Eur J Med Chem (1993) 28, 585–591

Synthesis of 4-amino/hydroxy-6-methylthio-1/2-(2',2'-diethoxyethyl)-1*H*/2*H*-pyrazolo[3,4-*d*]pyrimidines and their antiallergic activity*

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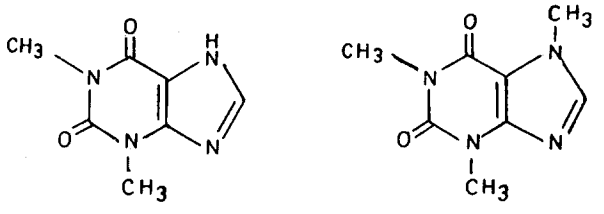
(Received 13 August 1992; accepted 16 February 1993)

Summary — The synthesis of a series of 4-amino/hydroxy-6-substituted-1/2-(2',2'-diethoxyethyl)-1*H*/2*H*-pyrazolo[3,4-*d*]pyrimidines by alkylation of substituted pyrazolo[3,4-*d*]pyrimidines has been described, and their antiallergic activity evaluated in mice. The 2 most active compounds **4b** and **5b** were also tested in the rat. The most active compound **4b** showed non-significant antihistaminic or antiserotoninergic activity.

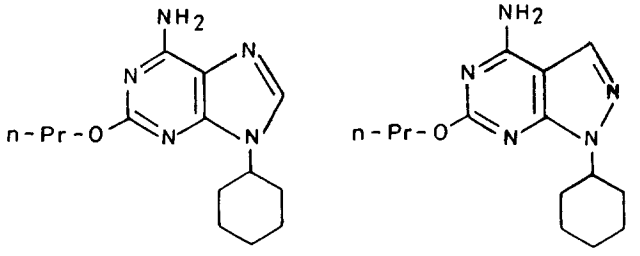
4-amino/hydroxy-pyrazolo[3,4-*d*]pyrimidines / anti-allergic activity

Introduction

Disodium chromoglycate (DSCG) remains a drug of choice for the treatment of allergy [1]. Since DSCG is not orally absorbed, increasing interest has been shown in developing orally active DSCG-like compounds [2, 3]. Due to the antiallergic properties [4, 5] of methylxanthines such as theophylline (**I**) and caffeine (**II**), efforts have been made to determine the structural basis for the biochemical properties of these compounds in order to produce more potent antiallergic drugs [6]. The usefulness of theophylline however, is impaired by numerous gastrointestinal CNS and CVS side effects [7]. A recent study [8] carried out on the treatment of acute asthma with aminophylline (ethylenediamine salt of theophylline) has further questioned its usefulness. On the other hand, a large number of 2,9-disubstituted adenines, including 9-cyclohexyl-2-*n*-propoxy-9*H*-adenine (BB-1502, **III**) have shown good bronchodilatory activity [9].



I–II



III–IV

These observations, coupled with our continued interest [10] in pyrazolo[3,4-*d*]pyrimidine compounds, have led us to synthesize pyrazolo[3,4-*d*]pyrimidine (**IV**) an analog of BB-1502 (**III**) to determine its antiallergic activity [11]. The observation of modest antiallergic activity of these pyrazolo[3,4-*d*]pyrimidines [11] in mice has provided impetus to continue our efforts in this area.

*CDRI communication No 4901.

The present report describes the synthesis and antiallergic activity of a large number of 4,6-disubstituted-1,2-(2',2'-diethoxyethyl)-1*H*/2*H*-pyrazolo[3,4-*d*]pyrimidines.

Chemistry

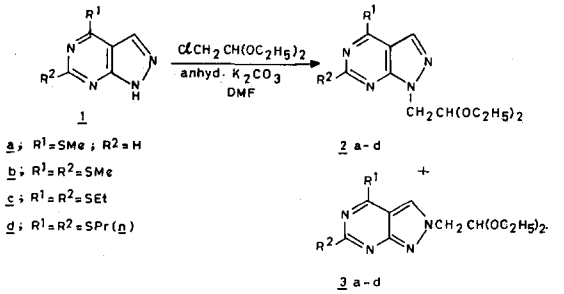
Condensation of 4,6-bis(methylthio)-1*H*-pyrazolo-[3,4-*d*]pyrimidine [12, 13] **1b** with chloroacetaldehyde diethyl acetal in dimethyl formamide in the presence of anhydrous K_2CO_3 gave a mixture of 2 isomeric *N*-1-(**2b**) and *N*-2-(**3b**) alkylated products (scheme 1). The structure of these isomeric products was determined by ^{13}C-NMR spectroscopy [14] after separation by column chromatography.

4-Amino-6-alkylthio-1-(2',2'-diethoxyethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidines **4a–d** and 4-amino-6-alkylthio-2-(2',2'-diethoxyethyl)-2*H*-pyrazolo[3,4-*d*]pyrimidines **5a–d** were synthesized by treatment of methanolic ammonia with **2a–d** or **3a–d**, respectively (scheme 2). Treatment of **2b–d** or **3b–d** with aqueous alkali gave 6-alkylthio-4(5*H*)-oxo-1-(2',2'-diethoxyethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidines **6b–d** and 6-alkylthio-4(5*H*)-oxo-2-(2',-diethoxyethyl)-2*H*-pyrazolo[3,4-*d*]pyrimidines **7b–d** respectively (scheme 2). Since BB-1502 (**III**) and its pyrazolo-analog (**IV**) both contained an alkoxy moiety in the pyrimidine ring, it was considered worthwhile to produce 4-amino-6-methoxy-1-(2',2'-diethoxyethyl)-1*H*-pyrazolo-[3,4-*d*]pyrimidine **10** and its corresponding *N*-2-analog **11** *via* intermediates **8** and **9** respectively (scheme 3).

