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Quinoxaline chemistry. Part XVII. Methyl [4-(substituted 2-quinoxalinyloxy) phenyl] acetates and ethyl *N*-{[4-(substituted 2-quinoxalinyloxy) phenyl] acetyl} glutamates analogs of methotrexate: synthesis and evaluation of in vitro anticancer activity

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Abstract

Fourteen out of 21 quinoxaline derivatives described in the present paper were selected at NCI for evaluation of their in vitro anticancer activity. Preliminary screening showed that some derivatives exhibited a moderate to strong growth inhibition activity on various tumor panel cell lines between 10^{-5} and 10^{-4} M concentrations. Interesting selectivities were also recorded between 10^{-8} and 10^{-6} M for the compounds **9** and **13**.

Keywords: Anticancer activity; Quinoxaline derivatives

1. Introduction

Quinoxaline derivatives continue to be a standing interest of our research group. So far a great deal of data concerning the anticancer activity of more than 300 compounds constitute a library for the antifolic analogs [1–14]. During our investigation we have taken into account as antifolate models either the classical methotrexate or the non-classical trimetrexate as well as the corresponding dideazafolic derivatives. In this context we have proved that bioisosteric replacement of pteridine ring with 6(7)-trifluoromethylquinoxaline affords a good substrate for the biological activity in the series of the classical antifolate analogs, whereas this was so in a few cases of the series of non-classical ones. Another aspect being considered was also the bioisosteric replacement of 2-NH group with an oxygen that in some cases was of relevance in the anticancer activity [6–8]. Recently, our interest focused on the homologation of quinoxaline analogs of classical type on the side of both amino and carboxamide groups of the aminobenzoylglutamic moiety linked at position 2 [11]. This homologation produced very few examples of active compounds in anticancer test at NCI. However, for a development of the knowledge about the pharmacophoric pattern we thought to replace the 2-NH bridge in the above-cited series with oxygen on the ground that very recently American authors have found that quinoxalines bearing a 2-(4-substituted phenoxy) substituent were endowed with potent antitumor activity [15]. Thus, the list of compounds **1–21** (Fig. 1) was prepared and the results of their activity are now presented.

2. Chemistry

The quinoxaline derivatives **1–21** were obtained by a convergent synthesis outlined in Scheme 1. The key intermediate chloroquinoxalines **22a–f**, already known as referenced in the experimental section, were reacted with methyl 4-hydroxyphenylacetate (**23**) to give the esters (**1–6**) among which **2–6** were converted into the corresponding acids (**7–11**) on alkaline hydrolysis (see Fig. 1). This process failed in the case of compound **1** that, even under the mild conditions used, alternatively gave the products **24** (10%) **25** (60%), and **26** (25%) instead of the expected acid. This result clearly indicated that a displacement of *O*-side chain had

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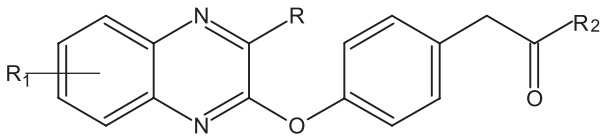


1-21

Compd	R	R_1	R_2
1	H	6,7-F	OMe
2	Ph	H	OMe
3	2-Th	H	OMe
4	COOEt	H	OMe
5	COOEt	7-CF_3	OMe
6	COOEt	6,7-F	OMe
7	Ph	H	OH
8	2-Th	H	OH
9	COOH	H	OH
10	COOH	7-CF_3	OH
11	COOH	6,7-F	OH
12	Ph	H	NH-gluEt
13	2-Th	H	NH-gluEt
14	CONHgluEt	H	NH-gluEt
15	COOH	7-CF_3	NH-gluEt
16	CONHgluEt	7-CF_3	NH-gluEt
17	Ph	H	NH-gluH
18	2-Th	H	NH-gluH
19	CONHgluH	H	NH-gluH
20	COOH	7-CF_3	NH-gluH
21	CONHgluH	7-CF_3	NH-gluH

Fig. 1. List of compounds prepared.

occurred in a similar fashion to the case of 6-trifluoromethyl-3-phenyl-2-phenoxyquinoxaline derivatives previously observed by us [7] restoring the quinoxalinone (**26**) which underwent nucleophilic substitution by an ethoxide anion at both positions 7 and 2 to give (**25**) and to a lesser extent at position 2 to give (**24**). This displacement of fluorine atom under basic conditions was analogous to that previously observed by us in the case of the preparation of 6,7-difluorobenzofuroxane [16] and in more general cases of reactivity of 6,7-difluoroquinolinones with bases [17]. Compound **26** has been reported in two patents [18,19] and mentioned in the preparation of 6,7-difluoro-2-chloroquinoxaline (**22d**) [20], but no physical data were described for both. Now, compounds **26** and **22d** are described in detail in this paper. The compounds **24** and **25** were obtained for the first time and fully characterized. Analogously, the alkaline hydrolysis of compound **5** gave along with the expected acid (**10**) also the compound **27** (10%) thus confirming a partial displacement of the *O*-side chain. When this reaction was carried out for a time longer than 1.5 h, **27** was the sole compound isolated. Conversion of the acids (**7–10**) into the corresponding glutamates carrying out the reaction with diethyl L-glutamate hydrochloride in dry DMF and in the presence of TEA and diethylcyanophosphonate, gave the following results. Compounds **7** and **8** yielded the glutamates (**12** and **13**) while the acids (**9** and **10**) in an identical run, slightly modifying the reactants ratio, behave differently. In the case of **9** we obtained only the diglutamate (**14**) in poor yield (14%), whereas in the case of **10** we were able to isolate either the mono- (**15**) or the diglutamate (**16**) in 1:1 ratio in 40% yield. This fact seems to confirm our previous observations that when an extra carboxylic group is present on quinoxaline ring, formation of the amide can take place without any selectivity. Alkaline hydrolysis of **12**, **13**, **15** and **14**, **16** proceeded normally and gave the corresponding acids (**17–21**).

