ELSEVIER

Il Farmaco 53 (1998) 150–159

IL FARMACO

Quinoxaline chemistry
Part 10. Quinoxaline 10-oxa-analogues of trimetrexate (TMQ) and of 5,8-dideazafolic acid. Synthesis and evaluation of in vitro anticancer activity

Gabriella Vitale, Paola Corona, Mario Loriga, Giuseppe Paglietti *

Istituto di Chimica Farmaceutica e Tossicologica, Università di Sassari, Via Muroni 23, 07100 Sassari, Italy

Received 5 November 1997; accepted 7 January 1998

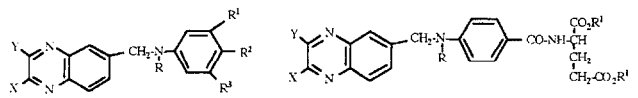
Abstract

Among twenty-eight novel compounds (twenty-two 2,3-disubstituted-6-[(substituted-phenoxy)methyl-quinoxalines and six 4-[(2,3-disubstituted-quinoxalin-6-yl)methoxy]benzoylglutamates) only thirteen were selected at NCI for evaluation of their in vitro anticancer activity. The results have shown that compounds **3l,c,b,e** and **4b** were endowed with significantly high values of percent tumor growth inhibition on several tumor cell lines at 10^{-4} M, while compound **3t** was characterized by a high selectivity, being still strongly inhibiting on three cell lines at 10^{-5} M. Comparison of the presently observed activity with that of the previously described aza-analogues confirms that the effected isosteric substitution is highly valuable in some cases.

Keywords: Anticancer agents; Quinoxaline derivatives; Trimetrexate analogues; 5,8-Dideazafolic acid analogues

1. Introduction

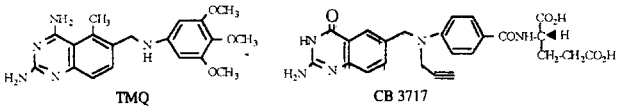
In a previous paper [1] we have reported the preparation of eighteen quinoxalines bearing a variously substituted phenylaminomethyl group on position 6 of the ring and other more or less lipophilic substituents on positions 2 and 3 of formulae **1** and **2**, in order to discover if



1 **2** (R^1 = Et,H)

N = Ph,OMe; Y = Cl,OMe,NH_2
R = H,Propargyl; R^1 = H,OMe;
R^2 = H,OMe,F,CN; R^3 = H,OMe;

structural analogy with both TMQ and CB 3717 might display in vitro anticancer activity.



TMQ CB 3717

Among these, twelve compounds were selected at the National Cancer Institute (NCI), Bethesda, MD, USA; they exhibited moderate to strong cell-growth inhibition at a concentration of 10^{-4} M. Now, in connection with this research program [2–5], we have prepared a new series of quinoxalines (**3a–v**) and (**4a–f**) where the nitrogen at position 10 has been replaced by an oxygen in order to investigate if this type of isosteric substitution might improve both selectivity and sensitivity in the in vitro anticancer activity test.

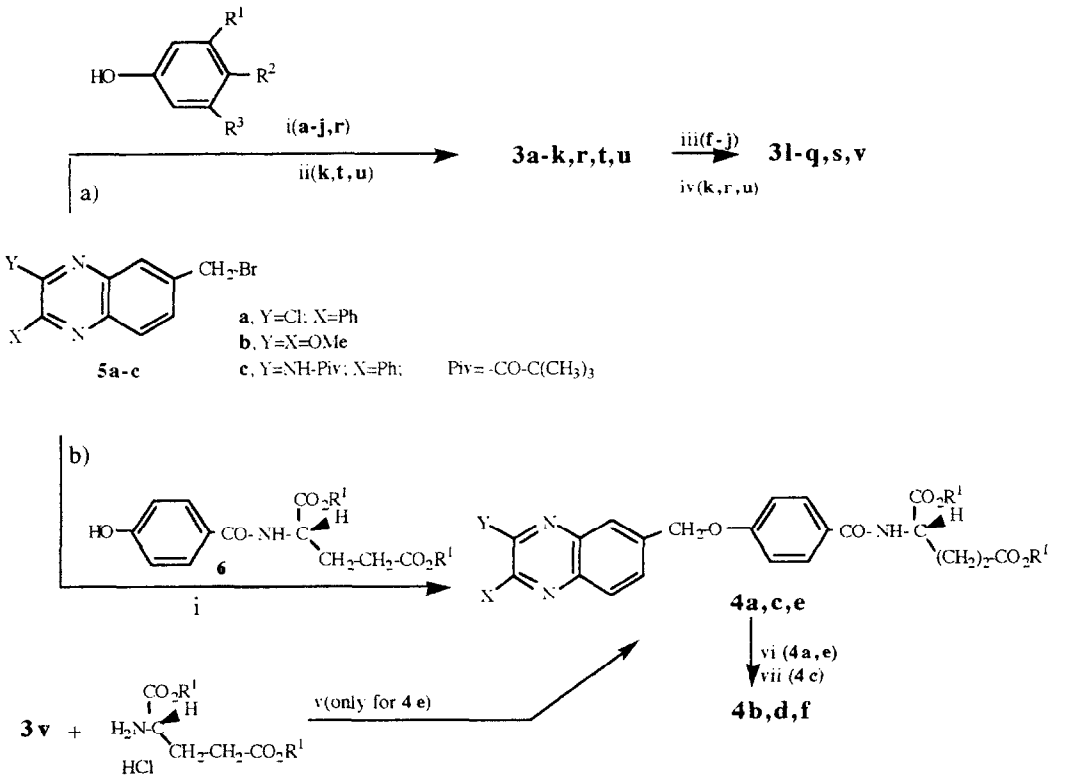
Examples of this type are recurrent in the literature in the series of quinazoline analogues of both trimetrexate and 5,8-dideazafolates which displayed interesting and potent inhibitory activity against dihydrofolate reductase (DHFR) and thymidylate synthase (TS) enzymes strongly involved in the build-up of tumor cells [6–8].

2. Chemistry

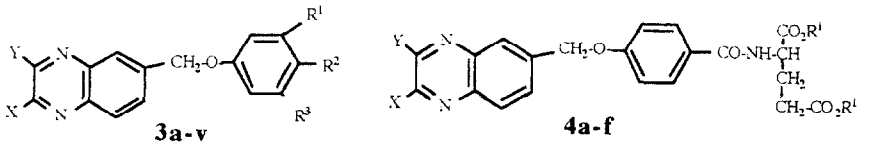
The 6-(phenoxy)methylquinoxalines **3a–v** and 4-[(quinoxalin-6-yl)methoxy]benzoylglutamic derivatives **4a–f** were prepared according to the reactions of Scheme 1. The bromethylquinoxalines **5a–c**, obtained as described in a previous paper [1], were reacted with the suitable substituted phenols (Fig. 1) in dimethylformamide (DMF) and in the presence of one mole equivalent of either Cs_2CO_3 at room temperature or $CsHCO_3$ at 70°C to give compounds **3a–**

* Corresponding author.

