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Quinoxaline chemistry. Part 14. 4-(2-Quinoxalylamino)-phenylacetates and 4-(2-quinoxalylamino)-phenylacetyl-L-glutamates as analogues–homologues of classical antifolate agents. Synthesis and evaluation of in vitro anticancer activity

Sandra Piras, Mario Loriga, Giuseppe Paglietti *

Dipartimento Chimico Tossicologico, Università di Sassari, Via Muroni, 23, 07100 Sassari, Italy

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Abstract

Among a new series of 26 4-(3-substituted-2-quinoxalylamino)phenylacetates and 4-(3-substituted-2-quinoxalylamino)-phenylacetyl-L-glutamates, eight were selected at NCI for evaluation of their in vitro anticancer activity. The results obtained in comparison with the corresponding nor-compounds series seem to indicate that this type of homologation is not helpful. © 2002 Elsevier Science S.A. All rights reserved.

Keywords: Anticancer activity; Quinoxalines derivatives; (Quinoxalylamino)-phenylacetates and (quinoxalylamino)-phenylacetyl-L-glutamate

1. Introduction

Bioisosteric replacement of the pteridine ring in the classical antifolates with quinoxaline has proved to be a good substrate for the biological activity as both in vitro anticancer and anti-DHFR [1–8]. Among the various types of quinoxalines designed for this purpose we have considered the effect of homologation in both *para* positions of the benzoyl-glutamate moiety. The series of quinoxalines bearing at 2 position the aminomethylbenzoylglutamate moiety has just appeared [9]. In this report we describe the synthesis of compounds **3**–**28** where homologation takes place on the side of the carboxylic moiety. In the heterocyclic ring we have placed the usual substitutions that in previous cases had shown the most similarities with the classical antifolates and they are reported in Fig. 1.

2. Chemistry

The starting material for the preparation of compounds **3**–**28** is represented by the chloroquinoxalines **1a**–**f** of Scheme 1, which were all obtained according to previously reported procedures [1,3,9–12]. Nucleophilic attack by ethyl *p*-aminophenylacetate gave compounds **3**–**8** in good yields. Conversion by alkaline hydrolysis into the acids **9**–**14** was necessary for elongation of side chain with diethyl L-glutamate in the presence of diethylcyanophosphonate and triethylamine to give the esters **15**–**20**. In the case of the esters **6**,**7** and **8** saponification occurred also at the ester group in C-3 to give the acids **12**, **13** and **14** which only in the case of **12** and **13** underwent further amidification with diethyl L-glutamate at carboxy group in position 3 to give the ester amides **27**, **28**. In the end the desired acids **21**–**26** were obtained by alkaline hydrolysis in hydroalcoholic medium.

3. Experimental

Melting points are uncorrected and were recorded on a Kofler or an Electrothermal melting point apparatus.

* Correspondence and reprints.
E-mail address: paglieti@ssmain.uniss.it (G. Paglietti).

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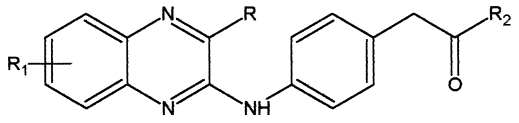
UV spectra are qualitative and were recorded in nm in ethanol solution with a Perkin–Elmer Lambda 5 spectrophotometer. IR spectra (Nujol mulls) were recorded with Perkin–Elmer 781 instrument. ^{1}H NMR spectra were recorded at 200 MHz with a Varian XL-200 instrument using TMS as internal standard. Elemental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

3.1. Chemistry

3.1.1. Intermediates

The intermediate chloroquinoxalines necessary for this work were known and prepared according to the data of the literature as follow: **1a** [10], **1b** [11], **1c** [3], **1d** [12], **1e** [9], and **1f** [1].



3-28

Compd	R	R_1	R_2
3	Ph	H	OEt
4	2-Th	H	OEt
5	H	6,7-F	OEt
6	COOEt	H	OEt
7	COOEt	7-CF_3	OEt
8	COOEt	6,7-F	OEt
9	Ph	H	OH
10	2-Th	H	OH
11	H	6,7-F	OH
12	COOH	H	OH
13	COOH	7-CF_3	OH
14	COOH	6,7-F	OH
15	Ph	H	GluEt
16	2-Th	H	GluEt
17	H	6,7-F	GluEt
18	COOH	H	GluEt
19	COOH	7-CF_3	GluEt
20	COOH	6,7-F	GluEt
21	Ph	H	GluH
22	2-Th	H	GluH
23	H	6,7-F	GluH
24	COOH	H	GluH
25	COOH	7-CF_3	GluH
26	COOH	6,7-F	GluH
27	COGluEt	H	GluEt
28	COGluEt	7-CF_3	GluEt

Fig. 1. Compounds **3**–**28** obtained according to Scheme 1.

