One-pot synthesis of antifungal active 9-substituted-3-aryl-5H,13aH-quinolino [3,2-f][1,2,4]triazolo [4,3-b][1,2,4]triazepines

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The title compounds, 9-substituted-3-aryl-5H,13aH-quinolino[3,2-f][1,2,4]triazolo[4,3-b][1,2,4]triazepines 8a-j, are synthesized from 5-aryl-3,4-diamino-1,2,4-triazoles 5 and 2-chloro-3-formylquinolines 7 using catalytic amount of p-TsOH and N,N-dimethylformamide as an energy transfer medium using microwave heating as well as solvent using oil-bath heating at 80°C affords novel 9-substituted-3-aryl-5H,13aH-quinolino[3,2-f][1,2,4]triazolo[4,3-b][1,2,4]triazepines. The products are obtained in good to moderate yields and are in a state of high purity.

Heterocyclic compounds are highly essential to life as they play a vital role in the metabolism of all living cells, eg. pyrimidines and purines are the bases of genetic material DNA; the essential amino acids like pyroline, histidine and tryptophan; vitamin and co-enzyme precursors such as thiamine, riboflavin, pyridoxine, folic acid and biotin; the B12 and E families of vitamin. Heterocycles constitute a unique class of compounds, whether natural or man-made, show interesting biological activities and are often present as key components in the biological processes. Generally, the biological activities of these heterocycles are because of the presence of one or more basic heterocyclic moieties. Most common heterocyclic moieties include triazoles, triazepines etc. For instance, chemistry of quinolines 1,2 and their derivatives have gained increasing attention. Particularly, substituted quinolines have been shown found to be effective as antibacterial, antitumor, antinecancerous, antiinflammatory agents etc. Triazepines have been found to posses insecticidal, antiviral activities etc. While substituted triazoles exhibit antibacterial, antimycobacterial, antifungal activities etc. In order to study the combined effect of these heterocyclic moieties in a single frame work, we wish to report a mild and efficient procedure for the synthesis of 9-substituted-3-aryl-5H,13aH-quinolino[3,2-f][1,2,4]triazolo[4,3-b][1,2,4]triazepines in the presence of catalytic amount of p-TsOH and N,N-dimethylformamide as an energy transfer medium as well as solvent using microwave heating as well as oil-bath heating from 5-aryl-3,4-diamino-1,2,4-triazoles and 2-chloro-3-formylquinolines. Some of the synthesized compounds were evaluated for antifungal activity. The synthesis and cytokinine production inhibition of triazolo-triazepines has been recently patented.

Results and Discussion

The required precursor, 5-aryl-3,4-diamino-1,2,4-triazoles 5, were prepared from methyl benzoates 1 which were then converted to aroyl hydrazides 2 on refluxing with hydrazine hydrate in ethanol followed by conversion to aroyl thiosemicarbazides 3 (ref. 14) on heating with potassium thiocyanate in aq. HCl. Aroyl thiosemicarbazides were then converted to 2-amino-5-aryl-1,3,4-thiadizoles 4 which were then converted to 5-aryl-3,4-diamino-1,2,4-triazoles 5 on refluxing with hydrazine hydrate in ethanol as shown in Scheme I, while 2-chloro-3-formyl quinolines 6, were prepared by Vilsmeier Haack reaction of acetanilides/substituted acetanilides 6 as illustrated in Scheme II. The synthesized precursors were then used for the synthesis of title compounds 8a-j in the presence of p-TsOH and N,N-dimethylformamide using both microwave and oil-bath heating.

It was found that N,N-dimethylformamide and p-TsOH has not yet been used for the condensation of 2-chloro-3-formyl quinolines 7 with 5-aryl-3,4-diamino-1,2,4-triazoles 5 followed by cyclizaton to afford 9-substituted-3-aryl-5H,13aH-quinolino[3,2-f][1,2,4]triazolo[4,3-b][1,2,4]triazepines 8 which is outlined in Scheme III. While carrying out the reaction, it was found that the two amino groups were
Scheme I

