2(3)-Aryl-thio(oxy)-methylquinoxaline Derivatives: A New Class of P-Glycoprotein-Mediated Drug Efflux Inhibitors

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Abstract: A series of quinoxalines variously substituted, namely 3-arylthiomethyl-1,6-dimethylquinoxalin-2-ones (**6a-f**), 3-arylthiomethyl-1-benzyl-7-trifluoromethylquinoxalin-2-ones (**8a-g**) and 2-arylthiomethyl-3-benzyloxy-6-trifluoromethylquinoxalines (**10a**,**b**,**e-h**), were synthesized and compared with previous arylphenoxymethylquinoxalines (**1a-f**, **2a-f** and **3a-b**). The purpose was to verify whether the replacement of oxygen with sulphur atom and the insertion of different substituents on the phenyl side chain were able to improve the capability to inhibit the Pgp pump and restore 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}). Furthermore, 2,3-bis(aryloxymethyl)-6-trifluoromethylquinoxalines (**13a-c**) were designed with the objective to evaluate the capability of the double side chain to potentiate the antiproliferative activity of the drugs tested. Biological assays showed that title compounds were, in general, endowed with good activity as Pgp inhibitors. In particular compound **3a**, bearing 2-CONHPh substituent on phenoxymethyl side chain, resulted the most effective, while the double side chain (compound **13c**) gives the ability to inhibit a different MRP pump (a membrane glycoprotein named mrp). Furthermore, we can conclude that replacement of oxygen with sulphur atom did not improve the biological activity.

Key Words: 2(3)-aryl-thio(oxy)-methylquinoxalines, antiproliferative activity, multidrug resistance, P-glycoprotein inhibitors, MRP pump, doxorubicin, vincristine, etoposide.

INTRODUCTION

A significant problem in anticancer chemotherapy is related to the development of resistance to a broad range of unrelated antiproliferative agents [1-2]. Tumor cell lines experimentally selected for resistance to one specific agent often displayed cross-resistance to other drugs [3-4]. The overexpression of drug transport proteins is one of the known mechanisms for this multidrug resistance (MDR) [5-6]. A series of multidrug resistance-related proteins (MRP) have been recently discovered. MRP are energy-dependent efflux pumps, localized at the cell surface, that can transport a broad range of structurally unrelated compounds out of the cell.

Among the MRP, P-glycoprotein (Pgp) is known to be expressed in virtually every type of tissue [7-8] and numerous studies have demonstrated that it is often overexpressed in tumor cells from patients undergoing chemotherapy [9-11]. Furthermore, Pgp pump is involved in blood-intestine and blood-brain barriers. Although its physiological role is not completely known, recent studies have shown that the -Pgp inhibition seems to be not essential for viability or fertility on mice [12].

Some MDR modulators as verapamil and cyclosporin analogues have been evaluated in clinical trials [13-18], but their lack of selectivity in antagonizing Pgp pump increased systemic toxicity of the drugs due to inhibition of MRP1 (which is not overexpressed in tumors). More interesting results have been obtained with newer inhibitors as XR9576 and LY335979 which have demonstrated improved Pgp selectivity [19-20].

On the ground of the observation reported by Smith *et al.* that 3-phenoxymethylquinoxalin-2-one derivatives selectively antagonize the P-glycoprotein (Pgp) [21] to the detriment of MRP-1, we recently designed two series of 1,6-dimethyl-3-phenoxymethylquinoxalin-2-ones (**1a-f**) and 1-benzyl-3-phenoxymethyl-7-trifluoromethylquinoxalin-2-ones (**2a-f**), and a series of 3-benzyloxy-2-phenoxymethyl-6-trifluoromethylquinoxalines (**3a-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 related to the overexpression of the MRP [22].

We concluded that *in vitro* data showed that those series of quinoxalines and quinoxalin-2-ones were able to potentiate the antiproliferative activity of Doxo and VCR in drug-resistant human nasopharyngeal carcinoma KB^{MDR} and KB^{V20C}. Compound (**3a**) turned out to be the most potent

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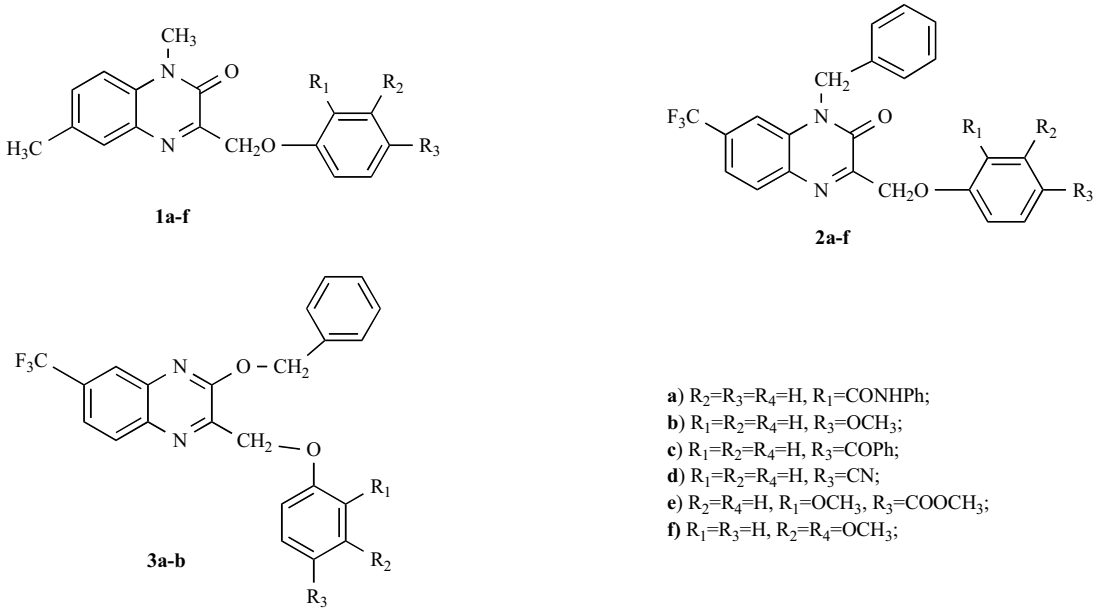


