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Resistance-Modifying Agents. Part 7: 2,6-Disubstituted-4,8-dibenzylaminopyrimido[5,4-*d*]pyrimidines that Inhibit Nucleoside Transport in the Presence of α_1-Acid Glycoprotein (AGP)†

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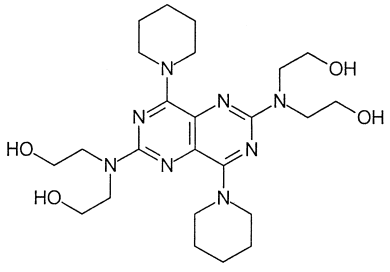
Abstract—The synthesis and biological evaluation of potent 4,8-dibenzylaminopyrimidopyrimidine nucleoside transport inhibitors, with reduced binding to α_1-acid glycoprotein, is reported. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Antimetabolite drugs which act by inhibition of de novo purine and pyrimidine biosynthesis play an important role in the chemotherapy of cancer,[1,2] clinically relevant examples including methotrexate, 5-fluorouracil (5FU) and the classical thymidylate synthase (TS) inhibitor raltitrexed. Tumour resistance to antimetabolites may arise via a number of mechanisms, one of which involves the salvage of extracellular nucleosides and nucleobases via carrier-mediated plasma membrane transport, resulting in repletion of nucleotide pools.[3–5]

Potentiation of the in vitro and in vivo cytotoxicity of a number of antimetabolite drugs has been observed with the cardiovascular and antithrombotic drug dipyridamole **1**,[6–8] which is an inhibitor of nucleoside and nucleobase transport.[9,10] However, the disappointing clinical activity of **1** has prevented the development of this agent as a resistance-modifier in cancer chemotherapy.[11,12]



1

Dipyridamole binds avidly to α_1-acid glycoprotein (AGP, orosomucoid), an acute phase protein, the plasma levels of which may be elevated in cancer patients,[13,14] and, as a consequence, AGP reduces the effective concentrations of the drug below those required to inhibit salvage pathways.[15,16] Thus, binding to AGP may account for the failure of **1** to enhance the activity of antimetabolite anticancer drugs in clinical trials. It is likely that a derivative of **1** which exhibited potent nucleoside transport inhibitory activity, but which did not bind to AGP, would have potential clinical application as a modulator of antimetabolite cytotoxicity in the therapy of cancer.

As part of an ongoing program to develop novel pyrimidopyrimidines as resistance-modifiers in cancer chemotherapy, we have conducted studies designed to elucidate structure–activity relationships for binding to

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†For part 6 see Curtin, N. J.; Bowman, K. J.; Turner, R. N.; Huang, B.; Loughlin, P. J.; Calvert, A. H.; Golding, B. T.; Griffin, R. J.; Newell, D. R. *Br. J. Cancer* **1999**, *80*, 1738.

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AGP. In this letter we describe the synthesis and preliminary biological evaluation of a new series of 4,8-dibenzylaminopyrimidopyrimidines, which are at least as potent as **1** as inhibitors of thymidine transport, but which retain activity in the presence of supraphysiological concentrations of AGP.

