Quinazoline Antifolate Thymidylate Synthase Inhibitors: Difluoro-Substituted Benzene Ring Analogues

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The synthesis of a series of new C^2-methyl-N^{10}-alkylquinazoline-based thymidylate synthase (TS) inhibitors containing difluorinated *p*-aminobenzoate rings is described. Derivatives of the N^{10}-propargyl and N^{10}-methylquinazoline antifolates were prepared with 2',3'-, 2',5'-, and 2',6'-difluoro substitution. The synthesis of the 2',5'-difluoro analogues involved oxidation of the difluoronitrotoluene to 2,5-difluoro-4-nitrobenzoic acid followed by glutamation, reduction, and alkylation (propargyl bromide or MeI) to the diethyl *N*-(4-(alkylamino)-2,5-difluorobenzoyl)-L-glutamates. For the synthesis of the 2',3'- and 2',6'-difluoro compounds a new route was devised starting from methyl 4-((*tert*-butoxycarbonyl)amino)-2,6-difluorobenzoate and its 2,3-substituted counterpart. Treatment with NaH and then an alkyl halide introduced the N^{10}-substituent. The methyl ester was hydrolyzed and the resulting acid was condensed with diethyl L-glutamate. The secondary amine was liberated using CF_3CO_2H and coupled with 6-(bromomethyl)-3,4-dihydro-2-methyl-4-oxoquinazoline to yield the antifolate diesters. Final deprotection with mild alkali completed the synthesis in each case. The target compounds were tested as inhibitors of partially purified L1210 TS and also examined for their inhibition of the growth of L1210 cells in culture. Compared to their nonfluorinated parent compounds all the difluoro analogues were poorer inhibitors of TS. The greatest loss of enzyme activity was seen in the N^{10}-propargyl analogues which contained one of the fluorine atoms ortho to the amine substituent. This loss was less apparent in the N^{10}-methyl derivatives. Despite this lower inhibition of TS the majority of new compounds have equivalent cytotoxicity to their nonfluorinated predecessors.

Introduction

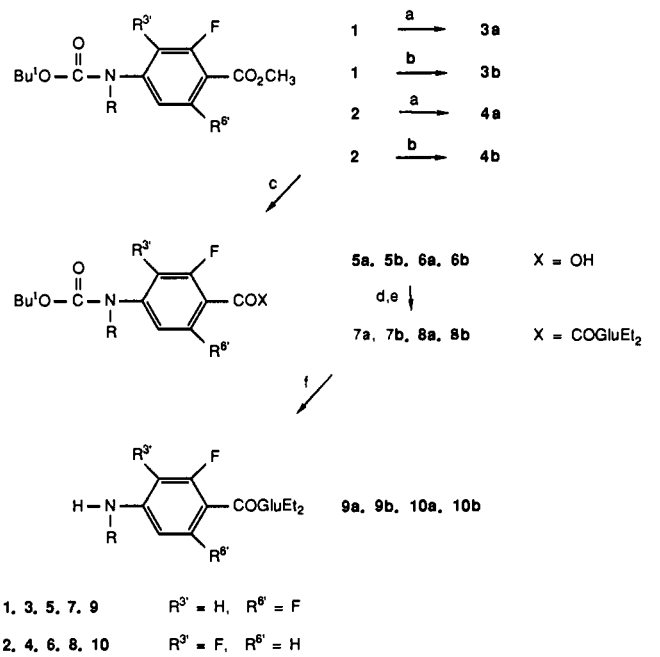
As part of an extensive study of the potential of analogues of N^{10}-propargyl-5,8-dideazafolic acid[1,2] as anticancer agents acting via inhibition of thymidylate synthase (TS, EC 2.1.1.45), we have investigated compounds modified by fluorine substitution into the benzoyl residue. We have reported earlier[3] on the improved TS inhibitory activity and L1210 cell growth inhibitory activity of 2'-fluoro-N^{10}-propargyl-5,8-dideazafolic acid and its 2-desamino, 2-desamino-2-methyl, 2-desamino-2-(hydroxymethyl) and 2-desamino-2-methoxy analogues over their parent benzoyl ring counterparts. We have also reported the 2'-fluoro and 3'-fluoro analogues[4] of a series of variously N^{10}-substituted 2-methyl-5,8-dideazafolic acids.[5]

In view of the improved activity conferred by the 2'-fluoro substituent we have investigated various difluorinated analogues retaining this substituent, namely 2',6'-, 2',3'-, and 2',5'-difluorinated derivatives. It was also of interest to explore the extent of the interaction between the 2'-fluoro substituent and the amide hydrogen atom in these difluorinated analogues. This interaction was seen in the ^{1}H-NMR spectra of the 2'-fluoro derivatives and was postulated as contributing to the favourable activity of these compounds against TS.[3,4]

Chemistry

Methyl 4-((*tert*-butyloxycarbonyl)amino)-2,6-difluorobenzoate (**1**)[6] and methyl 4-((*tert*-butyloxycarbonyl)amino)-2,3-difluorobenzoate (**2**)[6] were the respective intermediates in the syntheses of the target 2',6'- and 2',3'-difluoro derivatives (**20a,b** and **21a,b**). The syntheses from **1** and **2** of the intermediates **9a,b** and **10a,b** are shown in Scheme I. The synthesis of **9a** illustrates the general procedure. Compound **1** was treated with strong base (NaH) followed by propargyl bromide to give **3a**, thus introducing the N^{10}-propargyl substituent. Liberation of the carboxyl function using alkaline hydrolysis gave **5a** which was converted into the acid chloride upon treatment with oxalyl chloride and *N*,*N*-dimethylformamide (DMF). After coupling with diethyl glutamate, the *tert*-butyloxy-

Scheme I[a]



[a] (a) NaH, propargyl bromide, DMF; (b) NaH, MeI, DMF; (c) NaOH, H_2O, EtOH; (d) oxalyl chloride, DMF, CH_2Cl_2; (e) diethyl glutamate hydrochloride, Et_3N; (f) CF_3CO_2H.

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(1) Jones, T. R.; Calvert, A. H.; Jackman, A. L.; Brown, S. J.; Jones, M.; Harrap, K. R. A Potent Antitumour Quinazoline Inhibitor of Thymidylate Synthase: Synthesis, Biological Properties and Therapeutic Results in Mice. *Eur. J. Cancer* **1981**, *17*, 11-19.
(2) Synonyms: CB 3717; ICI 155387; NSC 327182; *N*-[4-[*N*-[(2-amino-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic acid

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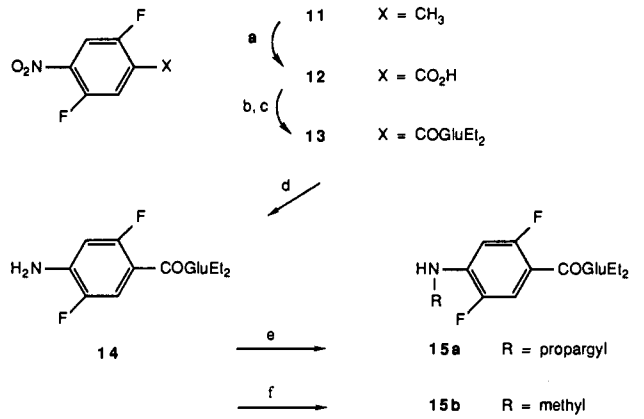
Table I. Preparation of Difluorinated Antifolate Esters

compd	(difluorobenzoyl)-glutamate ester	quinazoline	base	solvent	reaction temp, °C	% yield	mp, °C	formula[a]	mass spectra m/z, M+
17a	9a	16[b]	Proton Sponge	DMA	50	85	120–124	$C_{29}H_{30}F_2N_4O_6$	568
17b	9b	16	Proton Sponge	DMA	50	18[c]	257–259	$C_{27}H_{30}F_2N_4O_6$	544
18a	10a	16	Cs_2CO_3	CH_3CN	70	14	160–162	$C_{29}H_{30}F_2N_4O_6$	568
18b	10b	16	Cs_2CO_3	CH_3CN	60	69	157–161	$C_{27}H_{30}F_2N_4O_6$	545[d]
19a	15a	16	Cs_2CO_3	CH_3CN	80	30	57–60	$C_{29}H_{30}F_2N_4O_6 \cdot 0.5H_2O$	568
19b	16b	16	Cs_2CO_3	CH_3CN	80	7.7	160–165	$C_{27}H_{30}F_2N_4O_6$	544
24	9b	23[e]	Proton Sponge	DMA	50	68	54–57	$C_{33}H_{40}F_2N_4O_8$	659[d]