Fig. (1). Chemical structures of quinoxalinones and quinoxalines previously synthesized: 1,6-dimethyl-3-phenoxymethylquinoxalin-2-ones (**1a-f**), 1-benzyl-3-phenoxymethyl-7-trifluoromethylquinoxalin-2-ones (**2a-f**), and 3-benzyloxy-2-phenoxymethyl-6-trifluoromethylquinoxalines (**3a-b**).

quinoxaline derivative [22]. On the contrary, **3a** was ineffective in potentiating the antiproliferative activity of etoposide in KB^{7D} cells. The latter result is likely due to the fact that the KB^{7D} subclone has a different MRP from that of the KB^{V20C} subclone bearing the MDR phenotype.

Based on these premises, we have now prepared a new series of quinoxalines variously substituted and modified in order to improve the SAR (structure activity relationship) analysis (Fig. **2**). 3-Arylthiomethyl-1,6-dimethylquinoxalin-2-ones (**6a-f**), 3-arylthiomethyl-1-benzyl-7-trifluoromethyl-quinoxalin-2-ones (**8a-g**) and 2-arylthiomethyl-3-benzyloxy-6-trifluoromethylquinoxalines (**10a**,**b**,**e-h**) were designed with the aim of verifying whether the replacement of oxygen with sulphur atom was able to potentiate the biological activity. These new derivatives keep the most interesting substituents on the phenyl side chain of the previous series (CONHPh, $COOCH_3$ and OCH_3) or bear new substituents in order to extend the casuistry by introduction of either an halogen (Cl), a potent electron-drawing group (CF_3) or an alkyl group ($CH(CH_3)_2$). Furthermore, the phenyl side chain was also substituted with either 2-naphthyl or 2-pyrimidyl rings in order to evaluate both the steric hindrance and the effect of the nitrogen atoms, respectively, on the biological activity.

Finally, 2,3-bis(aryloxymethyl)-6-trifluoromethylquinoxalines (**13a-c**) were designed with the objective to evaluate the capability of the double side chain to potentiate the antiproliferative activity of the drugs tested.

CHEMISTRY

In Scheme (**1**) is reported the procedure used for the synthesis of 3-arylthiomethyl-1,6-dimethylquinoxalin-2-ones (**6a-f**). The known 3-bromomethyl-1,6-dimethylquinoxalin-2-one (**4**) [22] was condensed with the appropriate thiophenol (**5a**,**b**,**d**,**f**) in a heterogenic mixture of chloroform and water and in presence of NaOH and benzyltriethylammonium chloride, to afford in good yield (58-87%) the 3-arylthiomethyl-1,6-dimethylquinoxalin-2-one derivatives (**6a**, **b**,**d**,**f**). Compounds (**6c**,**e**) were in turn obtained by condensation of **4** with **5c**,**e** in anhydrous DMF and KOH or Cs_2CO_3, respectively (95 and 40% yield).

The 3-arylthiomethyl-1-benzyl-7-trifluoromethylquinoxalin-2-ones (**8a-g**) and 2-arylthiomethyl-3-benzyloxy-6-trifluoromethylquinoxaline (**10a**,**b**,**e-h**) were obtained in 34-96% of yield by condensation of the known 1-benzyl-3-bromomethyl-7-trifluoromethylquinoxalin-2-one (**7**) and 2-benzyloxy-3-bromomethyl-7-trifluromethylquinoxaline (**9**) [22] with the appropriate thiophenol (**5a-f**) or **5a**,**b**,**e-h** respectively, in a heterogenic mixture of chloroform and water and in presence of NaOH and benzyltriethylammonium chloride (Schemes **2-3**).

Finally, 2,3-bis(aryloxymethyl)-6-trifluoromethylquinoxaline (**13a-c**) were prepared (67-77 yield) reacting the known 2,3-bis(bromomethyl)-6-trifluoromethylquinoxaline (**11**) [23] with the appropriate phenol (**12a-c**) in a heterogenic mixture of chloroform and water and in presence of NaOH and benzyltriethylammonium chloride (Scheme **4**).

