Eur. J. Med. Chem. 35 (2000) 529−534
© 2000 Éditions scientifiques et médicales Elsevier SAS. All rights reserved
S0223523400001471/SCO

Preliminary communication

New γ-fluoromethotrexates modified in the pteridine ring: synthesis and in vitro immunosuppressive activity

Yoshitsugu Kokuryo, Takuji Nakatani*, Makoto Kakinuma, Mikio Kabaki, Kyozo Kawata, Akira Kugimiya, Kenji Kawada, Mitsunobu Matsumoto, Ryuji Suzuki, Mitsuaki Ohtani

Shionogi Research Laboratories, Shionogi & Co. Ltd., Fukushima-ku, Osaka 553-0002, Japan

Received 4 October 1999; revised 15 November 1999; accepted 2 December 1999

Abstract – Our continuing program to develop new antifolate drugs useful against rheumatoid arthritis led us to modify the pteridine ring of γ-fluoromethotrexate. Pyrrolopyrimidine derivatives **1*e*** and **1*t*** were found to exhibit potent suppressive effects on the responses of both T and B cells to mitogens, although tetrahydropyridopyrimidine derivatives **2*e*** and **2*t*** and quinazoline derivatives **3*e***, **3*t*** and **4*e*** showed very weak suppressive activities. Thus, conversion of the pteridine ring of γ-fluoromethotrexate to a pyrrolopyrimidine ring led to a new potential antirheumatic compound. © 2000 Éditions scientifiques et médicales Elsevier SAS

γ-fluoromethotrexate / antirheumatic agent / antifolate drug

1. Introduction

The antifolate methotrexate (MTX, *figure 1*) has been used clinically to treat various types of cancers for more than three decades. MTX has also been demonstrated to be effective for managing rheumatoid arthritis (RA) [1–3] and has recently become a major therapeutic drug in the United States and Europe. In spite of such a long and distinguished history as an antineoplastic and immunosuppressive drug, the extreme toxicity of MTX during continuous usage severely limits its clinical effectiveness for the treatment of RA.

In a previous paper we reported that modification of the phenyl ring of γ-fluoromethotrexate (FMTX) led to new antifolates useful for RA treatment [4]. These derivatives were not metabolized to polyglutamates due to the presence of the strongly electronegative fluorine atom and hence exhibited much less toxicity [5–8], while retaining potent immunosuppressive and antirheumatic activities. The studies also demonstrated that an in vitro inhibitory test against responses of both T and B cells to mitogens was useful for evaluating the potential of MTX as an antirheumatic drug.

We next wanted to clarify the effect of modifying the pteridine ring of FMTX. Since several anticancer MTX derivatives (**1**–**4**) modified in the pteridine ring were reported to be potent inhibitors of typical folate metabolism-related enzymes [9–16], these antifolates were selected as parent compounds for fluorine-containing analogues.

* Correspondence and reprints: takuji.nakatani@shionogi.co.jp

2. Chemistry

Fluorine-containing analogues of these parent compounds were prepared starting from enantiomerically pure L-*erythro*- or L-*threo*-γ-fluoroglutamic acid (FGlu) which were obtained by a practical method using aminoacylase [17]. Syntheses of **1*e***, **1*t***, **2*e***, **2*t***, **3*e*** and **3*t*** were performed efficiently by coupling the isopropyl esters of FGlu **5*e*** and **5*t*** with carboxyl derivatives **1a** [9], **2a** [11] or **3a** [18] at a late stage of the synthetic procedure as shown in *figure 2*. Since the yield of the coupling was extremely low in the case of **4*e***, the protected L-*erythro*-FGlu derivative **5*e*** was coupled with the carboxyl derivative **4a** [15] at an early stage of the synthesis and later treated with bromomethyl quinazolinone. Racemization at the hydrolysis step could be retarded completely using barium hydroxide as reported previously [4].

3. Biological results and discussion

The immunological activities of these fluorinated compounds were examined by their in vitro effects on the

