ELSEVIER

Il Farmaco 53 (1998) 594–601

IL FARMACO

Quinoxaline chemistry
Part 12. 3-Carboxy-2[phenoxy]-6(7)substituted quinoxalines and *N*-[4-(6(7) substituted-3-carboxyquinoxalin-2-yl)hydroxy] benzoylglutamates. Synthesis and evaluation of in vitro anticancer activity

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

Dipartimento Farmaco Chimico Tossicologico, Università di Sassari, via Muroni 23/a, 07100 Sassari, Italy

Received 23 July 1998; accepted 20 October 1998

Abstract

Thirty quinoxalines bearing a substituted phenoxy or hydroxybenzoylglutamate group on position 2, a carboethoxy or carboxy group on position 3 and a trifluoromethyl group on position 6 or 7 of the heterocycle were prepared in order to evaluate the in vitro anticancer activity. Screening over 21 compounds selected at the National Cancer Institute (Bethesda, MD) showed that only few derivatives exhibited a moderate growth inhibition activity on various tumor panel cell lines at 10^{-4} molar concentration. The acid derivatives showed no growth inhibition activity. The results obtained in this series seem to indicate that in general carboxy or carboethoxy groups close to O-link with phenyl or benzoyl glutamates on position 2 are detrimental for anticancer activity.

Keywords: Anticancer agents; Quinoxaline derivatives; Phenoxyquinoxalines

1. Introduction

In two previous papers we have reported the synthesis and biological activities of quinoxaline derivatives[1,2] of compounds **A** and **B** (Fig. 1) as both classic and non-classic antifolate analogous agents. The results obtained from their screening have shown an interesting tumor growth inhibition on various cell lines between 10^{-5} and 10^{-4} molar concentrations. Some of these compounds still exhibited significant values of percent growth inhibition at the most diluted concentrations (10^{-8}–10^{-6} mol).

In particular from these series two compounds (**A-1** and **B-1**) (Fig. 2) emerged for an in deep in vivo investigation of anticancer activity at the National Cancer Institute (NCI), (Bethesda, MD).

In this context we also considered the effect of a replacement of the nitrogen link of the side chain on position 2 of the quinoxaline ring with an oxygen as in the series of compounds **C** and the results of their anticancer activity have been recently reported [3] (Fig. 3).

Moreover, for the first time we have also provided evidence that anticancer activity was associated in some cases with moderate to strong DHFR (rat or bovine liver) inhibitory activity. With this in mind we have now prepared compounds **1–30** in order to discover if the concomitant presence of a carboxy/carboethoxy group in position 3 and an O-link in position 2 with both substituted phenyl and benzoylglutamate side chains might reproduce similar or better results than those found for **B-1**. Thus, the choice of the substituents replicate our previous observations [1–3] (Fig. 4).

2. Chemistry

The previously described chloroquinoxalines (**31a–c**) [2] were reacted with the appropriate substituted phenols (**32**) to give compounds **1–6**, **13–18** and **20–25** or with diethyl *p*-hydroxybenzoylglutamate (**33**) [4] to give the ester **27–29** in fair yields (Table 1) according to Scheme 1. The acid **7–12**, **19**, **26** and **30** were obtained by alkaline hydrolysis.

3. Experimental

3.1. Chemistry

Melting points (m.p.) are uncorrected and were recorded on a Kofler or an electrothermal melting point apparatus.

* Corresponding author. Tel.: + 39-079-228719; fax: + 39-079-228720; e-mail: chimfarm@ssmain.uniss.it

0014-827X/98/$ - see front matter
PII S0014-827X(98)00075-5

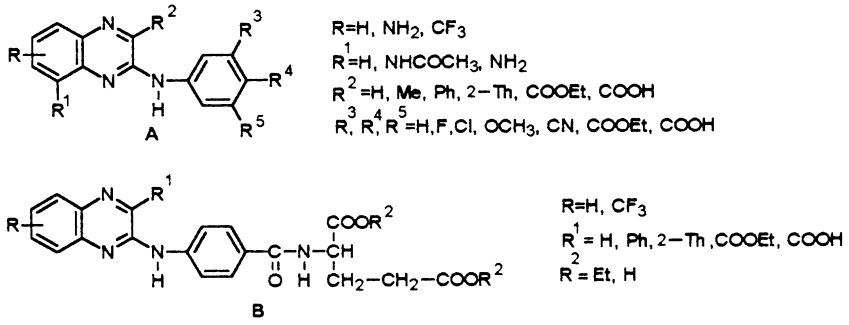


Fig. 1. Compounds **A** and **B**.

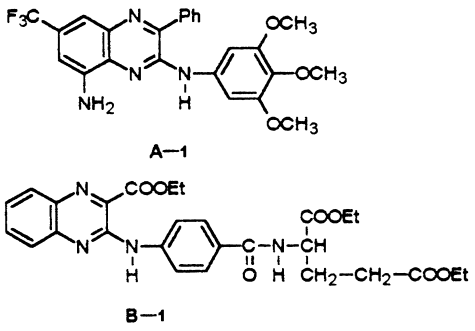


Fig. 2. Compounds **A-1** and **B-1**.

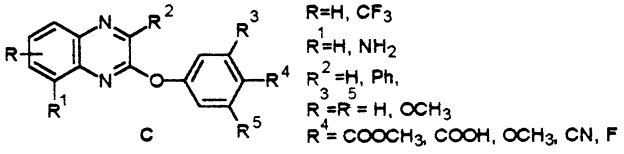


Fig. 3. Compound **C**.