3.1.2. General procedure for the preparation of the esters *3–8*

A mixture of equimolar amounts (2 mmol) of chloroquinoxalines (**1a**–**f**) and commercially available (Aldrich) ethyl 4-aminophenylacetate in ethanol (10 ml) was refluxed for 13 h (compounds **5**–**7**), for 24 h (compounds **4**, **8**) and for 72 h (compound **3**). On cooling the yellow–orange precipitates were collected and washed with ethanol to give **3**–**8** as crude products which were recrystallized from ethanol. Yields, m.p. values, analytical and spectroscopic (IR, UV, ^{1}H NMR) data are reported in Table 1.

3.1.3. General procedure for the preparation of the acids *9–14*

A mixture of the ester (**3**–**8**) (0.7 mmol) in ethanol (10 ml) and 2M NaOH (5 ml) in the case of compounds **3**, **4** or 1M NaOH (5 ml) in the case of compounds **5**–**8** was stirred at room temperature for 2.5 h (**3**, **4**) and under reflux for 4 h (**5**–**8**). On evaporation of the solvent, the mixture was taken up with water and made acidic with 2 M HCl. The red–orange products (**9**–**14**) were collected and washed with water. Yields, m.p. values, analytical and spectroscopic data are reported in Table 1.

3.1.4. General procedure for the preparation of the esters *15–20* and isolation of compounds *27*, *28*

An equimolar mixture (1.2 mmol) of compounds (**9**–**11**), diethyl L-glutamate hydrochloride and diethyl cyanophosphonate in the presence of 2 mole equiv. of TEA, and a ratio of 1:2:2 and 4 mole equiv. of TEA in the case of **12**–**14**, was stirred under nitrogen at room temperature for 2 h. The resulting solution was poured into a mixture of ethyl acetate and benzene in 3:1 ratio (60 ml). The organic phase was shaken with water (50 ml), then with saturated sodium carbonate aqueous solution (60 ml), rewashed with water (50 ml) and, if necessary, with saturated sodium chloride aqueous solution. Eventually, after drying over anhydrous sodium sulfate, on evaporation of the solvent compounds **15**–**17**, **27**, **28** were obtained as yellow–orange solids. From the alkaline mother liquors, on standing compounds **18**–**20** were separated as pure yellow solids. Yields, m.p. values, analytical and spectroscopic data are reported in Table 1.

3.1.5. General procedure for the preparation of the acids *21–26*

A suspension of the ester (**15**–**20**) (1 mmol) in a mixture of ethanol (10 ml) and 1 M NaOH (5 ml) was stirred at room temperature for 4 h. The red–orange solution formed was evaporated in vacuo and taken up with water then made acidic with 2 M HCl. A solid from yellow to red was collected and thoroughly washed with water and eventually dried. Yields, m.p.

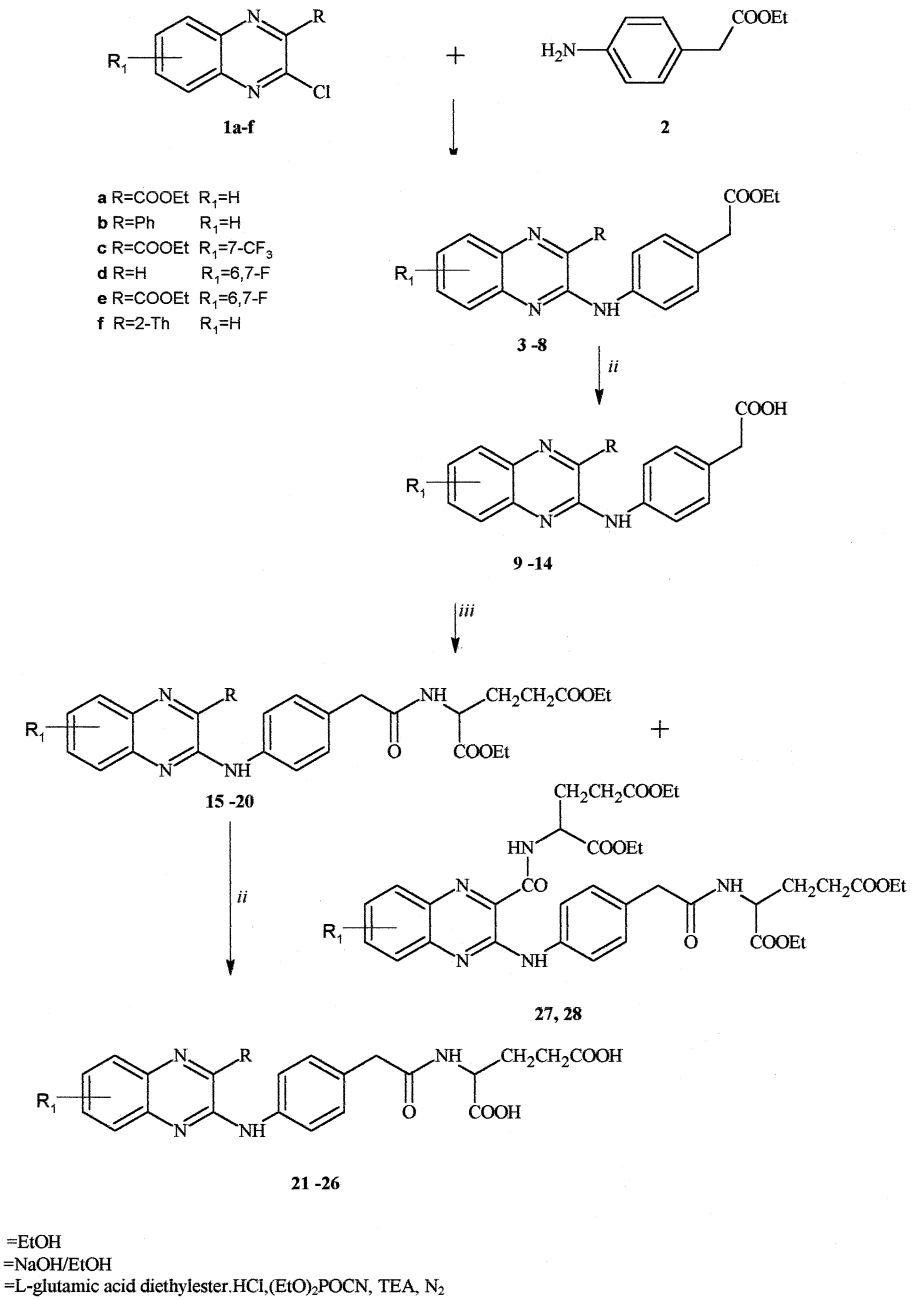
Table 1
Melting point, yield, analytical and spectroscopic (IR, UV, ^{1}H NMR) data of the compounds of Fig. 1

