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ARTICLE *in* BIOORGANIC & MEDICINAL CHEMISTRY · APRIL 2002

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Synthesis and Enzymatic Evaluation of Pyridinium-Substituted Uracil Derivatives as Novel Inhibitors of Thymidine Phosphorylase

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Received 21 March 2001; revised 19 July 2001; accepted 15 August 2001

Abstract—A series of water soluble N(1)- and C(6)-substituted uracil pyridinium compounds were prepared as potential inhibitors of thymidine phosphorylase (TP). The C(6)-uracil substituted derivatives were the most active. 1-[(5-Chloro-2,4-dihydroxypyrimidin-6-yl)methyl]pyridinium chloride, was identified as the best inhibitor being 5-fold more potent than the known inhibitor, 6-amino-5-bromouracil.

Introduction

The growth and metastasis of solid tumours is dependent on the formation of new blood vessels (angiogenesis).[1] Many solid tumours are able to promote angiogenic signals via gene-expression pathways.[2] This leads to the production of angiogenic growth factors such as platelet-derived endothelial cell growth factor (PD-ECGF).[3] Substantially increased levels of PD-ECGF have been reported within several tumour types relative to surrounding non-neoplastic regions.[4] In addition, recent studies have shown that PD-ECGF may also play a significant role in tumour metastasis[5] and the inhibition of hypoxia-induced apoptosis within certain tumours.[6] Furukawa and co-workers have demonstrated that PD-ECGF is identical to thymidine phosphorylase (TP, dThdPase, EC 2.4.2.4).[7]

TP is an important enzyme involved in the biochemical catabolic/salvage pathway of pyrimidine-based 2-deoxyribonucleosides. Its principal function is the reversible phosphorolysis of thymidine to thymine and 2-deoxyribose-1-phosphate (Scheme 1). The enzyme is also known to activate 5-fluorouracil (5-FU) and its many prodrugs, the first step in their metabolism to 5-fluoro-2′-deoxyribonucleotides which act as thymidylate synthetase inhibitors.[8] TP derives its angiogenic activity by chemotactic stimulation of endothelial cell migration from tissue surrounding the tumour.[9] The precise mechanisms are not fully understood but are thought to involve either the down-regulation of extracellular thymidine levels, which are inhibitory to angiogenesis,[10] or via the production of angiogenic 2-deoxyribose (the dephosphorylated form of 2-deoxyribose-1-phosphate).[11] The role of TP in tumour development has led to its identification as a potentially important chemotherapeutic target in the treatment of cancer.[2,8] It has been proposed that specific inhibition of TP could significantly reduce tumour growth by disruption of angiogenesis or metastasis and promotion of apoptosis. Early examples of TP inhibitors consist of simple 5/6-substituted uracil derivatives,[12] the most potent being 6-amino-5-bromouracil (**6A5BU**).[13] Recently, iminopyrrolidinyl-substituted uracils and multi-substrate alkylphosphonate analogues have also been identified as TP inhibitors.[8,14,15] In this paper we wish to report the syntheses of a novel series of bicyclic pyridinium-substituted uracil and thymine analogues and their in vitro TP *Escherichia coli* inhibitory activity.[16]

Results and Discussion

Chemistry

The compounds prepared were categorised into two groups according to the position at which the pyridinium group was substituted on the uracil/thymine

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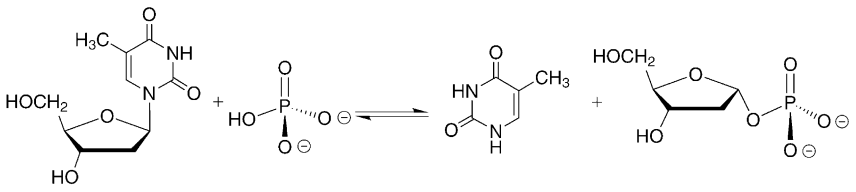
0968-0896/02/$ - see front matter
PII: S0968-0896(01)00309-1



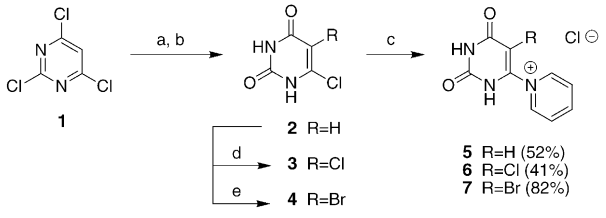
Scheme 1. The reversible phosphorolysis of thymidine catalysed by thymidine phosphorylase (TP).

heterocyclic ring. They were either C(6)-substituted (**5**–**7**, **11**–**15** and **21**) or N(1)-substituted derivatives (**25** and **27**).

Compounds **5**–**7**, where the pyridinium group was directly fused to the uracil ring, were prepared from 2,4,6-trichloropyrimidine **1** (Scheme 2). 6-Chlorouracil **2** was prepared by base-catalysed hydrolysis of **1**[17]. Initial attempts to synthesise pyridinium **5** devised by Schmidt and co-workers[18] using pyridine in chlorobenzene resulted in low yields. However, the omission of chlorobenzene and the use of pyridine as solvent afforded **5** in moderate yield (52%). Ring halogenation of **2** using either NCS/AcOH[19] or bromine water[20] gave 5-halo substituted derivatives **3** and **4**, respectively. By heating at reflux in pyridine, these compounds were converted to the novel pyridinium analogues **6** and **7**.

