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SYNTHESIS OF 1/2-BENZYL-4,6-DISUBSTITUTED 1H/2H-PYRAZOLO [3,4-d]PYRIMIDINES AND THEIR ANTILEISHMANIAL AND ANTIVIRAL ACTIVITIES +

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Regioselective benzylation of 4,6-bis(methylthio)-1H-pyrazolo[3,4-*d*]pyrimidine followed by treatment with different amines yielded the corresponding 4-aminoalkyl-6-methylthio-2-(benzyl)-2H-pyrazolo[3,4-*d*]pyrimidines (**4a-4g**) in fairly good yields. Oxidation of 4-amino-6-methylthio-2-(benzyl)-2H-pyrazolo[3,4-*d*]pyrimidine (**4a**) with $KMnO_4$ in glacial acetic acid afforded the corresponding 6-methylsulphonyl derivative (**5**) which on treatment with aqueous NaOH finally yielded 4-amino-6-(7H)-oxo-2-(benzyl)-2H-pyrazolo[3,4-*d*]pyrimidine (**6**). Of the compounds tested for biological activities, 4-amino(2-pyridyl)-2-benzyl-6-methylthio-2H-pyrazolo[3,4-*d*]pyridine (**4f**) exhibited 100% antiviral activity against Ranikhet disease virus (RDV) at 1μg/mL and the compounds (**4a-4d**) and (**4g**) showed 45% to 60% activity against RDV.4-(*p*-Chlorophenylethylamine)-6-methylthio-2-(benzyl)-2H-pyrazolo[3,4-*d*]pyrimidine (**4e**) and 4-amino-6(7H)-oxo-2-(benzyl)-2H-pyrazolo[3,4-*d*]pyrimidine (**6**) showed 60% and 55% inhibition respectively of amastigotes of *Leishmania donovani* in hamster at 100 mg/ml.

Pyrazolo[3,4-*d*]pyrimidine nucleosides[1-3] attracted the attention of medicinal chemists because of antibiotic properties of tubercidin[4], toyocamycin[5], sangivamycin[6], cadeguomycin[7], and kanagawamycin[8]. 9-Alkylated purines which can be classified as non classical nucleosides, are known to bind to the enzyme adenosine deaminase[9]. In recent years several non classical nucleosides have been synthesized and some of these have shown high order of biological activities. 9-Cyclohexyl-2-n-propoxy-9H-adenine is a potent bronchodilator[10], 9-(2-fluorobenzyl)-6-methylamino-9H-purine exhibits anticonvulsant activity[11] and6-dimethylamino-9-(4-methyl-benzyl)-2-trifluoromethyl-9H-purine is an antiviral agent[12]. We have found that **1** and **3** exhibited antileishmanial activity *in vitro*[13,14]. It is interesting that 4,6-bis-(ethylthio)-1-methyl-IH-pyrazolo[3,4-*d*]pyrimidine (**2**) potentiates the activity of phleomycin against *E. coli*[15]. We have extended these studies[17-20] and we now report further transformations of the compound **3** where new compounds (**4a-4g** and **6**) have been synthesized by chemical manipulation of 4-SMe and 6-SMe groups.

Regioselective benzylation of 4,6-bis(methylthio)-IH-pyrazolo[3,4-*d*]pyrimidine[16] with benzyl chloride in the presence of sodium hydride produced predominantly 2-benzyl-4,6-bis(methylthio)-2H-pyrazolo[3,4-*d*]pyrimidine (**3**)[14] in good yield. Treatment of **3** with methanolic ammonia, benzylamine, furfurylamine, *p*-aminophenylethylamine, *p*-chlorophenylethylamine,2-(pyridyl)methylamine, and4-(pyridyl)methylamine separately afforded the corresponding 4-aminoalkyl-6-methylthio-2-(benzyl)-2H-pyrazolo[3,4-*d*]pyrimidines (**4a-4g**) respectively in fair yields.

