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Sulfamide antifolates inhibiting thymidylate synthase: synthesis, enzyme inhibition and cytotoxicity★✪

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Synthesis and biological evaluation are described of seven new analogues (3–9) of two potent thymidylate synthase inhibitors, 10-propargyl-5,8-dideazafolate (1) and its 2-methyl-2-deamino congener ICI 198583 (2). While the new compunds 3 and 4 were analogues of 1 and 2, respectively, containing a *p*-aminobenzenesulfonyl residue in place of the *p*-aminobenzoic acid residue, the remaining 5 new compounds were analogues of 4 with the L-glutamic acid residue replaced by glycine (5), L-valine (6), L-alanine (7), L-phenylglycine (8) or L-norvaline (9). The new analogues were tested as inhibitors of thymidylate synthases isolated from tumour (Ehrlich carcinoma), parasite (*Hymenolepis diminuta*) and normal tissue (regenerating rat liver) and found to be weaker inhibitors than the parent 10-propargyl-5,8-dideazafolic acid. Selected new analogues, tested as inhibitors of growth of mouse leukemia L 5178Y cells, were less potent than the parent 10-propargyl-5,8-dideazafolic acid. Substitution of the glutamyl residue in compound 4 with L-norvaline (9) resulted in only a 5-fold stronger thymidylate synthase inhibitor, but a 40-fold weaker cell growth inhibitor.

Thymidylate synthase (EC 2.1.1.45) catalyzes the C(5) methylation of 2′-deoxyuridylate (dUMP) in a concerted transfer and reduction of the one-carbon group (at the aldehyde oxidation level) of N^5,^{10}-methylenetetrahydrofolate, and with concomitant production of dihydrofolate and thymidylate. The *in vivo* folate cofactor and the product of the

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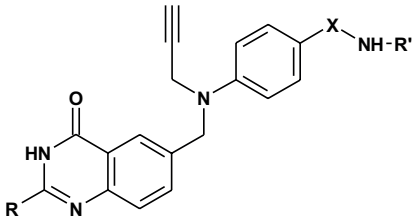
Abbreviations: DMA, dimethylacetamide; DMF, dimethylformamide; LSIMS, liquid secondary ion spectroscopy; mp, melting point.

reaction are usually in their γ-oligoglutamate forms, preventing transport through the cell membrane (Carreras & Santi, 1995). The sequential addition of L-glutamic acid residues in a γ-carboxyl peptide linkage to folate cofactors (and their analogues) is catalyzed by an ATP·Mg^{2+}-dependent enzyme, folylpolyglutamate synthetase (EC 6.3.2.17) (Synold *et al.,* 1996). As the sole *de novo* source of thymidylate synthesis in cells, it is a target in anticancer, antiviral, antifungal and antiprotozoan chemotherapy (Heidelberger *et al.,* 1983; Rathod, 1997; Georgopapadakou & Walsh, 1996; Takemura & Jackman, 1997).

The cofactor analogues, 10-propargyl-5,8-dideazafolic acid **1** (Fig. 1) and 2-deamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic acid **2** (ICI 198583; Fig. 1) are potent inhibitors of



Compound			
Abbreviation (No.)	R	X	R'
pddPteGlu (**1**)	NH_2	CO	Glu
CH_3pddPteGlu; ICI 198583 (**2**)	CH_3	CO	Glu
pddPteSO_2Glu (**3**)	NH_2	SO_2	Glu
CH_3pddPteSO_2Glu (**4**)	CH_3	SO_2	Glu
CH_3pddPteSO_2Gly (**5**)	CH_3	SO_2	Gly
CH_3pddPteSO_2Val (**6**)	CH_3	SO_2	Val
CH_3pddPteSO_2Ala (**7**)	CH_3	SO_2	Ala
CH_3pddPteSO_2PhGly (**8**)	CH_3	SO_2	PhGly
CH_3pddPteSO_2NVal (**9**)	CH_3	SO_2	Nva

Figure 1. Structures of 10-propargyl-5,8-dideazafolic acid (1), 2-deamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic acid (2; ICI 198583) and their analogues.

thymidylate synthase, their cytotoxic activity being strongly dependent on intracellular metabolism by folylpolyglutamate synthetase to polyglutamated derivatives, well retained within cells and more potent as inhibitors of the enzyme (Rosowsky, 1992). As dependence of antifolate cytotoxicity on polyglutamation may display some disadvantages, such as (i) lack of activity in tumours expressing a low level of, or an altered, folylpolyglutamate synthetase, (ii) prolonged normal tissue toxicities caused by polyglutamate retention or (iii) lack of activity due to an increased activity of γ-glutamyl hydrolases, new non-polyglutamatable folate-based thymidylate synthase inhibitors are being sought (Takemura & Jackman, 1997).

To learn more about the effect of the *p*-aminobenzoylglutamate residue of $N^{5,10}$-methylenetetrahydrofolate analogues on interaction with thymidylate synthase, and seeking non-polyglutamatable inhibitors of the enzyme, seven new compounds, (*N*-[*p*-[*N*-(3,4-dihydro-2-amino-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzenesulfonyl]-L-glutamic acid (**3**), *N*-[*p*-[*N*-(3,4-dihydro-2-methyl--4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzenesulfonyl]-L-glutamic acid (**4**), γ-glycine (**5**), -L-valine (**6**), -L-alanine (**7**), -L-phenylglycine (**8**) and -L-norvaline (**9**)), have been synthesized and tested as inhibitors of thymidylate synthase and tumour cell growth. The compounds **3** and **4** are analogues of the two thymidylate synthase inhibitors **1** and **2**, respectively,in which a *p*-aminobenzenesulfonyl residue replaces the *p*-aminobenzoic acid. The compounds **5–9**, analogues of **4**, have been additionally modified by replacing the L-glutamic acid residue with glycine (**5**), L-valine (**6**), L-alanine (**7**), L-phenylglycine (**8**) or L-norvaline (**9**).

MATERIALS AND METHODS

Chemistry: General

2-Amino-6-bromomethyl-3,4-dihydro-4-oxoquinazoline hydrobromide (Calvert *et al.,*

1980), 6-bromomethyl-3,4-dihydro-2-methyl-4-oxyquinazoline (Marsham *et al.,* 1990) and appropriate amino acid *tert*-butyl ester hydrochlorides (Roeske, 1963) were prepared as described. The *p*-nitrobenzenesulfonyl chloride was from Merck (# 820885) and propargyl bromide from Fluka (# 81830). Tetrahydrofuran, dioxane, diethyl ether and triethylamine were distilled over sodium and stored over sodium wire. Dimethylformamide (DMF) was azeotropically distilled and stored, like dimethylacetamide (DMA), over activated (250°C) 4 Å molecular sieves. The hydrogenolysis catalyst was 10% Pd/C used at 20% of the substrate weight. Reactions were monitored, and homogeneity of products checked, by TLC on silica gel 60 (Merck, # 5553) in the following systems: (A) 8% CH_3OH in $CHCl_3$; (B) 20% CH_3OH in $CHCl_3$; (C) 35% acetone in $CHCl_3$; (D) 5% acetone in $CHCl_3$ and (E) acetone. Spots were visualised with the chlorine-tolidine reagent. Fully protected compounds (**16–34**) were purified by low-pressure column chromatography on silica gel 60 (Merck, # 7736). Melting points (uncorrected) were determined on a Boëtius heating block. ^{1}H NMR spectra were recorded in [$(CH_3)_2SO$-d_6] on a Brucker WM 250 spectrometer. Chemical shifts are expressed as δ (ppm), and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; br s, broad singlet; m, multiplet. LSIMS mass spectra were obtained on an AMD 604 Intectra GmbH mass spectrometer.