The structures of the described compounds have been characterized by the whole of analytical and spectroscopic data. In particular in the case of 6,7-difluoro derivatives the ^{1}H NMR spectrum shows that 5 and 8 protons do not resonate as singlets but were splitted into doublets of doublets by *ortho, meta*-coupling with fluorine atoms in accordance with the literature reports [21].

3. Experimental

Melting points are uncorrected and were recorded on a Kofler or an electrothermal melting point apparatus. UV spectra are qualitative and were recorded in nanometer 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 ±0.4% of the theoretical values.

3.1. Chemistry

3.1.1. Intermediates

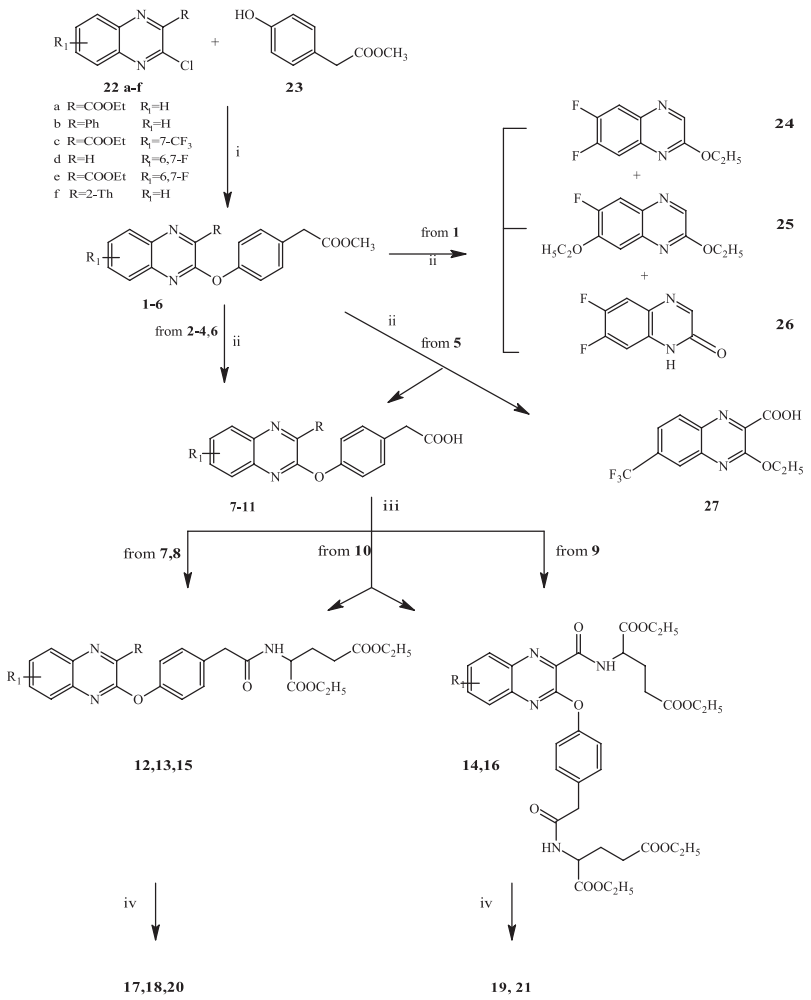
The intermediate chloroquinoxalines necessary for this work were known and prepared according to the data of the literature as follows: **22a** [22], **22b** [23], **22c** [3], **22e** [9], and **22f** [1]. Compound **22d** was previously mentioned in a paper [24] without reporting any data and has been obtained by us from 6,7-difluoroquinoxalinone (**26**) as described below.

*3.1.1.1. 6,7-Difluoroquinoxalin-2(1H)-one (**26**).* A mixture of equimolar amounts (7 mmol) of 4,5-difluoro-1,2-diamino benzene, obtained as described [20], and glyoxylic acid in ethanol (20 ml) was refluxed under stirring for 1 h. On cooling, a precipitate was collected and washed with the same solvent to give **26** (0.70 g, 70% yield), m.p. 277–282 °C (from ethanol). Analysis for $C_8H_4F_2N_2O$: C, H, N.

ν_{max} (nujol) cm^{-1}: 3150, 1680.

λ_{max} (EtOH) nm: 342, 273, 223, 205.

