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Bioorganic & Medicinal Chemistry 13 (2005) 3513–3518
Bioorganic & Medicinal Chemistry

Syntheses of novel heterocycles as anticancer agents

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Received 12 November 2004; revised 22 February 2005; accepted 25 February 2005
Available online 6 April 2005

Abstract—Several pteridine analogues **4–13**, **23–26** have been synthesized and tested in vitro against three cancer cell lines, MCF7 (breast), NCI-H460 (lung) and SF-268 (CNS). All tested pteridines can serve as novel templates for the anticancer chemotherapy and can serve as new leads in cancer chemotherapy.

1. Introduction

The pharmacological approach to neoplastic disease has made some impressive gains since 1940 when the antileukemic activity of nitrogen mustard was discovered during World War II. Cancer has been recognized as a disease of aberrant cellular proliferation, with traditional cancer therapies aiming to exploit the proliferation machinery. As such, they demonstrate only partial selectivity for tumour cells over normal cells in proliferation tissue in the gut and bone marrow.

It is now generally accepted that a neoplastic transformation is related to genes alteration or oncogene activation, allowing progress in the development of new treatments for malignant diseases, both by revealing the pathobiology of the disease and the discovery of new drugs. Moreover, the role of many proteins has been identified as novel targets in cancer therapy[1] allowing the design of more selective agents.

The classical anticancer agent methotrexate (MTX) which is a pteridine analog [2] owes its cytotoxicity by inhibiting the enzyme dihydrofolate reductase (DHFR). DHFR catalyzes NADPH dependent reduction of 7,8-dihydrofolate to 5,6,7,8-tetrahydrofolate. It also catalyzes reduction of folate to 7,8-dihydofolate by NADPH. Without the function of DHFR, cells are deprived of key metabolic intermediates needed to form nucleotides and ultimately nucleic acids required for proliferation of cells.[3–7]

Bearing in mind the importance of folate metabolism in the proliferation of cells, we have synthesized several pteridines analogues (**4–13**, **23–26**) as anticancer agents. Many heterocycles such as quinoline, benzimidazole, isoxazole and uracil have showed interesting cytotoxic activity[1] and different mode of action in cancer therapy when compared with pteridines.[2] Keeping in view all of the above facts, we have synthesized hybrid compounds having pteridine along with different heterocyclic rings in key positions, 2 and 4. Moreover, in aqueous solution pteridine adds one or two molecules of water reversibly across the 3,4-double bond or 5,6- and 7,8-double bonds, respectively. The equilibrium ratio in favour of the hydrated or anhydrous adducts varies in acidic or basic environment.[8] This property is of great importance since it has the potential to increase bioavailability of these molecules.

We have synthesized several 2- and 4-substituted pteridine having different heterocycles in position 2 linked through a thio ether group (**4–13**, **26**) and an alkylamino group on position 4 (**23–25**) in order to establish structure activity relationship. All synthesized compounds were tested in vitro against three cancer cell lines, MCF7 (breast), NCI-H460 (lung) and SF-268 (CNS). All tested pteridines are potential novel templates for anticancer chemotherapy and can serve as new clinical leads in cancer chemotherapy.

Keywords: Heterocycles; Pteridine; Pyrimidine; Uracil; Anticancer.
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doi:10.1016/j.bmc.2005.02.055

2. Chemistry

The reaction of **1** with glyoxal and 2,3-butanedione in methanol gave 4-hydroxy-2-mercaptopteridine **2** and 4-hydroxy-2-mercapto-6,7-dimethylpteridine **3**. Both mercapto-pteridines were reacted with 2-(chloromethyl)-quinoline in methanolic sodium hydroxide to give compounds **4** and **5**. Similarly, both mercapto-pteridines were also reacted with 2-(chloromethyl)benzimidazole in methanolic sodium hydroxide to afford compounds **6** and **7** and with 4-bromo-phenacylbromide yielded compounds **8** and **9**. Further reaction of 3,5-dimethyl-4-(chloromethyl)isoxazole in methanolic sodium hydroxide with **2** and **3** afforded compounds **10** and **11**. Refluxing 1,2-dibromoethane, compound **2** and K_2CO_3 in DMF yielded compound **12** which was treated further with pyrrolidine to yield **13** (Scheme 1).

The synthesis of 2-hydroxy-4-mercapto-5,6-diaminopyrimidine **15** was achieved through the reaction of 2,4-dihydroxy-5,6-diaminopyrimidine **14** and phosphorus pentasulfide in refluxing pyridine. Further cyclization of **15** with 2,3-butanedione in methanol afforded **19**. Compounds **20**–**22** were obtained by reacting commercially available 2,4,5,6-tetraaminopyrimidine **16**, 2,4-dimercapto-5,6-diaminopyrimidine **17** and 2-mercapto-4-hydroxy-5,6-diaminopyrimidine **18**, respectively, with 2,3-butanedione in methanol. Further reactions of **19**–**21** with ethanolamine in refluxing DMF yielded **23**–**25**. Reaction of 4-hydroxy-2-mercapto-6,7-dimethylpteridine **22** and 2-(chloromethyl)uracil in methanolic sodium hydroxide gave compound **26** (Scheme 2).