N-(p-*Nitrobenzenesulfonyl)amino acid* tert-*butyl esters (10–15). General procedure.* To a stirred solution of an appropriate amino acid *tert*-butyl ester hydrochloride (1 mM) and *p*-nitrobenzenesulfonyl chloride (1 mM) in tetrahydrofuran (2 ml) cooled to –10°C, *N*-methylmorpholine (2 mmol) was added dropwise during 10 min. Stirring was continued at –10°C for 10 min and at 20°C for 1 h, whereupon TLC (system A) showed the absence of starting material. *N*-Methylmorpholine hydrochloride was filtered off and the filtrate evaporated to dryness. The resulting residue was crystallised from diethyl ether/hexane. Yields and melting points of the products **10–15**, chromatographically homogeneous in system A, are given in Table 1.

Table 1. *N*-(*p*-Nitrobenzenesulfonyl)amino acid *tert*-butyl esters (10–15) and *N*-(*p*-aminobenzenesulfonyl)amino acid *tert*-butyl esters (16–21)

Compd. No.	Scale mM	Yield %	mp °C	Formula	*Anal.* Calcd (Found) C	H	N
10	15	92	72–73	$C_{19}H_{28}N_2O_8S$	51.34 (51.12)	6.35 (6.31)	6.30 (6.24)
11	10	94	138–140	$C_{12}H_{16}N_2O_6S$	45.56 (45.21)	5.10 (5.02)	8.86 (8.58)
12	10	93	91–93	$C_{15}H_{22}N_2O_6S$	50.27 (50.34)	6.19 (6.28)	7.82 (7.80)
13	10	95	90–92	$C_{13}H_{18}N_2O_6S$	47.26 (47.12)	5.49 (5.22)	8.48 (8.39)
14	15	93	138–140	$C_{18}H_{20}N_2O_6S$	55.09 (55.06)	5.14 (5.12)	7.14 (7.17)
15	10	96	100–102	$C_{15}H_{22}N_2O_6S$	50.27 (50.34)	6.19 (6.22)	7.82 (7.87)
16	7	98	110–111	$C_{19}H_{30}N_2O_6S$	55.05 (55.25)	7.29 (7.21)	6.76 (6.54)
17	9	96	141–143	$C_{12}H_{18}N_2O_4S$	50.33 (50.42)	6.34 (6.27)	9.78 (9.54)
18	10	97	171–172	$C_{15}H_{24}N_2O_4S$	54.86 (55.02)	7.37 (7.21)	8.53 (8.33)
19	9	95	141–144	$C_{13}H_{20}N_2O_4S$	51.98 (51.78)	6.71 (6.88)	9.33 (9.43)
20	12	97	162–164	$C_{18}H_{22}N_2O_4S$	59.65 (59.51)	6.12 (6.18)	7.73 (7.84)
21	9	85	157–160	$C_{15}H_{24}N_2O_4S$	54.86 (54.62)	7.37 (7.44)	8.53 (8.67)

N-(p-*Aminobenzenesulfonyl)amino acid* tert-*butyl esters (16–21). General procedure.* To a solution of an *N*-(*p*-nitrobenzenesulfonyl)amino acid *tert*-butyl ester (1 mM) in EtOH (10 ml), Pd/C was added and the suspension stirred under hydrogen until TLC (system A) showed absence of starting material (from 45 min to 2 h). The catalyst was filtered off and the filtrate evaporated to dryness. The resulting white crystalline powders were used in the next reactions. Yield and analytical data of the products **16–21**, chromatographically homogeneous in system A, are given in Table 1.

Di*-tert-*butyl* N-*[p-(prop-2-ynylamino)benzenesulfonyl]*-L-*glutamate (22). A mixture of **16** (2.86 g, 6.6 mM), $CaCO_3$ (0.80 g, 8 mM) and propargyl bromide (0.64 ml, 8 mM) in DMF (13 ml) was stirred in the dark at 20°C for 72 h. The mixture was poured into chloroform (200 ml), salts filtered off, the chloroform evaporated and ethyl acetate (200 ml) added. The organic phase was washed with H_2O (2 × 300 ml) and brine (200 ml), dried over Na_2SO_4 and the solvent then removed *in vacuo*. The resulting oil, dissolved in chloroform (20 ml), was purified on a column [gel (60 g); 5 cm i.d. × 8 cm L], by elution with chloroform (50 ml), 1% acetone in chloroform (50 ml) and 2% acetone in chloroform (250 ml) to give an oily residue. This was crystallized from chloroform/hexane; 1.64 g (54.6%); mp 127–128°C; ^{1}H NMR, δ: 1.24, 1.38 (2s, 18H, 2^{t}Bu), 1.72 (m, 2H, Glu $^\beta$CH$_2$), 2.22 (m, 2H, Glu, $^\gamma$CH$_2$), 3.09 (t, J = 2.4 Hz, 1H, C≡CH), 3.61 (m, 1H, Glu $^\alpha$CH), 3.92 (dd, J = 6.0 Hz, 2.4 Hz, 2H, CH_2C≡C), 6.67 (d, J = 8.7 Hz, 2H, Ph: 3′-H and 5′-H), 6.84 (t, J = 5.9 Hz, 1H, CH_2-NH), 7.46 (d, J = 8.7 Hz, 2H, Ph: 2′-H and 6′-H), 7.73 (d, J = 9.0 Hz, 1H, SO_2-NH). *Anal.* Calcd. for $C_{22}H_{32}N_2O_6S$: C, 58.39; H, 7.13; N, 6.19. Found: C, 58.47; H, 7.02; N, 5.26.

tert-*Butyl* N-*[p-(prop-2-ynylamino)benzenesulfonyl]glycinate (23).* A mixture of **17** (2.86 g, 10 mM), $CaCO_3$ (1.13 g, 11 mM) and propargyl bromide (0.86 ml, 11 mM) in DMF (10 ml) was stirred at 27°C for 96 h and then worked up as for **22**. The resulting oil, dissolved in 2% acetone in chloroform, was purified on a column [gel (110 g), 5 cm i.d. × 13 cm L], using a gradient of 2.00–3.25% acetone in chloroform as eluent, to yield an oily residue. This was crystallized from chloroform/diethyl ether/hexane (1:4:5); 2.36 g (73%); mp 114–115°C; ^{1}H NMR, δ: 1.27 (s, 9H, ^{t}Bu), 3.24 (t, J = 2.4 Hz, 1H, C≡CH), 3.47 (d, J = 5.2 Hz, 2H, Gly CH_2), 4.36 (dd, J = 6.0 Hz, 2.3 Hz, 2H, CH_2C≡C) 6.78 (t, J = 6.0 Hz, 1H, NH-CH_2-C≡C), 6.88 (d, J = 8.7 Hz, 2H, Ph: 3′-H and 5′-H), 7.57 (d, J = 8.7 Hz, Ph: 2H, 2′-H and 6′-H), 7.71 (t, J = 8.9 Hz, 1H, SO_2-NH). *Anal.* Calcd. for $C_{15}H_{20}N_2O_4S$: C, 55.54; H, 6.21; N, 8.64. Found: C, 55.28; H, 6.37; N, 8.76.