Comp.	m.p. (°C) [a]	Yield (%)	Analysis for	IR (nujol) (ν_{max} cm^{-1})	UV (EtOH) (λ_{max} nm)	^{1}H NMR, δ_H (J in Hz) [b]
3	65–68 (a)	50	$C_{24}H_{21}N_3O_2$	3380, 1710	370, 270, 211, 193	[A] 7.99–7.77 (2H, m, arom.), 7.76–7.44 (7H, m, arom), 7.72 (2H, d, $J = 8.4$, H-2′,6′, 7.29 (2H, d, $J = 8.4$, H-3′,5′, 4.14 (2H, q, CH_2), 3.60 (2H, s, CH_2), 1.26 (3H, t, CH_3)
4	69–71 (a)	51	$C_{22}H_{19}N_3O_2S$	3400, 1720	385, 276, 196	[A] 7.98–7.92 (1H, m, arom.), 7.86–7.72 (1H, m, arom.), 7.77 (2H, d, $J = 8.6$, H-2′,6′), 7.31 (2H, d, $J = 8.6$, H-3′,5′), 7.29–7.25 (1H, m, arom.), 4.15 (2H, q, CH_2), 3.62 (2H, s, CH_2), 1.27 (3H, t, CH_3)
5	130–133 (a)	62	$C_{18}H_{15}F_2N_3O_2$	3380, 1720	382, 332, 283, 208	[A] 8.34 (1H, s, H-3), 7.64 (1H, dd[p], $J = 11.2$ and 8.0, H-8), 7.63 (2H, d, $J = 8.4$, H-2′,6′), 7.49 (1H, dd, $J = 8.0$ and 11.2, H-5), 7.29 (2H, d, $J = 8.4$, H-3′,5′), 4.19 (2H, q, CH_2), 3.63 (2H, s, CH_2), 1.29 (3H, t, CH_3)
6	78–80 (a)	62	$C_{21}H_{21}N_3O_4$	3460, 3320, 1730, 1700	425, 290, 222, 206	[A] 10.33 (1H, s, NH), 8.04 (1H, d, $J = 8.2$, arom.), 7.88 (2H, d, $J = 8.4$, H-2′,6′), 7.86–7.67 (2H, m, arom.), 7.52–7.45 (1H, m, arom.), 7.32 (2H, d, $J = 8.4$, H-3′,5′), 4.60 (2H, q, CH_2), 4.17 (2H, q, CH_2), 3.62 (2H, s, CH_2), 1.54 (3H, t, CH_3), 1.27 (3H, t, CH_3)
7	112–115 (a)	68	$C_{22}H_{20}F_3N_3O_4$	3340, 1725, 1690	427, 304, 287, 219	[A] 10.39 (1H, s, NH), 8.14 (1H, d, $J = 8.8$, H-5), 8.08 (1H, s, H-8), 7.86 (2H, d, $J = 8.4$, H-2′,6′), 7.64 (1H, dd, $J = 1.8$ and 1.8, H-6), 7.34 (2H, d, $J = 8.4$, H-3′,5′), 4.61 (2H, q, CH_2), 4.17 (2H, q, CH_2), 3.63 (2H, s, CH_2), 1.54 (3H, t, CH_3), 1.31 (3H, t, CH_3)
8	72–74 (a)	49	$C_{21}H_{19}F_2N_3O_4$	3280, 1730, 1690	426, 288, 219	[A] 10.34 (1H, s, NH), 7.81 (2H, d, $J = 8.6$, H-2′,6′), 7.78 (1H, dd,[p] 11.0 and 8.0 H-8), 7.51 (1H, dd, $J = 11$ and 8.0, H-5), 7.32 (2H, d, $J = 8.6$, H-3′,5′), 4.59 (2H, q, CH_2), 4.21 (2H, q, CH_2), 3.63 (2H, s, CH_2), 1.53 3H, t, CH_3), 1.27 (3H, t, CH_3)
9	200–203	85	$C_{22}H_{17}N_3O_2$	1720	392, 285, 206	[A] 8.00–7.72 (2H, m, arom.), 7.75 (2H, d, $J = 8.4$, H-2′,6′), 7.70–7.35 (7H, m, arom.), 7.28 (2H, d, $J = 8.4$, H-3′,5′), 7.09 (1H, s, NH), 3.66 (2H, s, CH_2)
10	174–177	95	$C_{20}H_{15}N_3O_2S$	1720	388, 277, 196	[A] 7.96–7.72 (2H, m, arom.), 7.78 (d, $J = 8.2$, H-2′,6′), 7.66–7.42 (4H, m, arom.), 7.32 (2H, d, $J = 8.2$, H-3′,5′), 7.29–7.23 (1H, m, arom.), 3.66 (2H, s, CH_2)