Scheme II

Scheme III

R= C₆H₅, 2-ClC₆H₄, 4-ClC₆H₄, CH₂C₆H₅, (4-NO₂)C₆H₄

8a: R= C₆H₅, R’= H
8b: R= (4-NO₂)C₆H₄, R’= H
8c: R= 2-ClC₆H₄, R’= 3-CH₃
8d: R= (4-NO₂)C₆H₄, R’= 3-CH₃
8e: R= 2-ClC₆H₄, R’= 4-OCH₃
8f: R= CH₂C₆H₅, R’= 4-CH₃
8g: R= C₆H₅, R’= 4-CH₃
8h: R= 4-ClC₆H₄, R’= 4-OCH₃
8i: R= (4-NO₂)C₆H₄, R’= 4-OCH₃
8j: R= (4-NO₂)C₆H₄, R’= 4-CH₃
present at 4 and 5-position of 5-aryl-3,4-diamino-1,2,4-triazole 5 but the amino group at C-5 position was involved in tautomerization giving tautomeric form as shown in Scheme IV. It was supported by the fact that only -C-NH₂ group can form Schiff’s base with 2-chloro-3-formyl quinolines 7 while H₂N-N-group containing compounds are analogous to hydrazino type compounds which can displace chlorine easily of 2-chloro-3-formyl quinolines 7. So, it was only the amino group at C-2 position of 5-aryl-3,4-diamino-1,2,4-triazole 5 which can form Schiff’s base with 2-chloro-3-formyl quinolines 7 while N-NH₂ of 5 cannot form Schiff’s base. Moreover, in the resulting product 8, the H-atom at 5-position (NH) can form hydrogen bond with all the “R” groups present at C-3 position. Free amino group can displace chlorine easily while imino group cannot displace chlorine easily. These are the evidences which support that displacement occurs via NH₂-N-and condensation occurs via -C-NH₂.

Since p-TsOH is non-toxic, inexpensive and easily available reagent, the reaction of 5-phenyl-3,4-diamino-1,2,4-triazole 5 with 2-chloro-3-formyl quinoline as an energy transfer medium was carried out and isolated 8a in 60% yield. Similar reaction was also carried out with different substrates under conventional conditions at 80°C and were able to isolate 58% yield. To check the generality of the reaction, this procedure was also applied to other substrates and found good to moderate yields. The results are summarized in Table I. Thus, this represents a rapid, mild, cost-effective and green procedure for the synthesis of 9-substituted-3-aryl-5H,13aH-quinolino[3,2-f][1,2,4]triazolo[4,3-b][1,2,4]-triazepines.

In order to optimize the reaction conditions, different reactions were tried with varying amount of 2-chloro-3-formylquinolines 7a and 5-phenyl-3,4-diamino-1,2,4-triazole 5a which were selected as test substrates. It was found that for 5 mmole of each of 5-phenyl-3,4-diamino-1,2,4-triazole 5a, 2-chloro-3-formylquinoline 7a, 200 mg of p-TsOH, 2.5 mmole of N,N-dimethylformamide as an energy transfer medium under microwave heating and as solvent under oil-bath heating was required to give maximum yield under mild conditions. To select the optimum power level, reaction with test substrates was carried out at different power levels from 80-900 W. It was found that 640 W was selected as the optimum power level as far as yield and reaction times are concerned. At low power level, the reaction remains incomplete whereas, at high power level, low yields of products were obtained which may be due to decomposition. Under oil-bath heating, 80°C was selected as the optimum reaction temperature.

<table>
<thead>
<tr>
<th>Compd</th>
<th>Microwave (MW)</th>
<th>Oil-bath heating at 8°C (Δ)</th>
<th>m.p.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Time (min)</td>
<td>Yield (%)</td>
<td>Yield (%)</td>
</tr>
<tr>
<td>8a</td>
<td>6</td>
<td>60[a]</td>
<td>23</td>
</tr>
<tr>
<td>8b</td>
<td>4</td>
<td>75[b]</td>
<td>13</td>
</tr>
<tr>
<td>8c</td>
<td>8</td>
<td>66[a]</td>
<td>22</td>
</tr>
<tr>
<td>8d</td>
<td>6</td>
<td>70[a]</td>
<td>11</td>
</tr>
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<td>8e</td>
<td>13</td>
<td>78[b]</td>
<td>20</td>
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<td>8f</td>
<td>7</td>
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<tr>
<td>8g</td>
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<td>68[a]</td>
<td>26</td>
</tr>
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<td>8h</td>
<td>8</td>
<td>80[a]</td>
<td>18</td>
</tr>
<tr>
<td>8i</td>
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<td>10</td>
</tr>
<tr>
<td>8j</td>
<td>10</td>
<td>76[a]</td>
<td>12</td>
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[a] Products were purified by crystallization from ethyl acetate. [b] Products were purified by passing through column of alumina and elution with ethyl acetate: pet.ether.
Antifungal activity