tert-*Butyl* N-*[p-(prop-2-ynylamino)benzenesulfonyl]*-L-*valinate (24).* A mixture of **18** (3.12 g, 9.5 mM), $CaCO_3$ (1.05 g, 10.5 mM) and propargyl bromide (0.81 ml, 10.5 mM) in DMF (10 ml) was stirred at 20°C for 96 h and then worked up as for **22**. The resulting oil, dissolved in chloroform, was purified on a column [gel (110 g), 5 cm i.d. × 13.5 cm L] using a gradient of 0.5–1.0% acetone in chloroform as eluent to give an oily residue. This was crystallized from chloroform/acetone/hexane (1:4:4); 2.5 g (73%); mp 112.5–114°C; ^{1}H NMR, δ: 1.08 (d, J = 6.8 Hz, 6H, CH(CH_3)$_2$), 1.26 (s, 9H, ^{t}Bu), 2.32 (m, 1H, Val $^\beta$CH), 3.22 (t, J = 2.4 Hz, 1H, C≡CH), 4.35 (d, J = 6.0 Hz, 2H, CH_2C≡C), 4.62 (m, 1H, Val $^\alpha$CH), 6.56 (t, J = 6.0 Hz, 1H, HN-CH_2), 6.67 (d, J = 8.7 Hz, Ph: 2H, 3′-H and 5′-H), 7.46 (d, J = 8.7 Hz, 2H, Ph: 2′-H and 6′-H), 7.74 (d, J = 8.8 Hz, 1H, SO_2-NH). *Anal.* Calcd. for $C_{18}H_{26}N_2O_4S$: C, 58.99; H, 7.15; N, 7.64. Found: C, 60.21; H, 7.02; N, 7.63.

tert-*Butyl* N-*[p-(prop-2-ynylamino)benzenesulfonyl]*-L-*alaninate (25).* A mixture of **19** (3.0 g, 10 mM), $CaCO_3$ (1.10 g, 11 mM) and propargyl bromide (0.86 ml, 11 mM) in DMF (10 ml) was stirred at 20°C for 100 h and then worked up as for **22**. The resulting oil, dissolved in chloroform (10 ml), was purified on

a column [gel (100 g), 5 cm i.d. × 12.5 cm L], and eluted with chloroform (100 ml) and a gradient of 1–10% ethyl acetate in chloroform to give a light yellow oily residue. This slowly crystallized at low temperature to give 2.6 g (77%); mp 117.5–119°C; ^{1}H NMR, δ: 1.12 (d, J = 7.2 Hz, 3H, Ala CH_3), 1.21 (s, 9H, ^{t}Bu), 3.23 (t, J = 2.4 Hz, 1H, C≡CH), 3.62 (m, 1H, Ala $^\alpha$CH), 4.35 (d, J = 6.0 Hz, 2H, CH_2C≡C), 6.71 (t, J = 6.0 Hz, 1H, NH-CH_2), 6.85 (d, J = 8.7 Hz, 2H, Ph: 3′-H and 5′-H), 7.57 (d, J = 8.7 Hz, 2H, Ph: 2′-H and 6′-H), 7.82 (d, J = 8.5 Hz, 1H, SO_2-NH). *Anal.* Calcd. for $C_{18}H_{26}N_2O_4S$: C, 56.78; H, 6.55; N, 8.28. Found: C, 56.87; H, 6.45; N, 8.19.

tert-*Butyl* N-*[p-(prop-2-ynylamino)benzenesulfonyl]*-L-*phenylglycinate (26).* A mixture of **20** (3.62 g, 10 mM), $CaCO_3$ (1.10 g, 11 mM) and propargyl bromide (0.86 ml, 11 mM) in DMF (10 ml) was stirred at 20°C for 55 h and then worked up as for **22**. The resulting oil, dissolved in a mixture of CH_2Cl_2 and $CHCl_3$ (1:1) (10 ml), was purified on a column [gel (140 g), 5 cm i.d. × 14.5 cm L), by elution with chloroform (100 ml) and a gradient of 1.5–3.5% acetone in chloroform to give an oily residue. This was crystallized from acetone-chloroform/hexane (10:1:22); 2.48g (62%); mp 159–160°C; ^{1}H NMR, δ: 1.23 (s, 9H, ^{t}Bu), 3.28 (t, J = 2,3 Hz, 1H, C≡CH), 3.96 (m, 1H, Phg CH^α), 4.36 (d, J = 6.0 Hz, 2H, CH_2C≡C), 6.81 (t, J = 6.0 Hz, 1H, NH-CH_2), 6.88 (d, J = 8.7 Hz, 2H, Ph: 3′-H and 5′-H), 6.96 (d, J = 9.0, SO_2-NH), 7.29 (m, 3H, Ph), 7.48 (d, J = 6.3 Hz, 2H, Ph). *Anal.* Calcd. for $C_{21}H_{24}N_2O_4S$: C, 62.98; H, 6.04; N, 6.99. Found: C, 62.91; H, 6.17; N, 7.08.

tert-*Butyl* N-*[p-(prop-2-ynylamino)benzenesulfonyl]*-L-*norvalinate (27).* A mixture of **21** (3.28 g, 10 mM), $CaCO_3$ (1.10 g, 11 mM) and propargyl bromide (0.86 ml, 11 mM) in DMF (10 ml) was stirred in 29°C for 96 h and then worked up as for **22**. The resulting oil, dissolved in 0.5% acetone in $CHCl_3$ (10 ml) was purified on a column [gel (120 g), 5 cm i.d. × 14 cm L], by elution with a gradient of 0.5–2.5% acetone in chloroform to give an oily residue. This slowly crystallized from diethyl ether/chloroform/hexane (1:1:2); 2.62 g (72%); mp 89.5–92°C; ^{1}H NMR, δ: 0.76 (t, J = 7.2 Hz, 3H, $^\delta$$CH_3$), 1.20 (m, 11H, ^{t}Bu and $^\gamma$$CH_2$), 1.42 (m, 2H, $^\beta$$CH_2$), 3.20 (t, J = 2.2 Hz, 1H, C≡CH), 3.51 (m, 1H, Nva $^\alpha$CH), 4.34 (d, J = 6.0 Hz, 2H, CH_2C≡C), 6.65 (d, J = 8.7 Hz, 2H, Ph: 3′-H and 5′-H), 6.82 (t, J = 6.0 Hz, 1H, NH-CH_2), 6.97 (d, J = 9.0, SO_2-NH), 7.68 (d, J = 8.7 Hz, 2H, Ph: 2′-H and 6′-H). *Anal.* Calcd. for $C_{16}H_{26}N_2O_4S$: C, 58.99; H, 7.15; N, 7.64. Found: C, 59.17; H, 6.89; N, 7.52.