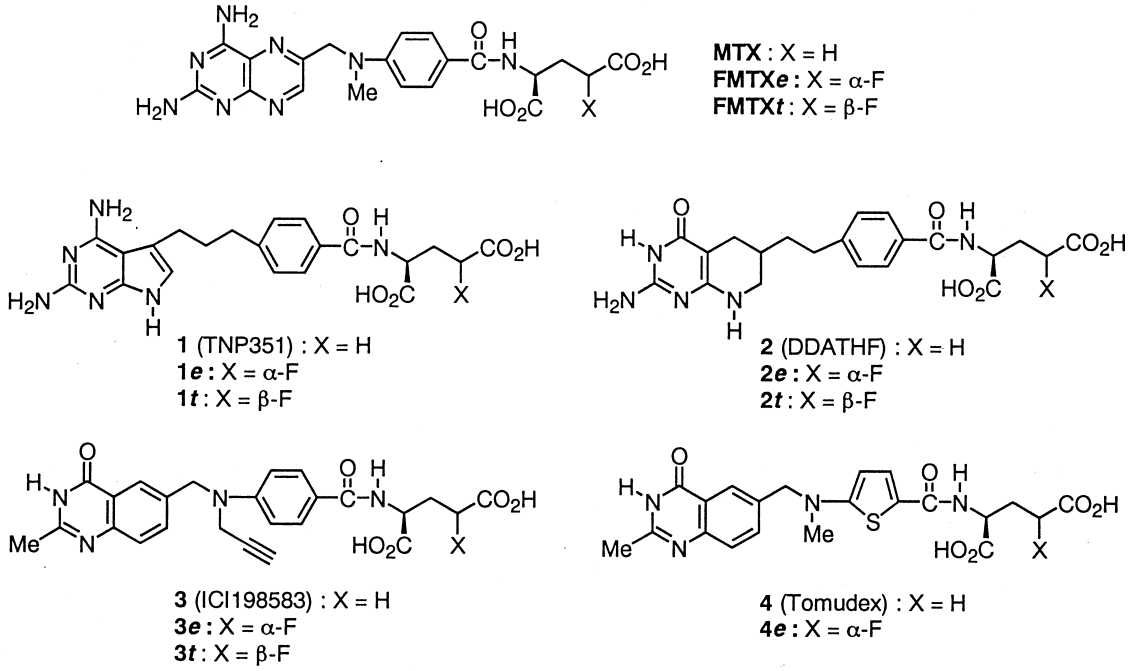


Figure 1.

responses of both T and B cells to mitogens, concanavalin A (Con A) and lipopolysaccharide (LPS), respectively [19]. As shown in *table I*, pyrrolopyrimidine derivatives **1*e*** and **1*t*** exhibited potent suppressive activities. On the other hand, much less active than MTX were tetrahydropyridopyrimidine derivatives **2*e*** and **2*t*** and quinazoline derivatives **3*e***, **3*t*** and **4*e***. As deduced from our previous study, these fluorinated compounds were not considered to have been converted to their polyglutamates [4]. Namely, the modification to pyrrolopyrimidine type derivatives left intact their activities in spite of the decreased polyglutamylation ability. Therefore, in order to develop less toxic antirheumatic agents with potent immunosuppressive activities but decreased polyglutamylation ability, modification of the pteridine ring, especially, its conversion to a pyrrolopyrimidine ring appears to be useful. The next step would be to conduct in vivo antirheumatic evaluations.

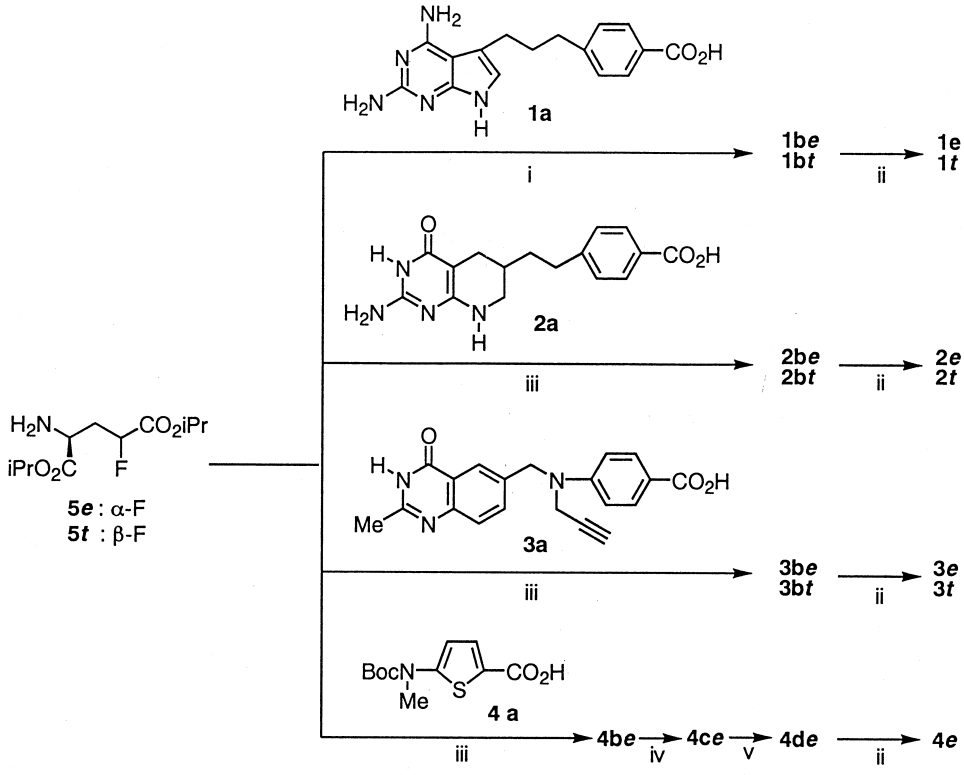
4. Experimental protocols

4.1. Chemistry

Merck Silica gel 60 or a Merck Lobar column was used for column chromatography. Melting points are uncorrected. ^{1}H-NMR spectra were determined at 200 or 300 MHz. Exact mass was determined from high-resolution fast atom bombardment mass spectra (HR-FABMS). Drying of an organic phase over anhydrous Na_2SO_4 is simply indicated by the word 'dried'.

