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QUINOXALINE CHEMISTRY. PART 8. 2-[ANILINO]-3-[CARBOXY]-6(7)-SUBSTITUTED QUINOXALINES AS NON CLASSICAL ANTIFOLATE AGENTS. SYNTHESIS AND EVALUATION OF *IN VITRO* ANTICANCER, ANTI-HIV AND ANTIFUNGAL ACTIVITY

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Summary – Thirty quinoxalines bearing a substituted anilino group on position 2, a carboethoxy or carboxy group on position 3 and a trifluoromethyl group on position 6 or 7 of the heterocycle were prepared in order to evaluate *in vitro* anticancer activity. Preliminary screening performed at NCI showed that most derivatives exhibited a moderate to strong growth inhibition activity on various tumor panel cell lines between 10^{-5} and 10^{-4} molar concentrations. Interesting selectivities were also recorded between 10^{-8} and 10^{-6} M for a few compounds. One single compound exhibited good activity against *Candida albicans*.

INTRODUCTION

Isosteric replacement of the heterocyclic nuclei of methotrexate and trimetrexate with quinoxaline represents our current research program devoted to envisage new potential antifolate derivatives[1-4]. In this context we have so far described the compounds of formula **1, 2, 3** (Fig. 1) many of which exhibited very promising *in vitro* total growth inhibition activity and a few have been selected for in depth *in vivo* screening[5,6]. In particular, we have discovered and recently reported that when the phenyl group in position 3 of quinoxaline ring has been replaced by a carboethoxy group and in position 2 we placed an aminobenzoylglutamate moiety (formula **3** R=CO_2Et; R^1=H; R^2=Et) the percent tumor growth inhibition was markedly high and accompanied with an interesting anti-HIV and very promising antifungal activity[7]. On the other hand, the ester was more active than the corresponding acid which in turn was superior in antifungal (*Candida albicans*) test.

On the basis of these findings we have now prepared the esters **4a-o** and the acids **5a-o** of Fig. 2 in order to verify the influence of both carbethoxy and carboxy group in the series of 2-anilino derivatives previously described[3].

CHEMISTRY

The previously described chloroquinoxalines **6-8**[7] were reacted with the appropriate anilines (**9**) in refluxing ethanol to give the desired esters **4a-o** in fair yields (Table I) according to Scheme I. The acids **5a-o** were obtained after alkaline hydrolysis of **4a-o.**

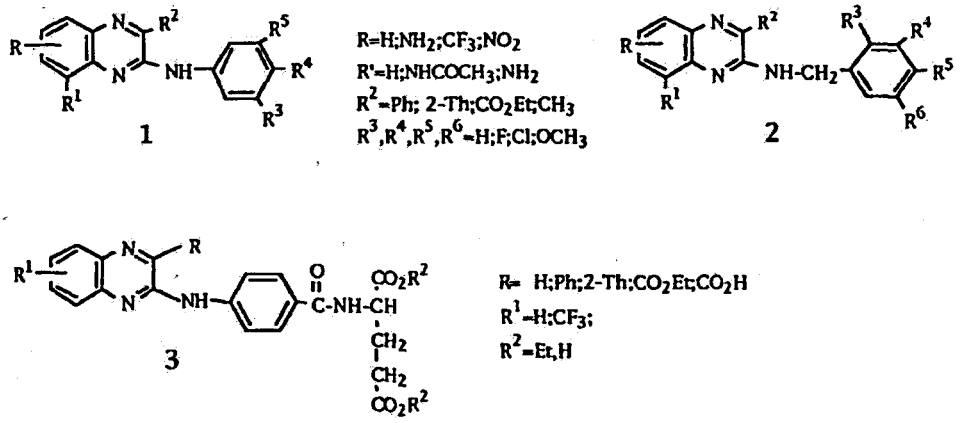
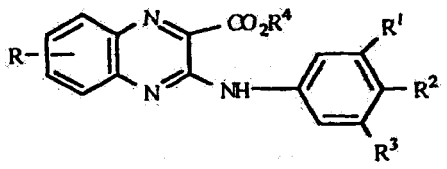


Fig. 1



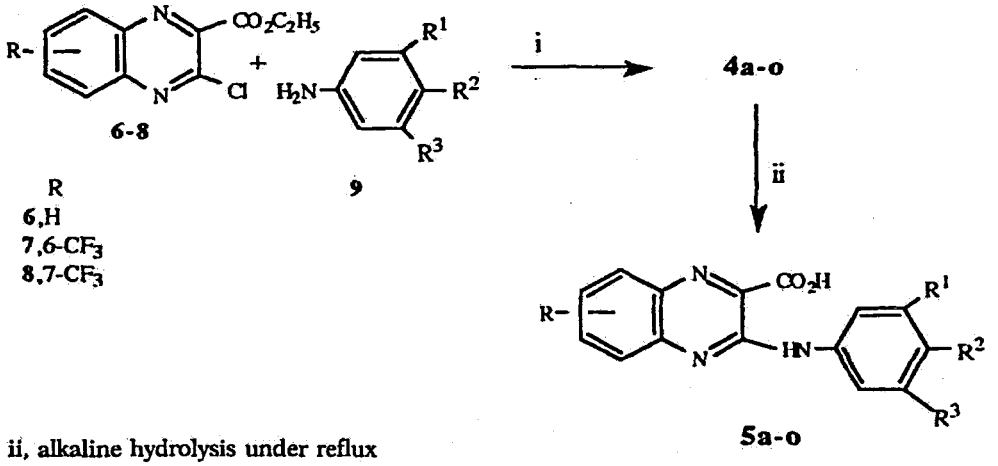
4a-o R^4=Et

5a-o R^4=H

Compd 4,5	R	R^1	R^2	R^3
a	H	H	OMe	H
b	H	OMe	OMe	H
c	H	OMe	OMe	OMe
d	H	Cl	Cl	H
e	H	H	F	H
f	7-CF_3	H	OMe	H
g	7-CF_3	OMe	OMe	H
h	7-CF_3	OMe	OMe	OMe
i	7-CF_3	Cl	Cl	H
j	7-CF_3	H	F	H
k	6-CF_3	H	OMe	H
l	6-CF_3	OMe	OMe	H
m	6-CF_3	OMe	OMe	OMe
n	6-CF_3	Cl	Cl	H
o	6-CF_3	H	F	H

