

# Novel Synthesis of Tetrazolopyrrolopyrimidines as Antifungal Agents

Shah Rina<sup>1</sup>, Shah Nirmal M<sup>2</sup>, Ramani Vivek<sup>2</sup>

Department of Chemistry, M. G. Science Institute, Navrangpura, Ahmedabad 380 009, India

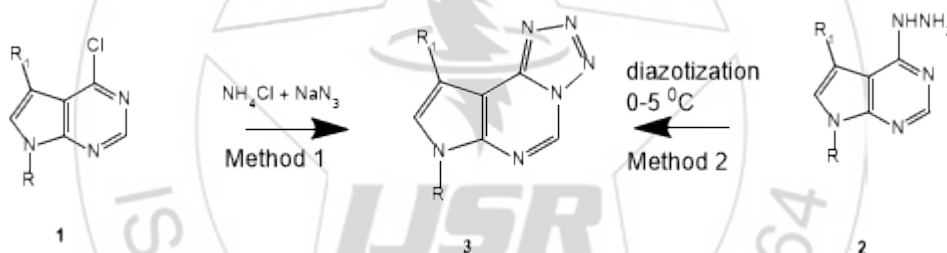
**Abstract:** Synthesis of Tetrazolopyrrolopyrimidines 7, 9-disubstituted 7H-pyrrolo [4, 3-e] tetrazolo [1, 5-c] pyrimidines 2 of biological and synthetic interests has been conducted via novel route from 5, 7-disubstituted 3-amino-4-iminopyrrolo [2, 3-d] pyrimidines 1 by diazotization using sodium nitrite in acetic acid as nitrogen donor moiety.

**Keywords:** Tetrazolopyrrolopyrimidines, pyrrolo [2, 3-d] pyrimidines, diazotization, antifungal agents

## 1. Introduction

Various pharmaceutical activities attributed to tetrazolopyrimidine derivatives such as antibacterial [1], analgesic [2] antiinflammatory [2] anticonvulsant [3] antiulcer [4] antiallergic [4] anticancer [5, 6] antifolate [7] antihypertensive [8] antimalarial [9] antitumor [10] and antifungal activities [11]. Moreover tetrazolopyrimidines have been identified as latent amine functionality generating pyrimidineamines of biological interest, [1, 12, 13, 14]. Earlier we have reported the synthesis of

Tetrazolopyrrolopyrimidines via two different classical methods [1, 11, 12]. The first synthetic method involved the nucleophilic displacement such as azidolysis of 4-chloropyrrolopyrimidine using sodium azide and ammonium chloride in DMSO under heating condition and the second method involved the diazotization of 4-hydrazinopyrazolopyrimidines which were obtained by hydrazinolysis of 4-chloropyrazolopyrimidines [1, 12, 13]. Baraldiet al reported the synthesis of pyrazolo [4, 3-e] tetrazolo [1, 5-c] pyrimidines from 3-amino-4-iminopyrazolopyrimidine [15].



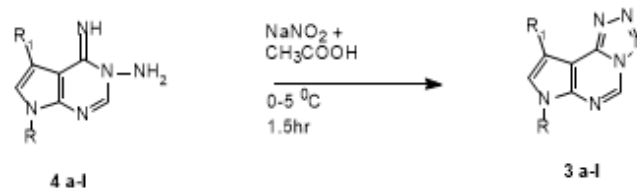
So far synthesis of pyrrolo [4, 3-e] tetrazolo [1, 5-c] pyrimidines have not been attempted from 3-amino-4-iminopyrrolo [1, 5-c] pyrimidines. In continuation of our interest in the synthesis of tetrazolopyrrolopyrimidines we would like to introduce novel route for the construction of tetrazole ring onto the existing pyrrolopyrimidine ring, forming 7, 9-disubstituted 7H-pyrrolo [4, 3-e] tetrazolo [1, 5-c] pyrimidines 3 by diazotization of 5, 7-disubstituted 3-amino-4-iminopyrrolo [2, 3-d] pyrimidines 4.

5, 7-Disubstituted 3-amino-4-iminopyrrolo [2, 3-d] pyrimidines 4, prepared from 1-substituted 2-amino-3-cyanopyrroles by the treatment with triethyl orthoformate, followed by hydrazine hydrate, have been reacted with sodium nitrite in acetic acid at 0-5 °C under stirring condition for 1.5 hour to give required 7, 9-disubstituted 7H-pyrrolo [4, 3-e] tetrazolo [1, 5-c] pyrimidines 3.