4.1.1. N-[4-[3-(2,4-Diamino-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-1-methylpropyl]benzoyl]-(αS,γR)-γ-fluoroglutamic acid α,γ-diisopropyl ester **1b*e***

To a suspension of **1a** (0.40 mmol) and **5*e***·HCl (125 mg, 0.44 mmol) in DMF 2 mL was added a solution of DPPA (218 mg, 0.79 mmol) in DMF 2 mL at 0 °C and the mixture was stirred for 15 min. After addition of triethylamine (159 mg, 1.57 mmol) in DMF 2 mL, stirring was continued for 30 min at 0 °C and then for 72 h at room temperature. The slurry was filtered off and the filtrate was concentrated to a residue. The residue was chromatographed on silica gel using 20:1 $CHCl_3$/MeOH to afford 130 mg (61%) of **1b*e*** as a white powder. M.p. 91–93 °C. $[\alpha]_D^{25}$ –2.6 (c = 1.0, DMSO). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.16–1.21 (m, 12H), 1.81–2.06 (m, 2H), 2.24–2.34 (m, 2H), 2.67 (t, *J* = 8.1 Hz, 2H), 2.71 (t, *J* = 8.4 Hz, 2H), 4.47–4.54 (m, 1H), 4.85–5.17 (m, 3H), 5.35 (brs, 2H), 5.92 (brs, 2H), 6.41 (brs, 1H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.79 (d, *J* = 8.1 Hz, 2H), 8.82 (d, *J* = 7.8 Hz,



(i) DPPA, Et_3N, DMF; (ii) $Ba(OH)_2$, aq EtOH; (iii) DEPC, Et_3N, DMF; (iv) TFA; (v) bromomethylquinazolinone, DMA. Yields are as follows: **1*e***: 61% for i, 51% for ii. **1*t***: 58% for i, 46% for ii. **2*e***: 31% for iii, 58% for ii. **2*t***: 80% for iii, 83% for ii. **3*e***: 79% for iii, 48% for ii. **3*t***: 82% for iii, 73% for ii. **4*e***: 99% for iii, 67% for iv, 64% for v, 60% for ii.

Figure 2.

1H), 10.38 (brs, 1H). IR (KBr): 3 381, 3 187, 2 981, 2 935, 2 860, 1 736, 1 608, 1 576, 1 547, 1 490, 1 427, 1 375, 1 286, 1 227, 1 103 cm^{-1}. HR-FABMS *m/z* 543.2744 $(M + H)^+$ (calcd. for $C_{27}H_{36}O_5N_6F$ *m/z* 543.2731).

Table I. In vitro mitogen responses.

	Mitogen response[a]	
Compound	T cell[b]	B cell[c]
MTX	13.40	27.5
1*e*	5.67	8.08
1*t*	14.2	18.6
2*e*	1 413	2 926
2*t*	1 864	4 724
3*e*	2 301	1 124
3*t*	10 440	5 240
4*e*	3 165	1 152

[a] IC_{50} (nM); [b]Inhibitory activity against Con A-stimulated T cell proliferation; [c]Inhibitory activity against LPS-stimulated B cell proliferation.

4.1.2. N-[4-[3-(2,4-Diamino-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-1-methylpropyl]benzoyl]-(αS,γR)-γ-fluoroglutamic acid α,γ-diisopropyl ester **1bt**

The procedure described for the preparation of **1be** was used (58%). **1bt**: a white powder. M.p. 95–97 °C. $[\alpha]_D^{25}$ +1.4 (c = 1.0, DMSO). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.14–1.18 (m, 12H), 1.81–1.85 (m, 2H), 2.23–2.45 (m, 2H), 2.66 (t, *J* = 8.4 Hz, 2H), 2.69 (t, *J* = 8.1 Hz, 2H), 4.50–4.57 (m, 1H), 4.83–4.94 (m, 2H), 5.11–5.32 (m, 1H), 5.32 (brs, 2H), 5.89 (brs, 2H), 6.41 (brs, 1H), 7.30 (d, *J* = 8.1 Hz, 2H), 7.76 (d, *J* = 8.7 Hz, 2H), 8.73 (d, *J* = 7.8 Hz, 1H), 10.36 (brs, 1H). IR (KBr): 3 384, 3 193, 2 981, 2 935, 2 860, 1 736, 1 608, 1 576, 1 549, 1 491,

1 427, 1 375, 1 284, 1 228, 1 105 cm^{-1}. HR-FABMS *m/z* 543.2735 $(M + H)^+$ (calcd. for $C_{27}H_{36}O_5N_6F$ *m/z* 543.2731).