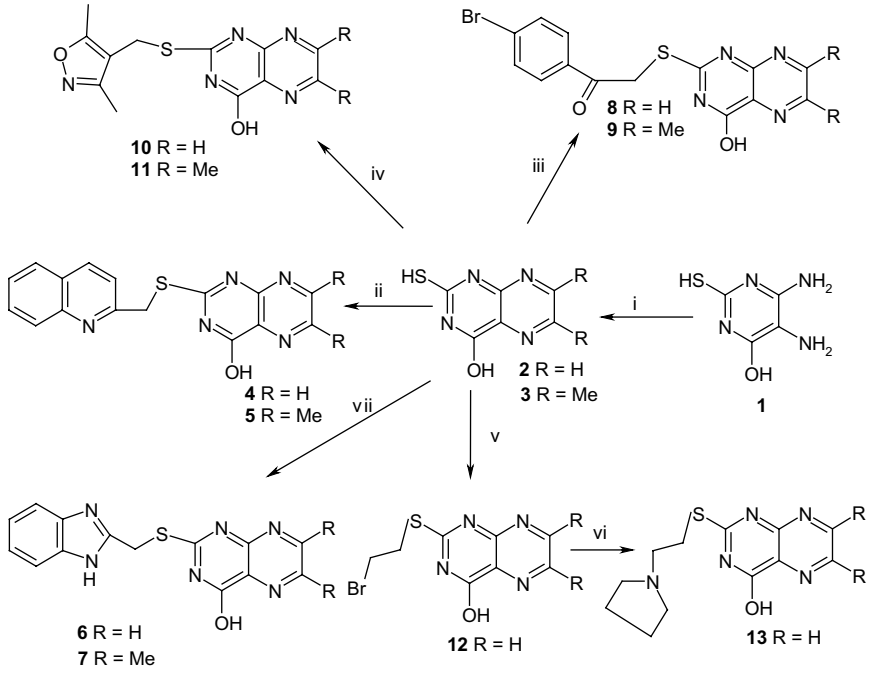
3. Anticancer screening

3.1. Methodology of the in vitro cancer screening

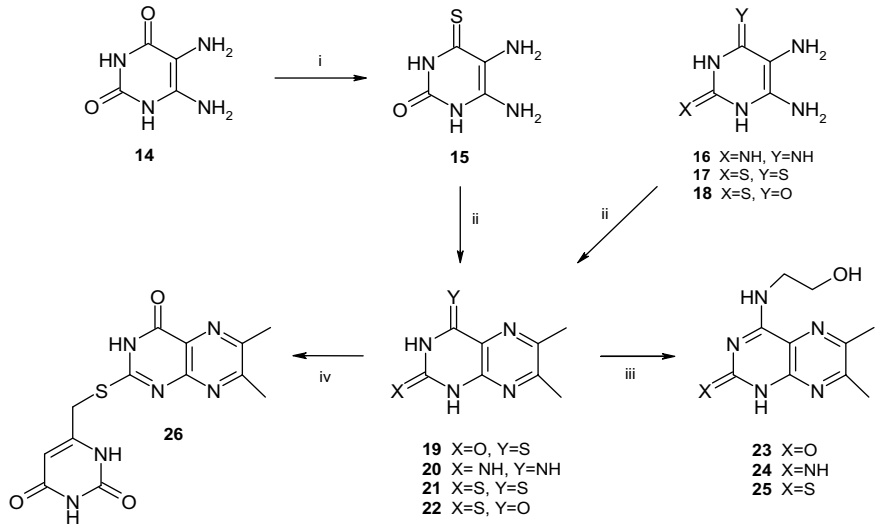
Experimental compounds were solubilized in dimethyl sulfoxide (DMSO) at 400-times the desired maximum tests concentration (maximum final DMSO concentration of 0.25%) and stored frozen. Compounds were then diluted with complete media with 0.1% gentamicin sulfate (5 μL of test sample in 100% DMSO was added to 565 μL of complete medium). 20 μL of this solution was then dispensed into test wells containing 50 μL of cell suspension to yield a test concentration of 1.00E−04 M.[9–11] Two standard drugs were used as a standard and tested against each cell line: NSC 19893 (5-FU) and NSC 123127 (Adriamycin). All compounds were tested for primary anticancer assay against three cell lines, MCF7 (breast), NCI-H460 (lung) and SF-268 (CNS). Results of each test agents is reported as percentage growth of the treated cells when compared with untreated control cells. Compounds which reduce the growth of any of the cell line by 32% or less (negative number indicate kills) is considered in vitro active.