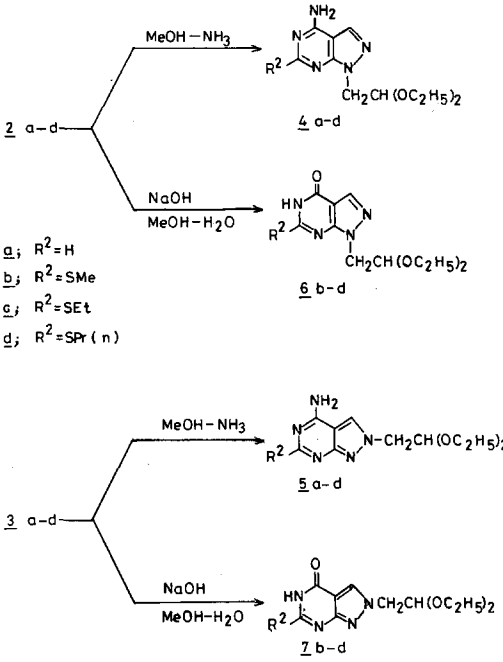
The known compound, 9-(2',2'-diethoxyethyl)-6-amino-9*H*-purine **12** was also prepared [15] for comparison of its antiallergic activity with the isomeric pyrazolo[3,4-*d*]pyrimidine compound **4a**.

Biological results and discussion

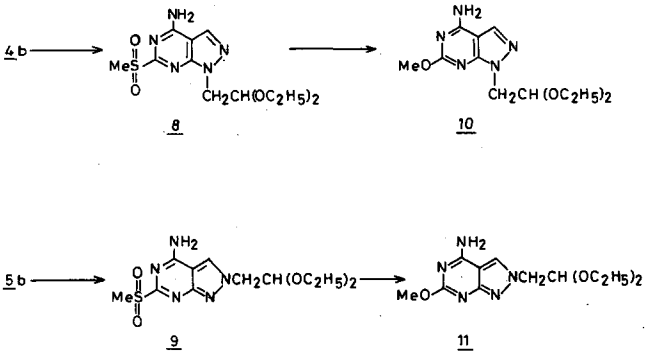
The inhibition of passive cutaneous anaphylaxis (PCA) in mice or rats by oral administration of test



Scheme 1.



Scheme 2.



Scheme 3.

compounds (50 mg/kg) is presented in tables I and II. The results shown in tables I and II clearly indicate that the presence of a methylthio function at the 6-position increases the anti-PCA activity as compared to a less bulky hydrogen or more bulky ethylthio or propylthio group. Also, replacement of the methylthio group by a methoxy group in the *N*-1 or *N*-2-series caused a decrease in activity. Compound **4b** was the most active of the *N*-1 series (table I) and **5b** of the *N*-2 series (table II). Therefore these 2 compounds were also tested in rats and were found to have significant antiallergic activity comparable to that of

Table I. Effect of test compounds (*po*) on passive cutaneous anaphylaxis in mice/rats.

Compound No	*R¹*	*R²*	*Mice/rats*	*% Inhibition of anaphylaxis (PCA) (mean ± SE)*
2a	SMe	H	Mice	62 ± 0.8
2b	SMe	SMe	Mice	63.5 ± 1.3
2c	SEt	SEt	Mice	50 ± 1.6
2d	SPr (*n*)	SPr (*n*)	Mice	30 ± 1.8
4a	NH_2	H	Mice	59.5 ± 1.7
4b	NH_2	SMe	Mice	71.7 ± 0.9
4b	NH_2	SMe	Rats	81.5 ± 2.2
4c	NH_2	SEt	Mice	40.5 ± 1.2
4d	NH_2	SPr (*n*)	Mice	43.5 ± 1.3
6b	OH	SMe	Mice	62.7 ± 1.3
6c	OH	SEt	Mice	42 ± 1.8
6d	OH	SPr (*n*)	Mice	49.5 ± 1.8
8	NH_2	SO_2Me	Mice	53.2 ± 1.3
10	NH_2	OMe	Mice	41.5 ± 1.2
12	–	–	Mice	60.7 ± 1.1
DSCG (ip)	–	–	Mice	78 ± 1.1
DSCG (ip)	–	–	Rats	80 ± 1.6

Table II. Effect of test compounds (*po*) on passive cutaneous anaphylaxis in mice/rats.

Compound No	*R¹*	*R²*	*Mice/rats*	*% Inhibition of anaphylaxis (PCA) (mean ± SE)*
3a	SMe	H	Mice	54 ± 1.7
3b	SMe	SMe	Mice	46 ± 1.2
3c	SEt	SEt	Mice	50 ± 2.2
3d	SPr (*n*)	SPr (*n*)	Mice	48.7 ± 1.4
5a	NH_2	H	Mice	55 ± 1.2
5b	NH_2	SMe	Mice	68 ± 1.8
5b	NH_2	SMe	Rats	85.3 ± 1.8
5c	NH_2	SEt	Mice	40 ± 0.8
5d	NH_2	SPr (*n*)	Mice	52.7 ± 1.4
7b	OH	SMe	Mice	63 ± 1.3
7c	OH	SEt	Mice	55 ± 1.3
7d	OH	SPr (*n*)	Mice	50.7 ± 1.5
9	NH_2	SO_2Me	Mice	27 ± 0.9
11	NH_2	OMe	Mice	58 ± 1.7

the standard drug disodium cromoglycate (DSCG) administered ip in similar doses since it is not absorbed orally. The most active compound **4b** was also tested at other doses and results are shown in table III.

There was significant protection of mast cell degranulation induced by non-immunological (compound **48–80** and dextran) and immunological (egg albumin) methods by compound **4b**. Compound **5b** (10 mg/kg po x 4 d) however, showed 64% protection of mast cell degranulation induced by compound **48–80**. The results are shown in table IV ($n = 3$).

Table III. Anti-PCA activity of **4b** in the rat.

Dose (mg/kg po)	*% Inhibition of PCA (mean ± SE)*
10	49.5 ± 2.3
25	74.0 ± 2.0
100	87.3 ± 1.4

Table IV. Effect of **4b** (10 mg/kg *po* x 4 d) and DSCG (10 mg/kg ip x 4 d) on mast cell degranulation.