Compound **11**, the first in a series of methylene bridged bicyclic compounds, was prepared from 6-(chloromethyl)uracil **8** and neat pyridine at room temperature (Scheme 3).[21] Novel C(6)-methylenepyridinium derivatives (**12**–**15**) were prepared by ring halogenation of compound **8** using either NCS/AcOH[18] to give chloro compound **9**,[22] or NBS/AcOH to give the novel bromo adduct **10**. Compounds **12** and **13** were synthesised by reacting **9** or **10** with neat pyridine at room temperature, whereas the aminopyridinium compounds **14** and **15** were prepared by reacting **9** with 2- and 3-aminopyridine, respectively, in refluxing chlorobenzene.

The thymine derivative **21** was prepared from 5,6-dimethylpyrimidine-2,4-dione **16** (Scheme 4). The first step involved selective oxidation of the 6-methyl group to the corresponding aldehyde **17**, followed by reduction with $NaBH_4$ to give the hydroxymethyl compound **18** in excellent yield (85%).[23,24] Ring and side-chain chlorination in refluxing $POCl_3$ gave **19**, which upon acid-catalysed hydrolysis afforded the chloromethyl compound **20**[24] in good yield. Nucleophilic substitution of the chloro group of **20** with neat pyridine at room temperature gave the pyridinium compound **21**.

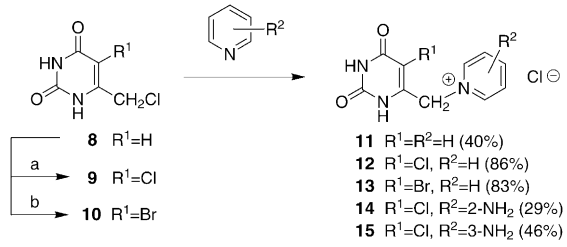


Scheme 2. Synthesis of pyridinium derivatives **5**–**7**. Reagents and conditions: (a) NaOH (aq), Δ; (b) concd HCl, rt (74%); (c) pyridine, Δ; (d) NCS, AcOH, Δ (40%), (e) Br_2, H_2O, 60 °C (71%).

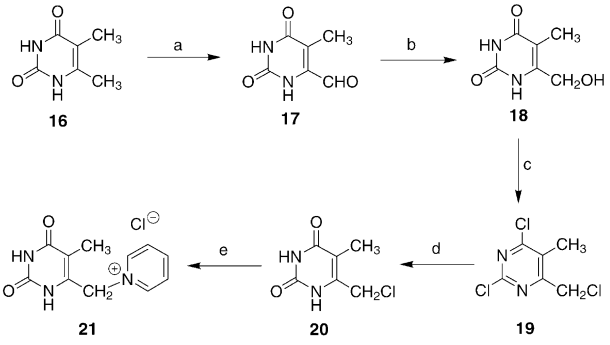
N(1)-Alkylpyridinium thymine analogues **25** and **27** were prepared by two different synthetic routes from thymine **22** (Scheme 5). Here, various synthetic approaches were undertaken in the preparation of N(1)-methylpyridinium compound **25**, but resulted in low yields (>10%).[25–28] An alternative synthetic route was thus used in which the N(1)-chloromethyl adduct **24** was prepared in situ from **23**,[29] which was subsequently quenched with pyridine to give the novel pyridinium compound **25**. The synthesis of N(1)-ethylpyridinium derivative **27**[30] (Scheme 5) and was achieved in two steps via 1-(2-bromoethyl)thymine **26**.[31]

Evaluation as inhibitors of TP

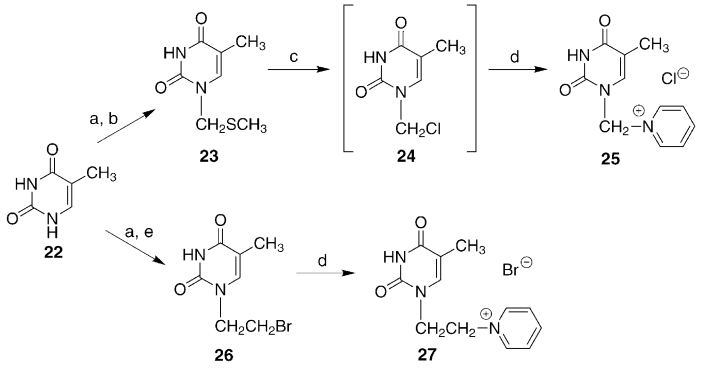
All pyridinium compounds prepared were tested for their TP inhibitory activity against the known inhibitor 6-amino-5-bromouracil (**6A5BU**)[13] using a modification of a standard TP assay described by Evrard and co-workers (Table 1).[32] The IC_{50} values were obtained using *Escherichia coli* TP (which shows 34% homology in their amino acid sequence, and 69% homology of the active site of human TP).[33] The data show that the N(1)-alkyl-substituted thymine analogues (**25** and **27**)



Scheme 3. Synthesis of methyleneperidinium derivatives **11**–**15**. Reagents and conditions: (a) NCS, AcOH, 60 °C (82%); (b) NBS, AcOH, 60 °C (71%).