0014-827X/98/$19.00
PII S0014-827X(98)00003-2



Scheme 1. i, DMF, Cs_2CO_3 at room temperature for 6 h; ii, DMF, $CsHCO_3$ at 70°C for 2 h; iii, EtOH and 2 M HCl under reflux for 7 h; iv, a mixture of EtOH and 1 M NaOH at 70°C; v, DMF, $(EtO)_2POCN$, N_2, TEA at room temperature for 2 h; vi, a mixture of EtOH and 1 M NaOH at room temperature for 3 h; vii, a mixture of EtOH and 1 M NaOH aqueous solution under reflux for 4 h.



Compd	X	Y	R^1	R^2	R^3
3a	Ph	Cl	OMe	OMe	OMe
3b	Ph	Cl	OMe	H	OMe
3c	Ph	Cl	H	OMe	H
3d	Ph	Cl	H	CN	H
3e	Ph	Cl	H	F	H
3f	Ph	NH-Piv	OMe	OMe	OMe
3g	Ph	NH-Piv	OMe	H	OMe
3h	Ph	NH-Piv	H	OMe	H
3i	Ph	NH-Piv	H	CN	H
3j	Ph	NH-Piv	H	F	H
3k	Ph	NH-Piv	H	COOMe	H
3l	Ph	NH_2	OMe	OMe	OMe
3m	Ph	NH_2	OMe	H	OMe
3n	Ph	NH_2	H	OMe	H
3o	Ph	NH_2	H	CN	H
3p	Ph	NH_2	H	F	H
3q	Ph	NH_2	H	COOH	H
3r	Ph	Cl	H	COOMe	H
3s	Ph	OEt	H	COOH	H
3t	OMe	OMe	OMe	OMe	OMe
3u	OMe	OMe	H	COOMe	H
3v	OMe	OMe	H	COOH	H

Compd	X	Y	R^1
4a	Ph	Cl	Et
4b	Ph	OEt	H
4c	Ph	NH-Piv	Et
4d	Ph	NH_2	H
4e	OMe	OMe	Et
4f	OMe	OMe	H

Fig. 1. Compounds obtained according to Scheme 1.

k,r,t,u in fair yields (route (a)). The amino derivatives **3l–p** were successively obtained by acidic hydrolysis in refluxing ethanol, whereas the alkaline saponification of the esters **3k,r,u** yielded the acids **3q,s,v**. The attainment of **3s** is further confirmation that during the alkaline hydrolysis the chlorine is nucleophilic and is displaced by the ethoxide anion, as previously observed in a similar case [1]. The quinoxalinylmethoxybenzoylglutamates **4a,c** were obtained from the intermediates **5a,c** and the diethyl *p*-oxybenzoylglutamate **6** [10] (route (b)), while compound **4e** was in turn obtained by condensation of the acid **3v** with diethyl L-glutamate hydrochloride. Alkaline hydrolysis of **4c,e** yielded the desired acid (**4d,f**), while in the attempt to obtain **4b** we came across an ethanolysis analogous to that for **3s** mentioned above.

Characterization of the described compounds followed according to the analytical and spectroscopic data (Table 1). In particular it is to be noted that the 2,3-dimethoxyquinoxaline derivatives **3t,u,v** and **4e,f** exhibited a set of very complex absorptions in the region 330–290 nm in the UV spectrum in ethanol, with two very fine maxima at 327 and 313 nm due to the strong conjugation in the heterocyclic system, with a pattern of both absorption wavelength and intensity identical to those recorded by us for the spectrum (not reported) of the well-known 2,3-dimethoxy-6-methylquinoxaline [9] that exhibited maxima at 329, 323, 315, 310, 303 and 247 nm.

3. Experimental

3.1. Chemistry

Melting points are uncorrected and were recorded on a Kofler or an electrothermal melting point apparatus. UV spectra are qualitative and were recorded in nm for solutions in ethanol with a Perkin-Elmer Lambda 5 spectrophotometer. IR spectra are for nujol mulls and were recorded on Perkin-Elmer 781 instruments. ^{1}H NMR spectra were recorded at 200 MHz with a Varian XL-200 instrument using tetramethylsilane (TMS) as internal standard. Elemental analyses were performed at the Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, Università di Padova (Padua). The analytical results for C, H and N were within ±0.4% of the theoretical values.

3.1.1. Intermediates

Most of the quinoxalines necessary to obtain the starting material (**5a–c**) were known compounds and have been purposely prepared according to the procedures described in a previous paper [1]. Diethyl *p*-hydroxybenzoylglutamate was prepared according to the indications of the literature [10].

*3.1.2. General procedure for preparation of the 6-(phenoxy)methylquinoxalines **3a–k,r,t,u***

A mixture of equimolar amounts (1 mmol) of **5a–c**, the suitably substituted phenol (Fig. 1) and cesium carbonate, in anhydrous DMF (15 ml), was stirred at room temperature for 6 h. In the case of phenols (**3k,t,u**), cesium hydrogencarbonate was used and the mixture heated at 70°C for 2 h. Then water was added to complete precipitation of a solid that was collected and washed up with water and eventually dried. Compounds **3g,j**, which separated as oils, were extracted with chloroform. The organic phase, dried over anhydrous sodium sulfate and evaporated in vacuo, gave solid compounds. Purification methods, yields, melting points, analytical and spectroscopic data are reported in Table 1.

*3.1.3. General procedure for preparation of the derivatives **3l–q,s,v***

(i) A mixture of **3f–j** (0.5 mmol), dissolved or suspended in a mixture (ratio 1:1) of EtOH/2 M HCl (10 ml), was refluxed for 7 h. On cooling, or after dilution with water, a solid was soon formed. After collection, the amines **3l–p** were washed with water and eventually dried. Purification methods, yields, melting points, analytical and spectroscopic data are reported in Table 1.

(ii) A mixture of compounds **3k,r,u** (0.5 mmol) was treated as follows:

3k suspended in a mixture (ratio 1:1) of EtOH/2 M NaOH aqueous solution (14 ml) was heated at 70°C for 18 h; **3r,u** suspended in a mixture (ratio 3:3:1) of EtOH/H_2O/2 M NaOH aqueous solution (14 ml) was heated at 70°C for 7 h; on cooling, the mixture was diluted with water and made acidic with 2 M HCl aqueous solution to pH = 4–5 to precipitate a solid that was collected and washed with water. Purification methods, yields, melting points, analytical and spectroscopic data of the acids **3,q,s,v** are reported in Table 1.