4. Results and discussion

Compound **4** has shown 67% growth inhibition against MCF7 (breast) cell line, 62% against NCI-H460 (lung) cell lines and 61% against SF-268 (CNS) cell line. Similarly compound **5** has exhibited 53% growth inhibition against NCI-H460 (lung) cell lines. Similarly, compound **11** has shown 57% growth against MCF7 (breast) cell line, 66% growth against NCI-H460 (lung) cell lines



Scheme 1. Reagents: (i) glyoxal, 2,3-butanedione, methanol; (ii) 2-(chloromethyl)quinoline, NaOH, methanol; (iii) 4-bromophenacylbromide, NaOH, methanol; (iv) 3,5-dimethyl-4-(chloromethyl)isoxazole, NaOH, methanol; (v) 1,2-dibromoethane, K_2CO_3, DMF; (vi) pyrrolidine, K_2CO_3, DMF; (vii) 2-(chloromethyl)benzimidazoles, NaOH, methanol.



Scheme 2. Reagents: (i) P_2S_5, pyridine; (ii) 2,3-butadione, methanol, reflux; (iii) ethanolamine, DMF, 140 °C; (iv) 6-(chloromethyl)uracil, NaOH, methanol.

Table 1. Percentage growth of the treated cells

Compound	Breast (MCF7) growth %	Lung (NCI-H460) growth %	CNS (SF-268) growth %
4	67	62	61
5	87	53	84
6	68	93	71
7	114	100	79
8	73	103	89
9	124	145	91
10	106	101	93
11	57	66	62
13	92	103	99
23	96	110	112
24	80	62	97
25	69	103	96
26	93	122	115

Concentration used in testing (molar units): 1.000E−4.

and 62% growth against SF-268 (CNS) cell line. Compound **24** has shown 62% growth against NCI-H460 (lung) cell lines. These results suggest that quinoline heterocycles and isooxazoles play an important role in inhibiting growth of cancer cell lines. Similarly, compound **24** having an amino group on position 2 and ethanolamine on position 4 has shown some inhibiting effect on the growth of lung cancer cell line. These pteridine analogues can serve as novel templates for anticancer chemotherapy and can be new leads in cancer chemotherapy. Further optimization of these identified chemical leads can possibly lead to more active molecules against cancer (Table 1).

5. Conclusion

In this paper, we have described pteridine compounds as potential anticancer agents and identified analogues in particular **4**, **5**, **11** and **24** that can be novel templates for lead optimization purpose in cancer chemotherapy. Furthermore, pteridines form covalent hydrate adducts with water and in equilibrium with the unsolvated adduct. Such property will allow pteridine analogues to form covalent hydration adducts in biological fluid (water) and increase bioavailability of such molecules.

6. Experimental

6.1. Instrumentations and general materials

All reactions were carried out under a nitrogen atmosphere unless otherwise stated. Solvents were of reagent grade and used as supplied commercially. ^1H and ^{13}C NMR spectra were recorded on a Varian 300 MHz spectrometer. Melting points were recorded on a Reichert Thermovar apparatus (350 °C). IR spectra were recorded as a thin film on an AVATAR 360 FT-IR spectrometer. Microanalyses were performed by A.W.R. Saunders at the University East Anglia. Electron spray mass spectrometry spectra were recorded by Richard Evans at the University East Anglia. Compounds **1**, **16**, **17**, **18** and **20** are commercially available intermediates and compounds **2**, **3**, **15**, **19**, **21** and **22** were synthesized following the literature procedures.[12,13]

6.2. 2-Mercapto-pteridine-4-ol and 2-mercapto-6,7-dimethylpteridine-4-ol (2,3)

4,5-Diamino-6-hydroxyl-2-mercapto-pyrimidine (**1**) (1.50 g, 0.01 mmol) and glyoxal or 2,3-butadione (0.86 g, 2.0 mL, 0.01 mmol) were refluxed in methanol (100 mL) for 3.5 h. The solution was cooled and a brown solid filtered and dried under vacuum to yield 2-mercapto-pteridine-4-ol (**2**) and 2-mercapto-6,7-dimethyl-pteridin-4-ol (**3**), respectively.[12,13]

6.3. 2-(Quinolin-2-ylmethylsulfanyl)-pteridin-4-ol (4)