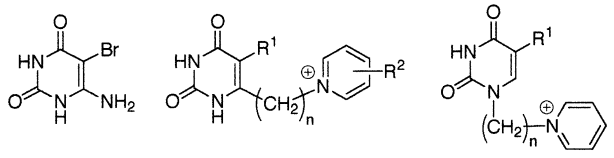
Scheme 4. Synthesis of methylenepyridinium derivative **21**. Reagents and conditions: (a) SeO_2, AcOH, Δ (70%); (b) $NaBH_4$, rt (85%); (c) $POCl_3$, Δ (91%); (d) concd HCl, MeOH, Δ (74%); (e) pyridine, rt (43%).



Scheme 5. Synthesis of thymine analogues **25** and **27**. Reagents and conditions: (a) $(Me_3Si)_2NH$, Me_3SiCl, Δ; (b) CH_3SCH_2Cl, CH_3CN, Δ (67%); (c) SO_2Cl_2, DCM, −78 °C; (d) pyridine, rt; (e) $BrCH_2CH_2Br$, DMF, 80 °C (28%).

and the 6-pyridinium substituted derivatives (**5**–**7**) were not as active as the reference inhibitor **6A5BU**. However, with the exception of the 5-unsubstituted uracil analogue **11**, the 6-methylenepyridinium compounds were more active than **6A5BU**. The most active compound **12** displays a 5-fold increase in potency over **6A5BU**. Derivatives **12**–**15** and **21** containing the additional monomethylene bridging unit between the heterocyclic rings were the most potent in the series. This unit will increase molecular flexibility allowing the inhibitor to bind to residues within the active site of TP (in contrast, **5**–**7** exist as planar compounds). In addition, the above observations seem to support the fact that the uracil N(1) group may be important for binding within the enzyme's active site[33] and that the best TP inhibitors are the 6-substitituted uracil analogues.[8,12–14,34] Moreover, amino substitution of the pyridinium ring in compounds **14** and **15** resulted in a 4-fold increase in potency.

Table 1. Inhibition of TP by pyridinium-substituted uracil analogues and **6A5BU**



6A5BU **A** **B**

Compound	Type	R^1	R^2	n	IC_{50} (mM)[a]
5	**A**	H	H	0	>1
6	**A**	Cl	H	0	>1
7	**A**	Br	H	0	>1
11	**A**	H	H	1	>1
12	**A**	Cl	H	1	0.23
13	**A**	Br	H	1	0.50
14	**A**	Cl	2-NH_2	1	0.50
15	**A**	Cl	3-NH_2	1	0.30
21	**A**	CH_3	H	1	0.50
25	**B**	CH_3	—	1	>1
27	**B**	CH_3	—	2	>1
6A5BU	—	—	—	—	1.10[b]

[a]IC_{50} values represent the result of the means of three assay experiments and are accurate to ±10%.
[b]IC_{50} value obtained was approximately 30-fold greater than that reported in the literature using different assay techniques.[8,13,15]

Halogen substitution at the 5-position of the uracil ring also confers significant inhibitory activity, with **12**–**15** being more active than **11** (an observation which agrees with other literature reports).[8,12–14] It has been suggested that this may be due to the increased acidity of the uracil N(1)H group within these compounds as a result of the halogen's electron-withdrawing capability—increasing the polarity of this group may strengthen hydrogen bonding of uracil with residues in the active site.[12a] This observation, coupled with the fact that thymine derivative **21** is at least twice as potent as uracil derivative **11**, may also support the proposed existence of lipophilic interactions of the inhibitor with the hydrophobic region of the pyrimidine-binding pocket.[33,35]

Conclusion

A series of water soluble N(1)- and C(6)-substituted uracil pyridinium compounds have been prepared, the most potent of which are the novel 6-methylenepyridinium derivatives **12**–**15** and **21**. These compounds represent the only heteroaromatic ring substituted uracil-based inhibitors of thymidine phosphorylase (TP). In agreement with literature reports, structure–activity relationships show that C(5)-halogeno substitution and additional 'side-group' uracil substitution at the C(6) position [vs N(1)-substitution] are favorable. The most active pyridinium compound in the series was 1-[(5-chloro-2,4-dihydroxypyrimidin-6-yl)methyl]pyridinium chloride **12**.

Experimental

General methods

The ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-EX270 spectrometer at 270 and 67.5 MHz, respectively. Chemical shifts are expressed in ppm (δ). The NMR spectra were obtained in deuterium oxide (D_2O) unless otherwise stated. Melting points were determined using a Gallenkamp capillary tube apparatus and are uncorrected. Mass spectra and elemental analyses were performed at the Department of Chemistry (University of Manchester). All solvents used were

dried via standard procedures. The reactions were performed under a dry nitrogen atmosphere.

1-(2,4-Dihydroxypyrimidin-6-yl)pyridinium chloride (5). 6-Chlorouracil[17] **2** (0.50 g, 3.41 mmol) was dissolved in pyridine (10 mL) and heated at reflux for 6 h. On cooling, the orange solid formed was filtered, washed with dichloromethane (30 mL) and dried. The product was recrystallised from methanol to give 0.51 g (52%) of the title compound **5**. Mp >300 °C (lit.[18] >300 °C). ^{1}H NMR δ 6.27 (1H, s, uracil–CH), 8.33 (2H, t, J = 7.1 Hz, 2×pyr–CH), 8.89 (1H, t, J = 8.4 Hz, pyr–CH), 9.26 (2H, d, J = 8.3 Hz, 2×pyr–CHN$^+$). MS (ES$^+$) 190 (cation M$^+$).