Oxidation of 4-amino-6-methylthio-2-(benzyl)-2H-pyrazolo[3,4-*d*]pyrimidine (**4a**) with $KMnO_4$ in glacial acetic acid afforded 4-amino-6-methylsulphonyl 2-(benzyl)-2H-pyrazolo[3,4-*d*]pyrimidine (**5**). Treatment of **5** with aq. sodium hydroxide finally furnished 4-amino-6(7H)-oxo-2-(benzyl)-2H-pyrazolo[3,4-*d*]pyrimidine (**6**). The IR, UV,NMR and mass spectral data of the compounds were in complete agreement with the assigned structures.

Experimental

For general direction see earlier papers in the series[13].

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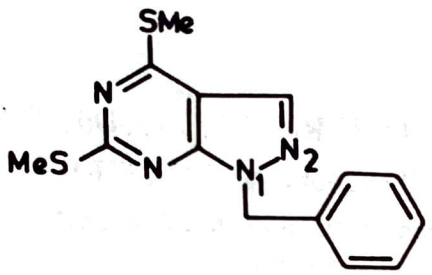

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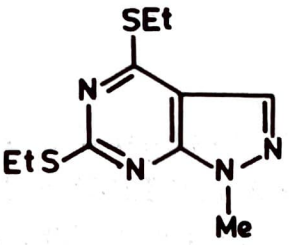

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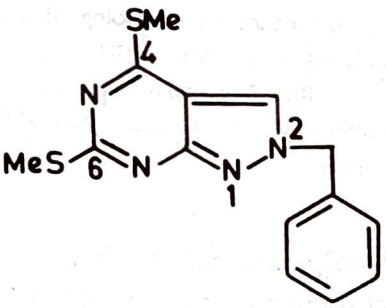

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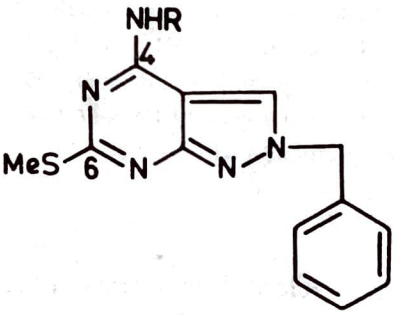


4

4a R = H

4b R = CH_2–Ph

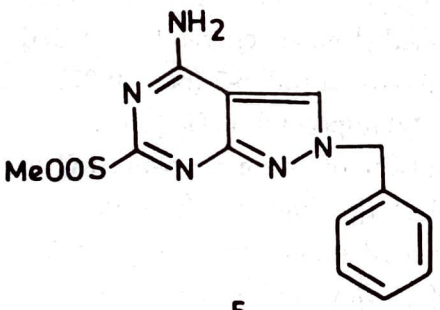
4c R = CH_2–(2-furyl)

4d R = CH_2–CH_2–(4-NH_2-Ph)

4e R = CH_2–CH_2–(4-Cl-Ph)

4f R = CH_2–(2-pyridyl)

4g R = CH_2–(4-pyridyl)



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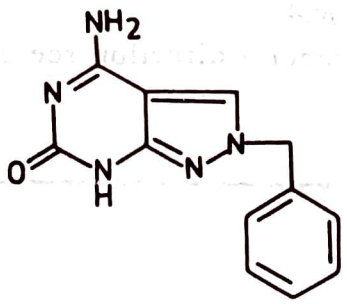

6

4-Alkylamino/arylamino-2-benzyl-6-methylthio-2H-pyrazolo [3,4- *d*] pyrimidines (4a-4g)

A mixture of 4,6-bis(methylthio)-2-benzyl-2H-pyrazolo[3,4-*d*]pyrimidine (3)[14] and the appropriate amines was heated at 120° in a steel bomb for 10-12 hr. The solvent and excess of reagent from the resulting mixture were removed *in vacuo*. The crude product, thus obtained, was chromatographed over SiO_2 column. Elution of the column with varying proportion of $CHCl_3$ and MeOH afforded compounds (4a-4g). The yield, m.p., spectral data and elemental analyses of the compounds are given below.