A solution of 2-mercapto-pteridine-4-ol (**2**, 0.9 g, 5.0 mmol), 2-(chloromethyl)quinoline (1.07 g, 5.0 mmol) and NaOH (0.08 g, 2.0 mmol) in methanol (50 cm^3) were stirred for 12 h at room temperature. The

separated solid was recrystallized from methanol to give the title compound (0.55 g, 37%) as a light yellow solid (Found: C, 54.79; H, 2.91; N, 20.32. $C_{16}H_{11}N_5OS\cdot 1.5H_2O$ requires C, 55.18; H, 3.18; N, 20.10%); mp 208–210 °C; ν_{max} (Nujol)/cm^{-1} 1250, 1380, 1460, 1590, 1680 (C=N); δ_H (300 MHz, DMSO-d_6) 4.65 (2H, s, C(10)H$_2$), 7.45 (1H, d, C(12)H), 7.60–7.85 (2H, m, C(15)H, C(16)H), 7.85 (1H, d, C(17)H), 8.00 (1H, d, C(14)H), 8.25 (1H, d, C(13)H), 8.40 (1H, d, C(7)H), 8.75 (1H, d, C(6)H); δ_C (75 MHz, DMSO-d_6) 37.97 (C-10), 122.88 (C-4a), 127.44 (C-12), 127.96 (C-15), 129.01 (C-13a), 129.55 (C-14), 130.87 (C-17), 133.10 (C-16), 137.92 (C-13), 141.37 (C-6), 148.21 (C-7), 149.54 (C-17a), 157.59 (C-11), 161.06 (C-8a), 171.57 (C-4), 173.106 (C-2); *m*/*z* (ES) M$^+$ (322.3).

6.4. 6,7-Dimethyl-2-(quinolin-2-ylmethylsulfanyl)-pteridin-4-ol (5)

Compound **5** was prepared as described before to give the title compound (**3**, 0.8 g, 57.2%) as a light yellow solid (Found: C, 56.12; H, 3.72; N, 18.48. $C_{18}H_{15}N_5OS\cdot 2H_2OC$ requires C, 56.05; H, 3.92; N, 18.16%); mp 215–217 °C; ν_{max} (Nujol)/cm^{-1}, 1174, 1296, 1376, 1489, 1495, 1521, 1549, 1652, 2338, 2361, 3648; δ_H (300 MHz, DMSO-d_6) 2.60 (6H, s, C(9)H$_3$, C(10)H$_3$), 4.60 (2H, s, C(12)H$_2$), 7.45 (1H, d, C(14)H), 7.60–7.80 (2H, m, C(17)H, C(18)H), 7.85 (1H, d, C(19)H), 8.00 (1H, d, C(16)H), 8.25 (1H, d, C(15)H); δ_C (75 MHz, DMSO-d_6) 22.59 (C-9), 23.56 (C-10), 37.89 (C-12), 122.82 (C-4a), 127.35 (C-14), 127.95 (C-17), 128.98 (C-15a), 129.61 (C-16), 129.74 (C-19), 130.78 (C-18), 137.83 (C-15), 148.23 (C-7), 149.07 (C-6), 155.90 (C-19a), 157.66 (C-13), 166.28 (C-8a), 171.11 (C-4), 171.58 (C-2); *m*/*z* (ES) M$^+$ (350.3), M^{2+} (351.4).

6.5. 2-(1*H*-Benzimidazol-2-ylmethylsulfanyl)-pteridin-4-ol (6)

A solution of 2-mercapto-pteridine-4-ol (**2**, 0.9 g, 5.0 mmol), 2-(chloromethyl)benzimidazole (0.8 g, 5.0 mmol) and NaOH (0.08 g, 2.0 mmol) in methanol (50 cm^3) was stirred for 16 h at room temperature. The solvent was removed under reduced pressure and the crude product was recrystallized from methanol to give the title compound (0.8 g, 51.6%) as a yellow solid (Found: C, 49.51; H, 4.02; N, 25.19. $C_{14}H_{10}N_6OS\cdot 1.5H_2O$ requires C, 49.84; H, 4.18; N, 24.91%); mp 210–214 °C; ν_{max} (Nujol)/cm^{-1} 3200–3400 (OH, NH), 1680 (C=N), δ_H (300 MHz, DMSO-d_6) 4.81 (2H, s, C(10)H$_2$), 7.0–7.25 (2H, m, C(14)H, C(15)H), 7.45 (2H, m, C(13)H, C(16)H), 8.65 (1H, d, C(7)H), 8.85 (1H, d, C(6)H); δ_C (75 MHz, DMSO-d_6) 29.09 (C-10), 116.02 (C-4a), 122.8 (C-16), 133.50 (C-13), 139.98 (C-14), 142.14 (C-15), 142.27 (C-12a), 149.73 (C-16a), 151.21 (C-7), 152.97 (C-11), 157.20 (C-6), 168.82 (C-8a), 169.78 (C-4), 177.45 (C-2); *m*/*z* (ES) M$^+$ (311.2).