1-(5-Chloro-2,4-dihydroxypyrimidin-6-yl)pyridinium chloride (6). 5,6-Dichlorouracil **3** was prepared in a 40% yield from 6-chlorouracil **2** and NCS as described by West and Barrett.[19] Compound **3** (0.10 g, 0.55 mmol) was dissolved in pyridine (2 mL) and heated at reflux for 6 h. On cooling, the yellow solid formed was filtered, washed with dichloromethane (30 mL) and dried to give 0.07 g (41%) of the pyridinium compound **6**. Mp >300 °C. ^{1}H NMR δ 8.35 (2H, t, J = 7.1 Hz, 2×pyr–CH), 8.90 (1H, t, J = 8.2 Hz, pyr–CH), 9.26 (2H, d, J = 8.4 Hz, 2×pyr–CHN$^+$); ^{13}C NMR δ 98.0, 127.6, 143.1, 148.4, 156.6, 157.5, 164.2. MS (ES$^+$) 224, 226 (cation M$^+$).

1-(5-Bromo-2,4-dihydroxypyrimidin-6-yl)pyridinium chloride (7). 5-Bromo-6-chlorouracil[20] **4** (0.20 g, 0.89 mmol) was dissolved in pyridine (5 mL) and heated at reflux for 5.5 h. On cooling, the yellow solid formed was filtered, washed with dichloromethane (30 mL) and dried to afford 0.20 g (82%) of the title compound **7**. Mp >300 °C. ^{1}H NMR δ 8.33 (2H, t, J = 7.1 Hz, 2×pyr–CH), 8.89 (1H, t, J = 8.4 Hz, pyr–CH), 9.26 (2H, d, J = 8.3 Hz, 2×pyr–CHN$^+$); ^{13}C NMR δ 97.7, 127.7, 143.0, 148.2, 156.4, 157.3, 164.2. MS (ES$^+$) 268, 270 (cation M$^+$).

5-Chloro-6-(chloromethyl)-2,4-dihydroxypyrimidine (9). Compound **9** was prepared from 6-(chloromethyl)uracil **8** and NCS in 82% yield. Mp 272–273 °C (lit.[22] 270–275 °C). ^{1}H NMR (DMSO-d_6) δ 4.49 (2H, s, CH_2Cl), 11.60 (1H, s, NH), 11.75 (1H, s, NH).

5-Bromo-6-(chloromethyl)-2,4-dihydroxypyrimidine (10). A solution of 6-(chloromethyl)uracil **8** (1.0 g, 6.2 mmol) in glacial acetic acid (15 mL) and acetic anhydride (1 mL) was heated to 80 °C for 30 min. The temperature of the solution was lowered to 60 °C and *N*-bromosuccinimide (NBS, 1.4 g, 7.8 mmol) was added. The reaction mixture was stirred at this temperature for 3 h. On cooling, the suspension was poured into iced water (50 mL), and the white solid was filtered and dried to give 1.1 g (71%) of **10**. Mp 260–261 °C. ^{1}H NMR (DMSO-d_6) δ 4.47 (2H, s, CH_2Br), 11.62 (1H, s, NH), 11.68 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 41.4, 97.4, 149.0, 150.3, 160.3. MS (CI) 127, 161, 206, 238 (M$^+$). Anal. calcd for $C_5H_4BrClN_2O_2$: C, 25.08; H, 1.68; N, 11.70. Found: C, 25.53; H, 1.52; N, 11.62%.

1-[(2,4-Dihydroxypyrimidin-6-yl)methyl]pyridinium chloride (11). 6-(Chloromethyl)uracil **8** (0.10 g, 0.62 mmol) and pyridine (5 mL) was stirred for 12 h at room temperature. The cloudy suspension obtained was concentrated in vacuo to give a gray–green solid which was recrystallised (MeOH) to afford 0.06 g (40%) of the title compound **11**. Mp 280–282 °C (lit.[21] 280–282 °C) ^{1}H NMR δ 5.62 (1H, s, uracil–CH), 5.73 (2H, s, NCH_2N^+), 8.20 (2H, t, J = 7.0 Hz, 2×pyr–CH), 8.72 (1H, t, J = 9.0 Hz, pyr–CH), 8.96 (2H, d, J = 7.0 Hz, 2×pyr–CHN$^+$). MS (ES$^+$) 204 (cation M$^+$).