Chemical Synthesis

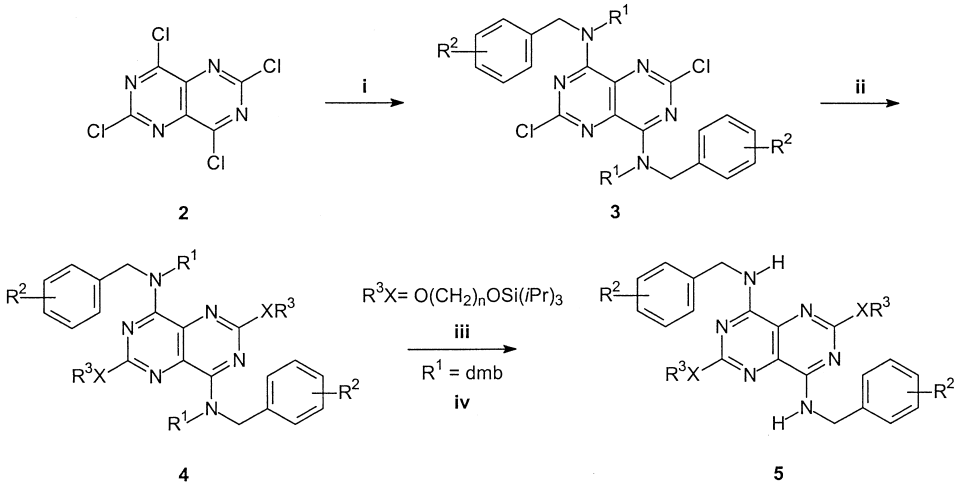
The required pyrimidopyrimidines were initially prepared analogously to **1**, following a two step literature procedure starting from tetrachloropyrimidopyrimidine **2**.[17,18] Thus, treatment of **2** with 4 equivalents of the required benzylamine at room temperature to give **3**, followed by 4 equivalents of the appropriate amine or alkoxide at elevated temperature (reactivity order: 4≅8 >2≅6) afforded the target compounds. However, while suitable for the preparation of analogues bearing *N*-methylbenzylamino substituents at the 4/8 positions (**4**, R^1 = Me), analogous reactions with benzylamines afforded low product yields and necessitated extensive product purification. This was attributed to side reactions arising from deprotonation of the relatively acidic 4,8-dibenzylamino functionality (**3**, R^1 = H) under the basic conditions and elevated temperatures employed in the second displacement step.

Protection of the vulnerable benzylamino NH substituent was achieved with the 3,4-dimethoxybenzyl (dmb) protecting group. This was introduced by reacting the appropriate benzylamine with 3,4-dimethoxybenzaldehyde in dry ethanol, and reducing the resultant imine with sodium borohydride in methanol.[19] Treatment of **2** with the requisite *N*-dmb-protected benzylamine at room temperature, gave the 4,8-disubstituted derivatives (**3**, R^1 = dmb) in excellent yield. Substitution at the 2,6-positions by the required amine or mono-triisopropylsilyl protected diol was then achieved without incident to furnish **4**. Where necessary, removal of the triisopropylsilyl groups at the 2,6-positions was effected with TBAF, and the 4,8-dmb protecting groups were finally removed on treatment with TFA or DDQ at room temperature, affording the target pyrimidopyrimidines **5** in good overall yield.[20]

Results and Discussion

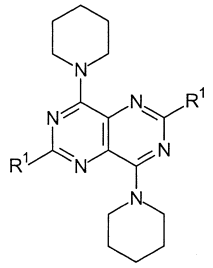
Although a potent inhibitor of nucleoside and nucleobase transport, dipyridamole exhibits poor aqueous solubility, and being hydrophobic and weakly basic (pK_a = 6.4), the drug binds avidly to AGP. In order to address these problems we first sought to identify novel pyrimidopyrimidines that retain the potency of **1**, but which do not exhibit significant binding to AGP. Accordingly, extensive structure–activity studies have been conducted with regard to substituents at the 2,6- and 4,8-positions of this heterocycle, and these will be reported subsequently in a full paper. Initially, the effect of replacing the 2,6-amino functions of dipyridamole with alkoxy groups was investigated, in the expectation that this modification would reduce the basicity of the molecule and attenuate binding to AGP. This indeed proved to be the case, as exemplified by the 2,6-bis-(2,3-dihydroxypropoxy) derivative **6**, where inhibition of ^{3}H-thymidine transport at an inhibitor concentration of 10 μM was not significantly reduced in the presence of 5 mg/mL of AGP, an AGP concentration which ablates the activity of **1** under the same conditions (Table 1). Unfortunately, this structural modification also resulted in approximately a 30-fold reduction in potency (IC_{50} value = 9.3 μM), as compared with **1** (IC_{50} value = 0.37 μM), and the introduction of other alkoxy substituents at the 2,6-positions did not markedly enhance potency.