*3.1.4. General procedure for preparation of the 4-[(quinoxalin-6-yl)methoxy]benzoylglutamates **4a,c,e***

(i) A mixture of equimolar amounts (1 mmol) of **5a,c**, diethyl 4-hydroxy-L-glutamate prepared as described in Ref. [10] and cesium carbonate in anhydrous DMF (10–15 ml) was stirred at room temperature for 6 h. Then it was diluted with water to give a solid formed by the compounds **4a,c**. After collection, followed by washing with water, purification was carried out as indicated in Table 1 which also reports the yields, melting points, analytical and spectroscopic data.

(ii) Diethyl cyanophosponate (0.13 g, 0.80 mmol) in DMF (2 ml) and TEA (0.16 g, 1.50 mmol) in DMF (2 ml) were added under a continuous stream of nitrogen to a mixture of **3v** (0.25 g, 0.73 mmol) and diethyl L-glutamate hydrochloride (0.19 2 g, 0.2 mmol) in DMF (10 ml). The mixture was stirred for 2 h and then poured onto a mixture (ratio 3:1) of ethyl acetate and benzene (32 ml). The organic phase was washed with water (50 ml), saturated sodium carbonate solution (60 ml), water (50 ml) and saturated sodium chloride solution (60 ml), in that order, and eventually dried over anhydrous sodium sulfate. On evaporation, compound **4e** was obtained as an oily residue and purified as indicated in

Table 1
Melting points, yields, analytical and spectroscopic (IR, UV, ^{1}H NMR) data for the compounds of Fig. 1 and Scheme 1

Compound	M.p. (°C) [a]	Yield (%)	Analysis for C, H, N	IR ν_{max}(nujol) (cm^{-1})	UV λ_{max}(EtOH) (nm)	^{1}H NMR, δ_H (J in Hz) Solvent: [A] = $CDCl_3$, [B] = $CDCl_3$–DMSO-d_6 (3:1), [C] = DMSO-d_6
3a	153–154 (a)	59	$C_{24}H_{21}ClN_2O_4$		341, 264 infl, 247, 207	[A] 8.18 (1H, d, $J_{7,8}$ = 8.6, H-8), 8.14 (1H, d, $J_{5,7}$ = 2.2, H-5), 7.87 (1H, dd [c], $J_{7,8}$ = 8.6 and $J_{7,5}$ = 2.2, H-7), 7.88–7.82 (2H, m, H-2″,6″), 7.59–7.51 (3H, m, H-3″,4″,5″), 6.28 (2H, s, H-2′,6′), 5.29 (2H, s, CH_2O), 3.85 (6H, s, 3′,5′-OCH_3), 3.80 (3H, s, 4′-OCH_3)
3b	162–164 (a)	72	$C_{23}H_{19}ClN_2O_3$		339, 264 infl, 247, 207	[A] 8.16 (1H, d, $J_{8,7}$ = 8.6, H-8), 8.12 (1H, d, $J_{5,7}$ = 2.2, H-5), 7.92–7.82 (3H, m, H-2″,6″ + dd [c], H-7), 7.60–7.52 (3H, m, H-3″,4″,5″), 6.20 (2H, d, J = 2.0, H-2′,6′), 6.12 (1H, d, J = 2.0, H-4′), 5.28 (2H, s, CH_2), 3.78 (6H, s, 3′,5′-OCH_3)
3c	149–150 (a)	54	$C_{22}H_{17}ClN_2O_2$		340, 264 infl, 248, 205	[A] 8.16 (1H, d, $J_{8,7}$ = 8.6, H-8), 8.11 (1H, d, $J_{5,7}$ = 2.2, H-5), 7.91–7.83 (3H, m, H-2″,6″ + dd [c], $J_{7,8}$ = 8.6 and $J_{7,5}$ = 2.2, H-7), 7.60–7.52 (3H, m, H-3″,4″,5″), 6.95 (4H, q, J = 9.2, H-2′,3′,5′,6′), 5.27 (2H, s, CH_2), 3.77 (3H, s, 4′-OCH_3)
