Synthesis of Variously Substituted 3-Phenoxymethyl Quinoxalin-2-Ones and Quinoxalines Capable to Potentiate *In Vitro* the Antiproliferative Activity of Anticancer Drugs in Multi-Drug Resistant Cell Lines

Antonio Carta[a,*], Mario Loriga[a], Sandra Piras[a], Giuseppe Paglietti[a], Paolo La Colla[b,*], Bernardetta Busonera[b], Gabriella Collu[b], and Roberta Loddo[b]

[a]*Dipartimento Farmaco-Chimico-Tossicologico, Università degli Studi di Sassari, Via Muroni 23/a, 07100 Sassari, Italy,* [b]*Dipartimento di Scienze e Tecnologie Biomediche, Sezione di Microbiologia e Virologia Generale e Biotecnologie Microbiche. Università degli Studi di Cagliari, Cittadella Universitaria, 09042 Monserrato (Cagliari), Italy*

Abstract: Two series of 1,6-dimethyl-3-phenoxymethylquinoxalin-2-ones and 1-benzyl-3-phenoxymethyl-7-trifluoromethylquinoxalin-2-ones, and a series of 2-benzyloxy-3-phenoxymethyl-7-trifluoromethylquinoxaline were synthesized. Their capability to restore/potentiate the antiproliferative activity of clinically useful drugs, such as doxorubicin (Doxo), vincristine (VCR) and etoposide (VP16), in drug-resistant human nasopharyngeal carcinoma KB cells (KB^{WT}, KB^{MDR}, $KB^{7D}$and KB^{V20C}) was evaluated. *In vitro* data show that many quinoxalin-2-ones and quinoxalines potentiate the antiproliferative activity of Doxo and VCR in tumor-derived MDR cell lines. In this series, **17a** turned out to be the most potent quinoxaline derivative in potentiating the antiproliferative activity of doxorubicin and vincristine against KB^{MDR} and KB^{V20C} resistant cell lines, respectively.

Key Words: 2-Quinoxalinones, quinoxalines, antiproliferative activity, multidrug resistance, P-glycoprotein inhibitors, doxorubicin, vincristine, etoposide.

INTRODUCTION

Quinoxaline derivatives display a wide range of interesting chemical and biological (antibacterial, antiviral, anticancer, antifungal, anthelmintic, insecticidal) properties [1]. Their interest in medicinal chemistry is continuous and, very likely, far to come to an end.

Oxidation of one or both nitrogen atoms of the quinoxaline ring, to give *mono*- and *di*- N-oxide quinoxaline derivatives, has been shown to lead to diversified biological activities [2] (animal growth promoting, antibacterial, hypoxia-selective, antimycobacterial, antitubercular, antiprotozoal, anticandida). In the last decade many 2-oxo and 2,3-dioxo derivatives of this heterocyclic system have been synthesized and their antibacterial, antiproliferative and antifungal activities reported [3-4]. More recently, within a series of over 150 quinoxalin-2-ones we observed different biological activity according to the substituent introduced [5] (structure **1,** Fig. **1**).

Furthermore, the recent observation by Smith *et al.* that new quinoxalin-2-ones antagonize the P-glycoprotein (Pgp) [6], prompted us to further investigate these compounds in order to: i) obtain additional data for SAR studies and, ii) establish whether also our new derivatives could behave as Pgp inhibitors. In this context, we designed two series of 1,6-dimethyl-3-phenoxymethylquinoxalin-2-ones and 1-benzyl-3-phenoxymethyl-7-trifluoromethylquinoxalin-2-ones, and a series of 2-benzyloxy-3-phenoxymethyl-7-trifluoromethylquinoxaline (structure **7a-f**, **14a-f** and **17a-b**, Fig. **1**) and evaluated whether they were able to potentiate the antiproliferative activity of doxorubicin (Doxo), vincristine (VCR) and etoposide (VP16) in human tumor derived cell lines carrying the MDR or MRP phenotype. The latter sustain energy-dependent efflux pumps, localized at the cell surface, that prevent the accumulation of certain drugs within cancer cells and are responsible for at least one of the mechanisms that confer multidrug resistance. The substrates of Pgp are a wide variety of structurally and functionally unrelated anticancer drugs, such as doxorubicin, the vinca alkaloids vincristine and vinblastine, the topoisomerase II inhibitor etoposide, to which MDR cells show a particular pattern of cross-resistance.

The *in vitro* antiproliferative activity of our derivatives was compared to that of 2-(4-benzyl-3-oxo-3,4-dihydro-quinoxalin-2-ylmethoxy)-*N*-phenylbenzamide (**2**) (Fig. **1**), the most interesting Pgp antagonist of the series reported by Smith [6], either using the drugs alone or in combination with doxorubicin, vincristine or etoposide.

CHEMISTRY

The commercially available 2-nitro-4-methylaniline (**3**) was reacted with CH_3I in anhydrous DMSO (made alkaline by KOH) to afford 2-nitro-4,*N*-dimethylaniline (**4**). Hydrogenation of **4** gave the not isolated amine (**4a**) that was condensed with an ethanolic solution of ethyl bromopyruvate

*Address correspondence to these authors at the Dipartimento Farmaco Chimico Tossicologico, *via* Muroni 23/a, 07100 Sassari-Italy; Tel: 079-228722; Fax: 079-228720; E-mail: acarta@uniss.it

Paolo La Colla, Dipartimento di Scienze e Tecnologie Biomediche, Cittadella di Monserrato, Università di Cagliari, 09042 Monserrato, Cagliari-Italy; Tel: 070-6754147; Fax: 070-6754210;
E-mail: placolla@unica.it

1573-4064/06 $50.00+.00

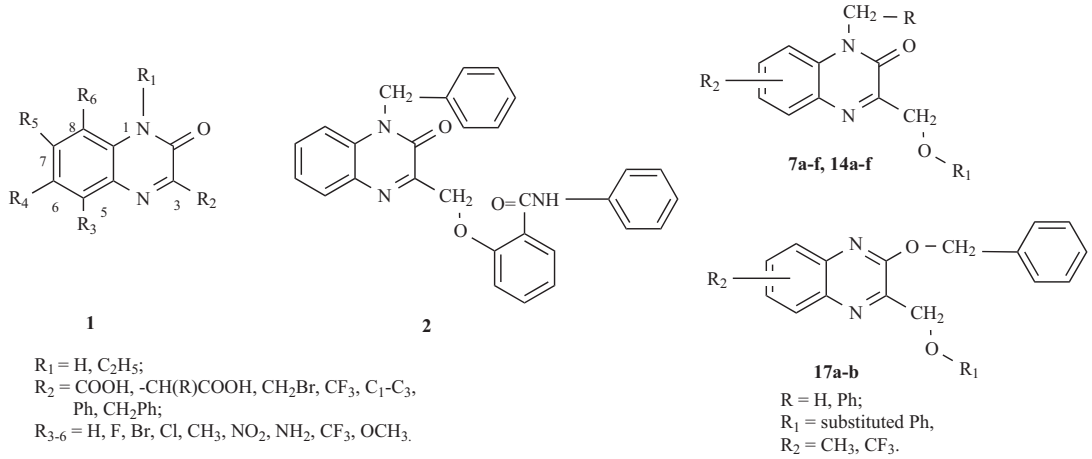
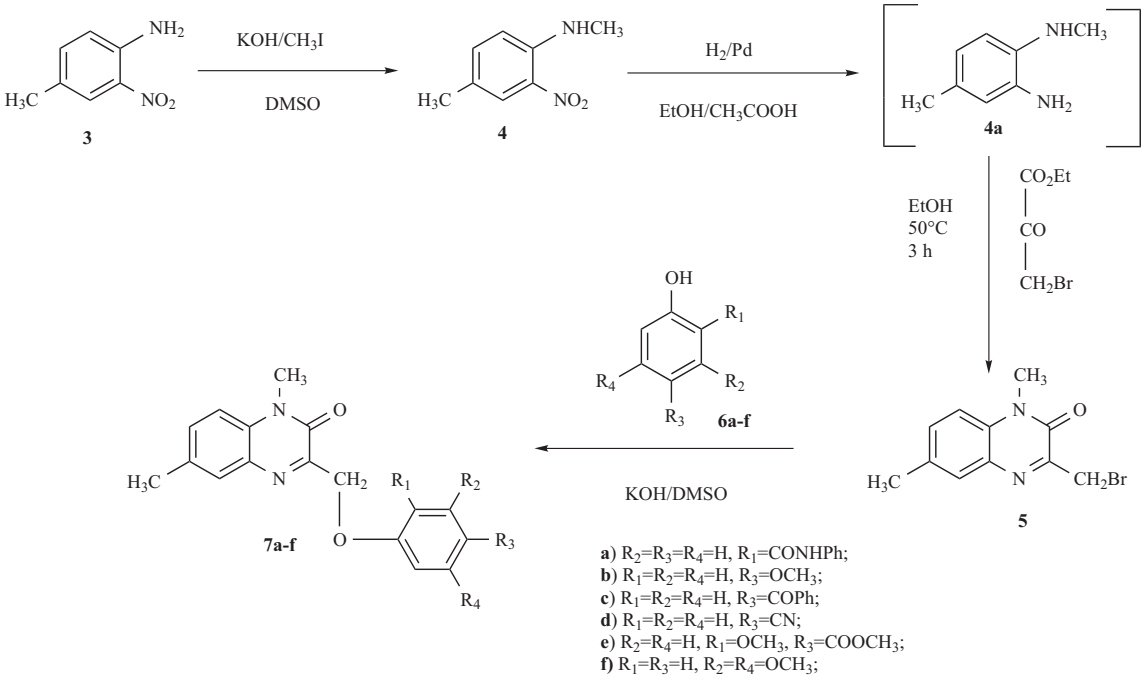


