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Quinoxaline chemistry. Part 16. 4-Substituted anilino and 4-substituted phenoxymethyl pyrrolo[1,2-a]quinoxalines and *N*-[4-(pyrrolo[1,2-a]quinoxalin-4-yl)amino and hydroxymethyl]benzoyl glutamates. Synthesis and evaluation of in vitro biological activity

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Abstract

Twenty eight pyrrolo[1,2-a]quinoxalines bearing at position 4 various substituents related to the moieties present in classical and non classical antifolic agents were prepared and evaluated in vitro for antiproliferative activity. In an in vitro screening performed at NCI, several compounds emerged as potent antiproliferative agents at concentrations ranging between 10 and 100 μM. Interestingly, some of these compounds proved active also against bovine and murine DHFR (Farmaco 53 (1998) 480). More recently, a compound of classical antifolate type has been reported to be a potent inhibitor of hDHFR in vitro (Farmaco 58 (2003) 51). We then synthesized new derivatives that, in our hands, were endowed with in vitro antiproliferative activities as low as 3.4 μM against a panel of cell lines derived from hematological and solid tumours. In addition, a complete screening of cytotoxicity, antiretroviral HIV-1 and antimicrobial activity has been carried out.

Keywords: Potential antifolate agents; Antiproliferative agents; Pyrrolo[1,2-a]quinoxalines

1. Introduction

In an attempt to develop new anticancer agents targeted at human dihydrofolate reductase (DHFR) and tymidylate synthase (TS), two specific enzymes involved in the biosynthesis of the nucleic acids, we designed and synthesized more than three hundred quinoxaline derivatives [1–11]. This has been done on the ground that the quinoxaline ring can bioisosterically replace the pteridine ring in both classical and non classical antifolates. In an in vitro screening performed at NCI, several compounds emerged as potent antiproliferative agents at concentrations ranging between 10 and 100 μM. Interestingly, some of these compounds proved active also against bovine and murine DHFR[7]. More recently, a compound of classical antifolate type has been reported to be a potent inhibitor of hDHFR in vitro[11]. These encouraging results prompted us to develop new structure-based compounds with the aim of finding more selective antifolic agents.

Thus, we took into account the preparation of compounds **1**–**28** (Fig. 1), which are structurally related to the previously described 6(7)-trifluoromethylquinoxalines bearing at position 2 both classical and non-classical antifolic moieties [1–11]. In particular, we considered the annulation of the pyrrole ring at the 3,4 positions of quinoxaline in order to mimic the lipophilic character, or the steric hindrance, of the phenyl group at

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position 3 which characterized the previously reported compounds endowed with the best antiproliferative activity. A number of the above compounds were selected and evaluated at NCI for anticancer activity in vitro. In addition, a complete screening for cytotoxicity, antiretroviral (HIV-1) and antimicrobial activity, was carried out in our laboratories.

2. Chemistry

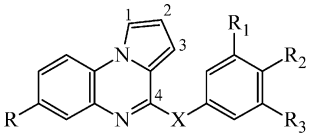
The preparation of the compounds was accomplished according to the reactions described in Scheme 1.

The chloropyrrolo[1,2-a]quinoxalines **29a**,**b**, prepared as described later, underwent nucleophilic attack by the corresponding anilines **30**, **32** of Fig. 1 in refluxing 1-propanol to give the anilinopyrrolo[1,2-a]quinoxalines **1**–**8** and **21**–**22**. Compounds **11**–**18** and **25**, **26** were instead obtained by nucleophilic displacement of chloromethyl derivatives **29c**,**d** with the corresponding phenols **31**–**33** of Fig. 1 carrying out the reaction at 70 °C in DMF and in the presence of one mole equivalent of Cs_2CO_3 for 6 h. In the case of **14**, **18**, **25** and **26** cesium hydrogen carbonate was alternatively used and heating at 70 °C was prolonged for 2 h. The desired acids **9**, **10**, **19**, **20**, **23**, **24**, **27**, **28** were obtained on alkaline hydrolysis of the parent esters in good yields. The intermediate halides **29a**–**c** were known compounds and have been purposely prepared according to the indications of Rault et al. [12]. The synthesis of **29d** was instead achieved adapting the Rault's indications for an intramolecular ring closure of the new amide **36** (Scheme 2), in refluxing phosphorous oxychloride. The latter was obtained from the unknown intermediate **35** in turn prepared via reduction with palladised charcoal and hydrazine hydrate in ethanol of **34** previously described by Campiani et al. [13]. Elucidation of the compounds' structures was achieved by the whole of the elemental analyses and spectroscopic data (Table 1). The UV spectra in ethanol showed very fine structure, as reported by Cheeseman and Tuck [14]. In particular, it is important to note that compounds **1**–**4**, **9**, **19**, **21**, **23**, which do not bear any substituent at position 7, exhibited a set of six maxima in the region of 360–260 nm endowed with hyperchromic effect when in para position to 4-anilino group exists a possibility of conjugation with a carbonyl group as in the case of compounds **4**, **9**, **19**, **21**, **23**. This fine structure collapses when an electrowithdrawing group as trifluoromethyl was present in position 7 of pyrroloquinoxaline ring. All compounds which bear a CH_2O–Ph at position 4 showed a flat maximum around 340–320 nm with the most intense peaks between 260 and 250 nm.

3. Antiproliferative, anti-HIV-1 and antimicrobial assays

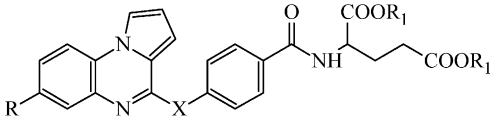
All the compounds of Fig. 1 and **29c**,**d** were submitted to NCI. Its scientific committee incontrovertibly selected the following **1**, **2**, **4**, **5**, **6**, **10**, **11**, **15**, **21**, **25** for the evaluation of their antiproliferative activity according to the in vitro disease oriented antitumour screening program, against a panel of 60 tumour cell-lines [15]. The activity of each compound, calculated from dose-response curves provided by NCI, is shown in Tables 2 and 3.

In Table 3 the response parameters refer to drug concentrations that produced 50% growth inhibition ($-\log GI_{50}$), total growth inhibition ($-\log TGI$) and 50% cytotoxicity ($-\log LC_{50}$) respectively, and were