3d	231–233 (b)	67	$C_{22}H_{14}ClN_3O + 0.125H_2O$	2220	340, 250, 205	[C] 8.21 (1H, d, $J_{8,7}$ = 8.6, H-8), 8.16 (1H, d, $J_{5,7}$ = 1.8, H-5), 7.98 (1H, dd, $J_{7,8}$ = 8.6 and $J_{7,5}$ = 1.8, H-7), 7.87–7.80 (2H, m, H-2″,6″), 7.82 (2H, d, J = 8.8, H-2′,6′), 7.62–7.55 (3H, m, H-3″,4″,5″), 7.28 (2H, d, J = 8.8, H-3′,5′), 5.54 (2H, s, CH_2)
3e	167–169 (a)	47	$C_{21}H_{14}ClFN_2O$		339, 264 infl, 247, 209	[A] 8.17 (1H, d, $J_{8,7}$ = 8.6, H-8), 8.10 (1H, d, $J_{5,7}$ = 1.6, H-5), 7.87–7.82 (3H, m, H-2″,6″ + dd [c], $J_{7,8}$ = 8.6 and $J_{7,5}$ = 1.6, H-7), 7.56–7.53 (3H, m, H-3″,4″,5″), 7.00–6.95 (4H, m, H-2′,3′,5′,6′), 5.29 (2H, s, CH_2)
3f	178–182 (a)	57	$C_{29}H_{31}N_3O_5$	3390, 1700	342, 244, 206	[A] 8.15 (1H, d, $J_{5,7}$ = 2.2, H-5), 8.10 (1H, d, $J_{8,7}$ = 8.8, H-8), 7.82–7.74 (3H, m, H-2″,6″ + H-7), 7.63–7.54 (3H, m, H-3″,4″,5″), 6.28 (2H, s, H-2′,6′), 5.26 (2H, s, CH_2), 3.85 (6H, s, 3′,5′-OCH_3), 3.80 (3H, s, 4′-OCH_3), 1.18 (9H, s, $C(CH_3)_3$)
3g	132–136 (g)	75	$C_{28}H_{29}N_3O_4$	3310, 1660	344, 244, 206	[A] 8.24 (1H, d, $J_{5,7}$ = 2.2, H-5), 8.10 (1H, d, $J_{8,7}$ = 9.2, H-8), 7.80–7.72 (3H, m, H-2″,6″ + H-7), 7.64–7.52 (3H, m, H-3″,4″,5″), 6.19 (2H, d, J = 2.0, H-2′,6′), 6.13 (1H, d, J = 2.0, H-4′), 5.26 (2H, s, CH_2), 3.78 (6H, s, 3′,5′-OCH_3), 1.18 (9H, s, $C(CH_3)_3$)
3h	60	70	$C_{27}H_{27}N_3O_3$	3300 br, 1670	343, 244, 244 sh, 205	[A] 8.11 (1H, d, $J_{5,7}$ = 2.2, H-5), 8.05 (1H, d, $J_{8,7}$ = 9.0, H-8), 7.82–7.71 (3H, m, H-2″,6″ + H-7), 7.62–7.48 (3H, m, H-3″,4″,5″), 6.89 (4H, q, J = 8.8, H-2′,6′,3′,5′), 5.25 (2H, s, CH_2), 3.77 (3H, s, OCH_3), 1.18 (9H, s, $C(CH_3)_3$)
3i	125–127 (c)	52	$C_{27}H_{24}N_4O_2$	3380, 3220, 2220, 1660	342, 249, 205	[A] 8.13–8.09 (2H, m, H-8 + H-5), 7.78–7.72 (3H, m, H-2″,6″ + H-7), 7.66–7.54 (5H, m, H-3″,4″,5″ + H-2′,6′), 7.06 (2H, d, J = 8.8, H-3′,5′), 5.36 (2H, s, CH_2), 1.18 (9H, s, $C(CH_3)_3$)
3j	[d]	98	$C_{26}H_{24}FN_3O_2$		342, 244, 206	[A] 8.20–8.00 (2H, m, H-8 + H-5), 7.80–7.70 (3H, m, H-2″,6″ + H-7), 7.62–7.50 (5H, m, H-3″,4″,5″ + H-2′,6′), 7.00–6.92 (2H, m, H-3′,5′), 5.26 (2H, s, CH_2O), 1.17 (9H, s, $C(CH_3)_3$)
3k	146–147 (c)	71	$C_{28}H_{27}N_3O_4$	3480, 3260, 1710, 1660	342, 255, 212	[A] 8.13 (1H, d, $J_{5,7}$ = 2.2, H-5), 8.11 (1H, d, $J_{8,7}$ = 9.0, H-8), 8.01 (2H, d, J = 8.6, H-2′,6′), 7.78–7.70 (3H, m, H-2″,6″ + H-7), 7.60–7.50 (3H, m, H-3″,4″,5″), 7.03 (2H, d, J = 8.6, H-3′,5′), 5.35 (2H, s, CH_2), 3.89 (3H, s, OCH_3), 1.18 (9H, s, $C(CH_3)_3$)
3l	82–85 (c)	90	$C_{24}H_{23}N_3O_4$		366, 257, 207	[A] 8.02 (1H, d, $J_{8,7}$ = 8.6, H-8), 7.85 (1H, d, $J_{5,7}$ = 2.2, H-5), 7.84–7.76 (3H, m, H-2″,6″ + H-7), 7.65–7.55 (3H, m, H-3″,4″,5″), 6.40 (2H, br s, NH_2 [b]), 6.27 (2H, s, H-2′,6′), 5.21 (2H, s, CH_2), 3.85 (6H, s, 3′,5′-OCH_3), 3.79 (3H, s, 4′-OCH_3)

