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Quinoxaline chemistry. Part 13: 3-carboxy-2-benzylamino-substituted quinoxalines and *N*-[4-[(3-carboxyquinoxalin-2-yl) aminomethyl]benzoyl]-L-glutamates: synthesis and evaluation of in vitro anticancer activity

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Abstract

Among a new series of 28 3-carboxy or carbethoxy quinoxalines bearing a substituted benzylamino or *N*-[4-(aminomethyl)benzoyl]glutamate group on position 2 of the ring and various substituents at C-6, 7 positions, 21 were selected at the National Cancer Institute for evaluation of their in vitro anticancer activity. The results obtained seem to confirm that the carboxy or carbethoxy group on position 3 is not helpful, with a few exceptions, for the anticancer activity. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Anticancer activity; Quinoxalines derivatives; Benzylaminoquinoxalines

1. Introduction

Our continuous efforts to develop potential chemotherapeutic anticancer drugs among quinoxaline derivatives as analogues of both classical and non-classical antifolate agents have so far produced some interesting compounds, which received attention at the National Cancer Institute (NCI), Bethesda, USA, for in depth in vivo investigation [1–4]. In line with these results, we designed new quinoxalines bearing novel combinations of substituents in order to get a closer structure–activity relationship.

In this paper we report the synthesis of a series of compounds **1**–**28** listed in Fig. 1, which can be considered as homologues of the previously described [2,3] 2-[anilino] and 2-[4-(aminobenzoyl)-L-glutamate]-3,6-disubstituted quinoxalines, in order to verify the influence of the methylene linkage in this series. Preliminary results of their anticancer activity, as well as of their preparation, have been communicated recently [5,6].

In particular, we have taken into account the presence of a carboxy or carbethoxy group on position 3 of quinoxaline, which in a previous series led to the discovery of compounds endowed with anticancer, anti-HIV, anti Candida activity [2].

2. Chemistry

Compounds **1**–**11** and **23**–**25** of Fig. 1 were obtained according to the sequence of reactions outlined in Scheme 1.

Nucleophilic displacement of chloroderivatives **31a**–**c** with the appropriate benzylamines was carried out in refluxing ethanol, whereas in the case of diethyl *N*-[4-(aminomethyl)benzoyl]-L-glutamate, this reaction was performed in DMF and in the presence of Cs_2CO_3.

It is interesting to note that in two cases (**31a**, **b**), along with the expected compounds **4** and **8**, a concomitant formation of the amides **29**, **30** was observed.

Alkaline hydrolysis of the esters **1**–**11** and **23**–**25** gave good yields of the corresponding acids **12**–**22** and **26**–**28** (Table 1).