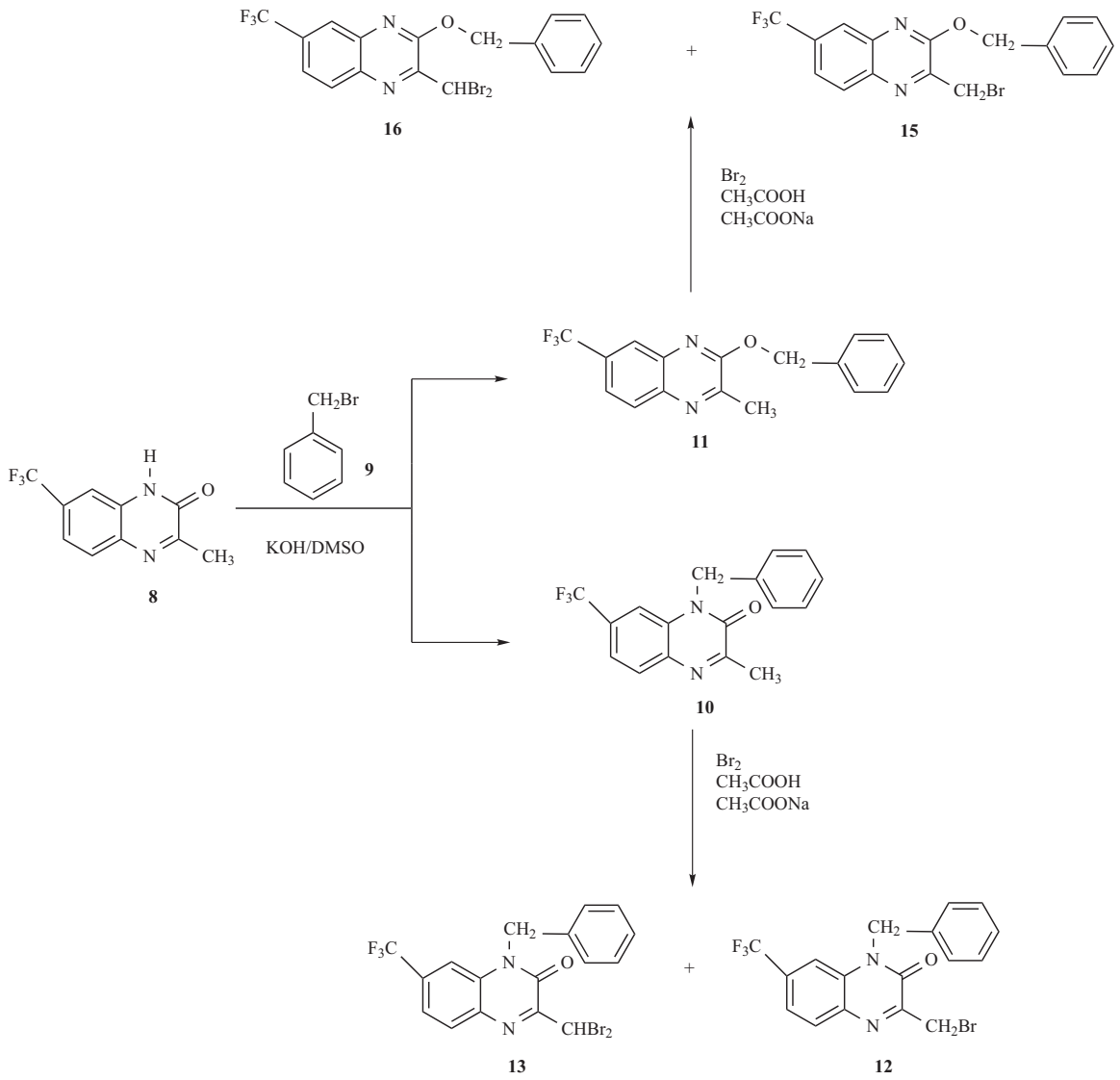
Fig. (1). Chemical structures of: quinoxalinones previously synthesized (**1**), reference compound (**2**), and new derivatives (**7a-f**, **14a-f** and **17a-b**).

to obtain the bromomethyl intermediate (**5**). Condensation of **5** with the appropriate phenol (**6a-f**), in anhydrous DMSO (made alkaline by KOH), afforded the desired 1,6-dimethyl-3-phenoxymethylquinoxalin-2-ones (**7a-f**) (Scheme **1**).

The starting compound 3-methyl-7-trifluoromethyl-quinoxalin-2-one (**8**), necessary for the synthesis of both the intermediates 1-benzyl-3-bromomethyl-7-trifluomethyl-quinoxalin-2-one (**12**) and 2-benzyloxy-3-bromomethyl-7-trifluoromethylquinoxaline (**15**), was prepared following the procedure previously described by us [7]. The latter were then submitted to benzylation with benzyl chloride in anhydrous DMF (made alkaline by KOH), to afford a mixture of 1-benzyl-3-methyl-7-trifluomethylquinoxalin-2-one (**10**) and 2-benzyloxy-3-methyl-7-trifluoromethylquinoxaline (**11**), which were separated by chromatography. Halogenation of both the intermediates **10** and **11** with bromine and CH_3COONa in glacial acetic acid afforded the desired bromomethylquinoxalinone and quinoxaline derivatives **12** and **15** accompanied with both the dibromomethyl derivative **13** and **16** respectively (Scheme **2**).



Scheme (1). Synthesis of 1,6-dimethyl-3-phenoxymethylquinoxalin-2-ones (**7a-f**).



Scheme (2). Synthesis of 1-benzyl-7-trifluoromethyl intermediate **12**, and 2-benzyloxy-7-trifluoromethyl intermediate **15**.