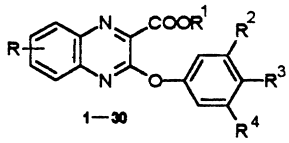
UV spectra are qualitative and were recorded in nanometers for a solution in ethanol with a Perkin Elmer Lambda 5 spectrophotometer. IR spectra are for Nujol mulls and were recorded on Perkin Elmer 781 instruments. ^{1}H NMR spectra were recorded at 200 MHz with a Varian XL-200 instrument using TMS as an internal standard. Elemental analyses were performed at the Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, University of Padua, Italy. The analytical results for C, H, N were within ± 0.4% of the theoretical values.

3.1.1. Intermediates

The intermediate chloroquinoxalines necessary for this work were obtained as previously described [2]. Diethyl p-hydroxybenzoylglutamate was prepared according to the indications in the literature [4].

*3.1.2. General procedure for the preparation of the 3-carboxyethyl 2-phenoxyquinoxalines (**1–6, 13–18, 20–25**)*

A mixture of equimolar amounts (15 mmol) of 2-chloroquinoxaline **31a–c** [2] and the suitably substituted phenol in



Compd.	R	R^1	R^2	R^3	R^4
1	H	Et	OMe	OMe	OMe
2	H	Et	OMe	H	OMe
3	H	Et	H	OMe	H
4	H	Et	H	CN	H
5	H	Et	H	F	H
6	H	Et	H	COOMe	H
7	H	H	OMe	OMe	OMe
8	H	H	OMe	H	OMe
9	H	H	H	OMe	H
10	H	H	H	CN	H
11	H	H	H	F	H
12	H	H	H	COOH	H
13	6-CF_3	Et	OMe	OMe	OMe
14	6-CF_3	Et	OMe	H	OMe
15	6-CF_3	Et	H	OMe	H
16	6-CF_3	Et	H	CN	H
17	6-CF_3	Et	H	F	H
18	6-CF_3	Et	H	COOMe	H
19	6-CF_3	H	H	COOH	H
20	7-CF_3	Et	OMe	OMe	OMe
21	7-CF_3	Et	OMe	H	OMe
22	7-CF_3	Et	H	OMe	H
23	7-CF_3	Et	H	CN	H
24	7-CF_3	Et	H	F	H
25	7-CF_3	Et	H	COOMe	H
26	7-CF_3	H	H	COOH	H
27	H	Et	H	CO-glu-Et	H
28	6-CF_3	Et	H	CO-glu-Et	H
29	7-CF_3	Et	H	CO-glu-Et	H
30	H	H	H	CO-glu-H	H

Fig. 4. Compounds **1–30** obtained according to Scheme 1.

Table 1
Melting points, yields, analytical and spectroscopic (IR, UV, ^{1}H NMR) data of compounds of Fig. 4 and Scheme 1