BIOLOGICAL ASSAYS

Test compounds were evaluated *in vitro* against the doxorubicin-resistant nasopharyngeal carcinoma cell line (KB^{MDR}) obtained by transfection of wild type (KB^{wt}) cells with a retroviral vector carrying the human MDR-1 gene and maintained under uninterrupted treatment with doxorubicin [24-25]. Compounds (**13a-c**) were also evaluated against (KB^{wt}), KB^{V20C}, and KB^{7D}. KB^{V20C} cell line were obtained under uninterrupted treatment with vincristine. These cells

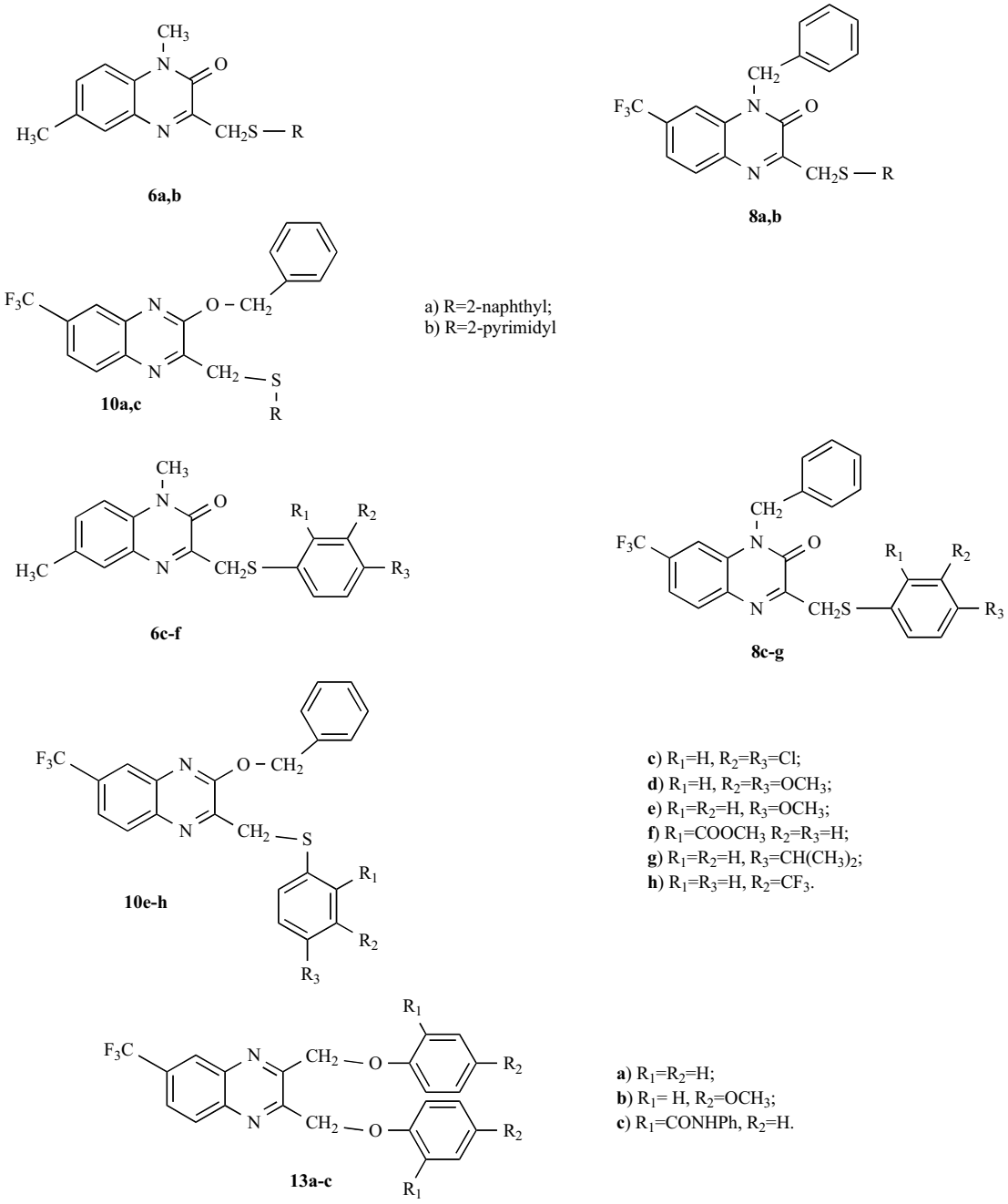


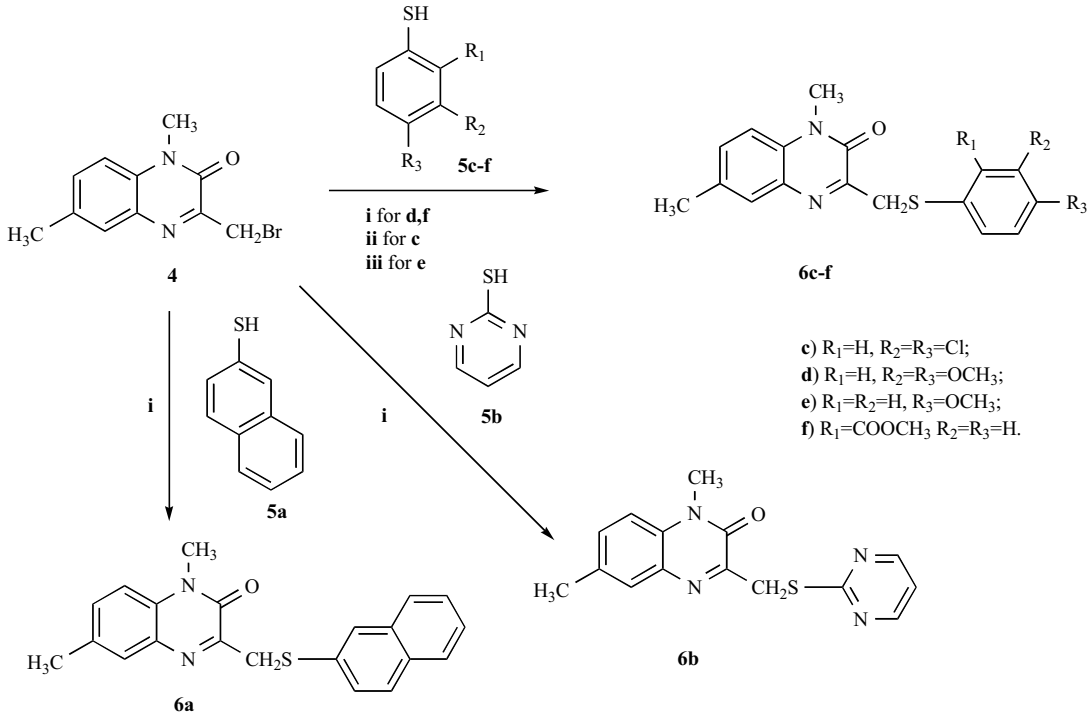
Fig. (2). Chemical structures of the new series of quinoxaline deivatives synthesized: 3-arylthiomethyl-1,6-dimethylquinoxalin-2-ones (**6a-f**), 3-arylthiomethyl-1-benzyl-7-trifluoromethylquinoxalin-2-ones (**8a-g**), 2-arylthiomethyl-3-benzyloxy-6-trifluoromethylquinoxalines (**10a**,**b**,**e-h**) and 2,3-bis(aryloxymethyl)-6-trifluoromethylquinoxalines (**13a-c**).