Finally, condensation of the bromomethyl quinoxalinone **12** with the same set of phenols (**6a-f**) and of the bromomethyl quinoxaline **15** with the phenols (**6a**,**b**), catalyzed by benzyltriethylammonium chloride (BTAC) and NaOH, afforded the desired 1-benzyl-3-phenoxymethyl-7-trifluoromethylquinoxalin-2-ones (**14a-f**) and 2-benzyloxy-3-phenoxymethyl-7-trifluoromethylquinoxaline (**17a**,**b**), respectively (Scheme **3**).

BIOLOGICAL ASSAYS

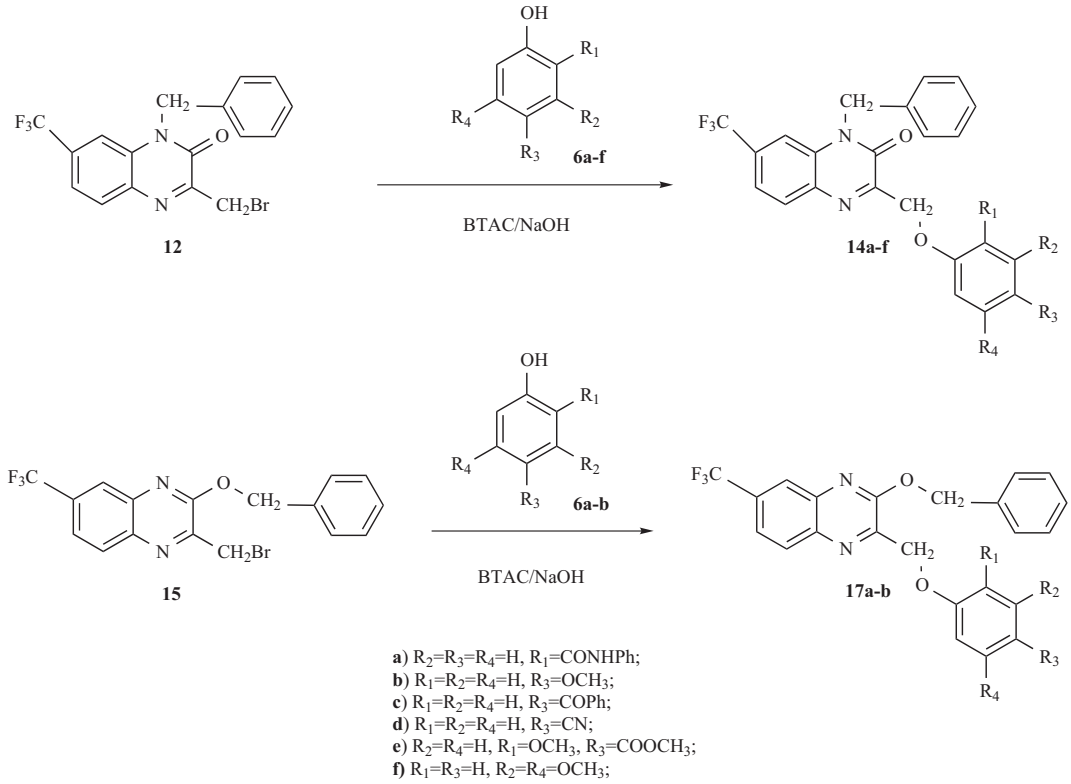
Test compounds were evaluated *in vitro* against the wild type nasopharyngeal carcinoma cell line (KB^{WT}) and the following drug resistant subclones.

(i) KB^{MDR}, obtained by transfection of wild type KB cells with a retroviral vector carrying the human MDR-1 gene and maintained under uninterrupted treatment with doxorubicin [8-9].

(ii) KB^{V20C}, selected under uninterrupted treatment with vincristine. These cells possess an MDR phenotype [10-11] related to the over-expression of the MDR-1 gene.

(iii) KB^{7D}, selected under uninterrupted treatment with etoposide, a topoisomerase II inhibitor in clinical use [12]. Their drug-resistance is due to the over-expression of the MRP gene, which codes for a membrane glycoprotein (mrp). These cells also express altered levels of topoisomerase II.

The objective was to evaluate the capability of test compounds to potentiate the antiproliferative activity of vincristine, etoposide and doxorubicin against the drug



a) $R_2=R_3=R_4=H$, $R_1=CONHPh$;
b) $R_1=R_2=R_4=H$, $R_3=OCH_3$;
c) $R_1=R_2=R_4=H$, $R_3=COPh$;
d) $R_1=R_2=R_4=H$, $R_3=CN$;
e) $R_2=R_4=H$, $R_1=OCH_3$, $R_3=COOCH_3$;
f) $R_1=R_3=H$, $R_2=R_4=OCH_3$;

Scheme (3). Synthesis of 1-benzyl-7-trifluoromethyl-3-phenoxymethylquinoxalin-2-ones (**14a**-**f**) and 2-benzyloxy-7-trifluoromethyl-3-phenoxymethylquinoxalines (**17a**,**b**).

resistant subclones. The antiproliferative activity of the reference drugs in wt and drug-resistant subclones are shown in Table **1**. Under our experimental conditions, KB^{MDR} cells express a Pgp responsible for the efflux of the structurally unrelated reference drugs, vincristine, etoposide and doxorubicin, to which they result 71-, >167- and 19-fold less susceptible than KB^{WT}. On the other hand, over-expression of the MDR-1 gene makes KB^{20C} cells 29-, 13 and 6-fold less susceptible to vincristine, etoposide and doxorubicin, respectively. Finally, over-expression of the MRP gene renders KB^{7D} cells 7-, 150- and 39-fold less susceptible to vincristine, etoposide and doxorubicin, respectively.

RESULTS AND DISCUSSION

The reference compound 2-(4-benzyl-3-oxo-3,4-dihydroquinoxalin-2-ylmethoxy)-*N*-phenylbenzamide (**2**), 1,6-dimethyl-3-phenoxymethylquinoxalin-2-ones (**7a-f**), 1-benzyl-7-trifluoromethyl-3-phenoxymethylquinoxalin-2-ones (**14a-f**), 2-benzyloxy-7-trifluoromethyl-3-phenoxymethylquinoxalines (**17a**,**b**), their bromo intermediates **5**, **12**, **13**, **15** and **16** and doxorubicin were tested for antiproliferative activity against the multidrug-resistant KB^{MDR} cells. The results, summarized in Table **1**, show that, when tested alone, the quinoxalinones **5**, **7a**, **7c**-**e** and the quinoxaline **17a** are

Table 1. Antiproliferative Activity of Vincristine, Etoposide and Doxorubicin Against KB^{WT} Nasopharyngeal Carcinoma Cells and KB^{MDR}, KB^{V20C} and KB^{7D} Subclones

Compds	[a]CC_{50} [μM]			
	KB^{WT}	KB^{MDR}	KB^{V20C}	KB^{7D}
Vincristine	0.007 ±0.0005	0.5 ±0.03 (71)	0.2 ±0.1 (29)	0.05 ±0.01 (7)
Etoposide	0.6 ±0.1	>100 (>167)	8 ±0.2 (13)	90 (150)
Doxorubicin	0.07 ±0.02	1.3 ±0.05 (19)	0.4 ±0.05 (6)	2.7 ±0.2 (39)

[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.

( ) Fold increase in resistance to antitumor drugs

totally unable to affect cell proliferation at concentrations as high as 100 μM.

Compound **2** and about half of the newly synthesized derivatives show CC_{50}s ranging between 56 and 13 μM, whereas only the compounds **7b**, **7f** and **16** show antiproliferative activity in the low μM range (5 μM). When used alone, doxorubicin shows a CC_{50} of 1.3 μM, whereas it is 3-fold more potent when used in combination with a 1 μM concentration of the reference compound **2** or of the quinoxaline **17a**. Interestingly, when doxorubicin is used in combination with a 10 μM concentration of **2** or **17a**, it turns out to be 33- and 16-fold more potent than when used alone, respectively, very likely as a consequence of the Pgp inhibition exerted by the quinoxaline derivatives. When used at 10 μM, most of the other title compounds are substantially unable to potentiate the antiproliferative activity of doxorubicin against KB^{MDR} cells.