Compound	M.p. (°C)[a]	Yield	Analysis	IR (Nujol) (ν_{max} cm^{-1})	UV (EtOH) (λ_{max} nm)	^{1}H NMR, δ_H J (Hz)[b]
1	156–158 (a)	86	$C_{20}H_{20}N_2O_6$	1730	300, 245, 205	[A] 8.14 (1H, dd, H-5[c]), 7.79–7.67 (3H, m, arom.), 6.54 (2H, s, H-2′,6′), 4.58 (2H, q, CH_2CH$_3$), 3.89 (3H, s, 4′-OMe), 3.85 (6H, s, 3′,4′-OMe), 1.49 (3H, t, CH$_2$CH_3)
2	95–97 (a)	89	$C_{19}H_{18}N_2O_5$	1730	303, 243, 204	[A] 8.14 (1H, dd, H-5[c]) 7.20–7.66 (3H, m, arom.), 6.46 (2H, d, $J_{2',4'}$ 2.0, H-2′,6′), 6.41 (1H, d, $J_{4',2'}$ 2.0, H-4′), 4.57 (2H, q, CH_2CH$_3$), 3.80 (6H, s, 3′,5′ OMe), 1.48 (3H, t, CH$_2$CH_3)
3	65–67 (a)	95	$C_{18}H_{16}N_2O_4$	1730	300, 285, 245, 204	[A] 8.13 (1H, dd, H-5[c]), 7.74–7.63 (3H, m, arom.), 7.21 (2H, d, $J_{2',3'}$, 9.0, H-2′,6′), 6.97 (2H, d, $J_{3',2'}$, 9.0, H-3′,5′), 4.57 (2H, q, CH_2CH$_3$), 3.86 (3H, s, OMe), 1.49 (3H, t, CH$_2$CH_3)
4	125–128 (a)	87	$C_{18}H_{13}N_3O_3$	2250, 1730	332, 244, 204	[A] 8.19 (1H, dd, H-5[c]), 7.80–7.70 (5H, m, arom.), 7.43 (2H, d, $J_{3',2'}$ 8.6, H-3′,5′), 4.58 (2H, q, CH_2CH$_3$), 1.48 (3H, t, CH$_2$CH_3)
5	114–116 (a)	67	$C_{17}H_{13}FN_2O_3$	1730	332, 300, 243, 205	[A] 8.15 (1H, dd, H-5[c]), 7.80–7.64 (3H, m, arom.), 7.36–7.10 (4H, m, H-2′,3′,4′,5′), 4.58 (2H, q, CH_2CH$_3$), 1.49 (3H, t, CH$_2$CH_3)
6	113–115 (a)	66	$C_{19}H_{16}N_2O_5$	1730	333, 300, 244, 204	[A] 8.16 (1H, dd, H-5[c]), 8.16 (2H, d, $J_{2',3'}$ 8.8, H-2′,6′), 7.78–7.66 (3H, m, arom.), 7.36 (2H, d, $J_{3',2'}$ 8.8, H-3′,5′), 4.58 (2H, q, CH_2CH$_3$), 3.95 (3H, s, COOMe), 1.48 (3H, t, CH$_2$CH_3)
7	162–163 (a)	76	$C_{18}H_{16}N_2O_6$	3450, 1740	338, 324, 242, 228, 206	[A] 8.14 (1H, dd, H-5[c]), 7.84–7.72 (3H, m, arom.), 6.56 (2H, s, H-2′,6′), 5.90 (1H, brs, COOH[d]), 3.89 (3H, s, 4′-OMe), 3.84 (6H, s, 3′,5′-OMe)
8	141–143 (a)	72	$C_{17}H_{14}N_2O_5$	3400, 1730	340, 324, 243, 225, 205	[A] 8.13 (1H, dd, H-5[c]), 7.84–7.75 (3H, m, arom.), 6.47–6.42 (3H, m, H-2′,4′,6′), 3.80 (6H, s, 3′,5′-OMe), 3.52 (1H, brs, COOH[d])
9	147–149 (a)	96	$C_{16}H_{12}N_2O_4$	1720	340, 325, 278, 242 229, 205	[B] 8.08 (1H, dd, H-5[c]), 7.80–7.64 (3H, m, arom.), 7.20 (2H, d, $J_{2',3'}$ 8.6, H-2′,6′), 6.96 (2H, d, $J_{3',2'}$ 8.6, H-3′,5′), 4.09 (1H, s, COOH[d]), 3.85 (3H, s, OMe)
10	159–160 (b)	58	$C_{16}H_9N_3O_3$	2220, 1720	338, 324, 243, 204	[B] 8.10 (1H. dd, H-5[c]), 7.96–7.76 (3H, m, arom.), 7.50 (2H, d, $J_{2',3'}$ 8.4, H-2′,6′), 7.35 (2H, d, $J_{3',2'}$ 8.4, H-3′,5′) 5.90 (1H, brs, COOH[d]
11	162–164 (b)	72	$C_{15}H_9FN_2O_3$	1720	338, 324, 242, 230, 205	[B] 8.11 (1H, dd, H-5[c]), 7.85 (3H, m, arom.), 7.32–7.14 (4H, m, H-2′,3′,5′,6′), 6.48 (1H, brs, COOH[d])
12	210–213 (a)	94	$C_{16}H_{10}N_2O_5$	1720	340, 324, 243, 205	[B] 8.10 (3H, d, $J_{2',3'}$ 8.4, H-2′,6′ + 1H, arom.), 7.38 (2H, d, $J_{3',2'}$ 8.4, H-3′,5′), 4.97 (1H, brs, COOH[d])
13	110–113 (a)	93	$C_{21}H_{19}F_3N_2O_6$	1740	338, 325, 242, 205	[A] 8.46 (1H, s, H-5), 7.92 (2H, as, H-7,8), 6.53 (2H, s, H-2′,6′), 4.60 (2H, q, CH_2CH$_3$), 3.90 (3H, s, 4′-OMe), 3.86 (6H, s, 3′,5′-OMe), 1.51 (3H, t, CH$_2$CH_3)
14	73–75 (a)	39	$C_{20}H_{17}F_3N_2O_5$	1740	338, 325, 242, 203	[A] 8.45 (1H, s, H-5), 7.91 (2H, as, H-7,8), 6.44 (3H, m, H-2′,6′,4′), 4.58 (2H, q, CH_2CH$_3$), 3.81 (6H, s, 3′,5′-OMe), 1.49 (3H, t, CH$_2$CH_3)
15	68–72 (b)	79	$C_{19}H_{15}F_3N_2O_4$	1740	338, 331, 280, 243, 228, 204	[A] 8.44 (1H, s, H-5), 7.87 (2H, as, H-7,8), 7.21 (2H, d, $J_{2',3'}$ 9.0, H-2′,6′), 6.99 (2H, d, $J_{3',2'}$ 9.0, H-3′,5′), 4.59 (2H, q, CH_2CH$_3$), 3.86 (3H, s, 4′-OMe), 1.50 (3H, t, CH$_2$CH_3)
16	89–90 (a)	74	$C_{19}H_{12}F_3N_3O_3$	1740	336, 326, 242, 204	[A] 8.49 (1H, s, H-5), 7.98–7.85 (2H, m, H-7,8), 7.80 (2H, d, $J_{2',3'}$ 8.6, H-2′,6′), 7.44 (2H, d, $J_{3',2'}$ 8.6, H-3′,5′), 4.59 (2H, q, CH_2CH$_3$) 1.50 (3H, t, CH$_2$CH_3)
17	67–68 (a)	62	$C_{18}H_{12}F_4N_2O_3$	1730	331, 324, 241, 205	[A] 8.46 (1H, s, H-5), 7.88 (2H, m, H-7,8), 7.21 (4H, m, H-2′,3′,5′,6′), 4.59 (2H, q, CH_2CH$_3$) 1.50 (3H, t CH$_2$CH_3)
18	72–75 (a)	49	$C_{20}H_{15}F_3N_2O_5$	1730	338, 326, 243, 203	[A] 8.47 (1H, s, H-5), 8.18 (2H, d, $J_{2',3'}$ 8.6, H-2′,6′), 7.89 (2H, m, H-7,8), 7.37 (2H, d, $J_{3',2'}$ 8.6. H-3′,5′), 4.59 (2H, q, CH_2CH$_3$), 3.96 (3H, s, COOMe), 1.50 (3H, t, CH$_2$CH_3)
19	142–145 (a)	67	$C_{17}H_9F_3N_2O_5$	1700, 1750	334, 320, 240, 204	[B] 8.42 (1H, s, H-5), 8.15 (2H, d, $J_{2',3'}$ 8.4, H-2′,6′), 7.95–7.87 (2H, m, H-7,8), 7.38 (2H, d, $J_{3',2'}$ 8.4, H-3′,5′), 6.20–5.20 (2H, brs, 3,4′-COOH[d])
20	156–158 (a)	90	$C_{21}H_{19}F_3N_2O_6$	1730	340, 334, 245,205	[A] 8.26 (1H, d, $J_{5',6'}$ 8.8, H-5), 8.12 (1H, d, $J_{8,6}$ 2.0, H-8), 7.85 (H, dd, $J_{6,5}$ 8.6 and $J_{6,8}$ 2.0, H-6), 6.51 (2H, s, H-2′,6′), 4.60 (2H, q, CH_2CH$_3$), 3.90 (3H, s, 4′-OMe), 3.86 (6H, s, 3′,5′-OMe), 1.50 (3H, t, CH$_2$CH_3)

