SYNTHESES OF 3-SUBSTITUTED 6-AMINO-2-METHYLPYRIMIDO-[4,5-*e*][1,2,4]TRIAZIN-8-ONES (7-SUBSTITUTED 6-METHYL-6-AZA-PTERINS) AS INHIBITORS OF DIHYDROPTERIDINE REDUCTASE FROM HUMAN BRAIN

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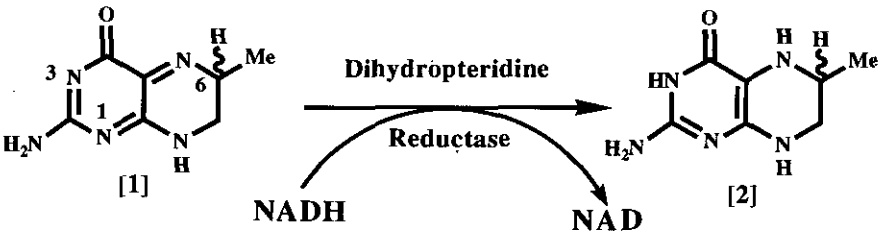
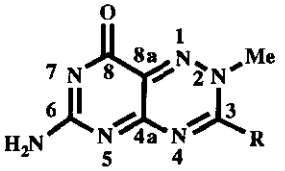
Abstract - Condensation of 5,5-dibromo-2-aminopyrimidine-4,6-dione with S-methyl-, S-benzyl- and S-*p*-carboxybenzyl- 2-methylisothiosemicarbazide hydrobromides provided 3-methylthio-, 3-benzylthio- and 3-*p*-carboxybenzylthio-6-amino-2-methylpyrimido[4,5-*e*][1,2,4]triazin-8-ones. Similar condensations with 1-amino-1-methylguanidinium bromide and 2,3-dimethylamidrazone hydrochloride gave 3-amino- and 3-methyl- 6-amino-2-methylpyrimido[4,5-*e*][1,2,4]triazin-8-one. The 3-thio derivatives hydrolyse in aqueous solution to the same 2-methylpyrimido[4,5-*e*][1,2,4]triazine-3,8-dione. The ionization and ^{13}C nmr spectra are consistent with the stated structures. These triazinones are not substrates for dihydropteridine reductase from human brain but are inhibitors of the reductase and their K_i values are reported.

Dihydropteridine reductase is a cytosolic enzyme that recycles the reduced pteridine cofactor for the aromatic amino acid hydroxylases[1,2] as well as other hydroxylases.[3] It catalyses the reduction of *quinonoid* 7,8-dihydro(6*H*)pterins (eg **1**) to the corresponding 5,6,7,8-tetrahydro(3*H*)pterins (eg **2**) while using NADH as the hydride source (Scheme 1).[1,4] We have succeeded in expressing the human dihydropteridine reductase in *E. coli* [5] and have obtained large quantities of the enzyme. We have also obtained crystals of the enzyme-NADH binary complex for X-ray analysis.[6] In order to obtain the structure of the ternary complex, enzyme-pterin-NADH, we required an azaheterocycle whose structure was very similar to a pterin substrate such as *quinonoid* 6-methyl-7,8-dihydro(6*H*)pterin [1], which did not "turn over" on the enzyme and which inhibited the enzyme by binding at the active site. By exposing the crystals to a solution of the inhibitor we should be able to infuse the inhibitor into the *quinonoid* pterin binding site to form the ternary complex in the crystals.

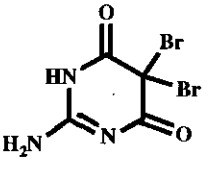


Scheme 1

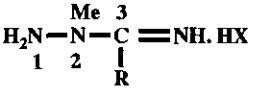
We chose to study a series of 6-amino-2-methylpyrimido[4,5-*e*][1,2,4]triazin-8-ones [**3**] because their structures are very similar to the effective substrate [**1**] and because they were unlikely to be reduced by the proposed enzyme. The active site of the reductase is known to tolerate small and large substituents on C7 of structure [**1**] without serious effects on enzyme activity.[2,7] The 6-amino-2-methylpyrimido[4,5-*e*][1,2,4]triazin-8-ones are indeed 6-azapterins.



