PURINE ALKYLATING AGENTS 2, 6-DIAMINO-8-HALO ALKYL PURINES (POTENTIAL ANTI-MALARIALS)

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Abstract

Haloalkyl purines i.e. 2, 6-diamino-8-(chloromethyl) purine **10**; 2, 6-diamino-8-(chloromethyl) purine **17**; 2, 6-diamino-8-(chloropropyl) purine **18**; were synthesized by two distinct but facile methods. The N-alkylating potential of 2, 6-diamino-8-(chloromethyl) purine was investigated. The following products 2, 6-diamino-8-(N-benzyl-N-methyl) purine **11**; 2, 6-diamino-8-(4-nitro anilinomethyl) purine **13**; 2, 6-diamino-8-(piperidino-N-methyl) purine **14**; 2,6-diamino-8-(3,4,5-trimethoxyanilino-methyl) purine **12**; of N-alkylation are reported. Antifolate activities obtained for these compounds are also reported. 2, 6-diamino-8-(3, 4, 5-trimethoxyanilino methyl) purine demonstrated significant biological activity ($ID_{50} = 1 \times 10^{-4}$ m). Most antifols are also antimalarials e.g. trimethoprim and pyrimethamine, and therefore these 8-haloalkyl purines may be effective antimalarials if modified by appropriate reagents.

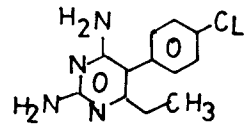
Introduction

The fight against malaria has a long history. This disease retarded the progress of exploration work in colonial Africa, and is the predominant disease in tropical and sub-tropical parts of the world. The health hazard posed by malaria has overwhelmed the genuine efforts made at various levels to combat the disease.

Two factors, that is the plasmodium parasite and the mosquito, are of principal concern in dealing with malaria. Chemotherapy for the plasmodium, and the use of insecticides, mosquito predators and elimination of mosquito breeding grounds are measures used all over the world to combat malaria. Is it possible to produce a new generation of mosquitoes (female) that are sterile and will not feed on human blood?

The syntheses of purines **10**, **17** and **18** and their possible evaluation as alkylators are the major concern of this paper. Alkylation of proteins in DNA [1-3] by purine C_8 substituents (e.g. carboxy, halogeno methyl, **10**) was proposed by Giner-Sorella *et al.* [4]. The majority of work in this area was carried out on adenine purines. 2, 6-diamino-8-halo alkyl purines **10**, **17**, **18** (made in the present work) may form covalent bonds with biological nucleophiles (-SH, NH_2, -OH) and may interfere with the utilization of folic acid, **5** (antifolates) by plasmodium parasites [5, 6].

The common structural component **2**, seen in the antifolates, trimethoprim, **3**, (antimalarial, antibiotic), pyrimethamine, **1**, (antimalarial), [7-10] and tetroxoprin **4** [11] is present in these novel purines **10**, **17**, and **18**.



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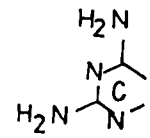

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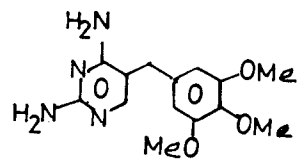

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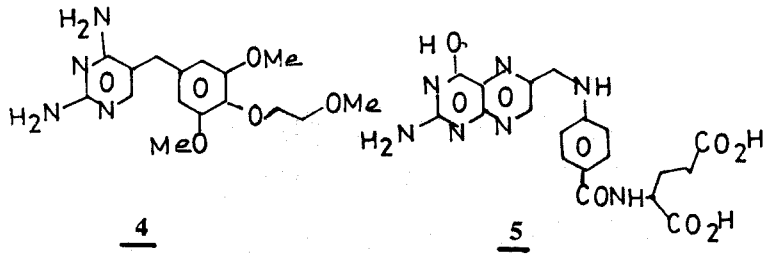
Keywords: Purines; Anti-Malarials