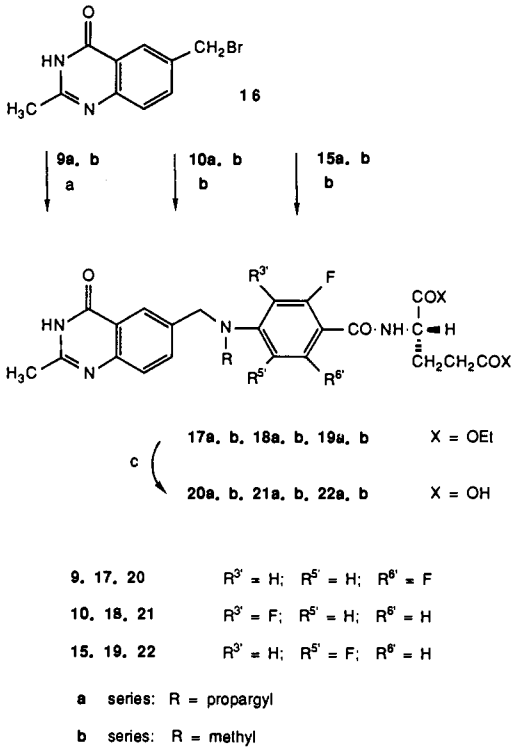
[a] Anal. C, H, N. F. [b] Reference 5. [c] Unreacted 9b (64%) was recovered. [d] $[MH]^+$, FAB. [e] Reference 9.

Scheme II[a]



[a] (a) $Na_2Cr_2O_7$, H_2SO_4, HOAc; (b) oxalyl chloride, DMF, CH_2Cl_2; (c) diethyl glutamate hydrochloride, Et_3N; (d) Fe, HOAc; (e) propargyl bromide, K_2CO_3, DMF; (f) methyl iodide, Cs_2CO_3, MeCN.

Scheme III[a]



[a] (a) Proton Sponge, DMA; (b) Cs_2CO_3, MeCN; (c) NaOH, H_2O, EtOH.

carbonyl protecting group of **7b** was removed by treatment with trifluoroacetic acid to give the intermediate **9a**.

Although methyl 4-((*tert*-butyloxycarbonyl)amino)-2,5-difluorobenzoate[6] is potentially a suitable intermediate for the synthesis of **22a** and **22b** using the same route as for its 2′,6′- and 2′,3′-difluoro analogues, the yield in its published synthesis from *N*-(*tert*-butyloxycarbonyl)-2,5-difluoroaniline was low. A different approach (Scheme II) was therefore used to make the desired intermediates **15a** and **15b**. 2,5-Difluorotoluene was nitrated para to methyl, giving a high yield of 2,5-difluoro-4-nitrotoluene (**11**). Oxidation of **11** with sodium dichromate in acetic acid gave the carboxylic acid **12** which was coupled via its acid chloride to diethyl L-glutamate to give **13**. Reduction of the nitro group using iron powder in HOAc, previously shown to occur without any accompanying defluorination,[3] gave **14**. Propargylation in the presence of K_2CO_3 proved more difficult with this weakly basic amine than in the synthesis of previous analogues[3,4] and may account for the relatively low yield of **15a**. Cs_2CO_3 which has proved superior to other alkali metal carbonates in promoting alkylation reactions[7] was therefore used in the alkylation of **14** to give **15b**.

As in the synthesis of 3′-fluoroantifolates[4] the difluorinated intermediates described here were too weakly basic for the standard coupling conditions, using $CaCO_3$, to be applied to their coupling with the (bromomethyl)-quinazoline **16**, and other bases were used (Scheme III). Thus the reaction of **9b** with 6-(bromomethyl)-3,4-dihydro-2-methyl-4-oxoquinazoline (**16**),[5] using Proton Sponge[8] as base, gave diester **17a**, which on alkaline hydrolysis yielded the dicarboxylic acid (**20a**). The N^{10}-methyl analogue (**20b**) was similarly prepared. The

(3) Jackman, A. L.; Marsham, P. R.; Thornton, T. J.; Jones, T. R.; Bishop, J. A. M.; O'Connor, B. M.; Hughes, L. R.; Calvert, A. H.; Jones, T. R. Quinazoline Antifolate Thymidylate Synthase Inhibitors: 2′-Fluoro-N^{10}-propargyl-5,8-dideazafolic Acid and Derivatives with Modifications in the C2 position. *J. Med. Chem.* **1990**, *33*, 3067–3071.

(4) Marsham, P. R.; Jackman, A. L.; Oldfield, J.; Hughes, L. R.; Thornton, T. J.; Bisset, G. M. F.; O'Connor, B. M.; Bishop, J. A. M.; Calvert, A. H. Quinazoline Antifolate Thymidylate Synthase Inhibitors: Benzoyl Ring Modifications in the C2-Methyl Series. *J. Med. Chem.* **1990**, *33*, 3072–3078.

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(6) Thornton, T. J.; Jarman, M. Metallation of *N*-(Pivaloyl)- and *N*-(*tert*-Butoxycarbonyl)difluoroanilines: Regiocontrol by Fluorine in the Synthesis of 4-Methoxycarbonyl Derivatives. *Synthesis* **1990**, 295–299.

(7) Kruizinga, W. H.; Kellogg, R. M. Preparation of Macrocyclic Lactones by Ring Closure of Cesium Carboxylates. *J. Am. Chem. Soc.* **1981**, *103*, 5183–5189.

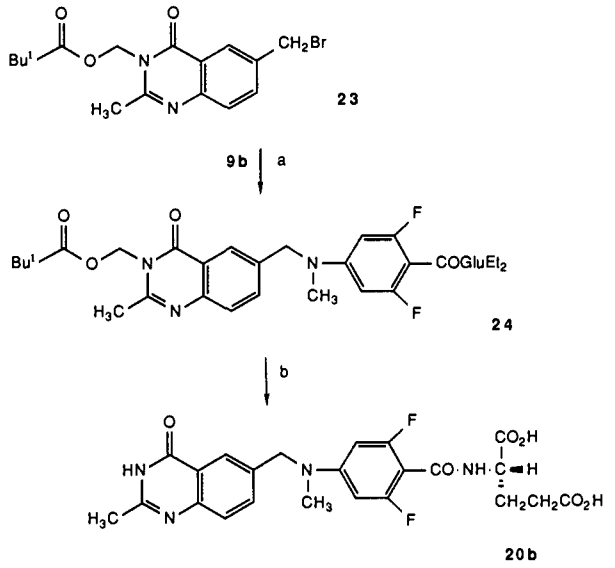
(8) 1,8-Bis(*N*,*N*-dimethylamino)naphthalene. Aldrich Chemical Co. Ltd., The Old Brickyard, New Road, Gillingham, Dorset SP8 4JL, U.K.

