Synthesis of Certain Alkenyl Purines and Purine Analogs as Inhibitors of Tumor Necrosis Factor Alpha (TNFα)

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The preparation of 2-penten-1-yl and 3-methyl-2-buten-1-yl derivatives of adenine **2a,b**, 7-deazaadenine **2c,d**, 2-aminopurine **4a,b** and **5a,b**, 4-aminopyrazolo[3,4-*d*]pyrimidine **7a,b** and 7-amino-v-triazolo-[4,5-*d*]pyrimidine **8a-10a** and **8b-10b** is described. The synthesis of compounds **2a-d** was accomplished by the functional group transformation reaction, whereas the synthesis of **4a-8a** and **4b-8b** was performed by the alkylation of the sodium salt of the heterocycles with alkenyl bromides. These alkenyl derivatives prepared as congeners of pentoxifylline (methylxanthine) were evaluated for their anti-tumor necrosis factor α activity in human monocytic leukemia cells. Only compounds **7a** and **7b** exhibited significant activity and a poor toxicity profile in this assay. In peripheral blood mononuclear cells, compounds **7a** and **7b**, inhibited tumor necrosis factor α production in a dose dependent manner.

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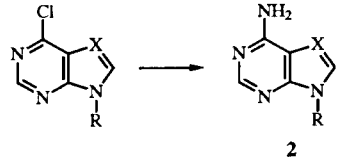
Tumor necrosis factor alpha, a mononuclear phagocytic cell derived protein, was originally described as a product of activated macrophages and shown to display tumoricidal activity [1-4]. Extensive research during the last few years has made it apparent that tumor necrosis factor α is a highly pleiotropic cytokine [5] and may play a role in tumorigenesis, septic shock, multiple sclerosis, cachexia, inflammation, autoimmunity, and other immunological and pathological reactions [6]. There are two forms of tumor necrosis factor α, a type II membrane protein of relative molecular mass 26,000 (26 kilodaltons) and a soluble, 17 kilodaltons form generated from the cell-bound protein by proteolytic cleavage. Several different types of tumors have been described in which tumor necrosis factor α acts as an autocrine growth factor, including leukemias, ovarian tumors, renal cell carcinoma, breast adenocarcinoma, neuroblastoma, and glioblastoma [7-11]. These tumors express tumor necrosis factor α and its receptors and furthermore proliferate in response to the cytokine. Agents that can block the production of tumor necrosis factor α in these different tumor cell types may have potential as antitumor agents.

Cytokines such as tumor necrosis factor α and interleukin-1β also play a central role in the regulation of the immune system and they have been implicated in inflammatory processes as well as in the pathogenesis of many diseases [12,13] Tumor necrosis factor α, first associated with tumor regression and with cachexia accompanying chronic invasive diseases, is now established as an immune modulator in normal and chronic inflammation situations as well as with septic shock [14]. Deregulated production of tumor necrosis factor α in humans is thought to contribute to the development of diseases such as cancer-associated cachexia [15], endotoxic shock [16], graft *versus* host disease [17], autoimmunity [18] and rheumatoid arthritis [19,20]. Therefore agents that can inhibit the production or maturation of tumor necrosis factor α and interleukin-1β in these different indications may have excellent therapeutic potential.

Since the role of tumor necrosis factor α and interleukin-1,β in the development of the septic shock syndrome and other ailments has been recognized [21], attempts have been made to suppress the production of these pathogenetic factors. Phosphodiesterase inhibitors [22] are potential agents for blocking the cytokine pathway due to their ability to suppress tumor necrosis factor production *via* elevation of intracellular adenosine 3',5'-cyclic phosphate [23]. The suppression of tumor necrosis factor α production by adenosine and certain xanthine derivatives (*e.g.* pentoxifylline) [24] by inhibition of phosphodiesterase activity has recently been demonstrated [25]. In the present study we describe the synthesis of 2-penten-1-yl and 3-methyl-2-buten-1-yl derivatives of certain purines and purine analogs, and their effect on tumor necrosis factor α production in human monocytic leukemia cells.