4.1.3. N-[4-[3-(2,4-Diamino-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-1-methyl-propyl]benzoyl]-(αS,γR)-γ-fluoroglutamic acid **1e**

To a suspension of **1b*e*** (130 mg, 0.24 mmol) in ethanol 5 mL, was added a solution of barium hydroxide octahydrate (315 mg, 1.0 mmol) in water 5 mL at 0 °C. After being stirred for 5 h at room temperature, the ethanol was evaporated. The mixture was brought to pH 3 by adding 1 N HCl solution. The precipitate was filtered off and washed with water and then dried in vacuo at 40 °C to afford 56 mg of **1*e*** (51%) as a white powder. M.p. > 250 °C. $[\alpha]_D^{25}$ +14.4 (c = 1.0, DMSO). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.81–2.06 (m, 2H), 2.24–2.34 (m, 2H), 2.67 (t, *J* = 8.1 Hz, 2H), 2.71 (t, *J* = 8.4 Hz, 2H), 4.47–4.54 (m, 1H), 4.85–5.17 (m, 1H), 5.35 (brs, 2H), 5.92 (brs, 2H), 6.41 (brs, 1H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.79 (d, *J* = 8.1 Hz, 2H), 8.82 (d, *J* = 7.8 Hz, IH), 10.38 (brs, 1H). IR (KBr): 3 348, 3 199, 2 933, 2 859, 1 641, 1 542, 1 498, 1 457, 1 394 cm^{-1}. HR-FABMS *m/z* 459.1808 $(M + H)^+$ (calcd. for $C_{21}H_{24}O_5N_6F$ *m/z* 459.1792).

4.1.4. N-[4-[3-(2,4-Diamino-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-1-methyl-propyl]benzoyl]-(αS,γS)-γ-fluoroglutamic acid **1t**

The procedure described for the preparation of **1*e*** was used (46%). **1*t***: a white powder. M.p. > 250 °C. $[\alpha]_D^{25}$ +11.4 (c = 1.0, DMSO). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.83–1.86 (m, 2H), 2.16–2.50 (m, 2H), 2.69 (t, *J* = 7.4 Hz, 2H), 2.74 (t, *J* = 8.0 Hz, 2H), 4.53–4.58 (m, 1H), 4.87–4.94 (m, 1H), 5.55 (brs, 2H), 6.22 (brs, 2H), 6.47 (brs, 1H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.82 (d, *J* = 8.0 Hz, 2H), 8.65 (d, *J* = 8.2 Hz, IH), 10.56 (brs, 1H). IR (KBr): 3 356, 3 207, 2 931, 2 860, 1 647, 1 542, 1 500, 1 458, 1 394 cm^{-1}. HR-FABMS *m/z* 459.1794 $(M + H)^+$ (calcd. for $C_{21}H_{24}O_5N_6F$ *m/z* 459.1792).

4.1.5. N-[4-[2-(2-Amino-4-hydroxy-5-deaza-5,6,7,8-tetrahydro-6-pteridinyl)ethyl]benzoyl]-(αS,γR)-γ-fluoroglutamic acid α,γ-diisopropyl ester **2be**

To a solution of **2a** (718 mg, 2.3 mmol) and **5*e***·HCl (984 mg, 3.5 mmol) in DMF 14.5 mL were added diethyl phosphocyanidate (DEPC) (423 mg, 2.6 mmol) and triethylamine (1 041 mg, 10.3 mmol) below 0 °C. The mixture was stirred at 0 °C for 0.5 h and then at room temperature for 15 h. The mixture was poured into saturated $NaHSO_3$ solution and extracted with EtOAc. The organic solution was washed with brine, dried and concentrated. The residue was chromatographed on silica gel using 18:1 $CHCl_3$/MeOH to afford 554 mg (31%) of **2b*e*** as white crystals. M.p. 121–123 °C. $[\alpha]_D^{25}$–6.2 (c = 1.0, DMSO). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.14–1.20 (m, 12H), 1.21–3.21 (m, 11H) 4.46–4.52 (m, 1H), 4.84–5.15 (m, 3H), 5.94 (brs, 2H), 6.24 (brs, 1H), 7.31 (d, *J* = 8.1 Hz, 2H), 7.78 (d, *J* = 7.8 Hz, 2H), 8.81 (d, *J* = 8.1 Hz, 1H), 9.73 (brs, 1H). IR (KBr): 3 359 (br), 2 981, 2 927, 2 852, 1 737, 1 645, 1 540, 1 463, 1 375, 1 340, 1 305, 1 218, 1 105 cm^{-1}. HR-FABMS *m/z* 568.2553 $(M + Na)^+$ (calcd. for $C_{27}H_{36}O_6N_5FNa$ *m/z* 568.2547). Analysis $C_{27}H_{36}FN_5O_6 \cdot 1.2H_2O$ (% calculated/found): 57.17/57.25 (C); 6.82/6.87 (H); 3.35/3.48 (F); 12.35/12.22 (N); 20.31/20.18 (O).

