Effect of the Substituent at C-6 on the Biological Activity of 2,4-Diamino-5-(1-adamantyl)pyrimidines†

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The synthesis and biological activity of 2,3-diamino-5-(1-adamantyl)- (**1**), 2,4-diamino-5-(1-adamantyl)-6-methyl- (**2**), and 2,4-diamino-5-adamantyl-6-ethylpyrimidine (**3**) have been described earlier.[1-3] It has been observed that the growth inhibitory potency for mouse mammary adenocarcinoma (TA3) cells in culture increases with the increasing size of the substituent at carbon 6. Similarly, **2** was 33 times more inhibitory for the purified dihydrofolate reductase from cultured Sarcoma 180 cells than **1**. It was therefore of interest to see how a further extension of the alkyl chain at carbon 6 affected the biological activity of diamino-5-(1-adamantyl)pyrimidines.

Two new compounds have been synthesized: 2,4-diamino-5-(1-adamantyl)-6-propylpyrimidine (**4**) and 2,4-diamino-5-(1-adamantyl)-6-butylpyrimidine (**5**). The synthesis was carried out essentially as described previously for the corresponding 6-methyl and 6-ethyl derivatives[1,2] by condensation of guanidine with ethyl 2-(1-adamantyl)-3-oxohexanoate (**6**) or with ethyl 2-(1-adamantyl)-3-oxoheptanoate (**9**). The 2-amino-4-hydroxypyrimidines (**8** and **9**) thus obtained were converted to 2,4-diaminopyrimidines by chlorination of the 4-hydroxy group, followed by amination. To improve the solubility in aqueous media, the final products were isolated in the form of their ethyl sulfonate salts.[2]

The diaminopyrimidines were tested as growth inhibitors of mouse mammary adenocarcinoma cells (TA3) *in vitro* using the procedure described earlier.[2] The study of the inhibition of purified dihydrofolate reductase from Sarcoma 180 was performed as described elsewhere.[3]

The results of the biological experiments with five diaminopyrimidines are summarized in Table I. Compound **3** as a free base or as its ethyl sulfonate salt is the strongest growth inhibitor of TA3 cells (ID_{50} 0.002 μM), exceeding the inhibitory potency of methotrexate (ID_{50} 0.008 μM).[2] With increasing length of the alkyl chain the growth inhibitory potency decreases the 6-butyl derivative **5** being about as strong an inhibitor as **1**. Neither the growth inhibition of cells *in vitro* nor the inhibition of dihydrofolate reductase seem to be affected by the formation of the ethyl sulfonate salt.

All pyrimidines tested, with the exception of the ethyl-substituted derivative, were found to be competitive inhibitors of dihydrofolate reductase. The 6-ethyl derivative **3** may be assumed to be also competitive; however, due to the extremely tight binding of this compound to the enzyme this could not be shown by the kinetic inhibition analysis. Figure 1 shows that the inhibition of dihydrofolate reductase by this compound is stoichiometric when dihydrofolate is the substrate.[4] Werkheiser studied the stoichiometric inhibition of dihydrofolate reductase by aminopterin and concluded that in the case of such inhibition the K_i can be estimated to be 10^{-10}-10^{-11} M.[4] Thus a K_i of such magnitude may be postulated for **3**.

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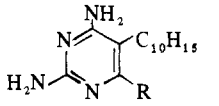
Table I. Effect of Substituent at C-6 on the Biological Activity of 2,4-Diamino-5-adamantylpyrimidines



	R	Form of compd	ID_{50},[a] μM	K_i,[b] μM
1	H	Free base	0.33[c]	0.2[c]
2	CH_3	Free base	0.006[c]	0.006[c]
2a	CH_3	Ethyl sulfonate salt	0.007[d]	0.009
3	C_2H_5	Free base	0.002	
3a	C_2H_5	Ethyl sulfonate salt	0.002[d]	0.0001[e]
4	C_3H_7	Ethyl sulfonate salt	0.04	0.014
5	C_4H_9	Ethyl sulfonate salt	0.40	0.06
		Pyrimethamine[f]	1.2	0.09

[a] Assay system: mouse mammary adenocarcinoma cells (TA3) (see ref 2). [b] Assay system: purified dihydrofolate reductase from Sarcoma-180 cells (see ref 3). [c] These values were reported previously (see ref 2 and 3) and are presented here for comparison with those of the new compounds. [d] These values were reported previously to be 0.001 and 0.0002 μM, respectively (see ref 2); reexamination of the earlier experiments resulted in new values reported here. [e] Stoichiometric inhibitor; K_i can be estimated to be 0.0001-0.00001 (see ref 4). [f] Pyrimethamine [2,4-diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine] is a known inhibitor of dihydrofolate reductase; see J. J. Burchall and G. H. Hitchings, *Mol. Pharmacol.*, **1**, 126 (1965). The ID_{50} and K_i values were determined in the test systems used in this laboratory and are reported here for comparison with the new compounds.