With a view to increasing potency in the 2,6-dialkoxypyrimidopyrimidine series without restoring AGP binding, the effect of replacing the 4,8-piperidino groups of **1** with other amines was investigated. The introduction of amino or simple alkylamino substituents in the

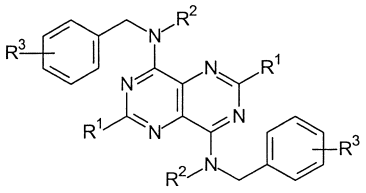


Scheme 1. General synthesis of pyrimidopyrimidines. Reagents and yields: (i) appropriate benzylamine, K_2CO_3, THF, 25 °C, 75–90%; (ii) where X = O: R^3OH, NaH, THF, reflux, where X = NR^4: R^3NHR^4, THF, 120–150 °C, 40–60%; (iii) TBAF, THF, 25 °C, 60–80%; (iv) TFA, 25 °C or DDQ in CH_2Cl_2/H_2O, 25 °C, 50–70%. dmb = 3,4-Dimethoxybenzyl.

Table 1. Inhibition of ^{3}H-thymidine uptake into L1210 cells by selected pyrimidopyrimidines[22]



A



B

No.	Structure	R^1	R^2	R^3	% Inhibition at 1 μM (no. of determinations in parentheses)		
					Inhibitor alone	+ 5mg/mL AGP[a]	Reduction (%)
1	**A**	$N(CH_2CH_2OH)_2$	—	—	89 ± 5[b] (9)	4 ± 11 (9)	96
6	**A**	$OCH_2CH(OH)CH_2OH$	—	—	37 ± 17 (21)[c]	29 ± 4 (3)	22
7	**B**	$N(CH_2CH_2OH)_2$	H	H	38 ± 1 (3)	21 ± 5 (3)	45
8	**B**	$N(CH_2CH_2OH)_2$	H	4-OMe	56 ± 10 (7)	46 ± 8 (7)	18
9	**B**	$N(CH_2CH_2OH)_2$	H	$3,4\text{-}(OMe)_2$	71 ± 5 (9)	53 ± 7 (9)	26
10	**B**	$N(CH_2CH_2OH)_2$	Me	4-OMe	75 ± 10 (12)	40 ± 20 (10)	47
11	**B**	$NHCH_2CH(OH)CH_3$	H	4-OMe	57 ± 11 (15)	32 ± 6 (7)	45
12	**B**	$NHCH_2CH(OH)CH_3$	H	$3,4\text{-}(OMe)_2$	85 ± 7 (9)	73 ± 7 (9)	14
13	**B**	$NHCH_2CH(OH)CH_3$	Me	4-OMe	39, 33 (2)	10,3, 3 (3)	86
14	**B**	$NHCH_2CH(OH)CH_3$	Me	$3,4\text{-}(OMe)_2$	73 ± 9 (9)	57 ± 12 (7)	22
15	**B**	$O(CH_2)_2OH$	H	4-OMe	40 ± 19 (11)	28 ± 25 (5)	30
16	**B**	$O(CH_2)_2OH$	H	$3,4\text{-}(OMe)_2$	57 ± 6 (6)	44 ± 8 (3)	23
17	**B**	$O(CH_2)_3OH$	H	4-OMe	66 ± 4 (6)	49 ± 6 (5)	26
18	**B**	$O(CH_2)_2OH$	Me	4-OMe	73 ± 7 (6)	17 ± 20 (5)	77
19	**B**	$O(CH_2)_3OH$	Me	4-OMe	69 ± 19 (8)	32 ± 10 (8)	53

[a]α_1-Acid glycoprotein.
[b]Standard deviation.
[c]Determined at 10 μM.