(continued)

Table 1 (*continued*)

Compound	M.p. (°C)[a]	Yield	Analysis	IR (Nujol) (ν_{max} cm^{-1})	UV (EtOH) (λ_{max} nm)	^{1}H NMR, δ_H J (Hz)[b]
21	124–125 (a)	80	$C_{20}H_{17}F_3N_2O_5$	1720	340, 332, 244, 204	[A] 8.25 (1H, d, $J_{5,6}$ 8,6, H-5), 8.12 (1H, d, $J_{8.6}$ 2.0, H-8), 7.86 (1H, dd, $J_{6,5}$ 8.6 and $J_{6,8}$ 2.0, H-6), 6.44 (3H, as, H-2′,4′,6′), 4.59 (2H, q, CH_2CH$_3$), 3.81 (6H, s, 3′,5′-OMe), 1.49 (3H, t, CH$_2$CH_3)
22	107–108 (a)	72	$C_{19}H_{15}F_3N_2O_4$	1730	336, 332, 245, 204	[A] 8.24 (1H, d, $J_{5,6}$ 8.7, H-5), 8.07 (1H, d, $J_{8.6}$ 1.8, H-8), 7.82 (1H, dd, $J_{6,5}$ 8.7 and $J_{6,8}$ 1.8, H-6), 7.20 (2H, d, $J_{2',3'}$ 9.0, H-2′,6′), 6.99 (2H, d, $J_{3',2'}$ 9.0, H-3′,5′), 4.59 (2H, q, CH_2CH$_3$), 3.86 (3H, s, OMe), 1.50 (3H, t, CH$_2$CH_3)
23	122–124 (a)	92	$C_{19}H_{12}F_3N_3O_3$	2240, 1730	340, 332, 244, 204	[A] 8.30 (1H, d, $J_{5,6}$ 8.6, H-5), 8.09 (1H, d, $J_{8,6}$ 1.8, H-8), 7.89 (1H, dd, $J_{6,5}$ 8.6 and $J_{6,8}$ 1.8, H-6), 7.82 (2H, d, $J_{2',3'}$ 8.8, H-2′,6′), 7.44 (2H, d, $J_{3',2'}$ 8.8, H-3′,5′), 4.60 (2H, q, CH_2CH$_3$), 1.50 (3H, t, CH$_2$CH_3)
24	107–109 (a)	84	$C_{18}H_{12}F_4N_2O_3$	1730	340, 332, 243, 206	[A] 8.26 (1H, d, $J_{5,6}$ 8.8, H-5), 8.07 (1H, d, $J_{8,6}$ 1.8, H-8), 7.84 (1H, dd, $J_{6,5}$ 8.8 and $J_{6,8}$ 1.8, H-6), 7.29–7.12 (4H, m, H-2′,3′,5′,6′), 4.60 (2H, q, CH_2CH$_3$), 1.50 (3H, t, CH$_2$CH_3)
25	106–107 (a)	76	$C_{20}H_{15}F_3N_2O_5$	1740, 1730	340, 331, 245, 204	[A] 8.28 (1H, d, $J_{5,6}$ 8.8, H-5), 8.18 (2H, d, $J_{2',3'}$, 8.8, H-2′,6′), 8.07 (1H, d, $J_{8,6}$ 2.2, H-8), 7.86 (1H, dd, $J_{6,5}$ 8.8 and $J_{6,8}$ 2.2, H-6), 7.37 (2H, d, $J_{3',2'}$ 8.8, H-3′,5′), 4.60 (2H, q, CH_2CH$_3$), 3.96 (2H, s, COOMe), 1.50 (3H, t, CH$_2$CH_3)
26	164–166 (a)	77	$C_{17}H_9F_3N_2O_5$	1750, 1720	336, 324, 244, 205	[B] 8.28 (1H, d, $J_{5,6}$ 8.8, H-5), 8.14 (2H, d, $J_{2',3'}$ 8.6, H-2′,6′), 8.05 (1H, s, H-8), 7.88 (1H, d, $J_{6,5}$ 8.8, H-6), 7.39 (2H, d, $J_{3',2'}$ 8.6, H-3′,5′), 5.60–4.80 (2H, brs, 3,4′-COOH[d])
27	134–136 (a)	91	$C_{27}H_{29}N_3O_8$	3300, 1760, 1730, 1640	336, 308, 244, 205	[A] 8.19–8.15 (1H, m, H-5), 7.95 (2H, d, $J_{2',3'}$ 8.6, H-2′,6′), 7.77–7.67 (3H, m, H-6,7,8),7.37 (2H, d, $J_{3',2'}$ 8.6, H-3′,5′), 7.12 (1H, d, J 7.4, NH-CH[a]), 4.89– 4.70 (1H, m, NHCHCH$_2$), 4.58 (2H, q, CH_2CH$_3$), 4.26 (2H, q, CH_2CH$_3$), 4.13 (2H, q, CH_2CH$_3$), 2.63–2.06 (4H, m, CH_2CH$_2$), 1.48 (3H, t, CH_3CH$_2$), 1.32 (3H, t, CH_3CH$_2$), 1.24 (3H, t, CH_3CH$_2$)
28	125–127 (a)	45	$C_{28}H_{28}F_3N_3O_8$	3300, 1740, 1640	338, 333, 242, 204	[A] 8.47 (1H, d, $J_{5,7}$ 1.8, H-5), 7.97 (2H, d, $J_{2',3'}$ 8.6, H-2′,6′), 7.90–7.85 (2H, m, H-7,8), 7.38 (2H, d, $J_{3',2'}$ 8.6, H-3′,5′), 7.16 (1H, d, J 7.2, NH[d]), 4.90–4.78 (1H, m, NHCHCH$_2$), 4.56 (2H, q, CH_2CH$_3$), 4.27 (2H, q, CH_2CH$_3$), 4.14 (2H, q, CH_2CH$_3$), 2.68–2.11 (4H, m, CH$_2$CH$_2$), 1.50 (3H, t, CH$_2$CH_3), 1.33 (3H, t, CH$_2$CH_3), 1.25 (3H, t, CH$_2$CH_3)
29	90–92 (a)	76	$C_{28}H_{28}F_3N_3O_8$	3300, 1740, 1640	340, 332, 244, 204	[A] 8.28 (1H, d. $J_{5,6}$ 8.6, H-5), 8.07 (1H, d, $J_{8,6}$ 2.0, H-8), 7.99 (1H, d. $J_{2',3'}$ 8.8, H-2′,6′), 7.86 (1H, dd, $J_{6,5}$ 8.6 and $J_{6,8}$ 2.0, H-6), 5.37 (2H, d, $J_{3',2'}$ 8.8, H-3′,5′), 7.17 (1H, d, J 7.4, NHCH[d]), 4.90–4.78 (1H, m, NHCHCH$_2$), 4.57 (2H, q, CH_2CH$_3$), 4.27 (2H, q, CH_2CH$_3$), 4.14 (2H, q, CH_2CH$_3$), 2.60–2.10 (4H, m, CH$_2$CH$_2$), 1.50 (3H, t, CH$_2$CH_3), 1.33 (3H, t, CH$_2$CH_3), 1.25 (3H, t, CH$_2$CH_3)
30	162–163 (a)	92	$C_{21}H_{17}N_3O_8$	1730	336, 324, 244, 204	[B] 8.21–8.11 (2H, m, arom.), 8.02 (2H, d, $J_{2',3'}$ 8.6, H-2′,6′), 7.78–7.68 (3H, m, arom. + CONH d, obscured), 7.35 (2H, d, $J_{2',3'}$ 8.6, H-3′,5′), 5.95–5.60 (3H, m, 3 COOH[d]), 4.67–4.65 (1H, m, NHCHCH$_2$), 2.60–2.09 (4H, m, CH$_2$CH$_2$)