Fig. 2

SCHEME



6-8 **9**

R
6,H
7,6-CF_3
8,7-CF_3

i, refluxing ethanol; ii, alkaline hydrolysis under reflux

EXPERIMENTAL

A) CHEMISTRY

Mp's are uncorrected and were recorded on a Kofler or a Electrothermal melting point apparatus. UV spectra are qualitative and were recorded in nm for solution in ethanol with a Perkin-Elmer Lambda 5 spectrophotometer. IR spectra are for Nujol mulls and were recorded on Perkin-Elmer 781 instruments. ^{1}H NMR spectra were recorded at 200 MHz with a Varian XL-200 instrument using TMS as internal standard. Elemental analyses were performed at Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, Università di Padova. The analytical results for C,H,N were within ± 0,4% of the theoretical values.

INTERMEDIATES

The intermediate chloroquinoxalines necessary for this work, as well as the quinoxalinones required for their preparation, were obtained as previously described[7].

General procedure for preparation of 3-ethoxycarbonyl-2-(R^1,R^2,R^3)substituted anilinoquinoxalines (4a-o)

A mixture of the suitable 2-chloroquinoxaline (6-8) (3 mmol) and the appropriate commercially available aniline suspended in ethanol (10 ml) was stirred under reflux for 13 h. On cooling the formed precipitates were filtered off and washed with ethanol. The yellow to red-orange products were further purified by recrystallization from ethanol-water. Yields, m.p.s, analytical and spectroscopic data are reported in Table I.

General procedure for preparation of 2-(R^1,R^2,R^3) substituted anilinoquinoxaline-3-carboxylic acids (5a-o).

A suspension of ester (4a-o) (1 mmol) in a mixture of ethanol (10 ml) and 1M NaOH aqueous solution (5 ml) was stirred under reflux for 1 h. On cooling, the precipitate, constituted by the sodium salts of 5a-o, was collected and washed with ethanol. The solids, slightly soluble in water, were dissolved in a mixture of water ethanol and the resulting solution, made acidic with 2M HCl aqueous solution, gave semi-solid precipitates or dusts of the acids (5a-o) that were collected and thoroughly washed with water. Yields, m.p.s, analytical and spectroscopic data are reported in Table I.

B) PHARMACOLOGY

Evaluation of anticancer and anti HIV activity was performed on 30 compounds referring to structures 4,5a-o of Fig. 2 and Table I at the National Cancer Institute of Bethesda, following the known[8] *in vitro* disease-oriented antitumor screening program against a panel of 60 human tumor cell lines and anti-HIV drug testing system[9]. The anticancer activity of each compound is deduced from dose-response curves and is presented in three different Tables according to the data provided by NCI. Antifungal activity was investigated at the Institute of Microbiology and Virology of Sassari University.

In Table II the response parameters GI_{50},**TGI** and LC_{50} refer to the concentration of the agent in the assay that produced 50% growth inhibition, total growth inhibition, 50% cytotoxicity respectively and are expressed as Mean Graph Midpoints.

In Table III we reported the activities of those compounds which showed percent growth inhibition greater than 40% on

TABLE I - Yields, analytical and spectroscopic data of the compounds **4a-o** and **5a-o**

