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Synthesis and Anti-Inflammatory Activities of N^4,N^5-disubstituted-3-methyl-1H-pyrazolo[3,4-*c*]pyridazines

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Abstract—The synthesis and anti-inflammatory activity of 4,5-dihydroxy-3-methyl-1H-pyrazolo[3,4-*c*] pyridazine (**4**), 4,5-dichloro-3-methyl-1H-pyrazolo[3,4-*c*]pyridazine (**5**), 4,-benzoyloxy-3-methyl-1-benzoyl-1H-pyrazolo[3,4-*c*]pyridazin-5yl benzoate (**6**), 3-methyl-N^4,N^5-bis(4-methylphenyl)-1H-pyrazolo[3,4-*c*]pyridazine-4,5-diamine (**7**), 4{[5-(4-carboxyanilino)-3-methyl-1H-pyrazolo[3,4-*c*]pyridazin-4yl}amino}benzoic acid (**8**), *N*-[5-(benzoylamino)-3-methyl-1H-pyrazolo[3,4-*c*]pyridazin-4-yl]benzamide (**9**) and 3-methyl-N^4,N^5-bis[4-(1H-benzimidazol-2yl)phenyl]-1H-pyrazolo[3,4-*c*]pyridazine-4,5-diamine (**10**) are being reported.

Introduction

During the various investigations[1,2] directed towards the synthesis of heterocyclic annelated pyridazines as building block for the preparation of potential biologically active compounds pyrazolo[3,4-*d*]pyridazine systems become an object of interest.[3,4] Generally pyrazolopyridazines have shown good antimicrobial, anti-inflammatory and analgesic activities. An attempt to synthesize[5] pyrazolo[3,4-*d*]pyridazines which are of chemical and biological interest[6,7] have utilized the pyrazoles with appropriate ortho functional groups as starting material except one report.[8] *N*-substituted pyrazolo derivatives have potent analgesic and anti-inflammatory activity.[9]

These reports prompted us to synthesize substituted pyrazolopyridazine derivatives, which have been of great biological interest. Such types of compounds have shown a great reactivity and numerous biological activities. The compounds have been screened for anti-inflammatory activity.

Chemistry

5-Methyl-2,4-dihydro-3H-pyrazol-3-one (**1**) was obtained from the condensation of acetoacetic ester and hydrazine hydrate in ethanol.[10] Reaction of **1** with hydrazine hydrate in ethanol in acidic medium[11,12] gave 5-hydrazino-3-methyl-1-H-pyrazole (**2**). Compound **2** was converted into 2-ethoxy-*N*-(5-methyl-2,4-dihydro-3H-pyrazol-3yl-idene)-2-oxoethanehydrazonic acid (**3**) when treated with oxalic acid in sodium acetate and ethanol. 4,5-Dihydroxy-3-methyl-1H-pyrazolo[3,4-*c*]pyridazine (**4**) was synthesized by cyclizing using $SnCl_4$ in nitrobenzene to produce the desired compound. In the PMR spectrum of (**4**) one proton singlet at δ 6.8 and δ 5.2 of hydroxyl protons of C-3 and C-4 and δ 6.3 of proton of nitrogen have been observed. Three-proton singlet of the methyl protons has been observed at δ 2.1. Molecular ion peak in the mass spectrum of compound is observed at (*m*/*z*) 166 (M^+). Other important peaks have been observed at (*m*/*z*) 137 ($C_5H_5N_4O$), 108 ($C_4H_4N_4$), and 81($C_3H_3N_3$) (Scheme 1).

4,5-Dichloro-3-methyl-1H-pyrazolo[3,4-*c*]pyridazine (**5**) has been synthesized by refluxing 4,5-dihydroxy-3-methyl-1H-pyrazolo[3,4-*c*]pyridazine (**4**) with phosphorous oxychloride for 50 h. Mass spectrum of the compound has shown the molecular ion peak at (*m*/*z*) 202 and 200. Other important peaks have been observed at (*m*/*z*) 168 ($C_6H_5N_4Cl$), 134 ($C_6H_6N_4$), 105 ($C_5H_5N_3$) and 80 ($C_4H_4N_2$).