Some of the synthesized compounds were screened for antifungal activity against *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus* species and *Pencillium notatum* species by paper disc technique against two concentrations 500 μg/mL and 1000 μg/mL. The zone of inhibition after 24 hr of incubation at 28 ± 2°C was compared with that of standard fluconazole. The screening data indicated that the compounds 8a, 8b, 8c and 8d showed excellent activity against *Aspergillus niger* 1000 μg Conc. and *Pencillium notatum* species at 500 μg as well as 1000 μg concentrations whereas, these compounds showed good to moderate activity against *Aspergillus flavus* and *Rhizopus* species at both the concentrations as shown in Table II. The medium used for evaluation of antifungal activity was potato dextrose agar-agar medium.

Preparation of the medium

Potato dextrose agar-agar medium was prepared as below:

Potato= 250 g, Dextrose= 10 g, Agar-agar= 20 g, and Distilled water = 100 mL

Sliced potatoes were taken with 500 mL of distilled water in a pan and boiled for half an hour till a spoon when placed on a slice can pierce into it. Filter it while hot and broth was again taken in a pan with rest of the distilled water. Dextrose dissolved in distilled water and weighed agar-agar was added to the broth and heated it to boil. The medium thus obtained was sterilized in pressure cooker for 30 min. and few drops of streptomycin were added to prevent it from any bacterial contamination.

Table II — Antifungal activity of synthesized compounds 9-Substituted-3-aryl-5H, 13aH-quinolino [3,2-f][1,2,4]triazolo [4,3-b][1,2,4]triazepines

<table>
<thead>
<tr>
<th>Compd</th>
<th>Concentration (µg/mL)</th>
<th>Zone of inhibition in mm (%)</th>
<th>Aspergillus niger</th>
<th>Aspergillus flavus</th>
<th>Rhizopus species</th>
<th>Pencillium notatum species</th>
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<tr>
<td></td>
<td>500µg</td>
<td>1000µg</td>
<td>500µg</td>
<td>1000µg</td>
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<tr>
<td>8a</td>
<td></td>
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<tr>
<td></td>
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<td>(53.33)</td>
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<td>(63.33)</td>
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<tr>
<td>8c</td>
<td></td>
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<td>43</td>
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<td>(40.00)</td>
<td>(71.66)</td>
<td>(70.66)</td>
<td>(53.33)</td>
</tr>
<tr>
<td>8d</td>
<td></td>
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<td>40</td>
<td>43</td>
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<td>(56.66)</td>
<td>(66.66)</td>
<td>(71.66)</td>
<td>(81.66)</td>
</tr>
<tr>
<td>Fluconazole</td>
<td></td>
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<td>48</td>
<td>35</td>
<td>42</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(70.00)</td>
<td>(80.00)</td>
<td>(58.33)</td>
<td>(70.00)</td>
</tr>
</tbody>
</table>

Fluconazole

(70.00) | (80.00) | (58.33) | (70.00) | (63.33) | (70.00) | (86.66) | (90.00)

% = \frac{C \times 100}{I}

Where, I= inhibition

C= diameter of zone of micro-organisms in check
T = diameter of the disc

The zone of inhibition was measured after 24 hr, fluconazole (500 µg/mL and 1000 µg/mL) was used as control standard.

**Experimental Section**

General. All the melting points were determined on a Tempo melting point apparatus and are uncorrected. 

1H NMR spectra were recorded on a Bruker DPX-200 NMR spectrometer (200 MHz) in CDCl$_3$ + DMSO-d$_6$ using tetramethylsilane as an internal standard and IR spectra were recorded using KBr disc on a Perkin Elmer FTIR spectrophotometer. The mass spectral data was obtained on a JEOL JMS-D 300 spectrometer. The elemental analysis were performed on a simple CHNS-932 Analyser (Leco). The reactions were carried out in domestic microwave oven LG-MS-255R with maximum output power of 900 W and by using pre-heated oil-bath.