possess an MDR phenotype [26-27], related to the over-expression of the MDR-1 gene. KB^{7D} cell lines were obtained under uninterrupted treatment with etoposide, a topoisomerase II inhibitor in clinical use [28]. Their drug-resistance is due to the over-expression of a MRP gene, which codes for a membrane glycoprotein (mrp). These cells also express altered levels of topoisomerase II.

The antiproliferative activity of the reference drugs along with the reference compound of the precedent series (**3a**) and a selected new derivative **13c** in wt and drug-resistant subclones are shown in Table **1**.

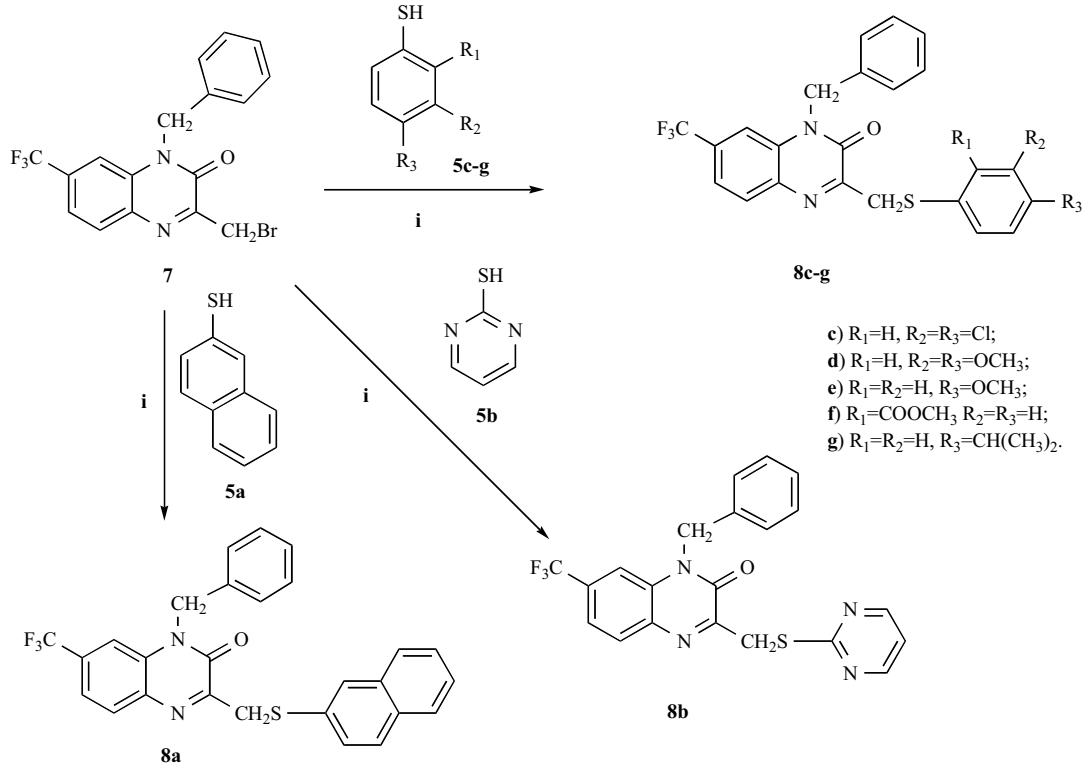
RESULTS AND DISCUSSION

The reference compound 3-benzyloxy-6-trifluoromethyl-2-(2-benzamidephenoxymethyl) quinoxaline (**3a**), the reference drug (Doxorubicin), and the new series 3-arylthio-methyl-1,6-dimethylquinoxalin-2-ones (**6a-f**), 3-arylthio-methyl-1-benzyl-7-trifluoromethylquinoxalin-2-ones (**8a-g**), 2-aryl-



