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Note

Synthesis and antifungal activity of 4-substituted-3,7-dimethyl-pyrazolo[3,4-*e*][1,2,4]triazine

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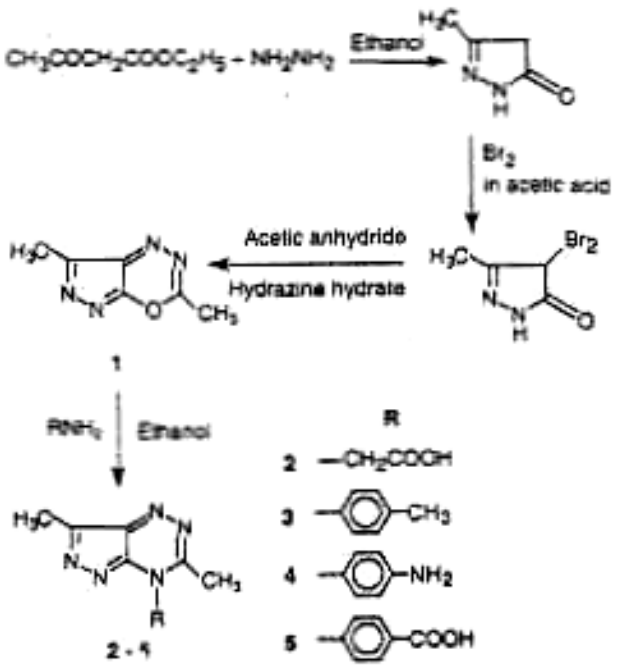
A convenient synthesis and antifungal activity of four compounds namely 2-(3,7-dimethyl-4*H*-pyrazolo[3,4-*e*][1,2,4]triazin-4-yl)acetic acid **2**, 3,7-dimethyl-4-(4-methylphenyl-pyrazolo[3,4-*e*][1,2,4]triazine **3**, 4-(3,7-dimethyl-4*H*-pyrazolo[3,4-*e*][1,2,4]triazin-4-yl)aniline **4** 4-(3,7-dimethyl-4*H*-pyrazolo[3,4-*e*][1,2,4]triazin-4-yl)benzoic acid **5** have been reported.

The chemistry of condensed heterocyclic systems especially containing triazine moiety has been largely investigated[1-9] because they are effective in many pharmacological areas. Their derivatives possess a great number of biological activities such as antidepressants[10], antitumor[11], antiparasitic[12], antifungal[13], bactericidal[14,15], antiallergic[16], antiinflammatory[17], antiviral[18,19] and antihypertensive effects[20,21]. Several compounds containing 1,2,4-triazine moiety are known for their wide applications in agriculture as herbicides and plant protecting agents[22].

3,7-Dimethylpyrazolo[4,3-*e*]oxadiazine[23] **1** the key intermediate in the synthesis of *N*-substituted-3,7-dimethylpyrazolo[3,4-*e*][1,2,4]triazine has been synthesized by the action of ethyl acetoacetate and hydrazine hydrate in absolute ethanol and produced a good yield of white coloured 3-methyl pyrazol-5-one. This pyrazole derivative was brominated at position-4 by using bromine in acetic acid as brominating agent to give 4,4-dibromo-3-methylpyrazol-5-one. This compound was then cyclized with acetic anhydride and hydrazine hydrate while refluxing for 6 hr to give **1** in 80% yield. Compounds **2 – 5** were obtained by the condensation of **1** with glycine, *p*-toluedine, *p*-phenylenediamine and 4-amino benzoic acid, respectively (**Scheme I**). Characterization of the compounds has been carried out by their mass and IR spectra.