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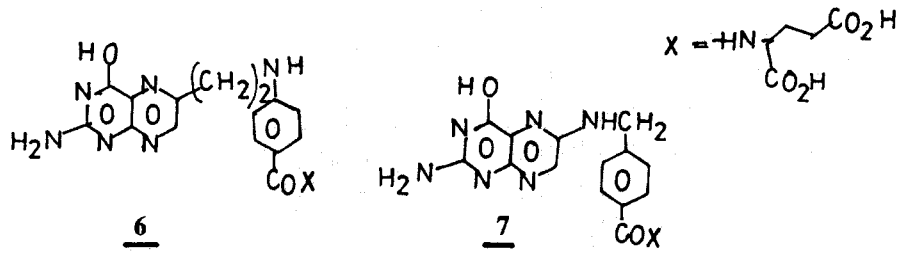
Experimental Section

Rationale

Previous designs for most antifols were aimed at lengthening the spacer or bridge portion by one carbon e.g. **6** and interchanging the N_{10} and C_9 positions in folic acid e.g. **7**. In the present work, the pteridine ring system is compressed to purine without appreciable loss in the base strength (pKa purine = 5.00, pKa pteridine = 5.32) and C_8 is substituted by a haloalkyl pendant group.

The compound **10** showed a high alkylating potential explainable by the benzylic character of the C_8 methylene attached to the halogen atom p-Nitro-aniline (a weak nucleophile); benzylamine; 3,4,5-trimethoxyamine and piperidine were all readily alkylated by **10**. The alkylating potential of these C_8 haloalkyl pendants may carry over to proteins with reactive nucleophiles.



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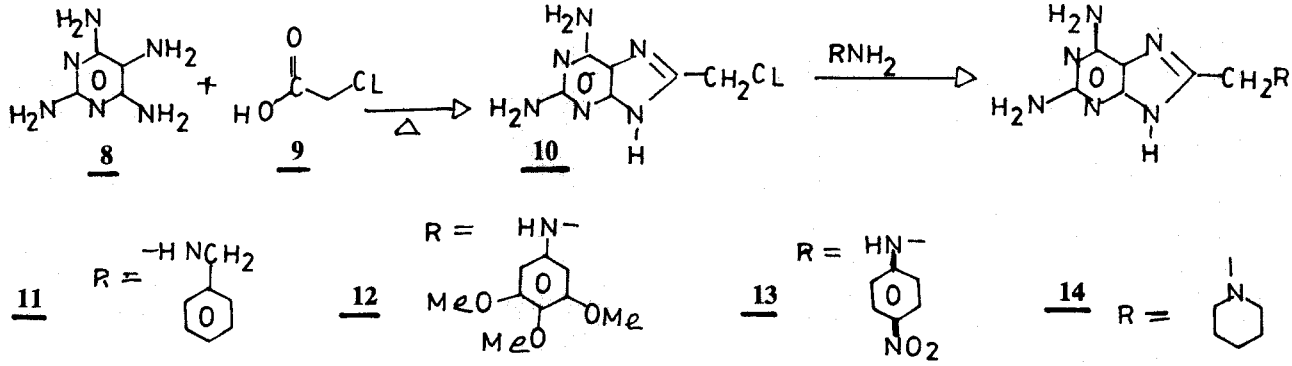
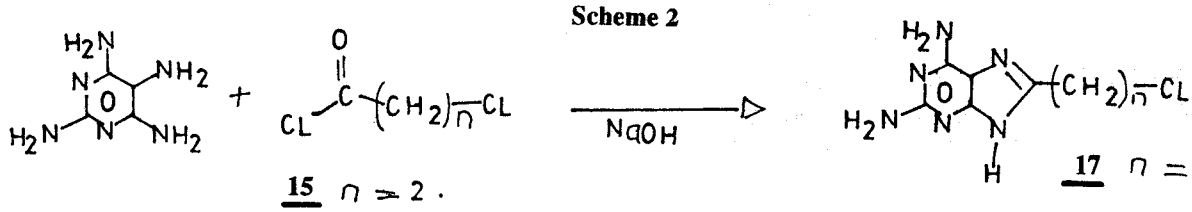
Scheme 1