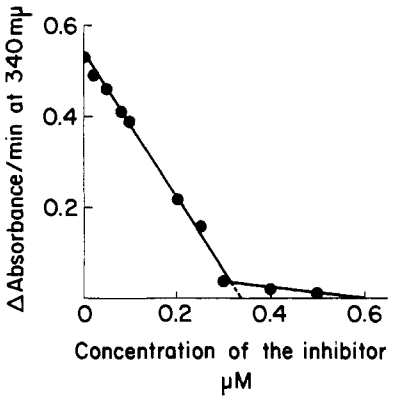


Figure 1. Titration of dihydrofolate reductase with 2,4-diamino-5-(1-adamantyl)-6-ethylpyrimidine. The assay mixture contained in a total volume of 1 ml: 50 μmol of citrate buffer pH 6.0, 0.1 μmol of NADPH, 0.1 μmol of dihydrofolate, and the enzyme. The enzymatic reaction was followed by measuring the change of absorbance at 340 mμ in Cary 14 recording spectrophotometer.

It is of interest that, whereas in the case of **1** and **2** the K_i values are numerically equal to the ID_{50} values, this relation does not hold in the case of the ethyl (**3**), propyl (**4**), and butyl (**5**) derivatives. All these compounds appear to be stronger inhibitors of the purified enzyme than of the cell growth. This may indicate that the uptake of the analogs by the cell may be decreasing with the increasing length of the 6 substituent to a larger extent than does the affinity to dihydrofolate reductase.

Experimental Section

Microanalyses were performed by G. I. Robertson, Jr., of Florham Park, N. J. Where analyses were within ±0.4% of the calculated value, the symbols of the elements are indicated. Melting points were determined on a Fisher-Johns apparatus. The column used for vpc was 4 ft SGR on HMDS treated Chromosorb W. Uv spectra were done with a Perkin-Elmer 202 spectrophotometer.

Ethyl 2-(1-Adamantyl)-3-oxohexanoate (6). This compound was prepared according to the procedure described previously for

the preparation of ethyl aceto(1-adamantyl)acetate.[5] β-Oxohexanoate[6] (35.9 g, 0.227 mol) and 1-adamantol (34.5 g, 0.200 mol) were dissolved in 600 ml of pentane at 5°. BF_3 was passed over the mixture for about 1 hr while maintaining the temperature at 5°. Subsequently the reaction mixture was allowed to reach 15° and then it was cooled down to 5°. After neutralization with KOH the solid was separated by filtration. The filtrate eventually gave an oil which was distilled at 20–40 μm: yield 15.4 g (22%); 92% pure by vpc.

2-Amino-4-hydroxy-5-(1-adamantyl)-6-(1-propyl)pyrimidine (8). Guanidine hydrochloride (1.27 g, 13.3 mmol) was neutralized with a solution of sodium ethoxide (0.615 g, 26.8 mg-atom of Na in 100 ml of absolute EtOH). After cooling, **6** (3.89 g, 13.3 mmol) was added and the reaction mixture refluxed for 5 days. The work-up is the same as that previously described for 2-amino-4-hydroxy-5-(1-octyl)-6-methylpyrimidine.[2] Recrystallization from absolute EtOH–charcoal and washing with Et_2O gave 1.2 g (31%): uv max (absolute EtOH) 293, 229 mμ; uv min 253 mμ; mp 298–299°. *Anal.* ($C_{17}H_{25}N_3O$) C, H, N.

2,4-Diamino-5-(1-adamantyl)-6-(1-propyl)pyrimidine Ethyl Sulfonate (4). The above compound **8** (1.7 g) was converted to its 4-Cl derivative (mp 238–240°) by $POCl_3$–PCl_5 as previously described for the preparation of 2-amino-4-chloro-5-(1-propyl-6-methyl)-pyrimidine.[3] This material was dried in a desiccator for 2 hr and mixed in a bomb with absolute EtOH (150 ml) saturated with NH_3 at 0°. The mixture was heated at 150° for 24 hr and then evaporated to dryness. The residue was stirred with 0.1 *N* NaOH for 2 hr. The solid (1.3 g) was collected, washed with distilled H_2O, and dissolved in the minute amount of hot absolute EtOH which contained ethylsulfonic acid (700 mg). After stirring for a few hours, Et_2O was added to precipitate the product (700 mg). Recrystallization from THF gave 300 mg of pyrimidine: mp 212–214°; uv max (absolute EtOH) 288 mμ; uv min 262 mμ. *Anal.* ($C_{19}H_{32}N_4O_3S$) C, H, N, S.

Ethyl β-Oxoheptanoate (10). This compound was prepared according to the method described for preparation of ethyl β-oxohexanoate[6] starting with Mg turnings (12.65 g, 0.52 g-atom), ethyl acetoacetate (67.3 g, 0.52 mol), and valeryl chloride (63.4 g, 0.53 mol). The product was obtained in 59% yield (52.9 g, 95% pure by vpc) by distillation at 128–134° (40 mm) [lit.[7] bp 110–125° (20 mm)]. The identity of this compound was established by ir and nmr spectra.