[a] Purification procedure: (a) crystallized from ethanol; (b) crystallized from methanol; (c) crystallized from a mixture of methanol and water; (d) crystallized from acetic acid; (p) partially obscured by other resonances.
[b] Solvent: [A] = $CDCl_3$; [B] = $CDCl_3$/DMSO-d_6 (3:1).
[c] $J_{5,6}$ = 8.4 and $J_{5,7}$ = 1.8 Hz.
[d] Exchanges with H_2O.

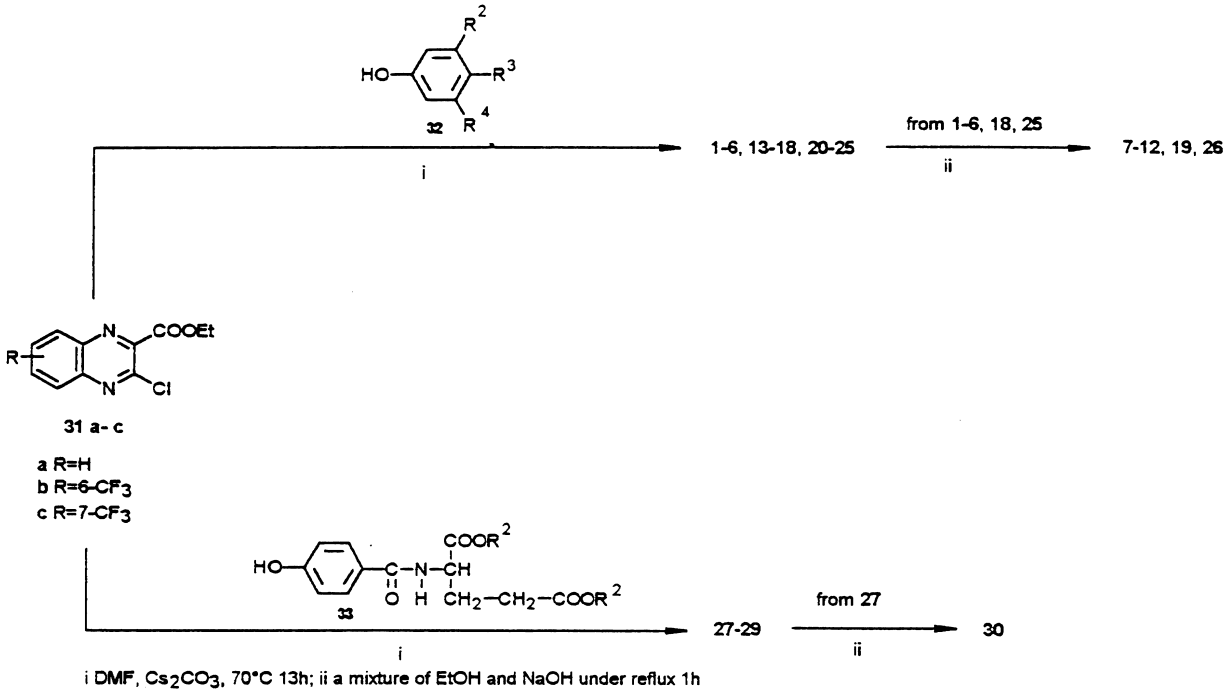
the presence of cesium carbonate, in anhydrous DMF (10 ml) was heated at 70°C under stirring for 13 h. Then water was added to complete precipitation of a solid that was collected and dried. Purification methods, yields, melting points, analytical and spectroscopic data are reported in Table 1.