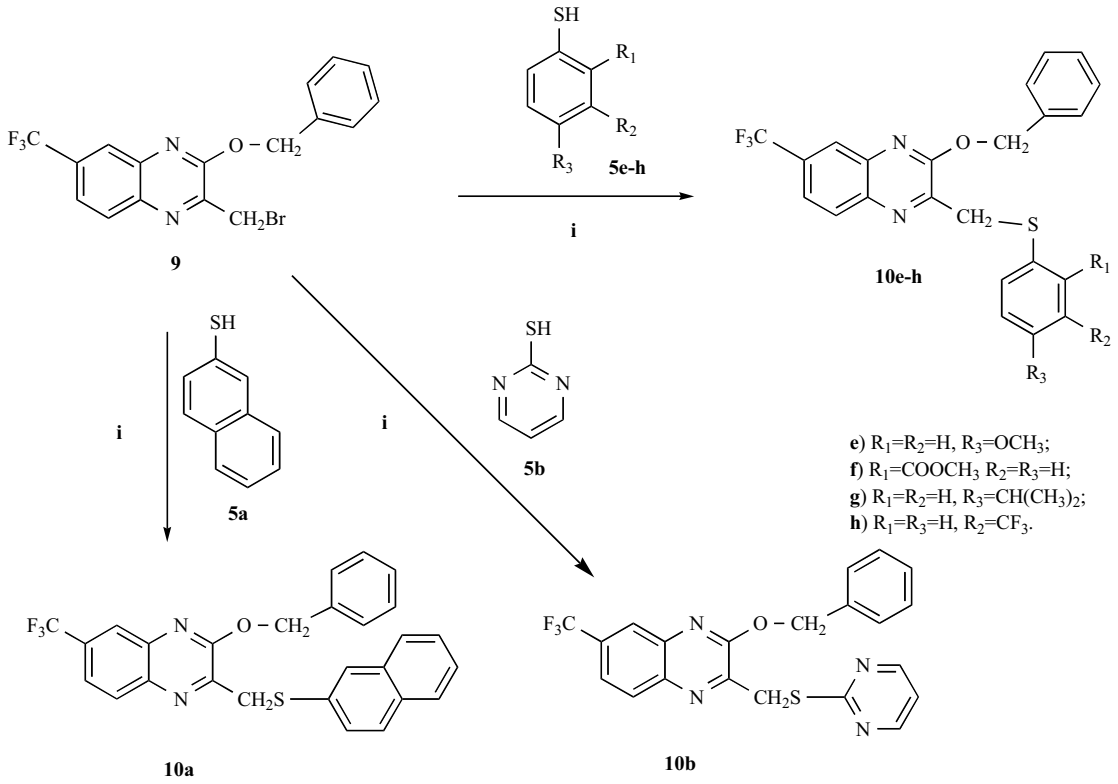
i) $CHCl_3$; NaOH, benzyltriethylammonium chloride, H_2O, 50 °C , 10 h; **ii**) DMF anhydrous, KOH, r.t., 0.5 h; **iii**) DMF anhydrous, $CsHCO_3$, 70 °C, 2.5 h.

Scheme (1). Synthesis of 3-arylthiomethyl-1,6-dimethylquinoxalin-2-ones (**6a-f**).



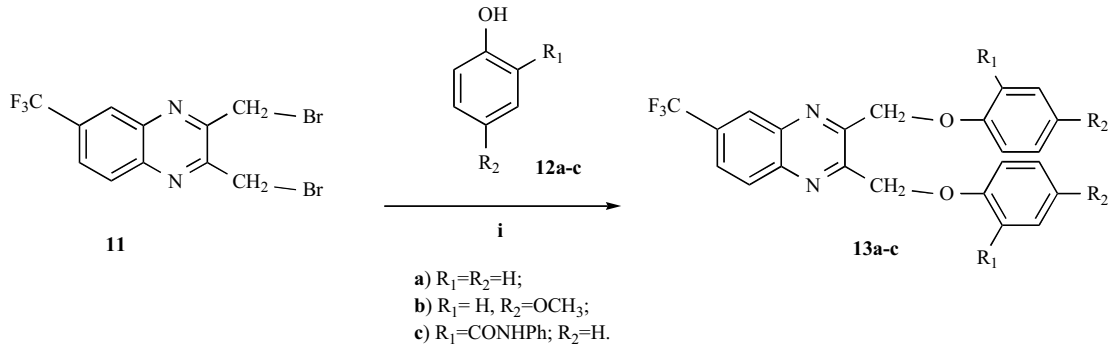
i) $CHCl_3$, NaOH, benzyltriethylammonium chloride, H_2O, 50 °C , 8-22 h.