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Table 1
Melting points, yields, 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]
1	118–120 (a)	70	$C_{21}H_{23}N_3O_5$	3380, 1680	416, 311, 262, 207	[A] 8.31 (1H, s, NHCH$_2$), 8.00 (1H, d, J = 8.4 Hz, arom.), 7.78–7.62 (2H, m, arom.) 7.50–7.38 (1H, m, arom), 6.70 (2H, s, H-2′,6′), 4.75 (2H, d, J = 5.2 Hz, CH$_2$NH), 4.53 (2H, q, CH$_2$CH$_3$), 3.86 (6H, s, 3′,5′-OCH$_3$), 3.84 (3H, s, 4′-OCH$_3$), 1.49 (3H, t, CH$_3$CH$_2$)
2	115–116 (a)	75	$C_{20}H_{27}N_3O_4$	3380, 1680	417, 311, 262, 219, 204	[A] 8.28 (1H, t, NHCH$_2$), 7.99 (1H, d, J = 8.4 Hz, arom.), 7.76–7.61 (2H, m, arom.), 7.44–7.36 (1H, m, arom.), 7.02 (1H, s, H-2′) 7.01 (1H, dd, H $_{6',5'}$ = 8.8 and $J_{6',5'}$ = 2.0 Hz, H-6′), 6.85 (1H, d, $J_{5',6'}$ = 8.8 Hz, H-5′), 4.75 (2H, d, J = 5.4 Hz, CH$_2$NH), 4.52 (2H, q, CH$_2$CH$_3$), 3.89 (3H, s, OCH$_3$), 3.88 (3H, s, OCH$_3$), 1.48 (3H, t, CH$_3$CH$_2$)
3	121–123 (a)	40.82	$C_{19}H_{19}N_3O_3$	3380, 1680	418, 311, 262, 219, 201	[A] 8.26 (1H, t, NHCH$_2$), 7.95 (1H, d, J = 8.4 Hz, arom.), 7.74–7.60 (2H, m, arom.), 7.45–7.40 (1H, m, arom.), 7.37 (2H, d, J = 8.2 Hz, H-3′,5′), 6.89 (2H, d, J = 8.2 Hz, H-2′,6′), 4.75 (2H, d, J = 4.8 Hz, CH$_2$NH), 4.51 (2H, q, CH$_2$CH$_3$), 3.80 (3H, s, OCH$_3$), 1.47 (3H, t, CH$_3$CH$_2$)
4	143–145 (a)	32	$C_{18}H_{15}Cl_2N_3O_2$	3380, 1680	412, 311, 261, 218, 203	[A] 8.41 (1H, t, NHCH$_2$), 8.01 (1H, d, J = 8.4 Hz, arom.), 7.70–7.64 (2H, m, arom.), 7.53 (1H, d, $J_{2',6'}$ = 2.0 Hz, H-2′), 7.67–7.56 (2H, m, arom.), 7.28 (1H, dd, $J_{6',5'}$ = 8.8 and $J_{6',2'}$ = 2.0 Hz, H-6′), 4.79 (2H, d, J = 6.0 Hz, CH$_2$NH), 4.55 (2H, q, CH$_2$CH$_3$), 1.52 (3H, t, CH$_3$CH$_2$)
5	143–145 (a)	73.17	$C_{18}H_{16}FN_3O_2$	3400, 1720	415, 311, 261, 219	[A] 8.33 (1H, t, NHCH$_2$), 8.00 (1H, d, J = 8.2 Hz, arom.) 7.75–7.60 (2H, m, arom.), 7.44–7.36 (2H, m, H-3′, 5′, and 1H arom.), 7.07–6.99 (2H, m, H-2′,6′), 4.79 (2H, d, J = 5.4, CH$_2$NH), 4.53 (2H, q, CH$_2$CH$_3$), 1.49 (3H, t, CH$_3$CH$_2$)
6	125–127 (a)	65.2	$C_{22}H_{22}F_3N_3O_5$	3380, 1720	419, 263, 208	[A] 8.42 (1H, t, NHCH$_2$), 8.11 (1H, d, $J_{5,6}$ = 8.6 Hz, H-5), 8.00 (1H, d, $J_{8,6}$ = 2.0 Hz, H-8), 7.57 (1H, dd, $J_{6,5}$ = 8.6 and $J_{6,8}$ = 2.0 Hz, H-6), 6.68 (2H, s, H-2′,6′), 4.75 (2H, d, J = 5.4 Hz, CH$_2$NH), 4.54 (2H, q, CH$_2$CH$_3$), 3.87 (6H, s, 3′,5′-OCH$_3$), 3.85 (3H, s, 4′-OCH$_3$), 1.50 (3H, t, CH$_3$CH$_2$)
7	95–96 (a)	81.4	$C_{21}H_{20}F_3N_3O_4$	3380, 1680	420, 304, 262, 215, 204	[A] 8.40 (1H, t, NHCH$_2$), 8.06 (1H, d, $J_{5,6}$ = 8.6 Hz, H-5), 8.00 (1H, d, $J_{8,6}$ = 2.0 Hz, H-8), 7.56 (1H, dd, $J_{6,5}$ = 8.6 and $J_{6,8}$ = 2.0 Hz, H-6), 7.00 (1H, d, $J_{5',6'}$ = 8.8 Hz, H-5′), 6.98 (1H, s, H-2′), 6.86 (1H, dd, $J_{6',5'}$ = 8.8 and $J_{6',2'}$ = 2.0 Hz, H-6′), 4.74 (2H, d, J = 5.4 Hz, CH$_2$NH), 4.54 (2H, q, CH$_2$CH$_3$), 3.89 (3H, s, 4′-OCH$_3$), 3.88 (3H, s, 3′-OCH$_3$), 1.49 (3H, t, CH$_3$CH$_2$)
8	106–107 (a)	52	$C_{20}H_{18}F_3N_3O_3$	3380, 1680	420, 263, 216,	[A] 8.39 (1H, t, NHCH$_2$), 8.09 (1H, d, $J_{5,6}$ = 8.2 Hz, H-5), 8.00 (1H, s, H-8), 7.55 (1H, dd, $J_{6,5}$ = 8.2 and $J_{6,8}$ = 1.8 Hz, H-6), 7.37 (2H, d, J = 8.6 Hz, H-3′, 5′), 6.90 (2H, d, J = 8.6 Hz, H-2′, 6′), 4.74 (2H, d, J = 5.6 Hz, CH$_2$NH), 4.53 (2H, q, CH$_2$CH$_3$), 3.81 (3H, s, 4′-OCH$_3$), 1.49 (3H, t, CH$_3$CH$_2$)
9	139–141 (a)	45.45	$C_{19}H_{14}Cl_2F_3N_3O_2$	3380, 1700	414, 261, 215, 204	[A] 8.53 (1H, t, NHCH$_2$), 8.11 (1H, d, $J_{5,6}$ = 8.8 Hz, H-5), 7.97 (1H, d, $J_{8,6}$ = 1.8 Hz, H-8), 7.58 (1H, dd, $J_{6,5}$ = 8.8 and $J_{6,5}$ = 1.8 Hz, H-6), 7.51 (1H, d, $J_{2',6'}$ = 1.6 Hz, H-2′), 7.42 (1H, d, $J_{5',6'}$ = 8.2 Hz, H-5′), 7.26 (1H, dd, $J_{6',5'}$ = 8.2 and $J_{6',2'}$ = 1.6 Hz, H-6′), 4.79 (2H, d, J = 5.8 Hz, CH$_2$NH), 4.56 (2H, q, CH$_2$CH$_3$), 1.51 (3H, t, CH$_3$CH$_2$)
10	106–108 (a)	65	$C_{19}H_{15}F_4N_3O_2$	3360, 1700	417, 304, 261, 216	[A] 8.45 (1H, t, NHCH$_2$), 8.10 (1H, d, $J_{5,6}$ = 8.8 Hz, H-5), 7.98 (1H, d, $J_{8,6}$ = 1.8 Hz, H-8), 7.56 (1H, dd, $J_{6,5}$ = 8.8 and $J_{6,8}$ = 1.8 Hz, H-6), 7.48–7.37 (2H, m, H-3′,5′), 7.13–7.01 (2H, m, H-2′,6′), 4.79 (2H, d, J = 5.6 Hz, CH$_2$NH), 4.54 (2H, q, CH$_2$CH$_3$), 1.50 (3H, t, CH$_3$CH$_2$)
11	148–150 (a)	68.75	$C_{21}H_{21}F_2N_3O_5$	3360, 1680	413, 304, 258, 208	[B] 8.41 (1H, t, NHCH$_2$), 7.85-7.70 (1H, m, H-5), 7.55–7.40 (1H, m, H-8), 6.72 (2H, H-2′,6′), 4.70 (2H, d, NHCH$_2$), 4.45 (2H, q, CH$_2$CH$_3$), 3.83 (6H, s, 3′,5′-OCH$_3$), 3.76 (3H, s, 4′-OCH$_3$), 1.46 (3H, t, CH$_3$CH$_2$)
12	155–158 (a)	81.08	$C_{19}H_{19}N_3O_5$	3400, 1680	386, 300, 258, 208	[A] 8.38 (1H, t, NHCH$_2$), 7.86 (1H, d, J = 8.2 Hz, arom.), 7.82–7.68 (2H, m, arom.), 7.52–7.42 (1H, m, arom.), 6.69 (2H, s, H-2′,6′), 4.78 (2H, d, J = 5.6 Hz, CH$_2$NH), 3.86 (6H, s, 3′,5′-OCH$_3$), 3.84 (3H, s, 4′-OCH$_3$)
13	125–128 (a)	74.07	$C_{18}H_{17}N_3O_4$	3340, 1680	388, 304, 259, 204	[A] 8.33 (1H, t, NHCH$_2$), 7.86 (1H, d, J = 8.8 Hz, arom.), 7.80–7.65 (2H, m, arom.), 7.50–7.40 (1H, m, arom.), 7.01 (1H, d, $J_{2',6'}$ = 1.6 Hz, H-2′), 6.99 (1H, dd, $J_{6',5'}$ = 6.4 and $J_{6',2'}$ = 1.8 Hz, H-6′), 6.84 (1H, d, J = 8.8 Hz, H-5′), 4.78 (2H, d, J = 5.6 Hz, CH$_2$NH), 3.88 (3H, s, OCH$_3$), 3.87 (3H, s, OCH$_3$)