8 9 10 11 12 13 14

Scheme 2

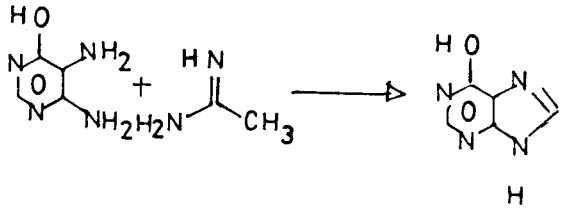


15 n = 2.
16 n = 3.
17 n = 2.
18 n = 3.

Chemistry

The syntheses of purines have been approached from a number of routes. A brief review of these methods of syntheses is presented.

a. Use has been made of acetamidine for purine synthesis e.g.

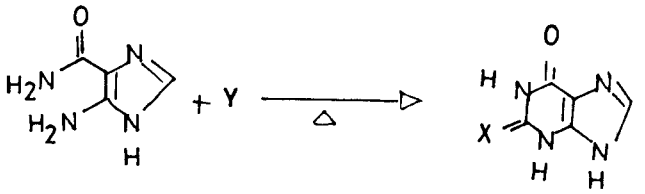


Ref [12]

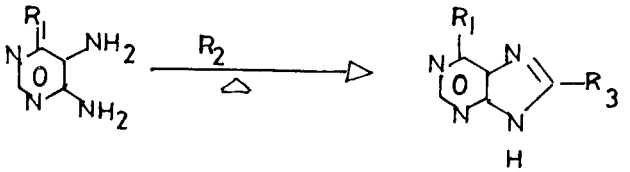
The following 8-substituted purines were obtained by this method.

No	2	4	8	Reaction Time in Min	Yield%
1	H	OH	Me	60	56
2	OH	OH	Me	60	54
3	SH	SH	Me	30	65/85
4	SH	NH_2	Me	25	66
5	SH	OH	Me	30	77/66
6	H	OH	Ph	70	50/78
7	OH	OH	Ph	40	80

b. (i) Suitable imidazoles have been condensed with the following regents at 170° - 180° to give purines e.g.



(ii) Alternatively, pyrimidines can be condensed with reagents like hydroxy esters, etc. to give purines e.g.



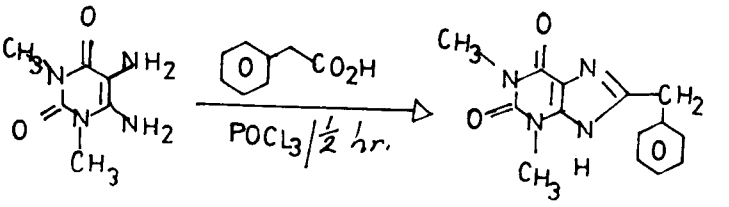
Ref [13]

$R_2 = HOCH_2CO_2Et$,

(33% Product Yield)

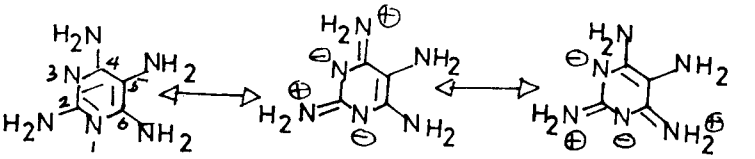
(65% Product Yield) (36% Product Yield)

C. The reaction of diamino pyrimidine and acid in a condensing solvent (e.g $POCL_3$) under reflux has been known to give purines e.g.



Ref [14]

The 5-amino group of 2, 4, 5, 6-tetra amino pyrimidines is the most highly nucleophilic group. This can be explained by the operative tautomerism in 2, 4, 5, 6-tetra amino pyrimidine system e.g.