1-20

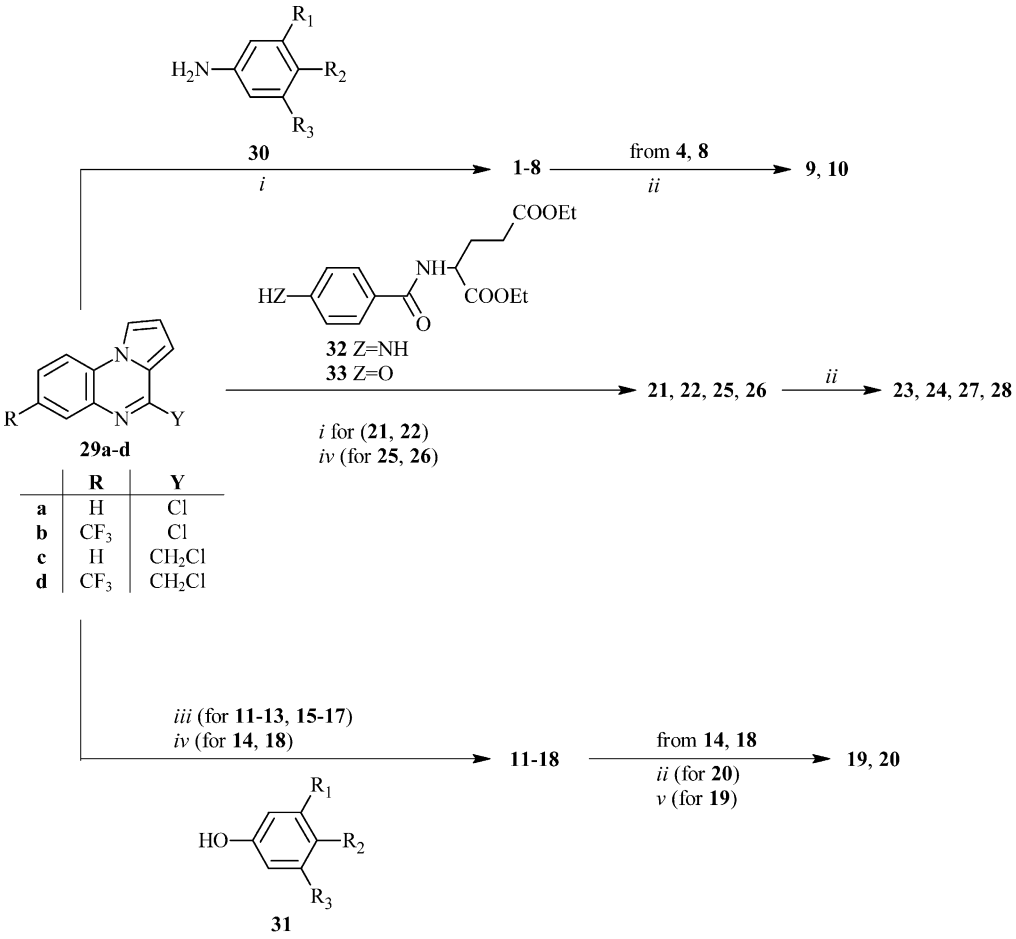
Compd	X	R	R_1	R_2	R_3
1	NH	H	OCH_3	OCH_3	OCH_3
2	NH	H	OCH_3	OCH_3	H
3	NH	H	H	OCH_3	H
4	NH	H	H	COOEt	H
5	NH	CF_3	OCH_3	OCH_3	OCH_3
6	NH	CF_3	OCH_3	OCH_3	H
7	NH	CF_3	H	OCH_3	H
8	NH	CF_3	H	COOEt	H
9	NH	H	H	COOH	H
10	NH	CF_3	H	COOH	H
11	CH_2O	H	OCH_3	OCH_3	OCH_3
12	CH_2O	H	OCH_3	H	OCH_3
13	CH_2O	H	H	OCH_3	H
14	CH_2O	H	H	COOEt	H
15	CH_2O	CF_3	OCH_3	OCH_3	OCH_3
16	CH_2O	CF_3	OCH_3	H	OCH_3
17	CH_2O	CF_3	H	OCH_3	H
18	CH_2O	CF_3	H	COOEt	H
19	CH_2O	H	H	COOH	H
20	CH_2O	CF_3	H	COOH	H



21-28

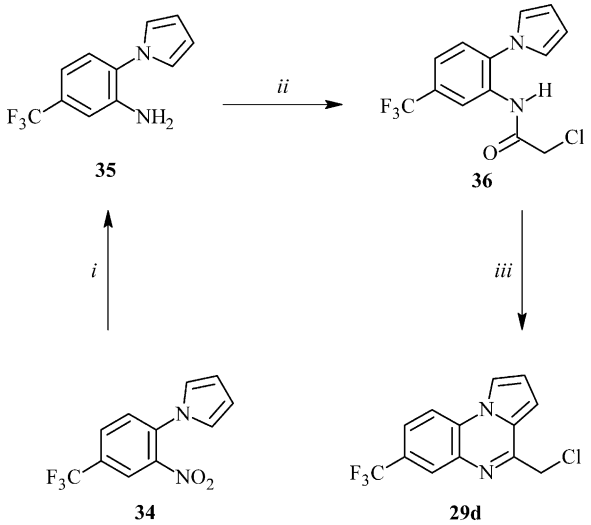
Compd	X	R	R_1
21	NH	H	Et
22	NH	CF_3	Et
23	NH	H	H
24	NH	CF_3	H
25	CH_2O	H	Et
26	CH_2O	CF_3	Et
27	CH_2O	H	H
28	CH_2O	CF_3	H

Fig. 1.



	R	Y
a	H	Cl
b	CF_3	Cl
c	H	CH_2Cl
d	CF_3	CH_2Cl

Scheme 1. *i*: PropOH, reflux, 2-10h; *ii*:EtOH, 1M NaOH, reflux, 4h; *iii*: Cs_2CO_3, DMF, 70 °C, 6h; *iv*:$CsHCO_3$, 2h; *v*: EtOH, 1M NaOH, 70 °C, 4h



Scheme 2. *i*: NH_2-NH_2 H_2O, Pd/C 10%, EtOH, reflux, 1.5h *ii*: $ClCOCH_2Cl$, pyridine, dioxane, reflux, 4h *iii*: $POCl_3$, reflux, 4h

expressed as mean graph midpoints, according to the data provided by NCI. Table 2 reports the activities of those compounds which showed a growth inhibition greater than 40% on subpanel cell lines at both 10^{-4} and 10^{-5} M. Based on the significant cytotoxicity for MT-4 cells shown in antiviral assays (Table 4) by compounds **2**, **10**, **13**, **19**, **29c**,**d**, we studied in detail their potential antiproliferative activity against a panel of cell lines derived from hematological (Table 5) and solid tumours (Table 6). In addition to the antiretroviral activity against HIV-1 (Table 4), title compounds were evaluated in vitro against representative strains of Gram-positive and Gram-negative bacteria (*S. aureus*, *Salmonella* spp.), of mycobacteria (*M. fortuitum*, *M. smegmatis* ATCC 19420 and *M. tuberculosis* ATCC 27294), of yeast and moulds (*C. albicans* ATCC 10231 and *A. fumigatus*), but their results have been omitted because none of them resulted active.

4. Results and discussion

From the data reported in Table 3 we can deduce that the average inhibitory activity of test compounds at