Synthesis of the alkenyl derivatives of adenine and 7-deazaadenine was accomplished starting from the corresponding chloro derivatives. Thus, the chloro derivatives **1a-1d** required were synthesized as reported earlier [26]. Treatment of **1a-1d** with methanolic ammonia at elevated

Scheme 1

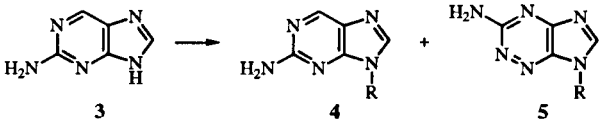


2

a) X = N; R = -CH_2CH=$CHCH_2CH_3$
b) X = N; R = -CH_2CH=$C(CH_3)_2$
c) X = CH; R = -CH_2CH=$CHCH_2CH_3$
d) X = CH; R = -CH_2CH=$C(CH_3)_2$

temperature gave the desired amino derivatives **2a-2d** in 64-73% yield (Scheme 1). The alkenyl derivatives of 2-aminopurine were synthesized starting from 2-aminopurine itself. Thus, the reaction of 2-aminopurine (**3**) with 1-bromo-2-pentene (predominantly the *Z* isomer) in dry *N,N*-dimethylformamide (DMF) in the presence of sodium hydride afforded a product which was a mixture of two positional isomers (Scheme 2); These isomers

Scheme 2



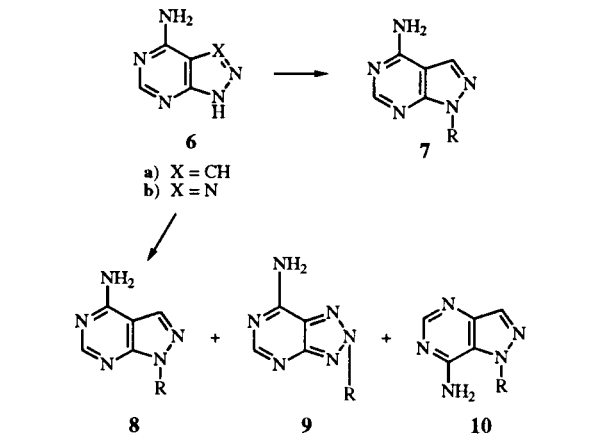
3 **4** **5**

a) R = -CH_2CH=$CHCH_2CH_3$
b) R = -CH_2CH=$C(CH_3)_2$

were separated by silica gel column chromatography. The major component 2-amino-9-[(*Z*)-2-penten-1-yl]purine (**4a**) was isolated in a 53% yield, whereas the minor component 2-amino-7-[(*Z*)-2-penten-1-yl]purine (**5a**) was isolated in 15% yield. The site of alkylation was established by comparison of the uv absorption maxima of **4a** and **5a** to that of the corresponding known nucleosides [27]. Similarly, alkylation of **3** with 4-bromo-2-methyl-2-butene gave the positional isomers **4b** and **5b** in 47% and 16% yields, respectively.

The alkenyl derivatives of 4-aminopyrazolo[3,4-*d*]-pyrimidine (**6a**) and 7-amino-v-triazolo[4,5-d]pyrimidine (**6b**) were synthesized starting from the corresponding amino base itself. Thus, the reaction of **6a** with either 1-bromo-2-pentene or 4-bromo-2-methyl-2-butene in DMF in the presence of sodium hydride afforded mainly one isomer (Scheme 3). The products **7a** and **7b** were isolated in 37% and 28% yields, respectively. The site of alkylation of these compounds was established by comparison of the uv absorpton maxima to that of the known

Scheme 3



6 **7**

a) X = CH
b) X = N

8 **9** **10**

a) R = -CH_2CH=$CHCH_2CH_3$
b) R = -CH_2CH=$C(CH_3)_2$

corresponding ribonucleosides [28]. Alkylation of **6b** with 1-bromo-2-pentene in DMF in the presence of sodium hydride gave a mixture of three positional isomers **8a, 9a,** and **10a** (Scheme 3). These isomers were separated by silica gel column chromatography. The N3 and N2 isomers, compounds **8a** and **9a,** were obtained in 25% and 24% yield, respectively, where as the N1 isomer **10a** was obtained in only 12% yield. Again the site of alkylation in these isomers was assigned on the basis of the comparison of the uv spectral data to that of the literature data of the known methyl derivatives [29]. Similarly, treatment of **6b** with 4-bromo-2-methyl-2-butene gave **8b, 9b,** and **10b** in 27%, 25% and 11% yields, respectively.