When doxorubicin is used in combination with a 100 μM concentration of the test compounds unable to affect per se the cell proliferation, the most convincing doxorubicin potentiators, in terms of absolute efficacy / dose-response effect, are, in addition to quinoxaline **17a**, the quinoxalin-2-one derivatives **7c**, **7d, 7e** and the bromo intermediate **5**.

Compounds **2** and **17a**, which were the most effective derivatives in potentiating the antiproliferative activity of doxorubicin, were also tested against the KB^{V20C} and KB^{7D} subclones, which over-express the MDR-1 and MRP genes, respectively, thus mediating the efflux of the reference

Table 2. Antiproliferative Activity, in KB^{MDR} Cells, of the Reference Compound 2, Quinoxalin-2-Ones (5, 7a-f, 12,13, 14a-f) and Quinoxalines (15, 16 and 17a,b), Alone and in Combination with Doxorubicin

Test Compds	[a][CC_{50}]			
	*TC alone	Doxorubicin in combination with		
		*TC 1 μM	*TC 10 μM	*TC 100 μM
2	28	0.5 (3)	0.04 (33)	-
5	>100	0.9	0.5 (3)	<0.02 (>65)
7a	>100	1.1	0.6 (3)	0.09 (14)
7b	5	1.0	-	
7c	>100	1.0	0.2 (7)	0.04 (33)
7d	>100	1.2	0.6 (2)	<0.02 (>65)
7e	>100	1.3	0.7 (2)	0.05 (26)
7f	4	1.1	-	-
12	30	0.8	0.5 (3)	-
13	13	1.2	1.3	-
14a	18	1.0	0.3 (4)	-
14b	56	1.2	0.6 (2)	-
14c	33	1.3	0.7 (2)	-
14d	23	1.2	0.6 (2)	-
14e	32	1.0	0.5 (3)	-
14f	13	1.2	-	-
15	27	1.3	0.6 (2)	-
16	5	1.3	-	-
17a	>100	0.5 (3)	0.08 (16)	<0.02 (>65)
17b	36	0.9	0.4 (3)	-
Doxorubicin	1.3			

[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 for three independent determinations. Variation among triplicate samples was less than 15%.

*TC = Test compounds

( ) Fold increase in susceptibility to doxorubicin

Table 3. Antiproliferative Activity, in KB^{V20C} Cells, of the Reference Compound 2 and of the Quinoxaline 17a, Alone and in Combination with Vincristine (VCR)

Test Compds	[a][CC_{50}]			
	[*]TC alone	Vincristine in combination with		
		[*]TC 0.8 μM	[*]TC 4 μM	[*]TC 20 μM
2	>100	0.02 (10)	0.003 (67)	0.001 (200)
17a	>100	0.09 (2)	0.01 (20)	0.004 (50)
Vincristine	0.2			

[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 for three independent determinations. Variation among triplicate samples was less than 15%.

[*]**TC** = Test compounds

( ) Fold increase in susceptibility to vincristine

antitumor drugs. The results show that both **2** and **17a** potentiate the activity of vincristine against KB^{V20C} cells, in a clear dose-dependent manner (Table **3**).

On the contrary, both the compounds **2** and **17a** are ineffective in potentiating the antiproliferative activity of etoposide in KB^{7D} cells, even when used at 100 μM (Table **4**). The latter result is likely due to the fact that the KB^{7D} subclone has a membrane glycoprotein (MRP) different from that of the KB^{V20C} subclone bearing the MDR phenotype.

It is noteworthy that, when used alone, both the compounds **2** and **17a** were devoid of antiproliferative activity against KB^{V20C} and KB^{7D} subclones, whereas the reference compound **2** showed antiproliferative activity against the KB^{MDR} subclone. Therefore, it was interesting to check in a single experiment the antiproliferative activity of the two quinoxaline derivatives against the three KB subclones, KB^{WT} and MT-4 cells. The latter, according to our experience, are among the cell lines most susceptible to the cytotoxicity exerted by numerous compounds through a variety of mechanisms. As shown in Table **5**, the quinoxaline **17a** turned out devoid of antiproliferative activity against all cell lines, whereas the quinoxalinone **2** is somewhat inhibitory to the multiplication of both KB^{MDR} and MT-4 cells. The reason for these results is not immediately apparent.

Similarly elusive remains the correlation between the structure of the various compounds and their different capability to affect the KB^{MDR} proliferation, when used alone. In fact, with the exception of the phenylmethoxy quinoxalinones **7b** and **7f** (CC_{50} = 5 μM), the others 6-methyl quinoxalinones (**5**, **7a, 7c-e**) are unable to affect cell proliferation at concentrations as high as 100 μM, whereas, with the exception of **17a**, the others 7-trifluoromethyl derivatives (**12**, **13**, **14a-f** and **17b**) show CC_{50} values in the range 13-56 μM.

As far as it concerns the potentiating effect of our compounds on the reference antitumor drugs used in the experiments, we must point out a similar activity of the quinoxaline **17a** in comparison with the reference compound **2**, but our compound showed to be much less toxic. Structure-activity relationship (SAR) put in evidence that the shift of benzyl group from N to O associated with the introduction of a CF_3 at position 7 contributes to maintain the activity but lowering the toxicity. On the other hand, comparing the reference compound **2** with the our compounds (**7a-f**, **14a-f** and **17a-b**) we have observed that the sole introduction of CF_3 at position 7 (compare **2** and **14a**), or the simultaneous introduction of a methyl group at position 6 and the substitution of a benzyl with a methyl group on the N atom (compare **2** and **7a-f**), is not sufficient

Table 4. Antiproliferative Activity, in KB^{7D} Cells, of the Reference Compound 2 **and of the Quinoxaline** 17a, **Alone and in Combination with Etoposide**

Test Compds	[a][CC_{50}]			
	[*]TC alone	Etoposide in combination with		
		[*]TC 0.8 μM	[*]TC 4 μM	[*]TC 20 μM
2	>100	86	60	55
17a	>100	>100	>100	>100
Etoposide	90			

[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 for three independent determinations. Variation among triplicate samples was less than 15%.

[*]**TC** = Test compounds

Table 5. Antiproliferative Activity of the Reference Compound 2 and of the Quinoxaline Against KB^{WT} Nasopharyngeal Carcinoma cells, KB^{MDR}, KB^{V20C} and KB^{7D} Subclones and MT-4 Cells

Compds	[a][CC_{50}]				
	KB^{WT}	KB^{MDR}	KB^{V20C}	KB^{7D}	MT-4
2	>100	28	>100	>100	16
17a	>100	>100	>100	>100	>100

[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 for three independent determinations. Variation among triplicate samples was less than 15%.

to maintain the activity. Noteworthy, substitution of 2-CONHPh with 4-OCH_3 leads to loss of biological activity (compare **17a** and **17b**).

In conclusion, the quinoxaline derivative **17a** proves to be at least as active as the reference compound **2** in inhibiting the activity of Pgp, thus restoring the antiproliferative activity of doxorubicin and vincristine against KB^{MDR} and KB^{V20C} cell lines, respectively. For this reason **17a** can be considered a new lead compound, which could be further investigated to optimize its activity. SAR studies could suggest that a bioisosteric replacement of oxygen atom with both sulfur and NH might be hopefully considered. On the other hand the phenyl moiety would be easily replaced with more complex aryl or heteroaryl group, thus allowing to observe the influence on the biological activity of more or less flexible substituents.