Di*-tert-*Butyl* N-*[4-[N-(2-amino-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]*-N-*prop-2-ynylamino]benzenesulfonyl]*-L-*glutamate (28). A mixture of 2-amino-6-bromomethyl-3,4-dihydro-4-oxoquinazoline hydrobromide (0.40 g, 1.1 mM), $CaCO_3$ (0.11 g, 1.1 mM) and **22** (0.45 g, 1 mM) in DMA (2.5 ml) was stirred at 20°C in the dark for 96 h. The mixture was diluted with chloroform (100 ml), salts were filtered off, chloroform was evaporated and ethyl acetate (100 ml) added. The organic phase was washed with H_2O (3×50 ml), 25% NH_4OH-brine (9:1, 3×30 ml), H_2O (30 ml) and brine (30 ml), dried over Na_2SO_4 and the solvent removed *in vacuo*. The resulting oil, dissolved in chloroform (10 ml), was purified on a column [gel (40 g), 4 cm i.d. × 6 cm L], by elution with 10% acetone in chloroform (100 ml), 2% methanol in chloroform (25 ml), 4% methanol in chloroform (50 ml) and 6% methanol in chloroform (100 ml) to give an oily residue. This was crystallized from ethyl acetate/hexane; 0.32 g (52%); mp 123°C; ^{1}H NMR, δ: 1.17, 1.37 (2s, 18H, 2^{t}Bu), 1.70 (m, 2H, $^\beta$$CH_2$), 2.23 (m, 2H, $^\gamma$$CH_2$), 3.21 (t, J = 2.4 Hz, 1H, C≡CH), 3.62 (m, 1H, $^\alpha$CH), 4.30 (d, J = 6.0 Hz, 2H, CH_2C≡C), 4.67 (s, 2H, ArCH_2N<), 6.50 (br s, 2H, NH_2), 6.88 (d, J = 8.7, 2H, Ph: 3′-H and 5′-H), 7.16 (d, J = 8.0 Hz, 1H, quinazoline 8-H), 7.50 (m, 3H, Ph: 2′-H and 6′-H, quinazoline 7-H), 7.81 (m, 2H, quinazoline 5-H and SO_2-NH). *Anal.* Calcd. for $C_{31}H_{39}N_5O_7S$: C, 59.50; H, 6.28; N, 11.19. Found: C, 59.22; H, 6.26; N, 10.97.

Di*-tert-*butyl* N-*[4-[N-(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]*-N-*prop-2-ynylamino]benzenesulfonyl]*-L-*glutamate (29). A mixture of **22** (0.45 g, 1 mM), 6-bromomethyl-3,4-dihydro-2-methyl-4oxoquinazoline (0.28 g, 1.1 mM) and $CaCO_3$ (0.11 g, 1.1 mM) in DMA (2.5 ml) was stirred at 20°C in the dark for 160 h. Work-up as for **28** gave **29**; 0.323 g, (49%); mp 112–115°C; ^{1}H NMR, δ: 1.16, 1.37 (2s, 18H, 2^{t}Bu), 1.70 (m, 2H, $^{\beta}CH_2$) 2.22 (m, 2H, $^{\gamma}CH_2$), 2.32 (s, 2H, CH_3-Ar), 3.22 (t, J = 2.3 Hz, 1H, C≡CH), 3.62 (m, 1H, $^{\alpha}$CH), 4.35 (s, 2H, $CH_2C{\equiv}C$), 4.78 (s, 2H, Ar$\underline{CH_2}$N<), 6.87 (d, J = 8.7 Hz, 2H, Ph: 3′-H and 5′-H), 7.52 (d, J = 8.1 Hz, 1H, quinazoline 8-H), 7.68 (d, J = 8.1 Hz, 1H, quinazoline 7-H), 7.83 (d, J = 8.7 Hz, 2H, Ph: 2′-H and 6′-H), 7.98 (s, 1H, quinazoline 5-H), 8.14 (d, J = 8.5 Hz, 1H, SO_2-NH). *Anal.* Calcd. for $C_{32}H_{40}N_4O_7S$: C, 61.52; H, 6.45; N, 8.97. Found: C, 61.24; H, 6.63; N, 8.68.

tert-*Butyl* N-*[4-[N-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]*-N-*prop-2-ynylamino]benzenesulfonyl]glycinate (30).* A mixture of **23** (0.66 g, 2.2 mM), 6-bromomethyl-3,4-dihydro-2-methyl-4-oxoquinazoline (0.51 g, 2.0 mM) and $CaCO_3$ (0.20 g, 2.0 mM) in DMA (4 ml) and DMF (10 ml) was stirred at 20°C in the dark for 168 h. The mixture was diluted with chloroform (100 ml), salts filtered off, chloroform evaporated, and ethyl acetate (200 ml) added. The organic phase was washed with H_2O (4 × 40 ml), 25% NH_4OH/brine (9:1, 3 × 40 ml), 10% citric acid (3 × 30 ml), H_2O (30 ml) and brine (30 ml), dried over $MgSO_4$ and solvent removed *in vacuo*. The residue, dissolved in a mixture of DMF (2 ml) and ethyl acetate (1.5 ml), was purified on a column [gel (40 g), 4 cm i.d. × 8.5 cm L) using a gradient of 30–40% acetone in CCl_4 as eluent to give an oily residue. This was crystallized from acetone/hexane; 0.58 g (58%); mp 202–203°C; ^{1}H NMR, δ: 0.96 (s, 9H, ^{t}Bu), 2.33 (s, 2H, CH_3-Ar), 3.21 (t, J = 2.4 Hz, 1H, C≡CH), 3.47 (d, J = 5.0 Hz, 2H, Gly CH_2), 4.35 (d, J = 6.0, 2H, $CH_2C{\equiv}C$), 4.79 (s, 2H, ArCH_2N<), 5.82 (d, J = 8.7 Hz, 2H, Ph: 3′-H and 5′-H), 7.52 (d, J = 8.4 Hz, 1H, quinazoline 8-H), 7.57 (d, J = 8.9 Hz, 1H quinazoline 7-H), 7.68 (m, 3H, Ph; 2′-H and 5′-H, SO_2-NH), 7.97 (s, 1H, quinazoline 5-H), 12.2 (br s, lactam NH-). *Anal.* Calcd. for $C_{25}H_{28}N_4O_5S$: C, 60.47; H, 5.68; N, 11.28. Found: C, 60.63; H, 5.47; N, 11.12.