4-Amino-2-benzyl-6-methylthio-2H-pyrazolo[3,4-*d*]pyrimidine (4a)

Yield : 50%; m.p. 245°; MS: m/z 271 (M^+); PMR($CDCl_3$): 8.3 (s, 1H, H-3), 7.4-7.2 (m, 5H, Ar*H*), 6.9 (bs, 2H, NH_2)., 5.6(s, 2H, PhCH_2), 2.5 (s, 3H, SCH_3), [Found: C, 57.5; H, 4.7; N, 26.0. $C_{13}H_{13}N_5S$ requires C, 57.7; H, 4.7; N, 25.8%].

4-Benzylamino-2-benzyl-6-methylthio-2H-pyrazolo[3,4-*d*] pyrimidine (4b)

Yield: 65%; m.p.164°; MS : 361(M^+); PMR ($CDCl_3$ + DMSO-d_6) : 8.1 (s, 1H, H-3), 7.6-7.2 (m, 10H, Ar-*H*), 6.8 (m, 1H, N*H*), 5.6-5.4 (m, 4H, 2 × CH_2Ph), 2.4 (s, 3H, SCH_3). [Found : C, 66.6; H, 5.0; N, 19.6. $C_{20}H_{19}N_5S$ requires C, 66.5; H, 5.3; N, 19.3%].

2-Benzyl-4-furfurylamino-6-methylthio-2H-pyrazolo[3,4-*d*] pyrimidine (4c)

Yield : 55%; m.p. 176°; MS: m/z 351 (M^+); PMR($CDCl_3$): 8.1 (s, 1H, H-3), 7.3-7.1 (m, 5H, Ar-*H*), 6.2 (m, 3H, furan-*H*), 5.3 (s, 2H, -CH_2Ph), 2.5 (s, 3H, SCH_3). [Found : C, 61.6; H, 4.9; N, 20.1. $C_{18}H_{17}N_5OS$ requires C, 61.5; H, 4.8; N, 19.9%].

4-*p*-Aminophenylethylamino-2-benzyl-6-methylthio-2H-pyrazolo[3,4-*d*]pyrimidine (4d)

Yield : 48%; m.p.186°; MS: m/z 390 (M^+); PMR ($CDCl_3$ + DMSO-d_6) : 8.0 (s,1H,H-3), 7.7-7.4 (m, 9H, Ar-*H*), 5.3 (s, 2H, CH_2Ph), 3.7-3.4 (m, 2H, NH-CH_2), 3.1-2.9(m, 2H,NH_2-Ph-CH_2) 2.5 (s, 3H, SCH_3). [Found : C, 64.8; H, 5.6; N, 21.8. $C_{21}H_{22}N_6S$ requires C, 64.6; H, 5.6; N, 21.5%].

2-Benzyl-4-*p*-chlorophenylethylamino-6-methylthio-2H- pyrazolo[3,4-*d*]pyrimidine (4e)

Yield : 50%; m.p. 132°; MS: m/z 409 (M^+); PMR($CDCl_3$): 7.8 (s, 1H, H-3), 7.3- 6.9 (m, 9H Ar-*H*), 6.4-6.3 (M, 1H, N*H*), 5.2(s, 2H, - CH_2Ph), 3.9-3.6(m, 2H, NH-CH_2), 3.0-2.7 (m, 2H, CH_2-Ph-Cl), 2.5(s, 3H, SCH_3). [Found : C,61.5;H, 4.8; N, 16.9. $C_{21}H_{20}N_5ClS$ requires C, 61.6; H, 4.8; N, 17.1%].

2-Benzyl-4-(2-pyridyl)methylamino-6-methylthio-2H-pyrazolo[3,4-*d*]pyrimidine (4f)

Yield : 70%; m.p. 170°; MS: m/z 262 (M^+); PMR($CDCl_3$): 8.4(s, 1H, H-3), 8.2-8 (m, 4H, Py-*H*), 7.3-7.0 (m, 5H, Ar-*H*), 5.2 (s, 2H, Py- CH_2), 4.8-4.6 (m, 2H, CH_2-Ph), 2.5 (s, 3H, SCH_3). [Found : C, 63.1; H, 4.9; N, 23.2. $C_{19}H_{18}N_6S$ requires C, 62.9; H, 5.0; N, 22.9%].