Table II. Preparation and in Vitro Activities of Difluorinated Antifolate Diacids 20–22

compd	R^{10}	fluorinated pattern	% yield	mp, °C	formula[a]	inhibn of TS: IC_{50}, μM	inhibn of L1210 cell growth in culture IC_{50}, μM	L1210 cell growth in the presence of thymidine (% control)
20a	$CH_2C{\equiv}CH$	2′,6′-F_2	45	198–200	$C_{25}H_{22}F_2N_4O_6{\cdot}H_2O$	0.08	0.062	93
20b[b]	CH_3	2′,6′-F_2	77	260–264	$C_{23}H_{22}F_2N_4O_6{\cdot}1.25H_2O$	0.95	0.05	90
20b[c]	CH_3	2′,6′-F_2	90	255–259	$C_{23}H_{22}F_2N_4O_6{\cdot}H_2O$			
21a	$CH_2C{\equiv}CH$	2′,3′-F_2	50	152–154	$C_{25}H_{22}F_2N_4O_6{\cdot}2H_2O$	1.7	0.28	94
21b	CH_3	2′,3′-F_2	87	150–151	$C_{23}H_{22}F_2N_4O_6{\cdot}0.75H_2O$	1.0	0.14	
22a	$CH_2C{\equiv}CH$	2′,5′-F_2	90	137–140	$C_{25}H_{22}F_2N_4O_6{\cdot}0.5H_2O$	0.60	0.027	95
22b	CH_3	2′,5′-F_2	71	157–160	$C_{23}H_{22}F_2N_4O_6{\cdot}H_2O$	1.1	0.075	94
25a[d]	$CH_2C{\equiv}CH$			165	$C_{25}H_{24}N_4O_6{\cdot}2H_2O$	0.04	0.090	100
25b[d]	CH_3			254–257	$C_{23}H_{24}N_4O_6{\cdot}0.75H_2O$	0.30	0.11	100
26a[e]	$C_2C{\equiv}CH$	2′-F		228–230	$C_{25}H_{23}FN_4O_6$	0.02	0.027	97
26b[e]	CH_3	2′-F		224–226	$C_{23}H_{23}FN_4O_6{\cdot}H_2O$	0.12	0.019	98
27a[e]	$CH_2C{\equiv}CH$	3′-F		156–160	$C_{25}H_{23}FN_4O_6{\cdot}H_2O$	1.43	0.052	102
27b[e]	CH_3	3′-F		210–211	$C_{23}H_{23}FN_4O_6{\cdot}H_2O$	1.14	0.14	92

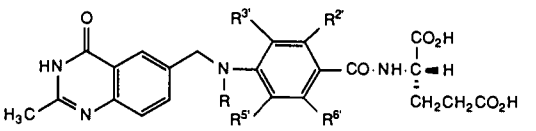
[a] Anal. C, H, N. F. [b] From 17b. [c] From 24. [d] Reference 5. [e] Reference 4.

Scheme IV[a]



[a] (a) Proton Sponge, DMA; (b) NaOH, H_2O, EtOH.

Chart I



compd	R	$R^{2'}$	$R^{3'}$	$R^{5'}$	$R^{6'}$
20a	propargyl	F	H	H	F
20b	methyl	F	H	H	F
21a	propargyl	F	F	H	H
21b	methyl	F	F	H	H
22a	propargyl	F	H	F	H
22b	methyl	F	H	F	H
25a	propargyl	H	H	H	H
25b	methyl	H	H	H	H
26a	propargyl	F	H	H	H
26b	methyl	F	H	H	H
27a	propargyl	H	F	H	H
27b	methyl	H	F	H	H

syntheses of **21a** and **21b**, starting from **2**, essentially paralleled those for **20a** and **20b**, respectively. However Cs_2CO_3 was used as base in the coupling reaction of **16** with both **10a** and **10b**. In the case of **10b**, 2 equiv of **16** gave a much improved yield of **21b**.

The coupling of **16** and **15a** did not occur in the presence of Proton Sponge but did so when Cs_2CO_3 was used. Both **19a** and **19b** were deprotected in the usual way to give **22a** and **22b**, respectively.

A large quantity (several grams) of **20b** was required for further studies (to be reported elsewhere). The need for column chromatography in the synthesis of its diethyl precursor (**17b**) coupled with its poor solubility in the applied solvent (CH_2Cl_2) hampered scale-up. To avoid this problem an N^3-((pivaloyloxy)methyl) (POM) protecting group was introduced. Alternately 6-(bromomethyl)-3,4-dihydro-2-methyl-4-oxo-3-((pivaloyloxy)methyl)quinazoline (**23**)[9] was coupled with **9b** to give **24**, a compound with enhanced solubility (Scheme IV). The deprotection step to **20b**, carried out in the usual way, occurred with simultaneous removal of the POM group.

Results and Discussion

The IC_{50} values of the new difluoroantifolates for the inhibition of partially purified L1210 TS and for growth inhibition of L1210 cells were obtained as described previously[3] and are shown in Table II. In the 2-desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic acid series the 2′,6′-difluoro derivative (**20a**) was respectively a 2-fold and 4-fold less potent inhibitor of TS (Table I) than its unsubstituted (**25a**) and 2′-fluoro-substituted (**26a**) counterparts. Compound **20a** had similar growth inhibition to **25a** toward L1210 cells although it was ~2-fold less potent than **26a**. The 2′,3′- and 2′,5′-substituted compounds (**21a** and **22a**) were respectively 85-fold and 30-fold less potent as TS inhibitors than **26a**. In contrast **21a** was only ~10-fold less cytotoxic than **26a**, while **22a** was equipotent. It therefore appears that the introduction of a second fluorine substituent into **26a** is only mildly detrimental to TS inhibition or L1210 cytotoxicity if located ortho to the carbonyl group. If the second fluorine substituent is located ortho to N^{10} as in **21a** or **22a**, these activities are similar to those of the monosubstituted 3′-fluoro derivative (**27a**) although the presence of two fluorine atoms on the same side of the ring (i.e. **21a**) is particularly detrimental to cytotoxicity. For these 3′-(5′-)fluorinated derivatives TS inhibition is poorer, yet L1210 cytotoxicity, generally speaking, is maintained relative to unsubstituted counterparts.

In the N^{10}-methyl series the pattern for TS inhibition and L1210 cytotoxicity is similar to that observed in the N^{10}-propargyl series. However, the loss in TS inhibition caused by additional 3′- (**21b**) or 5′-substitution (**22b**) is

(9) Hughes, L. R. Eur. Pat. Appl. 239 362 A2, 1987.

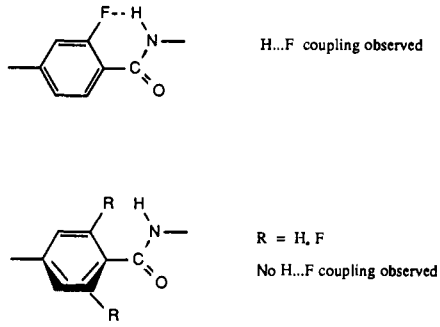


Figure 1. Proposed stereochemistry of the benzoyl ring moiety for 2′-fluoro- and 2′,6′-difluoro-substituted and unsubstituted N^{10}-propargyl-5,8-dideazafolic acids.

much more modest, this being only about 5-fold worse in each case than for the unsubstituted compound (**25b**) and 10-fold worse than for the 2′-fluoro-substituted compound (**26b**). Such a relatively smaller loss in inhibitory activity for N^{10}-methyl as opposed to N^{10}-propargyl derivatives has also previously been noted for the pairs of compounds **26b**, **27b** and **26a**, **27a**.[4] It would appear that an electron-withdrawing fluorine atom ortho to N^{10} has a more profound effect on a propargyl than on a methyl group on this nitrogen atom. This effect was also observed in the earlier 3′-monofluoro analogues[4] and can be rationalized in the light of recent X-ray crystallographic studies[10,11] of the ternary complex of CB3717,[2] 5-fluoro-2′-deoxyuridylate, and *Escherichia coli* TS (which has considerable homology with murine and human TS in the active site region). These studies indicate that the propargyl group fits into a specific solvent-lined pocket, thus suggesting a reason for the enhanced binding of the N^{10}-propargyl compound compared to its N^{10}-methyl analogue. The presence of an ortho ring fluorine atom appears to distort this region of the enzyme–inhibitor complex, possibly by acting as an additional hydrogen bond acceptor, to such an extent that this specific binding of an N^{10}-propargyl group no longer applies.