6.6. 2-(1*H*-Benzimidazol-2-ylmethylsulfanyl)-6,7-dimethyl-pteridin- 4-ol (7)

A solution of 2-mercapto-6,7-dimethyl-pteridin-4-ol (**3**, 1.04 g, 5.0 mmol), 2-(chloromethyl)benzimidazole (0.8 g, 5.0 mmol) and NaOH (0.08 g, 2.0 mmol) in methanol (50 cm^3) was stirred for 16 h at room temperature. The precipitated solid was recrystallized from methanol to give the title compound (1.2 g, 71%) as a yellow solid (Found: C, 50.51; H, 3.87; N, 22.23. $C_{16}H_{14}N_6OS\cdot 2H_2O$ requires: C, 51.33; H, 3.76; N, 22.44%); mp > 320 °C; ν_{max} (Nujol)/cm^{-1} 3100–3300 (OH, NH), 2900 (CH), 1680 (C=N); δ_H (300 MHz, DMSO-d_6) 2.45 (6H, br s, C(9)H$_3$, C(10)H$_3$), 4.45 (2H, s, C(12)H$_2$), 7.15 (2H, m, C(16)H, C(17)H), 7.45 (2H, m, C(15)H, C(18)H); δ_C (75 MHz, DMSO-d_6) 22.62 (C-9), 23.53 (C-10), 29.03 (C-12), 116.0 (C-4a), 119.0 (C-18), 122.68 (C-15), 129.8 (C-16) (C-17), 138.0 (C-18a), 140 (C-14a), 149.139 (C-13), 153.83 (C-7), 155.90 (C-6), 157.54 (C-8a), 170.84 (C-4), 171.48 (C-2); *m*/*z* (ES) M$^+$ (339.4), M^{2+} (340.3).

6.7. 1-(4-Bromo-phenyl)-2-(4-hydroxy-6,7-dimethyl-pteridin-2-ylsulfanyl)-ethanone (8)

A solution of 2-mercapto-pteridine 4-ol (**2**, 0.9 g, 5.0 mmol), 4-bromophenacylbromide (1.38 g, 5.0 mmol) and NaOH (0.08 g, 2.0 mmol) in methanol (50 cm^3) was stirred for 12 h at room temperature. The solid thus separated was recrystallized from methanol, to give the title compound (0.7 g, 37%) as a yellow solid (Found: C, 40.96; H, 2.19; N, 13.93. $C_{14}H_9BrN_4O_2S\cdot 2H_2O$ requires C, 40.69; H, 2.19; N, 13.65%); mp 180–183 °C; ν_{max} (Nujol)/cm^{-1} 1652 (C=N), 1700 (CO); δ_H (300 MHz, DMSO-d_6) 5.21 (2H, s, C(10)H$_2$), 8.25 (2H, d, C(13)H, C(17)H), 8.45 (2H, d, C(14)H, C(16)H); δ_C (75 MHz, DMSO-d_6) 38.93 (C-10), 128.51 (C-4a), 131.69 (C-13), (C-17), 132.97 (C-14), (C-16), 133.42 (C-12), 136.54 (C-15), 141.07 (C-7), 149.09 (C-6), 157.63 (C-8a), 170.85 (C-4), 172.11 (C-2), 195.87 (C-11); *m*/*z* (ES) M$^+$ (377.2), M^{2+} (379.1), M^{+3} (381.3).

6.8. 2-(4-Bromo-1-benzoyl-methylsulfanyl)-6,7-dimethyl-pteridine-4-ol (9)

Compound **9** was prepared as described before from **3** to give the title compound (61.6%) as a yellow solid (Found: C, 42.87; H, 2.90; N, 12.74. $C_{16}H_{13}BrN_4O_2S\cdot 2.5H_2O$ requires C, 42.68; H, 2.90; N, 12.44%); mp 228–231 °C (dec); ν_{max} (Nujol)/cm^{-1} 1170 (CO), 1652 (CN) cm^{-1}; δ_H (300 MHz, DMSO-d_6) 2.45 (6H, d s, C(10)H$_3$, C(9)H$_3$), 4.85 (2H, s, C(12)H$_2$), 7.8 (2H, d, C(15) H, C(19) H), 8.5 (2H, d, C(16) H, C(18) H); *m*/*z* (ES) M$^+$ (405.2), M^{2+} (407.2), M^{+3} (409.2).

6.9. 2-(3,5-Dimethyl-isoxazol-4-ylmethylsulfanyl)-pteridin-4-ol (10)

A solution of 2-mercapto-pteridine-4-ol (**2**, 0.9 g, 5.0 mmol), 3,5-dimethyl-4-(chlomethyl) isoxazol (0.72 g, 5.0 mmol) and NaOH (0.08 g, 2.0 mmol) in methanol (50 cm^3) was stirred for 16 h at room temperature. The solid thus separated was recrystallized from methanol to give the title compound (0.8 g, 55.5%) as a light yellow solid (Found: C, 46.65; H, 3.71; N, 22.64. $C_{12}H_{11}N_5O_2S\cdot H_2O$ requires C, 46.89; H, 3.60; N, 22.78%); mp 249–253 °C; ν_{max} (Nujol)/cm^{-1} 1457, 1377, 1680 (C=N), 2724; δ_H (300 MHz; DMSO-d_6)