Scheme (2). Synthesis of 3-arylthiomethyl-1-benzyl-7-trifluoromethylquinoxalin-2-ones (**8a-g**).



i) $CHCl_3$, NaOH, benzyltriethylammonium chloride, H_2O, 50 °C , 8-14 h.

Scheme (3). Synthesis of 2-arylthiomethyl-3-benzyloxy-6-trifluoromethylquinoxaline (**10a**,**b**,**e-h**).



i) $CHCl_3$, NaOH, benzyltriethylammonium chloride, H_2O, 50 °C , 8-14 h.

Scheme (4). Synthesis of 2,3-bis(aryloxymethyl)-6-trifluoromethylquinoxaline (**13a-c**).

thiomethyl-3-benzyloxy-6-trifluoromethylquinoxaline (**10a**, **b**,**e-h**) and 2,3-bis(aryloxymethyl)-6-trifluoromethyl-quinoxaline (**13a-c**) were tested for antiproliferative activity against KB^{MDR} cells. As showed in Table **2**, when doxorubicin is used in combination with 100 μM of **6b** or **8f**, (which were devoid of cytotoxicity against KB^{MDR} cells) it turns out to be 30- and 40-fold more potent than when used alone, respectively, even if reference compound (**3a**) resulted more potent (120-fold). Interestingly, derivative (**6d**) exhibited the same potency at 10 μM concentration, very likely as a consequence of the synergism of the Pgp inhibition and cytotoxicity (CC_{50} = 32 μM). Other compounds (**10a**,**b**,**e**), although more cytotoxic than **6d** (CC_{50} = 4.5-7 μM), proved to be ineffective in potentiating the antiproliferative activity of doxorubicin putting in evidence their inability in the Pgp inhibition.

A few *mono*-arylthiomethyl derivatives (**8a**) as well as the above **10a**,**b**,**e** and unexpectedly, all the *bis*-aryloxymethyl derivatives (**13a-c**) were totally unable to affect cell proliferation at concentration of 1 μM.

All the compounds were further evaluated in order to verify if they were effective against the drug resistant KB^{V20C} and KB^{7D} cells. Compounds (**6a-f**), (**8a-g**) and (**10a**,**b**,**e-h**)

Table 1. Antiproliferative activity of Vincristine, Etoposide, Doxorubicin, 3a and 13c 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	0.003 ± 0.0009	-	-	0.2 ± 0.03
Etoposide	2.2 ± 0.6	-	>100	-
Doxorubicin	0.2 ± 0.07	2.4 ± 0.6	-	-
3a	>100	>100	>100	>100
13c	>100	>100	95 ± 5	>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 (±SD) for three independent determinations.

Table 2. Antiproliferative Activity, in KB^{MDR} Cells, of the Reference Compound 3a, Quinoxalin-2-ones (6a-f, and 8a-g) and Quinoxalines (10a,b,e-h and 13a-c), 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
Doxorubicin	2.4	-	-	-
3a	>100	0.5	0.08 (30)	<0.02 (>100)
6a	20	0.5	0.2	-
6b	>100	0.6	0.9	0.08 (30)
6c	25	1.3	0.7	-
6d	32	0.6	0.06 (40)	-
6e	15	1.0	0.1	-
6f	34	0.6	0.2	-
8a	41	2.2	2.2	-
8b	12	0.7	-	-
8c	31	0.5	0.5	-
8d	89	0.6	0.7	-
8e	38	2.0	0.6	-
8f	>100	0.6	0.5	0.06 (40)
8g	13	0.3	-	-
10a	5.5	2.2	-	-
10b	7	2.8	-	-
10e	4.5	2.1	-	-
10f	60	0.6	0.2	-
10g	33	0.7	0.5	-
10h	27	0.7	0.5	-

(Table 2. Contd….)

Test Compds	[a][CC_{50}]			
	[*]TC alone	Doxorubicin in Combination With		
		[*]TC 1 μM	[*]TC 10 μM	[*]TC 100 μM
13a	26	7.0	-	-
13b	30	8.5	-	-
13c	>100	8.5		

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

[*]**TC** = Test compounds.

( ) Fold increase in susceptibility to Doxorubicin.

were not able to potentiate the antiproliferative activity of vincristine and etoposide at the cytotoxic concentration (data not reported). On the contrary, as summarized in Tables **3** and **4**, compounds (**13a-c**), which bear a double side chain, were in general active against KB^{V20C} and KB^{7D} cells. In particular derivative (**13c**), which was devoid of cytotoxicity, turns out to be most potent of the series resulting only slightly less effective than reference compound (**3a**) against KB^{V20C}.

Furthermore, it also emerged for its capability, although not high potency, to potentiate the antiproliferative activity of etoposide, very likely as a consequence of the membrane glycoprotein (mrp) inhibition.