^{1}H NMR ($CDCl_3$–DMSO-d_6 3/1) δ: 12.6 (1H, br s, NH); 8.14 (1H, s, H-3); 7.67 (1H, dd, $J_{H5,F6}$ = 10.4 Hz,



i , DMF and Cs_2CO_3;
ii, 2M NaOH / EtOH;
iii, L-glutamic acid diethylester hydrochloride, $(EtO)_2POCN$ and TEA;
iv, 1M NaOH / EtOH;

Scheme 1

$J_{H5,F7}$ = 8.2 Hz, H-5); 7.22 (1H, dd, $J_{H8,F7}$ = 10.6 Hz, $J_{H8,F6}$ = 7.6 Hz, H-8).

*3.1.1.2. 2-Chloro-6,7-difluoroquinoxaline (**22d**).* A mixture of **26** (3 g, 15 mmol) and $POCl_3$ (30 ml) was stirred under heating at 120 °C for 2 h. The obtained solution was taken up with water and ice and the formed solid was filtered off and washed with water to give **22d** (2.7 g, 90% yield), m.p. 92–94 °C (from EtOH). Analysis for $C_8H_3ClF_2N_2$: C, H, N.

λ_{max} (EtOH) nm: 321, 236, 204.

^{1}H NMR ($CDCl_3$) δ: 8.77 (1H, s, H-3); 7.88 (1H, dd, $J_{H5–F6}$ = 10.0 Hz and $J_{H5–F7}$ = 8.0 Hz, H-5); 7.78 (1H, dd, $J_{H8–F7}$ = 10.4 Hz and $J_{H8–F6}$ = 8.2 Hz, H-8).

*3.1.2. General procedure for the preparation of the esters **1–6***

A mixture of equimolar amounts (1.6 mmol) of chloroquinoxalines (**22a–f**) and commercially available (Aldrich) methyl 4-hydroxyphenylacetate (**23**) in the presence of Cs_2CO_3 in anhydrous dimethylformamide (3.5 ml) was stirred under heating at 70 °C for 13 h.

On cooling, the mixture was diluted with water and the precipitates were collected and washed with water to give **1–6** 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 **7–11** and isolation of compound **27***

A mixture of the ester **2–6** (2 mmol) in ethanol (20 ml) and 2 M NaOH (14 ml) was stirred at room temperature for 2.5 h (**2**), 24 h (**3** and **4**), and 1 h (**5** and **6**). The reaction mixture was diluted with water and after evaporation of the solvent was made acidic with 2 M HCl. The beige-yellow products (**7–11**) were collected and washed with water. Yields, m.p.

