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Synthesis and antimicrobial evaluation of isoxazole-substituted 1,3,4-oxidiazoles

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Abstract: Synthesis of N-(5-methylisoxazol-3-yl)-2-(5-aryl-1,3,4-oxidiazol-2-yl)acetamides 5a–k was achieved from readily available materials. The compounds were screened for their in vitro antimicrobial activity against representative bacterial and fungal strains. Compounds 5b, 5d and 5f exhibit good activity.

Keywords: 1,3,4-oxidiazole; antimicrobial activity; chloramine-T; isoxazole; oxidative cyclization.

Introduction

The emergence of microbial resistance to drugs is a widespread problem in the treatment of various infections. The identification of novel antibiotics for effective treatment of infections still remains a major challenge to medicinal chemists. The 1,3,4-oxidiazole moiety has become an important structural motif for the development of new drugs because of its biological activities including HIV integrase inhibition [1], anti-inflammatory [2], anticancer [3], antibacterial [4], anticonvulsant [5], analgesic [6], antitubercular [7], antifungal [8] and anti-allergic [9] activities. Some compounds having 1,3,4-oxidiazole derivatives currently used as drugs are raltegravir, an antiretroviral drug [10], zibotentan, an anticancer agent [11], fenadiazole, a hypnotic drug [12], nesapidil, an antihypertensive agent [13], and furamizole, an antibiotic [14], among others [15–24]. In continuation of our work on biologically active isoxazoles [25–27], we now report the synthesis of new compounds bearing 2,5-disubstituted 1,3,4-oxidiazoles that may be developed into practical drugs.

Results and discussion

Chemistry

Synthesis of N-(5-methylisoxazol-3-yl)-2-(5-phenyl-1,3,4-oxidiazol-2-yl)acetamides 5a–k is shown in Scheme 1. Condensation of 3-amino-5-methylisoxazole (1) with diethyl malonate in ethanol under reflux afforded ethyl 2-(5-methyl-3-isoxazolylcarbamoyl)acetate (2). Treatment of ester 2 with excess of hydrazine hydrate in ethanol furnished 3-hydrazyl-N-(5-methyl-3-isoxazolyl)-3-oxopropanamide (3). The hydrazide 3 was condensed with aromatic aldehydes in methanol to furnish (E/Z)-3-(2-benzylidenehydrazinyl)-N-(5-methylisoxazol-3-yl)-3-oxopropanamides 4a–k. Compounds 4a–k on treatment with chloramine-T underwent oxidative cyclization to give N-(5-methylisoxazol-3-yl)-2-(5-aryl-1,3,4-oxidiazol-2-yl)acetamides 5a–k.

The structures of compounds 3–5 were established based on IR, 1H NMR, 13C NMR, ESI-MS and analytical data. In particular, 1H NMR spectra of N-acylhydrazones 4a–k show characteristic double signals for each of the proton around the C=N bond, suggesting that these compounds exist in two isomeric forms. Two geometric isomers may be present in the ratio of 3:1; based on the integration values of proton signals. The results of the NMR experiments are discussed as follows.

Compound 4a was used for the analysis of nuclear Overhauser effect (nOe) and two-dimensional (2D)-1H NMR-13C heteronuclear multiple bond correlation (HMBC) spectra. In the nOe experiment, irradiation of the methylene protons (10-CH2) at δH 3.72 gives rise to an enhancement for both NH protons, which demonstrates that the methylene group is flanked by two amidic NH groups (Figure 1). The difference in nOe enhancement observed for the two amidic NH protons is due to their spatial arrangement. On irradiation of the NHCO proton at δH
11.07, only a signal for methylene protons is enhanced and irradiation of the olefinic proton $\text{NN}=\text{CH}$ at $\delta_H 7.93$ gives rise to nOe enhancement for phenyl ring protons and a hydrazine NH proton. Furthermore, irradiation of the other $\text{NN}=\text{C}$ proton at $\delta_H 11.49$ gives nOe enhancement for both the methylene protons of the major and minor isomers. There is also a strong enhancement for the olefinic proton of the major isomer while the proton of the minor isomer is unaffected. These results demonstrate that the compounds exist as a mixture of $E$- and $Z$-isomeric forms.