2.45 (3H, s, C(17)H_3), 2.8 (3H, s, C(16)H_3), 4.42 (2H, s, C(10)H_2), 8.85 (1H, d, C(7)H), 9.12 (1H, d, C(6)H), δ_C (75 MHz; DMSO-d_6) 10.93 (C-17), 12.23 (C-16), 23.28 (C-10), 112.22 (C-4a), 133.43 (C-11), 142.26 (C-15), 150.12 (C-7), 156.52 (C-6), 160.74 (C-8a), 166.84 (C-12), 167.62 (C-4), 167.80 (C-2); *m*/*z* (ES) M^+ (289.2), M^{1+} (290.2).

6.10. 2-(3,5-Dimethyl-isoxazol-4-ylmethylsulfanyl)-6,7-dimethyl-pteridin-4-ol (11)

Compound **11** was prepared as described before from **3** to give the title compound (1.1 g, 56%) as a yellow solid (Found: C, 46.57; H, 4.20; N, 19.37. $C_{14}H_{15}N_5O_2S\cdot 2.5H_2O$ requires C, 46.40; H, 4.17; N, 19.32%); mp 220–224 °C; ν_{max} (Nujol)/cm^{-1} 1298, 1400, 1489, 1520, 1539, 1554, 1635, 1652, 2849, 2975; δ_H (300 MHz; DMSO-d_6), 2.24 (3H, s, C(19)H_3), 2.45 (9H, m, C(18)H_3), C(9)H_3, C(10)H_3, 4.11 (2H, s, C(12)H_2); δ_C (75 MHz; DMSO-d_6) 10.90 (C-19), 12.12 (C-18), 22.56 (C-9), 23.05 (C-10), 23.62 (C-12), 112.75 (C-4a), 129.53 (C-13), 149.20 (C-17), 155.75 (C-7), 157.79 (C-6), 160.76 (C-8a), 167.76 (C-14), 170.95 (C-4), 171.46 (C-2); *m*/*z* (ES) M^+ (317.3).

6.11. 2-(2-Bromo-ethylsulfanyl)-pteridin-4-ol (12)

A solution of 2-mercapto-pteridine-4-ol (**2**, 0.9 g, 5.0 mmol), 1,2-dibromoethane (1.4 g, 5.0 mmol) and NaOH (0.08 g, 2.0 mmol) in methanol (50 cm^3) was stirred for 12 h at room temperature. The precipitated solid was recrystallized from DMF, to give the title compound (0.7 g, 48.2%) as a light yellow solid, mp 295 °C (Found: C, 33.52; H, 2.50; N, 10.44. $C_8H_7BrN_4OS$ requires C, 33.46; H, 2.46; N, 10.51%); ν_{max} (Nujol)/cm^{-1}, 1041, 1377, 1461, 1540, 1578, 1696, 2900; δ_H (300 MHz; DMSO-d_6) 3.85 (2H, t, C(10)H_2, J = 9.2 Hz), 4.45 (2H, t, C(11)H_2, J = 9.2 Hz), 8.65 (1H, d, C(7)H), 8.85 (1H, d, C(6)H); δ_C (75 MHz; DMSO-d_6) 27.85 (C-10), 49.98 (C-11), 132.51 (C-4a), 138.54 (C-7), 144.17 (C-6), 151.08 (C-8a), 158.67 (C-4), 166.79 (C-2); *m*/*z* (ES) M^+ (287), M^{2+} (289), M^{4+} (291).

6.12. 2-(Pyrrolidin-1-yl-ethylsulfanyl)-pteridin-4-ol (13)

A reaction mixture of **12** (0.287 g, 1.0 mmol), pyrrolidine (0.142 g, 2.0 mmol) and K_2CO_3 (0.20 g) in DMF (15 cm^3) were heated for 12 h. The reaction mixture was filtered and concentrated and the crude product recrystallized from methanol to give the title compound (0.20 g, 72%) as a yellow solid, mp 150–152 °C (Found: C, 51.85; H, 5.48; N, 25.38. $C_{12}H_{15}N_5OS$ requires C, 51.97; H, 5.45; N, 25.25%); ν_{max} (Nujol)/cm^{-1} 1398, 1436, 1506, 1528, 1554, 1635, 1652, 1684, 1699, 2361, 2361, 3628; δ_H (300 MHz; DMSO-d_6) 1.25–1.45 (4 H, m, C(14)H_2, C(15)H_2), 2.85–3.25 (8H, m, C(10)H_2 C(11)H_2, C(13)H_2, C(16)H_2), 7.88 (1H, d, C(7)H), 8.88 (1H, d, C(6)H); δ_C (75 MHz; DMSO-d_6) 25.14 (C-14), 26.02 (C-15), 46.04 (C-10), 47.80 (C-11), (C-13) (C-16),130.75 (C-4a), 138.54 (C-7),149.83 (C-6), 156.8 (C-8a), 158.67 (C-4), 167.51 (C-2); *m*/*z* (ES) M^+ (278.4).