Table 1 (*continued*)

Compound	M.p. (°C) [a]	Yield (%)	Analysis for C, H, N	IR ν_{max}(nujol) (cm^{-1})	UV λ_{max}(EtOH) (nm)	^{1}H NMR, δ_H (J in Hz) Solvent: [A] = $CDCl_3$, [B] = $CDCl_3$–DMSO-d_6 (3:1), [C] = DMSO-d_6
3m	208–212	93	$C_{23}H_{21}N_3O_3$	3330, 3075	365, 299, 257, 208	[B] 8.80 (2H, br s, NH_2 [b]), 8.01 (1H, d, $J_{8,7}$ = 8.4, H-8), 7.88 (1H, d, $J_{5,7}$ = 2.2, H-5), 7.86–7.77 (3H, m, H-2″,6″ + H-7), 7.70–7.61 (3H, m, H-3″,4″,5″), 6.16 (2H, d, J = 2.0, H-2′,6′), 6.09 (1H, d, J = 2.0, H-4′), 5.25 (2H, s, CH_2), 3.76 (6H, s, OCH_3)
3n	212–215	50	$C_{22}H_{19}N_3O_2$		363, 293, 258, 223, 209	[B] 8.85 (2H, br s, NH_2 [b]), 8.01 (1H, d, $J_{8,7}$ = 8.2, H-8), 7.87 (1H, d, $J_{5,7}$ = 2.2, H-5), 7.86–7.80 (3H, m, H-2″,6″ + H-7), 7.70–7.62 (3H, m, H-3″,4″,5″), 6.88 (4H, q, J = 8.0, H-2′,3′5′,6′), 5.24 (2H, s, CH_2), 3.75 (3H, s, OCH_3)
3o	223–235 (a)	87	$C_{22}H_{16}N_4O$	3420, 2240	366, 300 sh, 251, 207	[B] 8.80 (2H, br s, NH_2 [b]), 8.02 (1H, d, $J_{8,7}$ = 8.2, H-8), 7.89 (1H, d, $J_{5,7}$ = 2.2, H-5), 7.82–7.79 (3H, m, H-2″,6″), 7.72–7.58 (6H, m, H-3″,4″,5″ + H-7 + H-2′,6′), 7.16 (2H, d, J = 8.8, H-3′,5′), 5.39 (2H, s, CH_2)
3p	230–235	50	$C_{21}H_{16}FN_3O$	3450, 3300, 3150	366, 257, 220, 206	[B] 8.80 (2H, br s, NH_2 [b]), 8.01 (1H, d, $J_{8,7}$ = 8.6, H-8), 7.89 (1H, d, $J_{5,7}$ = 2.2, H-5), 7.83–7.78 (2H, m, H-2″,6″), 7.68–7.60 (4H, m, H-3″,4″,5″ + H-7), 7.02–6.98 (4H, m, H-2′,3′,5′,6′), 5.26 (2H, s, CH_2)
3q	289–293 (d)	91	$C_{22}H_{17}N_3O_3$	3480, 3300, 3260, 1670	367, 296, 256, 208	[C] 7.92 (2H, d, J = 7.8, H-2′,6′), 7.83 (1H, d, $J_{8,7}$ = 8.8, H-8), 7.82–7.75 (2H, m, H-2″,6″), 7.65 (1H, d, $J_{5,7}$ = 2.2, H-5), 7.60–7.53 (3H, m, H-3″,4″,5″), 7.44 (1H, d, $J_{7,8}$ = 8.8 and $J_{5,7}$ = 2.2, H-7), 7.15 (2H, d, J = 7.8, H-3′,5′), 6.64 (2H, s, NH_2 [b]), 5.37 2H, s, CH_2)
3r	215–217 (e)	42	$C_{23}H_{17}ClN_2O_3$	1710	340, 251,207	[A] 8.22 (1H, d, $J_{5,7}$ = 2.2, H-5), 8.14 (1H, d, $J_{8,7}$ = 8.2, H-8), 7.52 (2H, d, J = 8.6, H-2′,6′), 7.95–7.80 (3H, m, H-2″,6″ + H-7), 7.64–7.48 (3H, m, H-3″,4″,5″), 7.04 (2H, d, J = 8.2, H-3′,5′), 5.38 (2H, s, CH_2), 3.89 (3H, s, $COOCH_3$)
3s	195–198 (a)	81	$C_{24}H_{20}N_2O_4$	3500–2500 br, 1680 br	342, 330,254, 206	[B] 8.15–8.11 (2H, m, H-2″,6″), 8.05 (1H, d, $J_{5,7}$ = 2.2, H-5), 7.92 (2H, d, J = 7.8, H-2′,6′), 7.64 (2H, d, $J_{8,7}$ = 8.4, H-8), 7.58–7.42 (3H, m, H-3″,4″,5″), 7.07 (2H, d, J = 7.8, H-3′,5′), 5.36 (2H, s, CH_2), 4.62 (2H, q, CH_2CH_3), 1.50 (3H, t, CH_3CH_2)
3t	136–137 (a)	37	$C_{20}H_{22}N_2O_6$		327, 320, 313, 306, 300, 246, 207	[A] 7.85 (1H, d, $J_{5,7}$ = 1.4, H-5), 7.79 (1H, d, $J_{8,7}$ = 8.6, H-8), 7.55 (1H, dd, $J_{7,8}$ = 8.6 and $J_{7,5}$ = 1.4, H-7), 6.27 (2H, s, H-2′,6′), 5.17 (2H, s, CH_2), 4.16 (6H, s, 2,3-OCH_3), 3.84 (6H, s, 3′,5′-OCH_3), 3.80 (3H, s, 4′-OCH_3)
3u	152–154 (a)	92	$C_{19}H_{18}N_2O_5$	1715	327, 320, 313, 306, 300, 249, 209	[A] 8.00 (2H, d, J = 9.00, H-2′,6′), 7.84 (1H, d, $J_{5,7}$ = 2.00, H-5), 7.79 (1H, d, $J_{8,7}$ = 8.6, H-8), 7.54 (1H, dd, $J_{7,8}$ = 8.6 and $J_{7,5}$ = 2.0, H-7), 7.03 (2H, d, J = 9.0, H-3′,5′), 5.27 (2H, s, CH_2), 4.16 (3H, s, 3-OCH_3), 4.15 (3H, s, 2-OCH_3), 3.89 (3H, s, $COOCH_3$)
3v	235–237 (a)	67	$C_{18}H_{16}N_2O_5$	1680 br	327, 320, 313, 306, 300, 248, 208	[B] 7.98 (2H, d, J = 8.8, H-2′,6′), 7.84 (1H, d, $J_{5,7}$ = 1.8, H-5), 7.78 (1H, d, $J_{8,7}$ = 8.4, H-8), 7.56 (1H, dd, $J_{7,8}$ = 8.4 and $J_{7,5}$ = 1.8, H-7), 7.04 (2H, d, J = 8.8, H-3′,5′), 5.29 (2H, s, CH_2), 4.14 (6H, s, 2,3-OCH_3), 3.02 (1H, br s, COOH [b])
4a	145–147 (a)	78	$C_{31}H_{30}ClN_3O_6$	3280, 1730, 1630	340, 250, 205	[A] 8.18 (1H, d, $J_{8,7}$ = 8.8, H-8), 8.12 (1H, d, $J_{5,7}$ = 1.8, H-5), 7.93–7.86 (3H, m, H-2″,6″ + H-7), 7.82 (2H, d, J = 8.6, H-2′,6′), 7.55–7.52 (3H, m, H-3″,4″,5″), 7.05 (2H, d, J = 8.6, H-3′,5′), 6.97 (1H, d, J = 7.6, N*H*CH), 5.37 (2H, s, CH_2), 4.86–4.72 (1H, m, NHC*H*CH_2), 4.24 (2H, q, CH_2CH_3), 4.11 (2H, q, CH_2CH_3), 2.56–2.05 (4H, m, CH_2CH_2), 1.30 (3H, t, CH_2CH_3), 1.22 (3H, t, CH_2CH_3)

Table 1 (*continued*)