Table 1 (Continued)

14	162–164 (a)	94.6	$C_{17}H_{15}N_3O_3$	3300, 1720	387, 304, 259, 215	[B] 8.52 (1H, t, N*H*CH_2), 7.93 (1H, d, $J = 8.4$ Hz, arom.), 7.75–7.62 (2H, m, arom.), 7.45–7.38 (1H, m, arom.), 7.36 (2H, d, $J = 8.6$ Hz, H-3′,5′), 6.87 (2H, d, $J = 8.6$ Hz, H-2′,6′), 4.74 (2H, d, $J = 5.0$ Hz, C*H*$_2$NH), 3.80 (3H, s, OCH_3)
15	159–161 (a)	55	$C_{16}H_{11}Cl_2N_3O_2$	3340, 1700	382, 304, 258, 213, 205	[B] 8.63 (1H, t, N*H*CH_2), 7.96 (1H, d, $J = 8.4$ Hz, H-5), 7.70–7.60 (2H, m, arom.), 7.55 (1H, s, H-2′), 7.50–7.36 (2H, m, arom.), 7.33 (1H, dd, $J_{6',5'} = 8.4$ and $J_{6',2'} = 1.8$ Hz, H-6′), 4.80 (2H, d, $J = 6.0$ Hz, C*H*$_2$NH), 4.35 (1H, brs, COOH [c])
16	175–177 (a)	74.07	$C_{16}H_{12}FN_3O_2$	3400, 1700	388, 304, 259, 214	[B] 8.56 (1H, t, N*H*CH_2), 7.94 (1H, dd, $J_{5,6} = 8.6$ and $J_{5,7} = 1$ Hz, H-5), 7.67–7.64 (2H, m, arom.), 7.47–7.38 (3H, m, arom.), 7.07–6.98 (2H, m, arom.), 5.15 (1H, brs, COOH [c]), 4.79 (2H, d, $J = 5.4$ Hz, C*H*$_2$NH)
17	143–145 (a)	96.43	$C_{20}H_{18}F_3N_3O_5$	3440, 3360, 1680	393, 332, 258, 209	[A] 8.52 (1H, t, N*H*CH_2), 8.05 (1H, s, H-8), 7.99 (1H, d, $J_{5,6} = 8.4$ Hz, H-5), 7.60 (1H, dd, $J_{6,5} = 8.4$ and $J_{6,8} = 1.8$ Hz, H-6), 6.68 (2H, s, H-2′,6′), 4.77 (2H, d, $J = 5.4$, C*H*$_2$NH), 3.87 (6H, s, 3′,5′-OCH_3), 3.84 (3H, s, 4′-OCH_3)
18	127–128 (a)	96	$C_{19}H_{16}F_3N_3O_4$	3520, 3360, 1760	393, 258, 204	[A] 8.48 (1H, t, N*H*CH_2), 8.05 (1H, s, H-8), 7.98 (1H, d, $J_{5,6} = 8.8$ Hz, H-5), 7.59 (1H, dd, $J_{6,5} = 8.8$ and $J_{6,8} = 1.8$ Hz, H-6), 7.10–6.98 (2H, m, H-2′,6′), 6.85 (1H, d, $J_{5',6'} = 8.0$ Hz, H-5′), 4.76 (2H, d, $J = 5.4$ Hz, C*H*$_2$NH), 3.89 (3H, s, OCH_3), 3.88 (3H, s, OCH_3)
19	116–118 (a)	85.71	$C_{18}H_{14}F_3N_3O_3$	3360, 1700	393, 258, 213	[A] 8.48 (1H, t, N*H*CH_2), 8.05 (1H, s, H-8), 7.97 (1H, d, $J_{5,6} = 8.4$ Hz, H-5), 7.58 (1H, dd, $J_{6,5} = 8.8$ and $J_{6,8} = 1.8$ Hz, H-6), 7.36 (2H, d, $J = 8.6$ Hz, H-3′,5′), 6.89 (2H, d, $J = 8.6$ Hz, H-2′,6′), 4.77 (2H, d, $J = 5.8$ Hz, C*H*$_2$NH), 3.80 (3H, s, OCH_3)
20	270 (a)	90.91	$C_{17}H_{10}Cl_2F_3N_3O_2$	3380, 1750	387, 332, 257, 211, 203	[B] 9.13 (1H, t, N*H*CH_2), 8.10 (1H, d, $J_{5,6} = 8.2$ Hz, H-5), 7.89 (1H, s, H-8), 7.56 (1H, s, H-2′), 7.54 (1H, d, $J_{6,5} = 8.2$ Hz, H-6), 7.44 (1H, d, $J_{5',6'} = 8.2$ Hz, H-5′), 7.35 (1H, d, $J_{6',5'} = 8.2$ Hz, H-6′), 4.80 (2H, d, $J = 5.4$ Hz, C*H*$_2$NH)
21	162–163 (a)	69.44	$C_{17}H_{11}F_4N_3O_2$	3360, 1760	392, 257, 212	[A] 8.52 (1H, t, N*H*CH_2), 8.03 (1H, s, H-8), 7.99 (1H, d, $J_{5,6} = 8.4$ Hz, H-5), 8.60 (1H, d, $J_{6,5} = 8.6$ Hz, H-6), 7.44–7.37 (2H, m, H-3′,5′), 7.08–7.00 (2H, m, H-2′,6′), 4.81 (2H, d, $J = 5.4$ Hz, C*H*$_2$NH)