6.13. 2-Hydroxy-4-mercapto-5,6-diaminopyrimidine (15)

2,4-Dihydroxy-5,6-diaminopyrimidine (**14**) (2.0 g, 14.0 mmol) and phosphorus pentasulfide (7.0 g, mmol) were refluxed in pyridine (100 cm^3) for 3 h. The solvent was removed under reduced pressure and the residue dissolved in the minimum amount of dilute sulfuric acid. Upon standing in the refrigerator crystals precipitated and were filtered and dried under vacuum to give compound[12] (**15**) (1.54 g, 70%) as beige solid, δ_C (75 MHz, DMSO); 102.5 (C_5), 146.4 (C-6), 147.1 (C-2), 174.6 (C-4).

6.14. 2-Hydroxy-4-mercapto-6,7-dimethylpteridine (19)

2-Hydroxy-4-mercapto-5,6-diaminopyrimidine (**15**) (3.0 g, 19.0 mmol) and 2,3-butadione (2.0 cm^3, 1.2 equiv, 22.8 mmol) were refluxed in methanol (50 cm^3) for 4.0 h. The solution was cooled and part of the solvent removed to give compound[11] (**19**) (2.18 g, 56%) as a beige/brown solid, δ_H (300 MHz, DMSO) 3.39 (6H, br s, C(9)H_3, C(10)H_3); δ_C (75 MHz, DMSO) 21.32 (C-10), 22.33 (C-9), 127.91 (C-4a), 144.12 (C-6), 147.91 (C-2), 149.62 (C-8a), 158.82 (C-7), 190.83 (C-4).

6.15. 2,4-Diamino-6,7-dimethylpteridine (20)

Commercially available 2,4,5,6-tetraaminopyrimidine (**16**) (5.0 g, 21.0 mmol) and 2,3-butadione (2.76 cm^3, 1.5 equiv, 31 mmol) were refluxed in methanol (100 cm^3). After 3.5 h the solution was cooled and a brown solid filtered and dried under vacuum to give compound[11] (**20**) (3.17 g, 79%); δ_H (300 MHz, DMSO) 3.10 (3H, s, C(10)H_3), 2.51 (3H, s, C(9)H_3); δ_C (75 MHz, DMSO) 21.62 (C-9), 22.64 (C-10), 119.32 (C-4a), 144.61 (C-6), 150.92 (C-4), 155.74 (C-8a), 160.82 (C-7),163.05 (C-2).

6.16. 2,4-Dimercapto-6,7-dimethylpteridine (21)

2,4-Dimercapto-5,6-diaminopyrimidine (**17**) (5.0 g, 28.7 mmol) and 2,3-butadione (3.77 cm^3, 1.5 equiv, 43.1 mmol) were refluxed in methanol (100 cm^3) for 3.5 h. The solution was cooled and a brown solid filtered and dried under vacuum to yield compound[11] (**21**) (4.32 g, 68%); δ_H (300 MHz, DMSO) 2.51 (3H, s, C(9)H_3), 3.34 (3H, s, C(10)H_3); δ_C (75 MHz, DMSO) 21.62 (C-9), 22.43 (C-10), 130.61 (C-4a), 142.54 (C-6), 151.42 (C-8a), 159.71 (C-7), 172.32 (C-4), 187.51 (C-2).

6.17. 4-(2-Hydroxy-ethylamino)-6,7-dimethyl-1*H*-pteridin-2-one (23)

2-Hydroxy-4-mercapto-6,7-dimethylpteridine (**19**, 0.5 g, 2.4 mmol) was dissolved in hot dimethylformamide (DMF) (20 cm^3) and ethanolamine (0.2 cm^3, 1.5 equiv, 3.6 mmol) was added and the mixture refluxed for 5 h. The solution was cooled and left in the refrigerator to give the title compound (0.33 g, 60%) as a brown solid, mp 273–275 °C (Found: C, 51.14; H, 5.48; N, 29.68. $C_{10}H_{13}N_5O_2$ requires C, 51.06; H, 5.57; N, 29.77%); ν_{max} (Nujol)/cm^{-1} 1552, 1595, 1650 (C=N), 3383 (O–H); δ_H (300 MHz, DMSO) 3.33 (6H, s, C(9)H_3,

$C(10)H_3$), 3.49 (2H, br s, $C(12)H_2$), 3.54 (2H, br s, $C(13)H_2$); δ_C (75 MHz, DMSO) 21.1 (C-9), 22.24 (C-10), 42.81 (C-12), 59.13 (C-13), 118.64 (C-4a), 146.78 (C-6), 156.05 (C-7), 158.24 (C-4),160.34 (C-2); *m/z* (ES) M^{1+} (236).