The alkenyl derivatives of purines and purine analogs synthesized during this study were evaluated *in vitro* for their anti-tumor necrosis factor α activity in human monocytic leukemia cells in comparison with the known tumor necrosis factor α inhibitor, carbocyclic adenosine analog aristeromycin. The concentration (μg/ml) of the compound required to reduce the production of tumor necrosis factor α by 50% (IC_{50}) values along with the concentration of the compound required to inhibit the cell

Table 1

Effect of Alkenyladenine Analogs on Tumor Necrosis Factor Alpha Production in Human Monocytic Cell Line

Compound No.	Tumor Necrosis Factor Alpha Inhibition IC_{50} [a] μg/ml	Cytotoxicity TC_{50} [b] μg/ml
2a	>10	<2.34
2b	>10	<2.34
2c	>10	<2.34
2d	>10	<2.34
4a	>10	<2.34
4b	>10	3.59
5a	>10	18.42
5b	>10	<2.34
7a	2.6	6.9
7b	4.7	13.1
8a	>10	18.73
8b	>10	113.01
9a	>10	25.06
9b	>10	<2.34
10a	>10	27.7
10b	>10	7.73
aristeromycin	11.5	12.5

[a] IC_{50} is the concentration of the compound required to reduce the production of tumor necrosis factor alpha by 50%. [b] TC_{50} is the concentration of the compound required to inhibit the cell growth by 50%.

growth by 50% (TC_{50}) are tabulated in Table 1. The biological activity of these compounds was determined as described in the experimental section. Within the limits of biological variability, only two compounds, **7a** and **7b** exhibited significant activity, however they also produced a poor toxicity profile in this assay system. These two

compounds and aristeromycin were further evaluated for their ability to inhibit tumor necrosis factor α expression in peripheral blood mononuclear cells. The results from this study are presented in Figure 1. The compounds **7a** and **7b** inhibited tumor necrosis factor α in a dose dependent manner for at least 48 hours. Unlike in the human monocytic leukemia cells, these compounds were less toxic in the primary cells (Data not shown). These preliminary data suggest that compounds **7a** and **7b** are effective inhibitors of tumor necrosis factor α production and further improvements of these simple compounds might be useful in controlling tumor necrosis factor α expression and subsequent functions.