4,-Benzoyloxy-3-methyl-1-benzoyl-1H-pyrazolo[3,4-*c*]pyridazin-5yl benzoate (**6**) was synthesized by the reaction of 4,5-dihydroxy-3-methyl-1H-pyrazolo[3,4-*c*]pyridazine (**4**) with benzoyl chloride in sodium hydroxide. The IR

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spectrum of the compound has shown C=O stretching absorption band at 1735 cm^{-1} and C–N stretching band at 1418 cm^{-1}. PMR spectrum of compound has shown a multiplet centered at δ 7.8 for 15 aromatic protons and three-proton singlet was observed at δ 2.1. Mass spectral fragmentation has shown the base peaks at (*m*/*z*) 134 ($C_6H_6N_4$). The other major peaks in the mass spectrum of the compound have been found at *m*/*z* 478 (M^+), 374 ($C_{20}H_{14}N_4O_4$), 254 ($C_{13}H_{10}N_4O_2$), 149 ($C_6H_7N_5$), 134 ($C_6H_6N_4$), 105 (C_7H_5O), 80 ($C_4H_4N_2$) and 58 ($C_2H_6N_2$).

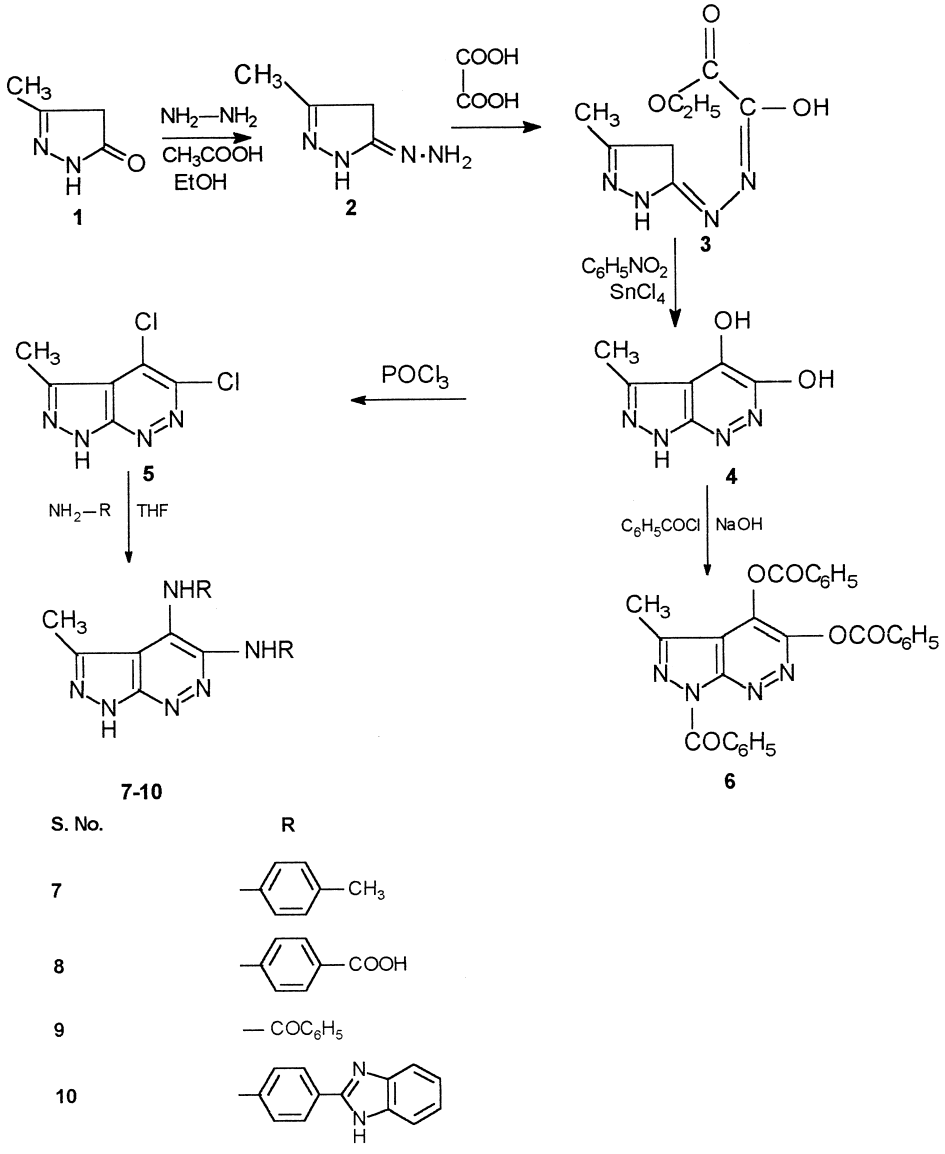
N^4,N^5-Diubstituted-3-methyl-1H-pyrazolo[3,4-*c*]pyridazines (**7**–**10**) were synthesized by the condensation of 4,5-dichloro-3-methyl-1H-pyrazolo[3,4-*c*]pyridazine (**5**) with *p*-toluidine, 4-amino benzoic acid, benzamide and *p*-phenylene diamine respectively. The structures of these compounds were established on the basis of their PMR and mass spectra. The PMR spectrum of compound **7** has shown an eight-proton multiplet centered at δ 7.2 for the aromatic protons. The other signals observed are a one-proton singlet at δ 6.5 for the N-H of pyrazole, two one-proton singlets at δ 6.1 and 5.8 for the N–H at position 5 and 4, a six-proton singlet at δ 2.4 for the methyl attached to the phenyl ring and three proton singlet at δ 2.1 of the methyl attached to the pyrazole ring. Mass spectrum of the compound has shown major peaks at *m*/*z* 344 (M^+), 254 ($C_{13}H_{14}N_6$), 164 ($C_6H_8N_6$), 149 ($C_6H_7N_5$), 134 ($C_6H_6N_4$), 107 (C_7H_9N), 91 (C_7H_7) 80 ($C_4H_4N_2$) and 58 ($C_2H_6N_2$).