Compd	M.p. °C	Yield %	Analysis for	ν_{max} cm^{-1} (Nujol)	λ_{max} nm (EtOH)	^{1}H-Nmr, δ (J in Hz)(*); solvent: A= $CDCl_3$; B= DMSO-d_6; C=$CDCl_3$+DMSO-d_6(1:1) D=$CDCl_3$+DMSOd_6(3:1)	
4a	127-128 (a)	85	$C_{18}H_{17}N_3O_3$	3310,3290, 1700	417,276,208	A	10.15 (1H, s, NH), 8.02 (1H, d, J 8.2, arom) 7.80 (2H, ad, J 9.0, H-3',5'), 7.78-7-65 (2H, m, arom), 7.49-7.44 (1H, m, arom), 6.96 (2H, ad, J 9.0, H-2',6'), 4.60 (2H, q, CH_2), 3.84 (3H, s, OMe), 1.53 (3H, t, Me)
4b	148-150	94	$C_{19}H_{19}N_3O_4$	3310,3290, 1700	296,223,205	A	10.21 (1H, s, NH), 8.03 (1H, d, J 8.4, arom), 7.78-7.66 (3H, m, arom), 7.50-7.36 (2H, m, arom), 6.91 (1H, d, J 8.6, H-5'), 4.60 (2H, q, CH_2), 3.97 (3H, s, OMe), 3.91 (3H, s, OMe), 1.54 (3H, t, Me)
4c	172-174	92	$C_{20}H_{21}N_3O_5$	3310,1690	295,225,208	A	10.31 (1H, s, NH), 8.04 (1H, d, J 8.6, arom) 7.78-7.76 (2H, m, arom), 7.56-7.44 (1H, m, arom), 7.28 (2H, as, H-2',6'), 4.61 (2H, q, CH_2), 3.94 (6H, s, 3',5'-OMe), 3.87 (3H, s, 4'-OMe), 1.55 (3H, t, Me)
4d	143-145	92	$C_{17}H_{13}Cl_2N_3O_2$	3310,3270, 3190,1700	415,292,222, 206	A	10.45 (1H, s, NH), 8.29 (1H, d, J 2.4, H-2'), 8.05 (1H, d, J 8.8, H-8), 7.88-7.74 (2H, m, arom), 7.67 (1H, dd, J 8.2 and 2.2, H-6'), 7.58-7.48 (1H, m, H-6), 7.41 (1H, d J 8.8, H-5'),4.60 (2H, q, CH_2), 1.54 (3H, t, Me)
4e	155-156	83	$C_{17}H_{14}FN_3O_2$	3310,3290, 3280,1695	424,285,222, 202	A	10.29 (1H, s, NH), 8.04 (1H, d, J 8.4, H-8), 7.90-7.70 (4H, m, arom), 7.52-7.45 (1H, m, arom), 7.13-7,05 (2H, m, arom), 4.60 (2H, q, CH_2), 1.53 (3H, t, Me)
4f	137-138	93	$C_{19}H_{16}F_3N_3O_3$	3310,3290, 1705	441,287,220, 203	A	10.21 (1H, s, NH), 8.11 (1H, d, J 8.2, H-5), 8.04 (1H, s, H-8), 7.77 (2H, d, J 9.0, H-3',5'), 7.60 (2H, dd, J 8.2 and 1.8, H-6), 6.97 (2H, d, J 9.0, H-2',6'), 4.61 (2H, q, CH2), 3.85 (3H, s, OMe), 1.53 (3H, t, Me)
4g	178-179	87	$C_{20}H_{18}F_3N_3O_4$	3310,3290, 1700	316,290,208	A	10.26 (1H, s, NH), 8.12 (1H, d, J 8.6-H-5), 7.99 (1H, s, H-8), 7.61 (1H, dd, J 8.6 and 1.8, H-6), 7.51 (1H, d, J 2.4, H-2'), 7.40 (1H, dd, J 8.6 and 2.4, H-6'), 6.92 (1H, d, J 8.6, H-5'), 4.61 (2H, q, CH_2), 3.92 (3H, s, OMe), 1.55 (3H, t, Me)
4h	189-191	89	$C_{21}H_{20}F_3N_3O_5$	3290,1710	310,291,206	A	10.37 (1H, s, NH), 8.15 (1H, d, J 8.8, H-5), 7.97 (1H, s, H-8), 7.65 (1H, dd, J 8.8 and 1.4, H-6), 7.21 (2H, s, H-2',6'), 4.62 (2H, q, CH2), 3.95 (6H, s 3',5'-OMe), 3.88 (3H, s, 4'-OMe), 1.56 (3H, t, Me)
4i	167-168	90	$C_{18}H_{12}Cl_2F_3N_3O_2$	3260,1700	416,304,287, 217	B	10.21 (1H, s, NH), 8.29 (1H, d, J 2.8, H-2'), 8.19 (1H, d, J 8.2, H-5), 8.10 (1H, a s, H-8), 7.90 (1H, dd, J 8.6 and 2.8, H-6'), 7.82 (1H, dd, J 8.2 and 2.2, H-6), 7.60 (1H, d, J 8.8, H-5'), 4.50 (2H, q, CH_2), 1.43 (3H, t, Me)
4j	155-156	81	$C_{18}H_{13}F_4N_3O_2$	3300,1700	429,283,218, 202	A	10.35(1H, s, NH), 8.14 (1H, d, 8.8, H-5), 8.05 (1H, s, H-8), 7.88-7.80 (2H, m, H-2',6'), 7.64 (1H, dd, J 8.8 and 1.8, H-6), 7.17 -7.05 (2H, m, H-3',5'), 4.62 (2H, q, CH_2), 1.55 (3H, t, Me)
4k	137-138	90	$C_{19}H_{16}F_3N_3O_3$	3310,3290, 1690	415,275,208	A	10.29 (1H, s, NH), 8.32 (1H, s, H-5), 7.81-7.52 (2H, m, H-7,8), 7.77 (2H, d, J 9.2, H-3',5'), 6.96 (2H, d, J 9.2, H-2',6'), 4.60 (2H, q, CH_2), 3.85 (3H,s,OMe), 1.53 (3H, t, Me)
4l	205-207	92	$C_{20}H_{18}F_3N_3O_4$	3270,1705	430,320,293, 206	A	10.34 (1H, s, NH), 8.34 (1H, s, H-5), 7.90-7.75 (2H, m, H-7,8), 7.59 (1H, d, J 8.8 and 2.4, H-6'), 6.92 (1H, d, J 8.8, H-5'), 4.61 (2H, q, CH_2), 3.96 (3H, s, OMe), 3.92 (3H, s, OMe), 1.54 (3H, t, Me)
4m	181-183	83	$C_{21}H_{20}F_3N_3O_5$	3280,1700	428,316,293, 224,207	A	10.44 (1H, s, NH), 8.36 (1H, s, H-5), 7.95-7.75 (2H, m, H-7,8), 7.24 (2H, s, H-2',6'), 4.62 (2H, q, CH_2), 3.94 (6H, s, 3',5-OMe), 3.88 (3H, s, 4'-OMe), 1.55 (3H, t, Me)
4n	162-164	78	$C_{18}H_{12}Cl_2F_3N_3O_2$	3260,1700	410,300sh,289, 219, 206	A	10.58 (1H, s, NH), 8.38 (1H, s, H-5), 8.25 (1H, d, J 2.4, H-2'), 7.95-7.82 (2H, m, H-5',6'), 7.67 1H, dd, J 8.6 and 2.4, H-7), 7.44 (1H, d, J 8.6, H-8), 4.61 (2H, q, CH_2), 1.54 (3H, t, Me)
4o	125-127	81	$C_{18}H_{13}F_4N_3O_2$	3280,1690	419,285,218, 202	A	10.43 (1H, s, NH), 8.36 (1H, s, H-5), 7.92- 7.81 (4H, m, arom), 7.16-7.07 (2H, m, H-3',5'), 4.61 (2H, q, CH_2), 1.55 (3H, t, Me)
5a	150-151	94	$C_{16}H_{13}N_3O_3$	3520,3320, 3280,1700	393,300sh,269, 200	C	10.41 (1H, s, NH), 7.97-7.93 (1H, m, arom), 7.81 (2H, d, J 9.0, H-3',5'), 7.72-7.70 (1H, m, arom), 7.53 (1H, m, arom), 6.93 (2H, d, J 9.0, H-2',6'), 4.44 (1H, br s, COOH), 3.81 (3H, s, 4'-OMe)
5b	152-153	95	$C_{17}H_{15}N_3O_4$	3280,1730	408,300sh,287, 207	D	10.41 (1H, s, NH), 8.00 (1H, d, J 8.2, H-8), 7.80-7.70 (3H, m, arom), 7.55-7.45 (1H, m, arom), 7.36 (1H, dd, J 8.8 and 2.2, H-6'), 6.90 (1H, d, J 8.8, H-5'), 4.88 (1H, br s, COOH), 3.96 (3H, s, OMe), 3.90 (3H, s, OMe)