EXPERIMENTAL SECTION

Chemistry

Melting points were determined by a Kofler hot stage or Digital Electrothermal apparatus, and are uncorrected. IR spectra were recorded as nujol mulls on a Perkin Elmer 781 spectrophotometer and are expressed in cm^{-1}. UV spectra are qualitative and were recorded in nm for ethanol solution with a Perkin-Elmer Lambda 5 spectrophotometer. ^1H NMR spectra were recorded on a Varian XL-200 (200 MHz) instrument, using TMS as internal standard. The chemical shift values are reported in ppm (δ) and coupling constants (*J*) in Hertz (Hz). Signal multiplicities are represented by: s (singlet), d (doublet), dd (double doublet), m (multiplet), and t (triplet). MS spectra were performed on combined HP 5790-HP 5970 GC/MS apparatus or with a combined Liquid Chromatograph-Agilent 1100 series Mass Selective Detector (MSD). Column chromatography was performed on silica gel (Merck 60, 70–230 mesh). The R_f values , and the progress of the reactions, were measured on aluminium backed TLC plates of silica gel 60 F254 (Merck, 0.2 mm) with the indicated eluent. Light petroleum refers to the fraction with bp 40-60 °C. The analytical results for C, H, N, were within ± 0.4 % of the theoretical values.

Intermediates

2-nitro-4-methylaniline (**3**), ethyl bromopyruvate, phenols (**6a-f**) and benzyl bromide (**9**) were commercially available, 7-trifluoromethyl-3-methylquinoxalin-2-one (**8**) was prepared following the procedure previously by us described [7]. 2-nitro-4,*N*-dimethylaniline (**4**), quinoxalinone and quinoxaline intermediates (**5**, **10**, **11**, **12** and **15)** were prepared following the procedure described below.

Preparation of 2-nitro-4,N-dimethylaniline (4)

1.0 g (6.6 mmol) of 2-nitro-4-methylaniline (**3**) was added to a solution of g 1.48 (26.4 mmol) of KOH in anhydrous DMSO (15 ml) and stirred at room temperature for 45 min. After that, the reaction mixture was cooled with an external ice bath and slowly added dropwise of g 0.82 (13.2 mmol) of CH_3I. After the addition was complete, the stirring was continued for an additional 45 min and then the reaction mixture was diluted with 100 mL of water. The resulting precipitate was filtered off, washed with water and dried, to afford the desired 2-nitro-4,N-dimethylaniline (**4**) as crystals in 95% yield, m. p. 78 °C (diethyl ether), TLC (diethyl ether-light petroleum 8:2): R_f 0.70; IR (nujol): ν 3400, 1640, 1570 cm^{-1}; UV (EtOH): λ_{max} 202, 234, 283, 442 nm; ^1H NMR ($CDCl_3$): δ 7.97 (1H, s, H-3), 7.31 (1H, d, J = 8.4 Hz, H-5), 6.75 (1H, d, J = 8.4 Hz, H-6), 3.01 (3H, d, J = 5.0 Hz, N-CH_3), 2.27 (3H, s, 4-CH_3). MS: *m/z* 166 (M^+); *Anal*. $C_8H_{10}N_2O_2$ (C,H,N).

Preparation of 3-bromomethyl-1,6-dimethylquinoxalin-2-one (5)

A solution of **3** (3.0 g, 19.8 mmol) in ethanol (100 mL) and acetic acid (2.5 mL) in the presence of 10% palladium-charcoal (0.30 g) was hydrogenated under 3 atm at room temperature for 1 h. After filtration of the catalyst, the mother liquors were evaporated to dryness to give 2.5 g of oily residue. The oil (intermediate **4a**) was added to a stirring solution of 3.5 g (18 mmol) of ethyl bromopyruvate in ethanol (20 mL) and the resulting mixture was stirred at 50°C for 3h. On cooling to room temperature a crude precipitate was formed and collected by filtration. The crystallization (diethyl ether) of the obtained solid afforded 1.8 g (56%) of **5**, m. p. 183-185 °C; TLC (diethyl ether-light petroleum 8:2): R_f 0.50; IR (nujol): ν 1640 cm^{-1}; UV (EtOH): λ_{max} 208, 236, 292, 364 nm; ^1H NMR ($CDCl_3$): δ 7.67 (1H, d, J = 1.4 Hz, H-5), 7.42 (1H, dd, J = 8.6 and 1.4, H-7), 7.23 (1H, d, J = 8.6, H-8), 4.66 (2H, s, CH_2-Br), 3.72 (3H, s, N-CH_3), 2.46 (3H, s, 6-CH_3). LC/MS: 267 (M+H), 269 (M+H), 289 (M+Na), 291 (M+Na); *Anal*. $C_{11}H_{11}BrN_2O$ (C,H,N).

Preparation of 1-benzyl-3-methyl-7-trifluoromethylquinoxalin-2-one (10) and 2-benzyloxy-3-methyl-7-trifluoromethylquinoxaline (11)

A mixture of equimolar amounts (8.8 mmol) of **8** [7] and benzyl chloride (**9**) in anhydrous DMA (30 ml) was added,

under stirring at room temperature, of 2.5 g (18 mmol) of KOH and the stirring was continued overnight. The mixture was then added to 100 mL of water and the crude precipitate formed was purified by chromatography on silica gel column, eluting with a 9:1 mixture of light petroleum/ethyl acetate. The quinoxaline derivative (**11**) moved faster in the eluate than the quinoxalinone derivative (**10**). Melting points, yields, analytical and spectroscopical data are reported below.

2-benzyloxy-3-methyl-7-trifluoromethylquinoxaline (**11**) was obtained in 27% yield; m. p. 108-110 °C (diethyl ether); TLC (light petroleum-ethyl acetate 95:5): R_f 0.61; IR (nujol): ν 1600 cm^{-1};UV (EtOH): λ_{max} 206, 244, 316, 332 nm; ^{1}H NMR ($CDCl_3$): δ 8.14 (1H, d, J = 1.6 Hz, H-8), 8.04 (1H, d, J = 8.6, H-5), 7.33 (1H, dd, J = 8.6 and 1.4, H-6), 7.56-7.50 (2H, m, H-2' + H-6'), 7.45-7.39 (3H, m, H-3' + H-4' + H-5'), 5.58 (2H, s, CH_2), 2.71 (3H, s, CH_3). LC/MS: 319 (M+H); *Anal.* $C_{17}H_{13}F_3N_2O$ (C,H,N).

1-benzyl-3-methyl-7-trifluoromethylquinoxalin-2-one (**10**) was obtained in 70% yield; m. p. 101-102 °C (diethyl ether); TLC (light petroleum-ethyl acetate 95:5): R_f 0.33; IR (nujol): ν 1664, 1612 cm^{-1}; UV (EtOH): λ_{max} 208, 230, 274, 332 nm; ^{1}H NMR ($CDCl_3$): δ 7.92 (1H, d, J = 8.8 Hz, H-5), 758-7.46 (2H, m, 2 aromatic-H), 758-7.46 (5H, m, 5 aromatic-H), 5.51 (2H, s, CH_2), 2.69 (3H, s, CH_3). LC/MS: 319 (M+H), 341 (M+Na), 357 (M+K); *Anal.* $C_{17}H_{13}F_3N_2O$ (C,H,N).

Preparation of 1-benzyl-3-bromomethyl-7-trifluromethyl-quinoxalin-2-one (12), 1-benzyl-3-dibromomethyl-7-trifluoromethylquinoxalin-2-one (13), 2-benzyloxy-3-bromomethyl-7-trifluromethylquinoxaline (15) and 2-benzyloxy-3-dibromomethyl-7-trifluoromethylquinoxaline (16)

To a stirred suspension of 1-benzyl-3-methyl-7-trifluomethylquinoxalin-2-one (**10**) or 2-benzyloxy-3-methyl-7-trifluoromethylquinoxaline (**11**) (4.7 mmol) and 0.33 g (4.0 mmol) of CH_3COONa in glacial acetic acid (28 mL), 0.75 g (4.7 mmol) of bromine were added dropwise. The reaction mixture was then heated to 80-90 °C for 50 min. On cooling to r.t., the acetic acid was removed *in vacuo* and the crude residue obtained was triturated with chloroform (3 X 20 mL). The chloroform extracts were dried over $MgSO_4$, filtered and evaporated. The crude solid obtained was chromatographed on silica gel column (eluent: 9:1 mixture of light petroleum/ethyl acetate for the mixture of **12**/**13** and 98:2 for the mixture of **15**/**16**). The *di*bromo derivatives (**13** and **16**) moved faster in the eluate than the *mono*bromo derivative (**12** and **15**). Melting points, yields, analytical and spectroscopical data are reported below.