tert-*Butyl* N-*[4-[N-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]*-N-*prop-2-ynylamino]benzenesulfonyl]*-L-*valinate (31).* A mixture of **24** (0.61 g, 1.7 mM), 6-bromomethyl-3,4-dihydro-2-methyl-4-oxoquinazoline (0.39 g, 1.5 mM) and $CaCO_3$ (0.15 g, 1.5 mM) in DMF (2 ml) was stirred at 20°C in the dark for 100 h. Work-up as for **30** gave crude product (1.06 g) which, dissolved in chloroform (2 ml), was purified on a column [gel (80 g), 5 cm i.d. × 9.5 cm L] using 15% ethyl acetate in chloroform (100 ml) and a gradient of 1.5–3.0% methanol in chloroform (600 ml) as eluents to give an oily residue. This was crystallized from ethyl acetate/chloroform/hexane (1:1:2); 0.50 g (61%); mp 111–114°C; ^{1}H NMR, δ: 0.94 (d, J = 6.8 Hz, 6H, $CH(CH_3)_2$), 1.28 (s, 9H, ^{t}Bu), 2.32 (s, 2H, CH_3-Ar), (s, 2H, CH_3-Ar), 2.33 (m, 1H, Val $^{\beta}CH(CH_3)_2$), 3.31 (t, J = 2.4 Hz, 1H, C≡CH), 4.36 (d, J = 6.0 Hz, 2H, $CH_2C{\equiv}C$), 3.65 (m, 1H, Val $^{\alpha}$CH), 4.20 (s, 2H, Ar$\underline{CH_2}$N<), 6.87 (d, J = 8.7 Hz, 2H, Ph: 3′-H and 5′-H), 7.76 (d, J = 8.7 Hz, 2H, Ph: 2′-H and 6′-H), 7.18 (d, J = 8.8 Hz, 1H, quinazoline 8-H), 7.48 (dd, J = 9.0, 1.5 Hz, 1H quinazoline 7-H), 7.78 (s, quinazoline 5-H), 8.14 (d, J = 9.0, 1H, SO_2-NH). *Anal.* Calcd. for $C_{28}H_{34}N_4O_5S$: C, 62.43; H, 6.36; N, 10.40. Found: C, 62.68; H, 6.09; N, 10.03.

tert-*Butyl* N-*[4-[N-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]*-N-*prop-2-ynylamino]benzenesulfonyl]*-L-*alaninate (32).* A mixture of **25** (0.69 g, 2.2 mM), 6-bromomethyl-3,4-dihydro-2-methyl-4-oxoquinazoline (0.51 g, 2.0 mM) and $CaCO_3$ (0.20 g, 2 mM) in DMA (4 ml) and DMF (1 ml) was stirred at 20°C in the dark for 168 h. Work-up

as for **30** gave a crude product (0.97 g) which, dissolved in a mixture of acetone (5.5 ml) and chloroform (3.5 ml), was then purified on a column [gel (60 g), 5 cm i.d. × 8 cm L], using 5% acetone in chloroform (50 ml), 10% acetone in chloroform (550 ml), and then, 3% methanol in chloroform (500 ml) as eluents to give an oily residue. This was crystallized from acetone/hexane; 0.44 g (43%); mp 140–142°C; H^1 NMR, δ: 1.12 (d, J = 7.2 Hz, 3H, $CHCH_3$), 1.21 (s, 9H, ^{t}Bu), 2.32 (s, 3H, CH_3-Ar), 3.23 (t, J = 2.0 Hz, 1H, HC≡C), 3.62 (m, 1H, $^\alpha$CH), 4.35 (d, J = 6.0 Hz, 2H, $CH_2C{\equiv}C$), 4.78 (s, 2H, Ar$\underline{CH_2}$N<), 6.45 (d, J = 8.7 Hz, 2H, Ph: 3′-H and 5′-H), 7.54 (d, J = 8.7 Hz, 2H, Ph: 2′-H and 6′-H), 7.65 (d, J = 8.9 Hz, 1H, quinazoline 8-H), 7.70 (dd, J = 9.1, 1.5Hz, 1H, quinazoline 7-H), 7.82 (d, J = 9.0 Hz, 1H, SO_2-NH), 7.97 (d, J = 1.5 Hz 1H, quinazoline 5-H), 12.10 (bs, 1H, lactam NH). *Anal.* Calcd. for $C_{26}H_{30}N_4O_5S$: C, 61.16; H, 5.92; N, 10.97. Found: C, 61.38; H, 6.09; N, 10.82.

tert-*Butyl* N-*[4-[N-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N-*prop-2-ynylamino]benzenesulfonyl]-*L-*phenylglycinate (33). A mixture of **26** (0.88 g, 2.2 mmol), 6-bromomethyl-3,4-dihydro-2-methyl-4-oxoquinazoline (0.51 g, 2.0 mM) and $CaCO_3$ (0.20 g, 2 mM) in DMF (3 ml) was stirred at 20°C in the dark for 168 h. Work-up as for **30** gave a crude product (1.36 g) which, dissolved in a mixture of chloroform, ethyl acetate and methanol (3.5 ml, 1.0 ml and 0.2 ml, respectively), was purified on a column [gel (80 g), 5 cm, i.d. × 10 cm L] using as eluents 10% ethyl acetate in chloroform (100 ml), 15% ethyl acetate in chloroform (100 ml), 20% ethyl acetate in chloroform (100 ml) and then, a gradient 1.0–2.5% methanol in chloroform, to give an oily residue. This was crystallized from acetone/hexane; 0.75 g (66%); mp 176–178°C; H^1 NMR, δ: 1.22 (s, 9H, ^{t}Bu), 2.32 (s, 3H, CH_3-Ar), 3.20 (t, J = 2.3 Hz, 1H, C≡CH), 3.96 (m, 1H, $^\alpha$CH), 4.32 (d, J = 6.0 Hz, 2H, $CH_2C{\equiv}C$), 4.76 (bs, 2H, Ar$\underline{CH_2}$NH<), 6.86 (d, J = 8.7 Hz, 2H, Ph: 3′-H, and 5′-H), 7.93 (d, J = 8.7 Hz, 2H, Ph: 2′-H, and 6′-H), 7.25–7.36 (m, 4H, Ph and quinazoline 8-H), 7.53–7.70 (m, 3H, Ph, quinazoline 7-H), 8.15 (d, J = 1.5 Hz, 1H, quinazoline 5-H). *Anal.* Calcd. for $C_{31}H_{32}N_4O_5S$: C, 65.02; H, 5.63; N, 9.78. Found: C, 65.11; H, 5.60; N, 9.81.

tert-*Butyl* N-*[4-[N-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N-*prop-2-ynylamino]benzenesulfonyl]-*L-*norvalinate (34). A mixture of **27** (0.75 g, 2.2 mM), 6-bromomethyl-3,4-dihydro-2-methyl-4-oxoquinazoline (0.51 g, 2.0 mM) and $CaCO_3$ (0.20 g, 2 mM) in DMA (4 ml) and DMF (1 ml) was stirred at 20°C in the dark for 168 h. Work up as for **30** furnished a crude product (1.11 g) which, dissolved in 2% methanol in chloroform (3 ml) was purified on a column [gel (54 g), 4 cm, i.d. × 10 cm L], using a gradient of 2.6–3.5% methanol in chloroform (500 ml) as eluent to give an oily residue. This was crystallized from acetone/hexane; 0.63 g (59%); mp 176–178°C; ^{1}H NMR, δ: 0.75 (t, 3H, CH_2CH_3), 1.22 (m, 11H, ^{t}Bu and $^\gamma CH_2$), 1.43 (m, 2H, $^\beta CH_2$), 2.32 (s, 2H, CH_3-Ar), 3.21 (t, J = 2.2 Hz, 1H, C≡CH), 3.51 (m, 1H, $^\alpha$CH), 4.34 (d, J = 6.0 Hz, 2H, $CH_2C{\equiv}C$), 6.65 (d, J = 8.7 Hz, 2H, Ph: 3′-H and 5′-H), 7.12 (d, J = 8.0 Hz, 1H, quinazoline 8-H), 7.50 (m, 3H, Ph: 2′-H, and 5′-H and quinazoline 7-H), 7.83 (m, 2H, quinazoline 5-H and SO_2-NH). *Anal.* Calcd. for $C_{28}H_{34}N_4O_5S$: C, 62.43; H, 6.36; N, 10.40. Found: C, 62.67; H, 6.12; N, 10.18.