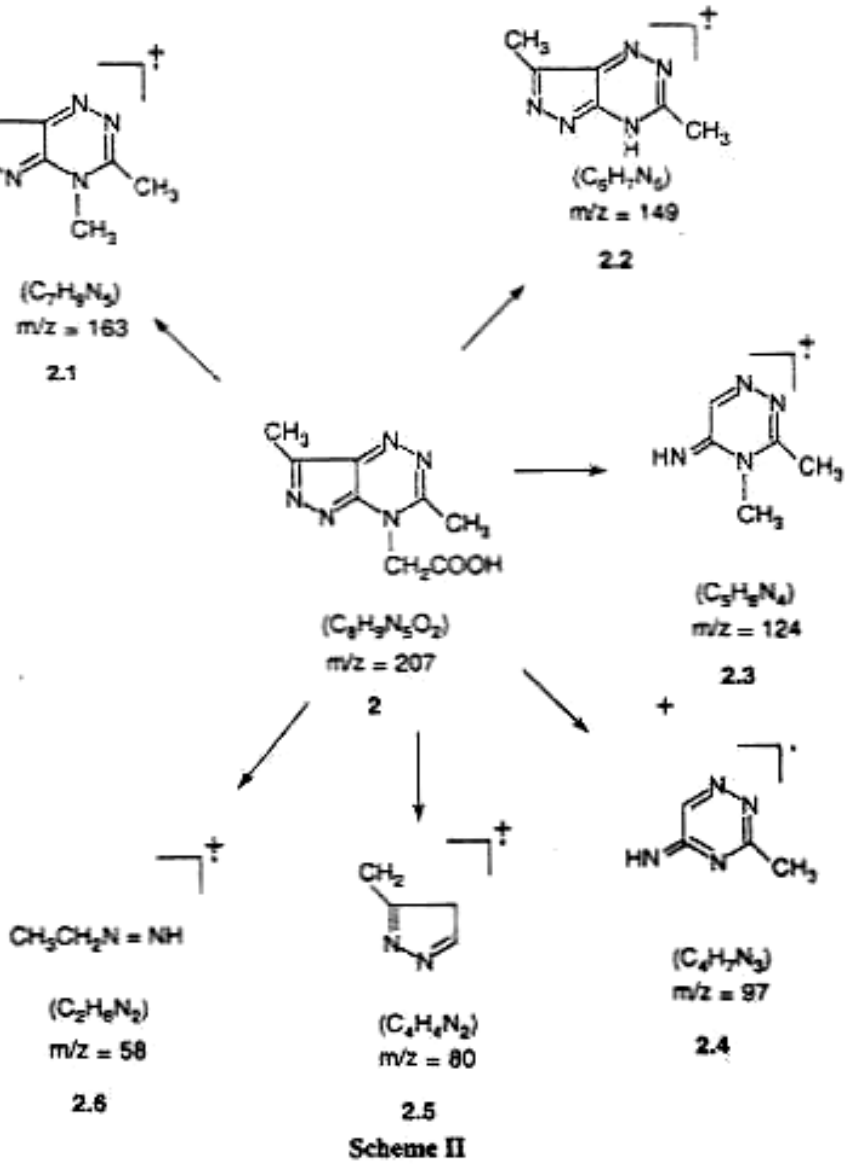


Scheme I

The common feature in the mass spectra of all the compounds is the appearance of the base peak at m/z 149. This fragment was due to the unsubstituted pyrazolo[3,4-*e*](1,2,4)triazine nucleus. The mass spectral analysis of **2** is given in **Scheme II**. The molecular ion peak was observed at m/z 207. Other major peaks were at m/z 163, 149, 124, 97, 80, 58 and 43. The first fragment at m/z 163 was due to the decarboxylation of the substituent at position 4. The peak at m/z 149 was assigned to the radical ion generated by the removal of the group at position 4. This was the base peak.

Antifungal activity

The antifungal activity of the compounds was carried out by the Poison food Technique. The compounds used were first tested in Potato Dextrose broth in concentration of 1:10 (10 mg/mL), 1:20 (5 mg/mL), 1:40 (2.5 mg/mL) and 1:100 (1 mg/mL) from the stock by dissolving 100 mg sample in 1 mL water. Results were noted after 3 days. Sterile distilled water was used for diluting the samples. Fungi used were *Alternaria sp, Aspergillus arvensis, Fusa*



Scheme II

rium moniliforme and *Helminthosporium orzae* in concentration of 0.100 mL/replication ($6\text{-}7\times10^4$ spores/ml). Percentage inhibition over control was calculated by inoculating 9 mm disc of fungus from 5-day old cultures on the poisoned agar medium poured in petriplates. Results were noted after 3 and 5 days. *Strepto-Penicillin* was added in broth and agar medium to prevent bacterial growth. Four replications of each treatment were maintained and the percentage inhibitions were calculated. The results are reported in **Table I**. Compounds **2** and **5** were more active at concentrations of 1:10 (10 mg/mL). The percentage inhibition of these compounds against various fungal species is given in **Table II**.

Experimental Section

2-(3,7-Dimethyl-4*H*-pyrazolo[3,4-*e*][1,2,4]triazin-4-yl)acetic acid 2. Compound **2** was synthesized by refluxing the mixture of **1** (4.5 g, 0.03 mole) and glycine (2.25 g, 0.03 mole) in ethanol (20 mL) for 3 hr on a water-bath. The reaction mixture was concentrated to precipitate out the desired compound. The product was recrystallized from methanol, yield 5.4 g (87%), mp 160°C; IR (KBr): 1324(C-N stretching), 1682(C=O stretching), 3512 cm^{-1} (O-H stretching); M/S (m/z): 207 (M^+), 149 ($C_6H_7N_5$), 124 ($C_5H_8N_4$), 97 ($C_4H_7N_3$), 80 ($C_4H_4N_2$), 58 ($C_2H_6N_2$) 43 (C_2H_5N). Anal: Calc. for $C_8H_9N_5O_2$: C, 46.37; H, 4.34; N, 33.81. Found: C, 45.80; H, 4.85; N, 32.98%.