Compound	*Comp **48–80***	*% Protection (mean ± SE)* *Dextran*	*Egg albumin*
4b	80 ± 2.5	67 ± 3.2	73 ± 2.5
DSCG	68 ± 1.5	57 ± 1.5	69 ± 2.0

Table V. Anti-histaminic and anti-serotonin activity of compound **4b** in guinea pig ileum.

Compound	*Concentration (μg/ml)*	*% Inhibition*	
		Histamine	*Serotonin*
4b	5.0	27	20
4b	10.0	47	44
Mepyramine	0.001	98	–
Cyproheptadine	5.0	–	90

The acute toxicity (ALD_{50}) values of compound **4b** and **5b** were 1000 mg and 681 mg/kg (ip) respectively. The anti-histaminic and anti-serotonin activity of compound **4b** in guinea pig ileum preparations is shown in table V.

Methods

Passive cutaneous anaphylactic (PCA) test in mice and rats

The PCA test was performed according to the procedure described by Kar *et al* [16]. A group of 4 male albino mice (20–25 g) per dose of compounds were passively sensitized by intradermal injection of 0.1 ml aliquots of rabbit hyperimmune anti egg albumin (EA) serum at dilution of 1 in 10 normal saline. Mice were injected 4 h later with 0.1 ml of 0.5% solution of Evans blue dye containing 1 mg EA. The animals were killed after 30 min and the coloured area of skin was measured both in control and treated groups. Results were expressed in terms of percentage inhibition of PCA as compared to control groups of animals receiving gum acacia in saline only. The test compounds (50 mg/kg po) were given 1 h before the antigen challenge. Disodium chromoglycate (DSCG) was used as reference standard.

The most active compounds were further tested in the PCA test in rats using the method described above. However, the latency period was 24 h instead of 4 h and rat homologous anti-egg albumin antibody was used.

Mast cell study

Ten ml normal saline was injected into the peritoneal cavity of normal and passively sensitised rats. After gentle massage, the peritoneal fluid was collected and transferred into the siliconised test tubes containing 7–10 ml RPMI-1640 medium (pH 7.2–7.4).

The cells were then washed 3 times by centrifugation at low speed (400–500 rpm) by discarding the supernatant and taking the pellet of mast cells into the medium. These cells were stained with 0.1% toluidine blue and observed under a microscope.

In the case of nonsensitised rats, 50 μl mast cells from the control and the treated group were treated with 0.5 μg/ml of compound **48–80** or 1 mg/ml dextran.

In the case of passively sensitised rats, the mast cells were challenged with 0.1 mg/ml egg albumin and incubated at 37°C in a water bath for 10 min, stained with toluidine blue and the percentage protection against degranulation counted under a microscope.

Determination of acute toxicity

The approximate LD_{50} (ALD_{50}) was studied in mice of either sex (20–25 g) by intraperitoneal administration of test compounds. Mice deprived of food for 16 h were divided into groups of 4 each and given intraperitoneal doses of 464 and 1000 mg/kg. Mortality over the next 24 h was recorded and the ALD_{50} calculated according to the method of Horn [17].

Guinea pig ileum preparation

The classical method of Magnus [18] was followed. Guinea pigs of either sex (350–450 g) were killed by stunning and bled by cutting their throats. The terminal segment (2–3 cm) of the ileum was suspended in an organ bath containing tyrode solution maintained at 37 ± 1°C saturated with air from a compressor. The test compound was added to the organ bath. Contractions of the ileal segment were recorded on a polygraph (Grass model-7). Contractions were induced by submaximal concentrations of histamine dihydrochloride (3 x 10^{-8} g/ml) and serotonin (6 x 10^{-7} g/ml).

Experimental protocols

Melting points were taken on Büchi 530 melting point apparatus and are uncorrected. The ^{1}H-NMR spectra were recorded on Perkin–Elmer R-30 (90 MHz) spectrometer. ^{13}C-NMR spectra were recorded on a Bruker WM 400 MHz NMR spectrometer at 100 MHz. Mass spectra were recorded on a Jeol JMS D 300 spectrometer at an ionization energy of 70 Ev. Infra-red spectra were taken on a Perkin–Elmer 157 grating infracord spectrometer. Column chromatography was performed on silica gel (60–120 mesh spectrochem). All compounds showed elemental analyses for C, H and N within 0.4% of the calculated values.

*General method for the N-1/N-2-alkylation of pyrazolo[3,4-d]pyrimidine **2** or **3***

A mixture of pyrazolo[3,4-*d*]pyrimidine (**1**, 10 mmol), DMF (30 ml), anhydrous K_2CO_3 (20 mmol) and chloroacetaldehyde diethyl acetal (12.5 mmol) was heated in an oil bath at 90°C for 16 h. The reaction mixture was cooled to room temperature, solid filtered and residue washed with DMF (2 x 10 ml). All DMF was removed at reduced pressure and the residue thus obtained was purified by column chromatography on silica gel with a mixture of ethyl acetate/hexane (15:85) to give pure **2** and **3**.

*1-(2',2'-Diethoxyethyl)-4-methylthio-1H-pyrazolo[3,4-d]pyrimidine **2a***
Yield: 20%, mp: 51°C (ethylacetate), MS: 282 (M^+, 8%), 103, ^{1}H-NMR ($CDCl_3$): 1.06 (t, J = 7 Hz, 6H, 2 x CH_2CH_3), 2.72 (s, 3H, SCH_3), 3.3–3.8 (m, 4H, 2 x OCH_2), 4.53 (d, J = 5.5 Hz, 2H, NCH_2), 5.02 (t, J = 5.5 Hz, 1H, C*H*-), 7.99 (s, 1H, H-3), 8.67 (s, 1H, H-6), ^{13}C-NMR ($CDCl_3$): 11.3 (SMe), 14.7 (2 x CH_2CH_3), 48.7 (NCH_2), 61.5 (2 x OCH_2), 99.4 (OCHO), 111.6, 131.0 (C-3), 150.8, 153.6, 164.9. Anal $C_{12}H_{18}N_4O_2S$ (C, H, N).