22	169–171 (a)	91.3	$C_{19}H_{17}F_2N_3O_5$	3440, 3340, 1700	390, 332, 253, 208	[A] 8.41 (1H, t, N*H*CH_2), 6.30 (1H, dd, $J = 10.2$ and $J = 8.4$ Hz, H-8), 4.80 (1H, dd, $J = 9.0$ and $J = 8.0$ Hz, H-5), 6.66 (2H, s, H-2′,6′), 8.73 (2H, d, $J = 5.4$ Hz, C*H*$_2$NH), 3.86 (6H, s, 3′,5′-OCH_3), 3.84 (3H, s, 4′-OCH_3)
23	182–184 (a)	48.78	$C_{28}H_{32}N_4O_7$	3400, 3300, 1730, 1700, 1630	414, 310, 261, 219, 204	[A] 8.43 (1H, t, N*H*CH_2), 8.00 (1H, d, $J = 8.4$ Hz, arom.), 7.80 (2H, d, $J = 8.0$ Hz, H-3′,5′), 7.66 (2H, m, arom.), 7.51 (2H, d, $J = 8.0$ Hz, H-2′,6′), 7.48–7.37 (1H, m, arom.), 7.02 (1H, d, $J = 7.4$ Hz, N*H*CH), 4.89 (2H, d, $J = 5.6$ Hz, C*H*$_2$NH), 4.87–4.74 (1H, m, NHC*H*CH_2), 4.54 (2H, q, C*H*$_2$$CH_3$), 4.23 (2H, q, C*H*$_2$$CH_3$), 4.10 (2H, q, C*H*$_2$$CH_3$), 2.60–2.04 (4H, m, CH_2CH_2), 1.50 (3H, t, C*H*$_3$$CH_2$), 1.30 (3H, t, C*H*$_3$$CH_2$), 1.21 (3H, t, C*H*$_3$$CH_2$)
24	160–161 (a)	50.63	$C_{29}H_{31}F_3N_4O_7$	3380, 3320, 1730, 1700, 1630	416, 304, 262, 216, 204	[A] 8.56 (1H, t, N*H*CH_2), 8.11 (1H, d, $J_{5,6} = 8.4$ Hz, H-5), 7.97 (1H, s, H-8), 7.82 (2H, d, $J = 8.2$ Hz, H-3′,5′), 7.57 (1H, dd[P], $J_{6,5} = 8.4$ and $J_{6,8} = 1.6$ Hz, H-6), 7.51 (2H, d, $J = 8.2$ Hz, H-2′,6′), 7.04 (1H, d, $J = 7.4$ Hz, N*H*CO), 4.89 (2H, d, $J = 6.2$, C*H*$_2$NH), 4.79 (1H, m, NHC*H*CH_2), 4.56 (2H, q, C*H*$_2$$CH_3$), 4.24 (2H, q, C*H*$_2$$CH_3$), 4.11 (2H, q, C*H*$_2$$CH_3$), 2.60–2.10 (4H, m, CH_2CH_2), 1.51 (3H, t, C*H*$_3$$CH_2$), 1.30 (3H, t, C*H*$_3$$CH_2$), 1.22 (3H, t, C*H*$_3$$CH_2$)
25	169–170 (a)	32	$C_{28}H_{30}F_2N_4O_7$	3360, 3300, 1730, 1700, 1630	412, 302, 258, 203	[A] 8.50 (1H, t, N*H*CH_2), 7.81 (2H, d, $J = 8.2$ Hz, H-3′,5′), 7.60 (1H, dd, $J = 10.2$ and $J = 8.6$ Hz, H-8), 7.89 (2H, d, $J = 8.4$ Hz, H-2′,6′), 7.42 (1H, dd, $J = 10.2$ and $J = 8.6$ Hz, H-5), 7.04 (1H, d, $J = 7.4$, N*H*CO), 4.85 (2H, d, $J = 5.8$ C*H*$_2$NH), 4.80 (1H, m, NHC*H*CH_2), 4.54 (2H, q, C*H*$_2$$CH_3$), 4.27 (2H, q, C*H*$_2$$CH_3$), 4.11 (2H, q, C*H*$_2$$CH_3$), 2.60–2.10 (4H, m, CH_2CH_2), 1.49 (3H, t, C*H*$_3$$CH_2$), 1.30 (3H, t, C*H*$_3$$CH_2$), 1.22 (3H, t, C*H*$_3$$CH_2$)
26	178–180	80	$C_{22}H_{20}N_4O_7$	3380, 3300, 1750, 1720, 1630	390, 304, 259, 216, 203	[B] 8.67 (1H, t, N*H*CH_2), 8.30 (1H, d, $J = 7.4$ Hz, N*H*CO), 7.93 (1H, d, $J = 8.2$ Hz, arom.), 7.87 (2H, d, $J = 8.2$ Hz, H-3′,5′), 7.80–7.60 (2H, m, arom.), 7.50 (2H, d, $J = 8.2$ Hz, H-2′,6′), 7.45–7.38 (1H, m, arom.), 5.52 (3H, brs, 3·COOH [c]), 4.87 (2H, d, $J = 4.8$ Hz, C*H*$_2$NH), 4.55 (1H, m, NHC*H*CH_2), 2.60–2.10 (4H, m, CH_2CH_2)