(to be continued)

TABLE I - (*continued*)

Compd	M.p. °C	Yield %	Analysis for	νmax cm-1 (Nujol)	λmax nm (EtOH)		^{1}H-Nmr, δ (J in Hz)(*); solvent: A= $CDCl_3$; B= DMSO-d_6; C=$CDCl_3$+DMSO-d_6(1:1) D=$CDCl_3$+DMSOd_6(3:1)
5c	173-174	87	$C_{18}H_{17}N_3O_5$	3500,3360, 3280,1670	389,300sh,273, 203	D	10.57(1H, s, NH), 7.99 (1H, d, J 8.8, arom), 7.73-7.68 (2H, m, arom), 7.56-7.45 (1H, m, arom), 7.31 (2H, s, H-2',6'), 4.52 (1H, br s, COOH), 3.91 (6H, s, 3',5'-OMe), 3.78 (3H, s, 4'-OMe)
5d	163-165	95	$C_{15}H_9Cl_2N_3O_2$ 0.25 H2O	3320,3280, 1710	392,294,216	D	10.78 (1H, s, NH), 8.34 (1H, s, H-2'), 8.03 (1H, d, J 8.4, arom), 7.90-7.75 (2H, m, arom), 7.70 (1H, m, arom), 7.60-7.50 (1H, m, arom), 7.43 (1H, d, J 8.4, H-5'), 5.02 (1H, br s, COOH)
5e	147-149	88	$C_{15}H_{10}FN_3O_3$	3340,1710	398,281,217	D	10.58(1H, s, NH), 8.01 (1H, d, J 8.4, H-8), 7.95-7.85 (2H, m, arom), 7.78-7.70 (2H, m, arom), 7.55-7.45 (1H, m, arom), 7.15-7.04 (2H, m, H-3',5'), 4.65 (1H, a s, COOH)
5f	140-141	72	$C_{17}H_{12}N_3O_3$	3500,3310, 3280,1690	415,304,284, 204	D	10.52 (1H, s, NH), 8.13 (1H, d, J 8.6, H-5), 8.02 (1H, s, H-8), 7.8 (2H, d, J 8.8, H-3',5'), 7.64 (1H, dd, J 8.6 and 1.4, H-6), 6.96 (2H, d, J 8.8, H-2',6'), 4.08 (1H, a s, COOH), 3.85 (3H, s, 4'-OMe)
5g	171-173	81	$C_{18}H_{14}F_3N_3O_4$	3540,3400, 1700	417,311,284, 206	D	10.54 (1H, s, NH), 8.12 (1H, d, J 8.4, H-5), 7.96 (1H, s, H-8), 7.64 (1H, d, J 8.4, H-6), 7.58 (1H, d, J 2.0, H-2'), 7.42 (1H, dd, J 8.6 and 2.0, H-6'), 6.94 (1H, d, J 8.6, H-5'), 4.00 (1H, br s, COOH), 3.91 (3H, s, OMe), 3.84 (3H, s, OMe)
5h	148-150	72	$C_{19}H_{16}F_3N_3O_5$ H2O	3530,3360, 3280,1700	414,384,307, 207	B	10.54 (1H, s, NH), 8.15 (1H, d, J 8.4, H-5), 8.02 (1H, s, H-8), 7.74 (1H, d, J 8.4, H-6), 7.30 (2H, s, H-2',6'), 3.86 (6H, s, 3',5'-OMe), 3.69 (3H, s, 4'-OMe), 3.53 (1H, a s, COOH)
5i	183-185	83	$C_{16}H_8Cl_2F_3N_3O_2$	3260,1680	399,298,214	D	10.87(1H, s, NH), 8.17 (1H, d, J 8.6, H-5), 8.08 (1H, s, H-8), 7.78-7.68 (3H, m, H-6,2',6'), 7.47 (1H, d, J 8.8, H-5'), 4.52 (1H, br s, COOH)
5j	166-168	95	$C_{16}H_9F_4N_3O_2$ 0.25 H2O	3630,3280, 3180,1700	404,295,279, 214	C	10.63 (1H, s, NH), 8.13 (1H, d, J 8.0, H-5), 8.03 (1H, s, H-8), 8.00-7.88 (2H, m, H-2',6'), 7.68 (1H, d, J 8.0, H-6), 7.25-7.10 (2H, m, H-3',5'), 5.10 (1H, br s, COOH)
5k	103-105	95	$C_{17}H_{12}F_3N_3O_3$	3500,3380, 3260,1700	407,305,282, 232,204	D	10.57(1H, s, NH), 8.31 (1H, s, H-5), 7.82-7.75 (2H, m, H-7,8), 7.80 (2H, d, J 9.0, H-3',5'), 6.95 (2H, d, J 9.0, H-2',6'), 5.12 (1H, br s, COOH), 3.84 (3H, s, 4'-OMe)
5l	**	86	$C_{18}H_{14}F_3N_3O_4$	3300,1710	395,301,269, 217	C	10.59 (1H, s, NH),8.22 (1H, s, H-5), 7.95-7.68 (2H, m, H-7,8), 7.67 (1H, s, H-2'), 6.35 (1H, d, 7.8, H-6'), 6.93 (1H, d, J 7.8, H-5'), 4.66 (1H, br s, COOH), 3.88 (3H, s, OMe), 3.82 (3H, s, OMe)
5m	***		$C_{19}H_{16}F_3N_3O_5$	3300,1750	303, 205	D	10.74 (1H, s, NH), 8.34 (1H, s, H-5), 7.95-7.80 (2H, m, H-7,8), 7.28 (2H, s, H-2',6'), 4.55 (1H, br s, COOH), 3.93 (6H, s, 3',5'-OMe), 3.84 (3H, s, 4'-OMe)
5n	176-178	90	$C_{16}H_8Cl_2F_3N_3O_2$	3360,3290, 1750	393,297,288, 214	D	10.97 (1H, s, NH), 8.35 (1H, s, H-5), 8.32 (1H, d, J 2.4, H-2'), 8.00-7.86 (2H, m, H-7,8), 7.73 (1H, dd, J 8.8 and 2.4, H-6'), 7.47 (1H, d, J 8.8, H-5'), 3.84 (1H, br s, COOH)
5o	179-181	86	$C_{16}H_9F_4N_3O_2$	3280,1750	398,280,227, 213	D	10.73 (1H, s, NH),8.32 (1H, s, H-5), 7.95-7.80 (4H, m, H-5,6,2',6'),7.18-7.02 (2H, m, H-3',5'), 5.28 (1H, br s, COOH)