11	205 (d)	87	$C_{16}H_{11}F_2N_3O_2$	3450, 3300, 3210, 3150, 1700	384, 281, 207	[B] 9.80 (1H, s, NH), 8.56 (1H, s, H-3), 7.88 (2H, d, $J = 8.4$, H-2′,6′), 7.64 (1H, dd, $J = 11.4$ and 8.2, H-8), 7.51 (dd, $J = 11.4$ and 8.2, H-5), 7.26 (2H, d, $J = 8.4$, H-3′,5′), 4.46 (1H, br s, COOH), 3.55 (2H, s, CH_2)
12	239–242 (a)	95	$C_{17}H_{13}N_3O_4$	3450, 3320, 1720, 1690	401, 290, 217, 208	[B] 10.59 (1H, s, NH), 8.00 (1H, d, $J = 8.0$, arom.), 7.87 (2H, d, $J = 8.4$, H-2′,6′), 7.82–7.70 (2H, m, arom.), 7.60–7.45 (1H, m, arom.), 7.30 (2H, d, $J = 8.4$, H-3′,5′), 4.60 (2H, br s, 2 COOH), 3.57 (2H, s, CH_2)
13	178–180	80	$C_{18}H_{12}F_3N_3O_4$	3350, 3200, 1710	403, 302, 204	[B] 10.70 (1H, s, NH), 8.14 (1H, d, $J = 8.4$, H-5), 8.05 (1H, d, $J = 1.8$, H-8), 7.87 (2H,d, $J = 8.6$, H-2′,6′), 6.65 (1H, dd, $J = 8.4$ and 1.8, H-6), 7.32 (2H, d, $J = 8.6$, H-3′,5′), 4.20 (2H, br s, 2 COOH), 3.59 (2H, s, CH_2)
14	162–163	93	$C_{17}H_{11}F_2N_3O_4$	3300, 1710	408, 290, 215	[B] 10.60 (1H, s, NH), 7.87 (1H, dd[p], $J = 11.2$ and 8.2, H-8), 7.80 (2H, d, $J = 8.2$, H-2′,6′), 7.62 (1H, dd, $J = 11.2$ and 8.2, H-5), 7.28 (2H, d, $J = 8.2$, H-5), 7.28 (2H, d, $J = 8.2$, H-3′,5′), 4.74 (2H, br s, 2 COOH), 3.56 (2H, s, CH_2)
15	140–143	30	$C_{31}H_{32}N_4O_5$	3260, 1710	371, 270, 208	[A] 8.26–7.75 (4H, m, arom.), 7.80 (2H, d, $J = 8.4$, H-2′,6′), 7.70–7.45 (4H, m, arom.), 7.28 (2H, d, $J = 8.4$, H-3′,5′), 7.11 (1H, s, NH), 6.15 (1H, d, $J = 7.8$, NH), 4.68–4.52 (1H, m, CH), 4.14 (2H, q, CH_2), 4.10 (2H, q, CH_2), 3.59 (2H, s, CH_2), 2.45–1.80 (4H, m, CH_2–CH_2), 1.29 (3H, t, CH_3), 1.23 (3H, t, CH_3)
16	159–162 (a)	74	$C_{29}H_{30}N_4O_5S$	3300, 1720	386, 277, 194	[A] 8.00–7.75 (3H, m, arom.), 7.85 (2H, d, $J = 8.6$, H-2′,6′), 7.70–7.45 (4H, m, arom.), 7.31 (2H, d, $J = 8.6$, H-3′,5′), 6.18 (1H, d, NH), 4.68–4.56 (1H, m, CH), 4.18 (2H, q, CH_2), 4.11 (2H, q, CH_2), 3.61 (2H, s, CH_2), 2.40–1.90 (4H, m, CH_2–CH_2), 1.26 (3H, t, CH_3), 1.23 (3H, t, CH_3)
17	169–170 (a)	73	$C_{25}H_{26}F_2N_4O_5$	3370, 3300, 1740, 1720, 1660	381, 281, 204	[A] 8.36 (1H, s, H-3), 7.70 (1H, dd[p], $J = 11.4$ and 8.0, H-8), 7.69 (2H, d, $J = 8.6$, H-2′,6′), 7.50 (1H, dd, $J = 11.4$ and 8.0, H-5), 7.28 (2H, d, $J = 8.6$, H-3′,5′), 7.17 (1H, s, NH), 6.29 (1H, d, $J = 8.0$, NH), 4.70–4.55 (1H, m, CH), 4.18 (2H, q, CH_2), 4.12 (2H, q, CH_2), 4.12 (2H, q, CH_2), 3.60 (2H, s, CH_2), 2.45–1.90 (4H, m, CH_2–CH_2), 1.27 (3H, t, CH_3), 1.23 (3H, t, CH_3)