Table 1 (Continued)

27	152–155	73.3	$C_{23}H_{19}F_3N_4O_7$	3460, 3280, 1700, 1630	390, 332, 256, 212	[B] 8.85 (1H, t, N*H*CH_2), 8.10 (1H, d, $J_{5,6}$ = 8.4 Hz, H-5), 8.00–7.90 (1H, m, arom.), 7.87 (2H, d, J = 8.4 Hz, H-3′,5′), 7.56 (1H, s, H-8), 7.49 (2H, d, J = 8.4 Hz, H-2′,6′), 5.35 (3H, brs, 3·COOH [c]), 4.87 (2H, d, J = 4.8 Hz, C*H*$_2$NH), 4.62 (1H, m, NHC*H*CH_2), 2.60–2.10 (4H, m, CH_2CH_2)
28	169–171	77.92	$C_{22}H_{18}F_2N_4O_7$	3320, 3260, 1740, 1700, 1640	385, 332, 252, 205	[B] 8.73 (1H, t, N*H*CH_2), 8.26 (1H, d, J = 5.4 Hz, N*H*CO), 7.87 (2H, d, J = 8.2 Hz, H-3′,5′), 7.74 (1H, dd, J = 10.2 and J = 8.2 Hz, H-8), 7.48 (2H, d, J = 8.2 Hz, H-2′,6′), 7.41 (1H, dd, J = 10.2 and J = 8.2 Hz, H-5), 6.16 (3H, brs, 3·COOH [c]), 7.83 (2H, d, CH_2NH), 4.56 (1H, m, NHC*H*CH_2), 2.50–2.00 (4H, m, CH_2CH_2)
29	154–156	15	$C_{23}H_{16}Cl_4N_4O$	3360, 3300, 1670	408, 309, 260, 219, 204	[A] 9.15 (1H, t, N*H*CH_2), 8.67 (1H, t, N*H*CH_2), 7.80 (1H, d, J = 8.4 Hz, arom.), 7.70–7.20 (9H, m, arom.), 4.77 (2H, d, J = 5.6 Hz, C*H*$_2$NH), 4.61 (2H, d, J = 6.4 Hz, C*H*$_2$NH)
30	90–92	21	$C_{26}H_{23}F_3N_4O_3$	3400, 3260, 1620	416, 308, 261, 217, 203	[A] 9.35 (1H, t, N*H*CH_2), 8.52 (1H, t, N*H*CH_2), 7.97 (1H, d, $J_{8,6}$ = 1.8 Hz, H-8), 7.85 (1H, d, $J_{5,6}$ = 8.4 Hz, H-5), 7.49 (1H, dd, $J_{6,5}$ = 8.6 and $J_{6,8}$ = 1.8 Hz, H-6), 7.37 (2H, d, J = 8.8 Hz, H-3′,5′), 7.30 (2H, d, J = 8.8 Hz, H-3″,5″), 6.90 (2H, d, J = 8.6 Hz, H-2′,6′), 6.88 (2H, d, J = 8.6 Hz, H-2″,6″), 4.73 (2H, d, J = 5.6 Hz, C*H*$_2$NH), 4.57 (2H, d, J = 5.8 Hz, C*H*$_2$NH), 3.81 (3H, s, OCH_3), 3.80 (3H, s, OCH_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] Exchanges with D_2O.

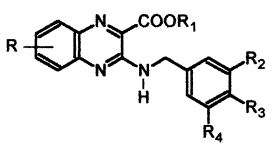
3. Experimental

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 nanometres 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 TMS as an internal standard. Elemental analyses were performed at the Dipartimento di Scienze Farmaceutiche, Laboratorio di Microanalisi, University of Padua, Italy. The analytical results for C, H, N were within $\pm 0.4\%$ of the theoretical values.

3.1.1. Intermediates

The intermediate chloroquinoxalines (**31a**, **b**) necessary for this work were obtained as described previously [7]. Compound **31c** is a new compound and its preparation is described below. Diethyl *N*-[4-(aminomethyl)benzoyl]-L-glutamate was prepared according to the indications of the literature [8].



1-28

Compound	R	R_1	R_2	R_3	R_4
1	H	Et	OMe	OMe	OMe
2	H	Et	OMe	OMe	H
3	H	Et	H	OMe	H
4	H	Et	Cl	Cl	H
5	H	Et	H	F	H
6	7-CF_3	Et	OMe	OMe	OMe
7	7-CF_3	Et	OMe	OMe	H
8	7-CF_3	Et	H	OMe	H
9	7-CF_3	Et	Cl	Cl	H
10	7-CF_3	Et	H	F	H
11	6,7-F	Et	OMe	OMe	OMe
12	H	H	OMe	OMe	OMe
13	H	H	OMe	OMe	H
14	H	H	H	OMe	H
15	H	H	Cl	Cl	H
16	H	H	H	F	H
17	7-CF_3	H	OMe	OMe	OMe
18	7-CF_3	H	OMe	OMe	H
19	7-CF_3	H	H	OMe	H
20	7-CF_3	H	Cl	Cl	H
21	7-CF_3	H	H	F	H
22	6,7-F	H	OMe	OMe	OMe
23	H	Et	H	CO-Glu-Et	H
24	7-CF_3	Et	H	CO-Glu-Et	H
25	6,7-F	Et	H	CO-Glu-Et	H
26	H	H	H	CO-Glu-H	H
27	7-CF_3	H	H	CO-Glu-H	H
28	6,7-F	H	H	CO-Glu-H	H

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

3.1.2. 2-Chloro-6,7-difluoro-3-ethoxycarbonyl-quinoxaline (**31c**)

A mixture of 3-ethoxycarbonyl-6,7-difluoroquinoxalin-2(1*H*)one [9] (1 g, 3.93 mmol) and $POCl_3$ (10 ml) was heated at 70°C while stirring for 5 h. After the removal of excess $POCl_3$ in vacuo, the residue was taken up with iced water and a solid was filtered off and washed thoroughly with water to give **31c** (0.9 g, 84% yield), m.p. 79–81°C. Anal. ($C_{11}H_7ClF_2N_2O_2$): C, H, N. IR: 1730 cm^{-1}. ^{1}H NMR δ: 7.95 (1H, dd, $J = 8.0$, 9.8 Hz, H-5), 7.83 (1H, dd, $J = 8.0$, 9.8 Hz, H-8), 4.58 (2H, q, $J = 7.0$, CH_2CH_3), 1.49 (3H, t, $J = 7.0$ Hz, CH_3CH_2).