4,8- positions resulted in compounds with very poor activity as inhibitors of thymidine transport (data not shown). However, the introduction of benzylamino groups (**7**) resulted in only approximately a 2-fold reduction in potency compared with **1**, and electron-donating substituents on the aryl ring of the benzylamino function enhanced activity, with **8** and **9** (IC_{50} values = 0.25 μM), proving at least equipotent with **1**. Crucially, and in contrast to **1**, the activity of **8** was not significantly reduced in the presence of AGP, and this was also observed for **12** which bears a 2-hydroxypropylamino substituent at the 2,6-positions. Although the reduced activity of compounds **9** and **11** in the presence of AGP was significant, this effect was modest compared with that observed for **1**. Taken together, these results suggest that AGP binding is not immutably associated with the presence of amino functions at the 2,6-positions.

Having identified substituted-benzylamino as an alternative to piperidino at positions 4- and 8, the effect of combining this functionality with simple hydroxyalkoxy substitution at the 2,6-positions was investigated. Thus, derivatives with a 2-hydroxyethoxy- (**15** and **16**) or 3-hydroxypropoxy- (**17**) substituent at the 2,6-positions, and a 4-methoxybenzylamino- (**15** and **17**) or 3,4-dimethoxybenzylamino- (**16**) group at the 4,8-positions, were prepared and evaluated. Although **17** was perhaps slightly more potent than **15** and **16**, all three compounds were less active than the corresponding 2,6-bis-(diethanolamino) derivative **8**, and this is consistent with the reduced potency of **6** compared with **8**. Addition of AGP had little or no effect on the inhibitory activity of compounds **15**, **16** or **17**. Finally, a comparison of the inhibitory activities of 4,8-dibenzylaminopyrimidopyrimidines with their *N*-methylbenzylamino counterparts suggests that the latter compounds may have a greater binding affinity for AGP. Thus, the activity of **10** is markedly reduced in the presence of AGP compared with that of compound **8**, where no significant reduction in activity is apparent. A similar relationship is also evident between the *N*-methylbenzylamino compounds **13**, **14**, **18** and **19**, and their respective benzylamino partners **11**, **12**, **15** and **17**. Although speculative, these observations suggest that a *tertiary*-amino function at pyrimidopyrimidine positions 4- and 8 may be a determinant of AGP binding, which would be consistent with the high binding affinity of dipyridamole (**1**).

Several of the more promising dibenzylaminopyrimidopyrimidines have been selected for in vitro and in vivo biological evaluation. Indeed, we have recently demonstrated that compound **8** (NU3076) potentiates the in vitro cytotoxicity of 5-FU, and the antifolate TS inhibitors CB3717 and nolatrexed in L1210 murine leukaemia cells.[21]