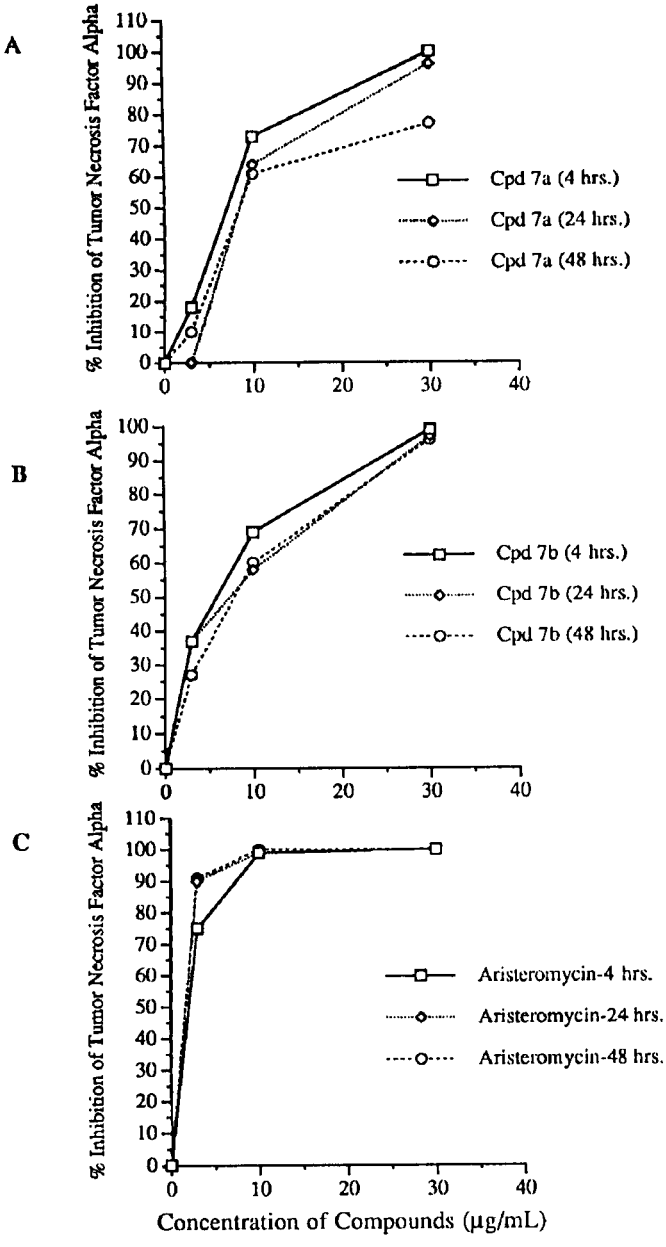


Figure 1

EXPERIMENTAL

Melting points (mp, uncorrected) were determined with a Thomas-Hoover capillary melting-point apparatus. Elemental analyses were performed by Quantitative Technologies Inc., Whitehouse, New Jersey. The presence of solvent as indicated by elemental analysis was verified by ^{1}H nmr spectroscopy. Thin layer chromatography (tlc) was performed on aluminum plates coated (0.2 mm) with silica gel $60F_{254}$ (EM Reagents). Silica gel (E. M. Science; 230-400 mesh) was used for flash column chromatography. All solvents and chemicals used were reagent grade and the solvent mixtures are in volumes. The detection of components on tlc was by uv light. Evaporations were conducted under diminished pressure with the bath temperature below 30°. Ultraviolet spectra (uv) were recorded with a Hewlett-Packard 8452 diode array spectrophotometer. Nuclear magnetic resonance (^{1}H nmr) spectra were recorded in dimethyl sulfoxide-d_6 at 400 MHz with a Bruker AM400 wide bore spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as the internal standard (key: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad).

9-[(*Z*)-2-Penten-1-yl]adenine (**2a**).

A mixture of 6-chloro-9-[(*Z*)-2-penten-1-yl]purine [26] (**1a**, 0.9 g, 4 mmoles) and methanolic ammonia (100 ml, saturated at 0°) was heated at 150° for 18 hours in a steel reaction vessel. The reaction vessel was cooled in an ice bath and carefully opened. The solvent was evaporated and the residue was purified by silica gel column (2 x 10 cm) chromatography. The product was eluted with dichloromethane containing 0-5% methanol. The appropriate fractions were collected and evaporated to give analytically pure **2a**, yield 0.55 g (67%), mp 142-143°; uv (methanol): λ max 262 nm (ε 16,200); ^{1}H nmr: δ 0.98 (s, 3 H, CH_3), 2.24 (m, 2 H, CH_2), 4.78 (d, 2 H, NCH_2), 5.60 (m, 2 H, CH=CH), 7.20 (s, 2 H, NH_2), 8.11, and 8.14 (2s, 2 H, C_2H and C_8H).

Anal. Calcd. for $C_{10}H_{13}N_5$: C, 59.09; H, 6.44; N, 34.46. Found: C, 59.38; H, 6.36; N, 34.37.

9-(-3-Methyl-2-buten-1-yl)adenine (**2b**).

In a similar manner to that described for the preparation of **2a**, reaction of **1b** [26] (0.55 g, 2.47 mmoles) with methanolic ammonia (100 ml) gave 0.32 g (64%) of **2b**, mp 164-166°; uv (methanol): λ max 262 nm (ε 16,200); ^{1}H nmr: δ 1.71 and 1.80 (2s, 6 H, $2CH_3$), 4.72 (d, 2 H, NCH_2), 5.40 (m, 1 H, =CH), 7.16 (s, 2 H, NH_2), 8.09, and 8.14 (2s, 2 H, C_2H and C_8H).

Anal. Calcd. for $C_{10}H_{13}N_5$: C, 59.09; H, 6.44; N, 34.46. Found: C, 59.48; H, 6.45; N, 34.36.

4-Amino-7-[(*Z*)-2-penten-1-yl]pyrrolo[2,3-*d*]pyrimidine (**2c**).

In a similar manner to that described for the preparation of **2a**, reaction of **1c** [26] (0.43 g, 1.94 mmoles) with methanolic ammonia (100 ml) gave 0.28 g (72%) of **2c**, mp 124-126°; uv (methanol): λ max 272 nm (ε 12,650); ^{1}H nmr: δ 0.95 (s, 3 H, CH_3), 2.23 (m, 2 H, CH_2), 4.74 (d, 2 H, NCH_2), 5.55 (m, 2 H, CH=CH), 6.53 (d, 1 H, C_5H), 6.92 (s, 2 H, NH_2), 7.08 (d, 1 H, C_6H), and 8.05 (s, 1 H,C_2H).