6.18. 2-(2-Amino-6,7-dimethyl-pteridin-4-ylamino)-ethanol (24)

Commercially available 2,4-diamino-6,7-dimethylpteridine (**20**, 0.5 g, 2.6 mmol) was dissolved in hot dimethylformamide (DMF) (30 cm^3) and ethanolamine (0.25 cm^3, 1.2 equiv, 3.4 mmol) was added and the mixture refluxed for 5 h. The solution was cooled and left in the refrigerator to give the title compound (0.38 g, 77%) as a beige solid, mp 258–260 °C (Found: C, 51.17; H, 6.16; N, 35.76. $C_{10}H_{14}N_6O$ requires C, 51.28; H, 6.08; N, 35.84%); ν_{max} (Nujol)/cm^{-1} 1593, 1658 (C=N), 3225 (O–H), 3348 (N–H); δ_H (300 MHz, DMSO) 3.42 (6H, s, $C(10)H_3$, $C(10)H_3$), 3.54 (2H, br s, $C(13)H_2$), 3.59 (2H, br s, $C(12)H_2$); δ_C (75 MHz, DMSO) 21.44 (C-9), 22.82 (C-10), 42.74 (C-12), 59.52 (C-13), 119.71 (C-4a), 145.36 (C-8a), 154.42 (C-6), 158.91 (C-7), 160.62 (C-4), 162.64 (C-2); *m/z* (ES) M^{1+} (235).

6.19. 2-(2-Mercapto-6,7-dimethyl-pteridin-4-ylamino)-ethanol (25)

2,4-Dimercapto-6,7-dimethylpteridine (**21**, 0.5 g, 2.2 mmol) was dissolved in hot dimethylformamide (DMF) (30 cm^3) and ethanolamine (0.2 cm^3, 1.3 equiv, 2.8 mmol) was added and the mixture refluxed for 5 h. The solution was cooled and left in the refrigerator to give the title compound (0.24 g, 43%) as an orange/brown solid, mp 175–177 °C (Found: C, 47.71; H, 5.16; N, 27.78. $C_{10}H_{13}N_5OS$ requires C, 47.79; H, 5.21; N, 27.87%); ν_{max} (Nujol)/cm^{-1} 1560 (C=N), 3175 (O–H), 3350 (N–H); δ_H (300 MHz, DMSO) 2.60 (6H, s, $C(10)H_3$, $C(9)H_3$), 3.50 (2H, br s, $C(13)H_2$), 3.62 (2H, br s, $C(12)H_2$); δ_C (75 MHz, DMSO) 21.42 (C-9), 22.81 (C-10), 44.22 (C-12), 61.02 (C-13), 145.25 (C-4a), 151.03 (C-8a), 157.28 (C-6), 161.01 (C-4), 183.04 (C-7), 200.01 (C-2); *m/z* (ES) M^{1+} (252), M−SH (218).

6.20. 6-(6,7-Dimethyl-4-oxo-3,4dihydro-pteridin-2-yl-sulfanylmethyl)-1*H*-pyrimidine-2,4-dione (26)

2-Mercapto-4-hydroxy-6,7-dimethylpteridine (**22**, 1.0 g, 4.8 mmol) was dissolved in methanolic sodium hydroxide (20 cm^3) and 2-(chloromethyl)uracil (0.92 g, 1.2 equiv, 5.7 mmol) was added and the mixture stirred for 16 h. The solid was filtered and recrystallized from DMF to give the title compound (0.93 g, 60%) as a beige solid, mp 307 °C (dec) (Found: C, 46.91; H, 3.56; N, 25.36. $C_{13}H_{12}N_6O_3S$ requires C, 46.98; H, 3.64; N, 25.29%); ν_{max} (Nujol)/cm^{-1} 1584, 1675 (C=N); δ_H (300 MHz, DMSO) 2.60 (6H, s, $C(10)H_3$, $C(9)H_3$) 3.9 (2H, s, $C(12)H_2$); δ_C (75 MHz, DMSO) 21.64 (C-9), 22.52 (C-10), 30.54 (C-12), 98.56 (C-18), 129.02 (C-14a), 148.44 (C-13), 151.62 (C-15), 154.64 (C-4), 155.33 (C-7), 156.51 (C-6), 164.46 (C-8a), 169.38 (C-2), 169.67 (C-17); *m/z* (ES) M^{1+} (333).

Acknowledgements

Both of us (P.M.S.C. and C.J.A.M.) are highly thankful to Pfizer for their financial support and to the NIH, USA for anticancer screening. We are also thankful to Ms. Anu Agarwal for her help in the preparation of the manuscript.

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