N-*[p-[N-(3,4-dihydro-2-amino-4-oxo-6-quinazolinyl)methyl]-*N-*prop-2-ynylamino]benzenesulfonyl]-*L-*glutamic acid (3)*, N-*[p-[N-(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N-*prop-2-ynylamino]benzenesulfonyl]-*L-*glutamic acid (4), -glycine (5), -*L*-valine (6), -*L*-alanine (7), -*L*-phenylglycine (8) and -*L*-norvaline (9). General procedure. A solution of each of **28–34** in trifluoroacetic acid (10 ml/mM) was stored for 1 h at room temp. TLC (system B) showed the absence of starting material. The acid was evaporated and diethyl ether (100 ml/mM)

Table 2. Sulfamide antifolates (3–9)

Compd. No.	Yield %[a]	mp °C	$[M+1]^+$	Formula	*Anal*. Calcd. (Found) C	H	N
3	92	169–172	514	$C_{23}H_{23}N_5O_7S \cdot 0.5\ CF_3COOH \cdot 0.1(C_2H_5)_2O \cdot 0.5\ H_2O$	49.97 (49.63	4.34 4.39	11.93 11.91)
4	91	140–142	513	$C_{24}H_{24}N_4O_7S \cdot 0.6\ CF_3COOH$	52.09 (52.13	4.27 4.18	9.64 9.55)
5	98	231–233	441	$C_{21}H_{20}N_4O_5S \cdot 0.4\ CF_3COOH \cdot 0.5\ H_2O$	52.88 (52.72	4.36 4.35	11.32 11.28)
6	98	212–215	483	$C_{24}H_{26}N_4O_5S \cdot 0.5\ CF_3COOH \cdot 0.4\ H_2O$	54.91 (54.85	5.03 5.07	10.25 10.17)
7	98	246	455	$C_{22}H_{22}N_4O_5S \cdot 0.3\ CF_3COOH \cdot 0.5\ H_2O$	54.53 (54.42	4.72 4.81	11.26 11.21)
8	97	215–217	517	$C_{27}H_{24}N_4O_5S \cdot 0.7\ CF_3COOH \cdot 0.5\ H_2O$	57.28 (57.15	4.35 4.38	9.41 9.45)
9	98	226–229	483	$C_{24}H_{26}N_4O_5S \cdot 0.6\ CF_3COOH \cdot 0.2\ H_2O$	61.14 (61.12	4.53 4.52	10.11 10.02)

a. Preparation in 0.5 mM scale

added. The white solid that precipitated was purified by six cycles of centrifugation-decantation-resuspension in diethyl ether. The product was dried *in vacuo* over KOH at 65°C overnight. Yields, analytical data and $[M+1]^+$ ions of **3–9** are given in Table 2, and ^{1}H NMR data in Table 3.

Biology

Cells. Mouse leukemia L5178Y cells were grown as reported earlier (Dzik *et al.,* 1996).

In vitro *cell growth inhibition.* The influence of each analogue on viability of exponentially growing cells and [^{14}C]leucine incorpo-

Table 3. ^{1}H NMR spectra of sulfamide antifolates (3–9)

	Chemical shifts δ											
		Propargyl										
Compd. No.	CH_3 (s)	H (t)	CH_2 (d)	CH^{α} (m)	CH_2^9 (s)	3',5' (d)	2',6' (d)	H^8 (d)	H^7 (dd)	H^5 (d)	SO_2-NH (d)	R
3	-	3.24	4.30	3.69	4.70	6.86	7.54	7.28	7.61	7.85	7.78	[a]
4	2.48	3.25	4.35	3.68	4.80	6.87	7.54	7.58	7.71	7.99	7.78	[b]
5	2.48	3.25	4.37	3.47	4.79	6.87	7.54	7.58	7.72	7.96	7.78	[c]
6	2.33	3.25	4.34	3.62	4.14	6.85	7.87	7.22	7.70	7.76	8.18	[d]
7	2.54	3.25	4.36	3.65	4.80	6.88	7.56	7.69	7.74	7.98	7.77	[e]
8	2.37	3.25	4.12	3.96	4.71	6.86	7.83	7.64	7.70	7.94	7.82	[f]
9	2.48	3.24	4.35	3.58	4.79	6.87	7.54	7.58	7.72	7.96	7.76	[g]

[a,b] 1.75 (m, 2H, Glu $^{\beta}CH_2$), 2.20 (t, J = 6.8, 2H, Glu $^{\gamma}CH_2$); [c] 3.47 (d, J = 6 Hz, 2H, Gly CH_2); [d] 1.07 [d, J = 6.4 Hz, 6H, $CH(CH_3)_2$], 2.34 [(m, 1H, Val $^{\beta}CH(CH_3)_2$]; [e] 1.12 (d, J = 7.3 Hz, 3H, Ala CH_3); [f] 7.25–7.36 (m, 4H, quinazoline H^8 and Phg Ph), 7.53–7.70 (m, 3H quinazoline H^7 and Phg Ph); [g] 0.76 (t, J = 7.3, 3H, Nva $^{\delta}CH_3$), 1.21(m, 2H, Nva $^{\gamma}CH_2$), 1.43 (q, J = 7.3, 2H, Nva $^{\beta}CH_2$).

ration was followed, and IC_{50} values determined as previously described (Dzik *et al.,* 1996).

Thymidylate synthase. Highly purified preparations of thymidylate synthase from mouse Ehrlich carcinoma cells (Jastreboff *et al.,* 1983), regenerating rat liver and the tapeworm *Hymenolepis diminuta* (Rode *et al.,* 1990) have been described in detail elsewhere.

Enzyme assay. Thymidylate synthase activity was assayed by monitoring release of tritium from [5-^{3}H]dUMP as previously described (Rode *et al.,* 1984), all measurements being done in triplicate. N^{10}-propargyl-5,8-dideazafolate and its analogues were added to the reaction mixture as neutral aqueous solutions.

Kinetic studies. To identify the type of inhibition involved, the effects of each analogue on the dependence of reaction rate on $N^{5,10}$-methylenetetrahydrofolate concentration, in the form of Lineweaver-Burk plots, were analyzed as previously reported (Rode *et al.,* 1984).

Statistically evaluated results. These are presented as means ± S.E.M. or means ± % difference between the mean and each of the two results, followed by the number of experiments (N) in parentheses.