Anal. Calcd. for $C_{11}H_{14}N_4$: C, 65.32; H, 6.98; N, 27.71. Found: C, 65.12; H, 6.94; N, 27.39.

4-Amino-7-(3-methyl-2-buten-1-yl)pyrrolo[2,3-*d*]pyrimidine (**2d**).

In a similar manner to that described for the preparation of **2a**, reaction of **1d** [26] (0.53 g, 2.39 mmoles) with methanolic ammonia (100 ml) gave 0.35 g (73%) of **2d**, mp 118-120°; uv (methanol): λ max 272 nm (ε 12,200); ^{1}H nmr: δ 1.69 and 1.78 (2s, 6 H, $2CH_3$), 4.68 (d, 2 H, NCH_2), 5.34 (m, 1 H, =CH), 6.52 (d, 1 H, C_5H), 6.90 (s, 2 H, NH_2), 7.08 (d, 1 H, C_6H), and 8.05 (s, 1 H, C_2H).

Anal. Calcd. for $C_{11}H_{14}N_4$: C, 65.32; H, 6.98; N, 27.71. Found: C, 65.26; H, 6.80; N, 27.52.

2-Amino-9-[(*Z*)-2-penten-1-yl]purine (**4a**) and 2-Amino-7-[(*Z*)-2-penten-1-yl]purine (**5a**).

2-Aminopurine (**3**, 0.5 g, 3.7 mmoles) was dried by coevaporation with dry *N,N*-dimethylformamide (DMF, 15 ml). The dried material was suspended in DMF (20 ml) to which sodium hydride (0.16 g, 4 mmoles, 60% dispersion in mineral oil) was added and the mixture was stirred at room temperature for 20 minutes with the exclusion of moisture. 1-Bromo-2-pentene (0.45 ml, 3.8 mmoles, predominanatly the *cis* isomer) was added and the reaction was continued at ambient temperature for 5 hours. The solvent was evaporated and the residue was coevaporated with toluene (2 x 25 ml). The crude product was dissolved in a mixture of dichloromethane and methanol and impregnated onto silica gel (5 g). The dried silica gel was loaded on the top of a silica gel column (2 x 15 cm). The two positional isomers were eluted using dichloromethane containing 0-3.5% methanol. The appropriate homogeneous fractions were collected and evaporated to give pure **4a** and **5a**.

2-Amino-9-[(*Z*)-2-penten-1-yl]purine (**4a**).

This compound was obtained in a 53% yield (0.4 g), mp 110-112°; uv (methanol): λ max 244 nm (ε 6850), 310 (9550); ^{1}H nmr: δ 0.99 (s, 3 H, CH_3), 2.22 (m, 2 H, CH_2), 4.70 (d, 2 H, NCH_2), 5.60 (m, 2 H, CH=CH), 6.38 (s, 2 H, NH_2), 8.08 (s, 1 H, C_4H), and 8.57 (s, 1 H, C_8H).

Anal. Calcd. for $C_{10}H_{13}N_5$: C, 59.09; H, 6.44; N, 34.46. Found: C, 59.15; H, 6.56; N, 34.10.

2-Amino-7-[(*Z*)-2-penten-1-yl]purine (**5a**).

This compound was obtained in a 15% yield (0.11 g), mp 204-206°; uv (methanol): λ max 256 nm (ε 7300), 322 (9400); ^{1}H nmr: δ 0.98 (s, 3 H, CH_3), 2.25 (m, 2 H, CH_2), 4.86 (d, 2 H, NCH_2), 5.63 (m, 2 H, CH=CH), 6.09 (s, 2 H, NH_2), 8.23 (s, 1 H, C_4H), and 8.55 (s, 1 H, C_8H).

Anal. Calcd. for $C_{10}H_{13}N_5$: C, 59.09; H, 6.44; N, 34.46. Found: C, 59.07; H, 6.56; N, 34.08.

2-Amino-9-(3-methyl-2-buten-1-yl)purine (**4b**) and 2-Amino-7-(3-methyl-2-buten-1-yl)purine (**5b**).

In a similar manner to that described for the preparation of **4a** and **5a**, alkylation of **3** (0.5 g, 3.7 mmoles) with 4-bromo-2-methyl-2-butene (0.43 ml, 3.7 mmoles) in dry DMF (20 ml) in the presence of sodium hydride (0.16 g, 4 mmoles) afforded **4b** and **5b**.