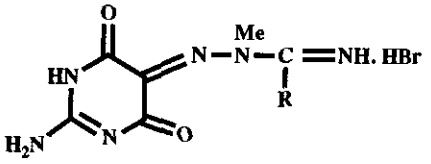
[**3a**] R = SMe
[**3b**] R = SCH_2Ph
[**3c**] R = $SCH_2C_6H_4$-*p*- CO_2H
[**3d**] R = NH_2
[**3e**] R = OH
[**3f**] R = Me



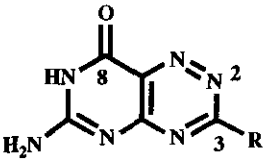
[**4**]



[**5a**] R = SMe, X = Br
[**5b**] R = SCH_2Ph, X = Br
[**5c**] R = $SCH_2C_6H_4$-*p*- CO_2H X = Br
[**5d**] R = NH_2, X = Br
[**5e**] R = Me, X = Cl



[**6**]



[**7a**] R = SEt, [**7b**] R = SCH_2Ph, [**7c**] R = NH_2

Pyrimido[4,5-*e*][1,2,4]triazin-8-ones have been known for some time,[8] notably through the works of E. C. Taylor, F. Yoneda, D. J. Brown, and S. Matsuura and their coworkers. Most of the compounds known have an oxo group at C6 as well as C8, some have aryl substituents at N2. Although Sugimoto and Matsuura[9] reported several 6-amino-8-oxo derivatives with amino and alkylthio groups at C3, none had an alkyl group at N2. No derivatives with the precise structures that we proposed to prepare have been reported. However we have successfully adopted the procedure developed by Sugimoto and Matsuura[9] to make the desired 2-methyl derivatives [**3**]. Condensation of 5,5-dibromo-2-aminopyrimidine-4,6-dione [**4**] with S-methyl- [**5a**], S-benzyl- [**5b**] and S-*p*-carboxybenzyl- [**5c**] 2-methylisothiosemicarbazide hydro-

bromide proceeded at room temperature in DMF to provide the 6-amino-2-methylpyrimido[4,5-*e*][1,2,4]triazin-8-ones [**3a-c**] respectively in high yields as the hydrobromide salts. A similar condensation using 1-amino-1-methylguanidinium bromide [**5d**] gave the hydrobromide of the 3,6-diamino compound [**3d**] in 86% yield. The 2-benzylthio derivative [**3b**] dissolved in boiling 3% aqueous ammonia solution, and after acidification with aqueous HBr gave the hydrobromide of the 3,6-dioxo derivative [**3e**]. At first we thought that all our products were the 1-amino-5-semicarbazidopyrimidine-4,6-diones [**6**] because they were all isolated as the hydrobromide salts. However, the elemental analyses, the isolation of the stable free bases and their elemental analyses, their pKa values, infrared and ^{13}C nmr spectra (see below and Experimental) all support the pyrimido[4,5-*e*][1,2,4]triazin-8-one structures.

Although the 3-thio derivatives [**3a**] and [**3b**] are good inhibitors (see below) they would not be satisfactory for making the ternary complex with the reductase because they are relatively unstable at near neutral pH and undergo hydrolysis to the oxo compound [**3e**] with half lives of 6 days and 26.4 hours respectively at 25°C. Also they may not survive irradiation during X-ray analysis. The amino and oxo compounds [**3d**] and [**3e**] would be unsatisfactory for preparing the ternary complex, although they are inhibitors, because of the possibility that they may bind at the active site in a manner that is different from the pterin substrate. We therefore synthesised the 3-methyl triazinone [**3f**] which is considerably more stable than [**3a**] and [**3b**] in solution and, as predicted, is an inhibitor of the enzyme.