*3.1.3. General procedure for the preparation of the 3-carboxy-2-phenoxyquinoxalines (**7–12**, **19**, **26**)*

A suspension of ester (**1–6**, **18**, **25**) (0.1 mmol) in a mixture of ethanol (10 ml) and 1 M NaOH aqueous solution (4 ml) was stirred under reflux for 1 h. On evaporation of the solvent, the mixture was diluted with water and made acidic with 2 M HCl aqueous solution to precipitate a solid that was collected and washed with water. Yields, melting points, analytical and spectroscopic data of the acids **7–12**, **19**, **26** are reported in Table 1.

*3.1.4. General procedure for preparation of the N[4-(6(7)substituted-3-carboxyquinoxalin-2-yl)hydroxy] benzoylglutamates (**27–29**)*

A mixture of equimolar amounts (1 mmol) of 2-chloroquinoxaline (**31a–c**) [2], diethyl-4-hydroxybenzoyl-l-gluta-



Scheme 1. (i) DMF, Cs_2CO_3, 70°C, 13 h; (ii) a mixture of EtOH and NaOH under reflux for 1 h.

mate [4] and cesium carbonate in anhydrous DMF (8 ml) was stirred at 70°C for 13 h. Then it was diluted with water to give a solid formed by the compounds **27–29**. 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.

*3.1.5. 4-(3-carboxyquinoxalin-2-yl)hydroxybenzoyl-glutamic acid (**30**)*

In an identical manner as for **7–12**, compound **30** was obtained in 92% yield. Spectroscopic data and melting points are reported in Table 1.

3.2. Pharmacology

Evaluation of anticancer and anti HIV activity was performed on 21 of 30 compounds (structures **1–8**, **10**, **15–18**, **20–25**, **27**, **30** of Fig. 2 and Scheme 1) at the NCI following the well known [5] in vitro disease-oriented anti-tumor screening program against a panel of 60 human tumor cell lines and anti HIV drug testing system [6]. No compound exhibited anti HIV activity. The anticancer activity 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 4 we reported the activities of those compounds which showed percent growth inhibition greater than 40% on subpanel cell lines at 10^{-4} molar concentration. The most diluted concentrations ($10^{-8} \div 10^{-5}$) were considered in the

Table 2
$-\log_{10}GI_{50}$, $-\log_{10}TGI$, $-\log_{10}LC_{50}$ mean graph midpoints (MG-MID) of in vitro inhibitory activity test for compounds **1–8**, **10**, **15–18**, **20–25**, **27** and **30** against human tumor cell lines[a]

Compound	$-\log_{10}GI_{50}$	$-\log_{10}TGI$	$-\log_{10}LC_{50}$
1	4.08	4.00	4.00
2	4.11	4.00	4.00
3	4.01	4.00	4.00
4	4.00	4.00	4.00
5	4.08	4.00	4.00
6	4.04	4.00	4.00
7	4.00	4.00	4.00
8	4.04	4.00	4.00
10	4.00	4.00	4.00
15	4.03	4.00	4.00
16	4.07	4.00	4.00
17	4.06	4.00	4.00
18	4.02	4.00	4.00
20	4.30	4.03	4.00
21	4.10	4.01	4.00
22	4.03	4.00	4.00
23	4.56	4.22	4.06
24	4.01	4.00	4.00
25	4.15	4.00	4.00
27	4.05	4.00	4.00
30	4.01	4.00	4.00

[a] MG-MID, mean graph midpoints: the average sensitivity of all cell lines toward the test agent; tumor cell lines from NCI.