*2-(2',2'-Diethoxyethyl)-4-methylthio-2H-pyrazolo[3,4-d]pyrimidine **3a***
Yield: 28%, mp: 58°C (ethylacetate), MS: *m/z* 282 (M^+, 21%), ^{1}H-NMR ($CDCl_3$): 1.13 (t, J = 7 Hz, 6H, 2 x CH_2CH_3), 2.71 (s, 3H, SCH_3), 3.3–3.8 (m, 4H, 2 x OCH_2), 4.45 (d, J = 5.5 Hz, 2H, NCH_2), 4.95 (t, J = 5.5 Hz, 1H, CH), 8.09 (s, 1H, H-3), 8.79 (s, 1H, H-6), ^{13}C-NMR ($CDCl_3$): 11.8 (SCH_3), 15.4 (2 x CH_2CH_3), 56.9 (NCH_2), 63.8 (2 x OCH_2), 100.8 (OCHO), 111.4, 124.3 (C-3), 154.3, 157.4, 167.2. Anal $C_{12}H_{18}N_4O_2S$ (C, H, N).

*4,6-Bis(methylthio)-1-(2',2'-diethoxyethyl)-1H-pyrazolo[3,4-d]pyrimidine **2b***
Yield: 31%, mp: 48°C (ethylacetate/hexane, 1:1), MS: *m/z* 328 (M^+, 21%), ^{1}H-NMR ($CDCl_3$): 1.14 (t, J = 7 Hz, 6H, 2 x CH_2CH_3), 2.50 (s, 3H, SCH_3), 2.56 (s, 3H, SCH_3), 3.1–4.1 (m, 4H, 2 x OCH_2), 4.50 (d, J = 5.5 Hz, 2H, NCH_2), 5.08 (t, J = 5.5 Hz, 1H, CH), 7.97 (s, 1H, H-3), ^{13}C-NMR ($CDCl_3$): 11.3 (q, SCH_3), 13.8 (q, SCH_3), 14.7 (q, 2 x CH_2CH_3), 48.5 (t, CH_2N), 61.5 (t, 2 x OCH_2), 99.4 (d, OCHO), 108.8 (s), 131.2 (d, C-3), 151.8 (s), 164.2 (s), 168.3 (s). Anal $C_{13}H_{20}N_4O_2S_2$ (C, H, N).

*4,6-Bis(methylthio)-2-(2',2'-diethoxyethyl)-2H-pyrazolo[3,4-d]pyrimidine **3b***
Yield: 36%, mp: 100°C (ethylacetate/hexane, 2:1), MS: *m/z* 328 (M^+, 26%), ^{1}H-NMR ($CDCl_3$): 1.06 (t, J = 7 Hz, 6H, 2 x CH_2CH_3), 2.57 (s, 3H, SCH_3), 2.61 (s, 3H, SCH_3), 3.1–4.0 (m, 4H, 2 x OCH_2), 4.28 (d, J = 5.5 Hz, 2H, CH_2N), 4.90 (t, J = 5.5 Hz, 1H, CH), 7.94 (s, 1H, H-3), ^{13}C-NMR ($CDCl_3$): 11.3 (q, SCH_3), 13.7 (q, SCH_3), 14.7 (q, 2 x CH_2CH_3), 56.3 (t, CH_2N), 63.3 (t, 2 x OCH_3), 100.4 (d, OCHO), 108.4 (s), 124.6 (d, C-3), 158.2 (s), 165.9 (s), 168.0 (s). Anal $C_{13}H_{20}N_4O_2S_2$ (C, H, N).

*4,6-Bis(ethylthio)-1-(2',2'-diethoxyethyl)-1H-pyrazolo[3,4-d]pyrimidine **2c***
Yield: 35% viscous liquid, MS: *m/z* 356 (M^+, 10%), ^{1}H-NMR ($CDCl_3$): 1.09 (t, J = 7 Hz, 6H, 2 x OCH_2CH_3), 1.41 (t, J = 7 Hz, 6H, 2 x SCH_2CH_3), 2.9–3.8 (m, 8H, 2 x SCH_2, 2 x OCH_2), 4.38 (d, J = 5.5 Hz, 2H, NCH_2), 4.91 (t, J = 5.5 Hz, 1H, CH), 7.74 (s, 1H, H-3), ^{13}C-NMR ($CDCl_3$): 14.4 (2 x CH_3), 15.0 (2 x CH_3), 23.2 (SCH_2), 25.2 (SCH_2), 48.8 (NCH_2), 61.9 (2 x OCH_2), 99.7 (OCHO), 109.3, 131.6 (C-3), 152.2, 164.4, 168.2. Anal $C_{15}H_{24}N_4O_2S_2$ (C, H, N).

*4,6-Bis(ethylthio)-2-(2',2'-diethoxyethyl)-2H-pyrazolo[3,4-d]pyrimidine **3c***
Yield: 40%, viscous liquid, MS: *m/z* 356 (M^+, 15%), ^{1}H-NMR ($CDCl_3$): 1.12 (t, J = 7 Hz, 6H, 2 x OCH_2CH_3), 1.39 (t, J = 7 Hz, 6H, 2 x SCH_2CH_3), 2.9–3.8 (m, 8H, 2 x SCH_2, 2 x OCH_2), 4.23 (d, J = 5.5 Hz, 2H, NCH_2), 4.82 (t, J = 5.5 Hz, 1H, CH), 7.75 (s, 1H, H-3), ^{13}C-NMR ($CDCl_3$): 14.1 (CH_3), 14.3 (CH_3), 14.9 (2 x CH_3), 23.3 (SCH_2), 24.9 (SCH_2), 56.5 (NCH_2), 63.7 (2 x OCH_2), 100.6 (OCHO), 108.8, 124.8 (C-3), 158.5, 166.0, 167.8. Anal $C_{15}H_{24}N_4O_2S_2$ (C, H, N).