CONCLUSIONS

In the light of the above, and previous [22] results, we can conclude that 2(3)-aryl-thio(oxy)-methylquinoxalines are, in general, endowed with good activity as Pgp inhibitors. Cell-based antitumor assays showed that the 2-CONHPh substituent on the phenyl side chain (compounds **3a** and **13c**) remains the most effective and when a double side chain containing this group (**13c**) is linked on both 2 and 3 positions, the derivative obtained acquires the ability to inhibit mrp pump and partially retains the capacity to inhibit the Pgp pump. Besides 2-pyrimidyl and 2-$COOCH_3$-phenyl side chains (compounds **6b** and **8f**) also showed a fair activity while all the other substituents, with the sole exception of two OCH_3 groups on the phenyl side chain (**6d**) were not effective. It is possible to conclude that for these series of quinoxalines *N*-phenyl-carboxamide and methyl carboxylate on the phenyl side chain are more effectives than halogen, methoxy, trifloromethyl or alkyl groups. On the other hand, the synergism of cytotoxicity and Pgp pump inhibition shown from **6d** put in evidence that two OCH_3 groups at the 3 and 4 position of the phenyl side chain are more effective of the single OCH_3 group at the 4 position (compound **6e**) which is evidently devoid of effectiveness against Pgp pump, thus although the cytotoxicity **6e** is higher than **6d**. For these reasons further modifications of the carbonyl moiety at 2 position of phenyl side chain of the quinoxaline derivatives can be considered as a new strategy to optimize the Pgp pump inhibition and activity. Furthermore, the simultaneous introduction of two (or more) methoxy (or alkoxy) groups and 2-carbonylic derivative groups on the phenyl side chain of the quinoxaline could improve the antiproliferative activ-

Table 3. Antiproliferative Activity, in KB^{V20C} Cells, of the Reference Compound 3a and *bis*-aryloxymethyl Derivatives (13a-c), Alone and in Combination with Vincristine

Test Compds	[a][CC_{50}]			
	[*]TC alone	Vincristine in Combination With		
		[*]TC 0.8 μM	[*]TC 4 μM	[*]TC 20 μM
Vincristine	0.2			
3a	>100	0.09	0.01 (20)	0.004 (50)
13a	41	0.2	0.1	0.02
13b	70	0.2	0.07	0.05
13c	>100	0.1	0.03 (7)	0.006 (33)

[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.

Table 4. Antiproliferative Activity, in KB^{7D} Cells, of the Reference Compound 3a and *bis*-aryloxymethyl Derivatives (13a-c), 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
Etoposide	>100			
3a	>100	>100	>100	>100
13a	35	>100	>100	56
13b	16	>100	>100	-
13c	95	>100	64	35 (>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 Etoposide.

ity of this class of compounds when utilized in combination with doxorubicin against doxorubicin-resistant tumor cell lines.

Finally, the replacement of oxygen with sulphur atom did not improve the biological activity.

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. ^{1}H 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) and m (multiplet). MS spectra were performed on 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 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

Quinoxalin-2-one intermediates 3-bromomethyl-1,6-dimethylquinoxalin-2-one (**4**) and 1-benzyl-3-bromomethyl-7-trifluoromethylquinoxalin-2-one (**7**) were prepared following the procedure previously described by us [22]. Quinoxaline intermediate 2,3-bis(bromomethyl)-6-trifluoromethylquinoxaline (**11**) was prepared following the procedure reported by Krajewski *et al*. [23]. Thiophenols (**5a-h**), phenols (**12a-c**) and benzyltriethylammonium chloride were commercially available.

General Procedure for Preparation of 3-arylthiomethyl-1,6-dimethylquinoxalin-2-ones (6a,b,d,f)

To a mechanically vigorously stirred solution of 1.12 mmol of 3-bromomethyl-1,6-dimethylquinoxalin-2-one (**4**) and 1.12 mmol of the appropriate thiophenol (**5a**,**b**,**d**,**f**) in 10 mL of chloroform, 1.7 mmol of NaOH and 0.112 mmol of benzyltriethylammonium chloride in 10 mL of water were added. After complete addition, the temperature of the heterogenic mixture was rised to 50 °C and the stirring continued for an additional 10 h. On cooling at room temperature, the chloroform layer was separated and the aqueous solution was extracted with chloroform. After combining the chloroform extracts, the resulting solution was washed with a saturated aqueous solution of NaCl and dried on anhydrous sodium sulfate. On evaporation of liquor mothers, the solid obtained was crystallized from diethyl ether. Melting points, yields, analytical and spectroscopical data are reported below.

1,6-dimethyl-3-((naphtalen-2-ylthio)methyl)quinoxalin-2-one (**6a**) was obtained in 58% yield; m. p. 168-169 °C (diethyl ether); TLC (diethyl ether-light petroleum 4:6): R_f 0.61; UV (EtOH): λ_{max} 210, 226, 284, 352 nm; ^{1}H NMR ($CDCl_3$): *δ* 7.98 (1H, s, H-5), 7.79-7.38 (7H, m, 7 naphthyl-H), 7.34 (1H, d, *J* = 8.2 Hz, H-7), 7.20 (1H, d, *J* = 8.2 Hz, H-8), 4.48 (2H, s, CH_2), 3.70 (3H, s, N-CH_3), 2.42 (3H, s, C_6-CH_3). LC/MS: 347 (M+H); *Anal. Calcd. For* $C_{21}H_{18}N_2OS$ (C,H,N): C, 72.80; H, 5.24; N, 8.09. *Found* C, 72.43; H, 5.49; N, 7.80.