Table 1 Ionization[a] of Pyrimido[4,5-*e*][1,2,4]triazin-8-ones in H_2O at 25°C

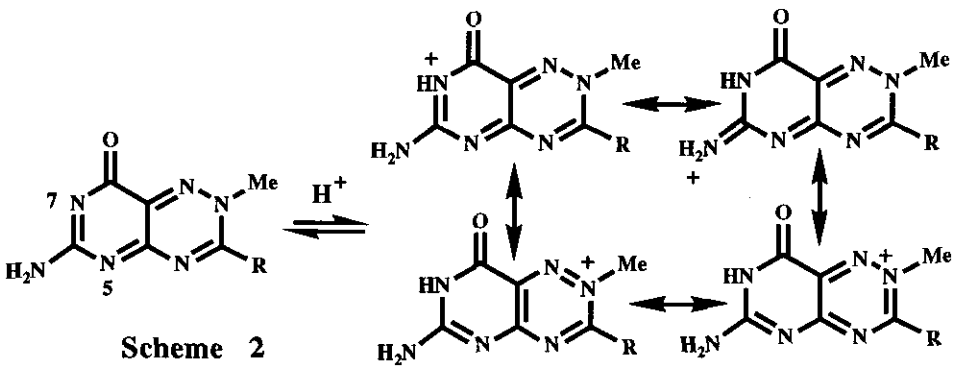
6-Amino-2-methylpyrimido-triazin-8-one				6-Aminopyrimido-triazin-8-one[b]		
	pKa	Species	λ nm[c]		pKa	Species
3-SMe [**3a**]	4.26	(+)	274	3-SEt [**7a**]	6.72	(-)
3-SCH_2Ph [**3b**]	4.72	(+)	272	3-SCH_2Ph[**7b**]	6.72	(-)
3-NH_2 [**3d**]	4.87	(+)	240	3-NH_2 [**7c**]	3.91	(+)
3-OH [**3e**]	1.43	(+)	240			
	6.11	(-)	370			
3-Me [**3f**]	4.32	(+)	270			

[a] Measured by standard procedures, *cf* The Determination of Ionization Constants by A. Albert and E. P. Serjeant, third edition, Chapman and Hall, London 1984; (+) for basic pKa and (-) for acidic pKa

[b] Taken from Ref. 9. [c] Analytical wavelength.

Ionization The ionization data for the 6-amino-2-methylpyrimido[4,5-*e*][1,2,4]triazin-8-one [**3a,b,d,e,f**] are in Table 1 together with the values for the 2-demethyl compounds [**7a-c**] for comparison taken from the literature.[9] The pKa values for **7a** and **7b** are for acidic ionization whereas the value for the 3-amino derivative [**7c**] is for basic ionization (ie protonation). The reason for thinking at first that products from the condensation were the uncyclised compounds [**6**] was that they formed stable salts and should

be strong bases [*cf* S-methylisothiouronium salts[10] have pKa 9.81 and guanidinium salts[11] have pKa 13.4). The products **[3a,b,d,f]** are weaker bases than **6** and although the uv maxima are not very different on protonation, the spectral changes are considerable (not shown). Structures such as **6** should show small, if any, uv spectral changes on protonation. The 2-methyl compounds **[3]** are stronger bases than the parent compounds **[7]** which is consistent with extended delocalisation of the positive charge in compounds **[3]** on protonation at N7 which does not occur in compounds **[7]** (Scheme 2). Similar structures can be drawn for protonation of **3** at N5.



Scheme 2

The acidic pKa of 6.11 for the oxo compound **[3e]** is consistent with those of **7a** and **7b**, the stronger acidity being attributed to electron attracting effect of the 3-oxo group. In this compound **[3e]** we found a second ionization which gives undoubtedly a basic pKa.

Table 2 ^{13}C Nmr Spectra[a] of 6-Amino-2-methylpyrimido[4,5-*e*][1,2,4]triazin-8-ones in DMSO-d_6

	2-CH_3	C3	C4a	C6	C8	C8a	Miscellaneous
3-SMe **[3a]** HBr	45.7	157.5	157.1	159.5	169.8	132.9	SCH_3 14.8
3-SCH_2Ph **[3b]** HBr	45.9	157.5	156.8	159.6	168.7	133.4	SCH_2[b] 35.6
3-Amino **[3d]** HBr	43.9	160.4	155.6	160.0	161.7	130.3	
3-OH **[3e]** HBr	41.3	156.0	147.6	156.6	158.3	126.0	
3-Me **[3f]**	45.3	162.6	159.0	165.8	168.5	133.8	3-CH_3 22.3
$H_2NN(Me)C(SMe)=NH$ HBr **[5a]**	SCH_3 14.4; 2-NCH_3 42.5; -C=N 171.0						
$H_2NN(Me)C(Me)=NH$ HCl **[5e]**	CH_3 16.4; 2-NCH_3 41.6; -C=N 164.2						

[a] Chemical shifts at 75.65 MHz relative to the central signal from DMSO at 39.5 ppm.