1-[(5-Chloro-2,4-dihydroxypyrimidin-6-yl)methyl]pyridinium chloride (12). 5-Chloro-6-(chloromethyl)uracil **9** (0.10 g, 0.51 mmol) and pyridine (5 mL) was stirred for 24 h at room temperature. The cloudy suspension obtained was concentrated in vacuo to give a white solid which was recrystallised (MeOH) to afford 0.12 g (86%) of the title compound **12**. Mp >300 °C. ^{1}H NMR δ 5.89 (2H, s, NCH_2N^+), 8.18 (2H, t, J = 7.1 Hz, 2×pyr–CH), 8.69 (1H, t, J = 9.1 Hz, pyr–CH), 8.97 (2H, d, J = 6.9 Hz, 2×pyr–CHN$^+$); ^{13}C NMR δ 57.5, 110.9, 127.5, 142.0, 144.3, 147.0, 150.7, 161.3. MS (ES$^+$) 238, 240 (cation M$^+$). Anal. calcd for $C_{10}H_9Cl_2N_3O_2$: C, 43.82; H, 3.31; N, 15.33; Cl, 25.87. Found: C, 43.80; H, 3.60; N, 15.05; Cl, 25.90%.

1-[(5-Bromo-2,4-dihydroxypyrimidin-6-yl)methyl]pyridinium chloride (13). 5-Bromo-6-(chloromethyl)uracil **10** (0.10 g, 0.42 mmol) and pyridine (3 mL) was stirred for 72 h at room temperature. The cloudy suspension obtained was filtered, washed with chloroform (25 mL) and dried to afford 0.11 g (83%) of pyridinium compound **13**. Mp >300 °C. ^{1}H NMR δ 5.80 (2H, s, NCH_2N^+), 8.17 (2H, t, J = 6.8 Hz, 2×pyr–CH), 8.65 (1H, t, J = 8.7 Hz, pyr–CH), 8.94 (2H, d, J = 5.9 Hz, 2×pyr–CHN$^+$); ^{13}C NMR δ 57.7, 109.3, 17.5, 141.7, 144.7, 147.0, 151.3, 161.4. MS (ES$^+$) 282, 284 (cation M$^+$). Anal. calcd for $C_{10}H_9BrClN_3O_2$: C, 37.70; H, 2.85; N, 13.19; Br, 25.08; Cl, 11.13. Found: C, 38.07; H, 2.90; N, 13.03; Br, 24.99; Cl, 10.96%.

2-Amino-1-[(5-chloro-2,4-dihydroxypyrimidin-6-yl)methyl]pyridinium chloride (14). 5-Chloro-6-(chloromethyl)uracil **9** (0.10 g, 0.51 mmol) and 2-aminopyridine (0.25 g, 2.66 mmol) were suspended in chlorobenzene (10 mL) and heated at reflux for 48 h. The solid obtained was filtered, washed with dichloromethane (30 mL) and dried. The crude solid was dissolved in ethanol/water/concd HCl (8:2:1, 50 mL), concentrated in vacuo and washed with hot methanol to yield 0.04 g (29%) of the title compound **14** as a black crystalline solid. Mp >300 °C. ^{1}H NMR (DMSO-d_6) δ 5.30 (2H, s, NCH_2N^+), 7.17 (1H, t, J = 6.6 Hz, pyr–CH), 7.44 (1H, d, J = 8.9 Hz, pyr–CH), 8.00 (1H, t, J = 7.4 Hz, pyr–CH), 8.12 (1H, d, J = 6.9 Hz, pyr–CHN$^+$), 11.40 (2H, s, NH_2), 11.53 (1H, s, NH), 11.78 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 49.4, 107.3, 115.5, 116.2, 127.3, 139.7, 142.9, 146.6, 149.7, 157.5. MS (ES$^+$) 95, 217, 239, 253 (cation M$^+$). Anal. calcd for $C_{10}H_{10}Cl_2N_4O_2$: C, 41.54; H, 3.49; N, 19.38. Found: C, 41.38; H, 3.95; N, 20.37%.

3-Amino-1-[(5-chloro-2,4-dihydroxypyrimidin-6-yl)methyl]pyridinium chloride (15). 5-Chloro-6-(chloromethyl)uracil **9** (0.10 g, 0.51 mmol) and 3-aminopyridine (0.25 g, 2.66 mmol) were suspended in chlorobenzene (10 mL) and heated at reflux for 16 h. A brown solid was obtained which was filtered, washed with dichloromethane (30 mL) and dried. The solid was dissolved in ethanol/water/concd HCl (8:2:1, 50 mL), concentrated in vacuo and washed with hot methanol to yield 0.07 g (46%) of the title compound as tan crystalline flakes. Mp >300 °C. ^{1}H NMR (DMSO-d_6) δ 5.55 (2H, s, NCH_2N^+), 7.70 (2H, m, 2×pyr–CH), 8.06 (1H, s, pyr–CHN^+), 8.12 (1H, d, J = 6.1 Hz, pyr–CHN^+); ^{13}C NMR (DMSO-d_6) δ 57.7, 109.6, 127.4, 128.6, 129.0, 132.1, 142.6, 149.0, 150.8, 160.2. MS (ES^+) 219, 253 (cation M^+). Anal. calcd for $C_{10}H_{10}Cl_2N_4O_2$: C, 41.54; H, 3.49; N, 19.38. Found: C, 41.18; H, 3.75; N, 18.92%.