It is also of interest to consider why 2′-fluoro substitution is beneficial whereas 2′,6′-difluoro substitution is slightly detrimental to TS inhibitory activity. A possible insight into the effects of placing fluorine ortho to the amide substituent comes from examining the amide hydrogen signal in the ^{1}H-NMR spectra of the variously fluorinated quinazoline antifolates (see the supplementary material). The NMR spectrum of compounds unsubstituted in the benzene ring (e.g. **25a,b**) shows the amide proton as a doublet with a coupling constant of about 7.6 Hz. In the presence of a single 2′-fluoro substituent the signal is now an apparent triplet due to a coupling (J = 6.4 Hz)[3] between the amide hydrogen atom and 2′-fluorine. A similar phenomenon is noticed here with the 2′,3′- (e.g. **21b**) and 2′,5′-substituted (e.g. **22a**) analogues with smaller NH–F coupling constants revealing the signals as double doublets, but is absent from the NMR spectrum of compounds substituted 2′,6′-difluoro and 3′-fluoro. It has been proposed[3] that there is an interaction between the 2′-fluorine and amide hydrogen atoms which holds this part of the molecule in a favorable conformation (in which the amide and phenyl ring are almost coplanar) for binding to TS. The benzoyl ring-unsubstituted compounds would also tend to favor the coplanar arrangement to maximize π overlap between the carbonyl and phenyl groups. However, a second ortho fluorine substituent in the 6′-position (**20a,b**) would interact repulsively with the carbonyl oxygen and tend to disfavor such a coplanar arrangement (see Figure 1). This would explain the observed lack of coupling between fluorine and amide hydrogen in these derivatives and also why 2′,6′-difluoro substitution is slightly detrimental to TS inhibitory activity.

The maintenance or improvement in cytotoxicity of most of the difluorinated and 3′-fluoro analogues does not relate to a change in the cytotoxic locus of action despite the fact that they are poorer inhibitors of TS than the parent compounds because the cytotoxicity of all these compounds is prevented by the coadministration of 10 μM thymidine at 10 × IC_{50} values. However, an increase in intracellular polyglutamate formation could account for the cytotoxicities seen either by an improvement in membrane transport or by substrate activity for glypolyglutamate synthetase.[12,13]

Experimental Section

General procedures are as given in ref 14 except as follows. Flash chromatography was carried out on Merck Kieselgel 60 (Art. 9385) unless otherwise stated. Petroleum ether refers to the fraction of bp 60–80 °C. Some of the NMR spectra were determined using a Bruker AM200 (200 MHz) spectrometer and some elemental analyses were determined at ICI Pharmaceuticals. Fast atom bombardment (FAB) mass spectra were determined on a VG MS9 spectrometer with Finnigan Incos data system using DMSO as solvent in a glycerol matrix. All mass spectra are EI unless otherwise denoted.

Methyl 4-(*N*-(*tert*-Butyloxycarbonyl)-*N*-prop-2-ynylamino)-2,6-difluorobenzoate (3a). Sodium hydride (184 mg of 55% suspension in oil, 4.2 mmol) was washed with hexane and suspended in dry DMF (8 mL) which was stirred in an argon atmosphere. Compound 1 (1.15 g, 4 mmol) was added. Once effervescence had subsided, propargyl bromide (0.49 mL, 4.4 mmol) was added slowly by syringe. The brown solution was allowed to stir for a further 2 h, diluted with EtOAc (100 mL), and extracted with H_2O (2 × 200 mL). The organic layer was dried and concentrated to give a brown oil (1.285 g, 99%). Chromatography by eluting with 2:1 CH_2Cl_2–petroleum ether gave pure **3a** as a white crystalline solid: mp 58–60 °C; NMR (90 MHz, $CHCl_3$) δ 1.49 (s, 9 H, t-Bu), 3.27 (m, 1 H, CH), 3.92 (s, 3 H, CO_2Me), 4.54 (d, 2 H, J = 2.3 Hz, CH_2), 7.28 (d, 2 H, $J_{H,F}$ = 10.7 Hz, H^3, H^5); MS (CI) m/z 326 $[MH]^+$. Anal. ($C_{15}H_{17}F_2NO_6$) C, H, N, F.

The experimental procedures for the synthesis of **3b**, **4a**, and **4b** were the same as for **3a** except that with **3b** and **4b** MeI was used instead of propargyl bromide.

Methyl 4-(*N*-(*tert*-Butyloxycarbonyl)-*N*-methylamino)-2,6-difluorobenzoate (3b). The compound was an oil (100%) which later solidified: mp 52–53 °C; NMR (250 MHz,

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$CDCl_3$) δ 1.51 (s, 9 H, t-Bu), 3.28 (s, 3 H, NCH_3), 3.94 (s, 3 H, OCH_3), 6.98 (dd, 2 H, $J_{H,F}$ = 11.8 Hz, H^3, H^5); MS *m/z* 301 $[M]^+$. Anal. ($C_{14}H_{17}F_2NO_4$) C, H, N, F.

Methyl 4-(*N*-(*tert*-Butyloxycarbonyl)-*N*-prop-2-ynylamino)-2,3-difluorobenzoate (4a). The compound was a waxy solid (99%): mp 47–48 °C; NMR (250 MHz, $CDCl_3$) δ 1.44 (s, 9 H, t-Bu), 2.25 (t, 1 H, *J* = 2.5 Hz, propargyl CH), 3.96 (s, 3 H, OCH_3), 4.40 (d, 2 H, *J* = 2.5 Hz, propargyl CH_2), 7.23 (ddd, 1 H, $J_{5,6}$ = 8.8 Hz, $J_{5,F-3}$ = 6.5 Hz, $J_{5,F-2}$ = 2.0 Hz, H^5), 7.72 (ddd, 1 H, $J_{6,5}$ = 8.8 Hz, $J_{6,F-2}$ = 7.2 Hz, J_{6F-3} = 2.2 Hz, H^6); MS (CI) *m/z* 326 $[MH]^+$. Anal. ($C_{16}H_{17}F_2NO_4$) C, H, N, F.

Methyl 4-(*N*-(*tert*-Butyloxycarbonyl)-*N*-methylamino)-2,3-difluorobenzoate (4b). The compound was an oil (98%): NMR (250 MHz, $CDCl_3$) δ 1.43 (s, 9 H, t-Bu), 3.24 (s, 3 H, NCH_3), 3.95 (s, 3 H, OCH_3), 7.07 (ddd, 1 H, $J_{5,F-2}$ = 1.9 Hz, $J_{5,F-3}$ = 6.5 Hz, $J_{5,6}$ = 8.8 Hz, H^5), 7.69 (ddd, 1 H, $J_{6,F-3}$ = 2.2 Hz, $J_{6,F-2}$ = 7.2 Hz, $J_{6,5}$ = 8.8 Hz, H^6); MS *m/z* 301 $[M]^+$. Anal. ($C_{14}H_{17}F_2NO_4$) C, H, N; F: calcd, 12.61; found, 12.16.