Table 1 (*Continued*)

Comp.	m.p. (°C) [a]	Yield (%)	Analysis for	IR (nujol) (ν_{max} cm^{-1})	UV (EtOH) (λ_{max} nm)	^1H NMR, δ_H (J in Hz) [b]
18	254–256 (a)	38	$C_{26}H_{28}N_4O_7$	3280, 1730	401, 295, 217	[C] 13.36 (1H, s, NH), 8.51 (1H, d, $J = 7.6$, NH), 8.01 (1H, d, $J = 7.8$, arom.), 7.86 (2H, d, $J = 8.6$, H-2′,6′), 7.72–7.58 (2H, m, arom.), 7.45–7.38 (1H, m, arom.), 7.26 (2H, d, $J = 8.6$, H-3′,5′), 4.32–4.20 (1H, m, CH), 4.08 (2H, q, CH_2), 4.05 (2H, q, CH_2), 3.45 (2H, s, CH_2), 2.38 (2H, t, CH_2), 2.06–1.90 (2H, m, CH_2), 1.17 (3H, t, CH_3), 1.16 (3H, t, CH_3)
19	236–240	10	$C_{27}H_{27}F_3N_4O_7$	1710	305, 220, 206	[B] 13.14 (1H, s, NH), 8.27–8.16 (2H, m, H-6,8), 7.92 (2H, $J = 7.8$, H-2′,6′), 7.52 (1H, d, $J = 9.0$, H-5), 7.30 (2H, d, $J = 7.8$, H-3′,5′), 4.43–4.26 (1H, m, CH), 4.14 (2H, q, CH_2), 4.10 (2H, q, CH_2), 3.52 (2H, s, CH_2), 2.40–1.90 (4H, m, CH_2–CH_2), 1.25 (3H, t, CH_3), 1.23 (3H, t, CH_3)
20	152–155 (a)	40	$C_{26}H_{26}F_2N_4O_7$	3280, 1730	404, 290, 210	[C] 13.44 (1H, s, 1H), 8.51 (1H, d, $J = 7.4$, NH), 8.04 (2H, d, $J = 10.8$ and 8.0, H-8), 7.82 (2H, d, $J = 8.0$, H-2′,6′), 7.68 (1H, dd, $J = 10.8$ and 8.0, H-5), 7.26 (2H, d, $J = 8.0$, H-3′,5′), 4.30–4.13 (1H, m, CH), 4.08 (2H, q, CH_2), 4.04 (2H, q, CH_2), 3.46 (2H, s, CH_2), 2.38 (2H, t, CH_2), 2.06–1.90 (2H, m, CH_2), 1.17 (6H, t, 2 CH_3)
21	160–163	95	$C_{27}H_{24}N_4O_5$	3380, 3240, 1700	368, 270, 208	[B] 7.68–7.58 (3H, m, arom.), 7.74 (2H, d, $J = 8.2$, H-2′,6′), 7.72 (6H, m, arom.), 7.29 (2H, d, $J = 8.2$, H-3′,5′), 7.11 (1H, d, $J = 7.6$, NH), 4.58–4.46 (1H, m, CH), 5.58 (2H, s, CH_2), 2.40–1.90 (4H, m, CH_2)
22	122–125	75	$C_{25}H_{22}N_4O_5S$	3400, 3260, 1710	387, 276, 193	[A] 7.95–7.73 (2H, m, arom.), 7.77 (2H, d, $J = 8.8$, H-2′,6′), 7.70–7.40 (4H, m, arom.), 7.32 (2H, d, $J = 8.8$, H-3′,5′), 7.30–7.26 (1H, m, arom.), 6.54 (1H, d, $J = 7.6$, NH), 4.60–4.50 (1H, m, CH), 3.60 (2H, s, CH_2), 2.50–1.90 (4H, m, CH_2–CH_2)