3.1.3. General procedure for the preparation of 2-benzylamino-3-ethoxycarbonylquinoxalines (**1**–**11**)

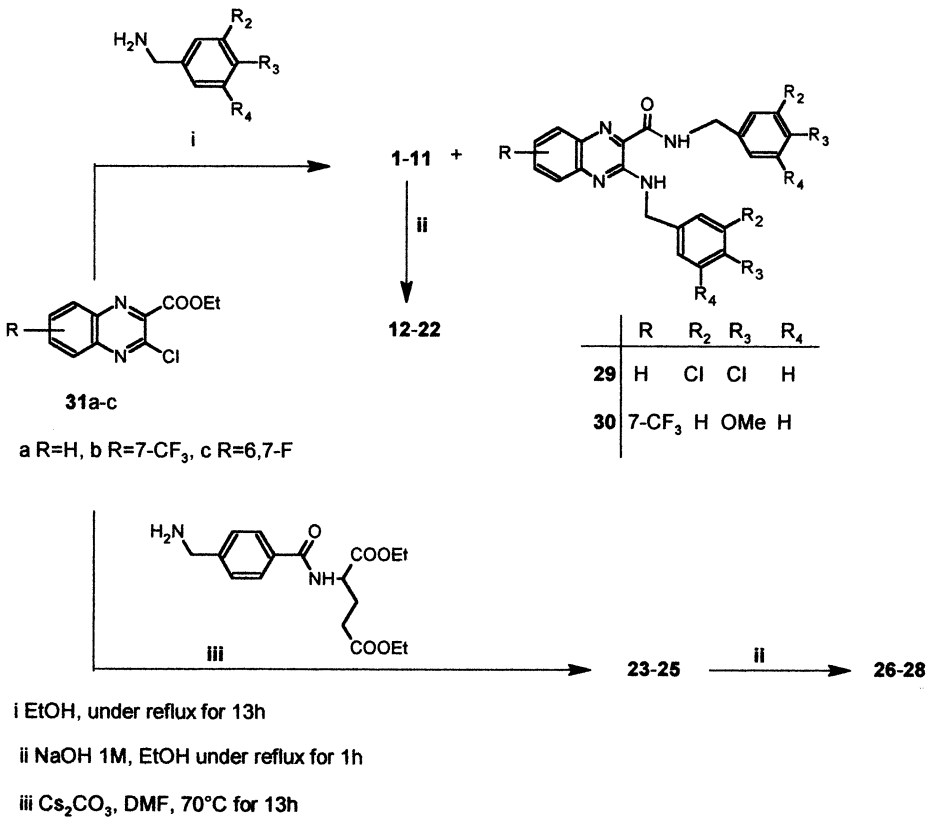
A mixture of equimolar amounts (2 mmol) of 2-chloro-quinoxalines (**31a**–**c**) and the appropriate commercially available substituted benzylamines suspended in ethanol (10 ml) was stirred under reflux for 13 h. After cooling, the yellow precipitates of compounds **1**–**11** were filtered off and washed with ethanol. Purification methods, yields, melting points, analytical and spectroscopic data are reported in Table 1. In the case of compound **4**, purification was accomplished by fractional recrystallization from ethanol, which also gave the amide **29** as a side compound in 15% yield. In the case of **8**, from the column chromatography on silica gel eluting with a mixture of petrol ether (b.p. 40–60°C) and ethyl acetate in the ratio of 95:5, we also isolated the amide **30** (21%).

3.1.4. General procedure for the preparation of 2-benzylamino-3-carboxy-quinoxalines (**12**–**22**)

A suspension of the ester (**1**–**11**) (1 mmol) in a mixture of ethanol (10 ml) and 1 M NaOH aqueous solution (5 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 and eventually dried. Yields, melting points, analytical and spectroscopic data of the acids **12**–**22** are reported in Table 1.

3.1.5. General procedure for the preparation of diethyl *N*-[4-[(3-ethoxycarbonylquinoxalin-2-yl)aminomethyl]-benzoyl]-L-glutamates (**23**–**25**)

A mixture of equimolar amounts (1 mmol) of 2-chloroquinoxaline (**31a**–**c**), diethyl *N*-[4-(aminomethyl)benzoyl]-L-glutamate, as prepared in Ref. [8], and cesium carbonate in anhydrous DMF (10 ml) was



Scheme 1.

stirred at 70°C for 13 h. On cooling, the mixture was diluted with water and the precipitates formed were collected and washed with water. The products **23**–**25** were further purified as indicated in Table 1, which also reports the yields, melting points, analytical and spectroscopic data.

*3.1.6. General procedure for the preparation of N-[4-[(3-carboxyquinoxalin-2-yl)aminomethyl]benzoyl]-L-glutamic acids (**26**–**28**)*

The acids **26**–**28** were obtained in an identical manner as for compounds **12**–**22**. Purification of the crude products was accomplished by recrystallization using the solvent indicated in Table 1, where yields, melting points and spectroscopic data (IR, UV, ^{1}H NMR) are reported.

4. Pharmacology

Evaluation of anticancer and anti-HIV activity was performed on 21 compounds referring to structures **1**–**3**, **6**–**10**, **12**–**14**, **16**–**21**, **23**, **24, 26** and **27** of Fig. 1 and Table 1 at the NCI, following the known [10] in vitro disease-oriented antitumour screening program, against a panel of 60 human tumour cell lines and an HIV drug-testing system [11]. No compound exhibited anti-HIV activity.

Table 2
$-\mathrm{Log}_{10}\,\mathrm{GI}_{50}$, $-\log_{10}\mathrm{TGI}$, $-\log_{10}\mathrm{LC}_{50}$ mean graph midpoints (MG-MID) [a] of in vitro inhibitory activity test for compounds **1**–**3**, **6**–**10**, **12**–**14**, **16**–**21**, **23**, **24**, **26**, **27** against human tumour cell lines [b]

Comp.	$-\mathrm{Log}_{10}\,\mathrm{GI}_{50}$	$-\mathrm{Log}_{10}\,\mathrm{TGI}$	$-\mathrm{Log}_{10}\,\mathrm{LC}_{50}$
1	4.38	4.01	4.00
2	4.02	4.00	4.00
3	4.08	4.00	4.00
6	4.29	4.02	4.00
7	4.27	4.00	4.00
8	4.15	4.00	4.00
9	4.46	4.07	4.00
10	4.50	4.03	4.00
12	4.03	4.00	4.00
13	4.04	4.00	4.00
14	4.11	4.01	4.00
16	4.21	4.01	4.00
17	4.67	4.20	4.03
18	4.51	4.06	4.00
19	4.59	4.14	4.01
20	4.78	4.45	4.16
21	4.62	4.20	4.03
23	4.09	4.01	4.00
24	4.50	4.23	4.09
26	4.00	4.00	4.00
27	4.00	4.00	4.00

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

Table 3
Percentage tumour growth inhibition recorded on subpanel cell lines at 10^{-4} M of compounds **1**, **2**, **3**, **6–10**, **12–14**, **16–21**, **23**, **24**, **26**, **27** [a]