(*) J of triplet and quartet corresponding to Me-CH_2 are not reported because well corresponding to the data of the literature; (**) Melts at 120 °C, and decomposes at 148 °C; (***) Melts at 170 °C, solidifies and remelted at 250 °C.

subpanel cell-lines at Molar concentration of 10^{-4} whereas in Table IV is reported the activity of those compounds which exhibited both significant selectivity and growth inhibition at 10^{-5} M. The most diluted concentrations (10^{-8}, 10^{-7}, 10^{-6} M) were considered in the case of compounds **4**, **5** which showed high selectivity (Table V).

In Table VI we reported the preliminary results of both anti-fungal activity against *Candida albicans* and anti-HIV protection.

RESULTS AND DISCUSSION

From the data of Table II we can deduce that the average sensitivity of all cell lines towards the tested agent, represented as Mean Graph Midpoints, falls in the range 10^{-5} -:- 10^{-4} Molar concentrations. Mean Graph Midpoints for compounds **4a-o** show that whereas GI_{50} was nearly

TABLE II - $-\log_{10}GI_{50}$, $-\log_{10}TGI$, $-\log_{10}LC_{50}$ Mean Graph Midpoints (MG-MID) of *in vitro* Inhibitory Activity Test for Compounds **4a-o** and **5a-o** against Human Tumor Cell Lines[a]

Compd	$-\log_{10}GI_{50}$	$-\log_{10}TGI$	$-\log_{10}LC_{50}$
4a	4.32	4.06	4.00
4b	4.06	4.00	4.00
4c	4.01	4.00	4.00
4d	4.32	4.04	4.00
4e	4.44	4.11	4.01
4f	4.23	4.03	4.00
4g	4.10	4.01	4.00
4h	4.00	4.00	4.00
4i	4.09	4.00	4.00
4j	4.13	4.01	4.00
4k	4.07	4.00	4.00
4l	4.02	4.00	4.00
4m	4.00	4.00	4.00
4n	4.08	4.00	4.00
4o	4.28	4.03	4.00
5a	4.21	4.01	4.00
5b	4.19	4.00	4.00
5c	4.23	4.01	4.00
5d	4.64	4.10	4.00
5e	4.51	4.04	4.00
5f	4.73	4.28	4.05
5g	4.53	4.07	4.00
5h	4.37	4.02	4.00
5i	5.14	4.62	4.23
5j	4.75	4.25	4.03
5k	4.54	4.06	4.00
5l	4.55	4.09	4.01
5m	4.61	4.21	4.03
5n	4.87	4.46	4.16
5o	4.79	4.20	4.01

(MG-MID): Mean Graph Midpoints, the average sensitivity of all cell lines towards the test agent; ([a]) from NCI

equal to both **TGI** and $\mathbf{LC_{50}}$ (**4b,c,g,h,i,l,m,n**) only a few subpanel cell-lines were sensitive. On the contrary in the series of compounds **5a-o** we recorded an high degree of inhibition activity between 10^{-5} -:- 10^{-4} Molar concentrations as expressed as Mean Graph Midpoints. The data of Table III were in line with the above observation. From the data of Table II and III we can establish a decreasing order of tumor growth inhibition activity for each compound (**5i>5n>5f>5m>5o>4e>5d>5l**) as expressed by both **TGI** and $\mathbf{LC_{50}}$. The most active compounds (**5i,5n**) were also the most cytotoxic as expressed as $\mathbf{LC_{50}}$.