This conclusion was further confirmed by 2D-1H-13C HMBC experiment. HMBC data unambiguously show that the connectivity, as expected, between protons and carbons of the major and minor isomers is intact, and the assignments of chemical shift values for compound 4a are as follows: correlation of H-6 ($\delta_H 2.3, 2.4$) with C$_2$ ($\delta_c 170.0, 170.1$) and C$_i$ ($\delta_c 96.7$); H$_2$ ($\delta_H 11.0, 11.1$) with C$_i$ ($\delta_c 96.6$) and C$_{16}$ ($\delta_c 166.3, 165.9$); H$_{13}$ ($\delta_H 11.5, 11.6$) with C$_{15}$ ($\delta_c 143.5, 147.3$) and C$_{10}$ ($\delta_c 43.0, 43.7$) and C$_{11}$ ($\delta_c 163.0$ and $169.1$) and H$_{10}$ ($\delta_H 7.9, 8.2$) with C$_{16}$ ($\delta_c 134.5$) and C$_{10}$ and C$_{11}$ ($\delta_c 127.3, 127.6$). The key points from HMBC data are the correlations between H$_2$, C$_i$, H$_{13}$, and H$_{10}$, C$_{8}$ and H$_{10}$, C$_{11}$, which indicate the absence of keto-enol tautomerism. On the basis of both 1D and 2D NMR data, it can be concluded that these compounds exist in the mixture of $Z$ and $E$ geometrical isomers.

The IR spectrum of $N$-(5-methylisoxazol-3-yl)-2-(5-phenyl-1,3,4-oxadiazol-2-yl)acetamide (5a) shows a characteristic band at 1237 cm$^{-1}$ due to C-O-C stretching vibration confirming the formation of 1,3,4-oxadiazole ring. 1H NMR spectrum of 5a does not exhibit signals due to the CH$=\text{N}$ proton, and the NH protons of the hydrazone, which are present in its precursor 4a at $\delta 7.93, 8.16, 11.49$ and 11.55, confirming the oxadiazole ring formation. The absence of azomethine carbon signals at $\delta 143.5$ and 147.3 in 13C NMR spectrum of 5a also supports the formation of the oxadiazole ring. The mass spectrum of 5a also agrees with its structure by exhibiting the protonated molecular ion [M+H]$^+$ peak at $m/z$ 285.

Antibacterial activity

Compounds 5a–k exhibit good antibacterial activity in comparison to the activity of the standard drug ciprofloxacin (Table 1). Some of the compounds exhibit excellent minimum inhibitory concentration values (MIC). Compounds 5b and 5f are highly active. The exceptional activity of compound 5d may be due to the presence of
nitro group on the phenyl ring. The remaining compounds 5a, 5c, 5e, 5h, 5i, 5j and 5k show moderate activity. However, the degree of inhibition varies both with the test compound and with the bacteria used in the present investigation.

**Antifungal activity**

Compounds 5a–k are significantly toxic toward all five pathogenic fungi and are lethal even at 100 μg/mL concentration when compared to the standard drug clotrimazole (Table 2). The activity data are indicated as a zone of inhibition at 100 μg/mL concentration. Compounds 5b and 5f exhibit high activity and they inhibit the growth of fungi to a remarkable extent, which may be due to the presence of chloro, nitro and bromo substituents on the benzene ring, besides the presence of isoxazole and oxadiazole skeletons. Compound 5d bearing a nitro group on the benzene ring shows good toxicity against the fungi used. Compounds 5a, 5c, 5e, 5g, 5h, 5i, 5j and 5k are moderate in their toxicity and they are less active compared to other compounds in the present study, but better than the standard drug clotrimazole. The degree of spore germination inhibition varies with test compounds and with the type of fungi.