4-(*N*-(*tert*-Butyloxycarbonyl)-*N*-prop-2-ynylamino)-2,6-difluorobenzoic Acid (5a). A mixture of **3b** (976 mg, 3 mmol), 1 N aqueous NaOH (6 mL), EtOH (15 mL), and H_2O (15 mL) was vigorously stirred for 18 h. After filtration through Celite, the pale yellow solution was concentrated to an oil. To this oil was added H_2O (15 mL) and enough 1 N HCl to bring the solution to pH 5. The product (964 mg) which precipitated as a pale brown solid was filtered off and washed with H_2O. Recrystallization from toluene/hexane gave white crystals (0.844 g, 91%): mp 138–141 °C; NMR (90 MHz, $CDCl_3$) δ 1.56 (s, 9 H, t-Bu), 2.36 (t, 1 H, *J* = 2.6 Hz, CH), 4.45 (d, 2 H, *J* = 2.6 Hz, CH_2), 7.12 (d, 2 H, $J_{H,F}$ = 10.4 Hz, H^3, H^5), 7.73 (br s, 1 H, CO_2H); MS *m/z* 312 $[MH]^+$. Anal. ($C_{15}H_{15}F_2NO_4$) C, H, N, F.

The experimental procedures for **5b**, **6a**, and **6b** were the same as for **5a**.

4-(*N*-(*tert*-Butyloxycarbonyl)-*N*-methylamino)-2,6-difluorobenzoic Acid (5b). The compound was obtained as white crystals (81%): mp 123.5–124.5 °C; MS (CI) *m/z* 288 $[MH]^+$; NMR (250 MHz, $CDCl_3$) δ 1.52 (s, 9 H, t-Bu), 3.30 (s, 3 H, CH_3), 7.03 (d, 2 H, $J_{H,F}$ = 10.8 Hz, H^3, H^5). Anal. ($C_{13}H_{15}F_2NO_4$) C, H, N, F.

4-(*N*-(*tert*-Butyloxycarbonyl)-*N*-prop-2-ynylamino)-2,3-difluorobenzoic Acid (6a). The compound was obtained as white crystals (90%): mp 130–132 °C; NMR (250 MHz, $CDCl_3$) δ 1.45 (s, 9 H, t-Bu), 2.26 (t, 1 H, *J* = 2.4 Hz, propargyl CH), 4.42 (d, 2 H, *J* = 2.4 Hz, propargyl CH_2), 7.27 (ddd, 1 H, $J_{5,6}$ = 8.8 Hz, $J_{5,F-3}$ = 6.5 Hz, $J_{5,F-2}$ = 1.9 Hz, H^5), 7.79 (ddd, 1 H, $J_{6,5}$ = 8.8 Hz, $J_{6,F-2}$ = 7.2 Hz, $J_{6,F-3}$ = 2.2 Hz, H^6); MS (CI) *m/z* 312 $[MH]^+$. Anal. ($C_{15}H_{15}F_2NO_4$) C, H, N, F.

4-(*N*-(*tert*-Butyloxycarbonyl)-*N*-methylamino)-2,3-difluorobenzoic Acid (6b). The compound was obtained as white crystals (88%): mp 155–157 °C; NMR (250 MHz, $CDCl_3$) δ 1.45 (s, 9 H, t-Bu), 3.26 (s, 3 H, NCH_3), 7.11 (dt, 1 H, $J_{5,F-2}$ = 2.0 Hz, $J_{5,F-3}$ and $J_{5,6}$ = 6.8 Hz, H^5), 7.76 (dt, 1 H, $J_{6,F-3}$ = 2.0 Hz, $J_{6,F-2}$ and $J_{6,5}$ = 6.8 Hz, H^6); MS *m/z* 288 $[MH]^+$. Anal. ($C_{13}H_{15}F_2NO_4$) C, H, N, F.

Diethyl *N*-(4-(*N*-(*tert*-Butyloxycarbonyl)-*N*-prop-2-ynylamino)-2,6-difluorobenzoyl)-L-glutamate (7a). To a stirred solution of **5a** (187 mg, 0.6 mmol) in dry CH_2Cl_2 (10 mL) was added oxalyl chloride (0.08 mL, 0.9 mmol) and DMF (a few drops) in an argon atmosphere. After 1 h the solution was concentrated to give a pale brown solid which was dissolved in CH_2Cl_2 (10 mL). This was added by syringe to a stirred solution of Et_3N (0.25 mL, 1.8 mmol) and diethyl glutamate hydrochloride (158 mg, 0.66 mmol) in CH_2Cl_2 (10 mL) in the absence of moisture and the temperature was maintained below 20 °C. The solution was stirred for a further 2 h and then washed with H_2O (100 mL), and the organic layer was dried and concentrated to give **7a** as an oil (298 mg, 100%): NMR (200 MHz, $CDCl_3$) δ 1.26 (t, 3 H, *J* = 7.1 Hz, CH_3), 1.33 (t, 3 H, *J* = 7.1 Hz, CH_3), 1.52 (s, 9 H, t-Bu), 2.13 (m, 2 H, Glu CH_2^β), 2.32 (t, 1 H, *J* = 2.5 Hz, propargyl CH), 2.44 (m, 2 H Glu CH_2^γ), 4.14 (q, 2 H, *J* = 7.1 Hz, ester CH_2), 4.25 (q, 2 H, *J* = 7.1 Hz, ester CH_2), 4.39 (d, 2 H, *J* = 2.5 Hz, propargyl CH_2), 4.84 (m, 1 H, CH^α), 6.72 (d, 1 H, *J* = 7.7 Hz, NH), 7.05 (d, 2 H, $J_{H,F}$ = 9.4 Hz, H^3 and H^5); MS (FAB) *m/z* 497 $[MH]^+$. Anal. ($C_{24}H_{30}F_2N_2O_7$) C, H, N, F.

The experimental procedures for **7b**, **8a**, and **8b** were as for **7a**.

Diethyl *N*-(4-(*N*-(*tert*-Butyloxycarbonyl)-*N*-methylamino)-2,6-difluorobenzoyl)-L-glutamate (7b). The compound was an oil (99%): NMR (250 MHz, $CDCl_3$) δ 1.26 (t, 3 H, *J* = 7.1 Hz, ester CH_3), 1.31 (t, 3 H, *J* = 7.1 Hz, ester CH_3), 1.51 (s, 9 H, t-Bu), 2.25 (m, 2 H, Glu CH_2^β), 2.45 (m, 2 H, Glu CH_2^γ), 3.27 (s, 3 H, NCH_3), 4.13 (q, 2 H, *J* = 7.1 Hz, ester CH_2), 4.25 (q, 2 H, *J* = 7.1 Hz, ester CH_2), 4.83 (m, 1 H, Glu CH^α), 6.75 (br d, 1 H, *J* = 7.5 Hz, amide NH), 6.97 (dd, 2 H, $J_{H,F}$ = 9.9 Hz, H^3, H^5); MS (FAB) *m/z* 473 $[MH]^+$. Anal. ($C_{22}H_{30}F_2N_2O_7$) C, H, N, F.

Diethyl *N*-(4-(*N*-(*tert*-Butyloxycarbonyl)-*N*-prop-2-ynylamino)-2,3-difluorobenzoyl)-L-glutamate (8a). The compound was an oil (89%): NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3 H, *J* = 7.1 Hz, ester CH_3), 1.32 (t, 3 H, *J* = 7.1 Hz, ester CH_3), 1.44 (s, 9 H, t-Bu), 2.24 (t, 1 H, *J* = 2.1 Hz, propargyl CH), 2.27 (m, 2 H, Glu CH_2^β), 2.44 (m, 2 H, Glu CH_2^γ), 4.13 (q, 2 H, *J* = 7.1 Hz, ester CH_2), 4.26 (q, 2 H, *J* = 7.1 Hz, ester CH_2), 4.40 (d, 2 H, *J* = 2.1 Hz, propargyl CH_2), 4.85 (m, 1 H, Glu CH^α), 7.29 (m, 2 H, H^5, NH), 7.79 (ddd, 1 H, $J_{6,5}$ = 9.0 Hz, $J_{6,F-2}$ = 7.2 Hz, $J_{6,F-3}$ = 2.2 Hz, H^6); MS *m/z* 496 $[M]^+$. Anal. ($C_{24}H_{30}F_2N_2O_7$) C, H, N, F.