*4,6-Bis(n-propylthio)-1-(2',2'-diethoxyethyl)-1H-pyrazolo[3,4-d]pyrimidine **2d***
Yield: 32%, viscous liquid, MS: *m/z* 384 (M^+, 14%), ^{1}H-NMR ($CDCl_3$): 1.08 (t, J = 7 Hz, 6H, 2 x CH_3), 1.12 (t, J = 7 Hz, 6H, 2 x CH_3), 1.8 (sextet, J = 7 Hz, 4H, 2 x $SCH_2CH_2CH_3$), 3.1–3.9 (m, 8H, 2 x SCH_2, 2 x OCH_2), 4.46 (d, J = 5.5 Hz, 2H, NCH_2), 5.02 (t, J = 5.5 Hz, 1H, CH), 7.86 (s, 1H, H-3), ^{13}C-NMR ($CDCl_3$): 13.1 (CH_3), 13.2 (CH_3), 15.0 (2 x CH_3), 22.3 ($CH_2CH_2CH_3$), 22.5 ($CH_2CH_2CH_3$), 30.2 (SCH_2), 32.3 (SCH_2), 48.7 (NCH_2), 61.6 (2 x OCH_2), 99.4 (OCHO), 108.9, 131.8 (C-3), 151.8, 164.3, 167.5. Anal $C_{17}H_{28}N_4O_2S_2$ (C, H, N).

*4,6-Bis(n-propyl-thio)-2-(2',2'-diethoxyethyl)-2H-pyrazolo[3,4-d]pyrimidine **3d***
Yield: 41%, viscous liquid; MS: *m/z* 384 (M^+, 18%), ^{1}H-NMR ($CDCl_3$): 1.03 (t, J = 7 Hz, 6H, 2 x CH_3), 1.1 (t, J = 7 Hz, 6H, 2 x CH_3), 1.8 (sextet, J = 7 Hz, 4H, 2 x $SCH_2CH_2CH_3$), 3.0–3.8 (m, 8H, 2 x SCH_2), 2 x OCH_2), 4.25 (d, J = 5.5 Hz, 2H, NCH_2), 5.0 (t, J = 5 Hz, 1H, CH), 7.66 (s, 1H, H-3), ^{13}C-NMR ($CDCl_3$): 13.3 (CH_3), 13.5 (CH_3), 15.0 (2 x CH_3), 22.4

($CH_2CH_2CH_3$), 22.5 ($CH_2CH_2CH_3$), 30.8 (SCH_2), 32.7 (SCH_2), 56.7 (NCH_2), 63.9 (2 x OCH_2), 100.8 (OCHO), 109.0, 124.9 (C-3), 158.6, 166.2, 168.1. Anal $C_{17}H_{28}N_4O_2S_2$ (C, H, N).

*General method for the preparation of 4-amino-1/2-(2',2'-diethoxyethyl)-1H/2H-pyrazolo[3,4-d]pyrimidines **4** or **5***

A mixture of 4,6-bis (methylthio)-1/2-(2',2'-diethoxyethyl)-1*H*/2*H*-pyrazolo[3,4-*d*]pyrimidine (**2** or **3**, 1 mmol) and methanolic ammonia (≈ 25 ml) was heated at 120–130°C in a steel bomb for 18–24 h. The reaction vessel was cooled in ice-water and contents were removed quantitatively with the help of methanol. All the solvent was removed and the residue was purified either by crystallization or column chromatography on silica gel followed by crystallization to give an analytically pure product (**4** or **5**).

*4-Amino-1-(2',2'-diethoxyethyl)-1H-pyrazolo[3,4-d]pyrimidine **4a***
Yield: 85%, mp: 121°C (chloroform/hexane, 3:1). IR (KBr): (NH_2), 3300, 3340 cm^{-1}, MS: *m/z* 251 (M^+, 7%), ^{1}H-NMR ($CDCl_3$): 1.03 (t, *J* = 7 Hz, 6H, 2 x CH_3), 3.2–3.8 (m, 4H, 2 x OCH_2), 4.34 (d, *J* = 5.5 Hz, 2H, NCH_2), 4.99 (t, *J* = 5.5 Hz, 1H, H-3), 7.5 (bs, 2H, NH_2), 8.08 (s, 1H), 8.16 (s, 1H). Anal $C_{11}H_{17}N_5O_2$ (C, H, N).

*4-Amino-1-(2',2'-diethoxyethyl)-6-methylthio-1H-pyrazolo[3,4-d]pyrimidine **4b***
Yield: 90%, mp: 143°C (chloroform/hexane, 2:1). IR (KBr): (NH_2) 3350 cm^{-1}, MS: *m/z* 297 (M^+, 10%), ^{1}H-NMR ($CDCl_3$): 1.04 (t, *J* = 7 Hz, 2 x CH_3), 2.5 (s, 3H, SCH_3), 3.0–4.0 (m, 4H, 2 x OCH_2), 4.3 (d, *J* = 5.5 Hz, 2H, NCH_2), 5.0 (t, *J* = 5.5 Hz, 1H, CH), 5.8 (bs, 2H, NH_2), 7.68 (s, 1H, H-3), ^{13}C-NMR (DMSO–d_6): 13.2 (SCH_3), 14.9 (2 x CH_3), 48.4 (NCH_2), 61.3 (2 x OCH_2), 97.7, 99.4, 132.2 (C-3), 154.1, 157.0, 168.4. Anal $C_{12}H_{19}N_5O_2S$ (C, H, N).

*4-Amino-1-(2',2'-diethoxyethyl)-6-ethylthio-1H-pyrazolo[3,4-d]pyrimidine **4c***
Yield: 91%, mp: 145°C (ethylacetate/hexane, 2:1). IR (KBr): (NH_2) 3320, 3350 cm^{-1}, MS: 312 [$(M + 1)^+$ 90%]. ^{1}H-NMR ($CDCl_3$): 1.09 (t, *J* = 7 Hz, 6H, 2 x CH_3), 1.38 (t, *J* = 7 Hz, 3H, SCH_2CH_3), 3.1 (q, *J* = 7 Hz, 2H, SCH_2CH_3), 3.2–3.8 (m, 4H, 2 x OCH_2), 4.34 (d, *J* = 5.5 Hz, 2H, NCH_2), 4.94 (t, *J* = 5.5 Hz, 1H, CH), 5.7 (brs, 2H, NH_2), 7.6 (s, 1H, H-3). Anal $C_{13}H_{21}N_5O_2S$ (C, H, N).