The lone pair of electrons on the nitrogen attached to C_5 does not contribute anything to the tautomerism and the C_5 amino group behaves like a primary amino group (it is Sp^3 hybridized all through the tautomeric process). The incoming amidine, or acid anhydride or chloride or amide, is expected to interact with the C_5 amino group intially before any other secondary reactions close up the imidazole ring of the purine. Of the very many approaches to the synthesis of 8-substituted purines the reaction systems outlined in schemes 1 and 2 were found to be the most appropriate. The reactions were fast and products were obtained in good yield.

Biological Evaluation

The antifolate activities obtained for these N-alkyl or N-alkyl-N-aralkyl purines are shown in Table I.

The significant antifolate activity demonstrated by 2,6,-diamino-8-(3,4,5-trimethoxy anilino methyl) purine can be attributed to the presence of the lipophilic moiety e.g. 3,4,5-trimethoxy aniline.

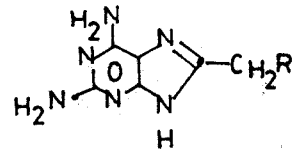


Table I

R	Compound No.	Highest Conc.	%Inhibition	%Yield	MP	R_f(nBuOH/ ACOH/H_2O) 4:5:1 V/V Refs [21, 12]
Benzylamino-	IME 652	$3x10^{-1}$M	8.2	50.00	300° (dec)	0.35
p-nitroanilino-	IME 6554	$1x10^{-4}$M	20.4	30.00	300° (dec)	0.30
Piperidino-	IME 656	$3x10^{-4}$M	0	41.14		0.66
3,4,5-trimeth-	Rp 9869	$1x10^{-3}$M	85.41	12.40	280°	0.46
Oxyanilino-	Nigerian patent	$1x10^{-4}$M	49.58			

2,6-diamino purine (sigma - no significant inhibition up to 3×10^{-4}M) Ref [15]

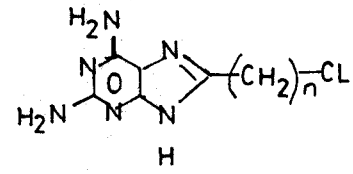


Table II

n	% Yield	Mp
1	32.20	300° (dec)
2	20.00	300° (dec)
3	31.32	259° - 262° (dec)

2,6-diamino-8-(N-benzy-N-methyl) purine **11** can be made more lipophilic by aromatic ring substitution and this may improve its biological activity. The 2,6 - diamino - 8 - (-haloalkyl) purines that were synthesized in this project have not been subjected to biological testing.

Materials and Methods

Infrared spectra were obtained on a nicolet model 700 FT-IR interfere meter and absorption frequencies were reported in CM^{-1}. Ultra Voilet spectra were taken on a Beckman DB-9. Melting points were determined with a Mel-temp. apparatus and were uncorrected.

The elemental analyses were supplied by Atlantic Micro Labs. Inc. Atlanta Georgia, U.S.A.

1. 2,6-Diamino-8-chloromethyl purine 10

2,4,5,6-Tetra - aminopyrimidine sulphate salt- (2.1g, 0.01 mole) was mixed with chloroacetic acid - (4.7g, 0.05 mole) by grinding in a mortar. The mixture was introduced into a 250 ml round bottom flask to which a water aspirator was attached. The flask was heated for 2 hours and allowed to cool. The contents of the flask were washed with diethylether (30 ml) for three consecutive times to remove excess chloroacetic acid. The residue was dissolved in 50ml of hot water and filtered (while hot). Fine crystals were obtained on cooling this filtrate. The crystalline solid recovered weighed 0.72g (32.2% yield).