4.1.6. N-[4-[2-(2-Amino-4-hydroxy-5-deaza-5,6,7,8-tetrahydro-6-pteridinyl)ethyl]benzoyl]-(αS,γS)-γ-fluoroglutamic acid α,γ-diisopropyl ester **2bt**

The procedure described for the preparation of **2b*e*** was used (80%). **2b*t***: white crystals. M.p. 116–118 °C. $[\alpha]_D^{25}$–2.1 (c = 1.0, DMSO). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.13–1.21 (m, 12H), 1.21–3.25 (m, 11H), 4.51–4.58 (m, 1H), 4.82–4.97 (m, 2H), 5.11–5.31 (m, 1H), 5.93 (brs, 2H), 6.26 (brs, 1H), 7.31 (d, *J* = 8.1 Hz, 2H), 7.76 (d, *J* = 7.8 Hz, 2H), 8.74 (d, *J* = 7.5 Hz, 1H), 9.69 (brs, 1H). IR (KBr): 3 357 (br), 2 981, 2 927, 2 852, 1 738, 1 639, 1 540, 1 464, 1 373, 1 342, 1 304, 1 219, 1 105 cm^{-1}. HR-FABMS *m/z* 568.2567 $(M + Na)^+$ (calcd. for $C_{27}H_{36}O_6N_5FNa$ *m/z* 568.2547).

4.1.7. N-[4-[2-(2-Amino-4-hydroxy-5-deaza-5,6,7,8-tetrahydro-6-pteridinyl)-ethyl]benzoyl]-(αS,γR)-γ-fluoroglutamic acid **2e**

The procedure described for the preparation of **1*e*** was used (58%). **2*e***: white crystals. M.p. > 250 °C. $[\alpha]_D^{25}$ +18.2 (c = 0.50, DMSO). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.80–3.15 (m, 10H), 4.25–5.05 (m, 3H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.82 (d, *J* = 8.4 Hz, 2H). IR (KBr): 3 369 (br), 2 925, 2 854, 1 702, 1 637, 1 502, 1 400, 1 349, 1 307 cm^{-1}. HR-FABMS *m/z* 462.1790 $(M + H)^+$ (calcd. for $C_{21}H_{25}O_6N_5F$ *m/z* 462.1788).

4.1.8. N-[4-[2-(2-Amino-4-hydroxy-5-deaza-5,6,7,8-tetrahydro-6-pteridinyl)-ethyl]benzoyl]-(αS,γS)-γ-fluoroglutamic acid **2t**

The procedure described for the preparation of **1*e*** was used (83%). **2*t***: white crystals. M.p. > 250 °C. $[\alpha]_D^{25}$–5.0 (c = 0.50, DMSO). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.80–3.15 (m, 10H), 4.30–5.20 (m, 3H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.91 (d, *J* = 8.4 Hz, 2H). IR (KBr): 3 372 (br), 2 924, 2 854, 1 714, 1 648, 1 502, 1 382, 1 348,

1 304 cm^{-1}. HR-FABMS *m/z* 484.1623 $(M + Na)^+$ (calcd. for $C_{21}H_{24}O_6N_5FNa$ *m/z* 484.1609).

4.1.9. N-[4-[N-[[3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl]methyl]-N-prop-2-ynylamino]benzoyl]-(αS,γR)-γ-fluoroglutamic acid α,γ-diisopropyl ester **3b*e***

To a solution of **3a** (100 mg, 0.29 mmol) and **5*e***·HCl (123 mg, 0.43 mmol) in DMF 2 mL were added DEPC (52 mg, 0.32 mmol) and triethylamine (160 mg, 1.58 mmol) below 0 °C. The mixture was stirred at 0 °C for 0.5 h and then at room temperature for 2 h. The mixture was poured into saturated $NaHSO_3$ solution and extracted with EtOAc. The organic solution was washed with brine, dried and concentrated. The residue was chromatographed on silica gel using 18:1 $CHCl_3$/MeOH to afford 120 mg (79%) of **3b*e*** as white crystals. M.p. 181–183 °C. $[\alpha]_D^{25}$+14.1 (c = 0.40, DMSO). ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.17 (d, *J* = 6.1 Hz, 6H), 1.18 (d, *J* = 6.3 Hz, 6H), 2.20–2.65 (m, 5H), 3.19 (s, 1H), 4.33 (s, 2H), 4.46–4.60 (m, 1H), 4.72–5.02 (m, 4H), 5.02–5.37 (m, 1H), 6.84 (d, *J* = 8.6 Hz, 2H), 7.53 (d, *J* = 8.2 Hz, 1H), 7.65–7.78 (m, 3H), 7.97 (s, 1H), 8.46 (d, *J* = 7.8 Hz, 1H), 12.17 (s, 1H). IR (KBr): 3 700–2 600, 1 740, 1 680, 1 610, 1 510, 1 380, 1 210, 1 110 cm^{-1}. Analysis $C_{31}H_{35}FN_4O_6$·0.3H_2O (% calculated/found): 63.75/63.49 (C); 6.14/6.02 (H); 3.25/3.04 (F); 9.59/9.87 (N).