2-Amino-9-(3-methyl-2-buten-1-yl)purine (**4b**).

This compound was obtained in a 47% yield (0.35 g), mp 204-206°; uv (methanol): λ max 244 nm (ε 5200), 310 (7600); 1H nmr: δ 1.71, 1.79 (2s, 6 H, $2CH_3$), 4.63 (d, 2 H, NCH_2), 5.37 (m, 1 H, =CH), 6.46 (s, 2 H, NH_2), 8.01 (s, 1 H, C_4H), and 8.56 (s, 1 H, C_8H).

Anal. Calcd. for $C_{10}H_{13}N_5$: C, 59.09; H, 6.44; N, 34.46. Found: C, 59.10; H, 6.42; N, 34.08.

2-Amino-7-(3-methyl-2-buten-1-yl)purine (**5b**).

This compound was obtained in a 16% yield (0.12 g), mp 216-217°; uv (methanol): λ max 256 nm (ε 4900), 322 (6350); ^{1}H nmr: δ 1.73 and 1.81 (2s, 6 H, $2CH_3$), 4.79 (d, 2 H, NCH_2), 5.39 (m, 1 H, =CH), 6.15 (s, 2 H, NH_2), 8.23 (s, 1 H, C_4H), and 8.53 (s, 1 H, C_8H).

Anal. Calcd. for $C_{10}H_{13}N_5$: C, 59.09; H, 6.44; N, 34.46. Found: C, 58.80; H, 6.29; N, 34.15.

1-[(*Z*)-2-Penten-1-yl]-aminopyrazolo[3,4-*d*]pyrimidine (**7a**).

4-Aminopyrazolo[3,4-*d*]pyrimidine (**6a**, 0.54 g, 4 mmoles) was dried by coevaporation with dry DMF (20 ml). The dried material was suspended in DMF (20 ml) to which sodium hydride (0.16 g, 4 mmoles) was added. The mixture was stirred at room temperature for 20 minutes before the addition of (*Z*)-1-bromo-2-pentene (0.5 ml, 4.2 mmoles). The reaction was continued for 5 hours and the solvent was evaporated. The residue was dissolved in dichloromethane (150 ml) and the organic phase was washed with water (100 ml). The aqueous layer was extracted with dichloromethane (75 ml) and the combined organic layer was dried (sodium sulfate) and evaporated. The residue was purified by silica gel column (2 x 10 cm) chromatography. The product was eluted with dichloromethane containing 0-4% methanol to yield **7a**, yield 0.3 g (37%), mp 160-162°; uv (methanol): λ max 262 nm (ε 10,950), 274 (12,500); ^{1}H nmr: δ 0.97 (s, 3 H, CH_3), 2.22 (m, 2 H, CH_2), 4.91 (d, 2 H, NCH_2), 5.55 (m, 2 H, CH=CH), 7.65 (s, 2 H, NH_2), 8.07 and 8.17 (2s, 2 H, C_3H and C_6H).

Anal. Calcd. for $C_{10}H_{13}N_5$: C, 59.09; H, 6.44; N, 34.46. Found: C, 59.16; H, 6.50; N, 34.17.

1-(3-Methyl-2-buten-1-yl)-4-aminopyrazolo[3,4-*d*]pyrimidine (**7b**).

In a similar manner to that described for **7a**, alkylation of **6a** (0.54 g, 4 mmoles) with 4-bromo-2-methyl-2-butene (0.46 ml, 4 mmoles) in DMF (20 ml) in the presence of sodium hydride (0.16 g, 4 mmoles) afforded 0.23 g (28%) of **7b**, mp 165-167°; uv (methanol): λ max 262 nm (ε 9100), 278 (10,300); ^{1}H nmr: δ 1.68 and 1.78 (2, 6 H, $2CH_3$), 4.85 (d, 2 H, NCH_2), 5.34 (m, 2 H, CH=CH), 7.65 (br s, 2 H, NH_2), 8.05, and 8.17 (2s, 2 H, C_3H and C_6H).

Anal. Calcd. for $C_{10}H_{13}N_5$: C, 59.09; H, 6.44; N, 34.46. Found: C, 59.07; H, 6.50; N, 34.06.