Table 1
The m.p., yields, analytical and spectroscopic (IR, UV, ^{1}H NMR) data

Compounds	m.p. (°C) [a]	Yields (%)	Analysis for	IR (nujol) (ν_{max} cm^{-1})	UV (EtOH) (λ_{max} nm)	^{1}H NMR[b], δ_H (J in Hz)
1	114–116 (a)	89	$C_{17}H_{12}F_2N_2O_3$	1730, 1580	325, 225, 204	[A] 3.69 (2H, s, CH_2); 3.74 (3H, s, CH_3); 7.22 (2H, d, J = 8.0, H-3′, 5′); 7.38 (2H, d, J = 8.0, H-2′, 6′); 7.60–7.45 (1H, m, H-5); 7.90–7.75 (1H, m, H-8); 8.67 (1H, s, H-3)
2	132–135 (a)	90	$C_{23}H_{18}N_3O_3$	1750, 1580	341, 242, 206	[A] 3.69 (2H, s, CH_2); 3.73 (3H, s, CH_3); 7.24 (2H, d, J = 8.4, H-3′, 5′); 7.40 (2H, d, J = 8.4, H-2′, 6′); 7.74–7.50 (6H, m, arom); 8.23–8.10 (2H, m, H-8 + arom)
3	159–161 (a)	98	$C_{21}H_{16}N_2O_3S$	1740, 1670, 1580	360, 266, 212	[B] 3.71 (2H, s, CH_2); 3.74 (3H, s, CH_3); 7.23–7.19 (2H, m, H3″, 4″); 7.32 (2H, d, J = 8.8, H-2′, 6′); 7.68–7.54 (3H, m, arom); 8.05 (1H, m, H-5″); 8.30 (1H, m, H-8)
4	86–87 (a)	92	$C_{20}H_{18}N_2O_5$	1750, 1580	302, 243, 206	[A] 1.48 (3H, t, CH_2CH_3); 3.69 (2H, s, CH_2); 3.74 (3H, s, $COOCH_3$); 4.57 (2H, q, CH_2CH_3); 7.26 (2H, d, J = 8.6, H-3′, 5′); 7.40 (2H, d, J = 8.6, H-2′, 6′); 7.80–7.62 (3H, m, arom); 8.20–8.10 (1H, dd, J = 7.6 and 1.2, H-8)
5	139–141 (a)	89	$C_{21}H_{17}F_3N_2O_5$	1730, 1580	332, 243, 206	[A] 1.50 (3H, t, CH_2CH_3); 3.70 (2H, s, CH_2); 3.74 (3H, s, $COOCH_3$); 4.58 (2H, q, CH_2CH_3); 7.24 (2H, d, J = 8.6, H-3′, 5′); 7.40 (2H, d, J = 8.6, H-2′, 6′); 7.86–7.80 (1H, dd, J = 8.6 and 1.8, H-6); 8.08 (1H, s, H-8); 8.25 (1H, d, J = 8.8, H-5)
6	78–80 (a)	60	$C_{20}H_{16}F_2N_2O_5$	1730	332, 228, 207	[A] 1.48 (3H, t, CH_2CH_3); 3.69 (2H, s, CH_2); 3.74 (3H, s, CH_3); 4.56 (2H, q, CH_2CH_3); 7.23 (2H, d, *J = 8.6, H-3′, 5′); 7.38 (2H, d,* J *= 8.6, H-2′, 6′); 7.60–7.45 (1H, m, H-5); 7.95–785 (1H, m, H-8)*
7	246–248	69	$C_{22}H_{16}N_2O_3$	1700, 1580, 1560	341, 243, 206	[B] 3.66 (2H, s, CH_2); 7.24 (2H, d, J = 8.6, H-3′, 5′); 7.40 (2H, d, J = 8.6, H-2′, 6′); 7.82–7.50 (6H, m, arom); 8.24–8.10 (2H, m, arom)
8	215–217	95	$C_{20}H_{14}N_2O_3S$	1715, 1620	361, 266, 212	[B] 3.66 (2H, s, CH_2); 7.26–7.21 (2H, m, H-3″, 4″); 7.30 (2H, d, J = 8.4, H-3′, 5′); 7.41 (2H, d, J = 8.4, H-2′, 6′); 7.70–7.50 (3H, m, arom); 8.00 (1H, m, H-5″); 8.29 (1H, m, H-8)
9	163–165	97	$C_{17}H_{12}N_2O_5$	1720, 1570	325, 242, 206	[B] 3.62 (2H, s, CH_2); 7.27 (2H, d, J = 8.4, H-3′, 5′); 7.41 (2H, d, J = 8.4, H-2′, 6′); 7.78–7.75 (3H, m, arom); 8.12–8.10 (1H, m, H-8)
10	120–121	89	$C_{18}H_{11}F_3N_2O_5$	3450, 1730, 1570	323, 207	[B] 3.66 (2H, s, CH_2); 7.25 (2H, d, J = 8.6, H-3′, 5′); 7.37 (2H, d, J = 8.6, H-2′, 6′); 7.82–7.74 (1H, m, H-6); 8.07 (1H, s, H-8); 8.25 (1H, d, J = 8.6, H-5)
11	161–163	77	$C_{17}H_{10}F_2N_2O_5$	3500, 1730, 1570	327, 206	[A] 3.66 (2H, s, CH_2); 7.21 (2H, d, J = 8.2, H-3′, 5′); 7.40 (2H, d, J = 8.2, H-2′, 6′); 7.60–7.45 (1H, m, H-5); 8.00–7.80 (1H, m, H-8)
12	139–142 (a)	65	$C_{31}H_{31}N_3O_6$	3300, 1730, 1650, 1540	341, 243, 206	[A] 1.35–1.20 (6H, m, $2CH_2CH_3$); 2.45–1.82 (4H, m, CH_2CH_2); 3.67 (2H, s, CH_2Ph); 4.22 –4.09 (4H, m, $2CH_2CH_3$); 4.62–4.75 (1H, m, CH); 6.28 (1H, d, J = 7.4, NH); 7.29 (2H, d, J = 8.4, H-2′, 6′); 7.80–7.55 (6H, m, arom); 8.25–8.10 (2H, m, arom)
13	177–180 (a)	39	$C_{29}H_{29}N_3O_6S$	3300, 1750, 1650, 1560	361, 266, 211	[A] 1.32–1.21 (6H, m, $2CH_2CH_3$); 2.40–1.90 (4H, m, CH_2CH_2); 3.68 (2H, s, CH_2Ph); 4.30–4.10 (4H, m, $2CH_2CH_3$); 4.59–4.72 (1H, m, CH); 6.31 (1H, d, J = 7.4, NH); 7.25–7.21 (2H, m, H-3″, 4″); 7.35 (2H, d, J = 8.6, H-3′, 5′); 7.39 (2H, d, J = 8.6, H-2′, 6′); 7.70–7.75 (3H, m, arom); 8.05 (1H, m, H-5″); 8.32 (1H, d, J = 8.2, arom)
14	105–108 (a)	14	$C_{35}H_{42}N_4O_{11}$	3300, 1730, 1670	331, 243, 205	[A] 1.42–1.20 (12H, m, $4CH_2CH_3$); 2.65–1.80 (8H, m, $2CH_2CH_2$); 3.66 (2H, s, CH_2Ph); 4.20–4.10 (4H, m, $2CH_2CH_3$); 4.30–4.20 (4H, m, $2CH_2CH_3$); 4.67–4.50 (1H, m, CH); 4.95–4.85 (1H, m, CH); 6.38 (1H, d, J = 7.2, NH); 7.28 (2H, d, J = 7.0, H-3′, 5′); 7.32 (2H, d, J = 7.0, H-2′, 6′); 7.85–7.70 (3H, m, arom); 8.13 (1H, dd, J = 8.6 and 1.4, H-8); 8.39 (1H, d, J = 7.8, NH)

(continued on next page)

Table 1
(continued)