## 2. Result and Discussion

Reaction of sodium nitrite with acetic acid generating nitrous acid serves as nitrogen donor moiety that first diazotized respective amines in turn found to undergo cyclization to form target tetrazolopyrrolopyrimidines 3. This path has been found to be step reducing and yield enhancing (81-87%) compared to other path. The formation of

tetrazolopyrrolopyrimidines 3 has been done on the basis of mixed melting point and spectral analysis. In IR spectra of compound 3 absence of bands at 3400-3150 and at 1648-1630  $\text{cm}^{-1}$  for the respective stretching and bending vibrations of NH in imino and amino functionalities and also at 2100  $\text{cm}^{-1}$  in  $\text{CHCl}_3$  supported the formation of tetrazole ring formation [16] and not the azide formation.  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ) of compound 3 gave signals at  $\delta$  8.3-7.1 in the form of multiplet responsible for aromatic protons. Mass spectrum of compound 3a gave molecular ion peak at 312. The fragmentation at  $m/z$  284, 257 and 256 obtained because of consecutive elimination of nitrogen and hydrogen cyanide or due to successive removal of two nitrogen molecules [2, 12, 16].



3, 4	R	R <sub>1</sub>	3, 4	R	R <sub>1</sub>
a	C <sub>6</sub> H <sub>5</sub>	C <sub>6</sub> H <sub>5</sub>	g	4-OCH <sub>3</sub> C <sub>6</sub> H <sub>4</sub>	4-FC <sub>6</sub> H <sub>4</sub>
b	C <sub>6</sub> H <sub>5</sub>	4-ClC <sub>6</sub> H <sub>4</sub>	h	4-OCH <sub>3</sub> C <sub>6</sub> H <sub>4</sub>	3-Cl, 4-FC <sub>6</sub> H <sub>3</sub>
c	C <sub>6</sub> H <sub>5</sub>	4-FC <sub>6</sub> H <sub>4</sub>	i	4-ClC <sub>6</sub> H <sub>4</sub>	C <sub>6</sub> H <sub>5</sub>
d	C <sub>6</sub> H <sub>5</sub>	3-Cl, 4-FC <sub>6</sub> H <sub>3</sub>	j	4-ClC <sub>6</sub> H <sub>4</sub>	4-ClC <sub>6</sub> H <sub>4</sub>
e	4-OCH <sub>3</sub> C <sub>6</sub> H <sub>4</sub>	C <sub>6</sub> H <sub>5</sub>	k	4-ClC <sub>6</sub> H <sub>4</sub>	4-FC <sub>6</sub> H <sub>4</sub>
f	4-OCH <sub>3</sub> C <sub>6</sub> H <sub>4</sub>	4-OCH <sub>3</sub> C <sub>6</sub> H <sub>4</sub>	l	4-ClC <sub>6</sub> H <sub>4</sub>	3-Cl, 4-FC <sub>6</sub> H <sub>3</sub>

### Antifungal activity

The antifungal activity of the synthesized compounds **3** was investigated *invitro* against two pathogenic representative such as *Candida albicans* (ATCCC 10231) and *Aspergillus niger* (NCCS1196) using the disc diffusion sensitivity method at 2 μg/mL in DMSO using dextrose agar media. The medium of petridish was prepared by following the method described by W. Bauer [17] and the solution of test organisms have been prepared by dissolving plant extract. Then 0.5 mL of the culture to be tested organisms was uniformly spreaded over the media. With the help of 6mm sterile borer the agar inoculation cups were scooped out. To each cup 2 μg/mL of test solution in DMSO was filled. Chloromeatazole at the same concentration was served as reference purpose and DMSO as negative control. The fungi sensitive Clortrimazole was served as reference purpose. The antifungal activity was measured as a diameter of zone of inhibition in mm. All compound **3** showed moderate to good activity among compound **3j** showed maximum activity comparable with Clortrimazole.

**Table:** Antifungal activity of 7, 9-disubstituted 7H-pyrrolo [4, 3-e] tetrazolo [1, 5-c] pyrimidines **3 a-i**

Compound <b>3</b>	<i>Candida albicans</i> (ATCCC 10231) Zone of inhibition in mm	<i>Aspergillus niger</i> (NCCS1196) Zone of inhibition in mm
a	17	16
b	22	24
c	15	19
d	21	22
e	17	18
f	15	16
g	19	20
h	18	22
i	23	22
j	29	28
k	20	21
l	23	22
Clortrimazole	30	29
DMSO	-	-

### 3. Experimental Section

Melting points were determined by electro thermal method in an open capillary tube and are uncorrected. The IR spectra were recorded in cm<sup>-1</sup> for I pellets on Bruker Alpha spectrophotometer. <sup>1</sup>H NMR spectra were taken on Varian 300 MHz spectrometer using CDCl<sub>3</sub> as a solvent and TMS as the internal reference standard. The chemical shifts are expressed in δ ppm. Mass spectra were recorded on LKB 9000 mass spectrometer. The purity of the compounds was confirmed by TLC using Silica G and the spots were detected by exposing them in iodine vapor.