Table 1

Comp.	M.p. (°C) [a]	Yield (%)	Analysis for	IR (Nujol)	UV (EtOH)	^{1}H NMR, δ_H (J in Hz)
				ν_{max} (cm^{-1})	λ_{max} (nm)	Solvent: [A] = $CDCl_3$ [B] = $CDCl_3$:DMSO-d_6 (3:1) [C] = $CDCl_3$:DMSO-d_6 (1:1)
1	218–220 [b]	82	$C_{20}H_{19}N_3O_3$	3485, 1633	350, 334, 320 (sh), 309, 264, 237, 208	[A] 11.31 (1H, br s, NH), 7.99–7.95 (1H, m, H-1), 7.82–7.77 (2H, m, H-6,9), 7.48–7.43 (2H, m, H-7, 8), 6.73–6.67 (3H, m, H-3, +H-2′6′), 6.20–6.17 (1H, m, H-2), 3.93 (3H, s, 4′-OCH_3), 3.63 (6H, s, 3′, 5′-OCH_3)
2	215–218 [c]	87	$C_{19}H_{17}N_3O_2$	3281, 1630	335 (sh), 313, 266, 231, 216, 205	[A] 11.19 (1H, br s, NH), 7.97 (1H, d, $J_{2',6'} = 1.6$ Hz, H-2′), 7.82–7.55 (3H, m, H-6, 9, 1), 7.46-7.42 (2H, m, H-8,7), 6.99–6.92 (2H, m, H-6′,5′), 6.68-6.65 (1H, m, H-3), 6.04–6.02 (1H, m, H-2), 3.98 (3H, s, OCH_3), 3.86 (3H, s, OCH_3)
3	230–232 [d]	77	$C_{18}H_{15}N_3O$	3446, 1630	348 (sh), 334, 308, 297, 264 (sh), 236, 212 (sh), 202	[B] 11.40 (1H, br s, NH), 8.24 (1H, s, H-1), 8.01–7.97 (1H, m, H-8), 7.80–7.76 (1H, m, H-9), 7.46–7.38 (4H, m, H-7,8+3′,5′), 7.07–7.03 (1H, m, H-3), 7.05 (2H, d, $J = 8.2$ Hz, H-2′, 6′), 6.88-6.85 (1H, m, H-2), 3.89 (3H, s, OCH_3)
4	125–127 [d]	73	$C_{20}H_{17}N_3O_2$	3443, 1709, 1629	356, 342, 328, 313, 302, 241, 213, 202	[A] 11.60 (1H, br s, NH), 8.15 (2H, d, $J = 8.4$ Hz, H-3′,5′), 8.03–8.02 (1H, m, H-1), 7.84–7.80 (2H, m, H-6,9), 7.52 (2H, d, $J = 8.2$ Hz, H-2′6′), 7.48-7.44 (2H, m, H-7,8), 6.70–6.68 (1H, m, H-3), 6.25–6.23 (1H, m, H-2), 4.42 (2H, q, CH_2CH$_3$), 1.44 (3H, t, CH_3CH$_2$)
5	194–196	52	$C_{21}H_{18}F_3N_3O_3$	3090, 1633	354, 336, 303, 240, 209	[A] 8.09–8.00 (2H, m, H-1,6), 7.84 (1H, d, $J_{9,8} = 8.4$ Hz, H-9), 7.70 (1H, dd, $J_{8,9} = 8.4$ Hz and $J_{8,6} = 1.6$ Hz, H-8), 6.80–6.75 (1H, m, H-3), 6.67 (2H, s, H-2′6′), 6.28–6.20 (1H, m, H-2), 3.93 (3H, s, 4′-OCH_3), 3.84 (6H, s, 3′, 5′-OCH_3)
6	131–133 [b]	77	$C_{20}H_{16}F_3N_3O_2$	3086, 1632	350 (sh), 340, 313, 240, 216, 202	[A] 11.42 (1H, br s, NH), 8.04–8.03 (2H, m, H-7,8), 7.90 (1H, d, $J_{9,8} = 8.0$ Hz, H-9), 7.66 (1H, dd, $J_{8,9} = 8.0$ Hz and $J_{8,6} = 2.4$ Hz, H-8), 7.08–6.90 (3H, m, H-2′,5′,6′), 6.80–6.73 (1H, m, H-3), 6.09–6.05 (1H, m, H-2), 3.98 (3H, s, OCH_3), 3.86 (3H, s, OCH_3)
7	202–204 [d]	76	$C_{19}H_{14}F_3N_3O$	3087, 1634	350 (sh), 338, 303, 243, 221, 210	[A] 11.40 (1H, br s, NH), 8.05–8.01 (2H, m, H-1,6), 7.85 (1H, dd, $J_{8,9} = 8.0$ Hz and $J_{8,6} = 2.4$ Hz, H-8), 7.65 (1H, d, $J_{9,8} = 8.0$ Hz, H-9), 7.34 (2H, d, $J = 8.8$ Hz, H-3′,5′), 7.01 (2H, d, $J = 8.8$ Hz, H-2′, 6′), 6.78-6.69 (1H, m, H-3), 6.18-6.00 (1H, m, H-2)
8	223–226 [b]	41	$C_{21}H_{16}F_3N_3O_2$	3092, 1728, 1604	356, 340, 311, 246, 216, 203	[A] 11.84 (1H, br s, NH), 8.18 (2H, d, $J = 8.6$ Hz, H-3′,5′), 8.10–8.05 (1H, m, H-1), 8.03 (1H, d, $J_{6,8} = 2.8$ Hz, H-6), 7.91 (1H, d, $J_{9,8} = 8.8$ Hz, H-9), 7.70 (1H, dd, $J_{8,9} = 8.8$ Hz and $J_{8,6} = 2.8$ Hz, H-8), 7.58 (2H, d, $J = 8.6$ Hz, H-2′,6′), 6.80-6.75 (1H, m, H-3), 6.36–6.30 (1H, m, H-2), 4.43 (2H, q, CH_2CH$_3$), 1.44 (3H, t, CH$_3$CH$_2$)
9	278–281	85	$C_{18}H_{13}N_3O_2$	3400, 1725, 1620	356, 340, 326, 303, 312, 240, 212, 202	[C] 11.40 (1H, br s, NH), 8.42–8.40 (1H, m, H-1), 8.13 (2H, d, $J = 8.8$ Hz, H-3′,5′), 7.93–7.91 (1H, m, H-3), 7.93–7.78 (2H, m, arom.), 7.80 (2H, d, $J = 8.8$ Hz, H-2′, 6′), 7.54–7.36 (2H, m, arom.), 6.96–6.86 (1H, m, H-2)
10	> 300	71	$C_{19}H_{12}F_3N_3O_2$	3435, 1701, 1604	355, 340, 311, 246, 216, 203	[B] 9.52 (1H, br s, NH), 8.30–8.10 (4H, m, arom.), 8.03–7.90 (3H, m, arom.), 7.61–7.48 (2H, m, H-1,3), 6.91–6.85 (1H, m, H-2)
11	135–137 [e]	64	$C_{21}H_{20}N_2O_4$	1606	346, 262, 248, 237 (sh), 225, 205	[A] 8.00–7.90 (2H, m, H-1,6), 7.90–7.82 (1H, m, H-9), 7.60–7.40 (2H, m, H-7,8), 7.19–7.13 (1H, m, H-3), 6.92–6.89 (1H, m, H-2), 6.39 (2H, s, H-2′, 6′), 5.38 (2H, s, CH_2), 3.81 (6H, s, 3′, 5′-OCH_3), 3.76 (3H, s, 4′-OCH_3)
12	90–92 [f]	86	$C_{20}H_{18}N_2O_3$	1599	346, 263 (sh), 248, 225, 207	[A] 8.00–7.86 (2H, m, H-1,6), 7.85 (1H, dd, $J_{9,8} = 8.0$ Hz and $J_{9,7} = 1.6$ Hz, H-9), 7.58–7.38 (2H, m, H-7,8), 7.14–7.10 (1H, m, H-3), 6.92–6.86 (1H, m, H-2), 6.29 (2H, d, $J_{2',4'} = 2.2$ Hz, H-2′, 6′), 6.22–6.18 (1H, m, H-4′), 5.36 (2H, s, CH$_2$), 3.75 (6H, s, 3′,5′-OCH_3)
13	78–80 [f]	62	$C_{19}H_{16}N_2O_2$	1612	337, 320 (sh), 264 (sh), 248, 226, 204	[A] 8.02–7.92 (2H, m, H-1,6), 7.76 (1H, dd, $J_{9,8} = 8.0$ Hz and $J_{9,7} = 1.6$ Hz, H-9), 7.60–7.40 (2H, m, H-7,8), 7.18–7.15 (1H, m, H-3), 7.02 (2H, d, $J = 9.0$ Hz, H-2′6′), 6.90–6.86 (1H, m, H-2), 6.82 (2H, d, $J = 9.2$ Hz, H-3′,5′), 5.36 (2H, s, CH$_2$), 3.75 (3H, s, OCH_3)
14	91–92 [f]	49	$C_{21}H_{18}N_2O_3$	1710, 1608	336, 257 (sh), 249, 226, 205, 198	[A] 8.06–7.91 (2H, m, H-1,6), 7.98 (2H, d, $J = 9.0$ Hz, H-3′,5′), 7.85 (1H, dd, $J_{9,8} = 7.8$ Hz and $J_{9,7} = 1.6$ Hz, H-9), 7.60–7.40 (2H, m, H-7,8), 7.10 (2H, d, $J = 9.0$ Hz, H-2′,6′), 7.15–7.00 (1H, m, H-3), 6.88 (1H, t, H-2), 5.45 (2H, s, CH$_2$), 4.32 (2H, q, CH$_2$CH$_3$), 1.35 (3H, t, CH$_3$CH$_2$)