23	218–220	83	$C_{21}H_{18}F_2N_4O_5$	3360, 3300, 1730, 1660	382, 281, 204	[B] 9.70(1H, s, NH), 8.52(1H, s, H-3), 7.94(1H, J,7.8, NH), 7,85(2H, d, J, 8.4, H-2',6'), 7:63(1H, dd, J 10.6 and 8.6, H-8), 7.50 (1H, dd, J 11.6 and 8.2, H-5), 7.28(2H, d, J 8.4, H-3',5'), 4.43–4,38(1H, m, CH), 3.53(2H, s,CH_2), 2.40–1.80(4H, m, CH_2–CH_2)
24	169–172	74	$C_{22}H_{20}N_4O_7$	3250, 1720, 1700	402, 291, 217, 205	[B] 10.93 (1H, s, NH), 8.42 (1H, s, $J = 5.8$, NH), 8.08–7.70 (3H, m, arom.), 7.86 (2H, d, $J = 6.8$, H-2′,6′), 7.58–7.46 (1H, m, arom.), 7.32 (2H, d, $J = 6.8$, H-3′,5′), 4.88 (3H, br s, 3 COOH), 4.40–4.25 (1H, m, CH), 1.45–1.80 (4H, m, CH_2–CH_2)
25	218–222	61	$C_{23}H_{19}F_3N_4O_7$	3310, 2250, 1700	389, 296, 209	[B] 9.76 (1H, s, NH), 8.10–7.95 (1H, m, arom.), 7.92 (2H, d, $J = 8.2$, H-2′,6′), 7.65–7.55 (2H, m, arom.), 7.32 (2H, d, $J = 8.2$, H-3′,5′), 4.60–4.45 (1H, m, CH), 2.50–1.90 (4H, m, CH_2–CH_2)
26	105–107 (dec.)	87	$C_{22}H_{18}F_2N_4O_7$	1710	405, 292, 203	[B] 10.54 (1H, s, NH), 8.35 (1H, d, $J = 6.6$, NH), 7.98–7.82 (2H, m, H-5,8), 7.78 (2H, d, $J = 7.8$, H-2′,6′), 7.30 (2H,d, $J = 7.8$, H-3′,5′), 5.70 (3H, br s, 3 COOH), 4.40–4.24 (1H, m, CH), 3.51 (2H, s, CH_2), 2.40–1.90 (4H, m, CH_2–CH_2)
27	111–115 (a)	20	$C_{35}H_{43}N_5O_{10}$	3300, 1730, 1660	419, 292, 222	[A] 11.20 (1H, s, NH), 8.92 (1H, d, $J = 7.6$, NH), 7.97 (2H, d, $J = 8.2$, H-2′,6′), 7.96–7.45 (4H, m, arom.), 7.29 (2H, d, $J = 8.2$, H-2′,6′), 6.18 (1H, d, $J = 7.6$, NH), 4.90–4.76 (1H, m, CH), 4.70–4.55 (1H, m, CH), 4.29 (2H, q, CH_2), 4.25–4.05 (6H, m, 3 CH_2), 2.60–1.80 (4H, m, CH_2–CH_2), 1.34 (3H, t, CH_3), 1.35–1.20 (9H, m, 3 CH_3)
28	Yellow oil	10	$C_{36}H_{42}F_3N_5O_{10}$	3320, 1720	400, 303, 215	[A] 11.24 (1H, s, NH), 8.40 (1H, d, $J = 7.8$, NH), 8.02 (1H, d, $J = 2.0$, H-8), 7.98 (1H, d, $J = 8.4$, H-5), 7.67 (2H, d, $J = 8.4$, H-2′,6′), 7.57 (1H, dd, $J = 8.6$ and 2.0, H-6), 7.25 (2H, d, $J = 8.4$, H-3′,5′), 6.12 (1H, d, $J = 7.2$, NH), 4.80–4.70 (1H, m, CH), 4.60–4.50 (1H, m, CH), 4.19 (2H, q, CH_2), 4.20–4.00 (6H, m, 3 CH_2), 3.54 (2H, s, CH_2), 2.50–1.80 (8H, m, 2 CH_2–CH_2), 1.48 (3H, t, CH_3), 1.20–1.10 (9H, m, 3 CH_3)