Compounds	m.p. (°C) [a]	Yields (%)	Analysis for	IR (nujol) (ν_{max} cm^{-1})	UV (EtOH) (λ_{max} nm)	^{1}H NMR[b], δ_H (*J* in Hz)
15	132–135 (b)	40	$C_{27}H_{26}F_3N_3O_8$	3300, 1730, 1650, 1580	325, 240, 228, 204	[A] 1.32–1.20 (6H, m, $2CH_2CH_3$); 2.50–1.90 (4H, m, CH_2CH_2); 3.66 (2H, s, CH_2Ph); 4.27–4.05 (4H, m, $2CH_2CH_3$); 4.60–4.56 (1H, m, CH); 6.32 (1H, d, *J* = 6.0, NH); 7.28 (2H, d, *J* = 8.8, H-3′, 5′); 7.40 (2H, d, *J* = 8.8, H-2′, 6′); 7.80 (1H, dd, *J* = 8.6 and 1.8, H-6); 8.08 (1H, s, H-8); 8.18 (1H, d, *J* = 8.8, H-5)
16	109–111 (b)	40	$C_{36}H_{41}F_3N_4O_{11}$	3300, 1730, 1660, 1580	331, 242, 204	[A] 1.37–1.15 (12H, m, $4CH_2CH_3$); 2.65–1.90 (8H, m, $2CH_2CH_2$); 3.66 (2H, s, CH_2Ph); 4.35–3.95 (8H, m, $4CH_2CH_3$); 4.75–4.52 (1H, m, CH); 5.00–4.86 (1H, m, CH); 6.30 (1H, d, *J* = 6.0, NH); 7.29 (2H, d, *J* = 7.0, H-3′, 5′); 7.38 (2H, d, *J* = 7.0, H-2′, 6′); 7.85 (1H, dd, *J* = 8.6 and 1.6, H-6); 8.08 (1H, s, H-8); 8.25 (1H, d, *J* = 8.4, H-5); 8.34 (1H, d, *J* = 7.8, NH)
17	187–190	95	$C_{27}H_{23}N_3O_6$	1730, 1700, 1650	342, 242, 207	[A] 2.45–1.90 (4H, m, CH_2CH_2); 3.65 (2H, s, CH_2CO); 4.55–4.49 (1H, m, CH); 7.22 (2H, d, *J* = 8.4, H-3′, 5′); 7.42 (2H, d, *J* = 8.4, H-2′, 6′); 7.80–7.50 (6H, m, arom); 8.30–8.10 (2H, m, arom)
18	212–214 (a)	84	$C_{25}H_{21}N_3O_6S$	3300, 1720, 1650	360, 266, 211	[B] 2.47–1.85 (4H, m, CH_2CH_2); 3.64 (2H, s, CH_2CO); 4.55–4.40 (1H, m, CH); 7.25–7.20 (2H, m, H-3″, 4″); 7.29 (2H, d, *J* = 8.8, H-3′, 5′); 7.48 (2H, d, *J* = 8.8, H-2′, 6′); 7.75–7.55 (3H, m, arom); 8.03–8.10 (1H, m, H-5″); 8.31–8.35 (1H, m, arom)
19	Oil	42	$C_{27}H_{26}N_4O_{11}$	3400, 1660	228, 193	[B] 2.44–1.80 (8H, m, $2CH_2CH_2$); 3.50 (2H, s, CH_2Ph); 4.54–4.15 (1H, m, CH); 4.75–4.50 (1H, m, CH); 7.25 (2H, d, *J* = 7.0, H-3′, 5′); 7.40 (2H, d, *J* = 7.0, H-2′, 6′); 7.90–7.60 (2H, m, arom); 8.11–8.07 (1H, m, arom); 8.37–8.34 (1H, m, arom)
20	105–108	72	$C_{23}H_{18}F_3N_3O_8$	1730, 1620	324, 240, 228, 205	[B] 2.50–1.90 (4H, m, $2CH_2CH_2$); 3.65 (2H, s, CH_2Ph); 4.60–4.50 (1H, m, CH); 6.90 (1H, d, *J* = 6.8, NH); 7.25 (2H, d, *J* = 8.6, H-3′, 5′); 7.42 (2H, d, *J* = 8.6, H-2′, 6′); 8.90–8.75 (1H, m, H-6); 8.07 (1H, s, H-8); 8.20 (1H, d, *J* = 8.8, H-5)
21	115–119	43	$C_{28}H_{25}F_3N_4O_{11}$	1640, 1580	330, 243, 206	[B] 2.60–2.10 (8H, m, $2CH_2CH_2$); 3.64 (2H, s, CH_2Ph); 4.55–4.40 (1H, m, CH); 4.85–4.70 (1H, m, CH); 7.26 (2H, d, *J* = 8.4, H-3′, 5′); 7.42 (2H, d, *J* = 8.4, H-2′, 6′); 8.30–8.05 (2H, m, arom); 8.70–8.60 (1H, m, arom); 8.90–9.01 (1H, m, NH)

[a] Purification procedure: (a) crystallized from ethanol and (b) flash chromatography petrol ether (40–60 °C)/ethyl acetate = 1/1.
[b] Solvent: [A] = $CDCl_3$; [B] = $CDCl_3$–DMSO-d_6.