[b] Aromatic carbons: C1 135.2, C2,6 129.4, C3,5 128.7 and C4 128.0.

Spectra The ^1H nmr spectra of the 6-amino-2-methylpyrimido[4,5-*e*][1,2,4]triazin-8-ones agree with the cyclic structure **[3]** but do not exclude the open structures **[6]**. The ^{13}C nmr spectra, on the other hand, provide further proof of the cyclic structures **[3]** (see Table 2). All ^{13}C resonances were assigned by inspection and by comparison with those known for pterins.[12] The important features that confirm the cyclic structures **[3]** instead of **6** are the chemical shifts of C3 which are at 156-162.6 ppm and are based on the fact that the lowest field signal is from C8. This is to be compared with the chemical shift of C3 in **5a** and **5e** which are at 171 and 164.2 ppm, and which are at lower field than any of the ^{13}C resonances of structures **[3]** other than C8.

The ir spectra of the 6-amino-2-methylpyrimido[4,5-*e*][1,2,4]triazin-8-ones are very characteristic and have been useful to demonstrtate that the free bases released from their salts had not undergone degradation.

Table 3. Inhibition of Dihydropteridine Reductase (human brain)[a] in 0.1 M Tris-HCl pH ~ 7.4 and 25°C

Inhibitor	Procedure	NADH μM	Ki (and Kis) μM	Inhibition Type
3-SMe **[3a]**	$K_3Fe(CN)_6$[b]	110	77 (±13) {134 (±17), 160 (±24)}	Competitive {Mixed}
3-SCH_2Ph **[3b]**	Peroxidase/H_2O_2[b] $K_3Fe(CN)_6$[b]	132 120	17 (±2) 24 (±3)	Competitive Competitive
3-$SCH_2C_4H_4CO_2H$ **[3c]**	$K_3Fe(CN)_6$[c]	120	10.2 (±1.4)	Competitive
3-NH_2 **[3d]**	$K_3Fe(CN)_6$[c]	120	36.2 (±8.2)	Competitive
3-OH **[3e]**	$K_3Fe(CN)_6$[b]	112	307 (±30) {144 (±42)}	Non-competitive {Competitive}
3-Me **[3e]**	$K_3Fe(CN)_6$[b]	120	163 (±20)	Competitive

[a] The substrate was *quinonoid* 6-methyl-7,8-dihydro(6*H*)pterin **[1]**, values in chain brackets are from alternative computations. [b] Using a single beam spectrophotometer. [c] Using a double beam spectrophotometer.

Enzyme inhibition All the 6-amino-2-methylpyrimido[4,5-*e*][1,2,4]triazin-8-ones inhibited dihydropteridine reductase from human brain when *quinonoid* 6-methyl-7,8-dihydro(6*H*)pterin **[1]** was the substrate at saturating concentrations of NADH. It should be noted that substrate inhibition occurred at concentrations above 95 μM. The initial rates of the enzymic reaction (Scheme 1) in the presence of varying concentrations of substrate **[1]** and inhibitors **[3a-f]** were used to calculate the inhibition constants. Computer programs[13,14] were used to evaluate the data which was run using separate equations for competitive, non-competitive, uncompetitive and mixed-inhibition. The parameters that gave the smallest standard errors and variances are listed in Table 3. The data of some compounds appear to favour competitive inhibition as would be expected from the close similarity of the structures of the inhibitors and the substrate. However some of the data could also be fitted for other types of inhibition [*cf* **3a** and **3e**] albeit with larger standard errors and variances. 2,6-Diamino-5-phenylazopyrimidin-4-one was not a substrate for the reductase but it was a poor inhibitor. This compound reduced the initial rate by 15 ±2% at inhibitor,

substrate [1] and NADH concentrations of 100, 80 and 130 μM respectively.