Anti-inflammatory activity

Compounds **4**–**10** were evaluated for anti-inflammatory activity against carragenin induced paw oedema in albino rats. All the compounds have exhibited activity in the range of 30–50%. The activities have been reported in Table 1.

Experimental

Melting points were taken in an electrically heated instrument and are uncorrected. Compounds were



Scheme 1.

Table 1. Anti-inflammatory activity of N^4,N^5-Diubstituted-3-methyl-1H-pyrazolo[3,4-*c*]pyridazines

Compound No.	Mean Difference	Percent Activity (100 mg/Kg)
4	26.14	36
5	22.01	45
6	24.35	39
7	21.19	32
8	17.73	49
9	23.18	40
10	25.63	40

routinely checked for their purity on silica gel G TLC plates and the spots were visualized by iodine vapors. IR spectra were recorded on Shimadzu 8201 PC FTIR spectrometer. PMR spectra were recorded on Bruker DRX 300 MHz FT NMR spectrometer using TMS as internal reference and chemical shift values are expressed in δ units. Mass spectra were run on Jeol SX-102 spectrometer.

4,5-Dihydroxy-3-methyl-1H-pyrazolo[3,4-*c*]pyridazine (4). 2-Ethoxy-*N*-(5-methyl-2,4-dihydro-3H-pyrazol-3yl-idene)-2-oxoethanehydrazonic acid (**3**) (9.2 g, 0.05 mol) was refluxed with nitrobenzene (30 mL) and $SnCl_4$ (10 g) for 5 h. The reaction was continued until a black jelly was settled on the bottom of round-bottomed flask. On completion of cyclization nitrobenzene was distilled out. The solid black material was shaken well with water and then filtered through suction. The resulting 4,5-dihydroxy-3-methyl-pyrazolo[3,4-*c*] pyridazine (**4**) was recrystallized from a mixture of methanol and water. Yield 6.52 g (80%). Mp 224 °C. IR (KBr) cm^{-1}: 3519 (O–H stretching) 3312 (N–H stretching). PMR (CD_3OD): δ 6.8 (s, 1H, OH), δ 6.3 (s, 1H, OH), 5.2 (s, 1H, NH), 2.1 (s, 3H, CH_3). M/S *(m/z)*: 166 (M^+), 137 ($C_5H_5N_4O$), 108 ($C_4H_4N_4$), 81 ($C_3H_3N_3$) and 56 ($C_2H_4N_2$). Anal. $C_6H_6N_4O_2$; calcd C, 43.37; H, 3.61; N, 33.76; found C, 43.14; H, 3.52; N, 34.14%.