4.1.10. N-[4-[N-[[3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl]methyl]-N-prop-2-ynylamino]benzoyl]-(αS,γS)-γ-fluoroglutamic acid α,γ-diisopropyl ester **3b*t***

The procedure described for the preparation of **3b*e*** was used (82%). **3b*t***: white crystals. M.p. 203–204 °C. $[\alpha]_D^{20}$ + 20.3 (c = 0.38, DMSO). ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.18 (d, *J* = 6.3 Hz, 6H), 1.23 (d, *J* = 6.2 Hz, 6H), 2.18–2.50 (m, 5H), 3.18 (s, 1H), 4.33 (s, 2H), 4.42–4.57 (m, 1H), 4.73 (s, 2H), 4.83–5.20 (m, 3H), 6.86 (d, *J* = 9.0 Hz, 2H), 7.54 (d, *J* = 8.3 Hz, 1H), 7.69 (dd, *J* = 2.0, 8.5 Hz, 1H), 7.75 (d, *J* = 8.8 Hz, 2H), 7.98 (d, *J* = 1.5 Hz, 1H), 8.55 (d, *J* = 8.2 Hz, 1H), 12.17 (s, 1H). IR (KBr): 3 700–2 600, 1 740, 1 680, 1 610, 1 510, 1 210, 1 110 cm^{-1}. Analysis $C_{31}H_{35}FN_4O_6$·0.3H_2O (% calculated/found): 63.75/63.80 (C); 6.14/6.08 (H); 3.25/3.18 (F); 9.59/9.52 (N).

4.1.11. N-[4-[N-[[3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl]methyl]-N-prop-2-ynylamino]benzoyl]-(αS,γR)-γ-fluoroglutamic acid **3*e***

The procedure described for the preparation of **1*e*** was used (48%). **3*e***: pale yellow crystals. M.p. 204–206 °C. $[\alpha]_D^{25}$ +5.9 (c = 1.0, DMSO). ^{1}H-NMR (200 MHz, DMSO-d_6) δ 2.10–2.68 (m, 5H), 3.19 (s, 1H), 4.33 (s, 2H), 4.56 (dd, *J* = 7.2, 13.4 Hz, 1H), 4.78 (s, 2H), 5.09 (ddd, *J* = 4.4, 7.3, 45.9 Hz, 1H), 6.84 (d, *J* = 9.0 Hz, 2H), 7.54 (d, *J* = 8.5 Hz, 1H), 7.62–7.85 (m, 3H), 7.97 (d, *J* = 1.9 Hz, 1H), 8.41 (d, *J* = 7.8 Hz, 1H), 12.16 (s, 1H). IR (KBr): 3 399, 3 286, 3 043, 2 929, 1 716, 1 604, 1 506, 1 307, 1 205 cm^{-1}. HR-FABMS *m/z* 517.1517 $(M + Na)^+$ (calcd. for $C_{25}H_{23}O_6N_4FNa$ *m/z* 517.1499).

4.1.12. N-[4-[N-[[3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl]methyl]-N-prop-2-ynylamino]benzoyl]-(αS,γS)-γ-fluoroglutamic acid **3*t***

The procedure described for the preparation of **1*e*** was used (73%). **3*t***: pale yellow crystals. M.p. 211–213 °C. $[\alpha]_D^{25}$ +7.8 (c = 1.0, DMSO). ^{1}H-NMR (200 MHz, DMSO-d_6) δ 2.03–2.67 (m, 5H), 3.18 (s, 1H), 4.33 (s, 2H), 4.40–4.64 (m, 1H), 4.64–5.16 (m, 3H), 6.86 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 8.3 Hz, 1H), 7.69 (dd, *J* = 1.6, 8.5 Hz, 1H), 7.77 (d, *J* = 8.8 Hz, 2H), 7.98 (d, *J* = 1.7 Hz, 1H), 8.47 (d, *J* = 8.1 Hz, 1H), 12.16 (s, 1H). IR (KBr): 3 409, 3 286, 3 041, 2 929, 1 718, 1 604, 1 506, 1 332, 1 205 cm^{-1}. HR-FABMS *m/z* 517.1494 $(M + Na)^+$ (calcd. for $C_{25}H_{23}O_6N_4FNa$ *m/z* 517.1499).