Acknowledgements

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19. In a typical preparation, a mixture of 3,4-dimethoxybenzylamine (1.80 mL, 12.0 mmol) and 3,4-dimethoxybenzaldehyde (2.00 g, 12.0 mmol) was refluxed in dry ethanol (20 mL) under N_2 for 1 h. Solvents were removed in vacuo, and the residual oil was triturated with methanol to give *N*-(3,4-dimethoxybenzyl)-*N*-3,4-dimethoxybenzylimine (3.69 g, 97%) as a white solid, mp 77–79 °C. Found C, 68. 31; H, 6.53; H, 4.18%. $C_{18}H_{21}NO_4$ requires C, 68.55; H, 6.71; N, 4.44%; δ_H (200 MHz, $CDCl_3$) 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.67 (s, 2H, ArCH_2), 7.05 (m, 6H, 6×Ar-H), 8.21 (s, 1H, ArCH=N); *m*/*z* (EI) 316 (MH^+), 315 (M^+, 18%), 151 ($[MeO]_2C_7H_7^+$, 100%). To a stirred solution of *N*-(3,4-dimethoxybenzyl)-*N*-3,4-dimethoxybenzylimine (3.50 g, 11.1 mmol) in dry methanol (20 mL) at 40 °C, was added sodium borohydride (0.42 g, 11.1 mmol) over a 20 min period. The mixture was refluxed for 1 h and the solvents were removed to afford *N*,*N*-bis-(3,4-dimethoxybenzyl)amine (3.10 g, 88%) as a white solid, mp 69–71 °C. Found C, 67.71; H, 7.13; N, 4.35%. $C_{18}H_{23}NO_4$ requires C, 68.12; H, 7.30; N, 4.41%; δ_H (200 MHz, $CDCl_3$) 1.51 (br s, 1H, NH), 3.68 (s, 4H, 2×ArCH_2), 3.81 (s, 6H, 2×OCH_3), 3.82 (s, 6H, 2×OCH_3), 6.78 (m, 6H, 6×Ar-H); *m*/*z* (EI) 317 (M^+, 7%), 166 ($[MeO]_2C_7H_5NH^+$), 151 ($[MeO]_2C_7H_5^+$, 100%).
20. A representative synthesis (compound **16**) is as follows: (A) a mixture of 2,4,6,8-tetrachloropyrimidopyrimidine (0.26 g, 0.98 mmol), *N*,*N*-bis-(3,4-dimethoxybenzyl)amine (1.24 g, 3.91 mmol) and potassium carbonate (0.59 g, 5.87 mmol) in THF (10 mL) was stirred at 25 °C for 1 h. Water (75 mL) was added and the resulting solid was collected, washed with water and dried in vacuo to give 2,6-dichloro-4,8-bis-(*N*,*N*-di-[3′,4′-dimethoxybenzyl]amino)pyrimidopyrimidine (0.55 g, 68%) as a yellow solid, mp 197–199 °C. Found C, 59.22; H, 4.91; N, 9.81%. $C_{42}H_{44}Cl_2N_6O_8{\cdot}1.0\ H_2O$ requires C, 59.36; H, 5.22; N, 9.89%; δ_H (200 MHz, $CDCl_3$) 3.78 (s, 12H, 4×OCH_3), 3.81 (s, 12H, 4×OCH_3), 4.77 (br s , 4H, 2×ArCH_2), 5.33 (br s, 4H, 2×ArCH_2), 6.78 (m, 12H, 4×$[MeO]_2$Ar-*H*); *m*/*z* (EI) 830, 832, 834 (M^+, 9:6:1, 16%), 679 (M^+-$[MeO]_2C_6H_3CH_2$), 529 (M^+-2×$[MeO]_2C_6H_3CH_2$), 151 ($[MeO]_2C_6H_3CH_2^+$, 100%). (B) Sodium hydride (0.07 g, 2.76 mmol) was added to a solution of 2-triisopropylsilyloxyethan-1-ol (0.55 g, 2.51 mmol) in dry THF (15 mL), and the mixture was stirred at 25 °C for 1 h. A solution of 2,6-dichloro-4,8-bis-(*N*,*N*-di-[3′,4′-dimethoxybenzyl]-amino)pyrimidopyrimidine (1.00 g, 0.84 mmol) in dry THF (15 mL) was added and the mixture was heated under reflux for 12 h. Water (30 mL) was added and the mixture was extracted with ethyl acetate (4×20 mL) and dried (Na_2SO_4). The yellow oil remaining after solvent removal was purified by chromatography on silica, employing petroleum ether (boiling range 