4,5-Dichloro-3-methyl-1H-pyrazolo[3,4-*c*]pyridazine (5). 4,5-Dihydroxy-3-methyl-1H-pyrazolo[3,4-*c*]pyridazine (**4**) (8.3 g, 0.05 mol) was refluxed with phosphorous oxychloride (200 mL) for 50 h. Excess $POCl_3$ was removed by vacuum distillation. The residual mixture was poured into crushed ice and on filtration **5** was obtained. Yield 9.6 g (95%). Mp 298 °C. M/S (*m/z*): 202(M^+), 168 ($C_6H_5N_4Cl$), 134 ($C_6H_6N_4$), 105 ($C_5H_5N_3$) and 80 ($C_4H_4N_2$).

4,-Benzoyloxy-3-methyl-1-benzoyl-1H-pyrazolo[3,4-*c*]pyridazin-5yl benzoate (6). 4,5-Dihydroxy-3-methyl-1H-pyrazolo[3,4-*c*]pyridazine (**4**) (11.6 g, 0.07 mol) was dissolved in 90 mL of 1% NaOH solution and to this solution benzoyl chloride (12.6 mL, 0.09 mol) was introduced. The mixture was shaken vigorously for about 30 min. When a solid substance was separated out 100 mL of water was added to this mixture and crude benzoate was filtered, washed with cold water. The compound was recrystallized from methanol and water. Yield 18 g (54%). Mp 88 °C. IR (KBr) cm^{-1}: 1735 (C=O stretching), 1418 (C–N stretching). PMR (DMSO-d_6): δ 7.8 (m, 15H, ArH), 2.1 (s, 3H, CH_3). CMR (DMSO-d_6): δ 200 (OCO), 180 (NCO), 158 (C-8), 151 (C-5 and C-3), 144, 143, 141 (aromatic), 82 (C-4), 76 (C-9), 21 (3-CH_3). M/S *(m/z)*: 478 (M^+), 374 ($C_{20}H_{14}N_4O_4$), 254 ($C_{13}H_{10}N_4O_2$), 149 ($C_6H_7N_5$), 134 ($C_6H_6N_4$), 105 (C_7H_5O), 80 ($C_4H_4N_2$) and 58 ($C_2H_6N_2$). Anal. ($C_{27}H_{18}N_4O_5$); calcd C, 67.78; H, 3.76; N, 11.71; found C, 68.31; H, 4.17; N 11.92%.

3-Methyl-N^4,N^5-bis(4-methylphenyl)-1H-pyrazolo[3,4-*c*]-pyridazine-4,5-diamine (7). 4,5-Dichloro-3-methyl-1H-pyrazolo[3,4-*c*]pyridazine (**5**) (2 g, 0.01 mole) was refluxed with *p*-toluedine (1.07 g, 0.01 mole) in tetrahydro furan for 12 h. After the completion of the reaction the reaction mixture was poured in cold water and filtered through suction. The crude material was recrystallized from a mixture of water and ethanol to give **7**. Yield 2.9 g (83%). PMR (CD_3OD): δ 7.2 (m, 8H,ArH), 6.5 (s, 1H, 1-NH), 6.1 (s, 1H, 5-NH), 5.8 (s, 1H, 4NH), 2.4 (s, 6H, Ar-CH_3), 2.1 (s, 3H, 3-CH_3). CMR (CD_3OD): δ 154 (C-8), 150 (C-5 and C-3), 144, 143, 141 (aromatic), 82 (C-4), 46 (C-9), 26 (Ar-CH_3), 21 (3-CH_3). M/S *(m/z)*: 344 (M^+), 254 ($C_{13}H_{14}N_6$), 164 ($C_6H_8N_6$), 149 ($C_6H_7N_5$), 134 ($C_6H_6N_4$), 107 (C_7H_9N), 91 (C_7H_7) 80 ($C_4H_4N_2$) and 58 ($C_2H_6N_2$). Anal.: ($C_{20}H_{20}N_6$); calcd C, 69.76; H, 5.81; N, 24.42; found C, 70.21; H, 6.32; N, 23.98%.