Table 1 (*Continued*)

Comp.	M.p. (°C) [a]	Yield (%)	Analysis for	IR (Nujol)	UV (EtOH)	^{1}H NMR, δ_H (J in Hz)
				ν_{max} (cm^{-1})	λ_{max} (nm)	Solvent: [A] = $CDCl_3$ [B] = $CDCl_3$:DMSO-d_6 (3:1) [C] = $CDCl_3$:DMSO-d_6 (1:1)
15	137–139 [e]	51	$C_{22}H_{19}F_3N_2O_4$	1588	338, 270 (sh), 252, 230, 207	[A] 8.26–8.22 (1H, m, H-1), 8.03–7.90 (2H, m, H-6,9), 7.74 (1H, dd, $J_{8,9}$ = 8.8 Hz and $J_{8,6}$ = 1.6 Hz, H-8), 7.28–7.18 (1H, m, H-3), 7.00–6.92 (1H, m, H-2), 6.67 (2H, s, H-2′, 6′), 5.38 (2H, s, CH_2), 3.82 (6H, s, 3′,5′,-OCH_3), 3.76 (3H, s, 4′-OCH_3)
16	129–130	76	$C_{21}H_{17}F_3N_2O_3$	1595	332, 266 (sh), 252, 229, 206	[A] 8.29–8.22 (1H, m, H-1), 7.98–7.90 (2H, m, H-6,9), 7.75 (1H, dd, $J_{8,9}$ = 8.8 Hz and $J_{8,6}$ = 1.6 Hz, H-8), 7.20–7.18 (1H, m, H-3), 6.96–6.90 (1H, m, H-2), 6.27 (2H, d, $J_{2',4'}$ = 1.6 Hz, H-2′, 6′), 6.10 (1H, d, $J_{4',2'}$ = 1.6 Hz, H-4′), 5.37 (2H, s, CH_2), 3.75 (6H, s, 3′,5′–OCH_3)
17	90–91	21	$C_{20}H_{15}F_3N_2O_2$	1630	389, 321, 254 (sh), 236, 214	[A] 8.26 (1H, a s, H-1), 7.95 (1H, d, $J_{6,8}$ = 2.8 Hz, H-6), 7.92 (1H, a s, H-9), 7.75 (1H, dd, $J_{8,9}$ = 8.8 Hz and $J_{8,6}$ = 1.8 Hz, H-8), 7.26–7.20 (1H, m, H-3), 7.01 (2H, d, J = 9.0 Hz, H-2′, 6′), 6.93 (1H, t, H-2), 8.15 (2H, d, J = 9.2 Hz, H-3′,5′), 5.36 (2H, s, CH_2), 3.74 (3H, s, OCH_3)
18	96–97 [g]	23	$C_{22}H_{17}F_3N_2O_3$	1708, 1607	336, 253, 232, 207	[A] 8.27 (1H, a s, H-1), 8.01-7.93 (2H, m, H-6,9), 7.99 (2H, d, J = 8.6 Hz, H-3′,5′), 7.78 (1H, dd, $J_{8,9}$ = 8.4 Hz and $J_{8,6}$ = 1.6 Hz, H-8), 7.21–7.19 (1H, m, H-3), 7.10 (2H, d, J = 8.8 Hz, H-2′, 6′), 6.96 (1H, t, H-2), 5.47 (2H, s, CH_2), 4.32 (2H, q, CH_2CH_3), 1.36 (3H, t, CH_3CH_2)
19	228–230 [g]	54	$C_{19}H_{14}N_2O_3$	1694, 1605	336, 249, 226, 205	[C] 8.93–8.86 (1H, m, H-1), 8.47 (1H, dd, $J_{6,7}$ = 8.2 Hz and $J_{6,8}$ = 1.6 Hz, H-6), 8.29 (1H, dd, $J_{9,8}$ = 8.4 Hz and $J_{9,7}$ = 1.6 Hz, H-9), 7.96 (2H, d, J = 8.8 Hz, H-3′,5′), 7.84–7.70 (1H, m, H-7), 7.70–7.64 (1H, m, H-8), 7.25 (2H, d, J = 8.6 Hz, H-2′, 6′), 5.89 (2H, s, CH_2), 4.67 (1H, br s, COOH)
20	218–220 [e]	75	$C_{20}H_{13}F_3N_2O_3$	1702, 1604	321, 236, 215	[C] 8.48 (1H, d, $J_{6,8}$ = 1.6 Hz, H-6), 8.39 (1H, d, $J_{9,8}$ = 8.6 Hz, H-9), 8.24 (1H, a s, H-1), 7.93 (2H, d, J = 8.8 Hz, H-3′,5′), 7.64 (1H, dd, $J_{8,9}$ = 8.8 Hz and $J_{8,6}$ = 1.6 Hz, H-8), 7.34–7.32 (1H, m, H-3), 7.14 (2H, d, J = 8.8 Hz, H-2′, 6′), 7.02 (1H, t, H-2), 5.57 (2H, s, CH_2)
21	168–170 [d]	75	$C_{27}H_{28}N_4O_5$	3324, 1758, 1738, 1601	356, 340, 312, 303, 272, 240, 210, 202	[A] 11.57 (1H, br s, NH), 8.02–8.00 (1H, m, H-1), 7.97 (2H, d, J = 8.4 Hz, H-3′,5′), 7.86–7.79 (2H, m, H-7,8), 7.64 (1H, d, J = 7.2 Hz, NHCO), 7.49 (2H, d, J = 8.4 Hz, H-2′, 6′), 7.46–7.43 (2H, m, H-6,9), 6.71–6.69 (1H, m, H-3), 6.45–6.35 (1H, m, H-2), 4.80–4.65 (1H, m, $NHCHCH_2$), 4.24 (2H, q, CH_2CH_3), 4.14 (2H, q, CH_2CH_3), 2.60–2.20 (4H, m, CH_2CH_2), 1.32 (3H, t, CH_3CH_2), 1.25 (3H, t, CH_3CH_2)
22	126–128 [b]	59	$C_{28}H_{27}F_3N_4O_5$	3299, 1727, 1712, 1606	340, 356, 311, 247, 216, 203	[A] 11.78 (1H, br s, NH), 8.12–8.06 (2H, m, H-1,8), 7.92 (2H, d, J = 7.8 Hz, H-3′,5′), 7.98–7.80 (1H, m, NHCO), 7.91–7.64 (2H, m, H-6,9), 7.49 (2H, d, J = 8.2 Hz, H-2′,6′), 6.80–6.60 (2H, m, H-2,3), 4.81–4.60 (1H, m, $NHCHCH_2$), 4.24 (2H, q, CH_2CH_3), 4.14 (2H, q, CH_2CH_3), 2.61–2.05 (4H, m, CH_2CH_2), 1.31 (3H, t, CH_3CH_2), 1.25 (3H, t, CH_3CH_2)
23	174–179	85	$C_{23}H_{20}N_4O_5$	3397, 3311, 1733, 1704, 1600	355, 340, 326, 312, 270, 241, 212, 202	[C] 11.58 (1H, br s, NH), 8.65 (1H, d, J = 7.4 Hz, NHCO), 8.48 (1H, a s, H-1), 8.21–8.17 (1H, m, H-6), 8.15 (2H, d, J = 7.8 Hz, H-3′,5′), 7.95–7.93 (1H, m, H-3), 7.84–7.78 (1H, m, H-9), 7.72 (2H, d, J = 8.2 Hz, H-2′, 6′), 7.58–7.38 (2H, m, H-7,8), 6.97 (1H, t, H-2), 4.59–4.51 (1H, m, $NHCHCH_2$), 2.48–2.00 (4H, m, CH_2CH_2)
24	183–186 [g]	72	$C_{24}H_{19}F_3N_4O_5$	1794, 1716, 1603	355, 339, 311, 247, 216, 204	[B] 10.85 (1H, br s, NH), 8.42–8.38 (1H, m, H-1), 8.33 (1H, a s, H-6), 8.19 (1H, d, $J_{9,8}$ = 8.8 Hz, H-9), 8.05 (2H, d, J = 8.2 Hz, H-3′,5′), 7.93 (2H, d, J = 8.8 Hz, H-2′6′), 7.88–7.86 (1H, m, H-3), 7.72–7.58 (1H, m, H-8), 6.93 (1H, t, H-2), 4.78 (2H, a s, 2·COOH), 4.70–4.55 (1H, m, NHC*H*CH_2), 2.47-2.00 (4H, m, CH_2CH_2)
25	72–73 [g]	78	$C_{28}H_{29}N_3O_6$	3290, 1731, 1607	341, 259 (sh), 249, 227, 205	[A] 8.01–7.93 (2H, m, H-1,6), 7.88 (1H, dd, $J_{9,8}$ = 7.2 Hz and $J_{9,7}$ = 1.2 Hz, H-9), 7.77 (2H, d, J = 8.4 Hz, H-3′,5′), 7.56–7.47 (2H, m, H-7,8), 7.12 (2H, d, J = 8.0 Hz, H-2′6′), 7.20-7.15 (1H, m, H-3), 6.91–6.88 (1H, m, H-2), 5.46 (2H, s, CH_2), 4.80–4.70 (1H, m, $NHCHCH_2$), 4.22 (2H, q, CH_2CH_3), 4.09 (2H, q, CH_2CH_3), 2.50–2.00 (4H, m, CH_2CH_2), 1.29 (3H, t, CH_3CH_2), 1.20 (3H, t, CH_3CH_2)
26	68–70	35	$C_{29}H_{28}F_3N_3O_6$	3310, 1733, 1605	332, 252, 231, 206	[A] 8.26 (1H, a s, H-1), 8.19 (1H, d, $J_{6,8}$ = 2.8 Hz, H-6), 7.96 (1H, d, $J_{9,8}$ = 10.4 Hz, H-9), 7.77 (2H, d, J = 8.6 Hz, H-3′,5′), 7.80–7.73 (1H, m, H-8), 7.19 (1H, d, J = 4.2 Hz, NHCO), 7.11 (2H, d, J = 8.8 Hz, H-2′6′), 6.96–6.92 (2H, m, H-2,3), 5.46 (2H, s, CH_2), 4.80–4.70 (1H, m, $NHCHCH_2$), 4.22 (2H, q, CH_2CH_3), 4.10 (2H, q, CH_2CH_3), 2.48–2.00 (4H, m, CH_2CH_2), 1.29 (3H, t, CH_3CH_2), 1.20 (3H, t, CH_3CH_2)