1-benzyl-3-dibromomethyl-7-trifluoromethylquinoxalin-2-one (**13**) was obtained in 40% yield; m. p. 103-105 °C (diethyl ether); TLC (light petroleum-ethyl acetate 9:1): R_f 0.46; IR (nujol): ν 1650, 1610 cm^{-1}; UV (EtOH): λ_{max} 208, 234, 248, 356 nm; ^{1}H NMR ($CDCl_3$): δ 8.11 (1H, d, J = 8.6 Hz, H-5), 7.64-7.52 (2H, m, 2 aromatic-H), 7.38-7.24 (5H, m, 5 aromatic-H), 7.21 (1H, s, $CHBr_2$), 5.54 (2H, s, CH_2). LC/MS: 498 (M+Na), 500 (M+Na); *Anal.* $C_{17}H_{11}Br_2F_3N_2O$ (C,H,N).

1-benzyl-3-bromomethyl-7-trifluoromethylquinoxalin-2-one (**12**) was obtained in 60% yield; m. p. 136-138 °C (diethyl ether); TLC (light petroleum-ethyl acetate 9:1): R_f 0.35; IR (nujol): ν 1660, cm^{-1}; UV (EtOH): λ_{max} 208, 232, 284, 348 nm; ^{1}H NMR ($CDCl_3$): δ 7.98 (1H, d, J = 8.8 Hz, H-5), 7.60-7.48 (2H, m, 2 aromatic-H), 7.38-7.26 (5H, m, 5 aromatic-H), 5.53 (2H, s, N-CH_2), 4.71 (2H, s, CH_2Br). LC/MS: 419 (M+Na), 421 (M+Na); *Anal.* $C_{17}H_{12}BrF_3N_2O$ (C,H,N).

2-benzyloxy-3-dibromomethyl-7-trifluoromethylquinoxaline (**16**) was obtained in 27% yield; m. p. 126-127 °C (diethyl ether); TLC (light petroleum-ethyl acetate 9:1): R_f 0.68; (nujol): ν 1580, cm^{-1}; UV (EtOH): λ_{max} 208, 232, 244, 284, 334 nm; ^{1}H NMR ($CDCl_3$): δ 8.25-8.16 (2H, m, H-5 + H-8), 7.81 (1H, dd, J = 8.2 and 1.6 Hz, H-6), 7.60-7.56 (2H, m, H-2' + H-6'), 7.55-7.32 (3H, m, H-3' + H-4'+ H-5'), 7.07 (1H, s, $CHBr_2$), 5.66 (2H, s, CH_2). LC/MS: 498 (M+Na), 500 (M+Na); *Anal.* $C_{17}H_{11}Br_2F_3N_2O$ (C,H,N).

2-benzyloxy-3-bromomethyl-7-trifluromethylquinoxaline (**15**) was obtained in 40% yield; m. p. 122-123 °C (diethyl ether); TLC (light petroleum-ethyl acetate 9:1): R_f 0.52; IR (nujol): ν 1580, cm^{-1}; UV (EtOH): λ_{max} 210, 232, 244, 290, 342 nm; ^{1}H NMR ($CDCl_3$): δ 8.16 (1H, s, H-8), 8.10 (1H, d, J = 8.6 Hz, H-5), 7.76 (1H, dd, J = 8.6 and 1.6 Hz, H-6), 7.60-7.50 (2H, m, H-2' + H-6'), 7.50-7.30 (3H, m, H-3' + H-4'+ H-5'), 5.64 (2H, s, N-CH_2), 4.73 (2H, s, CH_2Br). LC/MS: 397 (M+H), 399 (M+H), 397 (M+Na), 399 (M+Na); *Anal.* $C_{17}H_{12}BrF_3N_2O$ (C,H,N).

General procedure for preparation of 1,6-dimethyl-3-phenoxymethylquinoxalin-2-ones (7a-f)

To a stirred solution of 2.3 mmol of KOH in 10 mL of DMF anhydrous, 1.9 mmol of the opportune phenol (**6a-f**) were added and the stirring was continued, at r.t., for 30 min. After that, 1.9 mmol of 3-bromomethyl-1,6-dimethyl-quinoxalin-2-one (**5**) were added and the stirring continued for 24 h. The reaction mixture was then diluted with water (100 mL) and the crude precipitate obtained was washed, collected by filtration and crystallized (ethanol/water) to afford the desired (**7a-f**). Melting points, yields, analytical and spectroscopical data are reported below.

1,6-dimethyl-3-(2-benzamidephenoxymethyl)quinoxalin-2-one (**7a**) was obtained in 70% yield; m. p. 198-200 °C (methanol); TLC (diethyl ether-light petroleum 8:2): R_f 0.34; IR (nujol): ν 3320, 1640, 1600 cm^{-1}; UV (EtOH): λ_{max} 206, 232, 280, 352 nm; ^{1}H NMR (DMSO-d_6): δ 10.72 (1H, s, NH), 7.98 (1H, d, J = 8.2 Hz, H-7), 7.72-7.15 (11H, m, 11 aromatic-H), 5.56 (2H, s, CH_2), 3.65 (3H, s, N-CH_3), 2.29 (3H, s, 6-CH_3). LC/MS: 400 (M+H), 422 (M+Na), 423 (M+Na+H); *Anal.* $C_{24}H_{21}N_3O_3$ (C,H,N).

1,6-dimethyl-3-(4-methoxyphenoxymethyl)quinoxalin-2-one (**7b**) was obtained in 55% yield; m. p. 117-119 °C (methanol); TLC (diethyl ether-light petroleum 8:2): R_f 0.42; IR (nujol): ν 1640, 1600 cm^{-1}; UV (EtOH): λ_{max} 204, 230, 286, 352 nm; ^{1}H NMR ($CDCl_3$): δ 7.76 (1H, s, H-5), 7.40 (1H, d, J = 8.2 Hz, H-7), 7.24 (1H, d, J = 8.2 Hz, H-8), 7.03 (2H, d, J = 9.0 Hz, H-2' + H-6'), 6.83 (2H, d, J = 9.0 Hz, H-3' + H-5'), 5.31 (2H, s, CH_2), 3.77 (3H, s, OCH_3), 3.72 (3H,

s, N-CH_3), 2.45 (3H, s, 6-CH_3). LC/MS: 311 (M+H), 333 (M+Na), 349 (M+K); *Anal*. $C_{18}H_{18}N_2O_3$ (C,H,N).

1,6-dimethyl-3-(4-benzoylphenoxymethyl)quinoxalin-2-one (**7c**) was obtained in 42% yield; m. p. 142-144 °C (methanol); TLC (diethyl ether-light petroleum 8:2): R_f 0.32; IR (nujol): ν 1640, 1600 cm^{-1}; UV (EtOH): λ_{max} 208, 232, 292, 352 nm; ^{1}H NMR ($CDCl_3$): δ 7.83 (2H, d, *J* = 9.0 Hz, H-3' + H-5'), 7.77-7.70 (3H, m, 3 aromatic-H), 7.57-7.28 (5H, m, 5 aromatic-H), 7.13 (2H, d, *J* = 9.0 Hz, H-2' + H-6'), 5.45 (2H, s, CH_2), 3.74 (3H, s, N-CH_3), 2.46 (3H, s, 6-CH_3). LC/MS: 385 (M+H, 407 (M+Na), 423 (M+K); *Anal*. $C_{24}H_{20}N_2O_3$ (C,H,N).