According to Table IV compound **5i** mantained high inhibition activity at 10^{-5} Molar concentration in 39 over 60 subpanel cell-lines with particular significativity on leukemia, colon, breast and prostate cancer. A few other compounds were in decreasing order endowed with cell-line sensitivity at this concentration: compound **5n** (16 cell- lines over 60), **5o** (12 over 60), **4e** (11 over 60), **5j** (10 over 60), in the remaining compounds (**4d,g,h,o** and **5a,d,f,k**) we could only observe some cell-line sensitivity. The data of Table V show that some compounds (**4a,b,c,h**) and (**5a,b,d,e,g,h,i,k,l**) mantained a certain degree of tumor growth inhibition at the most diluted concentrations on some subpanel cell-lines as result of their cell-line selectivity.

Interestingly compound **4c** exhibited a promising antifungal activity against *Candida albicans* (MIC=1.9 μg/ml). Compound **4d** exhibited a moderate HIV protection (Table VI).

Structure-activity relationships show that between the series of the esters **4a-o** and the corresponding acids **5a-o** the last were in general superior in tumor growth inhibition activity. A positive influence of a trifluoromethyl group does not distinguish between the substitution positions in the quinoxaline ring.

Comparison of the activity of 3',4'-dichloro-3',4'-dimethoxy- and 3',4',5'-trimethoxyanilino quinoxalines (**4g,h,i,l,m,n**) and (**5g,h,i,l,m,n**) with the corresponding analogues **1** previously described[3] bearing hydrogen,phenyl,methyl as susbstituent on position 3 evidentiated that the tumor growth inhibition was of the same order of magnitude only for compounds (**5g,h,l,m**) whereas in the case of compounds **4** these were in general less active than their above cited analogues. On the contrary in the case of **5i** this compound was more active than the corresponding methyl and phenyl analogues.

In conclusion we can say that the electronegativity of the carboxyl group to some extent can positively influence the anticancer activity of these anilinoquinoxalines.

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TABLE III - Percent Tumor Growth Inhibition (GI) recorded on Subpanel/Cell Line at 10^{-4} Molar concentration of Compounds **4a-o** and **5a-o**