Table 1 (*Continued*)

Comp.	M.p. (°C)[a]	Yield (%)	Analysis for	IR (Nujol)	UV (EtOH)	^{1}H NMR, δ_H (J in Hz)
				ν_{max} (cm^{-1})	λ_{max} (nm)	Solvent: [A] = $CDCl_3$ [B] = $CDCl_3$:DMSO-d_6 (3:1) [C] = $CDCl_3$:DMSO-d_6 (1:1)
27	205–210	47	$C_{24}H_{21}N_3O_6$	1714, 1602	332, 248, 227, 205	[B] 8.83 (1H, a s, H-1), 8.60–8.42 (2H, m, arom.), 8.20 (1H, d, NHCO), 7.95–7.60 (5H, m, arom.), 7.28–7.14 (3H, m, arom.), 5.97 (2H, s, CH_2), 4.63–4.48 (1H, m, $NHCHCH_2$), 2.50–2.00 (4H, m, CH_2CH_2)
28	210–212[e]	65	$C_{25}H_{20}F_3N_3O_6$	1752, 1705, 604	321, 237, 215	[C] 8.48 (1H, a s, H-6), 8.39 (1H, d, $J_{9,8}$ = 8.6 Hz, H-9), 8.28 (1H, a s, H-1), 7.88 (2H, d, J = 8.8 Hz, H-3′,5′), 7.90–7.80 (1H, m, H-8), 7.39–7.36 (1H, m, H-3), 7.14 (2H, d, J = 8.8 Hz, H-2′,6′), 7.04 (1H, t, H-2), 5.58 (2H, s, CH_2), 4.57–4.42 (1H, m, $NHCHCH_2$), 2.39–1.95 (4H, m, CH_2CH_2)

[a] Purification procedure.
[b] Crystallizzation from MeOH/H_2O.
[c] Crystallization from CH_3CN.
[d] Crystallization from CH_3COCH_3.
[e] Crystallization from EtOH.
[f] Crystallization from MeOH.
[g] Crystallization from EtOH/H_2O; sh, shoulder.

NCI, represented as mean graph mid points, falls in the range $10^{-4.85}$–$10^{-4.00}$ molar concentration, corresponding to 14–100 μM range. Compound **2** was the most active; in fact, it is characterized by a significant GI_{50} (14.12 μM) and a good correlation between TGI (−4.41 = 38.90 μM) and LC_{50} (−4.12 = 75.85 μM). However, from the cytotoxicity test on mock-infected MT-4 cells (Table 4), additional five compounds (**13**, **19**, **22**, **29c**,**d**) exhibited lower CC_{50} values, which ranged from 6 μM, for the most active (**29c**), to 39 μM, for the least active (**22**).

The data in Table 2 are indicative of the wide spectrum of tumour growth inhibition at 10^{-4} and 10^{-5} M. A large number of derivatives (**2**, **4**, **5**, **6**, **10**, **11**, **15**, **21**, **25**) exhibit a wide spectrum activity against all subpanel cell lines (from 57 to 47 over 60 cell lines) and some of them possess percent tumour growth inhibition values at 10^{-5} M. In particular, compound **5** maintained these values over 31 cell lines, compound **11** over 21, compound **15** over 20, compound **6** over 16 and compound **4** over 11 cell lines, whereas compounds **1**, **2**, **10**, **21** and **25** showed very low percent growth inhibition values (below 40%). It is worth noting that the cell lines derived from leukemia, colon and breast cancer are the most affected.

Data in Table 4 show that none of the compound exhibited whatsoever significant anti-HIV-1 and in some case EC_{50} was coincident with CC_{50}. However, compounds **13**, **19**, **29c**,**d**, not tested at NCI, proved significantly cytotoxic in MT-4 cells, prompting an investigation of their potential antiproliferative activity against a panel of cell lines derived from hematological and solid tumours (Tables 5 and 6). Comparison of mean graph mid points of **5**, **6**, **10** and **15** with those of previously reported analogues bearing a phenyl group at position 3 of quinoxaline ring [1,3,7], reported in Table 7, shows that pyrrolo[1,2-a]quinoxaline derivatives are the most active. Structure–activity relationships allow to conclude that insertion of a pyrrole ring on the quinoxaline system increases the antiproliferative activity.