2-Benzyl-4-(4-pyridyl)methylamino-6-methylthio-2H-pyrazolo [3,4-*d*]pyrimidine (4g)

Yield : 40%; m.p. 90°; MS: m/z 362 (M^+); PMR($CDCl_3$): 8.4 (s, 1H, H-3), 8.2-8.0 (m, 4H, Py-*H*), 7.8-7.6 (m, 5H, Ar-*H*), 5.6 (m, 2H, Py-CH_2), 4.8 (s, 2H, CH_2-Ph), 2.5 (s, 3H, -SCH_3). [Found : C, 63.0; H, 5.0; N, 22.8. $C_{19}H_{18}N_6S$ requires C, 62.9; H, 4.9; N, 22.9%].

4-Amino-2-benzyl-6-methylsulphonyl-2H-pyrazolo[3,4-*d*]pyrimidine (5).

A solution of 4-amino-2-benzyl-6-methylthio-2H-pyrazolo [3,4- *d*]pyrimidine (4a) (1.5 g, 6 mmol) in 50% glacial acetic acid was cooled to 5°. To it was added $KMnO_4$ (2.0 g) in portions with stirring. Stirring was continued at ambient temperature for 2 hr. Excess of $KMnO_4$ in the resulting mixture was removed by H_2O_2 and the product extracted with $CHCl_3$ (4 × 10ml). The chloroform extract was washed with aq. $NaHCO_3$, water dried (anhy. Na_2SO_4) and the solvent removed *in vacuo*. The crude product, thus obtained, was chromatographed over SiO_2 (100 g). Elution of the column with $CHCl_3$: MeOH : MeCOMe (92 : 6 : 2, v/v) gave **5** (0.85 g., 50%); m.p. 250°; MS : m/z 304 (M^+); PMR($CDCl_3$ + DMSO-d_6) : 8.0 (s, 1H, H-3) 7.1-7.5 (m, 5H, Ar-*H*), 5.8 (m, 2H, CH_2-Ph), 3.4 (bs, 3H, SO_2-CH_3).

4-Amino-6-(7H)-oxo-2-benzyl-2H-pyrazolo[3,4-*d*]pyrimidine (6).

A mixture of **5** (1.5 g, 5 mmol) and 2N aq. NaOH (5 ml) at 65° was stirred for 2 hr. The resulting mixture was cooled, neutralized with dil. HCl and passed through ion exchange resin (IR-45°, OH form). The column was first eluted with

H_2O and then with MeOH. Removal of the solvent from MeOH eluate gave **6** (0.65 g, 54%); m.p. 260°; IR(KBr) : 1715 (C = O), 3400 (NH_2); MS : m/z 241 (M^+). [Found : C, 58.5, H, 4.2, N, 28.9. $C_{12}H_{11}N_5O$ requires C, 59.5, H, 4.2, N, 28.9%].

Antiviral assay

Ranikhet disease virus (RDV) was used for antiviral screening of the compounds. The strain of RDV, the haemagglutination test, the method of preparation of CAM culture and the optimal condition of the infection by the virus are described in earlier publication[21].

Aqueous solutions/suspensions (0.1 mg/ml) of **4a-4g** were separately incubated in CAM culture using 6 CAM culture sample along with 0.064 HA/ml of RSV. The cultures were incubated at 37° for 48 hr. The percentage inhibition of virus multiplication was assayed from HA titre of the nutrient fluid of CAM culture infected with RDV. The compound **4a** to **4g** exhibited 50, 45, 50, 60, 30, 100 and 45% inhibition respectively.

Antileishmanial activity

The compounds (**4a-4g**) and **6** were also evaluated for antileishmanial activity (*in vitro*) against *Leishmania donovani* and showed 35, 33, 22, 24, 58, 35, 25 and 60% inhibition respectively.

Acknowledgement

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