Panel/cell lines	**1**	**2**	**3**	**6**	**7**	**8**	**9**	**10**	**12**	**13**	**14**	**16**	**17**	**18**	**19**	**20**	**21**	**23**	**24**	**26**	**27**
Leukaemia																					
CCRF-CEM	74	*	*	nt	59	49	*	99	42	40	54	73	161	102	94	67	83	*	*	*	*
HL-60(TB)	92	*	*	110	66	81	85	96	45	*	51	83	146	nt	nt	nt	nt	nt	nt	nt	*
K-562	96	*	*	nt	69	100	56	91	40	*	51	64	132	113	127	147	128	*	60	*	*
MOLT-4	95	*	*	57	82	87	46	84	*	*	nt	59	126	133	136	153	144	*	*	*	44
RPMI-8226	95	*	*	137	53	55	*	87	45	*	117	76	nt	102	86	96	91	*	52	*	nt
SR	81	*	40	119	93	99	82	89	50	*	75	67	97	123	135	159	143	*	*	*	*
Non-small-cell lung cancer																					
A549/ATCC	76	*	44	58	71	69	88	74	40	42	50	65	123	96	102	200	154	*	80	*	*
EKVX	67	*	43	63	79	48	90	87	59	*	57	74	127	98	110	191	119	*	176	*	42
HOP-62	60	*	*	44	61	50	136	69	43	47	*	47	122	119	131	173	144	44	200	*	*
HOP-92	70	*	*	95	78	68	145	138	62	82	55	51	nt	92	121	179	141	47	127	*	*
NCI-H226	54	*	*	*	60	42	94	89	*	*	*	65	68	62	73	144	70	*	200	*	*
NCI-H23	41	*	45	64	59	54	78	97	*	*	46	65	92	95	112	193	109	41	97	*	*
NCI-H322M	nt	nt	nt	85	68	70	75	nt	nt	nt	76	79	122	99	115	171	107	*	61	*	*
NCI-H460	94	49	54	*	86	93	74	110	51	49	63	65	108	97	98	190	120	nt	nt	nt	*
NCI-H522	80	*	65	83	97	98	151	96	57	*	57	67	94	nt	nt	nt	nt	40	200	nt	*
Colon cancer																					
COLO 205	84	*	*	117	91	51	96	110	46	*	62	103	136	129	181	179	155	*	*	*	*
HCC-2998	67	*	43	68	nt	nt	nt	111	*	*	40	50	87	102	121	197	120	nt	nt	nt	*
HCT-116	79	*	66	90	78	65	99	77	*	*	69	79	200	115	159	200	200	*	*	*	*
HCT-15	79	*	41	75	61	53	82	87	40	54	126	145	99	99	116	171	105	*	57	*	*
HT29	87	*	51	66	92	64	104	87	49	*	45	60	nt	93	113	200	nt	*	nt	*	*
KM 12	80	*	67	81	71	76	95	62	44	*	60	72	111	96	122	174	96	*	86	*	*
SW-620	78	*	*	65	71	49	87	86	44	*	47	64	94	86	92	142	89	*	82	*	*
SNC cancer																					
SF-268	40	*	43	60	54	*	72	120	*	44	41	63	93	nt	nt	nt	nt	*	200	*	*
SF-295	68	*	*	48	71	45	121	57	*	*	77	90	156	92	95	165	108	*	200	*	*
SF-539	*	*	*	87	49	33	67	55	*	*	*	*	154	95	146	180	154	nt	nt	nt	*
SNB-19	nt	nt	nt	45	51	*	109	77	nt	nt	46	68	129	95	95	200	99	70	200	*	*
SNB-75	51	43	83	104	91	54	126	52	57	45	51	*	111	81	95	174	103	45	114	*	*
U251	82	*	58	nt	75	*	66	85	49	50	63	68	185	114	99	188	123	62	200	*	*
Melanoma																					
LOX IMVI	73	*	*	84	81	68	97	91	*	*	77	76	94	98	149	189	159	*	*	*	*
MALME-3M	90	*	*	nt	51	64	100	81	48	48	nt	nt	88	nt	nt	nt	nt	53	200	*	*
M14	nt	nt	nt	50	61	*	93	68	nt	nt	*	43	172	97	128	200	120	40	150	*	*
SK-MEL-2	nt	nt	nt	76	106	51	60	63	nt	nt	*	51	144	141	124	180	148	*	105	*	*
SK-MEL-28	62	*	*	74	nt	nt	nt	71	*	nt	41	*	98	64	87	184	78	*	69	*	*
SK-MEL-5	88	*	*	nt	93	65	93	89	50	45	65	85	nt	156	159	188	172	*	59	*	nt
UACC-257	56	*	*	49	65	54	69	85	*	*	*	40	121	87	99	133	111	nt	nt	*	*
UACC-62	73	*	53	67	57	*	93	90	57	*	43	48	200	149	142	187	166	*	162	*	*

Table 3 (Continued)