*4-Amino-1-(2',2'-diethoxyethyl)-6-n-propylthio-1H-pyrazolo-[3,4-d]pyrimidine **4d***
Yield: 81%, mp: 122°C (ethylacetate/hexane, 2:1), IR (KBr): (NH_2) 3325, 3450 cm^{-1}; MS: *m/z* 325 (M^+, 8%), ^{1}H-NMR ($CDCl_3$): 1.12 (unresolved t, *J* = 7 Hz, 9H, 3 x CH_3), 1.78 (sextet, *J* = 7 Hz, 2H, $SCH_2CH_2CH_3$), 3.15 (t, *J* = 7 Hz, 2H, SCH_2), 3.3–3.9 (m, 4H, 2 x OCH_2), 4.44 (d, *J* = 5.5 Hz, 2H, NCH_2), 5.06 (t, *J* = 5.5 Hz, 1H, CH), 5.6 (brs, 2H, NH_2), 7.8 (s, 1H, H-3). Anal $C_{14}H_{23}N_5O_2S$ (C, H, N).

*4-Amino-2-(2',2'-diethoxyethyl)-2H-pyrazolo[3,4-d]pyrimidine **5a***
Yield 82%, mp: 196°C (ethylacetate/hexane, 3:1), IR (KBr): (NH_2) 3320, 3360 cm^{-1}; MS: *m/z* 251 (M^+, 6%), ^{1}H-NMR (DMSO–d_6): 1.09 (t, *J* = 7 Hz, 6H, 2 x CH_3), 3.2–3.8 (m, 4H, 2 x OCH_2), 4.37 (d, *J* = 5.5 Hz, 2H, NCH_2), 4.91 (t, *J* = 5.5 Hz, 1H, CH), 7.4 (brs, 2H, NH_2), 8.07 (s, 1H, H-3), 8.32 (s, 1H, H-6). Anal $C_{11}H_{17}N_5O_2$ (C, H, N).

*4-Amino-2-(2',2'-diethoxyethyl)-6-methylthio-2H-pyrazolo[3,4-d]pyrimidine **5b***
Yield: 90%, mp: 169°C (chloroform/hexane, 2:1), IR (KBr): (NH_2) 3340 cm^{-1}; MS: *m/z* 297 (M^+, 24%), ^{1}H-NMR ($CDCl_3$): 1.05 (t, *J* = 7 Hz, 6H, 2 x CH_3), 2.5 (s, 3H, SCH_3), 3.1–4.0 (m, 4H, 2 x OCH_2), 4.22 (d, *J* = 5.5 Hz, 2H, NCH_2), 4.87 (s, 1H, CH), 7.0 (brs, 2H, NH_2), 8.16 (s, 1H, H-3). Anal $C_{12}H_{19}N_5O_2S$ (C, H, N).

*4-Amino-2-(2',2'-diethoxyethyl)-6-ethylthio-2H-pyrazolo[3,4-d]pyrimidine **5c***
Yield: 84%, mp: 170°C (ethylacetate/hexane, 3:1). IR (KBr): (NH_2) 3300, 3390 cm^{-1}, MS: *m/z* 312 [$(M + 1)^+$, 10%], ^{1}H-NMR ($CDCl_3$): 1.10 (t, *J* = 7 Hz, 6H, 2 x CH_3), 1.36 (t, *J* = 7 Hz, 3H, SCH_2CH_3), 3.14 (q, *J* = 7 Hz, 2H, SCH_2CH_3), 3.2–3.9 (m, 4H, 2 x OCH_2), 4.21 (d, *J* = 5.5 Hz, 2H, NCH_2), 4.77 (t, *J* = 5.5 Hz, 1H, CH), 6.0 (brs, 2H, NH_2), 7.72 (s, 1H, H-3). Anal $C_{13}H_{21}N_5O_2S$ (C, H, N).

*4-Amino-2-(2',2'-diethoxyethyl)-6-n-propylthio-2H-pyrazolo[3,4-d]pyrimidine **5d***
Yield: 76%, mp: 160°C (ethylacetate/hexane, 2:1), IR (KBr) (NH_2) 3320, 3400 cm^{-1}; MS: *m/z* 325 (M^+, 25%), ^{1}H-NMR ($CDCl_3$): 1.10 (t, *J* = 7 Hz, 9H, 3 x CH_3), 1.78 (sextet, *J* = 7 Hz, 2H, $SCH_2CH_2CH_3$), 3.20 (t, *J* = 7 Hz, 2H, SCH_2), 3.2–3.9 (m, 4H, 2 x OCH_2), 4.31 (d, *J* = 5.5 Hz, 2H, NCH_2), 4.93 (t, *J* = 5.5 Hz, 1H, CH), 6.08 (brs, 2H, NH_2), 7.90 (s, 1H, H-3). Anal $C_{14}H_{23}N_5O_2S$ (C, H, N).

*General method for the preparation of 1/2-(2',2'-diethoxyethyl)-6-methylthio-4(5H)-oxo-1H/2H-pyrazolo[3,4-d]pyrimidines **6** or **7***

A mixture of 4,6-bis(methylthio)-1/2-(2',2'-diethoxyethyl)-1*H*/2*H*-pyrazolo[3,4-*d*]pyrimidine (**2** or **3**, 1 mmol), 1 N NaOH solution (20 ml) and methanol (20 ml) was refluxed for 2–3 h. The reaction mixture was cooled to room temperature and neutralized with glacial acetic acid. All the solvents were removed by water aspirator. The residue was taken up in chloroform (50 ml) and organic layer was washed with water (3 x 15 ml) and dried over sodium sulphate. The solvent was removed at reduced pressure and the product purified by crystallization to give analytically pure product (**6** or **7**).