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

From hydrolysis of **5**, carried out as above, we obtained compound **27** as by-product (10% yield), m.p. 82–84 °C from ethanol. Analysis for $C_{12}H_9F_3N_2O_3$: C, H, N.

Table 2
–log GI_{50},–log TGI,–log LC_{50} mean graph midpoints (MG-MID) [a] of in vitro inhibitory activity test for compounds **3, 6, 7, 8, 9, 12,** and **13** against human tumor cell lines [b]

Compounds	–log GI_{50} = μM	–log TGI = μM	–log LC_{50} = μM
3	4.14 = 72.44	4.02 = 95.49	4.00 = 100
6	4.10 = 79.43	4.01 = 97.72	4.00 = 100
7	4.22 = 60.25	4.01 = 97.72	4.00 = 100
8	4.26 = 54.95	4.01 = 97.72	4.00 = 100
9	4.20 = 63.09	4.00 = 100	4.00 = 100
12	4.13 = 74.13	4.00 = 100	4.00 = 100
13	4.63 = 23.44	4.15 = 70.79	4.02 = 95.49

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

ν_{max} (nujol) cm^{-1}: 3450, 1720, 1570.
λ_{max} (EtOH) nm: 338, 324, 210.
^{1}H NMR ($CDCl_3$–DMSO-d6) δ: 8.20 (1H, d, *J* = 8.6 Hz, H-5); 8.15 (1H, s, H-8); 7.77 (1H, dd, *J* = 8.6 and 1.6 Hz, H-8); 5.20 (1H, br s, OH); 4.64 (2H, q, $\mathbf{CH_2}CH_3$); 1.51 (3H, t, $CH_2\mathbf{CH_3}$).

*3.1.4. Hydrolysis of compound **1** into 2-ethoxy-6,7-difluoroquinoxaline (**24**), 2,7-diethoxy-6-fluoroquinoxaline (**25**), and 6,7-difluoroquinoxalin-2(1H)-one (**26**)*

Compound **1** (0.25 g, 8.32 mmol), dissolved in a mixture of ethanol (8.3 ml) and 2 M NaOH (5.5 ml) was heated at 40 °C for 5 h. Then, the precipitated product was collected to give crude **24** (10% yield), m.p. 85–87 °C from ethanol. Analysis for $C_{10}H_8F_2N_2O$: C, H, N.

ν_{max} (nujol) cm^{-1}: 1575, 1513.
λ_{max} (EtOH) nm: 336, 322, 222, 207.
^{1}H NMR ($CDCl_3$) δ: 8.42 (1H, s, H-3); 7.76 (1H, dd, $J_{H5,F6}$ = 10.4 Hz, $J_{H5,F7}$ = 8.2 Hz, H-5); 7.56 (1H, dd,

Table 3
Percentage tumor growth inhibition recorded on subpanel cell lines at 10^{-4} M of compounds **3, 6, 7, 8, 9, 12,** and **13**

Panel/cell lines	Compounds						
	3	**6**	**7**	**8**	**9**	**12**	**13**
Leukemia							
CCRF-CEM	–	77	62	71	–	–	–
HL-60(TB)	–	89	63	70	71	–	–
K-562	–	102	51	50	81	58	42
MOLT-4	–	159	54	71	NT	–	–
RPMI-8226	–	108	57	90		–	–
SR	–	NT	83	89	66	–	–
Non-small cell lung cancer							
A549/ATCC		44	66	76	–	48	111
EKVX	40	51	111	85	–	–	76
HOP-62	72	–	40		–	77	180
HOP-92	124	42		87	–	NT	130
NCI-H226	100	67	87	67	–	108	104
NCI-H23	–	55	63	65	–	44	61
NCI-H322M	–	–		55	–	–	87
NCI-H460	–	–	71	72	–	–	64
NCI-H522	–	–	80	64	–	82	62
Colon cancer							
COLO 205	–	–	69	112	–	–	–
HCC-2998	–	NT	NT	65	–	45	61
HCT-116	–	90	54	69	–		89
HCT-15	–	–	62	88	–	–	–
HT29	–	62	64	84	–	–	–
KM12	–	46	64	78	–	47	40
SW-620	–	40	62	48	–	–	–
CNS cancer							
SF-268	69	–	75	60	–	84	153
SF-295	92	–	67	77	–	61	71
SF-539	77	–	62	55	–	96	147
SNB-19	69	–	49	54	–	72	136
SNB-75	172	43	60	108	–	89	NT
U251	73	–	66	89	–	56	103
Melanoma							
LOX IMVI	–	–	65	81	–	NT	68
MALME-3M	–	–	62	47	–	–	68
M14	–	NT	83	63	–	–	
SK-MEL-2	65	–	60	54	–	41	52
SK-MEL-28	–	–	52	66	–	–	
SK-MEL-5	–	91	66	58	–	60	50
UACC-257	–	–		41	–	–	43
UACC-62	–	47	113	63	–	46	62
Ovarian cancer							
IGROV1	–	–	75	60	–	NT	69
OVCAR-3	–	51	130	68	–	51	140
OVCAR-4	–	63	42	64	–	63	102
OVCAR-5	–	–		65	–	–	75
OVCAR-8	71	47	40	58	–	NT	177
SK-OV-3	103	–	–	–	–	69	NT