6-(Hydroxymethyl)-2,4-dihydroxy-5-methylpyrimidine (18). Compound **18** was prepared by the reduction of thymine-6-carboxaldehyde[23] **17** with $NaBH_4$ in 85% yield. Mp 225–226 °C (lit.[24] 223–224 °C). ^{1}H NMR (DMSO-d_6) δ 1.74 (3H, s, CH_3), 4.24 (2H, d, J = 5.3 Hz, CH_2O), 5.44 (1H, bs, OH), 10.20 (1H, bs, NH), 11.00 (1H, bs, NH).

6-(Chloromethyl)-2,4-dichloro-5-methylpyrimidine (19). 6-(Hydroxymethyl)-2,4-dihydroxy-5-methylpyrimidine **18** (0.25 g, 1.62 mmol) was suspended in phosphorus oxychloride (5 mL) and heated at reflux under nitrogen for 12 h. The solution was cooled and concentrated in vacuo to yield a dark red oil. Iced water (25 mL) was slowly added and the product was extracted with chloroform (3×25 mL). The organic phase was washed with brine, dried ($MgSO_4$), and evaporated to give 0.31 g (91%) of **19** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 2.47 (3H, s, CH_3), 4.59 (2H, s, CH_2Cl); ^{13}C NMR ($CDCl_3$) δ 14.0, 43.1, 127.6, 157.0, 163.5, 166.0. MS (EI) 84, 139, 176, 210 (M^+); AMM: 209.9517, $C_6H_5Cl_3N_2$ requires 209.9518.

6-(Chloromethyl)-2,4-dihydroxy-5-methylpyrimidine (20). 6-(Chloromethyl)-2,4-dichloro-5-methylpyrimidine **19** (1.1 g, 4.70 mmol) was dissolved in methanol (5 mL) and concd hydrochloric acid (1 mL). The mixture was heated at reflux for 2 h. The white solid formed was filtered, washed with dichloromethane (20 mL) and dried to afford 0.61 g (74%) of the title compound **20**. Mp 244–245 °C (lit.[24] 241–244 °C). ^{1}H NMR (DMSO-d_6) δ 1.82 (3H, s, CH_3), 4.43 (2H, s, CH_2Cl), 10.90 (1H, bs, NH), 11.19 (1H, bs, NH).

1-[(2,4-Dihydroxy-5-methylpyrimidin-6-yl)methyl]pyridinium chloride (21). 6-(Chloromethyl)-2,4-dihydroxy-5-methylpyrimidine **20** (0.08 g, 0.46 mmol) and pyridine (3 mL) was stirred for 48 h at room temperature. The creamy suspension obtained was concentrated in vacuo to yield a tan solid. Recrystallisation (MeOH) afforded 0.06 g (40%) of the title compound **21**. Mp >300 °C. ^{1}H NMR δ 2.03 (3H, s, CH_3), 5.77 (2H, s, NCH_2N^+), 8.16 (2H, t, J = 7.1 Hz, 2×pyr–CH), 8.67 (1H, t, J = 7.8 Hz, pyr–CH), 8.88 (2H, d, J = 5.9 Hz, 2×pyr–CHN^+); ^{13}C NMR δ 9.1, 57.5, 113.4, 128.5, 139.9, 144.0, 146.9, 151.9, 166.4. MS (ES^+) 218 (cation M^+). Anal. calcd for $C_{11}H_{12}ClN_3O_2$: C, 52.08; H, 4.77; N, 16.56; Cl, 13.98. Found: C, 51.75; H, 4.63; N, 16.22; Cl, 13.84%.

1-[(2,4-Dihydroxy-5-methylpyrimidin-1-yl)methylene]pyridinium chloride (25). 1-(Methylthiomethyl)thymine[27] **23** (0.25 g, 1.34 mmol) was dissolved in dry dichloromethane (5 mL) at −78 °C. Sulfuryl chloride (0.2 mL, 2.50 mmol) was added with stirring under a dry nitrogen atmosphere. The white suspension was slowly warmed to room temperature and dry pyridine (2 mL) was added. The yellow solution obtained was stirred for a further 24 h. The red solution afforded was concentrated in vacuo to give a dark red gum. Addition of chloroform resulted in the precipitation of a white solid which was filtered, washed with chloroform (25 mL) and dried to yield 0.14 g (42%) of the title compound **25**. Mp 225–227 °C. ^{1}H NMR δ 1.92 (3H, s, CH_3), 6.46 (2H, s, NCH_2N^+), 7.81 (1H, s, thymine–CH), 8.17 (2H, t, J = 7.1 Hz, 2×pyr–CH), 8.69 (1H, t, J = 7.9 Hz, pyr–CH), 9.07 (2H, d, J = 5.9 Hz, 2×pyr–CHN^+); ^{13}C NMR δ 10.9, 69.5, 112.4, 128.1, 139.9, 143.8, 147.6, 151.5, 166.0. MS (ES^+) 139, 218 (cation M^+). Anal. calcd for $C_{11}H_{12}ClN_3O_2$: C, 52.08; H, 4.77. Found: C, 47.40; H, 4.96%.