Diethyl *N*-(4-(*N*-(*tert*-Butyloxycarbonyl)-*N*-methylamino)-2,3-difluorobenzoyl)-L-glutamate (8b). The compound was an oil (53%): NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3 H, *J* = 7.1 Hz, ester CH_3), 1.32 (t, 3 H, *J* = 7.1 Hz, ester CH_3), 1.43 (s, 9 H, t-Bu), 2.26 (m, 2 H, Glu CH_2^β), 2.43 (m, 2 H, Glu CH_2^γ), 3.23 (s, 3 H, NH_3), 4.13 (q, 2 H, *J* = 7.1 Hz, ester CH_2), 4.25 (q, 2 H, *J* = 7.1 Hz, ester CH_2), 4.84 (m, 1 H, Glu CH^α), 7.11 (ddd, 1 H, $J_{5,F-2}$ = 1.8 Hz, $J_{5,F-3}$ = 7.7 Hz, $J_{5,6}$ = 8.6 Hz, H^5), 7.28 (dd, 1 H, $J_{NH,F-2}$ = 8.9 Hz, *J* = 7.6 Hz, amide NH), 7.77 (dt, 1 H, $J_{6,F-3}$ = 2.2 Hz, $J_{6,F-2}$ = 8.6 Hz, $J_{6,5}$ = 8.6 Hz, H^6); MS *m/z* 473 $[MH]^+$. Anal. ($C_{22}H_{30}F_2N_2O_7$) C, H, N, F.

Diethyl *N*-(2,6-Difluoro-4-(prop-2-ynylamino)benzoyl)-L-glutamate (9a). A mixture of **7a** (745 mg, 1.5 mmol) and CF_3CO_2H (7.2 mL) was stirred at 0 °C for 30 min. The solution was concentrated under vacuum to give a white crystalline solid (588 mg, 98%). Chromatography eluting with 5:1 CH_2Cl_2–EtOAc gave pure **9a**: mp 104–106 °C; NMR (200 MHz, $CDCl_3$) δ 1.25 (t, 3 H, *J* = 7.1 Hz, CH_3), 1.32 (t, 3 H, *J* = 7.1 Hz, CH_3), 2.09 (m, 2 H, CH_2^β), 2.28 (t, 1 H, *J* = 2.6 Hz, propargyl CH), 2.43 (m, 2 H, Glu CH_2^γ), 3.93 (d, 2 H, *J* = 2.6 Hz, propargyl CH_2), 4.13 (q, 2 H, *J* = 7.1 Hz, ester CH_2), 4.23 (q, 2 H, *J* = 7.1 Hz, ester CH_2), 4.83 (m, 1 H, CH^α), 6.20 (d, 2 H, $J_{H,F}$ = 11.0 Hz, H^3, H^5); 6.67 (br d, 1 H, *J* = 14.7 Hz, NH); MS *m/z* 396 $[M]^+$. Anal. ($C_{19}H_{22}F_2N_2O_5$) C, H, N, F.

The experimental procedures for **9b**, **10a**, and **10b** were as for **9a**.

Diethyl *N*-(2,6-Difluoro-4-(methylamino)benzoyl)-L-glutamate (9b). The compound gave white crystals (99%): mp 93–95 °C (from toluene); NMR (250 MHz, $CDCl_3$) δ 1.25 (t, 3 H, *J* = 7.1 Hz, ester CH_3), 1.30 (t, 3 H, *J* = 7.1 Hz, ester CH_3), 2.21 (m, 2 H, Glu CH_2^β), 2.43 (m, 2 H, Glu CH_2^γ), 2.84 (d, 3 H, *J* = 5.2 Hz, NCH_3), 4.12 (q, 2 H, *J* = 7.1 Hz, ester CH_2), 4.23 (q, 2 H, *J* = 7.1 Hz, ester CH_2), 4.24 (br, 1 H, amine NH), 4.82 (m, 1 H, Glu CH^α), 6.10 (d, 2 H, $J_{H,F}$ = 11.5 Hz, H^3, H^5), 6.67 (br, 1 H, amide NH); MS *m/z* 372 $[M]^+$. Anal. ($C_{17}H_{22}F_2N_2O_5$) C, H, N, F.

Diethyl *N*-(2,3-Difluoro-4-(prop-2-ynylamino)benzoyl)-L-glutamate (10a). The compound was a pale golden oil (98%) which solidified: mp 87–88 °C; NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3 H, *J* = 7.1 Hz, ester CH_3), 1.31 (t, 3 H, *J* = 7.1 Hz, ester CH_3) 2.23 (m, 2 H, Glu CH_2^β), 2.28 (t, 1 H, *J* = 2.3 Hz, propargyl CH), 2.41 (m, 2 H, Glu CH_2^γ), 4.04 (d, 2 H, *J* = 2.3 Hz, propargyl CH_2), 4.12 (q, 2 H, *J* = 7.1 Hz, ester CH_2), 4.24 (q, 2 H, *J* = 7.1 Hz, ester CH_2), 4.58 (bs, 1 H, amine NH), 4.83 (m, 1 H, Glu CH^α), 6.63 (dt, 1 H, $J_{5,F-3}$ and $J_{5,6}$ = 8.3 Hz, $J_{5,F-2}$ = 1.5 Hz, H^5), 7.14 (bdd, 1 H, $J_{NH,F-2}$ = 11.7 Hz, *J* = 7.6 Hz, amide NH), 7.76 (dt, 1 H, $J_{6,F-2}$ and $J_{6,5}$ = 8.3 Hz, $J_{6,F-3}$ = 2.1 Hz, H^6); MS *m/z* 396 $[M]^+$. Anal. ($C_{19}H_{22}F_2N_2O_5$), C, H, N, F.

Diethyl *N*-(2,3-Difluoro-4-(methylamino)benzoyl)-L-glutamate (10b). The compound was an oil (96%): NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3 H, *J* = 7.1 Hz, ester CH_3), 1.31 (t, 3 H, *J* = 7.1 Hz, ester CH_3) 2.23 (m, 2 H, Glu CH_2^β), 2.43 (m, 2 H, Glu CH_2^γ), 2.94 (d, 3 H, *J* = 5.1 Hz, NCH_3), 4.11 (q, 2 H, *J* = 7.1 Hz, ester CH_2), 4.24 (q, 2 H, *J* = 7.1 Hz, ester CH_2), 4.40 (br, 1 H, amine NH), 4.83 (m, 1 H, Glu CH^α), 6.49 (ddd, 1 H, $J_{5,F-2}$ = 1.3 Hz, $J_{5,F-3}$ = 7.5 Hz, $J_{5,6}$ = 8.6 Hz, H^5), 7.11 (dd, 1 H, $J_{NH,F-2}$ = 12.2

Hz, J = 7.6 Hz, amide NH), 7.74 (ddd, 1 H, $J_{6,F-3}$ = 2.1 Hz, $J_{6,F-2}$ = 10.4 Hz, $J_{6,5}$ = 8.6 Hz, H^6); MS m/z 373 (CI) [MH]$^+$. Anal. ($C_{17}H_{22}F_2N_2O_5$) C, H, N, F.

2,5-Difluoro-4-nitrotoluene (11). 2,5-Difluorotoluene (12.8 g, 0.1 mol) was added dropwise to stirred fuming nitric acid (50 mL), keeping the temperature between 0 and 10 °C. After 30 min, the solution was poured onto ice (100 g), the precipitate was extracted with CH_2Cl_2 (200 mL), and the extracts were washed with H_2O (2 × 250 mL), dried, decolorized (charcoal), and concentrated to give 11 as a golden oil (13.42 g, 78%): NMR (250 MHz, $CDCl_3$) δ 2.38 (t, 3 H, $J_{Me-H,F-2}$ = 2.0 Hz, $J_{6,Me-H}$ = 2.0 Hz, CH_3), 7.15 (dd, 1 H, $J_{6,F-5}$ = 10.9 Hz, $J_{6,F-2}$ = 6.0 Hz, H^6), 7.77 (dd, 1 H, $J_{3,F-2}$ = 8.5 Hz, $J_{3,F-5}$ = 6.2 Hz, H^3); MS m/z 173 [M]$^+$. Anal. ($C_7H_5F_2NO_2$) C, H, N, F.