5. Conclusion

In a first NCI screening, several compounds proved active in vitro as antiproliferative agents in the concentration range 10–100 μM. We then synthesized new derivatives that, in our hands, were endowed with in vitro antiproliferative activities as low as 3.4 μM against a panel of cell lines derived from hematological and solid tumours. In addition, a complete screening of cytotoxicity, antiretroviral HIV-1 activity has been carried out, but none of title compounds resulted active, nor were the compounds tested active as antimicrobial.

Table 2
Percent tumour growth inhibition recorded on subpanel cell lines at 10^{-4} and 10^{-5} M of compounds **1**, **2**, **4**–**6**, **10**, **11**, **15**, **21** and **25**

	1		2		4		5		6		10		11		15		21		25	
	Molar concentration (M)																			
	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}
Leukemia																				
CCRF-CEM	–	–	162	86	155	200	65	63	87	49	86	40	191	61	148	50	60	–	94	69
HL-60(TB)	70	–	162	101	197	200	95	91	116	–	99	–	148	58	106	42	–	–	99	47
K-562	–	–	98	–	56	45	–	45	118	52	113	–	183	71	143	57	76	–	75	43
MOLT-4	–	–	129	50	46	44	68	53	132	56	120	–	186	93	145	86	106	–	99	90
RPMI-8226	–	–	120	45	nt	–	–	48	121	–	111	43	191	82	nt	nt	98	–	nt	nt
SR	–	–	145	–	61	52	45	53	142	50	141	–	nt	nt	123	51	90	41	87	51
Non small cell lung cancer																				
A549/ATCC	–	–	145	–	53	–	–	44	126	–	85	–	74	39	93	–	90	–	48	–
EKVX	–	–	119	–	87	–	–	–	125	–	64	–	76	44	144	–	79	–	–	–
HOP-62	112	–	180	–	113	–	–	–	146	–	84	–	70	–	80	–	137	–	71	–
HOP-92	71	–	89	–	71	nt	48	47	134	–	61	–	91	56	78	45	93	–	63	–
NCI-H226	nt	nt	nt	nt	nt	–	nt	nt	nt	nt	nt	nt	nt	nt	119	51	nt	nt	–	–
NCI-23	56	–	200	–	59	–	47	–	147	–	79	–	80	67	77	56	91	–	82	–
NCI-H322M	nt	–	nt	–	58	–	nt	–	nt	–	nt	–	68	–	nt	nt	200	43	49	–
NCI-H460	–	–	185	–	53	–	–	57	162	48	154	–	79	–	85	–	97	–	44	–
NCI-H522	–	–	187	–	52	–	41	–	146	–	137	–	92	42	132	43	126	–	108	60
Colon cancer																				
COLO 205	–	–	200	–	nt	–	41	50	196	–	200	–	90	–	113	–	68	–	–	–
HCC-2998	–	–	198	–	73	–	–	–	197	–	139	–	61	–	113	–	88	–	–	–
HCT-116	–	–	166	–	46	–	–	–	179	–	118	–	93	–	158	–	93	–	75	–
HCT-15	43	–	169	–	64	43	49	48	118	46	94	–	76	63	94	46	88	–	64	–
HT29	–	–	96	57	56	42	–	51	176	53	154	48	75	–	96	–	99	–	89	–
KM12	–	–	177	–	58	43	46	55	145	49	87	–	180	45	78	–	83	–	92	–
SW-620	–	–	162	–	67	51	–	–	89	–	81	–	143	61	83	–	56	–	81	48
SNC cancer																				
SF-268	66	–	135	–	79	–	–	–	92	–	78	–	66	–	82	–	88	–	65	–
SF-295	60	–	152	–	83	–	62	71	156	48	98	–	84	–	96	55	116	–	60	–
SF-539	49	–	170	–	88	–	–	–	176	–	113	–	91	–	nt	nt	187	–	76	–
SNB-19	50	–	95	–	67	–	–	–	122	–	62	–	44	–	95	–	81	–	67	–
SNB-75	nt	–	nt	nt	147	–	–	47	135	48	109	–	–	–	52	–	145	–	99	–
U251	62	–	168	–	101	–	48	40	200	–	136	–	91	–	96	–	124	–	86	–
Melanoma																				
LOX IMVI	–	–	200	–	58	–	45	52	200	75	154	49	100	–	91	–	85	–	69	–
MALME-3M	–	–	200	–	42	–	–	–	138	–	53	–	81	–	130	–	89	–	–	–
M14	–	–	189	–	nt	–	56	48	174	–	109	–	87	–	121	–	70	–	59	–
SK-MEL-2	66	–	200	–	77	–	–	–	184	–	94	–	–	–	nt	nt	168	–	45	–
SK-MEL-28	–	–	195	–	51	–	–	–	96	–	69	–	105	43	72	–	75	–	43	–
SK-MEL-5	–	–	200	–	58	–	57	5	200	–	158	–	140	98	177	110	94	–	51	–
UACC-257	–	–	200	–	46	–	45	–	176	–	119	–	77	43	131	59	116	–	50	–

Table 2 (*Continued*)

	1		2		4		5		6		10		11		15		21		25	
	Molar concentration (M)																			
	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}
UACC-62	–	–	200	–	65	–	–	40	200	–	111	–	123	41	82	43	137	–	–	–
Ovarian cancer																				
IGROV1	61	–	135	–	63	–	–	–	88	–	91	–	88	–	82	–	90	–	82	–
OVCAR-3	–	–	153	–	57	–	–	–	132	–	87	–	79	–	137	51	45	–	60	–
OVCAR-4	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	134	50	91	47	nt	nt	59	–
OVCAR-5	–	–	83	–	40	–	47	–	124	–	71	–	41	–	90	–	76	–	–	–
OVCAR-8	66	43	199	–	72	–	47	–	105	–	66	–	94	–	94	–	109	–	66	–
SK-OV-3	76	–	127	–	103	–	–	–	103	–	78	–	67	–	90	–	147	–	44	–
Renal cancer																				
786-0	51	–	164	–	97	–	–	63	170	55	120	–	93	–	132	–	183	–	55	–
A498	–	–	126	–	66	–	40	46	170	–	75	–	55	–	45	–	60	–	–	–
ACHN	–	–	115	–	57	–	–	42	110	–	92	–	85	–	76	40	148	–	64	–
CAKI-1	–	–	105	–	46	–	–	–	96	–	66	–	97	–	75	–	93	–	–	–
RXF 393	110	–	159	–	128	–	55	81	163	46	122	–	–	–	41	–	157	40	74	–
SN12C	nt	–	nt	–	141	–	61	–	nt	nt	nt	–	51	–	110	–	nt	–	–	–
TK-10	78	–	155	–	84	–	–	45	126	41	94	–	74	–	113	–	121	–	47	–
UO-31	–	–	167	–	nt	–	–	–	122	–	nt	–	56	–	106	45	92	–	50	–
Prostate cancer																				
PC-3	–	–	200	–	78	–	48	43	171	47	103	–	63	–	94	–	172	–	70	–
DU-145	–	–	171	–	53	–	–	–	134	–	69	–	69	–	55	–	101	–	56	–
Breast cancer																				
MCF7	–	–	200	–	2	–	–	42	129	–	82	–	133	94	100	77	100	–	70	–
MCF7/ADR-RES	–	–	125	–	72	–	54	51	130	–	61	–	67	–	112	50	140	–	–	–
MDA-MB-231/ATCC	89	–	147	–	81	–	–	51	170	–	138	–	51	–	78	–	145	–	–	–
HS 578T	57	–	86	–	76	–	–	–	97	–	65	–	57	–	65	–	96	–	58	–
MDA-MB-435	–	–	200	–	99	–	57	51	161	–	82	–	192	–	93	–	97	–	54	–
BT-549	59	–	136	–	47	–	51	–	125	–	86	–	87	–	96	–	111	–	56	–
T-47D	127	127	169	85	123	51	109	100	133	41	113	–	–	55	67	78	158	140	70	41
MDA-N	–	–	200	–	94	92	41	–	173	–	74	–	93	–	88	–	98	–	50	–

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

Table 3
$-\log GI_{50}$, $-\log TGI$, $-\log LC_{50}$ mean graph midpoints (MG-MD) of in vitro inhibitory activity test for compounds **1**, **2**, **4**–**6**, **10**, **11**, **15**, **21** and **25**

Comp.	$-\log GI_{50}$	$-\log TGI$	$-\log LC_{50}$
1	4.16	4.04	4.00
2	4.85	4.41	4.12
4	4.51	4.14	4.09
5	4.25	4.02	4.00
6	4.82	4.34	4.07
10	4.54	4.10	4.01
11	4.70	4.14	4.03
15	4.75	4.13	4.01
21	4.60	4.15	4.03
25	4.33	4.00	4.00

MG-MID: mean graph midpoints, the average sensitivity of all cell lines towards the test agent from NCI.