**Table 1** Antibacterial activity of N-(5-methylisoxazol-3-yl)-2-(5-aryl-1,3,4-oxadiazol-2-yl)acetamides 5a–k.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Minimum inhibitory concentration (MIC)</th>
<th>Bacterial strains</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>P. aeruginosa</td>
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<tr>
<td>5a</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>5b</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>5c</td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>5d</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>5e</td>
<td></td>
<td>10</td>
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<tr>
<td>5f</td>
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<td>5g</td>
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<td>5h</td>
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<td>5i</td>
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</tr>
<tr>
<td>5j</td>
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<td>23</td>
</tr>
<tr>
<td>5k</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>30</td>
</tr>
</tbody>
</table>

*aNegative control (acetone) – no activity. bConcentration in μg/mL.

**Table 2** Antifungal activity of N-(5-methylisoxazol-3-yl)-2-(5-aryl-1,3,4-oxadiazol-2-yl)acetamides 5a–k.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Zone of inhibition (mm)</th>
<th>Fungal strains</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>A. niger</td>
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<tr>
<td>5a</td>
<td></td>
<td>52.5</td>
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<tr>
<td>5b</td>
<td></td>
<td>69.1</td>
</tr>
<tr>
<td>5c</td>
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<td>56.0</td>
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<td>75.0</td>
</tr>
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<td>5e</td>
<td></td>
<td>48.0</td>
</tr>
<tr>
<td>5f</td>
<td></td>
<td>63.2</td>
</tr>
<tr>
<td>5g</td>
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<td>55.8</td>
</tr>
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<td>5h</td>
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<td>47.2</td>
</tr>
<tr>
<td>5i</td>
<td></td>
<td>39.0</td>
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<td>51.0</td>
</tr>
<tr>
<td>5k</td>
<td></td>
<td>45.5</td>
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<tr>
<td>Clotrimazole</td>
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<td>26.5</td>
</tr>
</tbody>
</table>

*aNegative control (acetone) – no activity. bConcentration 100 μg/mL.
Conclusions

A simple and efficient protocol for the synthesis of isoxazolyl-2,5-disubstituted 1,3,4-oxadiazoles 5a–k with potential pharmaceutical properties is described. Compounds 4a–k exhibit characteristic 1H NMR spectra indicating the presence of E and Z geometrical isomers. The title compounds 5a–k were evaluated for antimicrobial activity. Compounds 5a, 5d and 5f show excellent antimicrobial activity.

Experimental

Melting points are uncorrected. Analytical thin-layer chromatography (TLC) analysis was performed on Merck precoated 60F254 silica gel plates. Visualization was done by exposure to UV light. IR spectra were recorded in KBr pellets on a Perkin-Elmer BX series Fourier-transform infrared (FT-IR) spectrometer. 1H NMR (400 MHz) and 13C NMR (DMSO-d6 with tetramethylsilane (TMS) as internal standard. ESI mass spectra were recorded on an Agilent liquid chromatography-mass selective detector (LC-MSD). EA were performed on Carlo Erba 106 and Perkin-Elmer model 240 analyzers.

Synthesis of ethyl 2-(5-methyl-3-isoxazolylcarbamoylethyl)acetate (2)

A mixture of 3-amino-5-methylisoxazole (1, 0.1 mmol) and diethyl malonate (0.1 mmol) in ethanol (20 mL) was heated under reflux for 12 h. The reaction was monitored by TLC analysis. After the mixture was concentrated and cooled, the separated solid product was filtered under suction, dried and crystallized from ethanol; yield 80%; mp 112–113°C; IR: νmax 3266, 1743, 1700 cm−1; 1H NMR (CDCl3): δ 1.30 (t, J = 8.0 Hz, 3H, OCH2-CH3), 2.41 (s, 3H, isoxazolyl-CH3), 3.52 (s, 2H, CH2), 4.25 (q, J = 8.0 Hz, 2H, OCH2-CH2), 6.70 (s, 1H, isoxazole-H), 10.19 (s, 1H, NH); 13C NMR (CDCl3): δ 12.6, 16.0, 42.0, 62.0, 96.6, 157.3, 163.4, 168.2, 170.1; MS: m/z 213, (M + H)+. Anal. Calcd for C8H12N2O4: C, 50.94; H, 5.70; N, 13.20. Found: C, 50.92; H, 5.68; N, 13.21.