2,5-Difluoro-4-nitrobenzoic Acid (12). To a solution of sodium dichromate (40.23 g, 0.13 mol) in AcOH (200 mL) was added 11 (17.31 g, 0.1 mol) and then concentrated H_2SO_4 (100 g), during 5 min. The dark solution became hot, and manual stirring was needed to break up the chromic oxide which rapidly formed. The dark green slurry was allowed to cool to room temperature, H_2O (800 mL) was added, and the product was extracted with EtOAc (2 × 500 mL). The combined organic extracts were washed with H_2O (400 mL), dried, and concentrated to give 12 (17.20 g, 85%): mp 147–148 °C (from EtOH–H_2O); NMR (250 MHz, acetone-d_6) δ 8.01 (dd, 1 H, $J_{6,F-5}$ = 10.8 Hz, $J_{6,F-2}$ = 5.7 Hz, H^6), 8.13 (dd, 1 H, $J_{3,F-2}$ = 9.4 Hz, $J_{3,F-5}$ = 5.9 Hz, H^3); MS m/z 203 [M]$^+$. Anal. ($C_7H_3F_2NO_4$) C, H, N, F.

Diethyl *N*-(2,5-Difluoro-4-nitrobenzoyl)-L-glutamate (13). To a solution of 12 (20.31 g, 0.1 mol) in CH_2Cl_2 was added DMF (a few drops) and oxalyl chloride (13.09 mL, 19.0 g, 0.15 mol). The mixture was stirred for 2.5 h in the absence of moisture and then concentrated to dryness. A solution of this acid chloride in CH_2Cl_2 (150 mL) was added during 15 min to a solution of diethyl L-glutamate hydrochloride (23.97 g, 0.1 mol) and Et_3N (29.27 mL, 0.1 mol) in CH_2Cl_2 (400 mL) with stirring in the absence of moisture while the temperature was maintained below 20 °C. After a further 1 h, the solution was extracted with H_2O (2 × 500 mL) and the organic phase concentrated to give 13 (38.6 g, 99.5%): mp 83 °C (from toluene–cyclohexane); NMR (250 MHz, $CDCl_3$) δ 1.25 (t, 3 H, J = 7.1 Hz, CH_3), 1.33 (t, 3 H, J = 7.1 Hz, CH_3), 2.26 (m, 2 H, Glu CH_2^β), 2.36 (m, 2 H, Glu CH_2^γ), 4.13 (q, 2 H, J = 7.1 Hz, ester CH_2), 4.27 (q, 2 H, J = 7.1 Hz, ester CH_2), 4.82 (m, 1 H, Glu CH^α), 7.56 (t, 1 H, J = 8.9 Hz, $J_{NH,F-2}$ = 8.9 Hz, NH), 7.91 (dd, 1 H, $J_{3,F-5}$ = 5.5 Hz, $J_{3,F-2}$ = 9.9 Hz, H^3), 8.03 (dd, 1 H, $J_{6,F-2}$ = 5.9 Hz, $J_{6,F-5}$ = 10.7 Hz, H^6); MS (CI) m/z 389 [MH]$^+$. Anal. ($C_{16}H_{18}F_2N_2O_7$) C, H, N, F.

Diethyl *N*-(4-Amino-2,5-Difluorobenzoyl)-L-glutamate (14). A mixture of 13 (9.71 g, 0.025 mol), iron powder (325 mesh, 6.14 g, 0.11 mol), and AcOH (50 mL) was stirred together for 2 h at 50 °C and cooled to 20 °C. CH_2Cl_2 (250 mL) and H_2O (500 mL) were added, and the mixture was filtered through Celite. The organic layer was washed with H_2O (500 mL), dried, and concentrated to afford 14 (7.63 g, 85%) as white crystals: mp 79–80 °C (from toluene–hexane); NMR (250 MHz, $CDCl_3$) δ 1.23 (t, 3 H, J = 7.1 Hz, CH_3), 1.30 (t, 3 H, J = 7.1 Hz, CH_3), 2.22 (m, 2 H, Glu CH_2^β), 2.41 (m, 2 H, Glu CH_2^γ), 4.11 (q, 2 H, J = 7.1 Hz, ester CH_2), 4.22 (br s, 2 H, NH_2), 4.23 (q, 2 H, J = 7.1 Hz, ester CH_2), 4.81 (m, 1 H, CH^α), 6.47 (dd, 1 H, $J_{3,F-5}$ = 7.0 Hz, $J_{3,F-2}$ = 12.9 Hz, H^3), 7.25 (dd, 1 H, J = 8.7 Hz, $J_{NH,F-2}$ = 12.0 Hz, NH), 7.69 (dd, 1 H, $J_{6,F-2}$ = 6.7 Hz, $J_{6,F-5}$ = 11.7 Hz, H^6); MS m/z 358 [M]$^+$. Anal. ($C_{16}H_{20}F_2N_2O_5$) C, H, N, F.

Diethyl *N*-(2,5-Difluoro-4-(prop-2-ynylamino)benzoyl)-L-glutamate (15a). To a solution of 14 (7.17 g, 0.02 mol) in DMF (180 mL) was added K_2CO_3 (2.76 g, 0.02 mol) and propargyl bromide (80% w/v solution in toluene, 4.46 mL, 0.04 mol). The mixture was stirred vigorously at 100 °C for 5 h and then concentrated, and the residue was partitioned between EtOAc (150 mL) and H_2O (2 × 250 mL). The dried organic phase was concentrated, and a solution in 1.5:1 petroleum ether–EtOAc was applied to a column of silica gel (Merck Art. 15111) which was eluted with the same solvent mixture. Concentration of the appropriate fractions yielded 15a as a pale orange oil (2.71 g, 34%): NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3 H, J = 7.1 Hz, CH_3), 1.31 (t, 3 H, J = 7.1 Hz, CH_3), 2.24 (m, 2 H, Glu CH_2^β), 2.30 (m, 1 H, propargyl CH), 2.41 (m, 2 H, Glu CH_2^γ), 4.02 (m, 2 H, propargyl CH_2), 4.12 (q, 2 H, J = 7.1 Hz, ester CH_2), 4.23 (q, 2 H, J = 7.1 Hz, ester CH_2), 4.65 (br s, 1 H, amine NH), 4.82 (m, 1 H, Glu CH^α), 6.49 (dd, 1 H, $J_{3,F-5}$ = 8.9 Hz, $J_{3,F-2}$ = 13.5 Hz, H^3), 7.27 (m, 1 H, amide NH), 7.70 (dd, 1 H, $J_{6,F-2}$ = 6.9 Hz, $J_{6,F-5}$ = 12.1 Hz, H^6); MS m/z 306 [M]$^+$. Anal. ($C_{16}H_{20}F_2N_2O_5$) C, H, N, F.