4.1.13. N-[5-[N-(tert-Butoxycarbonyl)-N-methylamino]-2-thenoyl]-(αS,γR)-γ-fluoroglutamic acid α,γ-diisopropyl ester **4b*e***

To a solution of **4a** (1.0 g, 3.89 mmol) and **5*e***·HCl (1.44 g, 5.06 mmol) in DMF 7 mL were added DEPC (0.70 g, 4.28 mmol) and triethylamine (1.57 g, 15.6 mmol) below 0 °C. The mixture was stirred at 0 °C for 0.5 h and then at room temperature for 2 h. The mixture was poured into saturated $NaHSO_3$ solution and extracted with EtOAc. The organic solution was washed with brine, dried and concentrated. The residue was chromatographed on silica gel using 2.5:1 AcOEt/hexane to afford 1.78 g (99%) of **4b*e*** as a pale yellow foam. $[\alpha]_D^{20}$–3.50 (c = 1.13, DMSO). ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.19 (d, *J* = 6.2 Hz, 12H), 1.51 (s, 9H), 2.20–2.50 (m, 2H), 3.34 (s, 3H), 4.52 (dd, *J* = 7.6, 13.9 Hz, 1H), 4.91 (septet, *J* = 6.2 Hz, 1H), 4.93 (septet, *J* = 6.2 Hz, 1H), 5.19 (ddd, *J* = 5.0, 6.8, 47.8 Hz, 1H), 6.63 (d, *J* = 4.3 Hz, 1H), 7.61 (d, *J* = 4.3 Hz, 1H), 8.61 (d, *J* = 8.0 Hz, 1H). IR (KBr): 3 700–3 150, 2 980, 1 740, 1 700, 1 380, 1 150, 1 110 cm^{-1}. Analysis $C_{22}H_{33}FN_2O_7$·0.1H_2O (% calculated/found): 53.89/53.87 (C); 6.82/6.75 (H); 3.87/3.81 (F); 5.71/5.64 (N); 6.54/6.44 (S).

4.1.14. N-[5-(Methylamino)-2-thenoyl]-(αS,γR)-γ-fluoroglutamic acid α,γ-diisopropyl ester **4c*e***

4b*e* (1.5 g, 3.26 mmol) was dissolved with stirring in CF_3CO_2H (8 mL). After being stirred for 14 h, the CF_3CO_2H was evaporated and the residue was partitioned between aqueous $NaHCO_3$ and $CHCl_3$. The or-

ganic layer was dried and concentrated to an oil. The oil was chromatographed on silica gel using 100:5 $CHCl_3$/EtOAc to afford 0.79 g (67%) of **4c*e*** as a brown amorphous mass. $[\alpha]_D^{25}$ + 3.2 (c = 1.0, DMSO). ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.19 (d, *J* = 5.5 Hz, 12H), 2.08–2.47 (m, 2H), 2.74 (d, *J* = 4.6 Hz, 3H), 4.48 (dd, *J* = 7.7, 14.3 Hz, 1H), 4.92 (septet, *J* = 6.2 Hz, 2H), 5.18 (ddd, *J* = 3.9, 6.9, 48.0 Hz, 1H), 5.81 (d, *J* = 4.1 Hz, 1H), 6.91 (q, *J* = 4.7 Hz, 1H), 7.47 (d, *J* = 4.2 Hz, 1H), 8.23 (d, *J* = 8.0 Hz, 1H). IR (KBr): 3 375, 3 261, 2 985, 2 937, 1 747, 1 730, 1 603, 1 556, 1 518, 1 489, 1 410, 1 373, 1 252, 1 232, 1 146, 1 107, 1 061 cm^{-1}. HR-FABMS *m/z* 411.1348 $(M + Na)^+$ (calcd. for $C_{17}H_{25}O_5N_2FSNa$ *m/z* 411.1366).

4.1.15. N-[5-[N-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-N-methylamino]-2-thenoyl]-(αS,γR)-γ-fluoroglutamic acid α,γ-diisopropyl ester **4d*e***

A mixture of **4c*e*** (0.30 g, 0.83 mmol), bromomethyldiaminopteridine (0.25 g, 1.0 mmol) and 2,6-lutidine (0.48 mL, 4.1 mmol) in DMF (5 mL) was stirred for 17 h at 50 °C. The cooled mixture was evaporated to dryness and the residue was partitioned between 1 N HCl and $CHCl_3$. The organic layer was washed with aqueous $NaHCO_3$, brine, dried and concentrated. The residue was chromatographed on silica gel using 4:1 EtOAc/hexane to afford 0.28 g (64%) of **4d*e*** as a white powder. M.p. 163–165 °C. $[\alpha]_D^{25}$ +3.2 (c = 1.0, DMSO). ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.18 (d, *J* = 6.2 Hz, 12H), 2.15–2.48 (m, 5H), 3.05 (s, 3H), 4.49 (dd, *J* = 7.6, 13.9 Hz, 1H), 4.65 (s, 2H), 4.90 (septet, *J* = 6.2 Hz, 1H), 4.91 (septet, *J* = 6.2 Hz, 1H), 5.17 (ddd, *J* = 4.8, 6.9, 47.8 Hz, 1H), 5.99 (d, *J* = 4.3 Hz, 1H), 7.53 (d, *J* = 4.2 Hz, 1H), 7.55 (d, *J* = 8.9 Hz, 1H), 7.66 (dd, *J* = 2.1, 8.4 Hz, 1H), 7.94 (d, *J* = 1.8 Hz, 1H), 8.31 (d, *J* = 8.0 Hz, 1H), 12.21 (s, 1H). IR (KBr): 3 354, 3 167, 3 076, 3 022, 2 981, 2 939, 2 895, 1 753, 1 728, 1 678, 1 620, 1 550, 1 433, 1 415, 1 377, 1 340, 1 321, 1 304, 1 234, 1 213, 1 147, 1 107 cm^{-1}. HR-FABMS *m/z* 583.2004 $(M + Na)^+$ (calcd. for $C_{27}H_{33}O_6N_4FSNa$ *m/z* 583.2002).