*1-(2',2'-Diethoxyethyl)-6-methylthio-4(5H)-oxo-1H-pyrazolo-[3,4-d]pyrimidine **6b***
Yield: 86%, mp: 121°C (ethylacetate/chloroform, 1:1), IR (KBr) (C=O) 1670 cm–^{1}, MS: *m/z* 298 (M^+, 12%), ^{1}H-NMR ($CDCl_3$): 1.13 (t, *J* = 7 Hz, 6H, 2 x CH_3), 2.68 (s, 3H, SCH_3), 3.2–3.9 (m, 4H, 2 x OCH_2), 4.46 (d, *J* = 5.5 Hz, 2H, NCH_2), 5.08 (t, *J* = 5.5 Hz, 1H, CH), 8.1 (s, 1H, H-3). Anal $C_{12}H_{18}N_4O_3S$ (C, H, N).

*1-(2',2'-Diethoxyethyl)-6-ethylthio-4(5H)-oxo-1H-pyrazolo-[3,4-d]pyrimidine **6c***
Yield: 75%, mp: 123°C (ethylacetate/chloroform, 1:1), IR (KBr) (C=O) 1680 cm^{-1}; MS: *m/z* 312 (M^+, 2%), ^{1}H-NMR ($CDCl_3$): 1.1 (t, *J* = 7 Hz, 6H, 2 x CH_3), 1.4 (t, *J* = 7 Hz, 3H, SCH_2CH_3), 3.17 (q, *J* = 7 Hz, 2H, SCH_2), 3.2–3.8 (m, 4H, 2 x OCH_2), 4.3 (d, *J* = 5.5 Hz, 2H, NCH_2), 4.9 (t, *J* = 5.5 Hz, 1H, CH), 7.8 (s, 1H, H-3). Anal $C_{13}H_{20}N_4O_3S$ (C, H, N).

*1-(2',2'-Diethoxyethyl)-4(5H)-oxo-6n-propylthio-1H-pyrazolo-[3,4-d]pyrimidine **6d***
Yield: 76%, mp: 106°C (ethylacetate/hexane, 3:1), IR (KBr) (C=O) 1665 cm^{-1}; MS: *m/z* 326 (M^+, 10%), ^{1}H-NMR ($CDCl_3$):

1.03 (t, J = 7 Hz, 3H, CH_3), 1.12 (t, J = 7 Hz, 6H, 2 x OCH_2CH_3), 1.84 (sextet, J = 7 Hz, 2H, $SCH_2CH_2CH_3$), 3.24 (t, J = 7 Hz, 2H, SCH_2), 3.3–3.8 (m, 4H, 2 x OCH_2), 4.38 (d, J = 5.5 Hz, 2H, NCH_2), 5.0 (t, J = 5.5 Hz, 1H, CH), 7.98 (s, 1H, H-3). Anal $C_{14}H_{22}N_4O_3S$ (C, H, N).

2-(2',2'-Diethoxyethyl)-6-methylthio-4(5H)-oxo-2H-pyrazolo-[3,4-d]pyrimidine ***7b***
Yield: 84%, mp: 181°C (ethylacetate), IR (KBr) (C=O) 1690 cm^{-1}, MS: *m/z* 298 (M^+, 5%), ^{1}H-NMR ($CDCl_3$): 1.16 (t, J = 7 Hz, 6H, 2 x CH_3), 2.7 (s, 3H, SCH_3), 3.3–4.0 (m, 4H, 2 x OCH_2), 4.37 (d, J = 5.5 Hz, 2H, NCH_2), 4.98 (t, J = 5.5 Hz, 1H, CH), 8.26 (s, 1H, H-3). Anal $C_{12}H_{18}N_4O_3S$ (C, H, N).

2-(2',2'-Diethoxyethyl)-6-ethylthio-4(5H)-oxo-2H-pyrazolo[3,4-d]pyrimidine ***7c***
Yield: 78%, mp: 185°C (ethylacetate/chloroform, 3:1), IR (KBr) (C=O) 1650 cm^{-1}, MS: *m/z* 312 (M^+, 19%), ^{1}H-NMR ($CDCl_3$): 1.15 (t, J = 7 Hz, 6H, 2 x CH_3), 1.40 (t, J = 7 Hz, 3H, SCH_2CH_3), 3.20 (q, J = 7 Hz, 2H, SCH_2), 3.2–3.8 (m, 4H, 2 x OCH_2), 4.2 (d, J = 5.5 Hz, 2H, NCH_2), 4.8 (t, J = 5.5 Hz, 1H, CH), 7.9 (s, 1H, H-3). Anal $C_{13}H_{20}N_4O_3S$ (C, H, N).

2-(2',2'-Diethoxyethyl)-4(5H)-oxo-6-n-propylthio-2H-pyrazolo-[3,4-d]pyrimidine ***7d***
Yield: 89%, mp: 195°C (ethylacetate/hexane, 4:1), IR (KBr) (C=O) 1660 cm^{-1}, MS: 326 (M^+, 19%), ^{1}H-NMR ($CDCl_3$): 1.03 (t, J = 7 Hz, 3H, $SCH_2CH_2CH_3$), 1.15 (t, J = 7 Hz, 6H, 2 x CH_3), 1.83 (sextet, J = 7 Hz, 2H, $SCH_2CH_2CH_3$), 3.28 (t, J = 7 Hz, 2H, SCH_2), 3.2–3.8 (m, 4H, 2 x OCH_2), 4.27 (d, J = 5.5 Hz, 2H, NCH_2), 4.88 (t, J = 5.5 Hz, 1H, CH), 8.08 (s, 1H, H-3). Anal $C_{14}H_{22}N_4O_3S$ (C, H, N).