### General Procedure for the synthesis of 7, 9-disubstituted 7H-pyrrolo [4, 3-e] tetrazolo [1, 5-c] pyrimidines:

To the ice cold solution [0-5 °C] of compound **4** [12, 18, 19] (0.001 mol) in acetic acid (10 mL) was added an ice-cold solution of sodium nitrite (0.21 g/5 mL H<sub>2</sub>O, 0.016 mol) under stirring condition over a period of 5 minutes. Then the stirring was continued for 1.5 h under same conditions. The reaction mixture was poured onto the crushed ice and the solid separated was filtered, washed with water, dried and recrystallized from dioxane to afford compound **3a** as white to off white crystals.

**7, 9-Diphenyltetrazolo [1, 5-c] pyrrolo-7H- [3, 2-e] pyrimidine 3a:** yield: 84 %, mp: 217-18 °C, IR (KBr): 1608, 1504 (C=C, C=N ring) cm<sup>-1</sup>, <sup>1</sup>H NMR (CDCl<sub>3</sub> d<sup>6</sup>): δ 8.0-7.1 (m, 12H, ArH), MS: 312 m/z (M<sup>+</sup>). Anal. Calcd for C<sub>19</sub>H<sub>12</sub>N<sub>6</sub>: C 69.22, H 3.87, N 23.71 %. [Found: C 69.01, H 3.70, N 23.83 %].

**7-(4-Fluorophenyl)-9-phenyltetrazolo [1, 5-c] -7H-pyrrolo [3, 2-e] pyrimidine 3b:** yield: 81 %, mp: 218-19 °C, IR (KBr): 1604, 1516 (C=C, C=N ring) cm<sup>-1</sup>, <sup>1</sup>H NMR (CDCl<sub>3</sub> d<sup>6</sup>): δ 8.01-7.21 (m, 11H, ArH), MS: 330 m/z (M<sup>+</sup>). Anal. Calcd for C<sub>18</sub>H<sub>11</sub>FN<sub>6</sub>: C 65.42, H 3.36, N 25.45 %. [Found: C 65.46, H 3.27, N 25.09 %].

**7-(4-Chlorophenyl)-9-phenyl-7H-tetrazolo [1, 5-c] -7H-pyrrolo [3, 2-e] pyrimidine 3c:** yield: 85%, mp: 218-20 °C, IR (KBr): 1612, 1493 (C=C, C=N ring) cm<sup>-1</sup>, <sup>1</sup>H NMR (CDCl<sub>3</sub> d<sup>6</sup>): δ 8.11-7.29 (m, 11H, ArH), MS: 347 m/z (M<sup>+</sup>). Anal. Calcd for C<sub>18</sub>H<sub>11</sub>ClN<sub>6</sub>: C 62.34, H 3.20, N 24.24 %. [Found: C 62.46, H 3.27, N 24.56 %].

**7-(3-Chloro-4-fluorophenyl)-9-phenyl-7H-tetrazolo [1, 5-c] -7H-pyrrolo [3, 2-e] pyrimidine 3d:** yield: 82%, mp: 214-15 °C, IR (KBr): 1604, 1492 (C=C, C=N ring) cm<sup>-1</sup>, <sup>1</sup>H NMR (CDCl<sub>3</sub> d<sup>6</sup>): δ 8.1-7.2 (m, 10H, ArH), MS: 365 m/z (M<sup>+</sup>). Anal. Calcd for C<sub>18</sub>H<sub>10</sub>ClFN<sub>6</sub>: C 59.27, H 2.76, N 23.04 %. [Found: C 59.53, H 2.51, N 22.87 %].

**7-Phenyl-9-(4-methoxyphenyl)tetrazolo [1, 5-c] -7H-pyrrolo [3, 2-e] pyrimidine 3e:** yield: 82 %, mp: 217-18 °C, IR (KBr): 1604, 1516 (C=C, C=N ring) cm<sup>-1</sup>, <sup>1</sup>H NMR (CDCl<sub>3</sub> d<sup>6</sup>): δ 8.0-7.15 (m, 11H, ArH), 3.9 (s, 3H, OCH<sub>3</sub>), MS: 342 m/z (M<sup>+</sup>). Anal. Calcd for C<sub>19</sub>H<sub>14</sub>N<sub>6</sub>O: C 66.65, H 4.12, N 24.55 %. [ Found: C 66.45, H 4.28, N 24.77 %].