Diethyl *N*-(2,5-Difluoro-4-(methylamino)benzoyl)-L-glutamate (15b). A mixture of 14 (3.58 g, 10 mmol), Cs_2CO_3 (3.26 g, 10 mmol), MeI (4.96 mL, 80 mmol), and CH_3CN (100 mL) was heated to 80 °C for 48 h and then filtered when cool. The filtrate was concentrated, and a solution of the residual oil in CH_2Cl_2 was chromatographed using 1.5:1 petroleum ether–EtOAc as eluant, to give 15b (1.77 g, 48%): mp 83 °C (from toluene–hexane); NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3 H, J = 7.1 Hz, ester CH_3), 1.31 (t, 3 H, J = 7.1 Hz, ester CH_3), 2.24 (m, 2 H, Glu CH_2^β), 2.43 (m, 2 H, Glu CH_2^γ), 2.92 (d, 3 H, J = 5.0 Hz, NCH_3), 4.12 (q, 2 H, J = 7.1 Hz, ester CH_2), 4.25 (q, 2 H, J = 7.1 Hz, ester CH_2), 4.48 (br, 1 H, amine NH), 4.83 (m, 1 H, Glu CH^α), 6.32 (dd, 1 H, $J_{3,F-2}$ = 13.7 Hz, $J_{3,F-5}$ = 6.9 Hz, H^3), 7.24 (m, 1 H, amide NH), 7.66 (dd, 1 H, $J_{6,F-5}$ = 12.4 Hz, $J_{6,F-2}$ = 7.0 Hz, H^6); MS m/z 372 [M]$^+$. Anal. ($C_{17}H_{22}F_2N_2O_5$) C, H, N, F.

Diethyl *N*-(2,6-Difluoro-4-(*N*-((3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl)prop-2-ynylamino)benzoyl)-L-glutamate (17a). A mixture of 9a (317 mg, 0.8 mmol), 6-(bromomethyl)-3,4-dihydro-2-methyl-4-oxoquinazoline[8] (16; 202 mg, 0.8 mmol), Proton Sponge (171 mg, 0.8 mmol), and dry DMF (2.5 mL) was stirred at 50 °C for 24 h. The brown slurry was partitioned between EtOAc (200 mL) and H_2O (2 × 200 mL), and the EtOAc solution was dried and evaporated. A solution of the crude product in CH_2Cl_2 was chromatographed using 93:7 CH_2Cl_2–EtOH as eluant to give 17a as a white amorphous solid: 386 mg (85%); mp 120–124 °C; MS m/z 568 [M]$^+$, (CI) m/z 569 [MH]$^+$. Anal. ($C_{29}H_{30}F_2N_4O_6$) C, H, N, F.

The experimental procedures for the other quinazoline antifolate esters were the same as for 17a, and details are given in Table I. The following esters were synthesized: **diethyl *N*-(2,6-difluoro-4-(*N*-((3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl)methylamino)benzoyl)-L-glutamate (17b), diethyl *N*-(2,3-difluoro-4-(*N*-((3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl)prop-2-ynylamino)benzoyl)-L-glutamate (18a), diethyl *N*-(2,3-difluoro-4-(*N*-((3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl)methylamino)benzoyl)-L-glutamate (18b), diethyl *N*-(2,5-difluoro-4-(*N*-((3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl)prop-2-ynylamino)benzoyl)-L-glutamate (19a), diethyl *N*-(2,5-difluoro-4-(*N*-((3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl)methylamino)benzoyl)-L-glutamate (19b), and diethyl *N*-(2,6-difluoro-4-(*N*-((3,4-dihydro-2-methyl-4-oxo-3-((pivaloyloxy)methyl)-6-quinazolinyl)methyl)methylamino)benzoyl)-L-glutamate (24).**

All diesters had NMR spectra consistent with the assigned structures.

***N*-(2,6-Difluoro-4-(*N*-((3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl)prop-2-ynylamino)benzoyl)-L-glutamic Acid (20a).** To a solution of the diester (17a; 386 mg, 0.679 mmol) in EtOH (5 mL) and H_2O (11.5 mL) was added 1 N NaOH (2.44 mL). The mixture was stirred for 5 h at room temperature and then filtered through a bed of Celite. The filtrate was acidified to pH 3.5 with 2 N HCl, and the resulting white precipitate was centrifuged at 2500 rpm/30 min and washed with 3 cycles of resuspension (H_2O, 30 mL)–centrifugation–decantation. After drying over P_2O_5 under vacuum, first at room temperature for 18 h and then at 64 °C for 3 h, 20a was obtained as a white amorphous solid: 163 mg, (45%); mp 198–200 °C; MS (FAB) m/z 511 [M − H]$^-$. Anal. ($C_{25}H_{22}F_2N_4O_6 \cdot H_2O$) C, H, N, F.

The experimental procedures for the other quinazoline antifolate acids are the same as for 20a, and details can be found in Table II. The following esters were synthesized: ***N*-(2,6-difluoro-4-(*N*-((3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl)methylamino)benzoyl)-L-glutamic acid (20b), *N*-(2,3-difluoro-4-(*N*-((3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl)prop-2-ynylamino)benzoyl)-L-glutamic acid (21a), *N*-(2,3-difluoro-4-(*N*-((3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl)methylamino)benzoyl)-L-glutamic acid (21b), *N*-(2,5-difluoro-4-(*N*-((3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl)prop-2-ynylamino)benzoyl)-L-glutamic acid (22a), and *N*-(2,5-difluoro-4-(*N*-((3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl)-**

methylamino)benzoyl)-L-glutamic acid (22b).

All free acids had correct elemental analyses (C, H, N, F) for the formulas listed in the table and NMR spectra consistent with the assigned structures.

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Supplementary Material Available: 250-MHz NMR spectral data for N^{10}-propargyl (Table III) and N^{10}-methyl (Table IV) fluorine-substituted antifolate esters and acids in DMSO-d_6 (2 pages). Ordering information is given on any current masthead page.

Structure–Activity Studies of Potassium Channel Opening in Pinacidil-Type Cyanoguanidines, Nitroethenediamines, Thioureas, and Ureas

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Potassium channel opening activity for pinacidil-type cyanoguanidines, nitroethenediamines, thioureas, and ureas, has been assessed through simultaneous measurement of spontaneous contractile activity and stimulation of $^{86}Rb^+$ efflux from rat portal veins loaded with $^{86}Rb^+$. The good correlation between these two effects suggests that the vasodilator activity of the compounds is directly attributable to an increased opening of potassium channels. The resulting quantitative in vitro data has been used to analyze the structure–activity relationships for potassium channel opening, allowing the biological activity to be rationalized in terms of a pharmacophore involving a hydrogen-bond-acceptor element, a hydrogen-bond-donor element, and a lipophilic binding group. A model for the binding of pinacidil-related compounds to their potassium channel receptor has been developed, and compounds designed to test this model have been synthesized and tested. Prototropic equilibria are implicated as playing a fundamental role in determining the hydrogen-bonding ability of the compounds, and conformational changes in the receptor are invoked to explain disparities in the chiral recognition of lipophilic groups in different compounds.

Following the discovery that the antihypertensive agent pinacidil[1,2] (19; Pindac; Leo, Eli Lilly) acts like cromakalim[3] (BRL 34915, Beecham) via the opening of potassium channels[4,5] and the promising clinical trials with this latter compound in both cardiovascular indications[6,7] and asthma,[8] much interest has been aroused in this new approach to developing drugs for smooth muscle relaxation.

Potassium channels play an important role in controlling cellular membrane potential and hence, in the case of smooth muscle cells, contractility.[9,10] Pinacidil and cromakalim are thought to exert their biological effects by increasing the open probability (P_{open}) of plasmalemmal potassium channels in smooth muscle.[11-13] When these channels are in their open state, intracellular potassium effluxes along its electrochemical gradient, causing the cell membrane to become hyperpolarized until it approaches the potassium equilibrium potential. This hyperpolarization prevents depolarization of the membrane and thereby inhibits the opening of voltage-operated calcium channels (VOCs);[14] in addition the compounds inhibit other pathways leading to increased cytosolic calcium, involving mechanisms which in part may also depend on membrane potential.[12,13,15] With insufficient intracellular calcium, smooth muscle cells are unable to contract, resulting in their relaxation.

Most of the structure–activity studies reported to date for potassium channel openers have relied upon in vivo blood pressure lowering data.[16,17] Such data is often very

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