Table 3
Percent tumor growth inhibition recorded on sub-panel cell lines at 10^{-4} M of compounds **1–6**, **15–18**, **20–25**, **27**

Panel/cell lines	**1**	**2**	**3**	**4**	**5**	**6**	**15**	**16**	**17**	**18**	**20**	**21**	**22**	**23**	**24**	**25**	**27**
Leukemia																	
CCRF-CEM	–[a]	–	–	–	–	64	61	60	47	51	nt	–	–	–	–	–	–
HL-60 (TB)	–	nt	–	–	–	nt	49	57	57	78	44	–	nt	–	–	–	–
K-562	–	–	–	–	–	–	40	55	–	45	81	–	–	–	63	–	nt
MOLT-4	–	–	–	–	–	96	71	64	46	42	–	–	–	–	–	–	–
RPMI-8226	–	–	–	–	–	–	68	60	49	–	–	–	–	–	–	–	–
SR	–	–	–	–	nt	nt	44	64	44	49	nt	nt	nt	54	–	–	–
Non-small cell lung cancer																	
A549/ATCC	–	42	–	–	–	–	42	47	40	–	67	50	–	nt	–	nt	–
EKVX	–	60	–	–	–	46	41	–	nt	–	72	–	56	178	–	48	–
HOP-62	98	56	–	–	44	–	–	nt	52	nt	84	60	–	199	–	112	59
HOP-92	79	84	–	51	73	44	–	–	–	40	74	78	53	137	–	81	49
NCI-H226	nt	–	–	–	–	–	41	–	nt	–	109	60	–	147	46	74	–
NCI-23	52	nt	nt	–	nt	nt	nt	nt	–	nt	nt	nt	nt	89	–	50	–
NCI-H322M	–	48	–	–	–	–	–	74	–	–	–	60	42	91	–	41	–
NCI-H460	–	60	–	–	–	–	59	46	47	44	73	62	–	63	–	68	–
NCI-H522	55	59	60	–	nt	nt	54	46	–	57	nt	nt	–	nt	–	nt	–
Colon cancer																	
COLO 205	–	53	–	–	–	–	–	–	–	–	118	41	–	105	–	–	–
HCC-2998	–	nt	–	–	nt	nt	42	55	–	–	nt	nt	nt	61	–	–	–
HCT-116	–	48	–	–	–	–	43	90	109	46	64	42	–	82	–	50	59
HCT-15	–	–	–	–	–	40	48	45	46	64	56	–	–	81	–	–	–
HT29	–	–	–	–	–	40	nt	nt	nt	nt	53	40	–	110	–	55	–
KM12	–	55	–	–	–	44	–	–	–	–	89	76	48	88	–	63	–
SW620	–	–	–	–	–	–	–	–	–	–	68	–	–	87	–	42	–
SNC cancer																	
SF-268	49	55	–	–	59	–	–	–	–	–	78	51	40	171	–	55	–
SF-295	75	45	–	–	57	–	–	–	–	–	51	55	–	176	–	67	–
SF-539	75	59	–	–	41	–	–	–	–	–	54	58	–	179	–	56	40
SNB-19	–	42	–	–	–	–	–	–	–	–	95	59	–	175	–	81	53
SNB-75	78	nt	–	–	99	89	–	–	–	–	115	111	41	197	–	76	101
U251	100	54	–	–	58	–	–	–	–	–	61	52	–	129	–	87	60
Melanoma																	
LOX IMVI	–	–	–	–	–	–	44	56	–	56	–	–	–	81	–	–	–
MALME-3M	–	–	–	–	–	–	41	–	–	43	83	68	61	nt	–	nt	–
M14	–	–	–	–	–	–	–	–	–	60	–	–	–	150	–	72	–
SK-MEL-2	–	–	–	–	–	–	–	51	–	–	–	–	–	189	–	82	–
SK-MEL-28	52	–	–	–	–	–	–	–	–	–	40	–	–	86	–	53	–
SK-MEL-5	–	47	–	–	–	–	43	nt	43	47	99	112	–	83	–	81	–
UACC-257	–	–	–	–	–	–	–	–	–	–	76	–	–	122	–	41	–
UACC-62	–	–	–	–	–	–	–	–	–	–	51	–	–	178	–	61	–
Ovarian cancer																	
IGROV1	–	44	–	–	–	–	–	–	–	–	59	65	45	193	–	64	–
OVCAR-3	–	–	–	–	–	–	47	–	–	–	44	–	–	nt	nt	nt	–
OVCAR-4	–	79	–	–	–	48	45	41	59	54	103	104	–	109	–	50	–
OVCAR-5	–	–	–	–	–	–	–	–	–	–	50	–	–	nt	nt	nt	–
OVCAR-8	–	–	–	–	–	–	–	–	–	–	68	44	–	145	–	–	79
SK-OV-3	nt	–	–	–	–	–	nt	nt	nt	nt	50	50	–	136	–	–	nt
Renal cancer																	
786-0	80	71	–	–	48	–	–	–	41	–	74	48	–	176	–	60	86
A498	nt	–	–	–	–	–	60	52	–	–	–	–	–	131	–	–	nt
ACHN	66	49	–	–	53	–	–	–	–	–	76	49	–	97	–	40	–
CAKI-1	54	nt	nt	69	89	–	–	–	–	–	nt	nt	62	125	–	48	–

(continued)

Table 3 (*continued*)

Panel/cell lines	**1**	**2**	**3**	**4**	**5**	**6**	**15**	**16**	**17**	**18**	**20**	**21**	**22**	**23**	**24**	**25**	**27**
RXF 393	nt	60	–	–	62	–	66	90	71	52	81	–	–	176	–	60	nt
SN12C	–	–	–	–	–	–	–	–	–	–	40	–	–	nt	nt	nt	–
TK-10	67	–	–	–	–	–	–	–	–	–	57	–	–	108	–	73	47
UO-31	–	–	–	–	–	–	–	46	56	–	–	–	–	86	–	–	–
Prostate cancer																	
PC-3	–	–	–	–	–	–	41	–	–	–	44	50	–	170	–	86	–
DU-145	–	44	–	–	–	–	–	–	–	–	74	53	–	167	–	69	–
Breast cancer																	
MCF7	–	–	–	–	–	–	42	46	45	47	42	–	42	167	–	71	–
MCF7/ADR-RES	–	–	–	–	–	–	–	–	–	–	74	–	–	136	–	44	65
MDA-MB-231/ATCC	88	48	–	nt	56	–	–	–	–	–	52	–	–	183	–	54	102
HS 578T	88	94	–	–	72	–	–	–	–	–	110	117	46	154	–	99	–
MDA-MB-435	–	–	–	–	–	–	–	–	–	–	44	–	–	88	–	58	–
BT-549	–	–	–	–	–	–	–	–	–	–	–	–	–	174	–	65	45
T-47D	–	–	–	–	–	–	41	58	63	–	–	–	–	97	–	–	–
MDA-N	41	–	–	–	–	–	–	–	–	–	–	–	–	–	nt	–	–

[a] –, below 40% growth inhibition; nt, not tested at this molar concentration.