Panel/Cell Line	Compound																												
	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4k	4l	4m	4n	4o	5a	5b	5c	5d	5e	5f	5g	5h	5i	5j	5k	5l	5m	5n	5o
Leukemia																														
CCRF-CEM		-	-	nt	-	-	-	-	53	nt	nt	-	-	59	117	74	73	59	86	111	nt	114	104	nt	nt	127	nt	93	nt	100
HL-60(TB)	47	-	-	79	-	50	-	-	51	-	-	-	-	68	nt	64	55	40	92	111	106	117	72	162	156	nt	129	99	87	91
K-562		-	-	54	-	58	-	-	81	48	nt	-	-	nt	142	62	-	-	92	103	138	100	74	158	131	95	94	94	nt	nt
MOLT-4		-	-	45	-	-	-	-	44	40	-	-	-	49	nt	54	65	-	94	95	154	115	nt	174	158	101	142	97	nt	117
RPMI-8226		-	-	61	-	40	-	-	-	nt	-	-	-	67	111	nt	62	69	86	94	nt	103	nt	nt	145	112	nt	103	112	93
SR	42	-	-	-	-	50	62	46	55	44	45	-	-	63	nt	70	48	-	73	105	148	112	nt	16"	nt	nt	124	100	101	126
Non-SmallCell Lung Cancer																														
A549/ATCC	72	-	-	60	57	66	-	-	61	109	-	-	-	54	79	41	42	-	93	98	nt	95	66	nt	nt	99	nt	87	193	95
EKVX	65	94	-	58	133	50	-	-	-	nt	-	-	-	47	58	67	63	84	96	87	118	87	82	178	112	89	88	101	150	104
HOP-62	182	-	-	132	160	124	170	-	74	69	74	94	-	56	51	-	72	76	121	82	184	87	74	198	175	86	113	137	200	nt
HOP-92	135	61	49	142	155	125	81	-	-	100	84	-	-	102	123	-	82	103	120	107	nt	111	108	nt	nt	104	nt	nt	200	144
NCI-H226	77	nt	nt	nt	97	83	-	104	nt	nt	-	-	-	-	63	nt	72	47	85	100	125	124	89	182	124	94	84	171	170	93
NCI-H23	68	45	45	67	109	70	44	-	-	79	48	-	-	50	56	52	55	55	133	99	182	111	74	199	154	102	131	180	173	155
NCI-H322M	57	-	-	55	-	45	-	-	-	-	65	-	-	-	66	47	-	nt	nt	84	101	113	85	150	109	71	84	nt	119	84
NCI-H460	82	-	-	91	76	81	-	-	-	77	nt	-	-	81	96	82	72	61	105	95	139	94	66	193	129	108	132	144	200	nt
NCI-H522	93	-	-	71	83	79	-	-	-	62	-	-	-	50	nt	52	-	66	120	108	166	96	82	184	165	nt	107	137	185	106
Colon Cancer																														
Colo 205	-	-	-	59	-	45	-	-	43	nt	73	-	-	-	50	90	-	-	108	114	148	120	81	185	150	96	92	195	200	159
HCC-2998	40	-	-	-	-	-	-	-	89	-	-	-	-	57	93	127	58	61	111	93	170	89	65	182	169	147	149	186	nt	nt
HCT-116	76	-	-	nt	95	63	-	-	61	48	66	-	-	61	51	64	45	43	111	95	171	102	89	200	163	87	110	183	200	132
HCT-15	-	-	-	-	61	-	-	-	51	-	-	-	-	50	67	83	72	85	150	104	155	93	83	187	115	99	97	179	200	nt
HT29	48	-	-	-	-	-	-	-	-	-	-	-	-	61	50	41	-	nt	nt	99	99	93	80	163	100	97	90	127	199	98
KM12	-	-	-	-	-	-	-	-	57	43	-	-	-	59	41	77	58	45	119	88	103	105	82	187	119	116	178	128	195	144
SW-620	-	nt	nt	nt	-	-	-	-	-	-	-	nt	-	57	55	83	44	-	89	87	110	77	58	172	108	96	100	130	200	114
CNS Cancer																														
SF-268	71	78	-	78	158	74	55	49	47	70	48	-	-	40	40	61	77	86	96	100	157	78	73	192	132	87	94	142	165	96
SF-295	130	44	48	nt	184	112	-	-	82	74	58	-	-	56	62	62	79	79	113	130	151	127	121	176	141	104	111	109	151	118
SF-539	138	-	-	65	146	105	50	-	65	57	-	-	-	-	128	-	48	43	153	127	nt	124	83	nt	nt	112	nt	107	199	191
SNB-19	105	-	-	91	128	89	94	-	103	87	62	-	-	40	nt	-	57	57	89	87	111	95	83	187	nt	nt	99	99	118	90
SNB-75	104	110	-	116	195	52	117	-	66	109	71	-	46	63	78	58	54	75	65	108	136	68	95	179	127	114	112	81	133	136
U251	92	57	42	89	124	93	-	nt	nt	nt	nt	nt	-	66	83	63	49	41	87	82	170	86	83	195	100	82	94	176	146	122
Melanoma																														
LOX IMVI	43	nt	nt	nt	51	-	-	-	-	45	74	43	-	-	47	64	50	63	94	96	148	93	92	199	130	89	95	183	199	138
Malme-3M	52	-	-	192	-	-	-	-	-	-	nt	-	-	-	49	48	74	49	92	91	154	91	65	169	153	90	99	105	172	124
M14	49	-	-	77	-	54	-	-	56	-	42	-	-	50	64	-	45	56	108	91	nt	92	86	nt	nt	85	nt	103	197	109
SK-MEL-2	78	-	-	53	115	56	nt	-	nt	-	-	nt	-	49	41	41	nt	nt	nt	113	196	150	166	198	164	81	171	154	180	159
SK-MEL-28	66	-	-	-	-	-	-	-	-	-	-	-	-	40	-	65	71	81	108	83	84	77	59	192	78	92	76	110	nt	80
SK-MEL-5	67	55	-	119	-	56	-	-	42	nt	-	-	-	-	68	84	96	68	134	133	121	148	70	189	96	98	141	94	130	134
UACC-257	72	nt	nt	nt	-	44	-	-	-	-	-	-	-	-	43	41	50	52	84	90	135	96	44	144	110	102	91	155	119	124
UACC-62	124	-	-	108	-	65	-	-	-	-	-	-	-	46	93	42	67	nt	nt	112	194	138	92	200	173	116	100	167	198	165
Ovarian Cancer																														
IGROV1	72	-	-	81	132	86	-	-	-	53	47	-	-	-	83	53	50	56	96	93	104	89	62	153	121	155	88	121	160	126
OVCAR-3	114	-	-	66	62	97	-	-	74	-	47	-	-	-	72	63	82	62	142	108	154	102	94	162	115	143	154	103	200	130
OVCAR-4	127	-	-	96	-	137	nt	-	nt	-	-	nt	-	51	106	-	nt	nt	nt	82	90	88	47	106	100	90	80	126	118	nt
OVCAR-5	53	-	-	41	-	41	-	-	-	-	-	-	-	-	46	-	-	-	75	67	127	67	-	189	127	107	76	88	196	103
OVCAR-8	58	nt	nt	80	159	-	-	-	58	68	-	-	-	40	67	57	-	-	80	96	143	86	67	194	106	92	95	117	194	107
SK-OV-3	nt	-	-	171	nt	nt	68	-	-	-	nt	-	-	51	51	60	64	54	106	nt	nt	nt	nt	nt	nt	113	nt	86	197	98
Renal Cancer																														
786-O	91	55	41	96	92	99	89	-	56	72	73	-	-	-	45	70	61	59	119	83	145	87	68	189	130	73	87	115	200	119
A498	106	nt	nt	nt	132	65	-	-	-	40	-	-	-	-	74	44	111	111	138	79	162	95	57	192	161	123	106	nt	186	131
ACHN	90	-	-	56	96	63	72	-	51	43	53	-	-	50	nt	68	66	49	92	90	89	99	61	119	92	nt	89	153	55	150
CAKI-1	120	-	-	116	nt	nt	106	-	69	-	-	-	-	-	40	84	95	109	115	121	99	139	100	187	113	86	86	127	111	113
RXF-393	156	nt	nt	nt	183	115	44	-	-	83	67	-	-	-	65	-	60	43	118	126	181	127	120	181	174	123	99	140	184	165
SN12C	117	-	nt	55	nt	nt	46	-	46	-	-	-	-	-	41	56	69	55	98	nt	98	120	64	183	107	88	85	150	nt	nt
TK-10	99	nt	nt	78	86	56	86	-	-	52	-	47	-	-	56	-	101	108	86	91	88	81	72	86	79	93	81	124	152	nt
UO-31	51	-	-	69	52	46	47	-	43	63	-	-	-	-	nt	78	nt	92	171	87	200	97	84	199	197	nt	150	200	199	163
Prostate Cancer																														
PC-3 (*GDP)	95	nt	nt	nt	-	73	-	-	-	-	-	-	-	57	79	45	46	45	82	104	127	91	nt	198	132	103	102	135	191	154
DU 145	66	-	-	45	56	-	-	-	62	-	47	-	-	40	-	57	48	-	86	85	96	88	80	nt	106	70	96	110	nt	nt
Breast Cancer																														
MCF7	-	-	-	55	-	-	-	-	-	-	-	-	-	-	53	-	65	nt	nt	116	136	103	71	113	125	84	94	92	104	113
MCF7/ADRRES	79	-	-	50	80	63	-	-	-	45	-	-	-	-	54	80	79	99	86	85	125	88	89	188	106	91	126	152	192	98
HS 578T	99	77	51	92	100	92		-	51	112	67	73	40	45	-	105	-	103	88	88	130	78	75	154	102	nt	102	126	149	111
MDA-MB-435	-	-	-	45	-	-	-	-	-	-	-	-	-	60	-	41	49	50	91	88	148	94	74	182	143	93	110	116	188	99
MDA-N	nt	-	-	62	nt	nt	-	-	50	-	-	-	-	-	-	52	48	42	95	nt	99	nt	89	156	111	77	90	143	112	103
BT-549	114	-	-	126	109	106	51	-	-	-	-	-	-	54	82	46	59	52	118	81	188	99	90	198	132	119	115	172	171	129
T-47D	75	-	-	69	111	49	-	-	-	41	51	59	-	-	43	68	67	92	112	93	nt	74	88	nt	nt	86	nt	111	152	90
MDAMB231/ATCC	137	-	-	-	198	132	66	-	61	nt	64	-	-	-	-	-	64	95	105	nt	99	126	115	149	122	64	82	130	142	115