(continued on next page)

Table 3
(continued)

Panel/cell lines	Compounds						
	3	**6**	**7**	**8**	**9**	**12**	**13**
Renal cancer							
786-0	71	43	–	56	–	88	173
A498	NT	NT	–	106	–	–	106
ACHN	43	–	69	68	–	42	143
CAKI-1	–	–	69	64	50	–	65
RXF 393	139	–	101	124	–	93	136
SN12C	–	47	60	54	–	–	76
TK-10	51	–	51	52	–	51	114
UO-31	–	–	85	80	–	67	114
Prostate cancer							
PC–3	–	60	67	68	–	77	52
DU-145	–	–	56	55	–	–	84
Breast cancer							
MCF-7	–	–	65	79	–	52	
MCF-7/ADR-RES	53	109	50	63	–	57	92
MDA-MB-231/ATCC	97	50	62		–	–	133
HS578T	70	–	77	62	–	81	106
MDA-MB-435	–	–	54	52	65	43	52
BT-549	69	–	90	96	–	40	74
T-47D	–	NT	48	120	–	100	127
MDA-N	NT	NT	NT	56	NT	–	NT

–, below 40% growth inhibition; NT, not tested at this molar concentration.

$J_{H8,F7}$ = 10.4 Hz, $J_{H8,F6}$ = 8.2 Hz, H-8); 4.51 (2H, q, $\mathbf{CH_2}CH_3$); 1.48 (3H, t, $CH_2\mathbf{CH_3}$).

The mother liquors on standing yielded another product constituted by **25** (60% yield), m.p. 57–58 °C from ethanol. Analysis for $C_{12}H_{13}FN_2O_2$: C, H, N.

ν_{max} (nujol) cm^{-1}: 1629, 1574.

λ_{max} (EtOH) nm: 338, 326, 214.

^{1}H NMR ($CDCl_3$) δ: 8.31 (1H, s, H-3); 7.63 (1H, d, J_{H-F} = 11.4 Hz, H-5); 7.21 (1H, d, J_{H-F} = 8.4 Hz, H-8); 4.49 (2H, q, $\mathbf{CH_2}CH_3$); 4.25 (2H, q, $\mathbf{CH_2}CH_3$); 1.55 (3H, t, $CH_2\mathbf{CH_3}$); 1.47 (3H, t, $CH_2\mathbf{CH_3}$).

The mother liquors on acidification gave crystals of **26** (25% yield), identical with an authentic sample as described above.

3.1.5. General procedure for the preparation of the esters ***12–16***

A mixture of equimolar amounts of compounds **7, 8**, and **10** (0.42 mmol) in anhydrous DMF (7.2 ml), diethyl L-glutamate hydrochloride and diethylcyanophosphonate in anhydrous DMF (0.6 ml) and in the presence of 2 mol equivalent of TEA was stirred under nitrogen at room temperature for 1.5 h.

The above reactants were used in a ratio of 1:2:2 and in the presence of fourfold mole equivalent of TEA in the case of **9** and **10.**

The resulting solution was poured into a mixture of ethyl acetate and benzene in 3:1 ratio (22 ml). The organic phase was shaken with water (35 ml), then with saturated sodium carbonate aqueous solution (42 ml), rewashed with water (35 ml) and, if necessary, with saturated sodium chloride aqueous solution (42 ml) and eventually dried over anhydrous sodium sulfate. On evaporation of the solvent, compounds **12–16** were obtained as crude products, which were recrystallized from ethanol.

Compounds **15** and **16** were both obtained in one time from compound **10,** and separated by flash chromatography eluting with a mixture of petroleum ether (40–60 °C)/ethyl acetate in a ratio of 1:1.

On the contrary from compound **9** only the diglutamate **14** was obtained. Yields, m.p. values, analytical and spectroscopic data are reported in Table 1.

3.1.6. General procedure for the preparation of the acids ***17–21***

A suspension of the proper ester (**12–16**) (61 mmol) in a mixture of ethanol (9.3 ml) and 1 M NaOH (2.9 ml) was stirred at room temperature for 3 h.

The ethanol was evaporated in vacuo and the residue taken up with water then made acidic with 2 M HCl. A solid (compounds **17–21,** respectively) from beige to yellow-orange was collected and washed with water.

If necessary compounds were recrystallized from ethanol. Yields, m.p. values, analytical and spectroscopic data are reported in Table 1.

Table 4
Percent growth inhibition recorded by compound **13** between 10^{-8} and 10^{-5} M concentrations

	10^{-8}	10^{-7}	10^{-6}	10^{-5}
Leukemia				
K-562		27	36	60
Non-small cell lung cancer				
A549/ATTC	31	30	36	94
HOP-62	31	30	36	94
Colon cancer				
HCT-116			21	58
CNS cancer				
SF-539				66
SNB-19				52
U251				47
Melanoma				
LOX IMVI			20	42
MALME-3M	31	33	37	40
Ovarian cancer				
OVCAR-3	15	32	27	65
OVCAR-4	46	50	48	42
OVCAR-5	22	29	26	51
OVCAR-8		18	37	86
Renal cancer				
786-0	15	16	28	83
ACHN	23	29	28	85
RXF 393	28	21	37	72
SN12C			26	51
Prostate cancer				
DU-145	27	39	35	52
Breast cancer				
MCF-7/ADR-RES	22	17	24	61
MDA-MB-231/ATCC	28	29	17	71
HS 578T				41
T-47D	47	65	39	61

4. Pharmacology

Twenty-one compounds of the list (Fig. 1) were submitted for in vitro anticancer evaluation to NCI. Among these 14 were selected by NCI for the in vitro assay. Only seven emerged from a preliminary screening on three-cell panel lines (MCF-7; NCI-H460; SF-288) and their evaluation is referred to the structures of **3, 6, 7, 8, 9, 12,** and **13.**

The protocol for anticancer activity is well documented [25] and it involves a panel of 60 human tumor cell lines.