40–60 °C):ethyl acetate (3:2) as eluent, to give the required 2,6-di-(2′-triisopropylsilyloxy)ethoxy-4,8-bis-(*N*,*N*-di-[3′,4′-dimethoxybenzyl]amino)pyrimidopyrimidine (0.40 g, 40%) as a white solid, mp 81–83 °C. Found C, 64.58; H, 8.26; N, 6.99%. $C_{64}H_{94}N_6O_{12}Si_2$ requires C, 64.29; H, 7.92; N, 7.03%; δ_H (200 MHz, $CDCl_3$) 0.90–0.95 (m, 42H, 2×Si[$CH(CH_3)_2$]$_3$) 3.72 (m, 16H, 4×OCH_3 and 2×SiOCH_2CH_2O), 3.80 (s, 12H, 4×OCH_3), 3.97 (t, 4H, 2×SiOCH_2CH_2O, J=5.4 Hz), 5.16 (br s, 8H, 4×ArCH_2), 6.76 (m, 12H, 12×Ar-H); *m*/*z* (EI) 1194 (M^+, 0.5%), 1151 (M^+-C_3H_7), 1043 (M^+-$[MeO]_2C_7H_5$), 151 ($[MeO]_2C_7H_5^+$, 100%). (C) To a solution of 2,6-di-(2′-triisopropylsilyloxy)ethoxy-4,8-bis-(*N*,*N*-di-[3′,4′-dimethoxybenzyl]-amino)pyrimidopyrimidine (0.40 g, 0.34 mmol) in THF (15 mL) was added tetrabutylammonium fluoride (1 M soln in dry THF, 1.36 mL, 1.36 mmol). The mixture was stirred for 12 h at room temperature, and solvents were removed in vacuo. The yellow residue was suspended in water (30 mL), stirred for 12 h, and the product was collected to give 2,6-di-(2′-hydroxyethoxy)-4,8-bis-(*N*,*N*-di-[3′,4′-dimethoxybenzyl]amino)pyrimidopyrimidine (0.20 g, 68%) as a white solid, mp 196–198 °C. Found C, 61.93; H, 6.40; N, 9.20%. $C_{46}H_{54}N_6O_{12}.0.2H_2O$ requires C, 62.32; H, 6.14; N, 9.48%; δ_H (200 MHz, d_6-DMSO) 3.54 (m, 4H, 2×HOCH_2CH_2O), 3.76 (s, 12H, 4×OCH_3), 3.81 (s, 12H, 4×OCH_3), 3.94 (t, 4H, 2×$HOCH_2$ CH_2O), 4.84 (t, 2H, 2×OH), 5.30 (br s, 8H, 4×ArCH_2), 6.96 (m, 12H, 12×Ar-H); *m*/*z* (EI) 882 (M^+, 0.2%), 731 (M^+-$[MeO]_2C_7H_5$), 151 ($[MeO]_2C_7H_5^+$, 100%). (D) A solution of 2,6-di-(2′-hydroxyethoxy)-4,8-bis-(*N*,*N*-di-[3′,4′-dimethoxybenzyl]amino)pyrimidopyrimidine (0.13 g, 0.15 mmol) in trifluoroacetic acid (2.5 mL) was stirred for 3 h at 25 °C. Excess trifluoroacetic acid was removed in vacuo and the residue was suspended in sat. $NaHCO_3$ solution (20 mL), and stirred for 12 h. The precipitated solid was collected by filtration and purified by chromatography on silica, with dichloromethane:methanol (98:2) as eluent, to furnish 2,6-di-(2′-hydroxyethoxy)-4,8-*N*,*N*-di-(3′,4′-dimethoxybenzyl)aminopyrimidopyrimidine (0.03 g, 36%) as a pale yellow solid, mp 216–218 °C; δ_H (200 MHz, d_6-DMSO) 3.80 (m, 6H, 2×OCH_3), 3.82 (s, 6H, 2×OCH_3), 4.41 (m, 4H, 2×HOCH_2CH_2O), 4.69 (m, 8H, 2×$HOCH_2CH_2O$ and 2×ArCH_2), 4.92 (t, 2H, 2×OH, J=5.3 Hz), 7.05 (m, 6H, 6×Ar-H), 8.51 (t, 2H, 2×NH); *m*/*z*

(EI) 582 (M^+, 67%), 166 ($[MeO]_2C_7H_5NH^+$), 151 ($[MeO]_2C_7H_5^+$, 100%).
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22. Thymidine uptake in murine L1210 leukaemia cells was assayed as described in ref 21. Briefly, the uptake of 100 μM thymidine by 10^6 cells was followed at 2 s intervals over a 12 s time course in the presence or absence of inhibitor in 1 or 5% (v/v) DMSO. In experiments to determine the effect of AGP, the uptake of thymidine was also measured in the presence of 5 mg/mL human AGP.