4{[5-(4-Carboxyanilino)-3-methyl-1H-pyrazolo[3,4-*c*]pyridazin-4yl}amino} benzoic acid (8). 4,5-Dichloro-3-methyl-1H-pyrazolo[3,4-*c*]pyridazine (**5**) (2 g, 0.01 mol) was refluxed with *p*-amino benzoic acid (1.37 g, 0.01 mole) for 12 h in tetrahydrofuran. On the completion of the reaction the reaction mixture was poured in cold water and water insoluble material was filtered through suction. The crude material was recrystallized from a mixture of water and ethanol giving the desired product. Yield 3.97 g (98%). IR (KBr) cm^{-1}: 3518 (O-H stretching), 3449 (N-H stretching), 3406 (N-H stretching), 1737 (C=O stretching). PMR (CD_3OD): δ 7.6 (m, 8H, ArH), 6.5 (s, 1H, 1NH), 6.2 (s, 1H, 5NH), 5.8 (s, 1H, 4NH), 2.3 (s, 3H, CH_3). CMR *(CD_3OD)*: δ 172 (CO), 154 (C-8), 151 (C-5 and C-3), 141, 143, 145 (Aromatic), 82 (C-4),78 (C-9), 21 (3-CH_3). M/S *(m/z)*: 404(M^+), 316 ($C_{18}H_{16}N_6$), 240 ($C_{12}H_{12}N_6$), 164 ($C_6H_8N_6$), 149 ($C_6H_7N_5$), 134 ($C_6H_6N_4$), 93 (C_6H_7N), 80 ($C_4H_4N_2$) and 58 ($C_2H_6N_2$). Anal. ($C_{20}H_{16}N_6O_4$); calcd C, 59.40; H, 3.96; N, 20.79; found: C, 60.31; H, 4.82; N, 18.17%.

***N*-[5-(Benzoylamino)-3-methyl-1H-pyrazolo[3,4-*c*]pyridazin-4-yl]benzamide (9).** 4,5-Dichloro-3-methyl-1H-pyrazolo-[3,4-*c*]pyridazine (**5**) (2 g, 0.01 mol) was refluxed with benzamide (1.21 g, 0.01 mol) for 12 h in tetrahydrofuran on a sand bath. The reaction mixture was filtered and the crude product was then recrystallized from the mixture of water and alcohol to give **9**. Yield 3.22 g (87%). IR (KBr) cm^{-1}: 3515 (N-H stretching), 1723 (C=O stretching), 1433 (C-N stretching). PMR (CD_3OD): δ 7.2 (m, 10H, ArH), 6.3 (s, 1H, 1-NH), 5.9 (s, 1H, 5-NH), 5.3 (s, 1H, 4-NH), 2.3 (s, 3H, CH_3). CMR *(CD_3OD)*: δ 182 (CO), 154 (C-8), 150 (C-5 and C-3), 141, 143, 145 (aromatic), 82 (C-4), 78 (C-9), 21 (3-CH_3).

M/S (*m*/*z*): 372 (M^+), 268 ($C_{13}H_{12}N_6O$), 164 ($C_6H_8N_6$), 151 ($C_7H_7N_2O$), 149 ($C_6H_7N_5$), 134 ($C_6H_6N_4$), 107 ($C_5H_5N_3$), 80 ($C_4H_4N_2$) and 58 ($C_2H_6N_2$). Anal. ($C_{20}H_{16}N_6O_2$); calcd C, 64.51; H, 4.30; N, 22.59; found C, 64.83; H, 5.21; N, 23.11%.

