful to the CSIR and the UGC, New Delhi for the award of Junior Research Fellowship to Khalid Hussain and Deepak K. Aneja. Thanks are also due to the RSC, CDRI Lucknow, India, for providing mass and elemental analyses.

Author details

1. Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra 136119, Haryana, India. 2. Department of Chemistry, Kurukshetra University, Kurukshetra 136119, Haryana, India. 3. Department of Microbiology, Kurukshetra University, Kurukshetra 136119, Haryana, India

Competing interests

The authors declare that they have no competing interests.

Received: 23 March 2011 Accepted: 18 July 2011 Published: 18 July 2011
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