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Novel Synthesis of Tetrazolopyrrolopyrimidines as Antifungal Agents

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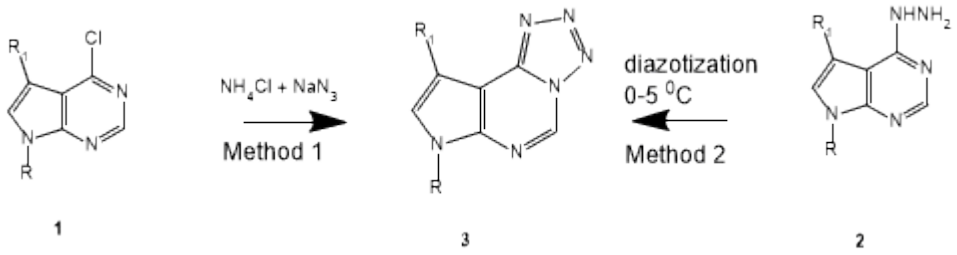
Abstract: ***Synthesis of Tetrazolopyrrolopyrimidines 7, 9-disubstituted7H-pyrrolo [4, 3-e] tetrazolo [1, 5-c] pyrimidines 2 of biological and synthetic interests has been conducted via novel route from 5, 7-disubstituted 3-amino-4-iminopyrrolo [2, 3-d] pyrimidines 1by diazotization using sodium nitrite in acetic acid as nitrogen donor moiety.***

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

1. Introduction

Various pharmaceutical activities attributted 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 syntheticmethod involved the nuclophilic displacement such as azidolysis of 4-chloropyrrolopyrimidine using sodium azide and ammonium chloride in DMSO under heating conditionand the second methodinvolved 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-iminopyrrazolopyrimidine [15] .



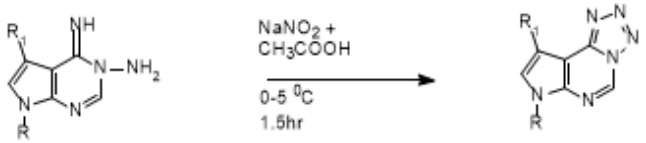
1 3 2

So far synthesis of pyrrolo [4, 3-e] tetrazolo [1, 5-c] pyrimidines have not been attempted from 3-amino-4-aminopyrrolo [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-disubstituted7H-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 ^{0}C under stirring condition for 1.5 hour to give required 7, 9-disubstituted7H-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 moietythat first diazotized respective amines inturn 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 cm^{-1} for the respective stretching and bending vibrations of NH in imino and amino functionalities and also at 2100 cm^{-1} in $CHCl_3$supported the formation of tetrazole ring formation [16] and not the azide formation. H^1 NMR($CDCl_3$)of compound **3** gave signals atδ 8-3-7.1in the form of multiplet responsible for aromatic protons. Mass spectram of compound **3a** gave molcular ion peak at 312. The fragmentation at m/z 284, 257 and 256 obtained because of consequitive elimination of nitrogen and hydrogen cyanide or due to successive removal of two nitrogen molecules [2, 12, 16] .



4 a-l 3 a-l

3, 4	R	R_1	3, 4	R	R_1
a	C_6H_5	C_6H_5	g	4-$OCH_3C_6H_4$	4-FC_6H_4
b	C_6H_5	4-ClC_6H_4	h	4-$OCH_3C_6H_4$	3-Cl, 4-FC_6H_3
c	C_6H_5	4-FC_6H_4	i	4-ClC_6H_4	C_6H_5
d	C_6H_5	3-Cl, 4-FC_6H_3	j	4-ClC_6H_4	4-ClC_6H_4
e	4-$OCH_3C_6H_4$	C_6H_5	k	4-ClC_6H_4	4-FC_6H_4
f	4-$OCH_3C_6H_4$	4-$OCH_3C_6H_4$	l	4-ClC_6H_4	3-Cl, 4-FC_6H_3

Antifungal activity

The antifungal activity of the synthesized compounds **3** was investigated *invitro*against two pathogenic representative such as *Candida albicans*(ATCCC 10231) and *Asppergillus niger*(NCCS1196) using the disc diffusion sensitivity method at 2µg/mLin DMSO using dextrose agar media, . The medium of petridishwas 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 2µg/mL of test solutionin DMSO was filled.Chlormeatazoleat the same concentrationwas served as reference purposeand DMSO as negetive 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 ompound **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 3	Candida albicans (ATCCC 10231) Zone of inhibtion in mm	Asppergillus niger(NCCS1196) Zone of inhibtion 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^{-1} for I pellets on BrukerAlpha spectrophotometer. ^1H NMR spectra were taken on Varian 300 MHz spectrometer using $CDCl_3$ as a solvent and TMS as the internal reference standard. The chemical shifts are expressed in δppm. 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 detectedby exposing them in iodine vapor.

General Procedure for the synthesis of 7, 9-disubstituted7H-pyrrolo [4, 3-e] tetrazolo [1, 5-c] pyrimidines:

To the ice cold solution [0-5 ^0C] 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_2O, 0.016 mol) under stirring conditon over a period of5 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 seperated was filtered, washed with water, dried and recrystallized from dioxane to afford compound **3**as white to off white crystals.