**General procedure for the synthesis of compounds 8a-j**

**Microwave heating**

A mixture of 5-phenyl-3,4-diamino-1,2,4-triazole 5 (5 mmole), 2-chloro-3-formylquinoline 7 (5 mmole), N,N-dimethylformamide (0.18 g, 2.5 mmole) as an energy transfer medium and catalytic amount of p-TsOH (200 mg) was taken in a 50 mL borosil beaker and mixed properly with the help of a glass rod (5 s). The mixture was then exposed to microwave irradiation for an appropriate time (monitored by TLC, shown in Table I) followed by cooling time of 5 s each at 640 W. After the completion of reaction, crushed ice was added and the solid obtained was filtered, washed with water and dried. The crude product was purified either by crystallization from ethyl acetate or by passing through a column of alumina and elution with ethyl acetate and petroleum ether.

The physical data of the synthesized compounds is given in Table I. The structures of the products were confirmed by IR, 1H NMR, mass spectral data and by elemental analysis.

**Spectral data of the synthesized compounds 8a-j**

3-Phenyl-5H,13aH-quinolino[3,2-f][1,2,4]triazo-lo-[4,3-b][1,2,4]triazepine 8a

This compound was obtained as orange coloured shining solid (ethyl acetate), m.p. 160-62°C; IR (KBr, $v_{max}$): 3100 (NH), 3040 (aromatic C-H), 1642 (C=N), 1406 cm$^{-1}$ (C-N); 1H NMR (CDCl$_3$+ DMSO-d$_6$): $\delta$ 7.10-7.34 (m, 4H, H arom), 7.47-7.69 (m, 5H, H arom and s, 1H buried N=CH), 8.01 (d, 1H, H arom), 8,35 (bs, 1H, Harom); MS: m/z (M$^+$) 292 Anal. Calcd. for C$_{18}$H$_{12}$N$_6$: C, 73.97; H, 4.10; N, 21.91. Found: C, 73.93; H, 4.06; N, 21.87%.

3-(4´-nitrophenyl)-5H,13aH-quinolino[3,2-f][1,2,4]triazo-lo-[4,3-b][1,2,4]triazepine 8b

This compound was obtained as brown coloured shining solid (ethanol), m.p. 210-12°C; IR (KBr, $v_{max}$): 3108 (NH), 3070 (aromatic C-H), 1638 (C=N), 1540 (NO$_2$), 1430 cm$^{-1}$ (C-N), 1H NMR (CDCl$_3$+ DMSO-d$_6$): $\delta$ 7.10-7.34 (m, 4H, H arom), 7.47-7.69 (m, 5H, H arom and s, 1H buried N=CH), 8.01 (d, 1H, H arom), 8,35 (bs, 1H, H arom); MS: m/z (M$^+$) 292 Anal. Calcd. for C$_{18}$H$_{12}$N$_6$: C, 73.97; H, 4.10; N, 21.91. Found: C, 73.93; H, 4.06; N, 21.87%.

9-(3´-methyl)-(2´-chlorophenyl)-5H,13aH-quinolino[3,2-f][1,2,4]triazo-lo-[4,3-b][1,2,4]triazepine 8c

This compound was obtained as yellow shining solid (through column), m.p. 172-74°C; IR (KBr, $v_{max}$): 3104 (NH), 3070 (aromatic C-H), 1638 (C=N), 1540 (NO$_2$), 1430 cm$^{-1}$ (C-N); 1H NMR (CDCl$_3$+ DMSO-d$_6$): $\delta$ 7.20 (m, 1H, H arom), 7.50-7.58 (m, 2H, H arom), 7.70-7.85 (m, 3H, H arom and s, 1H buried N=CH), 8.02 (d, 1H, H arom), 8.20-8.31 (m, 2H, Harom), 8.55 (bs, 1H, Harom); MS: m/z (M$^+$) 353 Anal. Calcd. for C$_{18}$H$_{12}$N$_6$: C, 73.18; H, 3.11; N, 26.62; O, 9.06. Found: C, 73.14; H, 2.97; N, 26.58; O, 9.02%.
N=CH), 7.98 (d, 1H, H_{arom}), 8.35 (bs, 1H, NH); MS: m/z (M') 340.5. Anal. Calcd. for C_{19}H_{12}N_{4}Cl: C, 66.96; H, 3.81; N, 18.79. Found: C, 66.92; H, 3.77; N, 18.72%.