1,6-dimethyl-3-(4-cianophenoxymethyl)quinoxalin-2-one (**7d**) was obtained in 91% yield; m. p. 62-64 °C (methanol); TLC (diethyl ether-light petroleum 8:2): R_f 0.31; IR (nujol): ν 2215, 1645, 1600 cm^{-1}; UV (EtOH): λ_{max} 208, 236, 280, 352 nm; ^{1}H NMR ($CDCl_3$): δ 7.69 (1H, d, *J* = 1.8 Hz, H-5), 7.58 (2H, d, *J* = 9.0 Hz, H-3' + H-5'), 7.44 (1H, dd, *J* = 8.4 and 1.8 Hz, H-7), 7.25 (1H, d, *J* = 8.4 Hz, H-8), 7.01 (2H, d, *J* = 9.0 Hz, H-2' + H-6'), 5.41 (2H, s, CH_2), 3.74 (3H, s, N-CH_3), 2.45 (3H, s, 6-CH_3). LC/MS: 306 (M+H), 328 (M+Na), 344 (M+K); *Anal*. $C_{18}H_{15}N_3O_2$ (C,H,N).

1,6-dimethyl-3-(4-carbometoxy-2-methoxyphenoxymethyl) quinoxalin-2-one (**7e**) was obtained in 84% yield; m. p. 268-270 °C (methanol); TLC (diethyl ether-light petroleum 8:2): R_f 0.18; IR (nujol): ν 1720, 1645, 1600 cm^{-1}; UV (EtOH): λ_{max} 208, 259, 290, 356 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 8.22 (1H, s, H-3'), 7.57 (1H, s, H-5), 7.51-7.46 (3H, m, H-7 + H-8 + H-5'), 7.09 (1H, d, *J* = 8.4 Hz, H-6'), 5.35 (2H, s, CH_2), 3.86 (3H, s, $COOCH_3$), 3.83 (3H, s, OCH_3), 3.67 (3H, s, N-CH_3), 2.41 (3H, s, 6-CH_3). LC/MS: 369 (M+H), 407 (M+K); *Anal*. $C_{20}H_{20}N_2O_5$ (C,H,N).

1,6-dimethyl-3-(3,5-dimethoxyphenoxymethyl)quinoxalin-2-one (**7f**) was obtained in 66% yield; m. p. 115-117 °C (methanol); TLC (diethyl ether-light petroleum 8:2): R_f 0.41; IR (nujol): ν 1645, 1600 cm^{-1}; UV (EtOH): λ_{max} 208, 230, 284, 352 nm; ^{1}H NMR ($CDCl_3$): δ 7.76 (1H, d, *J* = 1.8 Hz, H-5), 7.41 (1H, dd, *J* = 8.4 and 1.8 Hz, H-7), 7.23 (1H, d, *J* = 8.4 Hz, H-8), 6.28 (2H, d, *J* = 2.0 Hz, H-2' + H-6'), 6.11 (1H, d, *J* = 2.0 Hz, H-4'), 5.33 (2H, s, CH_2), 3.75 (6H, s, 2 OCH_3), 3.65 (3H, s, N-CH_3), 2.45 (s, 3H, 6-CH_3). LC/MS: 341 (M+H), 363 (M+Na); *Anal*. $C_{19}H_{20}N_2O_4$ (C,H,N).

General procedure for preparation of 1-benzyl-3-phenoxymethyl-7-trifluoromethylquinoxalin-2-ones (14a-f) and 2-benzyloxy-3-phenoxymethyl-7-trifluoromethyl-quinoxaline (17a,b)

To a stirred mixture of equimolar amounts of 1-benzyl-3-bromomethyl-7-trifluoromethylquinoxalin-2-one (**12**) and the suitable phenol (**6a-f**) (0.75 mmol), or of 2-benzyloxy-3-bromomethyl-7-triflurometylquinoxaline (**15**) with **6a**,**b**, in chloroform (10 mL), a solution of 1.12 mmol of NaOH and 0.075 mmol of benzyltriethylammonium chloride (BTAC) in 10 mL of water was added dropwise. The reaction mixture was then heated to 50 °C and the stirring continued for 10-40 h, as reported below. On cooling to r.t., the mother liquors were extracted with chloroform (3 X 20 mL). The chloroform extracts were dried over $MgSO_4$, filtered and evaporated. The crude solid obtained was purified by chromatography on silica gel column (eluent: 7:3 mixture of light petroleum/ethyl acetate) or by crystallization (methanol). Reaction times, melting points, yields, analytical and spectroscopical data are reported below.

1-benzyl-7-trifluoromethyl-3-(2-benzamidephenoxymethyl) quinoxalin-2-ones (**14a**) was obtained in 96% yield after 10 h under reflux; m. p. 176-178 °C (methanol); TLC (light petroleum-ethyl acetate 8:2): R_f 0.62; IR (nujol): ν 1660, 1600 cm^{-1}; UV (EtOH): λ_{max} 208, 230, 282, 340 nm; ^{1}H NMR ($CDCl_3$): δ 10.76 (1H, s, NH), 8.37 (1H, dd, *J* = 8.2 and 1.8 Hz, H-6), 7.75 (1H, d, *J* = 8.2 Hz, H-5), 7.62-7.14 (15H, m, 15 aromatic-H), 5.68 (2H, s, CH_2O), 5.55 (2H, s, N-CH_2). LC/MS: 552 (M+Na); *Anal*. $C_{30}H_{22}F_3N_3O_3$ (C,H,N).

1-benzyl-7-trifluoromethyl-3-(4-methoxyphenoxymethyl) quinoxalin-2-ones (**14b**) was obtained in 52% yield after 40 h under reflux; m. p. 145-147 °C (methanol); TLC (light petroleum-ethyl acetate 7:3): R_f 0.64; IR (nujol): ν 1650, 1600 cm^{-1}; UV (EtOH): λ_{max} 206, 230, 284, 340 nm; ^{1}H NMR ($CDCl_3$): δ 8.08 (1H, d, *J* = 8.0 Hz, H-6), 7.58-7.50 (2H, m, 2 aromatic-H), 7.36-7.26 (5H, m, 5 aromatic-H), 7.05 (2H, d, *J* = 8.6 Hz, H-2' + H-6'), 6.85 (2H, d, *J* = 8.6 Hz, H-3' + H-5'), 5.53 (2H, s, CH_2O), 5.40 (2H, s, N-CH_2), 3.78 (3H, s, CH_3O). LC/MS: 463 (M+Na), 479 (M+K); *Anal*. $C_{24}H_{19}F_3N_2O_3$ (C,H,N).

1-benzyl-7-trifluoromethyl-3-(4-benzoylphenoxymethyl) quinoxalin-2-ones (**14c**) was obtained in 57% yield after 20 h under reflux; m. p. 161-163 °C (methanol); TLC (light petroleum-ethyl acetate 7:3): R_f 0.55; IR (nujol): ν 1660 cm^{-1}; UV (EtOH): λ_{max} 208, 228, 284, 300 nm; ^{1}H NMR ($CDCl_3$): δ 8.05 (1H, d, *J* = 8.6 Hz, H-6), 7.85 (2H, d, *J* = 8.6 Hz, H-3' + H-5'), 7.78-7.75 (5H, m, 5 aromatic-H), 7.33-7.26 (5H, m, 5 aromatic-H), 7.15 (2H, d, *J* = 8.6 Hz, H-2' + H-6'), 5.54 (4H, s, CH_2O + N-CH_2). LC/MS: 515 (M+H); *Anal*. $C_{10}H_{14}ClN_3O$ (C,H,N).