EXPERIMENTAL

Elemental analyses were performed by the Australian National University Microanalytical Service. ^{1}H Nmr spectra were run on a Jeol FX90Q spectrometer and the ^{13}C nmr spectra were run on a Varian Gemini 300 spectrometer at 75.65 MHz (d ppm, J Hz). Ir spectra (ν in cm^{-1}) were in KBr discs using a Perkin Elmer 1600 Series FTIR spectrophotometer and uv spectra (λ in nm and ε in $M^{-1}cm^{-1}$)were measured on Varian Cary 219 and 2215 (double beam) and Perkin Elmer Lambda 1 (single beam) spectrophotometers thermostated at 25°C.

Syntheses

S-*Methyl-2*-N-*methylisothiosemicarbazide hydrobromide* [**5a**] was prepared from the sulphate salt[15] (3.36 g 10 mmol) in water (20 ml) by mixing with $BaBr_2$ (3.33 g, 10 mmol) in water (20 ml) and stirring at 25°C for 30 min. The insoluble $BaSO_4$ was removed, the filtrate was evaporated to dryness, the residue was extracted with EtOH, evaporated, extracted again with hot isoPrOH, filtered and Et_2O was added. The crystals were collected and recrystallised from EtOH-Et_2O to give the *hydrobromide* (2.34 g, 58.5%), mp 102-103°C (Anal. Calcd for $C_3H_9N_3S$. HBr: C, 18.01; H, 5.01; N, 21.00; S, 16.02; Br, 39.93. Found: C, 18.20; H, 5.30; N, 21.44; S, 15.89; Br, 39.56.); ^{1}H nmr δ (0.5M DCl): 2.62 (s, SCH_3) and 3.41 (s, NCH_3).

S-*Benzyl-2*-N-*methylisothiosemicarbazide hydrobromide* [**5b**] was prepared in 40% yield by adapting a previous procedure for making the non-methylated analogue[16] and using benzyl bromide. It had mp 148-149°C (Anal. Calcd for $C_9H_{13}N_3S$.HBr: C, 39.14; H. 5.11; N, 15.21; S, 11.61; Br, 28.93. Found: C, 38.89; H, 5.09; N, 15.12; S, 11.59; Br, 28.91.), ^{1}H nmr δ (D_2O): 3.39 (s, NCH_3); 4.40 (s, CH_2); 7.45 (s, C_6H_5); δ (DMSO-d_6): 4.09 (s, NCH_3); 4.65 (s, CH_2); 7.61-7.27 (m C_6H_5); 8.26 (br s, NH) and 9.62 (br s NH).

S-p-*Carboxybenzyl-2*-N-*methylisothiosemicarbazide hydrobromide* [**5c**] was prepared in 94% yield, mp 242-244°C (Anal. Calcd for $C_{10}H_{13}N_3O_2S$. HBr: C, 37.51; H, 4.41; N, 13.12; S, 10.01; Br, 24.95. Found: C, 37.96; H, 4.33; N, 12.97; S, 9.90; Br, 25.08.), ^{1}H nmr δ (D_2O): 3.41 (s, NCH_3); 4.50 (s, CH_2); 7.52, 7.61 and 7.97, 8.06 (AA'XX' system, $J_{AX} = J_{A'X'}$ 8.3, aromatic H).

2,3-Dimethylamidrazone hydrochloride [**5e**] was prepared by adding slowly acetamide ethyl ether hydrochloride[17] (7.41 g, 62 mmol) with stirring to a solution of methylhydrazine (2.85 g, 62 mmol) in ethanol (45 ml) cooled at -30°C (dry ice in CCl_4). The mixture was stirred at -30°C for 2 h and allowed to warm to 25°C. The *hydrochloride* separated as needles (2.19 g, 30%) which were washed with cold ethanol and recrystallised from isoPrOH. Evaporation of the filtrate *in vacuo* and recrystallisation of the residue from isoPrOH (40 parts) gave more *hydrochloride* . Total yield was 5.83 g (78.7%). It had mp 180-181°C

8.34; N, 31.91; Cl, 26.92. Found: C, 27.29; H, 8.01; N, 31.98; Cl, 27.02.); ^{1}H nmr δ (DMSO-d_6) 2.31 (s, CH_3); 3.32 (s, NCH_3); 5.60 (br s, NH_2) and 5.69 (br s, NH), and ir (ν) 3353 m, 3258 s, 3145 s, 1692 vs, 1617 m,1419 w and 1248 w.