4-Amino-1-(2',2'-diethoxyethyl)-6-methoxy-1H-pyrazolo[3,4-d]pyrimidine ***10***
To a solution of **4b** (297 mg, 1 mmol), in glacial acetic acid (5 ml) and water (5 ml) at 0°C was added solid $KMnO_4$ (316 mg, 2 mmol) in portions and the mixture stirred for 1 h at ambient temperature. The excess of $KMnO_4$ was decomposed by careful addition of H_2O_2 (30% aqueous solution). The colorless solution was extracted with chloroform, washed with aqueous $NaHCO_3$ solution, water and dried over anhydrous Na_2SO_4. Removal of the solvent gave a residue which was crystallized from a mixture of chloroform and hexane, to give sulfone **8** as a white solid, mp: 204°C (206 mg, 63%). ^{1}H-NMR ($CDCl_3$): 1.08 (t, J = 7 Hz, 6H, 2 x CH_3), 3.31 (s, 3H, SO_2CH_3), 3.2–3.9 (m, 4H, 2 x OCH_2), 4.47 (d, J = 5.5 Hz, 2H, NCH_2), 4.98 (t, J = 5.5 Hz, 1H, CH), 7.1 (brs, 2H, NH_2), 7.96 (s, 1H, H-3). This compound (165 mg, 0.5 mmol) was added to a solution of sodium methoxide (prepared by adding 83 mg Na to 10 ml MeOH) and reaction mixture was refluxed for 2 h. The reaction mixture was cooled, neutralized with acetic acid and solvent removed to give a white residue which was purified by column chromatography on silica gel with ethylacetate.

Yield: 123 mg (87%), mp: 191°C (chloroform/hexane, 3:1); IR (KBr) (NH_2) 3320, 3380 cm^{-1}, MS: *m/z* 282 [$(M + 1)^+$, 4%] ^{1}H-NMR ($CDCl_3$): 1.08 (t, J = 7.0 Hz, 2 x CH_3), 3.2–3.9 (m, 4H, 2 x OCH_2), 3.9 (s, 3H, OCH_3), 4.33 (d, J = 5.5 Hz, 2H, NCH_2), 4.99 (t, J = 5.5 Hz, 1H, CH), 5.7 (brs, 2H, NCH_2), 7.7 (s, 1H, H-3). Anal $C_{12}H_{19}N_5O_3$ (C, H, N).

4-Amino-2-(2',2'-diethoxyethyl)-6-methoxy-2H-pyrazolo[3,4-d]pyrimidine ***11***
A similar method as described above for **8** and **10** was used. Thus 4-amino-2-(2',2'-diethoxyethyl)-6-methanesulfonyl-2*H*-pyrazolo[3,4-*d*]pyrimidine **9**, was obtained in 53% yield, mp: 192°C (chloroform/hexane, 4:1), ^{1}H-NMR ($CDCl_3$): 1.1 (t, J = 7 Hz, 6H, 2 x CH_3), 3.43 (s, 3H, OCH_3), 3.2–3.9 (m, 4H, 2 x OCH_2), 4.44 (d, J = 5.5 Hz, 2H, NCH_2), 4.94 (t, J = 5.5 Hz, 1H, CH), 8.25 (s, 1H, H-3).

This sulfone on treatment with sodium methoxide gave **11** in 82% yield, mp: 183°C (chloroform/hexane, 3:1). IR (KBr): (NH_2), 3310, 3345 cm^{-1}, MS: *m/z* 282 [$(M + 1)^+$, 40%], ^{1}H-NMR ($CDCl_3$): 1.14 (t, J = 7 Hz, 6H, 2 x CH_3), 3.2–3.9 (m, 4H, 2 x OCH_2), 3.95 (s, 3H, OCH_3), 4.26 (d, J = 5.5 Hz, 2H, NCH_2), 4.85 (t, J = 5.5 Hz, 1H, CH), 6.4 (brs, 2H, NH_2), 7.9 (s, 1H, H-3). Anal $C_{12}H_{19}N_5O_3$ (C, H, N).

Acknowledgments

Financial support from the Office of Naval Research (Grant N-00014-82-G-0130) is gratefully acknowledged. Thanks are due to the staff of RSIC, Lucknow, India, for providing elemental analyses and spectral data and to Mr Amarnath for technical assistance.

References

1 Murphy S, Kelley HW (1987) *Drug Intell Clin Pharm* 21, 22–27
2 Suschitzsky JL, Sheard P (1984) *Progress in Medicinal Chemistry* (Ellis GP, West GB, eds) Elsevier Science, Amsterdam, vol 21, 1–51
3 Sangwan NK, Rastogi SN, Kar K (1987) *Eur J Med Chem* 22, 153–156
4 Orange RP, Kaliner MA, Laraia PJ, Austen KF (1971) *Fed Proc Am Soc Exp Biol* 30, 1725–1729
5 Lichtenstern LM, Margolis S (1968) *Science* 161, 902–903
6 Holland A, Jackson D, Chaplen P, Lunt E, Marshall S, Pain D, Wooldridge K (1975) *Eur J Med Chem* 10, 447–449
7 Hendeles L, Weinberger M (1980) *Eur J Res Dis* (suppl) (109) 61, 103–106
8 Littenberg B (1988) *J Am Med Assoc* 259, 1678–1684
9 Naito T, Nakagawa S, Okita Taka-Aki, Yamashita H, Yamasaki T, Kamei H, Tomatsu K, Imanishi H, Kawaguchi H (1982) *Chem Pharm Bull* 30, 2011–2019
10 Misra S, Jain S, Avasthi K, Bhakuni DS (1990) *Nucleosides Nucleotides* 9, 837–846
11 Deo K, Avasthi K, Ram Pratap, Bhakuni DS, Kar K (1989) *Ind J Chem* 288, 237–241
12 Falo EA, Hitchings GH (1956) *J Am Chem Soc* 78, 3143–3145
13 Taylor EC, Warrener RN, McKillop A (1966) *Angew Chem Int Ed Engl* 5, 309
14 Garg N, Avasthi K, Bhakuni DS (1989) *Synthesis* 876–878
15 Holy A, Votruba I, De Clercq E (1982) *Coll Czech Chem Commun* 47, 1392–1407
16 Kar K, Bajpai SK, Dhawan BN (1978) *Curr Sci* 47, 406–408
17 Horn HJ (1956) *Biometrics* 12, 311–316
18 Magnus F (1904) *Pflugers Arch Ges Physiol* 102, 123–125