Table I — Antifungal activity

Compd	Concentration			
	1:10 (10 mg/mL)	1:20 (5 mg/mL)	1:40 (2.5 mg/mL)	1:100 (1 mg/mL)
2	-	+	++	++
3	++	++	++	++
4	++	++	++	++
5	-	+	++	++
Control	++	++	++	++

\- no growth of fungal mycelium
\+ growth of fungal mycelium
++ growth of fungal mycelium with sporulation

Table II — Percentage decrease in inhibitory zone over control

Fungi	Compounds			
	2		3	
	3 days	5 days	3 days	5 days
Alternaria sp	38.4	28.0	5.1	1.2
Aspergillus awamori	36.4	32.2	63.6	54.3
Fusarium moniliforme	54.1	54.4	58.6	60.0
Helminthosporium oryzae	10.3	13.2	15.5	25.3

3,7-Dimethyl-4-(4-methylphenyl)pyrazolo[3,4-*e*][1,2,4]triazine 3. Compound 3 was synthesized by condensing a mixture of **1** (4.5 g, 0.03 mole) and *p*-toluidine (3.2 g, 0.03 mole) in ethanol (20 mL) under reflux conditions for 6 hr on a water-bath. The reaction mixture was concentrated to precipitate out the desired compound. The product was recrystallized from methanol, yield 5.2g (72%). mp 130°C. IR (KBr) cm^{-1} 1392 (C-N stretching), 2966 (C-H stretching). M/S (m/z): 239 (M^+), 225 ($C_{12}H_{11}N_3$), 200 ($C_{11}H_{12}N_4$), 186 ($C_{10}H_{10}N_4$), 149 ($C_8H_7N_3$), 97 ($C_4H_7N_3$), 92 (C_7H_8), 80 ($C_4H_4N_2$), 58 ($C_2H_6N_2$), 43 (C_2H_5N). Anal. Calc. for $C_{13}H_{13}N_5$: C, 65.27; H, 5.44; N, 29.28. Found: C, 65.30; H, 5.88; N, 29.12%.

4-(3,7-Dimethyl-4*H*-pyrazolo[3,4-*e*][1,2,4]triazin-4-yl)aniline 4. Compound 4 was synthesized by condensing equimolar amount of **1** (4.5 g, 0.03 mole) and *p*-phenylene diamine (3.2 g, 0.03 mole) in ethanol (20 mL) under reflux conditions for 6 hr. The temperature was maintained between 80 to 100°C. The reaction mixture was concentrated to precipitate out the desired compound. The product was recrystallized from methanol, yield 4.82 g (67%). mp 215°C. IR (KBr) cm^{-1} 1335 (C-N stretching), 3290 (NH_2 asymmetric); 3335 (NH_2 symmetric). M/S (m/z): 240 (M^+), 225 ($C_{12}H_{11}N_5$), 201 ($C_{10}H_{11}N_5$), 186 ($C_{10}H_{10}N_4$), 149 ($C_6H_7N_4$), 97 ($C_4H_7N_3$), 93 (C_6H_7N), 80 ($C_4H_4N_2$), 58 ($C_2H_6N_2$), 43 (C_2H_5N). Anal.: Calc. for $C_{12}H_{12}N_6$: C, 59.75; H, 5.00; N, 34.85. Found: C, 59.86; H, 5.98; N, 33.98%.

4-(3,7-dimethyl-4*H*-pyrazolo[3,4-*e*][1,2,4]triazin-4-yl)benzoic acid 5. Compound 5 was synthesized by condensing the mixture of **1** (4.5 g, 0.03 mole) and 4-aminobenzoic acid (4.11 g, 0.03 mole) in ethanol (20 mL) under reflux conditions for 6 hr on a water bath. The reaction mixture was concentrated to precipitate out the desired compound. The product was recrystallized from methanol, yield 3.9 g (47%), mp 146°C; IR (KBr) cm^{-1}: 1120 (C-O stretching), 1240 (C-N stretching), 1640 (C=O stretching), 2920 (C-H stretching), 3346 (O-H- stretching). M/S (m/z): 269 (M^+), 225 ($C_{12}H_{11}N_3$), 186 ($C_{10}H_{10}N_4$), 149 ($C_8H_7N_3$), 97 ($C_4H_7N_3$), 80 ($C_4H_4N_2$), 78 (C_6H_6), 58 ($C_2H_6N_2$), 43 (C_2H_5N). Anal. Calc. for $C_{13}H_{11}N_5O_2$: C, 57.99; H, 4.08; N, 26.02. Found: C, 58.27; H, 4.86; N, 25.48%.

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