3-Methyl-N^4,N^5-bis[4-(1H-benzimidazol-2yl)phenyl]-1H-pyrazolo[3,4-*c*] pyridazine-4,5-diamine (10). 4{[5-(4-Carboxyanilino)-3-methyl-1H-pyrazolo[3,4-*c*]pyridazin-4yl} amino} benzoic acid (**8**) (2 g, 0.005 mol) was refluxed with *p*-phenylene diamine (2 g, 0.02 mol) for 4 h in 4 N HCl. The reaction mixture was made alkaline by the addition of ammonia solution and filtered. The crude product was recrystallized from the mixture of water and ethanol. Yield 9.3 g (85%). PMR (DMSO-d_6): δ 7.9 (m, 8H, ArH), 7.3 (m, 8H, N–ArH), 6.5 (bs, 1H, NH), 6.2 (s, 1H, NH), 5.8 (bs, 1H, NH), 5.5 (s, 1H, NH), 2.0 (s, 3H, CH_3). CMR (DMSO-d_6): δ 156 (C-4′), 154 (C-8), 150 (C-5 and C-3), 145, 143, 141, 139 (aromatic), 128 (C-2′), 82 (C-4), 78 (C-9), 21 (3-CH_3). M/S (*m*/*z*): 548(M^+), 400 ($C_{20}H_{20}N_{10}$), 316 ($C_{18}H_{16}N_6$), 240 ($C_{12}H_{12}N_6$), 164 ($C_6H_8N_6$), 149 ($C_6H_7N_5$), 134 ($C_6H_6N_4$). 118 ($C_7H_6N_2$), 80 ($C_4H_4N_2$) and 58 ($C_2H_6N_2$). Anal. ($C_{32}H_{24}N_{10}$); calcd C, 70.07; H, 4.37; N, 25.54; found: C, 70.87; H, 4.35; N, 25.23%.

Anti-inflammatory activity

The activity was evaluated against carrageenin induced paw oedema in albino rats of either sex weighing 80–180 g each. Food and water was allowed ad-libidam prior to the experiments 0.05 mL of freshly prepared suspension of carrageenin (1.0%) in 0.9% of saline was injected beneath the planter aponeurosis of right paw of the rats by the method of Winter et. al. (1962). One group of five rats was kept as control and other groups were pretreated with the test drugs and the standard drug at a dose of 100 mg/kg per orally 1 h prior to the carrageenin. Rat paw was measured before and 3 h after the carrageenin treatment by the micro-pipette method as described by Buttle et al. (1957). The increase in the volume of the paw in each group was measured and percent anti-inflammatory activity was calculated by following formula:

$$\text{Percent anti inflammatory activity} = \left[1 - \frac{V_t}{V_c}\right] \times 100$$

where V_t and V_c are the volume of the paw edema in drug treated and control group respectively. The anti-inflammatory activity of compounds **4**–**10** is given in Table 1.

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References and Notes

1. Deeb, A.; Said, S. A.; Hamed, M. M.; Yosine, F. J. *Chim. Soc. (Taipei)* **1990**, *37*, 287.
2. Deeb, A.; Said, S. A. *Coll. Czech. Chem. Commun.* **1990**, *55*, 2795.
3. Tisler M.; Stanovnic B. In *The Chemistry of Heterocyclic Compounds*; Castle R. N., Ed.; John Wiley and Sons, Inc.: New York, 1973; Vol. 27, p 785.
4. Saeda, M.; Fawzy, M. M.; Jahine, H.; Abdei Magid, M.; Saad, R. R. J. *Chim. Soc (Taipei)* **1989**, *36*, 241.
5. Kaji, K.; Nigoshima, H.; Taliashi, K.; Oda, H. *Hetrocycles* **1984**, *22*(10), 2357.
6. Kaji K.; Nigoshima H.; Oda H.; Nagao S.; Wakabayashi Y.; Hirose Y.; Taniguchi M.; Ikai T., Japan Patent Kokai 71010, 1981; *Chem. Abstr.* **1981**, *95*, 127428n.
7. Lespagnol, A.; Lespagnol, C.; Willcomme, B. *Eur. J. Med. Chem.* **1974**, *9*, 51.
8. Frank Neumann, M.; Leclerc, G. *Tetrahedron Lett.* **1969**, *1063*.
9. Meier, K.; Ringier, B. H.; Druley, J. *Helv. Chim. Acta* **1954**, *37*, 523.
10. Vogel A.I. *Text Book of Practical Organic Chemistry*, 4th ed.; Longman Group: UK, 1978, p 575.
11. El Sekily, M.; Mancy, S.; Gross, B. *Carbohydr. Res.* **1983**, *122*, 151.
12. Metwally, M. A.; Amer, F. A. *Pharmazie* **1983**, *38*, 172.