RESULTS

Chemistry

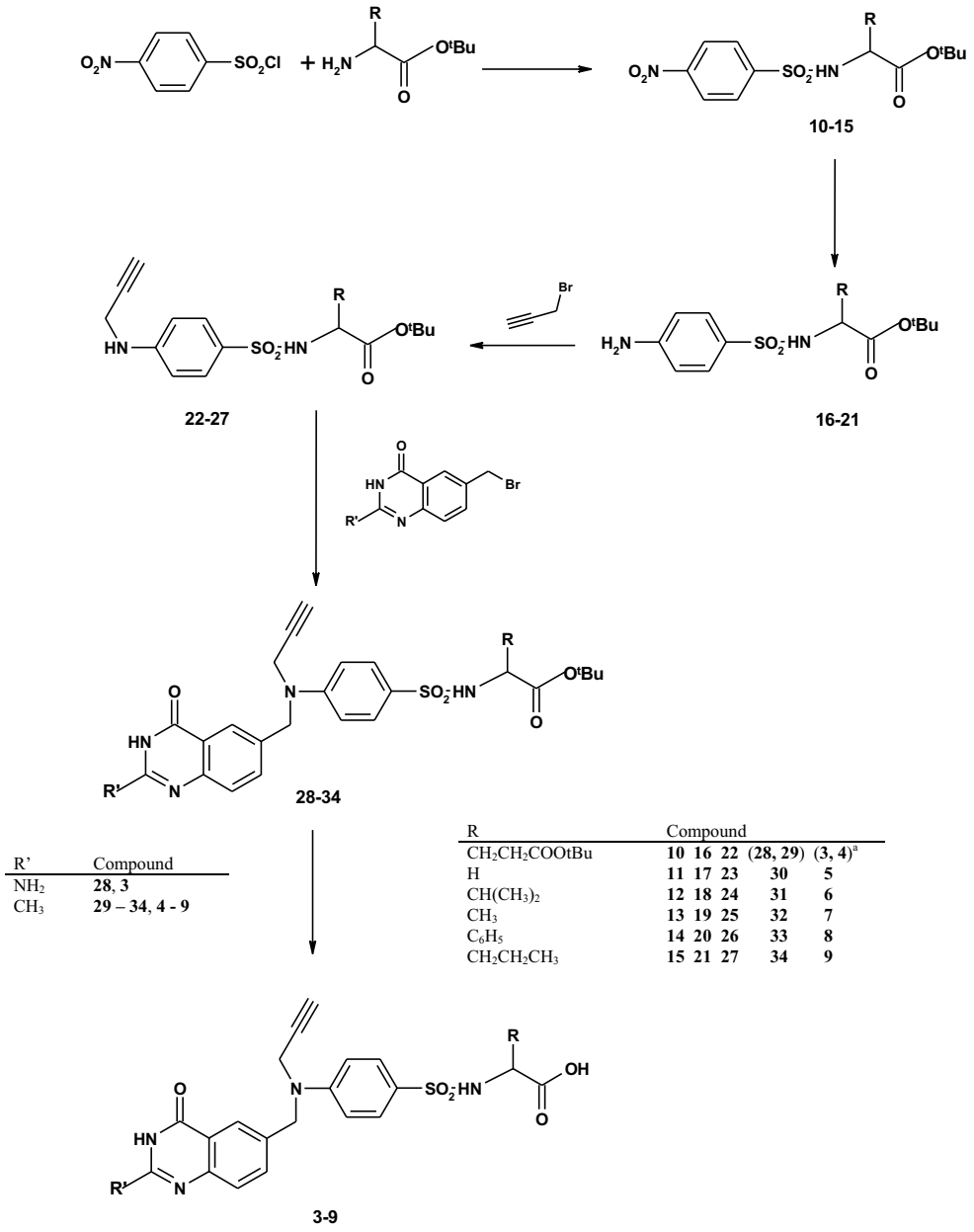
The synthetic route to compounds **3–9** (Fig. 2) started with *p*-nitrobenzenesulfonylchloride coupling with an appropriate amino acid *tert*-butyl ester to give the nitro derivatives (**10–15**) which, after catalytic hydrogenation, furnished *N*-(*p*-aminobenzenesulfonyl)amino acid *tert*-butyl esters (**16–21**). Their N-alkylation with propargyl bromide yielded secondary amines (**22–27**). The second N-alkylation with 2-amino-6-bromomethyl-3,4-dihydro-4-oxoquinazoline or 6-bromomethyl-3,4-dihydro-2-methyl-4-oxoquinazoline led to the antifolate esters (**28–34**). Final removal of the *tert*-butyl group with trifluoroacetic acid gave the appropriate antifolates (**3–9**). Purity of all compounds **3–34** was established by elemental analysis, and the structures of **22–34** and **3–9** were confirmed by ^{1}H NMR spectroscopy. The final products **3–9** were additionally characterised by FAB-mass spectrometry.

Biological evaluation

While replacement of CONH by SO_2NH in the parent pddPteGlu (**1**) resulted in 3–10-fold weaker inhibition of the enzyme (Table 4; compound **3**), the same modification of **2** led to a 5-fold loss in thymidylate synthase inhibition potency and an 8–9-fold decrease in cell growth inhibition potency (Tables 4–5; compound **4**). Substitution of the glutamyl residue in **4** with norvaline resulted in 2–5-fold stronger thymidylate synthase inhibition, but almost 4–5-fold weaker growth inhibition (Tables 4–5; compound **9**). Similar substitutions with glycine, alanine, valine and phenylglycine were either without a distinct effect (Table 4; compounds **7–8**, and **5** with regenerating rat liver enzyme) or lowered enzyme inhibitory potency (Table 4; compounds **6**, and **7** with the tumour and parasite enzyme). The analogues substituted with glycine and alanine caused 4–7-fold weaker cell growth inhibition than compound **4** (Table 5, compounds **5** and **7**).

DISCUSSION

pddPteGlu was the first thymidylate synthase-targeted antifolate tested as an antitumour drug in clinical trials (Rosowsky, 1992). Although it showed significant activity against a number of human tumours, undesirable side effects, such as liver toxicity and kidney toxicity, resulting from poor solubility, prevented its use (Harrap *et al.,* 1995). Replacement of the amino group at C(2) by a hy-



R'	Compound
NH_2	**28, 3**
CH_3	**29 – 34, 4 - 9**

R	Compound				
$CH_2CH_2COOtBu$	**10**	**16**	**22**	**(28, 29)**	**(3, 4)**[a]
H	**11**	**17**	**23**	**30**	**5**
$CH(CH_3)_2$	**12**	**18**	**24**	**31**	**6**
CH_3	**13**	**19**	**25**	**32**	**7**
C_6H_5	**14**	**20**	**26**	**33**	**8**
$CH_2CH_2CH_3$	**15**	**21**	**27**	**34**	**9**

Figure 2. Synthesis of the new analogues 3–9.

drogen or a methyl group led to several-fold weaker binding to thymidylate synthase, but with substantially increased solubility, with no distinct loss of antitumour activity (Jones *et al.,* 1989). The 2-methyl substitution resulted in particularly strong cell growth inhibition (Jackman *et al.,* 1991), presumably due to more efficient conversion of the 2-methyl derivative to non-effluxing long-chain polyglutamates, known to be stronger thymidylate synthase inhibitors. In accord with the foregoing, results presented here show also a 4–5-fold weaker inhibition of rat thymidylate synthase and unchanged cell growth inhibition resulting from the 2-methyl substitution in a pddPteGlu derivative (Tables 4–5, cf. pddPteSO_2Glu and CH_3pddPteSO_2Glu). It should be noted that the L5178Y cells used in our experiments were 2–20-fold less sensitive to growth inhibition than L1210 cells by several known antifolates (Kusakiewicz-Dawid *et al.,* 2002).