7-Amino-3-[(*Z*)-2-penten-1-yl]-v-triazolo[4,5-*d*]pyrimidine (**8a**), 7-Amino-2-[(*Z*)-2-penten-1-yl]-v-triazolo[4,5-*d*]pyrimidine (**9a**) and 7-Amino-1-[(*Z*)-2-penten-1-yl]-v-triazolo[4,5-*d*]pyrimidine (**10a**).

In a similar manner to that described for the preparation of **4a**, alkylation of **6b** (0.5 g, 3.67 mmoles) with (*Z*)-1-bromo-2-pentene (0.45 ml, 3.80 mmoles) in dry DMF (20 ml) in the presence of sodium hydride (0.17 g, 4.22 mmoles), evaporation of the solvent and silica gel column chromatography using 0-60% ethyl acetate in dichloromethane as the eluent afforded the isomers in the order listed.

7-Amino-3-[(*Z*)-2-penten-1-yl]-v-triazolo[4,5-*d*]pyrimidine (**8a**).

This compound was obtained in 25% yield (0.19 g), mp 174-176°; uv (methanol): λ max 276 nm (ε 13,200); ^{1}H nmr: δ 0.96

(s, 3 H, CH_3), 2.25 (m, 2 H, CH_2), 5.16 (d, 2 H, NCH_2), 5.63 (m, 2 H, CH=CH), 8.05 and 8.37 (2s, 2 H, NH_2), and 8.29 (s, 1 H, C_5H).

Anal. Calcd. for $C_9H_{12}N_6$: C, 52.92; H, 5.92; N, 41.15. Found: C, 52.87; H, 6.05; N, 40.79.

7-Amino-2-[(*Z*)-2-penten-1-yl]-v-triazolo[4,5-*d*]pyrimidine (**9a**).

This compound was obtained in 24% yield (0.18 g), mp 140-142°; uv (methanol): λ max 292 nm (ε 12,200); ^{1}H nmr: δ 0.95 (s, 3 H, CH_3), 2.23 (m, 2 H, CH_2), 5.26 (d, 2 H, NCH_2), 5.72 (m, 2 H, CH=CH), 8.00 and 8.17 (2br s, 2 H, NH_2), and 8.26 (s, 1 H, C_5H).

Anal. Calcd. for $C_9H_{12}N_6$: C, 52.92; H, 5.92; N, 41.15. Found: C, 53.03; H, 6.08; N, 40.80.

7-Amino-1-[(*Z*)-2-penten-1-yl]-v-triazolo[4,5-*d*]pyrimidine (**10a**).

This compound was obtained in 12% yield (0.09 g), mp 252-254°; uv (methanol): λ max 286 nm (ε 12,000); ^{1}H nmr: δ 0.98 (s, 3 H, CH_3), 2.37 (m, 2 H, CH_2), 4.99 (d, 2 H, NCH_2), 5.66 (m, 2 H, CH=CH), 8.47 (s, 1 H, C_5H), 8.80, and 9.13 (2s, 2 H, NH_2).

Anal. Calcd. for $C_9H_{12}N_6$•0.25 methanol: C, 52.34; H, 6.17; N, 39.60. Found: C, 52.22; H, 6.20; N, 39.33.

7-Amino-3-(3-methyl-2-buten-1-yl)-v-triazolo[4,5-*d*]pyrimidine (**8b**), 7-Amino-2-(3-methyl-2-buten-1-yl)-v-triazolo[4,5-*d*]-pyrimidine (**9b**) and 7-Amino-1-(3-methyl-2-buten-1-yl)-v-triazolo[4,5-*d*]pyrimidine (**10b**) .

In a similar manner to that described for the preparation of **4a,** alkylation of **6b** (0.5 g, 3.67 mmoles) with 4-bromo-2-methyl-2-butene (0.43 ml, 3.70 mmoles) in dry DMF (20 ml) in the presence of sodium hydride (0.17 g, 4.22 mmoles), evaporation of the solvent and silica gel column chromatography using 0-6% methanol in dichloromethane as the eluent afforded the isomers in the order listed.

7-Amino-3-(3-methyl-2-buten-1-yl)-v-triazolo[4,5-*d*]pyrimidine (**8b**).

This compound was obtained in 27% yield (0.20 g), mp 214-215°; uv (methanol): λ max 278 nm (ε 15,150); ^{1}H nmr: δ 1.71 and 1.83 (2s, 6 H, $2CH_3$), 5.11 (d, 2 H, NCH_2), 5.43 (m, 1 H, =CH), 8.04 and 8.37 (2br s, 2 H, NH_2), and 8.30 (s, 1 H, C_5H).