The anticancer activity is derived from dose–response curves and it is presented in three different Tables 2–4.

In Table 2 the response parameters (–log GI_{50}), (–log TGI), and (–log LC_{50}) refer to the concentration of the agent in the assay that produced 50% growth inhibition (GI), total growth inhibition (TGI) and 50% cytotoxicity (LC), respectively, and are expressed as mean graph midpoints.

In Table 3, we reported the activities of those compounds, which showed a percent GI greater than 40% on subpanel cell lines at 10^{-4} M concentration.

In Table 4, we reported the activity of compound **13** that exhibited a significant percentage GI at the most diluted concentrations (10^{-8}–10^{-5} M).

In Fig. 2, we reported the percent GI of compound **9** against leukemia HL-60 (TB), K-562 and SR cell lines in the range of 10^{-8}–10^{-4} M concentration.

5. Results of the in vitro pharmacological antitumor assays

The data of in vitro anticancer activity reported in Table 2 established that the average sensitivity of all cell lines towards the tested agents, represented as mean graph midpoints, falls in the range of $10^{-4.63}$–$10^{-4.00}$ M concentration and according to this the following decreasing order of activity was recorded **13 >8 >7 >9 >3 >12 >6.**

The data of Table 3 better indicate that the highest activities were recorded on all the panel cell lines for the compounds **7, 8, 12,** and **13,** whereas the compounds **3** and **6** exhibited a moderate range of both cell line sensitivity and percent tumor GI. Interestingly compound **9** (Fig. 2) showed to possess both selective and dose-dependent activity against leukemia subpanel cell lines (HL-60 (TB), K-562 and SR) in the range of 10^{-8}–10^{-4} M, the other cell lines being almost unaffected.

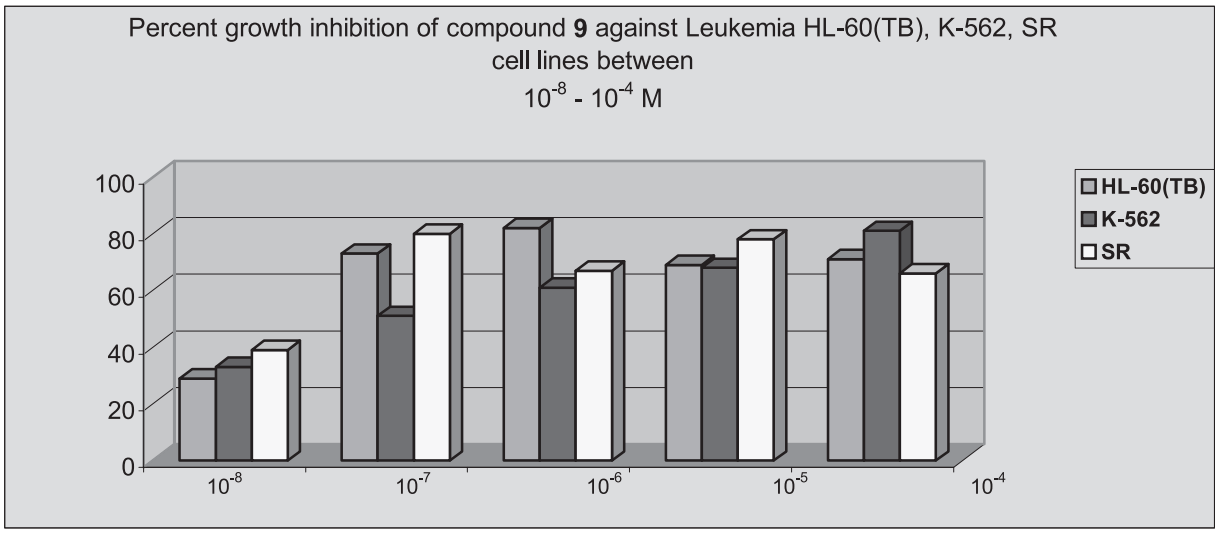


Fig. 2. Percent growth inhibition of compounds **9** against leukemia HL-60 (TB), K-562, and SR cell lines between 10^{-8} and 10^{-4}.

According to the data of Table 4 we can observe that compound **13** still exhibited significant percent tumor GI values at the most diluted concentrations (10^{-8}–10^{-5} M) whereas, in some case its activity seems to be dose dependent.

In conclusion we can say that this type of homologation does not present any significative improvement towards the previously encouraging reports [11] but at least two compounds emerged for the selectivity (**9**) and low toxicity (**13**).

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