UV λmax 290 nm at PH 11

IR cm^{-1} **KBr** 3415, 3337, 3267, 3154, (-NH_2; NH) 3020, 2971, 2809, (-CH_2-), 710 (-CH_2-Cl)

Elemental Analysis

Calculated C 30.71 H 4.72 N 35.81 Cl 15.11

Found C 30.99 H 4.92 N 36.68 Cl 15.42

Mol. Formula $C_6H_7N_6Cl2H_2O$

2. 2,6-Diamino-8-chloroethyl purine 17

2,4,5,6-Tetra-amino pyrimidine sulphate - (7g, 0.08 mole) was put in a 250 ml round - bottom flask and dissolved with 100 ml of 2M NaCH solution. An undetermined quantity of ice chips was added to this solution. The outside of the flask was also surrounded with ice. 3-chloropropionyl chloride (7ml, 0.08 mole, 2 equivalents)

was added to the flask in two disproportionate batches (4ml followed by 3ml later) and vigorous stirring was started with a magnetic stirrer. The flask was stoppered and the reaction was run until stirring became very difficult (because of the syrupy nature of the reaction mixture). This reaction took about 20 minutes. It was worked up by filtering with a buchner funnel attached to a powerful water aspirator (to provide suction pressure). The residue was dissolved in hot ammoniun hydroxide and filtered (hot). The crystals which were obtained weighed 2.06g (oven dried at 100°)
i.e. 20.0% yield.

UV λmax 300 nm at PH 11

IR cm^{-1} **KBr** 3400, 3344, 3203, 3147, ($-NH_2$, NH)
2956, 2886, 2745, ($-CH_2-$) 751 ($-CH_2-Cl$)

Elemental Analysis

Calculated	C 33.81	H 5.23	N 33.70	Cl 14.28
Found	C 33.59	H 5.31	N 33.61	Cl 14.20

Mol. Formula $C_7H_9N_6Cl.2H_2O$

3. 2,6-Diamino-8-chloropropyl purine 18

This compound was prepared in the same fashion as for 2,6-diamino-8-chloroethyl purine (using 2,4,5,6-tetra-amino pyrimidine sulphate 6g, 0.023 mole and 4-chlorobutyryl chloride (0.05 mole-2 equivalents). A dry weight of 1.66g (31.3% yield) of expected product was obtained after recrystallization.

UV λmax 292 nm at PH 11

IR cm^{-1} **KBr** 3492, 3386, 3344, 3154, ($-NH_2$, NH)
2985, 2942, 2816, ($-CH_2-$)

Elemental Analysis

Calculated C 39.26 H 5.32 N 34.35 Cl.14.15..... (a)
Found C 39.15 N 5.36 N 34.98 Cl.12.91.....(b)

$\frac{C}{N}$ ratio (a) = 1.14
(b) = 1.12

Mol. Formula $C_8H_{11}N_6ClH_2O$

4. 2,6-Diamino-8-(3,4,5-trimethoxy anilino) methyl purine 12

2,6-diamino-8-(chloromethyl) purine - (0.2g, 0.002 mole) was mixed with 3,4,5-trimethoxy aniline (0.24g, 0.002 mole) and introduced into a 50 ml round-bottom flask containing water. A reflux water condenser was connected to the flask and the set up refluxed for about 16 hours (the refluxing solution turned red) and filtered. The hot filtrate (pH 4) was allowed to cool. The yellowish crystals obtained from this weighed 0.19g (12.4% yield).

UV λmax = 287, 247 nm at PH 11

IR (cm^{-1}) **KBr** 3464, 3393, 3105, (NH_2, NH), 2985 2840, ($-CH_2-$), 1654, 1580, ($-CH_2$) 1513, 1492, 1456 **1231** (-O-Me), 1138, 1080, 808, 737.