Synthesis of 3-hydrazino-N-(5-methylisoxazol-3-yl)-3-oxopropanamide (3)

A mixture of compound 2 (0.1 mmol) in ethanol (15 mL) and hydrazine hydrate (0.5 mmol) (98%) was heated under reflux for 6 h, and the progress of the reaction was monitored by TLC. The excess ethanol was removed under reduced pressure and the residue of 3 was washed with cold water and cold methanol and crystallized from methanol; yield 92%; mp 152–153°C; IR: νmax 3211–3337, 3141, 3288, 3266, 1743, 1700 cm−1; 1H NMR (DMSO-d6): δ 2.35 (s, 3H, isoxazole-CH3), 3.19 (s, 2H, CH2), 4.26 (s, 2H, NH2), 6.57 (s, 1H, isoxazole-H), 9.13 (s, 1H, NH), 10.94 (s, 1H, NH); 13C NMR (DMSO-d6): δ 12.5, 42.5, 96.6, 158.3, 165.9, 166.1, 170.0; MS: m/z 199.15 (M + H)+. Anal. Calcd for C8H10N2O3: C, 46.0; H, 5.24; N, 23.35. Found: C, 45.89; H, 5.20; N, 23.30.

General procedure for the synthesis of 3-(2-arylideneydrazino)-N-(5-methyl-3-isoxazolyl)-3-oxopropanamides 4a–k

A mixture of hydrazide 3 (0.1 mmol) and an aromatic aldehyde (0.1 mmol) was heated under reflux in methanol (20 mL) in the presence of a catalytic amount of glacial acetic acid for 4–6 h. The progress of the reaction was monitored by TLC. The reaction mixture was cooled, and the separated solid was filtered and crystallized from methanol.

(E/Z)-3-(2-Benzylideneydrazino)-N-(5-methylisoxazol-3-yl)-3-oxopropanamide (4a) Yield 90%; mp 160–161°C; IR: νmax 3257, 3220, 1680, 1621 cm−1; 1H NMR (DMSO-d6): δ 2.33 and 2.36 (s, 3H, H3), 3.60 and 3.72 (s, 2H, H5), 6.57 and 6.60 (s, 1H, H7), 7.34 and 742 (m, 3H, H6, H7, H8, H9); 13C NMR (DMSO-d6): δ 12.6 (C6), 43.30 and 43.7 (C10), 96.6 (C16), 127.3, and 1276 (C7, C8, and C9), 129.2 and 129.3 (C12 and C13), 130.3 and 130.6 (C14, 134.5 (C15), 143.5 and 1473 (C16), 158.4 and 1586 (C17), 163.0 and 169.1 (C18), 165.9 and 166.3 (C19), 170.0 and 170.1 (C1); MS: m/z 287 (M + H)+. Anal. Calcd for C18H14ClN4O3: C, 58.73; H, 4.93; N, 19.57. Found: C, 58.72; H, 4.92; N, 19.56.

(E/Z)-3-(2-(4-Chlorobenzylideneydrazino)-N-(5-methylisoxazol-3-yl)-3-oxopropanamide (4b) Yield 92%; mp 178–180°C; IR: νmax 3255, 1678, 1618 cm−1; 1H NMR (DMSO-d6): δ 2.34 and 2.38 (2s, 3H, isoxazole-CH3), 3.41 and 3.70 (2s, 2H, CH2), 6.59 and 6.61 (2s, 1H, isoxazole-H), 6.80–730 (m, 4H, Ar-H), 8.01 and 8.20 (2s, 1H, N=CH), 10.92 and 10.96 (2s, 1H, NH), 11.20 and 11.28 (2s, 1H, NH); 13C NMR (DMSO-d6): δ 12.7, 43.1, 43.9, 95.7, 130.2, 131.9, 132.9, 139.8, 143.2, 1471, 1578, 158.2, 162.2, 165.6, 165.8, 169.9, 170.2, 171.4; MS: m/z 321 (M + H)+. Anal. Calcd for C18H13ClN4O3: C, 52.43; H, 4.09; N, 17.47. Found: C, 52.41; H, 4.08; N, 17.45.