[a] Purification procedure: (a), crystallized from ethanol; [p], partially obscured by other resonances.
[b] Solvent: [A] = $CDCl_3$; [B] = $CDCl_3$–DMSO-d_6 (3:1); [C] = DMSO-d_6.



Scheme 1.

values, analytical and spectroscopic data are reported in Table 1.

4. Pharmacology

The tested compounds **6**, **7**, **8**, **12**, **13**, **15**, **16**, and **20** of Fig. 1 and Scheme 1 were incontrovertibly selected by National Cancer Institute of Bethesda among the 26 submitted. Evaluation of anticancer activity was performed following the known [13] in vitro disease-oriented antitumour screening program against a panel of 60 human tumour cell lines. 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, 50% cytotoxicity, respectively, and are expressed as Mean Graph Midpoints. In Table 3 we reported the activities of those compounds which showed percent growth inhibition greater than 40% on subpanel cell-lines at 10^{-4} molar concentration. In Fig. 2 we reported the activities

Table 2
$-\log GI_{50}$, $-\log TGI$, $-\log LC_{50}$ mean graph midpoints (MG-MID) [a] of in vitro inhibitory activity test for compounds **6**, **7**, **8**, **12**, **13**, **15**, **16**, and **20** against human tumour cells lines [b]

Comp.	$-\log GI_{50} = \mu M$		$-\log TGI$	$-\log LC_{50}$
6	4.26	53.70	4.03	4.00
7	4.20	61.66	4.02	4.00
8	4.40	38.90	4.08	4.00
12	4.00	100.00	4.00	4.00
13	4.07	85.11	4.00	4.00
15	4.35	43.65	4.05	4.00
16	4.26	53.70	4.01	4.00
20	4.15	70.79	4.02	4.00

[a] (MG-MID) mean graph midpoints, the average sensitivity of all cell lines towards the test agent.
[b] From NCI.

of the compound **15** which showed percent growth inhibition greater than 40% on subpanel cell-lines at 10^{-5} molar concentration.

4.1. Results of the in vitro pharmacological anticancer assays

The data of in vitro anticancer activity reported in Table 2 established that the average sensitivity of all cell lines towards the tested agent, represented as Mean Graph Midpoints of GI_{50}, falls in the range of 38.90–100 μM, whereas the TGI is very close to LC_{50} which was identical for all compounds at the highest concentration (100.00 μM). However, from this table it is easy to see that the most active agent is compound **8** followed in decreasing order by **15** < **6** = **16** < **7** < **20** < **13**. From the data of Table 3 we can observe that compound **8** exhibited the largest sensitivity upon all subpanel cell lines (53 over 60 cell lines) recording an interesting selective percent growth inhibition for renal ACHN (191%), UO-31 (183%) and breast cancer MDA-MB-231/ATCC (144%) cell lines at 10^{-4} molar concentration. Compound **15** confirmed analogous sensitivity at lower level (37 over 60) and exhibited various selectivities at 10^{-5} molar concentration (Fig. 2). Compounds **12** and **13** which represent the products of hydrolysis of **6** and **7** appear to be devoid of intrinsic activity at all the concentrations examined with the only exception for **13** that in the leukemia SR32 cell line recorded % growth inhibition (58, 63, 68, 66) between 10^{-8} and 10^{-5} M, respectively. The fact of the observed inactivity for the free acids in this test is not surprising since we have often recorded this behaviour in similar cases [1–9], possibly because of their low solubility in the culture medium. Comparison of the activity as expressed as GI_{50} (Mean Graph-Midpoint) of the above described compounds with those of the previously reported analogous nor-derivatives was applicable in a few cases (**6**, **7**, **15**, **16**) (Table 4). From this table we can observe that only with the exception of compound **6** the nor derivatives resulted more active. A study of structure–activity relationships is excluded by the narrow number of derivatives tested. An observation has to be marked for the case of **8** that is the unique example of an active compound bearing two atoms of fluorine in the 6,7-positions of quinoxaline ring among all the compounds so far described by us. This substitution is somewhat profitable also in compound **20** with GI_{50} whose value was twice that of **8**. In conclusion the results obtained seem to indicate that this type of homologation of the aminobenzoyl-L-glutamate side chain and of its precursor is not profitable under the conditions examined.

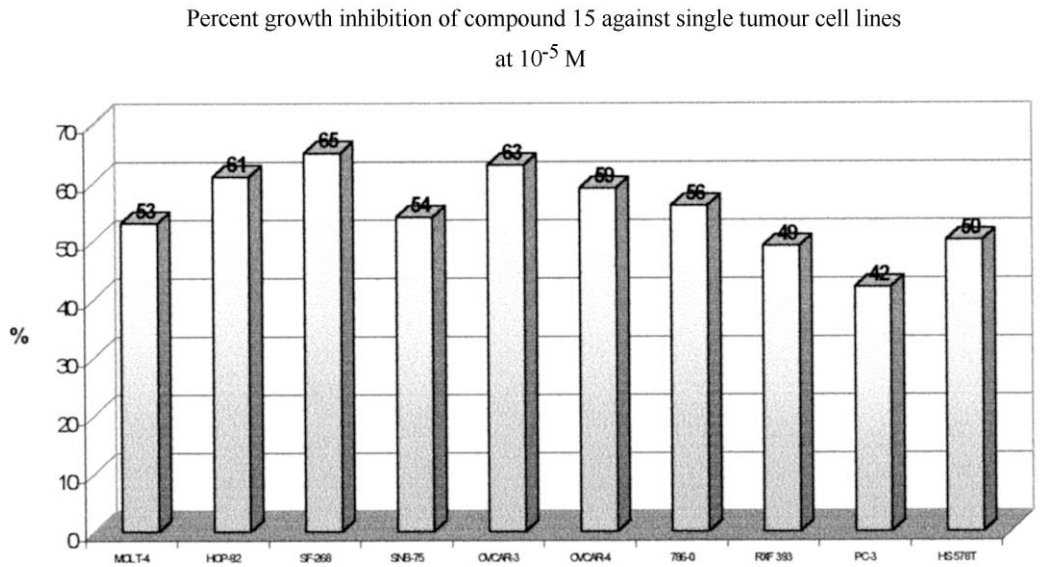


Fig. 2. Percent growth inhibition of compound **15** against single tumour cell lines at 10^{-5} M.