Elemental Analysis

Calculated	C 46.15	H 6.15	N 25.13
Found	C 46.37	H 5.96	N 24.89

Mol. Formula $C_{15}H_{19}C_3N_72,5H_2O$

5. (i) **2,6-Diamino-8-(N-benzyl-N.methyl amino) purine 11**

(ii) **2,6-Diamino-8-(4-nitro anilino-methyl) purine 13**

(iii) **2,6 - Diamino - 8 - (Piperidino - N - methyl amino) purine 14**

These were made in a similar fashion as for **12** by taking known weights of the reagents in water heated to reflux. The following weights of reagents were required.
(a) 0.43g, 0.002 mole of 2,6-diamino-(chloromethyl) purine-and 0.43g, 0.004 mole of benzylamine in 20ml of water for 5(i) (0.27g of product).
(b) 0.4g, 0.002 mole of 2,6-diamino-8-chloromethyl purine-and 0.55g, 0.004 mole of 4-nitroaniline in 20ml of water for 5(ii) (0.18g of product).
(c) 0.3g, 0.0015g mole of 2,6-diamino-8-(chloromethyl) purine - and 0.3 mole, 0.03 mole of piperidine in 20ml of water for 5(iii) (0.15g of product).

These reactions were worked up in a similar fashion as for **12**, by filtering the reaction mixture (hot) and allowing the filtrate to cool. The resulting crystalline solid was dried (oven-at reasonable temperatures - 100°).

2,6-Diamino-8-(N-benzyl-N-methyl amino) purine 11

IR (cm^{-1}) **KBr** 3478, 3388, 3253, 3062, 3027, 2851, 2830, 1668, 1618, 1569, 1484, 1449, 1266, 1160, 1069, 829, 787.

Elemental Analysis

Calculated	C 49.68	H 6.36	N 31.20
Found	C 50.46	H 6.12	N 30.21

Mol. Formula $C_{13}H_{15}N_75H_2O$

2,6-Diamino-8-(4.nitro anilino-methyl) purine 13

IR (cm^{-1}) **KBr** 3471, 3337, 3196, 1639, 1618, 1506, ($-NO_2-$), 696.

Elemental Analysis

Calculated	C 41.26	H 4.87	N 32.09
Found	C 41.36	H 4.61	N 31.63

Mol. Formula $C_{12}H_{12}O_2N_8,2.7H_2O$

2,6-Diamino-8-(Piperidino-N-methyl amino) purine 14

IR (cm^{-1}) **KBr** 3494, 3393, 3132, 3020, 2936, 2865, 2816, 1675, 1625, 1569, 1449, 1273, 1160, 1097, 865, 787.

Elemental Analysis

Calculated	C 41.37	H 7.84	N 30.72
Found	C 41.75	H 7.32	N 30.23

Mol. Formula $C_{11}H_{17}N_73.5H_2O$

Results and Discussion

The difference in the UV for the ethyl product i.e. n=2 can be attributed to the vertical stacking mechanism observed for these purines. The influence of chlorine on the absorption frequence is felt when the chlorine atom is 2 carbons away from the purine ring. This chlorine may be sitting on the purine ring (by a bending back mechanism of the 2 carbon chains). This brings about an enhancement of the absorption frequency. However, increment of the carbon chain by an additional carbon (i.e. n=3) pushes out the chlorine from the sphere of influence of the purine ring and the absorption frequency drops back. Vertical stacking has also been observed in anthocyanidines (Flavanoids) [16]. The UV absorptions are in accord with those of other related 2,6-8-trisubstituted purines [17, 18] and N-alkyl guanosines (purines) [22].

The infrared spectra of these novel compounds showed up as expected. The absorptions at 3464cm^{-1}, 3395cm^{-1} belong to NH_2 and 3105cm^{-1} is the N-H of the secondary amino groups. These absorptions slightly vary as can be seen with different nucleophiles. These IR absorptions can be compared to those of pteroylglutamic acid or pteroic acid [11]. It is interesting to note the semblance in the region 3500cm^{-1} - 3000cm^{-1} for both the purines and the pteridine derivatives. [19, 20].

The yields obtained in these reactions are reasonable and demonstrate an improvement on those reported for other related purines. It is therefore possible that other very reactive (aromatic) nucleophiles will be alkylated by these 8-halo-alkyl-purines.

Acknowledgements

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