7, 9-Diphenyltetrazolo [1, 5-*c*] pyrrolo-7*H*- [3, 2-*e*] pyrimidine3a: yield: 84 %, mp: 217-18^0C, ir (KBr):1608, 1504 (C=C, C=N ring) cm^{-1}, ^1H nmr ($CDCl_3$ d^6): δ 8.0-7.1 (m, 12H, ArH), MS: 312m/z (M^+). Anal. Calcd for $C_{19}H_{12}N_6$: 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*] -7*H*-pyrrolo [3, 2-*e*] pyrimidine3b: yield: 81 %, mp: 218-19 ^0C, ir (KBr):1604, 1516 (C=C, C=N ring) cm^{-1}, ^1H nmr ($CDCl_3$ d^6): δ 8.01-7.21 (m, 11H, ArH), MS: 330 m/z(M^+). Anal. Calcd for $C_{18}H_{11}FN_6$: C 65.42, H 3.36, N 25.45 %. [Found: C 65.46, H 3.27, N 25.09 %] .

7-(4-Chorophenyl)-9-phenyl-7*H*-tetrazolo [1, 5-*c*] -7*H*-pyrrolo [3, 2-*e*] pyrimidine3c: yield: 85%, mp: 218-20 ^0C, ir (KBr):1612, 1493 (C=C, C=N ring) cm^{-1}, ^1H nmr ($CDCl_3$ d^6): δ 8.11-7.29 (m, 11H, ArH), MS: 347 m/z(M^+). Anal. Calcd for $C_{18}H_{11}ClN_6$: C 62.34, H 3.20, N 24.24 %. [Found: C 62.46, H 3.27, N 24.56 %] .

7-(3-Choro-4-fluorophenyl)-9-phenyl-7*H*-tetrazolo [1, 5-*c*] -7*H*-pyrrolo [3, 2-pyrimidine3d: yield: 82%, mp: 214-15^0C, ir(KBr):1604, 1492 (C=C, C=N ring) cm^{-1}, ^1H nmr ($CDCl_3$ d^6): δ 8.1-7.2 (m, 10H, ArH), MS: 365m/z (M^+). Anal. Calcd for $C_{18}H_{10}ClFN_6$: 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*] -7*H*-pyrrolo [3, 2-*e*] pyrimidine3e: yield: 82 %, mp: 217-18 ^0C, ir (KBr):1604, 1516(C=C, C=N ring) cm^{-1}, ^1H nmr ($CDCl_3$ d^6): δ 8.0-7.15 (m, 11H, ArH), 3.9 (s, 3H, OCH_3), MS: 342 m/z (M^+). Anal. Calcd for $C_{19}H_{14}N_6O$: 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*] -7*H*-pyrrolo [3, 2-*e*] pyrimidine3f: yield: 83 %, mp: 238-39 ^0C, ir (KBr):1596, 1500 (C=C, C=N ring) cm^{-1}, ^1H nmr ($CDCl_3$ d^6): δ 8.1-7.3 (m, 10H, ArH), 3.95 (s, 6H, OCH_3), MS: 372 m/z(M^+). Anal. Calcd for $C_{20}H_{16}FN_6O_2$: C 64.50, H 4.33, N 22.57 %. Found: C 64.39, H 4.28, N 22.32 %.

7-(4-Fluoropheny)l-9-(4-methoxyphenyl)tetrazolo [1, 5-*c*] -7*H*-pyrrolo [3, 2-*e*] pyrimidine3g: yield: 82 %, mp: 247-48^{0}C, ir (KBr):1600, 1504(C=C, C=N ring) cm^{-1}, ^{1}H nmr ($CDCl_3$ d^6): δ 8.1-7.31 (m, 10H, ArH), 4.01 (s, 3H, OCH_3). MS: 360m/z (M^+). Anal. Calcd for $C_{19}H_{13}FN_6O$: 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*] -7*H*-pyrrolo [3, 2-*e*] pyrimidine3h: yield: 82 %, mp: 220-21 ^{0}C, ir (KBr):1608, 1504(C=C, C=N ring) cm^{-1}, ^{1}H nmr ($CDCl_3$ d^6): δ 8.3-7.3 (m, 9H, ArH), 3.95 (s, 3H, OCH_3), 383 m/z (M^+). Anal. Calcd for $C_{19}H_{12}ClFN_6O$**:** 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)-7*H*-tetrazolo [1, 5-*c*] -7*H*-pyrrolo [3, 2-*e*] pyrimidine3i: yield: 85 %, mp: 235-36^{0}C, ir (KBr):1608, 1508(C=C, C=N ring) cm^{-1}, ^{1}H nmr ($CDCl_3$ d^6): δ 8.0-7.2 (m, 11H, ArH), 347 m/z (M^+) Anal. Calcd for $C_{18}H_{10}ClN_6$**:** 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)-7*H*-tetrazolo [1, 5-*c*] -7*H*-pyrrolo [3, 2-*e*] pyrimidine3j: yield: 84 %, mp: 227-28 ^{0}C, ir (KBr):1612, 1497(C=C, C=N ring) cm^{-1}, ^{1}H nmr ($CDCl_3$ d^6): δ 8.25-7.3 (m, 10H, ArH), 365 m/z (M^+) Anal. Calcd for $C_{18}H_{12}ClFN_6$**:** C 59.27, H 2.76, N 24.24 %. [Found: C 59.07, H2.48, N 24.46 %] .

7, 9-Di(4-chlorophenyl)-7*H*-tetrazolo [1, 5-*c*] -7*H*-pyrrolo [3, 2-*e*] pyrimidine3k: yield: 87 % mp: 225-26^{0}C, ir (KBr):1607, 1502(C=C, C=N ring) cm^{-1}, ^{1}H nmr ($CDCl_3$ d^6): δ 8.2-7.1 (m, 11H, ArH), MS: 381 m/z (M^+). Anal. Calcd for $C_{18}H_{10}Cl_2N_6$: 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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