Compound	M.p. (°C) [a]	Yield (%)	Analysis for C, H, N	IR ν_{max} (nujol) (cm^{-1})	UV λ_{max} (EtOH) (nm)	^1H NMR, δ_H (*J* in Hz) Solvent: [A] = $CDCl_3$, [B] = $CDCl_3$–DMSO-d_6 (3:1), [C] = DMSO-d_6
4b	100	63	$C_{29}H_{27}N_3O_7$	3500–2500 br, 1710 br, 1640	346, 300 sh, 255, 206	[C] 8.50 (1H, d, $J_{8,7}$ = 8.6, H-8), 8.15–8.04 (2H, m, H-2″6″), 7.89 (2H, d, *J* = 8.8, H-2′,6′), 7.73 (1H, dd, $J_{7,8}$ = 8.6 and $J_{7,5}$ = 1.8, H-7), 7.62–7.50 (3H, m, H-3″,4″,5″), 7.17 (2H, d, *J* = 8.8, H-3′,5′), 5.45 (2H, s, CH_2O), 4.57 (2H, q, CH_2CH_3), 4.46-4.33 (1H, m, $NHCHCH_2$), 2.51 (1H, t, $CH_2CHCOOH$), 2.36 (2H, t, CH_2COOH), 2.20–1.88 (2H, m, $CHCH_2CH_2$), 1.43 (3H, t, CH_2CH_3)
4c	57–60 (i)	81	$C_{36}H_{40}N_4O_7$	3300, 1740, 1640	341, 249, 205	[A] 8.12 (1H, d, $J_{5,7}$ = 1.8, H-5), 8.10 (1H, d, $J_{8,7}$ = 8.6, H-8), 7.80 (2H, d, *J* = 8.8, H-2′,6′), 7.78–7.70 (2H, m, H-2″,6″), 7.74 (1H, dd, $J_{7,8}$ = 8.6 and $J_{7,5}$ = 1.8, H-7), 7.60–7.50 (3H, m, H-3″,4″,5″), 7.04 (2H, d, *J* = 8.8, H-3′,5′), 5.34 (2H, s, CH_2O), 4.85–4.75 (1H, m, $NHCHCH_2$), 4.23 (2H, q, CH_2CH_3), 4.10 (2H, q, CH_2CH_3), 2.55–2.05 (4H, m, CH_2CH_2), 1.30 (3H, t, CH_2CH_3), 1.25 (3H, t, CH_2CH_3), 1.22 (9H, s, $C(CH_3)_3$)
4d	222–225	63	$C_{27}H_{24}N_4O_6$	3500–2500 br, 1700 br	366, 300, 256, 207	[B] 8.22 (1H, d, *J* = 7.0, NH), 7.88 (2H, d, *J* = 8.4, H-2′,6′), 7.84–7.76 (4H, m, H-2″,6″ + H-6), 7.60–7.50 (3H, m, H-3″,4″,5″), 4.44 (1H, dd, *J* = 8.4 and 1.8, H-7), 7.04 (2H, d, *J* = 8.4, H-3′,5′), 6.13 (2H, s, NH_2), 5.31 (2H, s, CH_2), 4.58–4.45 (1H, m, $NHCHCH_2$), 4.05 (2H, a s, 2·COOH), 2.50–2.00 (4H, m, CH_2CH_2)
4e	110–112 (h)	66	$C_{27}H_{31}N_3O_8$	3280, 1730, 1640	327, 313, 306, 300, 249, 208	[A] 7.84 (1H, d, $J_{5,7}$ = 1.4, H-5), 7.82 (1H, d, $J_{8,7}$ = 8.4, H-8), 7.80 (2H, d, *J* = 8.8, H-3′,5′), 7.55 (1H, dd, $J_{7,8}$ = 8.4 and $J_{7,5}$ = 1.4, H-7), 7.04 (2H, d, *J* = 8.8, H-2′,6′), 6.92 (1H, d, NHCH), 5.27 (2H, s, CH_2O), 4.80 (1H, m, $NHCHCH_2$), 4.24 (2H, q, CH_2CH_3), 4.16 (6H, s, 2,3-OCH_3), 4.11 (2H, q, CH_2CH_3), 2.60–2.10 (4H, m, CH_2CH_2), 1.30 (3H, t, CH_3CH_2), 1.22 (3H, t, CH_3CH_2)
4f	64 (f)	71	$C_{23}H_{23}N_3O_8$	3500–2500 br, 1720 br, 1630	327, 320, 313, 306, 300, 249, 208	[B] 7.83 (2H, d, *J* = 8.6, H-2′,6′), 7.82 (1H, s, H-5), 7.54 (1H, dd, $J_{7,8}$ = 9.2 and $J_{7,5}$ = 1.4, H-7), 7.48 (1H, d, $J_{8,7}$ = 9.2, H-8), 7.02 (2H, d, *J* = 8.6, H-3′,5′), 5.26 (2H, s, CH_2O), 4.72 (1H, m, NH–C*H*–CH_2), 4.15 (6H, s, 2,3-OCH_3), 2.60–2.10 (4H, m, CH_2CH_2)

[a] Purification procedure: (a) crystallized from ethanol; (b) crystallized from acetonitrile; (c) crystallized from a mixture of ethanol and water; (d) crystallized from acetic acid; (e) crystallized from propanol; (f) crystallized from a mixture of diethyl ether and ethanol; (g) washed with diethyl ether; (h) washed with a mixture of petroleum ether at 40–60°C and diethyl ether; (i) from flash chromatography (eluant: mixture of diethyl ether and petroleum ether at 40–60°C).

[b] Exchanges with H_2O.

[c] Partially obscured by other resonances.

[d] Melting point not determined, product impure characterized as amine **2p** after hydrolysis.

Table 1which also reports the yields, melting points, analytical and spectroscopic data.

*3.1.5. General procedure for preparation of the acids **4b,d,f***

A mixture of the ester (**4a,c,e**) (0.4 g) suspended in a mixture of EtOH (6 ml)/1 M NaOH aqueous solution (3 ml) was stirred at room temperature for 3 h (**4a,e**) and for 4 h under reflux (**4c**). On evaporation of the solvent, the mixture was taken up with water and made acidic with 2 M HCl aqueous solution. The solids formed (**4b,d**) were collected and washed with water, whereas compound **4f** separated as an oil and was extracted with ether and purified as indicated in Table 1 which also reports the yields, melting points, analytical and spectroscopic data.

3.2. Pharmacology

Evaluation of anticancer and anti-HIV activity was performed on 13 (structures **3a–e,l,q,r,t,u,v** and **4a,e** of Fig. 1 and Scheme 1) out of the 28 compounds at NCI following the well-known [11] in vitro disease-oriented antitumor screening program against a panel of 60 human tumor cell lines and the anti-HIV drug testing system [12]. Compounds **3q,t** were not tested at 10^{-4} M because of solubility problems. No compound exhibited anti-HIV activity. The anticancer activity of each compound is deduced from dose–response curves and is presented in three different tables according to the data provided by NCI. In Table 2 the response parameters GI_{50}, TGI and LC_{50} refer to the concentration of the agent in the assay that produced 50% growth inhibition, total growth inhibition and 50% cytotoxicity, respectively, and are expressed as mean graph midpoints. In Table 3 we report the activities of those compounds which showed percent growth inhibition greater than 40% on subpanel cell lines at 10^{-4} M. In Table 4 we report the activities of those compounds which showed percent growth inhibition greater than 40% on subpanel cell lines at 10^{-5} M.

4. Results and discussion

The data of Table 2 show that the average sensitivity of all cell lines towards the tested agent, represented as mean graph midpoints, falls in the range $10^{-4.74}$–$10^{-4.00}$ M. Mean graph midpoints for the reported compounds also show that only GI_{50} was significant in the case of **3l**, the other compounds being placed in decreasing order of activity: **31** > **3c** > **3b** > **3e** > **4e** > **3a** > **3r** > **4a** > **3v** > **3u** > **3d**. Comparing these data with those of Table 3 we can clearly establish that compound **3l** was the most active in both series (56 out of 56 cell lines tested), endowed with the highest values of percent tumor growth inhibition which remained significant in some subpanel cell lines at 10^{-5} M (Table 4), while **4a,e** were the only interesting compounds in the series of quinoxaline analogues of 5,8-dideazafolate modified at position 10. Among the tested compounds, only **3d,u** exhibited a moderate sensitivity on a few cell lines, while in the other cases the values of tumor percent growth inhibition were sensibly high and spanned all subpanel cell lines.