1-[2-(2,4-Dihydroxy-5-methylpyrimidin-1-yl)ethyl]pyridinium bromide (27). 1-(2-Bromoethyl)thymine[31] **26** (0.65 g, 0.28 mmol) was dissolved in pyridine (2 mL) and stirred at room temperature for 48 h. The suspension was filtered and oven-dried to afford 0.43 g (49%) of the title compound **27** as a white solid. Mp 298–300 °C. ^{1}H NMR δ 1.83 (3H, s, CH_3), 4.39 (2H, t, J = 5.6 Hz, CH_2N), 4.97 (2H, t, J = 5.6 Hz, CH_2N^+), 7.37 (1H, s, thymine–CH), 8.10 (2H, t, J = 7.3 Hz, 2×pyr–CH), 8.62 (1H, t, J = 7.9 Hz, pyr–CH), 8.87 (2H, d, J = 5.3 Hz, 2×pyr–CHN^+). MS (ES^+) 153, 232 (cation M^+). Anal. calcd for $C_{12}H_{14}BrN_3O_2$: C, 46.17; H, 4.52; N, 13.46; Br, 25.60. Found: C, 46.02; H, 4.71; N, 13.17; Br, 24.82%.

TP Assays

Materials

E. coli TP (600 units/mL) was obtained from the Sigma Chemical Company. Spectrophotometric measurements were obtained using a Beckman 650 spectrophometer.

Methods

The compounds were prepared to the required final concentrations in a 1:1 substrate buffer mixture of thymidine (10 mM) and potassium dihydrogen phosphate (KH_2PO_4, 10 mM, pH = 8.4). To each test sample 1.5 units of TP was added. The samples (final volume = 300 μL) were incubated for 1 h at 37 °C, then quenched with ice-cold 0.5 M NaOH solution (700 μL). Inhibitory activity was determined by measuring the rate of thymidine to thymine conversion spectrophotometrically at an absorbance level of 300 nm. Thymine levels within the test samples (in nmoles/min) were

obtained by interpolation of the above readings via a linear standard curve generated using 0.2–1.0 mM solutions of thymine (Abs_{300} readings versus log[thymine] values were plotted to the best fit polynomial). The IC_{50} value was defined as the compound concentration required to decrease the Abs_{300} reading obtained using a control sample of TP in substrate buffer alone by 50%.

Acknowledgements

We are grateful to the Association of International Cancer Research (PEM) and the Medical Research Council (SDL) for financial support of this project. PEM thanks the American College of Radiology for Junior Investigator Award (11th International Chemical Modifiers Conference—Tumor Physiology and Cancer Treatment).