Table 4
Anti-HIV activity of compounds **1**–**28**, **29c**,**d**

Comp.	CC_{50} [a] MT-4	EC_{50} [b] (HIV-1)
1	> 100	> 100
2	31	> 31
3	> 100	> 100
4	= 100	> 100
5	57	> 57
6	27	> 27
7	> 100	> 100
8	> 100	> 100
9	= 100	> 100
10	15	> 15
11	55	> 55
12	> 100	> 100
13	16	> 16
14	> 100	> 100
15	100	> 100
16	> 100	> 100
17	52	> 52
18	> 100	> 100
19	8	> 8
20	> 100	> 100
21	48	> 48
22	39	> 39
23	> 100	> 100
24	> 100	> 100
25	> 100	> 100
26	68	> 100
27	95	> 95
28	> 100	> 100
29c	6	> 100
29d	18	> 100

[a] Compound concentration (μM) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method.
[b] Compound concentration (μM) required to achieve 50% protection of MT-4 cells from the HIV-1 induced cytopathogeneticy, as determined by the MTT method.

Table 5
Activity of compounds **2**, **13**, **10**, **19**, **29c**,**d** against hematological tumour-derived cell lines

Comp.	IC_{50} [a] (μM)		
	CCRF-CEM [b]	WIL-2NS [c]	CCRF-SB [d]
2	17.6	21.4	20.1
10	25.1	20.7	20.5
13	48.1	58.5	52.5
19	> 100	> 100	> 100
29c	3.4	10.3	3.5
29d	6.8	39.2	19.5

[a] Compound concentration required to reduce cell proliferation by 50%, as determined by the MTT method, under conditions allowing untreated controls to undergo at least three consecutive rounds of multiplication. Data represent mean values (±SD) for three independent determinations.
[b] $CD4^+$ human acute T-lymphoblastic leukaemia.
[c] Human splenic B-lymphoblastoid cells.
[d] Human acute B-lymphoblastic leukemia.

6. Experimental

Melting points were determined by a Kofler hot stage or Digital Electrothermal apparatus and are uncorrected. UV spectra are qualitative and were recorded in nm for solutions in ethanol with a Perkin–Elmer Lamba 5 spectrophotometer. Infrared spectra are for nujol mulls and were recorded using a Perkin–Elmer 781 spectrophotometer. ^1H NMR spectra were recorded on a Varian XL-200 (200 MHz) instrument, using TMS as internal standard Elemental analyses were performed by 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.

6.1. Chemistry

6.1.1. Intermediates

Compounds **29a**,**b** could not be obtained according to the data of the literature [13], but we performed their preparation using the procedure described by Rault et al. [12] for either **29a**,**b** and the previously described **29c**. The *p*-hydroxybenzoyl-L-glutamate diethyl ester (**33**) is not commercially available and was obtained according to the procedure described in the literature [16,17].

*6.1.1.1. 1-(2-Amino-4-trifluoromethylphenyl)pyrrole (**35**).* A mixture of 1-(2-nitro-4-trifluoromethyl)pyrrole [13] (**34**) (4.1 g, 16 mmol), and an excess of hydrazine hydrate (5.13 g 160 mmol) in ethanol (100 ml) and in presence of 10% palladised charcoal (0.4 g) was refluxed for 3 h. On cooling, the ethanolic solution was filtered off and evaporated in vacuo to give a crude solid, which was purified by flash chromatography on a silica gel column eluting with a mixture of ethyl acetate/petrol ether (b.p. 40–60 °C) in the ratio of 9:1. The fast moving

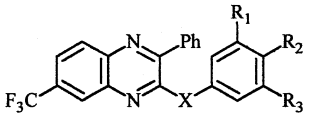
Table 6
Activity of compounds **2**, **13**, **10**, **19**, **29c**,**29d** against solid tumour-derived cell lines

Comp.	IC_{50} [a] (μM)				
	SK-MEL-28 [b]	MCF7 [c]	SKMES-1 [d]	HepG2 [e]	DU145 [f]
2	46.7	35.4	87.6	38.3	35.1
10	46.0	45.0	23.0	45.0	38.0
13	88.0	> 100	> 100	> 100	100
19	> 100	> 100	> 100	> 100	> 100
29c	30.4	28.1	21.6	37.5	23.7
29d	56.8	62.1	59.8	70.4	55.8

[a] Compound concentration required to reduce cell proliferation by 50%, as determined by the MTT method [19], under conditions allowing untreated controls to undergo at least three consecutive rounds of multiplication. Data represent mean values (±SD) for three independent determinations.
[b] Human skin melanoma.
[c] Human breast adenocarcinoma.
[d] Human lung squamous carcinoma.
[e] Human hepatocellular carcinoma.
[f] Human prostate carcinoma.

Table 7
Comparison based on mean graph activity between compounds **5**, **6**, **10**, **15** and the corresponding 3-phenyl analogues derivatives **37**–**40** previously described as cited [Ref.] reported below

Comp. (Ref.)	−log GI_{50} (μM)	−log TGI (μM)	−log LC_{50} (μM)
5	4.25 (56.2)	4.02 (95.5)	4.00 (100)
37 [1]	4.31(49)	4.11 (77.6)	4.02 (95.5)
6	4.82 (15.1)	4.34 (45.7)	4.07 (85.1)
38 [1]	4.66 (21.9)	4.18 (66.1)	4.00 (100)
10	4.54 (28.8)	4.10 (79.4)	4.01 (97.7)
39 [3]	4.70 (19.9)	4.22 (60.2)	4.04 (91.2)
15	4.54 (2.88)	4.10 (79.4)	4.01 (97.7)
40 [7]	4.15 (70.8)	4.00 (100)	4.00 (100)



Compd	X	R_1	R_2	R_3
37[1]	NH	OMe	OMe	OMe
38[1]	NH	OMe	OMe	H
39[3]	NH	H	COOH	H
40[7]	CH_2O	OMe	OMe	OMe

fraction gave on evaporation compound **35** (55% yield), m.p. 92–94 °C (MeOH). IR: 3380, 3300, 3206. UV: 333, 307, 229, 205. ^{1}H NMR ($CDCl_3$): δ 7.24 (1H, d, $J_{6',5'}$ = 8.4 Hz, H-6′), 7.04 (1H, d, $J_{3',5'} = 2.5$ Hz, H-3′), 7.02 (1H, dd, $J_{5',6'} = 8.8$ Hz and $J_{5',3'} = 2.5$ Hz, H-5′), 6.85 (2H, t, H-2, 5), 6.38 (2H, t, H-3, 4), 3.94 (2H, br s, NH_2).