6-Amino-2-methyl-3-methylthiopyrimido[4,5-e][1,2,4]triazin-8-one [**3a**]. The isothiosemicarbazide hydrobromide [**5a**] (1.2 g, 6mmol) was added slowly to a solution of 5,5-dibromo-2-aminopyrimidine-4,6-dione[9] [**4**] (1.14 g, 4 mmol) in an ice bath. The mixture was stirred at 25°C for 2 h and the solid that deposited was collected, washed with EtOH and recrystallised by dissolving in DMF at 25°C and adding Et_2O. The *hydrobomide* (1.0 g, 84%) had mp > 300°C (decomp.) (Anal. Calcd for $C_7H_8N_6OS$. 0.95 HBr. 0.25 DMF: C, 29.14; H, 3.38; N, 27.41; S, 10.04; Br, 23.77. Found: C, 29.27; H, 3.25; N, 27.25; S, 9.67; Br, 23.38.); ^{1}H nmr δ (0.5M DCl): 2.78 (s, SCH_3) and 4.21 (s, NCH_3); uv λ (ε): pH 2.62 (4 mM HCl) 273 (28100) and 345 (9500), pH 7.31 (0.1 M Tris-HCl): 272 (27400) and 343 (9000); ir (ν) : 3586 m, 3471 m, 3130 br m, 1730 s, 1675 s, 1564 vs, 1499 s, 1479 s, 1385 s, 1363 s, 1306 s and 1144 m. The *free base* was obatined when an aqueous suspension of the hydrobromide was treated with $NaHCO_3$ and the solid was collected and recrystallised from water. It had mp > 300°C (decomp.) (Anal. Calcd for $C_7H_8N_6OS$: C, 37.49; H, 3.60: N, 37.48; S, 14.30. Found: C, 37.55; H, 3.56; N, 37.56; S, 14.25.) and ir (ν): 3276 m, 3047 br m, 1653 s, 1640 m, 1580 vs, 1550 m, 1484 m, 1475 s, 1447 m, 1429 s, 1372 vs, 1338 s, 1316 m, 1183 m and 1104 m.

6-Amino-3-benzylthio-2-methylpyrimido[4,5-e][1,2,4]triazin-8-one [**3b**]. The *hydrobromide* was prepared at 25°C as above on a 1.6 mmol scale, but as the product remained in the DMF solution. Et_2O (30 ml) was added and the salt that separated was collected (an oil which solidified, 476 mg, 79%), washed with EtOH and recrystallised from DMF-Et_2O. It had mp > 300°C (decomp.) (Anal. Calcd for $C_{13}H_{12}N_6OS$. HBr: C, 40.95; H, 3.44; N, 22.04; S, 8.41; Br, 20.9. Found: C, 40.89; H, 3.08: N, 21.90: S, 7.93; Br, 21.35.) and before drying (Anal. Calcd for $C_{13}H_{12}N_6OS$. HBr. 0.5 H_2O: C, 40.01; H, 3.62; N, 21.53; S, 8.22; Br, 20.48. Found: C, 39.74; H, 3.35; N, 21.66; S, 8.04; Br, 20.36.); ^{1}H nmr δ (0.5 M DCl): 4.18 (s, NCH_3); 4.71 (s, CH_2) and 7.32-7.65 (m, aromatic H); δ (DMSO-d_6): 4.09 (s, NCH_3); 4.65 (s, CH_2); 7.27-7.61 (m, aromatic H); 8.26 and 9.62 (br s NH); uv λ (ε): pH 2.59, 274 (28200) and 345 (11000); pH 7.33, 274 (30900) and 343 (10700); ir (ν): 3114 br m, 1737 w, 1725 m, 1656 vs, 1569 vs, 1479s, 1387 s and 1313 m. The *free base* prepared as above had mp > 300°C (decomp.) and was sparingly soluble in MeOH, EtOH, DMF, MeCN and in H_2O slowly liberates benzylthiol (Anal. Calcd for $C_{13}H_{12}NOS$. 0.5 H_2O: C, 50.41; H, 4.23; N, 27.27; S, 10.35. Found: C, 50.43; H, 3.95; N, 27.66; S, 9.73.), ir (ν): 3323 m, 1669 m, 1601 vs, 1563 m, 1481 m, 1443 m, 1376 m, 1332 m, 1239 w, 1187 w, 822 w and 696 w.