4.1.16. N-[5-[N-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-N-methylamino]-2-thenoyl]-(αS,γR)-γ-fluoroglutamic acid **4*e***

The procedure described for the preparation of **1*e*** was used (60%). **4*e***: pale yellow crystals. M.p. 171–173 °C. $[\alpha]_D^{25}$–8.3 (c = 1.0, DMSO). ^{1}H-NMR (200 MHz, DMSO-d_6) δ 2.33 (s, 3H), 2.00–2.45 (m, 2H), 3.04 (s, 3H), 4.40–4.50 (m, 1H), 4.65 (s, 2H), 4.91 (m, 1H), 5.98 (d, *J* = 5.1 Hz, 1H), 7.52–7.55 (m, 2H,), 7.65 (m, 1H), 7.94 (s, 1H). IR (KBr): 3 390, 3 033, 2 927, 1 718, 1 671, 1 616, 1 494, 1 415, 1 342, 1 307, 1 222, 1 153, 1 093, 1 043 cm^{-1}. HR-FABMS *m/z* 499.1053 $(M + Na)^+$ (calcd. for $C_{21}H_{21}O_6N_4FSNa$ *m/z* 499.1064).

4.2. Biology

The effects on mitogen responses were determined as described in our previous report [19].

References

[1] Bannwarth B., Labat L., Moride Y., Scaeverbeke T., Drugs 47 (1994) 25–50.

[2] Furst D.E., Br. J. Rheumatol. 36 (1997) 1196–1204.

[3] Seitz M., Curr. Opin. Rheumatol. 11 (1999) 226–232.

[4] Kokuryo Y., Kawata K., Nakatani T., Kugimiya A., Tamura Y., Kawada K. et al., J. Med. Chem. 40 (1997) 3280–3291.

[5] Tsushima T., Kawada K., Shiratori O., Uchida N., Heterocycles 23 (1985) 45–49.

[6] Tsushima T., Kawada K., Ishihara S., Uchida N., Shiratori O., Higaki J., Hirata M., Tetrahedron 44 (1988) 5375–5387.

[7] McGuire J.J., Coward J.K., in: Blakley R.L., Benkovic S.J. (Eds.), Folates and Pterins Vol. 1, John Wiley & Sons, NY, 1984, pp. 135–190.

[8] Chabner B.A., Allegra C.J., Curt G.A., Clendeninn N.J., Baram J., Koizumi S., Drake J.C., Jolivet J., J. Clin. Invest. 76 (1985) 907–912.

[9] Miwa T., Hitaka H., Akimoto H., Nomura H., J. Med. Chem. 34 (1991) 555–560.

[10] Akimoto H., Hitaka T., Miwa T., Yukishige K., Kusanagi T., Ootsu K., Proc. Am. Assoc. Cancer Res. 32 (1991) 327.

[11] Taylor E.C., Harrington P.J., Fletcher S.R., Beardsley G.P., Moran R.G., J. Med. Chem. 28 (1985) 914–921.

[12] Cole J.T., Gralla R.J., Kardinal C.G., Rivera N.P., Proc. Am. Assoc. Cancer Res. 33 (1992) 413.

[13] Hughes L.R., Jackman A.L., Oldfield J., Smith R.C., Burrows K.D., Marsham P.R. et al., J. Med. Chem. 33 (1990) 3060–3067.

[14] Jackman A.L., Newell D.R., Gibson W., Jodrell D.I., Taylor G.A., Bishop J.A.M., Hughes L.R., Calvert A.H., Biochem. Pharmacol. 42 (1991) 1885–1893.

[15] Marsham P.R., Hughes L.R., Jackman A.L., Hayter A.J., Oldfield J., Wardleworth J.M. et al., J. Med. Chem. 34 (1991) 1594–1605.

[16] Jackman A.L., Taylor G.A., Gibson W., Kimbell R., Brown M., Calvert A.H., Judson I.R., Hughes L.R., Cancer Res. 51 (1991) 5579–5586.

[17] Kokuryo Y., Nakatani T., Kobayashi K., Tamura Y., Kawada K., Ohtani M., Tetrahedron Asymmetry 7 (1996) 3543–3551.

[18] Bisset G.M.F., Pawekzak K., Jackman A.L., Calvert A.H., Hughes L.R., J. Med. Chem. 35 (1992) 859–866.

[19] Yasui K., Tamura Y., Nakatani T., Horibe I., Kawada K., Koizumi K., Suzuki R., Ohtani M., J. Antibiotics 49 (1996) 173–180.