(E/Z)-3-(2-(4-Methoxybenzylideneydrazino)-N-(5-methylisoxazol-3-yl)-3-oxopropanamide (4c) Yield 93%; mp 172–173°C; IR: νmax 3230, 1675, 1622 cm−1; 1H NMR (DMSO-d6): δ 2.33 and 2.36 (2s, 3H, isoxazole-CH3), 3.40 and 3.72 (2s, 2H, CH2), 3.78 and 3.80 (2s, 3H, OCH3), 6.57 and 6.60 (2s, 1H, isoxazole-H), 6.91–7.36 (m, 4H, Ar-H), 7.90 and 8.13 (2s, 1H, N=CH), 11.07 and 11.10 (2s, 1H, NH), 11.10 and 11.56 (2s, 1H, NH); 13C NMR (DMSO-d6): δ 12.4, 43.0, 43.9, 55.4, 55.6, 96.5, 110.9, 111.2, 116.7, 116.9, 120.3, 120.6, 130.3, 130.4, 135.7, 143.8, 1475, 158.2, 159.8, 162.0, 163.8, 165.8, 166.3, 169.1, 170.2, 170.4; MS: m/z 317 (M + H)+. Anal. Calcd for C18H14ClN4O3: C, 56.96; H, 5.10; N, 17.71. Found: C, 56.94; H, 5.09; N, 17.69.
N=C=H), 9.60 and 9.62 (2s, 1H, NH), 11.22 and 11.25 (2s, 1H, NH); 13C NMR (DMSO-d6): δ 12.2, 42.9, 43.5, 96.1, 120.8, 131.2, 140.5, 142.9, 146.0, 169.7, 157.2, 163.0, 164.9, 165.2, 168.2, 169.0, 168.8, 170.0; MS: m/z 354 (M + Na)−

**General procedure for the synthesis of N-(5-methylisoxazol-3-yl)-2-(5-aryl-1,3,4-oxadiazol-2-yl)acetamides 5a–k**

A mixture of hydrazine 4 (0.1 mmol), chlorine-T (0.5 mmol) and ethanol (15 mL) was heated under reflux for 4–6 h. The progress of the reaction was monitored with TLC. Afterward, the mixture was poured into water and extracted with ethyl acetate. The extract was washed with water, dried over anhydrous sodium sulfate and concentrated under reduced pressure. After removal of the solvent, the crude product was passed over silica gel column. The product was eluted with 40% ethyl acetate in n-hexane.

N-(5-Methylisoxazol-3-yl)-2-(5-phenyl-1,3,4-oxadiazol-2-yl)acetamide (5a)

Yield 72%; mp 220–221°C; IR: v max 3125, 1659, 1237 cm−1; 1H NMR (DMSO-d6): δ 2.30 (3H, 3H, isoxazole-CH3), 3.42 (2s, 2H, CH2), 6.58 (s, 1H, isoxazole-H), 7.13–7.17 (m, 1H, Ar-H), 7.31–7.35 (m, 2H, Ar-H), 7.47–7.49 (m, 2H, Ar-H), 9.50 (s, 1H, NH); 13C NMR (DMSO-d6): δ 12.3, 42.6, 96.4, 126.8, 127.9, 128.1, 128.9, 129.2, 156.2, 164.2, 168.0, 169.4, 169.6, 170.5; MS: m/z 325 (M + H)+