Table 3
Percentage tumour growth inhibition recorded on subpanel cell lines at 10^{-4} M of compounds **6**, **7**, **8**, **12**, **13**, **15**, **16**, and **20** [a]

Panel/cell-lines	6	7	8	12	13	15	16	20
Leukaemia								
CCRF-CEM	*	*	78	*	*	*	*	147
HL-60(TB)	*	*	92			*	*	141
K-562	nt	nt	80	nt	nt	*	41	94
MOLT-4	*	*	nt	*	*	*	41	83
RPMI-8226	nt	nt	82	nt	*	*	*	nt
SR	*	*	93	*	54	*	61	141
Non small cell lung cancer								
A549/ATCC	77	*	56	*	*	53	57	*
EKVX	81	*	132	*	*	56	62	*
HOP-62	85	83	42	*	*	92	76	*
HOP-92	80	82	122	*	*	144	nt	109
NCI-H226	91	103	60	*	*	53	43	47
NCI-H23	67	92	122	*	*	84	79	*
NCI-H322M	54	*	68	*	*	*	60	*
NCI-H460	71	*	59	*	*	*	53	*
NCI-H522	54	68	139	*	*	83	81	82
Colon cancer								
COLO 205	*	*	83	*	*	*	*	*
HCC-2998	nt	nt	118	nt	nt	*	43	42
HCT-116	47	*	58	*	*	*	*	54
HCT-15	*	*	99	*	*	*	*	*
HT29	*	*	65	*	*	*	*	*
KM12	*	*	45	44	*	*	45	*
SW-620	*	*	74	*	*	*	46	58
SNC cancer								
SF-268	72	78	61	*	*	158	78	91
SF-295	89	59	56	*	*	133	71	*
SF-539	82	1222	*	*	*	92	89	48
SNB-19	134	91	70	*	*	81	83	*
SNB-75	163	143	57	*	*	133	99	117
U251	74	73	96	*	*	108	75	*
Melanoma								
LOX IMVI	48	*	92	*	*	*	nt	79
MALME-3M	82	*	45	*	*	63	67	53
M14	45	*	89	*	*	60	*	*
SK-MEL-2	52	60	103	*	*	111	64	127
SK-MEL-28	*	*	59	*	*	*	45	49
SK-MEL-5	67	43	72	*	*	46	71	47
UACC-257	48	*	75	*	*	*	54	78
UACC-62	74	67	72	*	*	62	58	63
Ovarian cancer								
IGROV1	65	47	nt	*	*	64	*	nt
OVCAR-3	46	*	73	*	*	50	86	40
OVCAR-4	57	91	65	*	*	53	78	*
OVCAR-5	*	*	*	*	*	51	*	*
OVCAR-8	67	90	96	*	*	69	nt	51
SK-OV-3	69	71	*	*	*	90	75	*
Renal cancer								
786-0	86	93	48	*	*	96	79	*
A498	105	92	nt	*	*	74	58	*
ACHN	68	83	191	*	*	72	50	*
CAKI-1	56	85	152	*	*	62	*	*
RXF 393	113	136	82	*	*	127	123	*
SN12C	44	*	56	*	*	45	44	59
TK-10	75	70	*	*	*	119	107	*
UO-31	142	*	183	*	*	*	*	*
Prostate cancer								
PC-3	67	*	63	*	*	75	64	*
DU-145	51	*	99	*	*	*	68	*
Breast cancer								
MCF7	58	51	78	*	*	44	73	53

Table 3 (*Continued*)

Panel/cell-lines	6	7	8	12	13	15	16	20
MCF7/ADR-RES	65	42	70	*	*	59	57	*
MDA-MB-231/ATCC	*	*	144	nt	nt	127	43	80
HS 578T	106	108	62	*	*	107	73	75
MDA-MB-435	*	*	59	*	*	*	65	43
BT-549	99	58	66	*	*	67	46	71
T-47D	69	*	69	*	*	*	111	110
MDA-N	*	*	74	*	*	*	47	47

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

Table 4
Comparison of mean graph activity between compounds **6**, **7**, **15**, **16** and the corresponding nor derivatives of the cited papers (Ref)

Comp. (Ref)	GI_{50}	TGI	LC_{50}
6	4.26	4.00	4.00
12 [3]	4.03	4.00	4.00
7	4.20	4.02	4.00
14 [3]	4.21	4.03	4.00
15	4.35	4.05	4.00
29 [3]	5.17	4.55	4.00
16	4.26	4.01	4.00
7 [3]	4.57	4.01	4.00

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