9-(3''-methyl)-3-(4''-nitrophenyl)-5H,13aH-quinolin-3,2-f\[1,2,4\]triazolo[4,3-b][1,2,4]triazepine 8d

This compound was obtained as yellow coloured shining solid (ethyl acetate), m.p. 204-206°C; IR (KBr, v max): 3115 (NH), 3060 (aromatic C-H), 2810 (C-H); 1H NMR (CDCl$_3$+DMSO-$d_6$): δ 2.32 (s, 3H, CH$_3$), 7.22 (m, 1H, H$_{arom}$), 7.52-7.68 (m, 1H, H$_{arom}$ and s, 1H buried N=CH), 7.72-7.82 (m, 3H, H$_{arom}$), 8.16-8.28 (m, 2H, H$_{arom}$), 8.35 (bs, 1H, NH); MS: m/z (M') 367. Anal. Calcd. for C$_{19}$H$_{12}$N$_{4}$: C, 62.62; H, 3.56; N, 25.82. Found: C, 62.54; H, 3.58; N, 25.84%.

9-(3''-methyl)-3-(4''-methoxy)-5H,13aH-quinolin-3,2-f\[1,2,4\]triazolo[4,3-b][1,2,4]triazepine 8e

This compound was obtained as yellow coloured shining solid (through column), m.p. 214-216°C; IR (KBr, v max): 3122 (NH), 3020 (aromatic C-H), 1640 (C=N), 1442 (C-N), 1440 (NO$_2$), 1275 (OCH$_3$); 1H NMR (CDCl$_3$+DMSO-$d_6$): δ 3.92 (s, 3H, OCH$_3$), 7.15-7.34 (m, 4H, H$_{arom}$), 7.42-7.60 (m, 3H, H$_{arom}$), 7.60 (s, 1H, N=CH), 7.97 (d, 1H, H$_{arom}$), 8.35 (bs, 1H, NH); MS: m/z (M') 356.5. Anal. Calcd. for C$_{19}$H$_{13}$O$_3$N$_7$: C, 63.95; H, 3.64; N, 17.95; O, 9.95. Found: C, 63.91; H, 3.60; N, 17.91; O, 9.90%.

9-(3''-methyl)-5-benzyl-3H,13aH-quinolin-3,2-f\[1,2,4\]triazolo[4,3-b][1,2,4]triazepine 8f

This compound was obtained as pale yellow shining solid (ethyl acetate), m.p. 215-217°C; IR (KBr, v max): 3110 (NH), 3022 (aromatic C-H), 2810 (C-H), 1510 (NO$_2$), 1421 cm$^{-1}$ (C-N); 1H NMR (CDCl$_3$+DMSO-$d_6$): δ 2.34 (s, 3H, CH$_3$), 7.11-7.34 (m, 4H, H$_{arom}$), 7.22 (m, 1H, H$_{arom}$), 7.60-7.72 (m, 1H, H$_{arom}$ and s, 1H buried N=CH), 7.75-7.86 (m, 3H, H$_{arom}$), 8.01 (d, 1H, H$_{arom}$), 8.36 (bs, 1H, NH); MS: m/z (M') 375. Anal. Calcd. for C$_{19}$H$_{13}$N$_{6}$Cl: C, 68.78; H, 4.45; N, 26.75. Found: C, 68.74; H, 4.41; N, 26.76%.

9-(3''-methoxy)-5-(4''-nitrophenyl)-3H,13aH-quinolin-3,2-f\[1,2,4\]triazolo[4,3-b][1,2,4]triazepine 8h

This compound was obtained as pale yellow shining solid (ethyl acetate), m.p. 220-222°C; IR (KBr, v max): 3100 (NH), 3020 (aromatic C-H), 1640 (C=N), 1442 (C-N), 1440 (NO$_2$), 1275 (OCH$_3$); 1H NMR (CDCl$_3$+DMSO-$d_6$): δ 3.92 (s, 3H, OCH$_3$), 7.22 (m, 1H, H$_{arom}$), 7.60-7.72 (m, 1H, H$_{arom}$ and s, 1H buried N=CH), 7.75-7.86 (m, 3H, H$_{arom}$), 7.99 (d, 1H, H$_{arom}$), 8.21-8.24 (m, 2H, H$_{arom}$), 8.36 (bs, 1H, H$_{arom}$); MS: m/z (M') 387. Anal. Calcd. for C$_{19}$H$_{10}$O$_2$N$_7$: C, 58.91; H, 3.35; N, 25.32; O, 12.40. Found: C, 58.87; H, 3.32; N, 25.31. O, 12.35%.

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References