2-(5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yI)-N-(5-methylisoxazol-3-yl)acetamide (5c) Yield 74%; mp 226–227°C; IR: νmax, 3215, 1658, 1261 cm−1; 1H NMR (DMSO-d6): δ 2.48 (s, 3H, isoxazole-CH3), 3.80 (s, 3H, OCH3), 6.20 (s, 2H, Ar-CH), 6.80–7.30 (m, 4H, Ar-H), 8.51 (s, 1H, Ar-OH), 9.62 (s, 1H, NH); 13C NMR (DMSO-d6): δ 12.2, 42.0, 56.1, 95.2, 124.2, 125.2, 129.0, 132.6, 132.8, 134.0, 155.8, 163.0, 165.8, 168.9, 170.4; MS: m/z 315 (M+H)+. Anal. Calcd for C14H11BrN4O3: C, 46.30; H, 3.05; N, 15.43. Found: C, 46.32; H, 3.06; N, 15.44.

N-(5-Methylisoxazol-3-yl)-2-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yI)acetamide (5d) Yield 70%; mp 250–251°C; IR: νmax, 3230, 1656, 1230 cm−1; 1H NMR (DMSO-d6): δ 2.40 (s, 3H, isoxazole-CH3), 4.05 (s, 2H, CH2), 6.40 (s, 1H, isoxazole-H), 7.40 (d, J = 8.0 Hz, 2H, Ar-H), 8.10 (d, J = 8.0 Hz, 2H, Ar-H), 9.32 (s, 1H, NH); 13C NMR (DMSO-d6): δ 12.8, 41.9, 95.6, 122.8, 132, 147.9, 155.5, 163.7, 167.2, 168.9, 170.0; MS: m/z 330 (M+H)+. Anal. Calcd for C15H14N4O4: C, 57.32; H, 4.49; N, 17.83. Found: C, 57.30; H, 4.48; N, 17.82.

Antibacterial activity

The antibacterial activity assay was performed by the broth dilution method [29] and expressed as minimum inhibitory concentration. The nutrient broth medium (HiMedia, 24 g) was suspended in distilled water (100 mL) and heated to boiling until it dissolved completely. The nutrient broth medium and test tubes were autoclaved at 15 lb/inch2 for 20 min. A set of sterilized test tubes with nutrient broth medium was prepared by dissolving the compound 20 g/mL. Agar plates were inoculated and grown for 48 h at 37°C. The growth of bacteria was measured as a turbidity index. Bacterial strains used were Pseudomonas aeruginosa (MTCC 741), Klebsiella aerogenes (MTCC 39), Chromobacterium violaceum (MTCC 2666) (Gram-negative) and Bacillus subtilis (MTCC 441), Bacillus sphaericus (MTCC 511) and Staphylococcus aureus (MTCC 96) (Gram-positive). Ciprofloxacin was used as the standard drug for comparison.

Antifungal activity

The antifungal activity assay was performed by the broth dilution method [30] and expressed as minimum inhibitory concentration. The nutrient broth medium (HiMedia, 24 g) was suspended in distilled water (100 mL) and heated to boiling until it dissolved completely. The nutrient broth medium and test tubes were autoclaved at a pressure of 15 lb/inch2 for 20 min. A set of sterilized test tubes with nutrient broth medium was autoclaved at 37°C for 20 h. Then, the tubes were measured for turbidity. Bacterial strains used were Pseudomonas aeruginosa (MTCC 741), Klebsiella aerogenes (MTCC 39), Chromobacterium violaceum (MTCC 2666) (Gram-negative) and Bacillus subtilis (MTCC 441), Bacillus sphaericus (MTCC 511) and Staphylococcus aureus (MTCC 96) (Gram-positive). Ciprofloxacin was used as the standard drug for comparison.
and the lids of the dishes were replaced. To each cup, 100 μg/mL concentration of the test solution 5a–k was added. Controls were maintained with acetone and clotrimazole (100 μg/mL). The treated mixtures and the controls were kept at room temperature for 72–96 h. The inhibition zone was measured as a diameter (mm). Three to four replicates were maintained for each treatment. The fungal strains Aspergillus niger (MTCC 282), Chrysosporium tropicum (MTCC 2821), Fusarium moniliforme (MTCC 18/48) and Carvularia lunata (MTCC 2030) were used.

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