1-benzyl-7-trifluoromethyl-3-(4-cianophenoxymethyl) quinoxalin-2-ones (**14d**) was obtained in 36% yield after 20 h under reflux; m. p. 144-146 °C (methanol); TLC (light petroleum-ethyl acetate 7:3): R_f 0.53; IR (nujol): ν 2220, 1660 cm^{-1}; UV (EtOH): λ_{max} 206, 236, 272, 338 nm; ^{1}H NMR ($CDCl_3$): δ 8.02 (1H, d, *J* = 8.6 Hz, H-6), 7.62-7.54 (4H, m, H-5 + H-8 + H-3' + H-6'), 7.30-7.26 (5H, m, 5 benzylic-H), 7.13 (2H, d, *J* = 8.8 Hz, H-2' + H-6'), 5.53 (2H, s, CH_2O), 5.50 (2H, s, N-CH_2). LC/MS: 436 (M+H), 458 (M+Na); *Anal*. $C_{24}H_{16}F_3N_3O2$ (C,H,N).

1-benzyl-7-trifluoromethyl-3-(4-carbomethoxy-2-methoxy-phenoxymethyl)quinoxalin-2-ones (**14e**) was obtained in 30% yield after 20 h under reflux; m. p. 114-116 °C (methanol); TLC (light petroleum-ethyl acetate 7:3): R_f 0.39; IR (nujol): ν 1720, 1650 cm^{-1}; UV (EtOH): λ_{max} 208, 258, 290, 340 nm; ^{1}H NMR ($CDCl_3$): δ 8.04 (1H, d, *J* = 8.0 Hz, H-6), 7.60-7.51 (4H, m, H-5 + H-8 + H-3' + H-5'), 7.38-7.26 (5H, m, 5 benzylic-H), 7.00 (1H, d, *J* = 8.0 Hz, H-6'), 5.57 (2H, s, CH_2O), 5.53 (2H, s, N-CH_2), 3.98 (3H, s, $COOCH_3$), 3.90 (3H, s, CH_3O). LC/MS: 521 (M+Na); *Anal*. $C_{26}H_{21}F_3N_2O_5$ (C,H,N).

1-benzyl-7-trifluoromethyl-3-(3,5-dimethoxyphenoxy-methyl)quinoxalin-2-ones (**14f**) was obtained in 77% yield after 10 h under reflux; m. p. 52-53 °C (methanol); TLC (light petroleum-ethyl acetate 7:3): R_f 0.59; IR (nujol): ν

1650, 1600 cm^{-1}; UV (EtOH): λ_{max} 210, 230, 280, 344 nm; ^1H NMR ($CDCl_3$): δ 8.07 (1H, d, *J* = 8.0 Hz, H-6), 7.60-7.54 (2H, m, H-5 + H-8), 7.40-7.24 (5H, m, 5 benzylic-H), 6.28 (2H, s, H-2' + H-6'), 6.13 (1H, s, H-4'), 5.53 (2H, s, CH_2O), 5.42 (s, 2H, N-CH_2), 3.77 (3H, s, CH_3O), 3.75 (3H, s, CH_3O). LC/MS: 493 (M+K); *Anal*. $C_{25}H_{21}F_3N_2O_4$

2-benzyloxy-3-(2-benzamidephenoxymethyl)-7-trifluoromethylquinoxaline (**17a**) was obtained in 96% yield after 10 h under reflux; m. p. 161-162 °C (methanol); TLC (light petroleum-ethyl acetate 9:1): R_f 0.13; IR (nujol): ν 3320, 1660 cm^{-1}; UV (EtOH): λ_{max} 212, 246, 269, 336 nm; ^1H NMR ($CDCl_3$): δ 10.80 (1H, s, NH), 8.35 (1H, dd, *J* = 8.0 and 2.0 Hz, H-6), 8.21 (1H, s, H-8), 7.86 (1H, d, *J* = 8.0 Hz, H-5), 7.70-7.16 (14H, m, 14 aromatic-H), 5.65 (2H, s, CH_2O), 5.63 (2H, s, CH_2O). LC/MS: 552 (M+Na); *Anal*. $C_{30}H_{22}F_3N_3O_3$ (C,H,N).

2-benzyloxy-3-(4-methoxyphenoxymethyl)-7-trifluoromethylquinoxaline (**17b**) was obtained in 94% yield after 20 h under reflux; m. p. 125-127 °C (methanol); TLC (light petroleum-ethyl acetate 9:1): R_f 0.34; IR (nujol): ν 1580 cm^{-1}; UV (EtOH): λ_{max} 206, 226, 244, 284, 328 nm; ^1H NMR ($CDCl_3$): δ 8.22-8.16 (2H, m, H-6 + H-8), 7.76 (1H, dd, *J* = 8.6 and 1.6 Hz, H-5), 7.60-7.48 (2H, m, H-2" + H-6"), 7.48-7.30 (3H, m, H-3" + H-4" + H-5"), 6.98 (2H, d, *J* = 9.0 Hz, H-2' + H-6'), 6.82 (2H, d, *J* = 9.0 Hz, H-3' + H-5'), 5.62 (2H, s, CH_2O), 5.36 (2H, s, CH_2O), 3.77 (3H, s, CH_3O). LC/MS: 441 (M+H); *Anal*. $C_{24}H_{19}F_3N_2O_3$ (C,H,N).

Biological assays

Compounds

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

Cells

Cell lines were purchased from American Type Culture Collection (ATCC). Solid tumor-derived cells were grown in RPMI-1640 medium supplemented with 10% FCS, 100 units/mL penicillin G and 100 μg/mL streptomycin. 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.

Antiproliferative Assays

Activity against solid-tumor-derived cells was evaluated in exponentially growing cultures seeded at 10^5 cells/mL and allowed to adhere for 16 hrs to culture plates before addition of the drugs. Cell viability was determined after 96 hrs at 37 °C by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [13].

REFERENCES

[1] Brown, D.J. In *The Heterocycles Compounds*: *The Quinoxalines Supplement II*; Taylor-Wipf, Ed.; John Wiley & Sons, Inc: Hoboken, **2004**.
[2] Carta, A.; Corona, P.; Loriga, M. *CMC*, **2005**, *12*, 2259, and references cited therein.
[3] Catarzi, D.; Colotta, V.; Varano, F.; Filacchioni, G.; Galli, A.; Costagli, C.; Carlà, V. *J. Med. Chem.,* **2001**, *44*, 3157.
[4] Catarzi, D.; Colotta, V.; Varano, F.; Calabri, F.R.; Filacchioni, G.; Galli, A.; Costagli, C.; Carlà, V. *J. Med. Chem.,* **2004**, *47*, 262.
[5] Carta, A.; Loriga, M.; Zanetti, S.; Sechi, L. *Farmaco*, **2003**, *58*, 1251.
[6] Lawrence, D.S.; Copper, J.E.; Smith, C.D. *J. Med. Chem*., **2001**, *44*, 594.
[7] Loriga, M.; Fiore, M.; Sanna, P.; Paglietti, G. *Farmaco*, **1995**, *50*, 289.
[8] Endicott, J.A.; Ling, V. *Annu. Rev. Biochem.* **1989**, *58*, 137.
[9] Twentyman, P.R. *Drug News Perspect.* **1993**, *6*, 647.
[10] Pastan, I.; Gottesman, M.M.; Ueda, K.; Lovelace, E.; Rutherford, A. V.; Willngham, M.C. *Proc. Natl. Acad. Sci. U.S.A*. **1988**, *85*, 4486.
[11] Chen, H.X.; Bamberger, U.; Heckel, A.; Guo, X.; Cheng, Y.C. *Cancer Res*. **1993**, *53*, 1974.
[12] Gaj, C.L.; Anyanwutaku, I.; Chang, Y.H.; Cheng, Y.C. *Biochem. Pharmacol*. **1998**, *55*, 1199.
[13] Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyster, J.; De Clercq, E.; *J. Virol. Methods,* **1988**, *20*, 309.

Received: 14 June, 2005 **Revised: 30 September, 2005** **Accepted: 01 October, 2005**