**7, 9-Di(4-methoxyphenyl)tetrazolo [1, 5-c] -7H-pyrrolo [3, 2-e] pyrimidine 3f:** yield: 83 %, mp: 238-39 °C, IR (KBr): 1596, 1500 (C=C, C=N ring) cm<sup>-1</sup>, <sup>1</sup>H NMR (CDCl<sub>3</sub> d<sup>6</sup>): δ 8.1-7.3 (m, 10H, ArH), 3.95 (s, 6H, OCH<sub>3</sub>), MS: 372 m/z (M<sup>+</sup>). Anal. Calcd for C<sub>20</sub>H<sub>16</sub>FN<sub>6</sub>O<sub>2</sub>: C 64.50, H 4.33, N 22.57 %. Found: C 64.39, H 4.28, N 22.32 %.

**7-(4-Fluorophenyl)-9-(4-methoxyphenyl)tetrazolo [1, 5-c] -7H-pyrrolo [3, 2-e] pyrimidine3g:** yield: 82 %, mp: 247-48°C, ir (KBr):1600, 1504(C=C, C=N ring) cm<sup>-1</sup>, <sup>1</sup>H nmr (CDCl<sub>3</sub> d<sup>6</sup>): δ 8.1-7.31 (m, 10H, ArH), 4.01 (s, 3H, OCH<sub>3</sub>). MS: 360m/z (M<sup>+</sup>). Anal. Calcd for C<sub>19</sub>H<sub>13</sub>FN<sub>6</sub>O: C 63.33, H 3.64, N 23.33 %. Found: C 63.10, H 3.44, N 23.43 %.

**7-(3-Chloro-4-fluorophenyl)-9-(4-methoxyphenyl)tetrazolo [1, 5-c] -7H-pyrrolo [3, 2-e] pyrimidine3h:** yield: 82 %, mp: 220-21 °C, ir (KBr):1608, 1504(C=C, C=N ring) cm<sup>-1</sup>, <sup>1</sup>H nmr (CDCl<sub>3</sub> d<sup>6</sup>): δ 8.3-7.3 (m, 9H, ArH), 3.95 (s, 3H, OCH<sub>3</sub>), 383 m/z (M<sup>+</sup>). Anal. Calcd for C<sub>19</sub>H<sub>12</sub>ClFN<sub>6</sub>O: C 59.61, H 3.16, N 21.96 %. Found: C 59.44, H 3.24, N 21.61 %.

**7-(4-Phenyl)-9-(4-Chlorophenyl)-7H-tetrazolo [1, 5-c] -7H-pyrrolo [3, 2-e] pyrimidine3i:** yield: 85 %, mp: 235-36°C, ir (KBr):1608, 1508(C=C, C=N ring) cm<sup>-1</sup>, <sup>1</sup>H nmr (CDCl<sub>3</sub> d<sup>6</sup>): δ 8.0-7.2 (m, 11H, ArH), 347 m/z (M<sup>+</sup>) Anal. Calcd for C<sub>18</sub>H<sub>10</sub>ClN<sub>6</sub>: C 62.35, H 3.21, N 24.23 %. Found: C 62.46, H 3.27, N 24.56 %.

**7-(4-Fluorophenyl)-9-(4-chlorophenyl)-7H-tetrazolo [1, 5-c] -7H-pyrrolo [3, 2-e] pyrimidine3j:** yield: 84 %, mp: 227-28 °C, ir (KBr):1612, 1497(C=C, C=N ring) cm<sup>-1</sup>, <sup>1</sup>H nmr (CDCl<sub>3</sub> d<sup>6</sup>): δ 8.25-7.3 (m, 10H, ArH), 365 m/z (M<sup>+</sup>) Anal. Calcd for C<sub>18</sub>H<sub>12</sub>ClFN<sub>6</sub>: C 59.27, H 2.76, N 24.24 %. [Found: C 59.07, H 2.48, N 24.46 %] .

**7, 9-Di(4-chlorophenyl)-7H-tetrazolo [1, 5-c] -7H-pyrrolo [3, 2-e] pyrimidine3k:** yield: 87 % mp: 225-26°C, ir (KBr):1607, 1502(C=C, C=N ring) cm<sup>-1</sup>, <sup>1</sup>H nmr (CDCl<sub>3</sub> d<sup>6</sup>): δ 8.2-7.1 (m, 11H, ArH), MS: 381 m/z (M<sup>+</sup>). Anal. Calcd for C<sub>18</sub>H<sub>10</sub>Cl<sub>2</sub>N<sub>6</sub>: C 56.70, 2.64, N 22.05 %. [Found: C 56.49, H 2.39, N 21.88 %] .

#### 4. Acknowledgement

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#### 5. Conclusion

Novel route for the synthesis of bioactive tetrazolo [1, 5-c] pyrrolo [3, 2-e] pyrimidines has been established from 3-amino-4-iminopyrrolo [2, 3-d] pyrimidines and studied their antifungal activity.

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