Table 2
$-\log_{10}GI_{50}$, $-\log_{10}TGI$ and $-\log_{10}LC_{50}$ mean graph midpoints (MG-MID) of in vitro inhibitory activity tests for compounds **3a–e,l,r,t u,v** and **4a,e** against human tumor cell lines [a]

Compound	$-\log_{10}GI_{50}$	$-\log_{10}TGI$	$-\log_{10}LC_{50}$
3a	4.21	4.01	4.00
3b	4.50	4.11	4.01
3c	4.64	4.11	4.01
3d	4.02	4.00	4.00
3e	4.46	4.10	4.01
3l	4.74	4.30	4.04
3r	4.17	4.03	4.00
3t [b]	5.06	5.04	5.01
3u	4.03	4.00	4.00
3v	4.13	4.01	4.00
4a	4.16	4.03	4.00
4e	4.32	4.03	4.00

MG-MID: mean graph midpoints, the average sensitivity of all cell lines toward the test agent.
[a] From NCI.
[b] Not tested at 10^{-4} M.

In conclusion, the limited number of compounds tested allows us to make a few observations on structure–activity relationships. In the case of 6-phenoxymethylquinoxaline derivatives the presence of a phenyl group in C-2, an amino group in C-3, and a methoxyl group in 3′,4′,5′ seems to increase the potency of the series. Replacing the NH_2 group with Cl in C-3, the best results were obtained by compounds **3b,c,e**, with very close mean graph midpoints (GI_{50}, TGI, LC_{50}) (Table 2), thus indicating that little influence may be attributed to the oxyphenyl counterpart. These compounds also exhibited interesting selectivity at 10^{-5} M in the leukemia, non-small cell lung (NSCL), melanoma, renal and breast cancer cell lines (Table 4). When both the phenyl and chlorine (or amino) groups were replaced by two methoxyl groups in C-2 (or C-3), compound **3t** (not tested at 10^{-4} M because of inadequate solubility) exhibited three very significant cell line selectivities, with high values of percent tumor growth inhibition at 10^{-5} M (melanoma SK-Mel-2, 165%; prostate cancer PC-3, 177%; breast cancer BT-549, 143%). This selectivity was mantained high at 137% in the melanoma SK-Mel-2 cell line at 10^{-6} M. On the contrary, compound **3v** exhibited moderate to high percent growth inhibition activity in all subpanel cell lines at 10^{-4} M, although to a lesser extent than the above-mentioned compounds. The only two results available for the series of 6-methyloxybenzoylglutamatequinoxalines (**4a,e**) seem to indicate a greater sensitivity for compound **4e** over all subpanel cell lines (53 out of 56 lines tested) while in comparison compound **4a** exhibited higher values of percent growth inhibition over a fewer cell lines (39 out of 60). From an examination of Table 3, according to the values recorded in each subpanel cell line it is evident that NSCL and central

Table 3
Percent tumor growth inhibition recorded on subpanel cell lines at 10^{-4} M of compounds **3a–e,l,r,u,v** and **4a,e** [a]

Panel/cell lines	3a	3b	3c	3d	3e	3l	3r	3u	3v	4a	4e
Leukemia											
CCRF-CEM			137		104	118			101	49	102
HL-60 (TB)	64	66	130		91	159	70		74	57	118
K-562	nt	nt	74	nt	nt	99	nt	42	60	70	79
MOLT-4		114	71		125	155	52	nt	50	41	90
RPMI-8226	43	70	63		51	148			nt	62	61
SR		141	80		148	146	45		nt	53	66
Non-small cell lung cancer											
A549/ATCC	43	69	60		88	123	53		43	62	43
EKVX	58	92	71		88	137	43			69	nt
HOP-62	89	177	189	73	189	nt	98	59			44
HOP-92	106	116	132		152	123	98	nt	71	122	88
NCI-H226	nt	nt	nt	nt	nt	140	nt		57	115	42
NCI-23	75	99	115		98	nt	71	47		85	48
NCI-H322M		53			59	128			48		55
NCI-H460	52	74	79		94	161				44	92
NCI-H522	92	158	134	43	159	148	129	78	49	67	122
Colon cancer											
COLO 205	49	119	106		91	200	55		44		183
HCC-2998		88	79		80	99			65		70
HCT-116	55	81	95		87	180	54	52	89		67
HCT-15			65		49	156			62		55
HT29		74	56		61	nt					40
KM12		67			72	174				49	41
SW-620		65	67		55	96			53		44
Central nervous system cancer											
SF-268	42	91	94	40	96	90	75	44	56	109	63
SF-295	82	118	123		140	119	68	54	98	71	63
SF-539	77	135	136	43	128	154	88		73	108	72
SNB-19	72	121	120		nt	81	52			101	55
SNB-75	74	157	78		145	123	133		121	104	64
U251	128	124	161	62	156	90	96	70	55	86	45
Melanoma											
LOX IMVI		74	53		52	179	43		68	48	54
MALME-3M	102	151	146		139	164	111				116
M14		42	82		59	200		45	62		101
SK-MEL-2	75	96	99		84	152	54		43	150	113
SK-MEL-28		45	65		59	138			60		77
SK-MEL-5	76	111	158		85	195	56				88
UACC-257		48	47			151					96
UACC-62	52	87	nt		83	185	48	nt	90		96
Ovarian cancer											
IGROV1	59	118	93		114	131	54	nt		68	65
OVCAR-3		48	53		76	146					138
OVCAR-4	48	84	101		77	158	61			98	nt
OVCAR-5	55	70	90		79	126					
OVCAR-8		102	73		80	117	72		85	119	45
SK-OV-3	nt	nt	nt	nt	nt	nt	nt	nt		69	
Renal cancer											
786-0	88	161	150	63	123	111	142		47	98	46
A498	100	117	109		152	115	48	nt	51	117	58
ACHN	76	97	90	73	116	120	69		54	56	90
CAKI-1	82	171	140		125	115	120		51		
RXF 393	nt	nt	nt	nt	nt	111	nt	nt	nt	113	104
SN12C	40	78	94		65	96	46		46	41	78
TK-10	99	129	126	60	105	147	63	40	nt	94	81
UO-31		62	61			145			44		63

Table 3 (*continued*)

Panel/cell lines	3a	3b	3c	3d	3e	3l	3r	3u	3v	4a	4e
Prostate cancer											
PC-3		85	80		92	142		nt	60	136	80
DU-145		68	46		92	110		nt			57
Breast cancer											
MCF7	62	64	69		66	130	47	nt		54	76
MCF7/ADR-RES	63	119	77		89	140	60	nt	53	86	nt
MDA-MB-231/ATCC	71	146	141		143	119	127	nt	41	99	nt
HS 578T	79	95	99	41	119	109	100	nt	nt	96	73
MDA-MB-435		56	64		52	134	55	nt	99		104
BT-549	66	129	161		108	132	48	nt	nt	62	70
T-47D	50	92	65		82	140		nt	47	77	100
MDA-N		72	66		57	200		nt	86		95

[a] Compounds **3q** and **3t** were not tested at this molar concentration.
Values not recorded are below 40% growth inhibition; nt = not tested.