*6.1.1.2. 2-Chloro-N-[(5-trifluoromethyl-2-pyrrol-1-yl)phenyl]acetamide (**36**).* Pyridine (0.38 g, 4.8 mmol) was added to a solution of 1-(2-amino-4-trifluoromethylphenyl)pyrrole (**35**) (0.99 g, 4.4 mmol) in dioxane (30 ml) followed by chloroacetyl cloride (0.50 g, 4.4 mmol). The reaction mixture was refluxed for 4 h. After removal of the solvent under reduced pressure, the residue was taken up with water and extracted with ethyl ether (2 × 20 ml). The organic extract was dried over anhydrous sodium sulfate and evaporated to dryness. The solid residue (85% yield) was recrystallized from methanol/water, m.p. 125–127 °C. IR: 3280, 1680. UV: 250, 234, 202. ^{1}H NMR ($CDCl_3$): δ 8.81 (1H, a s, H-3′), 8.44 (1H, br s, NH), 7.50 (2H, dd, $J_{5',3'} = 8.4$ Hz and $J_{5',6'} = 1.6$ Hz, H-5′), 7.44 (1H, d, $J_{3',4'} = 8.4$ Hz, H-6′), 6.82 (2H, t, H-2,5), 6.46 (2H, t, H-3,4), 4.11 (2H, s, CH_2).

*6.1.1.3. 4-Chloromethyl-7-trifluoromethylpyrrolo[1,2-a]quinoxaline (**29d**).* A mixture of the amide **36** (1.21 g, 4 mmol) and an excess of $POCl_3$ (6.17 g, 40 mmol) was refluxed for 4 h. On cooling, the solvent was removed in vacuo and the residue was taken up with water, made alkaline with $NaHCO_3$ and extracted with ethyl acetate. The organic layer was then washed out with water (24 ml) and eventually dried over anhydrous sodium sulfate. On evaporation of the solvent in vacuo, a crude product (68%) was obtained and recrystallized from methanol, m.p. 99–101 °C. UV 350, 253, 231, 209. ^{1}H NMR ($CDCl_3$): δ 8.27 (1H, d, $J_{6,8} = 1.6$ Hz, H-6), 7.80 (1H, d, $J = 2.4$ Hz, H-1), 7.95 (1H, d, $J_{9,8} = 8.8$ Hz, H-9), 7.77 (1H, dd, $J_{8,9} = 8.8$ Hz and $J_{8,6} = 1.6$ Hz, H-8), 7.14–7.12 (1H, m, H-3), 7.00–6.97 (1H, m, H-2), 4.86 (2H, s, CH_2Cl).

*6.1.2. General procedure for preparation of 4-anilino pyrrolo[1,2-a] quinoxaline (**1**–**8**)*

A mixture of equimolar amounts (0.5 mmol) of chloropyrrolo[1,2-a] quinoxaline [12] **29a** or **29b** and the corresponding substituted anilines **30** of Fig. 1 in 1-propanol was refluxed for 2/10 h. After cooling the solvent was removed under reduced pressure. The residue was taken up with acetone and filtered off.

The coloured products (**1**–**8**) were further purified as described in Table 1 which also contains yields, melting points, analytical and spectroscopic data.

*6.1.3. Diethyl N-[4-(pyrrolo[1,2-a]quinoxalin-4-yl)amino]benzoyl-L-glutamate (**21**) and diethyl N-[4-(7-trifluoromethylpyrrolo[1,2-a]quinoxalin-4-yl)amino]benzoyl-L-glutamate (**22**)*

A solution of equimolar amounts (0.5 mmol) **29a** or **29b** and the *p*-amino-benzoyl-L-glutamate (**32**) in 1-propanol (10 ml) was refluxed for 2 h. After cooling the solvent was evaporated in vacuo and the oily residues were taken up with acetone to give solid compounds. Purification methods, yields, melting points, analytical and spectroscopic data are reported in Table 1.

*6.1.4. General procedure for preparation of 4-phenoxymethylpyrrolo[1,2-a]quinoxalines (**11**–**18**)*

A mixture of equimolar amounts (0.7 mmol) of **29c**,**d** and the corresponding substituted phenols **31** of Fig. 1 in anhydrous DMF (5 ml) and in the presence of one mole equivalent of caesium carbonate, was stirred at 70 °C for 6 h. In the case of compounds **14** and **18**, caesium hydrogenocarbonate was alternatively used and the mixture heated at 70 °C for 2 h. After cooling, water was added to complete precipitation of the solids (**11**–**18**) which were filtered off and washed with water. Purification methods, yields, melting points, analytical and spectroscopic data are reported in Table 1.

*6.1.5. Diethyl N-[4-(pyrrolo[1,2-a]quinoxalin-4-yl)hydroxymethyl]benzoyl-L-glutamate (**25**) and diethyl N-[4-(7-trifluoromethylpyrrolo[1,2-a]quinoxalin-4-yl)hydroxymethyl]benzoyl-L-glutamate (**26**)*

A mixture of equimolar amounts (0.7 mmol) of **29c**,**d** and diethyl 4-hydroxy-benzoyl-L-glutamate [15,16] (**33**), dissolved in anhydrous DMF (6 ml) and in the presence of one mole equivalent of caesium hydrogencarbonate, was stirred at 70 °C for 2 h. After cooling water was added. A cream coloured solid precipitate (**25**) was first filtered then taken up with ether. In the case of compound **26** the acidic aqueous solution was extracted with chloroform. The organic phase, dried over anhydrous sodium sulfate, was evaporated in vacuo to give an oily residue that was further purified as reported in Table 1, in which are included yields, melting points, analytical and spectroscopic data.

*6.1.6. Hydrolysis of esters **4**, **8**, **14**, **18**, **21**, **22**, **25** and **26** into the acids **9**, **10**, **19**, **20**, **23**, **24**, **27** and **28***

A mixture of ester (**4**, **8**, **14**, **18**, **21**, **22**, **25**, **26**) (0.6 mmol) in ethanol (10 ml) and 1 M NaOH aqueous solution (5 ml) was refluxed for 4 h or heated at 70 °C in the case of compound **19**. On cooling, after removal of the solvent under reduced pressure, the residue was diluted with water and made acidic with 2 M HCl aqueous solution. The solid precipitate was filtered off and washed with water and eventually recrystallized. In the case of compound **23**, the acidic aqueous solution was extracted with chloroform. The organic phase, dried over anhydrous sodium sulfate and evaporated in vacuo gave a crystalline solid. Yields, melting points, analytical and spectroscopic data are reported in Table 1.

7. Material and methods

7.1. Compounds

Test compounds were solubilised in DMSO at 100 mM and then diluted into culture medium.

7.2. Cells

Cell lines were purchased from American Type Culture Collection (ATCC). Hematological tumor-derived cells were grown in RPMI-1640 medium supplemented with 10% FCS, 100 U/ml penicillin G and 100 μg/ml streptomycin. Solid tumor-derived cells were grown in their specific media supplemented with 10% FCS and antibiotics. Cell cultures were incubated at 37 °C in a humidified, 5% CO_2 atmosphere. The absence of mycoplasma contamination was checked periodically by the Hoechst staining method.

7.3. Antiproliferative assays

Exponentially growing cells were resuspended in growth medium containing serial dilutions of the drugs. Cell viability was determined after 96 h at 37 °C by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [18,19].

7.4. Virus

Human immunodeficiency virus type 1 (HIV-1) was obtained from supernatants of persistently infected H9/III_B cells. The HIV-1 stock solution had a titre of 1.0×10^7 50% cell culture infectious dose ($CCID_{50}$)/ml.

7.5. Antiviral assays

Activity of compounds against HIV-1 was based on inhibition of virus-induced cytopathogenicity in MT-4 cells acutely infected at a multiplicity of infection of 0.01.

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