*6-Amino-3-*p*-carboxybenzylthio-2-methylpyrimido[4,5-e][1,2,4]triazin-8-one* [**3c**]. The *hydrobromide* was prepared as above in 60% yield using **5c** and recrystallised from DMF-Et_2O. It had mp > 260°C (decomp.) (Anal. Calcd for $C_{12}H_{12}N_6O_3S$. HBr. 1.1 DMF: C, 41.09; H, 4.13; N, 19.67; S, 6.34; Br, 15.80. Found: C, 41.56; H, 3.98; N, 19.61; S, 6.06; Br, 16.33.); ^{1}H nmr δ (DMSO-d_6): 4.10 (s, NCH_3); 4.72 (s, CH_2); 7.57, 7.66 and 7.87, 7.97 (AA'XX' system, $J_{AX} = J_{A'X'}$ 8.4, aromatic H), 8.23 (br s, NH_2); 9.57 (s,

CO_2H); and ir (ν): 3423 s, 3111 br m, 1739 w, 1727 w, 1706 s, 1653 vs, 1576 s, 1476 s, 1380 s, 1313 m, 1249 m and 1118 m.

3,6-Diamino-2-methylpyrimido[4,5-e][1,2,4]triazin-8-one [**3d**]. Condensation as for **3a** and using **5d**[15] gave a solid (86%) which was washed with H_2O and had mp > 300°C (decomp.) (Anal. Calcd for $C_6H_7N_6O$. 0.55 HBr. 0.9 H_2O: C, 28.38; H, 3.71; N, 38.62; Br, 17.31. Found: C, 28.52; H, 3.95;N, 38.38; Br, 17.46.). The *free base* was obtained by stirring with aqueous $NaHCO_3$, recrystallised from water and had mp >300°C (decomp.) (Anal. Calcd for $C_6H_7N_7O$. 0.4 H_2O: C, 35.97; H, 3.92; N, 48.93. Found: C, 35.87; H, 3.88; N, 48.93.); uv λ (ε): pH 2.58, 232 (25200), 252 (23100) and 332 (8500); pH 7.33, 223 (18000), 250 (19100) and 326 (9400); ir (ν): 3332 s, 3106 br s, 1686 w, 1644 s, 1642 s, 1576 s, 1520 s, 1511 s, 1456 s and 1452 s.

6-Amino-2,3-dimethylpyrimido[4,5-e][1,2,4]triazin-8-one [**3f**]. Condensation of the amidrazone salt [**5e**] (0.99 g, 8 mmol) and **4** (1.71 g, 6 mmol) in DMF (15 ml) as above deposited crystals which were collected, washed with water, suspended in water, made alkaline with saturated aqueous $NaHCO_3$ and stirred at 25°C for 2 h. The solid was collected and recrystallised from water (100 parts) to give the *3-methylpyrimidotriazinone* (275 mg, 24%). It had mp > 300°C (decomp.) (Anal. Calcd for $C_7H_8N_6O$. 0.5 H_2O: C, 41.79; H, 4.51; N, 41.77. Found: C, 41.84; H, 4.57; N, 41.99.); ^1H nmr δ (DMSO-d_6) for HCl salt: 2.81 (s, 3-CH_3) and 4.26 (s, 2-CH_3) and for the *free base* 2.61 (s, 3-CH_3) and 4,02 (s, 2-CH_3); uv λ (ε): pH 1.4, 239 (6400), 266(8200), 298 (7600), 344 (7500); pH 7.56, 232 (5900), 277 (14000), 335 (6900); ir (ν): 3440 m, 3319 s, 3160 m, 1659 w, 1623 vs, 1507 s, 1487 s, 1369 m and 1350 m.