Ovarian cancer																					
IGROV1	64	*	*	40	59	49	130	112	*	40	45	67	nt	112	92	175	95	62	200	48	*
OVCAR-3	55	*	*	64	74	56	120	75	44	42	45	75	123	128	167	171	143	54	200	*	*
OVCAR-4	81	*	46	nt	57	60	143	102	46	41	nt	nt	nt	70	84	132	*	nt	nt	*	*
OVCAR-5	*	*	*	*	*	*	*	60	*	*	*	*	71	78	102	200	167	44	115	*	*
OVCAR-8	61	*	*	46	56	47	84	85	*	46	50	68	89	84	96	180	131	*	nt	*	*
SK-OV-3	54	*	48	nt	*	*	93	62	57	41	nt	nt	97	94	86	170	103	*	96	*	*
Renal cancer																					
786-D	56	*	46	75	41	*	161	85	49	*	64	73	123	91	123	200	200	*	200	*	*
A498	101	*	82	45	66	63	104	nt	*	51	*	*	127	116	135	178	135	*	*	*	*
ACHN	71	*	*	70	68	52	117	89	*	45	nt	nt	115	98	97	187	98	48	107	*	*
CAKI-1	57	*	*	*	*	*	133	74	40	47	*	61	110	96	94	176	96	nt	nt	nt	*
RXF 393	88	91	115	74	76	50	108	94	52	*	49	*	92	nt	nt	nt	24	nt	nt	nt	*
SN 12C	84	45	nt	46	nt	*	nt	91	53	51	*	*	189	112	122	200	174	*	56	*	nt
TK-10	*	54	80	*	46	56	77	79	*	*	*	*	141	nt	nt	nt	nt	*	161	*	*
UO-31	84	*	49	100	61	50	73	88	68	75	91	84	162	99	149	200	192	60	105	*	*
Prostate cancer																					
PC-3	98	*	nt	81	88	64	111	83	48	43	49	47	128	98	98	194	108	*	111	*	*
DU-145	*	*	*	62	nt	nt	nt	70	*	*	63	50	85	85	99	187	*	*	*	*	*
Breast cancer																					
MCF7	96	*	70	65	85	74	87	88	55	*	*	54	138	102	123	142	117	*	188	*	nt
MCF7/ADR-RES	132	*	57	51	74	56	69	95	49	85	82	98	nt	nt	nt	nt	nt	44	nt	*	nt
MDA-MB-231/ATCC	95	*	50	64	50	*	98	74	47	*	*	58	nt	94	100	174	111	100	86	*	nt
HS 578T	67	83	84	83	75	50	108	105	80	48	*	61	117	nt	nt	nt	nt	46	200	*	nt
MDA-MB-435	113	*	*	88	69	47	132	86	49	*	*	41	103	110	139	192	*	108	66	*	*
BT-549	126	*	*	114	63	60	81	nt	46	75	41	73	92	89	117	187	104	nt	nt	nt	nt
T-47D	77	*	54	49	74	55	74	105	41	*	64	46	106	85	99	170	100	nt	nt	nt	*
MDA-N	126	*	nt	96	70	41	65	91	47	*	41	43	153	93	115	190	113	*	*	*	*

[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	Comp.	Percentage tumour growth inhibition at the indicated molar concentration			
		10^{-8}	10^{-7}	10^{-6}	10^{-5}
Leukaemia					
RPMI-8226	**6**	48	29	29	21
HL-60(TB)	**7**	29	34	37	41
CCRF-CEM	**17**		33	54	34
HL-60(TB)	**17**	45	50	77	90
MOLT-4	**20**				40
MOLT-4	**21**	35			53
SR	**23**	46	53	39	54
RPMI-8226	**24**		24	71	108
Non-small-cell lung cancer					
NCI-H522	**8**	39	36	25	46
HOP-92	**10**	32			67
HOP-92	**13**			34	40
A549-ATCC	**24**				21
EKVX	**24**				27
HOP62	**24**				51
NCI-460	**24**				36
SNC cancer					
SNB-75	**9**	32	26	41	37
SF-268	**10**			27	45
U-251	**23**				44
SF-295	**24**			20	57
SF-539	**24**				69
SNB-19	**24**				62
SNB-75	**24**				56
Melanoma					
SK-MEL-5	**24**		23	31	47
Ovarian cancer					
OVCAR-4	**10**			27	46
IGROV1	**18**				44
OVCAR-3	**20**				54
OVCAR-3	**21**				54
OVCAR-8	**24**				52
Renal cancer					
RXF-393	**1**			24	42
UO-31	**21**				115
786-O	**24**				76
Breast cancer					
HS 578T	**17**		47	40	49
MDA-MB-435	**23**		21	21	82
MDA-MB-231/ATCC	**24**				63

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 that showed percentage growth inhibition greater than 40% on subpanel cell lines at molar concentration of 10^{-4}, whereas in Table 4, we report the activity of those compounds that exhibited a significant percentage growth inhibition at the most diluted concentrations (10^{-8} to 10^{-5}M).

5. Results and discussion

The data of Table 2 show that the average sensitivity of cell lines towards the tested agent, represented as mean graph midpoints, falls in the range 10^{-5}–10^{-4} M concentration. Mean graph midpoints for the reported

compounds also show that GI_{50} was significant and in the following decreasing order: **20** > **17** > **21** > **19** > **18** > **10** ≥ **24** > **9** > **1** > **6** > **7**, whereas mean graph midpoints for TGI and LC_{50} showed values near the highest concentrations with the exception of compounds **20** and **24**, which in turn exhibited the lowest values. The data of Table 3 are indicative of the wide range of percentage tumour growth inhibition recorded at 10^{-4} M concentration. From these it is easy to observe that only a few compounds (**2**, **23**, **26**, **27**) were almost devoid of cell-line sensitivity. In particular, the results for the acids **26** and **27** replicate previous observations, whereas in the in vitro test, the acidic functions from the glutamate moiety are somewhat unable to produce any observable activity, which on the contrary appears well evident in the corresponding esters. According to the data of Table 4, we can observe that some compounds still exhibited significant percentage tumour growth inhibition values and in particular compound **24** maintained these at the most diluted concentration spanning over 13 subpanel cell lines. Compound **17** maintained significant percentage tumour growth inhibition values against leukaemia HL-60(TB) and CCRF-CEM cell lines whereas compound **23** did so against leukaemia (SR), SNC (U-251) and breast (MDA-MB435) cancer cell lines. It is evident from the reported data that in some cases (Table 4) the percentage growth inhibition values recorded is not dose dependent and that in most cases (Table 3) the highest values of percentage growth inhibition (> 100%) are associated to high cytotoxicity.

Structure–activity relationships are limited to the members of the series selected at the NCI. However, comparison of the percentage growth inhibition values establishes that compounds **6**–**8** bearing a 7-trifluoromethyl group were superior to the corresponding **1**–**3** devoid of it, while in the 7-CF_3 substituted series the acids **17**–**21** exhibited a greater activity than the parent esters **6**–**10**.

Finally, comparison of mean graph midpoints of compounds listed in Table 2 with the previously mentioned 2-anilino [2] and 2-aminobenzoylglutamate [3] analogues clearly shows that with the exception of the compounds **1**, **6**, **7**, **17**, the insertion of a methylene bridge between the amino and the substituted phenyl moiety does not increase the cell-line sensitivity even though for most compounds in the series, the percentage growth inhibition was of the same order of magnitude with the widest non-selective anticancer activity at 10^{-4} M concentration.

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