Ethyl 2-(1-Adamantyl)-3-oxoheptanoate (7). The above compound **10** (10.6 g, 61.6 mmol) was added to a suspension of 1-adamantol (9.36 g, 61.6 mmol) in 160 ml of pentane at 5°. BF_3 was passed over the reaction mixture for 1.5 hr. The rest of the work-up is the same as that described for **6**. The yield was 6.47 g (34.3%), 96% pure by vpc.

2-Amino-4-hydroxy-5-(1-adamantyl)-6-(1-butyl)pyrimidine (9). Guanidine hydrochloride (2.02 g, 21.2 mmol) was neutralized with NaOEt (975 mg, 42.4 mg-atom in 150 ml of absolute EtOH). **7** (6.47 g, 21.2 mmol) was added to the solution of the guanidine and the reaction mixture refluxed for 93 hr. The volume of the mixture was reduced to one-third and poured into 300 ml of H_2O. Et_2O (150 ml) was added and the mixture stirred until the gum present fully solidified. The solid (3.0 g) was collected and washed with Et_2O, mp 260–262°. Recrystallization twice from 95% EtOH–charcoal and once from ethoxyethanol–charcoal gave 1.60 g: mp 265–270°; uv max (absolute EtOH) 292, 226 mμ; uv min 251 mμ. *Anal.* ($C_8H_{27}N_3O \cdot 0.25C_2H_5OC_2H_4OH$) C, H, N.

2,4-Diamino-5-(1-adamantyl)-6-(1-butyl)pyrimidine (5). The above compound 9 (800 mg) was chlorinated in the usual manner with $POCl_3$–PCl_5. The 4-chloro compound was collected and added immediately to a solution of 100 ml of absolute EtOH saturated with NH_3 at 0°. The mixture was heated at 150° for 24 hr. The work-up procedure was the same as described for **4**. The crude diaminopyrimidine was stirred for 2 days in EtOH containing ethylsulfonic acid and subsequently the product was precipitated with Et_2O. Recrystallization from H_2O yielded 200 mg of product: mp 204–205°; uv max (absolute EtOH) 290, 237 mμ; uv min 263 mμ. *Anal.* ($C_{20}H_{34}N_4O_3S$) C, H, N, S.

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Phenol–Piperazine Adducts Showing Anthelmintic Properties

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It has already been established that complexes formed from *tert*-butylphenols and piperazine are new entities displaying neither the toxicities nor the activities of their constituents,[1] the former being reduced and the latter enhanced to such a degree that complexes containing as little as 15% piperazine in combination with an inactive or barely active phenol can exhibit activity equal or even superior to that of piperazine itself. *This is all the more surprising since, in the case of the salts formed from piperazine and carboxylic acids, activity is generally considered as being directly linked to their piperazine content.*[2] The major advantage of certain of these carboxylates over the hydrate is their reduced toxicity and improved activity due to their solubilization exclusively in the intestinal milieu.[3]

Consideration of the results of work on *tert*-butylphenol–piperazine adducts prompted the following questions. (a) Phenols bearing substituents other than *tert*-butyl have been reported as anthelmintic.[4] What are the activities of their addition compounds with piperazine? Is there, for these complexes, any relation between the nature and site of the substitution on the phenyl group and anthelmintic properties? (b) The activity of salts formed from acids and piperazine is largely dependent on the amount of piperazine present in the adduct, but this rule does not hold for salts formed from phenols. Is then the binding of phenols to piperazine of the salt type, and, if not, what is the nature of this bond? Is there a relationship between the strength of this liaison and anthelmintic activity? The present work is an attempt to elucidate these points.

Physicochemical Study. In view of the small number of phenols already reported as having anthelmintic properties, we conducted a systematic investigation of the influence of Me, *i*-Pr, *tert*-Bu, OMe, Cl, NO_2, and of a second OH as substituents on the phenolic moiety.

With the monophenols, if the binding is not of the ionic (or salt) type it can be by hydrogen bridging or can correspond to a resonance hybrid between the two forms. As seen in Table I (I), the ir spectra of the monophenol adducts show, in place of the phenolic band at 3600 cm^{-1}, a narrow, intense band in the 3300-cm^{-1} region. This shift toward the lower wavelengths, together with the absence of a band in the 2500-2700-cm^{-1} region characteristic of amine salts, indicates a hydrogen bonding.[5] The adduct formed from *o*-*tert*-butylphenol presents a second interesting band at 3260 cm^{-1}. The presence of this, and of another at 3300 cm^{-1}, corresponds most probably to a mixture of isomeric adducts of the forms *Z* and *E*. These bands have already been described for *o*-*tert*-butylphenol.[6]

With the diphenols, both the meta and para derivatives give rise to adducts linking one phenolic hydroxyl to one