Anal. Calcd. for $C_9H_{12}N_6$: C, 52.92; H, 5.92; N, 41.15. Found: C, 52.91; H, 5.83; N, 41.10.

7-Amino-2-(3-methyl-2-buten-1-yl)-v-triazolo[4,5-*d*]pyrimidine (**9b**).

This compound was obtained in 25% yield (0.19 g), mp 148 150°; uv (methanol): λ max 292 nm (ε 11,200); ^{1}H nmr: δ 1.76 and 1.81 (2s, 6 H, $2CH_3$), 5.29 (d, 2 H, NCH_2), 5.51 (m, 1 H, =CH), 7.99 and 8.16 (2br s, 2 H, NH_2), and 8.27 (s, 1 H, C_5H).

Anal. Calcd. for $C_9H_{12}N_6$: C, 52.92; H, 5.92; N, 41.15. Found: C, 52.95; H, 5.88; N, 40.75.

7-Amino-1-(3-methyl-2-buten-1-yl)-v-triazolo[4,5-*d*]pyrimidine (**10b**).

This compound was obtained in 11% yield (0.08 g), mp 266-268°; uv (methanol): λ max 286 nm (ε 11,500); ^{1}H nmr: δ 1.72 and 1.86 (2s, 6 H, $2CH_3$), 4.94 (d, 2 H, NCH_2), 5.49 (m, 1 H, =CH), 8.47 (s, 1 H, C_5H), 8.79, and 9.11 (2br s, 2 H, NH_2).

Anal. Calcd. for $C_9H_{12}N_6$•0.25 methanol: C, 52.34; H, 6.17; N, 39.60. Found: C, 52.72; H, 5.88; N, 39.33.

Biological Procedures.

Cell line: Human monocytic leukemia cells were maintained in suspension cells culture medium containing 25 m*M* Hepes buffer, 2 m*M* L-glutamine, 50 units of penicillin per ml, 50 μg of streptomycin sulfate per ml, and 10% heat-inactivated fetal bovine serum in a humidified incubator containing 5% carbon dioxide. The cells were split 1:4 weekly.

Tumor Necrosis Factor α Assay.

Varying concentrations of compounds or control were added to human monocytic leukemia cells plated at a density of 1 x 10^6 cells/ml in 600 μl of media supplemented with 10% Fetal Bovine Serum in a 48 well plate. After one hour the cells were stimulated with 0.5 ug of lipopolysaccharide per ml. Six hours post-stimulation, supernatants were collected and assayed for tumor necrosis factor α production using a Biosource or R and D Systems cytokine detection Enzyme Linked Immunosorbent Assay kit according to the suppliers instructions.

Cytotoxicity Analysis.

The cytotoxicity of the compounds were assayed using the CellTiter 96™ Aqueous Non-Radioactive Cell Proliferation Assay (Promega). Briefly, viable cell number was determined by trypan blue staining and human monocytic leukemia cells were resuspended in media supplemented with 10% Fetal Bovine Serum. Eighty microliters of cell suspension (1.7 x 10^4 cells/well) was dispensed onto a 96-well microtiter plates. At this time 20 μl of drug (or control) was added to appropriate wells. Each concentration was assayed in quadruplicate. The plates were incubated at 37° in a humidified 5% carbon dioxide atmosphere for 4 days and Aqueous Non-radioactive Cell Proliferation assay was performed according to the manufacturer's instructions. The average absorbance of the sample was graphed for each concentration and the data obtained was used to calculate TC_{50} (the concentration of the compound required to inhibit the cell growth by 50%) for each compound.

Primary Cell Assays.

Primary human peripheral blood mononuclear cells were isolated as described by Ojwang *et al.* [30]. Isolated monocytes were added to 24-well cluster plates (Costar) at a concentration of 1 x 10^5 viable cells per well. Culture medium or test compound dilutions in culture medium were added to the wells, the plates were then incubated at 37° for one hour. Tumor necrosis factor α production was subsequently induced by stimulation with 100 μ/ml (final concentration) of lipopolysaccharide. The plates were incubated an additional 4, 24 or 48 hours. The supernatant fluids were then harvested and centrifuged to pellet cells or cellular debris, and the supernatant fluids were stored at -70° until they were assayed for the presence of tumor necrosis factor α using the commercially available Enzyme linked Immunosorbent Assay kits.

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