Table 4
Comparison of the inhibitory activity of compounds on some cell lines at the most diluted concentrations

Cell line	Compound	Percent tumor growth inhibition at the indicated molar concentration			
		10^{-8}	10^{-7}	10^{-6}	10^{-5}
Leukemia					
HL-60 (TB)	**2**	35	15	28	52
RPMI-8226	**2**	–	–	–	48
HL-60 (TB)	**3**	–	34	26	20
SR	**3**	23	41	35	11
HL-60 (TB)	**4**	21	25	38	29
NSCL					
NCI-H522	**3**	30	33	43	42
HOP-92	**5**	48	49	45	57
HOP-92	**20**	17	33	33	45
NCI H226	**23**	21	13	36	45
Colon cancer					
KM12	**3**	23	9	18	44
HCT-116	**17**	–	30	30	61
CNS					
SNB-75	**4**	81	44	72	47
SNB-19	**5**	33	14	29	41
SNB-75	**5**	41	–	20	47
Ovarian cancer					
OVCAR-4	**8**	13	36	54	94
OVCAR-4	**20**	–	–	–	112
Renal cancer					
CAKI-1	**4**	59	77	31	61
CAKI-1	**5**	64	25	48	70
A498	**15**	35	50	40	–
786-0	**20**	–	–	–	46
ACHN	**20**	–	–	–	55
Breast cancer					
BT-549	**8**	–	–	–	55
T-47D	**17**	–	–	–	63

case of compounds which showed a significant percent growth inhibition (Table 4).

4. Results and discussion

From the data of Table 2 we can deduce that the average inhibitory activity of the test agent, represented as mean graph midpoints, falls in the range $10^{-4,5}$–10^{-4} molar concentration. Mean graph midpoints show that only GI_{50} was significant in the case of compounds **20**, **23** and **25**, whereas both TGI and LC_{50} exhibited a low range of sensitivity.

The data of Table 3 show that at 10^{-4} M compounds **20**, **23** and **25** were the most active endowed with a broad range of sensitivity (48, 44 and 38 of 60 cell lines tested, respectively), followed in decreasing order of activity by **2** = **21** > **15** > **16** > **1** = **17** = **18** > **5** = **13** > **22** > **6** > **4**

= **24** > **23**. The tested acids **7**, **8**, **10** and **30** were not reported in Table 3 for they showed very low percent growth inhibition values (below 40%). Compounds listed in Table 4 exhibited no activity at 10^{-5} M, only **2**, **5** and **20** showed growth inhibition over a few cell lines (Table 4).

From the data of Table 4 we can observe that some compounds (**3**, **4**, **5**, **15**, **17**, **20** and **23**) exhibited interesting selectivity at the lowest concentrations (10^{-8}–10^{-5}). Compound **5** maintained almost an equal percent growth inhibition between 10^{-8} and 10^{-5} molar concentration against HOP 92, whereas compound **4** exhibited the highest values of inhibition at 10^{-8} M against SNB75.

In conclusion, it seems particularly interesting to note that among the three examples of active compounds (**20**, **23**, **25**) the association between the trifluoromethyl group with a lipophilic substituent on side chain still holds in this type of compounds as observed in the case of the corresponding aza analogues. On the other hand, it was disappointing that no one compound bearing benzoylglutamate moiety, despite the strict analogy with the model **B-1**. was active and in general the presence of carboxyethyl group was detrimental for the activity as a whole.

References

[1] M. Loriga, M. Fiore, P. Sanna, G. Paglietti, Quinoxaline chemistry. Part 4: 2-(*R*)-anilinoquinoxalines as non-classic antifolate agents. Synthesis, structure elucidation and evaluation of in vitro anticancer activity, Farmaco 50 (1997) 289–301.

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

[3] P. Corona, G. Vitale, M. Loriga, G. Paglietti, Quinoxaline chemistry. Part 11 – 3-Phenyl-2[phenoxy- and phenoxymethyl]6(7) or 6,8-substituted quinoxalines and *N*[4-(6(7)substituted or 6,8-disubstituted-3-phenylquinoxalin-2-yl)hydroxy or hydroxymethyl]benzoylglutamates. Synthesis and evaluation of in vitro anticancer activity and enzymatic inhibitory activity against dihydrofolate reductase (DHFR) and thymidylate synthase (TS), Farmaco 53 (1998) 480–493.

[4] E.I. Fairburn, B.I. Magerlein, L. Stubberfield, E. Stapert, D.I. Weisblat, Oxygen analogues of pteroic acid, J. Am. Chem. Soc. 76 (1954) 676–678.

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

[6] O.V. Weislow, R. Kiser, D. Fine, J. Bader, R.H. Shoemaker, M.R. Boyd, New Soluble–Formazan assay for HIV-1 cytophathic effect: application to high-flux screening of synthetic and natural products for AIDS antiviral activity, J. Natl. Cancer Inst. 81 (1989) 577–586.