nt=not tested; (-) Below 40% growth inhibition.

TABLE IV - Percent Growth Inhibition recorded at 10^{-5} Molar concentration for compounds **4d, 4e, 4g, 4h, 4o, 5a, 5d, 5f, 5i, 5j, 5k, 5n, 5o**

	Compound													
Panel/Cell Line	4d	4e	4g	4h	4o	5a	5d	5f	5g	5i	5j	5k	5n	5o
Leukemia														
CCRF-CEM													49	44
HL-60(TB)										68				
K-562										84	46		43	44
MOLT-4								55		67	51			
RPMI-8226					60							44	59	70
SR			46					46		89	51			
Non-SmallCell Lung Cancer														
A549/ATCC					40									38
EKVX										56				
HOP-18														
HOP-62			40							55				
HOP-92		42			47								43	
NCI-H226				59						46				
NCI-H23										62				
NCI-H322M														
NCI-H460	48						42			56				
NCI-H522		43								81	48			57
Colon Cancer														
Colo 205										41				
HCC-2998						84				74				
HCT-116										70			52	
HCT-15										52				
HT29										52				
KM12		47					48			60				59
KM20L2														
SW-620										55			45	
CNS Cancer														
SF-268										53				
SF-295		41								67	44		49	40
SF-539		46											41	
SNB-19		43								97	41		52	
SNB-75		77												
U251										81	57		49	
Melanoma														
LOX IMVI										50				
Mdme-3M														
M14														
M19-MEL														
SK-MEL-2									45	95			109	57
SK-MEL-28							43							
SK-MEL-5										47				
UACC-257														
UACC-62										66	41			
Ovarian Cancer														
IGROV1		42												
OVCAR-3										62	44			
OVCAR-4										57				
OVCAR-5														
OVCAR-8		45								69				
SK-OV-3						51								
Renal Cancer														
786-O										47				
A498														
ACHN										56			44	40
CAKI-1				50										
RXF-393		43												
SN12C										56				
TK-10														
UO-31										65				
Prostate Cancer														
PC-3 (°GDP)										75			76	46
DU 145									41	61				
Breast Cancer														
MCF7														
MCF7/ADR-RES										63			47	
HS 578T														
MDA-MB-435										68			68	55
MDA-N										62				
BT-549	41									80	41	56	44	41
T-47D														
MDAMB231/ATCC		86								63				

TABLE V - Comparison of the inhibitory activity of compounds **4a,b,c,k,h** on some cell-lines at alla concentrations examined

Cell-Line	Compd	Percent Tumor Growth Inhibition at the indicated Molar concentration		
		10^{-8}	10^{-7}	10^{-6}
Leukemia				
HL-60(TB)	**4a**			45
CCR-CEM	**4b**		41	
RPMI-8226	**4k**		40	
NSCL				
NCI H226	**4h**	52	38	54
HOP-92	**5d**			44
NCI-H23	**5i**			31
CNS				
SNB-19	**4a**			31
SNB-75	**4b**	27		28
SNB-75	**4c**	36	27	28
SNB-75	**5h**			42
SF 268	**5i**		24	45
UACC-62	**5i**			41
Ovarian-OVCAR-8	**5h**			39
Renal				
RXF-393	**5e**	40	36	
Caki-1	**5g**	26	33	29
Caki-1	**5h**	42	40	44
TK-10	**5i**	31	53	44
Prostate DU-145	**5f**	43	25	24
Breast Cancer				
TD-47	**4c**	25	41	33
MS 578T	**5a**			39
MCF7/ADR-RES	**5b**			36
BT-549	**5k**			45

TABLE VI - Antifungal and Anti-HIV Activity of compounds **4c,4d,4h,5c**

Compd	*Candida albicans*		*HIV*		
			% protection	Dose (Molar)	EC_{50}(Molar)
	MIC				
4c	1.9	µg/ml			
4d	-	-	56.92	$>2.00 \times 10^{-4}$	1.71×10^{-4}
4h	500	µg/ml			
5c	500	µg/ml			

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