Replacement of CONH by SO_2NH in CH_3pddPteGlu weakened inhibition of both the enzyme and cell growth (Tables 4–5). Several thymidylate synthase inhibitors have been synthesized, containing $-SO_2NH-$ or $SO_2N=$ sulfonamide group (Elslager *et al.,* 1984; Pendergast *et al.,* 1993; Varney *et al.,* 1992; Jones *et al.,* 1996). One of them, AG 331, entered clinical studies (Bavetsias & Jackman, 1998). Some members of a series of 2,4-diamino-6-quinazolinesulfonamides showed strong antimalarial activity (Elslager *et al.*, 1984) but were not tested against potential target enzymes. Recently, introduction of the sulfonamido (SO_2NH) link into benzoquinazoline derivatives inhibiting thymidylate synthase resulted in compounds with K_i values in the nM range (Pendergast *et al.,* 1993). Amongst nonpolyglutamable analogues of 2-deamino-2-methyl-10-propargyl-5,8-dideazafolic acid **2** (Fig. 1), that containing a phenyl ring with a 3-fluoromethyl group at the position 2 *vs*. N^{10}, bound with the glycyl residue *via* a sulfonamide bond, was as potent an enzyme inhibitor as the parent compound, but a weak cell growth inhibitor (Jones *et al.,* 1996).

Substitution of the glutamyl residue in CH_3pddPteSO_2Glu with different amino-acid residue (Ala, Gly,Val, PhGly or NVal) appears to offer new derivatives with moderate specificity, reflected by several-fold differences in inhibitory potency against thymidylate synthases of different origin (Table 4). Interestingly, while previously described similar substitutions of the glutamyl residue in pddPteGlu with Ala and Gly resulted in distinctly lower, relative to the parent com-

Table 4. Inhibition of Ehrlich carcinoma (Ehrlich c.), *Hymenolepis diminuta* (*H.d.)* and regenerating rat liver (RRL) thymidylate synthases by10-propargyl-5,8-dideazafolic acid (pddPteGlu; 1) and its 2-methyl-2-desamino analogue (ICI 198583; 2), sulphonamide analogues of 1 and 2 (PddPteSO_2Glu and CH_3pddPteSO_2Glu, respectively), and sulphonamide analogues of 2 with glutamyl residue substituted by glycyl (in CH_3pddPteSO_2Gly), valyl (in CH_3pddPteSO_2Val), alanyl (in CH_3pddPteSO_2Ala), phenylglycyl (in CH_3pddPteSO_2PhGly) or norvalyl (in CH_3pddPteSO_2NVal) residue; for structures see Fig. 1.

Compound				Ehrlich c. enzyme		*H.d.* enzyme		RRL enzyme	
No.	R	X	R'	K_i(μM)	K_i/K_m	K_i(μM)	K_i/K_m	K_i(μM)	K_i/K_m
pddPteGlu (**1**)	NH_2	CO	Glu[a]	0.008 ± 0.002(3)	0.00024	0.017[b]	0.00057	0.010[b]	0.00042
ICI 198583 (**2**)	CH_3	CO	Glu	0.010[c]		ND[d]		0.039[e]	0.0014
pddPteSO_2Glu (**3**)	NH_2	SO_2	Glu	0.082 ± 0.008(3)	0.0024	0.049±0.005(3)	0.0016	0.051±0.000(3)	0.0021
CH_3pddPteSO_2Glu (**4**)	CH_3	SO_2	Glu	0.192 ± 0.010(3)	0.0057	0.27±0.01(3)	0.0090	0.21±0.02(3)	0.0087
CH_3pddPteSO_2Gly (**5**)	CH_3	SO_2	Gly	0.303 ± 0.061(3)	0.0090	0.66[b]	0.022	0.16[b]	0.0067
CH_3pddPteSO_2Val (**6**)	CH_3	SO_2	Val	0.386 ± 0.074(3)	0.012	0.93[b]	0.031	0.29[b]	0.012
CH_3pddPteSO_2Ala (**7**)	CH_3	SO_2	Ala	0.184 ± 0.022(3)	0.0055	0.17[b]	0.0057	0.14[b]	0.0058
CH_3pddPteSO_2PhGly (**8**)	CH_3	SO_2	PhGly	0.150 ± 0.020(3)	0.0045	0.55[b]	0.018	0.22[b]	0.0092
CH_3pddPteSO_2NVal (**9**)	CH_3	SO_2	NVal	0.048 ± 0.001(3)	0.0014	0.14±0.01(3)	0.0047	0.085±0.013(3)	0.0035

[a]Amino-acid residue abbreviations: glutamic acid (Glu), glycine (Gly), valine (Val), alanine (Ala), phenylglycine (PhGly), norvaline (NVal); [b]Mean result of two experiments differing by not more than 20%. [c]Thymidylate synthase from murine leukaemia L1210 cells; Hughes *et al.* 1990; [d]Not determined. [e]Recombinant rat hepatoma thymidylate synthase; Kusakiewicz-Dawid *et al.* 2002.

Table 5. Inhibition of murine leukaemia L518Y cell growth by 10-propargyl-5,8-dideazafolic acid (pddPteGlu; 1) and its 2-methyl-2-desamino analogue (ICI 198583; 2), sulphonamide analogue of 2 (CH_3pddPteSO_2Glu, respectively), and sulphonamide analogues of 2 with glutamyl residue substituted by glycyl (in CH_3pddPteSO_2Gly), alanyl (in CH_3pddPteSO_2Ala), or norvalyl (in CH_3pddPteSO_2NVal) residue; for structures see Fig. 1.

Compound Abbreviation (No.)	R	X	R'	Cell growth inhibition [IC_{50} (μM)] Cell count	[^{14}C]Leu incorporation
pddPteGlu (**1**)	NH_2	CO	Glu	9.05 ± 1.24 (3)	8.3 ± 1.21 (3)
CH_3pddPteGlu; ICI 198583 (**2**)	CH_3	CO	Glu	6.9 ± 7% (2)	6.61 ± 15% (2)
CH_3pddPteSO_2Glu (**4**)	CH_3	SO_2	Glu	59.5 ± 2.5% (2)	54.6 ± 28% (2)
CH_3pddPteSO_2Gly (**5**)	CH_3	SO_2	Gly	210 ± 12% (2)	270 ± 25% (2)
CH_3pddPteSO_2Ala (**7**)	CH_3	SO_2	Ala	451 ± 3% (2)	399 ± 14% (2)
CH_3pddPteSO_2NVal (**9**)	CH_3	SO_2	NVal	290 ± 11% (2)	204 ± 4% (2)

pound, affinities for mammalian thymidylate synthase (Jones *et al.,* 1986), the same substitutions in the CH_3pddPteSO_2Glu molecule seemed to be without effect (Table 4). All the new analogues with the glutamyl residue replaced by different amino-acid residues, compared with the parent CH_3pddPteSO_2Glu, were several-fold weaker inhibitors of cell growth (cf. Jones *et al.,* 1986; Marsham *et al.,* 1995). This is not surprising, since these compounds cannot be substrates for folylpolyglutamate synthetase.

Of particular interest are the properties of the new analogue containing norvaline in place of the glutamate residue (CH_3pddPteSO_2NVal), being a several-fold stronger inhibitor of the three thymidylate synthases than CH_3pddPteSO_2Glu, and either only several-fold weaker (with the mammalian enzyme) or as potent (with tapeworm enzyme) inhibitor as the parent CH_3pddPteGlu (Table 4).

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