References and Notes

1. (a) Baillie, C. T.; Winslet, M. C.; Bradley, N. J. *Br. J. Cancer* **1995**, *72*, 257. (b) Battegay, E. J. *J. Mol. Med.* **1995**, *73*, 333.
2. Griffiths, L.; Stratford, I. J. *Br. J. Cancer* **1997**, *76*, 689.
3. (a) Miyazono, K.; Okabe, T.; Urabe, A.; Takaku, F.; Heidin, C. H. *J. Biol. Chem.* **1987**, *262*, 4098. (b) Brown, S. B.; Bicknell, R. *Biochem. J.* **1998**, *334*, 1.
4. (a) Bicknell, R.; Moghaddam, A.; Zhang, H. T.; Harris, A. L.; Hu, D. E.; Fan, T. P. *Proc. Am. Assoc. Cancer. Res.* **1994**, *35*, 567. (b) Griffiths, L.; Stratford, I. J. *Int. J. Radiation Oncology, Biol., Phys.* **1998**, *42*, 877.
5. Takao, S.; Akiyama, S.; Nakajo, A.; Yoh, H.; Kitazano, M.; Natsugoe, S.; Miyadera, K.; Fukushima, M.; Yamada, Y.; Aikou, T. *Cancer Res.* **2000**, *60*, 5345.
6. Kitazano, M.; Takebayashi, Y.; Ishitsuka, K.; Takao, S.; Tani, A.; Furukawa, T.; Miyadera, K.; Yamada, Y.; Aikou, T.; Akiyama, S.-I. *Biochem. Biophys. Res. Commun.* **1998**, *253*, 797.
7. Furukawa, T.; Yoshimura, A.; Sumizawa, T.; Haraguchi, M.; Akiyama, S.-I.; Fukui, K.; Ishizawa, M.; Yamada, Y. *Nature* **1992**, *356*, 668.
8. Cole, C.; Foster, A. J.; Freeman, S.; Jaffar, M.; Murray, P. E.; Stratford, I. J. *Anti-Cancer Drug Des.* **1999**, *14*, 383.
9. Moghaddam, A.; Zhang, H. T.; Fan, T. P.; Hu, D. E.; Lees, V. C.; Turley, H.; Fox, S.; Gatter, K. C.; Harris, A. L.; Bicknell, R. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, 998.
10. Finnis, C.; Dodsworth, N.; Pollitt, C. E.; Carr, G.; Sleep, D. *Eur. J. Biochem.* **1993**, *212*, 201.
11. (a) Haraguchi, M.; Miyadera, K.; Uemera, K.; Sumizawa, T.; Furukawa, T.; Yamada, K.; Akiyama, S.-I.; Yamada, Y. *Nature* **1994**, *368*, 198. (b) Dada, M. A.; Boshoff, C. H.; Comley, M. A.; Turley, H.; Schneider, J. W.; Chetty, R.; Gatter, K. C. *J. Clin. Pathol.* **1996**, *49*, 400.
12. (a) Baker, B. R. *Design of Active-site-directed Irreversible Enzyme Inhibitors*; J. Wiley: New York, 1967; p 79. (b) Baker, B. R.; Kelley, J. L. *J. Med. Chem.* **1970**, *13*, 458, and references therein. (c) Niedzwicki, J. G.; El Kouni, M. H.; Chu, S. H.; Cha, S. *Biochem. Pharmacol.* **1983**, *32*, 399.
13. Langen, P.; Etzold, G.; Bärwolff, D.; Preussel, B. *Biochem. Pharmacol.* **1967**, *16*, 1833.
14. (a) Shigeto, M.; Nitanda, T.; Furukawa, T.; Sumizawa, T.; Tani, A.; Nishimoto, K.; Akiba, S.; Miyadera, K.; Haraguchi, M.; Miyadera, K.; Yamada, Y.; Yoshida, H.; Kanzaki, T.; Akiyama, S.-I. *Cancer Res.* **1999**, *59*, 1911. (b) Fukushima, M.; Suzuki, N.; Emura, T.; Yano, S.; Kazuno, H.; Tada, Y.; Yamada, Y.; Asao, T. *Biochem. Pharmacol.* **2000**, *59*, 1227. (c) Takao, S.; Akiyama, S.; Nakajo, A.; Yoh, H.; Kitazono, M.; Natsugoe, S.; Miyadera, K.; Fukushima, M.; Yamada, Y.; Aikou, T. *Cancer Res.* **2000**, *60*, 5345.
15. (a) Gamboa, A. E.; Balzarini, J.; Esnouf, R.; De Clercq, E.; Camarasa, M.-J.; Pérez-Pérez, M.-J. *J. Med. Chem.* **2000**, *43*, 971. (b) Balzarini, J.; Degrève, B.; Gamboa, A. E.; Esnouf, R.; De Clercq, E.; Engelborghs, Y.; Camarasa, M.-J.; Pérez-Pérez, M.-J. *FEBS Lett.* **2000**, *483*, 181.
16. Ishikawa, F.; Miyazono, K.; Hellman, U.; Drexler, H.; Wernstedt, C.; Hagiwara, K.; Usuki, K.; Takaku, F.; Risau, W.; Heldin, C.-H. *Nature* **1989**, *338*, 557.
17. Cresswell, R. M.; Wood, H. C. S. *J. Chem. Soc.* **1960**, 4768.
18. Schmidt, A.; Kindermann, M. K.; Vainiotalo, P.; Nieger, M. *J. Org. Chem.* **1999**, *64*, 9499.
19. West, R. A.; Barrett, H. W. *J. Amer. Chem. Soc.* **1954**, *76*, 3146.
20. Pfleiderer, W.; Deiss, H. *Israel J. Chem.* **1968**, *6*, 603.
21. Nagpal, K. L.; Jain, P. C.; Srivastava, P. C.; Dhar, M. M.; Anand, N. *Indian J. Chem.* **1968**, *6*, 762.
22. Cornforth, J. W.; Huang, H. T. *J. Chem. Soc* **1948**, 1988.
23. Zee-Cheng, K.-Y.; Cheng, C. C. *J. Heterocycl. Chem.* **1967**, *4*, 163.
24. Johnson, T. B.; Chernoff, L. H. *J. Amer. Chem. Soc.* **1913**, *35*, 585.
25. Kundu, N. G.; Khatri, S. G. *Synthesis* **1985**, 323.
26. Ahmad, S.; Ozaki, S.; Nagase, T.; Iigo, M.; Tokuzen, R.; Hoshi, A. *Chem. Pharm. Bull.* **1987**, *35*, 4137.
27. Zagorodnii, S. G.; Malyshev, A. A.; Konstantinova, I. D.; Kuznetsov, S. A.; Miroshnikov, A. I. *Russ. J. Bioorg. Chem. (Engl. Transl.)* **1997**, *23*, 550.
28. Ozaki, S.; Nagase, T.; Tamai, H.; Mori, H.; Hoshi, A.; Iigo, M. *Chem. Pharm. Bull.* **1987**, *35*, 3894.
29. Edstrom, E. D.; Feng, X.; Tumkevicius, S. *Tetrahedron Lett.* **1996**, *37*, 759.
30. Shimidzu, T.; Murakami, A.; Konishi, Y.; Minami, M. *Bull. Chem. Soc. Jpn.* **1978**, *5*, 821.
31. Ciapetti, P.; Taddei, M. *Tetrahedron* **1998**, *54*, 11305.
32. Evrard, A.; Cuq, P.; Robert, B.; Vian, L.; Pèlegrin, A.; Cano, J.-P. *Int. J. Cancer* **1999**, *80*, 465.
33. Cole, C.; Marks, D. S.; Jaffar, M.; Stratford, I. J.; Douglas, K. T.; Freeman, S. *Anti-Cancer Drug Des.* **1999**, *14*, 411.
34. Focher, F.; Ubiali, D.; Pregnolato, M.; Zhi, C.; Gambino, J.; Wright, G. E.; Spadari, S. *J. Med. Chem.* **2000**, *43*, 2601.
35. Erion, M. D.; Stoeckler, J. D.; Guida, W. C.; Walter, R. L.; Ealick, S. E. *Biochemistry* **1997**, *36*, 11735.