6-Amino-2-methylpyrimido[4,5-e][1,2,4]triazine-3,8-dione [**3e**]. The 3-benzylthio compound [**3b**] (500 mg) was dissolved in 3% aqueous ammonia (40 ml), refluxed for 1.5 h, cooled and acidified with 47% aqueous HBr (15 ml) to give pH ~ 0. The solution was evaporated to dryness and gave the *hydrobromide* (273 mg, 79%) mp > 300°C (decomp.) (Anal. Calcd for $C_6H_6N_6O$. HBr: C, 26.20; H, 2.56; N, 30.55; Br, 29.05. Found: C, 26.30; H, 2.57; N,30.39; Br, 29.24.); mass spectrum by fast atom bombardment with glycerol gave m/z 195, ie molecular ion ($C_6H_7N_6O_2^+$); ^1H nmr δ (DMSO-d_6): 3.75 (s, NCH_3); uv λ (ε): 2 M HCl, 250 (28500) and 348 (12700); pH 4.0, 228 (54000) and 336 (10600); pH 10.0, 228 (54000) and 330 (10800); ir (ν): 3136 br m, 2785 br m, 2748 br m, 1748 m, 1710 s, 1669 m, 1615 s, 1594 s, 1577 s, 1504 vs, 1338s, and 1299 w. The *free base* was obtained by stirring with aqueous $NaHCO_3$ and had mp > 300°C (decomp.) (Anal. Calcd for $C_6H_6N_6O_2$: C, 37.12; H, 3.11; N, 43.29. Found: C, 36.82; H, 2.75; N, 43.21.) and ir (ν): 3272 br m, 2998 br m, 1718 m, 1699 vs, 1662 s, 1573 vs, 1507 s, 1501 s, 1437 m, 1400 m, 1342 s, 1279 m, and 1263 m.

Enzyme inhibition All initial rates were measured at 340 nm and the reaction ε value was 6620 $M^{-1}cm^{-1}$ when $K_3Fe(CN)_6$ was used to generate *quinonoid* 6-methyl-7,8-dihydro(6*H*)pterin [**1**] from the tetrahydropterin [**2**] [ie 6200 $M^{-1}cm^{-1}$ for the oxidation of NADH and 2 x 210 $M^{-1}cm^{-1}$ for the oxidation of 2 x $K_3Fe(CN)_6$ to 2 x $K_4Fe(CN)_6$].[1]

Kinetic runs were performed with about five concentrations of substrate (one set without inhibitor) and four concentrations of inhibitor, ie 20-25 runs for each inhibitor. Two procedures were used (i) with a single beam spectrophotmeter where the cuvette contained 1 M Tris-HCl pH ~ 7.4 (100 µl, final concn. 0.1 M), 300 mM $K_3Fe(CN)_6$ (100 µl, final concn.~ 300 µM), inhibitor in 4 mM HCl (50 µl at various appropriateconcentrations), 6-methyl-5,6,7,8-tetrahydropterin HCl in 4 mM HCl (100 µl, various concentrations from 3mµ to 75 µM, final concn. 3-75 µM), 220-264 mM NADH (50 µl, final concn 110-132 µM, see Table 3) and de-ionized H_2O to make a total of 1 ml in a thermostated cell holder at 25°C. The reaction was started by injecting dihydropteridine reductase[19] (2-5 µl, ~ 0.2 µg in 50 mM Tris-HCl pH 7.4 and 2mM DTT). Blank rates without addition of enzyme were run and the necessary corrections for the enzymic rates were made. When peroxidase-H_2O_2 was the oxidant to generate the substrate [1] the cuvettes contained 1 M Tris-HCl pH ~ 7.4 (100 µl), peroxidase (50 µl, 0.2 mg in 0.1 M Tris-HCl pH ~ 7.4), H_2O_2 (20 µl, 10 mM in 0.1 M Tris-HCl pH ~ 7.4), inhibitor, tetrahydropterin and NADH as above, all made up to 1 ml with de-ionized water. (ii) With a double beam spectrophotometer and using $K_3Fe(CN)_6$ as oxidant, the two cuvettes contained all the above ingredients. They were allowed to equilibrate (*ca* 0.5 min) and the enzyme was injected into one cuvette.

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