Table 4
Percent tumor growth inhibition recorded on subpanel cell lines at 10^{-5} M of compounds **3a–c,e,l,q,r,t,v** and **4a**

Panel/cell lines	3a	3b	3c	3e	3l	3q	3r	3t	3v	4a
Leukemia										
HL-60(TB)	41		42							57
K-562	nt	nt	50	nt			nt			46
MOLT-4	42				54					
RPMI-8226			50		53				nt	42
SR										49
Non-small cell lung cancer										
A549/ATCC										64
HOP-92	53				45	42		nt		
NCI-H322M					47					
NCI-H460			42							
NCI-H522		74	47	63			44			
Colon cancer										
HCT-116					62	nt				
Central nervous system cancer										
SF-539										
SNB-75									45	
Melanoma										
MALME-3M		46		51		nt	54			152
SK-MEL-2								165		
SK-MEL-28										
SK-MEL-5			59		42					
Renal cancer										
786-0		50	47			nt				
CAKI-1		42								
RXF 393	nt	nt	nt	nt			nt		nt	105
UO-31					47			nt		
Prostate cancer										
PC-3								177		149
Breast cancer										
MDA-MB-231/ATCC		57	43							
BT-549	43		55					143	nt	

Values not recorded are below 40% growth inhibition; nt = not tested at this molar concentration.

nervous system (CNS) cell lines were sensibly affected by our compounds. Comparison of these results with those available from the analogous 10-aza derivatives reported recently [1] shows that isosteric substitution of NH of the *N*-propargyl group in position 10 with oxygen as in **3b** and **3l** determined an increase in potency for the last compounds, while for **3u** the comparison was a little in favour of the aza analogues. The only comparison possible in the series of 4-[(quinoxalin-

6-yl)methoxy]benzoylglutamic derivatives between compounds **4a,e** with the corresponding 10-aza analogues (**5a** and **5e**) described in Ref. [1] showed that in the case of **4a**, which exhibited mean graph midpoints $GI_{50}=4.16$, $TGI=4.03$ and $LC_{50}=4.00$ compared with $GI_{50}=4.52$, $TGI=4.12$ and $LC_{50}=4.04$ for **5a**, the activity of **5a** was superior, while in the case of **4e** ($GI_{50}=4.32$, $TGI=4.03$, $LC_{50}=4.00$) a good overlap exists with the data reported for **5e** ($GI_{50}=4.35$, $TGI=4.12$, $LC_{50}=4.02$) in almost every subpanel cell line (expressed as percent tumor growth inhibition values), except in melanoma where the 10-aza derivative was superior.

References

[1] M. Loriga, G. Vitale, G. Paglietti, Quinoxaline chemistry. Part 9. Quinoxaline analogues of trimetrexate (TMQ) and 10-propargyl-5,8-dideazafolic acid (CB 3717) and its precursors. Synthesis and evaluation of in vitro anticancer activity, Farmaco 53 (1998) 139–149.

[2] M. Loriga, M. Fiore, P. Sanna, G. Paglietti, Quinoxaline chemistry. Part 4. 2-(R)-anilinoquinoxalines as non classical antifolate agents. Synthesis, structure elucidation and evaluation of in vitro anticancer activity, Farmaco 50 (1995) 289–301.

[3] M. Loriga, M. Fiore, P. Sanna, G. Paglietti, Quinoxaline chemistry. Part 5. 2-(R)-Benzylaminoquinoxalines as non classical antifolate agents. Synthesis and evaluation of in vitro anticancer activity, Farmaco 51 (1996) 559–568.

[4] M. Loriga, M. Piras, P. Sanna, G. Paglietti, Quinoxaline chemistry. Part 7. 2-[Aminobenzoates]- and 2-[aminobenzoylglutamate]-quinoxalines as classical antifolate agents. Synthesis and evaluation of in vitro anticancer, anti-HIV and antifungal activity, Farmaco 52 (1997) 157–166.

[5] M. Loriga, P.A. Moro, P. Sanna, G. Paglietti, Quinoxaline chemistry. Part 8. 2-[Anilino]-3-[carboxy]-6(7)-substituted quinoxalines as non classical antifolate agents. Synthesis and evaluation of in vitro anticancer, anti-HIV and antifungal activity, Farmaco 52 (1997) 531–537.

[6] J.E. Oatis Jr., J.B. Hynes Jr., Synthesis of quinazoline analogues of folic acid modified at position 10, J. Med. Chem. 20 (1977) 1393–1396.

[7] J.B. Hynes, S.A. Patil, R.L. Hagan, A. Cole, W. Kohler, J.H. Freisheim, Comparison of the biological effects of selected 5,8-dideazafolate analogues with their 2-desamino counterparts, J. Med. Chem. 32 (1989) 852–856.

[8] W.T. Mueller, G.K. Smith, S.J. Benkovic, J.B. Hynes, Inhibition of chicken liver 5-aminoimidazole-4-carboxamide ribonucleotide transformylase by 5,8-dideaza analogues of folic acid, Biochem. Pharmacol. 37 (1988) 449.

[9] F.H.S. Curd, D.G. Davey, G.J. Stacey, Synthetic antimalarials. Part XL. The effect of variation of substituents in 2-chloro-3-β-diethylaminoethylaminoquinoxaline, J. Chem. Soc. (1949) 1271–1277.

[10] E.I. Fairburn, B.J. Magerlein, L. Stubberfield, E. Stapert, D.I. Weisblat, Oxygen analogs of pteroic acid, J. Am. Chem. Soc. 76 (1954) 676–679.

[11] M.R. Boyd, Status of the NCI preclinical antitumor drug discovery screen, Princ. Pract. Oncol. 3 (10) (1989) 1–12.

[12] O.W. Weislow, R. Kiser, D. Fine, J. Bader, R.H. Shoemaker, M.R. Boyd, New soluble-formazan assay for HIV-1 cytopathic effects: application to high-flux screening of synthetic and natural products for AIDS antiviral activity, J. Natl. Cancer Inst. 81 (1989) 577–586.