1,6-dimethyl-3-((pyrimidin-2-ylthio)methyl)quinoxalin-2-one (**6b**) was obtained in 87% yield; m. p. 212-214 °C (diethyl ether); TLC (dichlorometane-acetone 8:2): R_f 0.80; UV (EtOH): λ_{max} 208, 236, 286, 352 nm; ^{1}H NMR ($CDCl_3$): *δ* 8.53 (2H, m, H-4' + H-6'), 7.67 (1H, s, H-5), 7.37 (1H, d, *J* = 8.6 Hz, H-7), 7.20 (1H, d, *J* = 8.6 Hz, H-8), 6.97 (1H, m, H-5'), 4.76 (2H, s, CH_2), 3.71 (3H, s, N-CH_3), 2.43 (3H, s, C_6-CH_3). LC/MS: 299 (M+H); *Anal. Calcd. For* $C_{15}H_{14}N_4OS$ (C,H,N): C, 60.38; H, 4.73; N, 18.78. *Found* C, 60.04; H, 4.98; N, 18.42.

1,6-dimethyl-3-((3,4-dimethoxyphenylthio)methyl)quinoxalin-2-one (**6d**) was obtained in 63% yield; m. p. 107-108 °C (diethyl ether); TLC (diethyl ether-light petroleum 8:2): R_f 0.29; UV (EtOH): λ_{max} 210, 234, 288, 356 nm; ^{1}H NMR ($CDCl_3$): δ 7.55 (1H, d, *J* = 1.6 Hz, H-5), 7.37 (1H, dd, *J* = 8.2 and 1.6 Hz, H-7), 7.20 (1H, d, *J* = 8.2 Hz, H-8), 7.07 (1H, dd, *J* = 8.4 and 2.0 Hz, H-6'), 7.02 (1H, d, *J* = 2.0 Hz, H-2'), 6.77 (1H, d, *J* = 8.4 Hz, H-5'), 4.27 (2H, s, CH_2), 3.85 (3H, s, O-CH_3), 3.79 (3H, s, O-CH_3), 3.71 (3H, s, N-CH_3), 2.44 (3H, s, C_6-CH_3). LC/MS: 357 (M+H); *Anal. Calcd. For* $C_{19}H_{20}N_2O_3S$ (C,H,N): C, 64.02; H, 5.66; N, 7.86. *Found* C, 64.30; H, 5.78; N, 8.03.

1,6-dimethyl-3-((2-carboxymethylphenylthio)methyl) quinoxalin-2-one (**6f**) was obtained in 76% yield; m. p. 130-131 °C (diethyl ether); TLC (diethyl ether-light petroleum 8:2): R_f 0.46; UV (EtOH): λ_{max} 208, 230, 292, 344 nm; ^{1}H NMR ($CDCl_3$): δ 7.92 (1H, dd, *J* = 8.0 and 1.6 Hz, H-7), 7.79 (1H, d, *J* = 8.0 Hz, H-8), 7.65 (1H, d, *J* = 1.6 Hz, H-5), 7.52-7.05 (4H, m, H-3' + H-4' + H-5' + H-6'), 4.44 (2H, s, CH_2), 3.89 (3H, s, O-CH_3), 3.70 (3H, s, N-CH_3), 2.44 (3H, s, C_6-CH_3). LC/MS: 355 (M+H); *Anal. Calcd. For* $C_{19}H_{18}N_2O_3S$ (C,H,N): C, 64.39; H, 5.12; N, 7.90. *Found* C, 64.11; H, 5.04; N, 8.21.

Preparation of 1,6-dimethyl-3-((3,4-dichlorophenylthio) methyl)quinoxalin-2-one (6c)

To a stirred mixture of 2.7 mmol of KOH and 2.24 mmol of 3,4-dichlorothiophenol in 10 mL of DMF anhydrous, stirred at room temperature for 30 min, 2.24 mmol of 3-bromomethyl-1,6-dimethylquinoxalin-2-one (**4**) were added and the stirring was continued for 24 h. The reaction mixture was then diluted with water (100 mL) and the crude precipitate obtained was collected by filtration, washed and crystallized (diethyl ether) to afford the desired **6c** in 95% of yield. M. p. 137-138 °C; TLC (diethyl ether-light petroleum 8:2): R_f 0.56; UV (EtOH): λ_{max} 204, 232, 260, 288, 352 nm; ^{1}H NMR ($CDCl_3$): δ 8.02 (1H, s, H-5), 7.64-7.60 (2H, m, H-7 + H-8), 7.39 (1H, dd, *J* = 8.8 and 1.8 Hz, H-6'), 7.31 (1H, s, H-2'), 7.21 (1H, d, *J* = 8.8 Hz, H-5'), 4.25 (2H, s, CH_2), 3.71 (3H, s, N-CH_3), 2.45 (3H, s, C_6-CH_3). LC/MS: 366 (M+H); *Anal. Calcd. For* $C_{17}H_{14}Cl_2N_2OS$ (C,H,Cl,N): C, 55.90; H, 3.86; Cl, 19.41; N, 7.67. *Found* C, 55.63; H, 4.11; Cl, 19.11; N, 7.50.

Preparation of 1,6-dimethyl-3-((4-methoxyphenylthio) methyl)quinoxalin-2-one (6e)

A mixture of 1.12 mmol of 3-bromomethyl-1,6-dimethylquinoxalin-2-one (**4**), 1.12 mmol of 3,4-dichlorothiophenol (**5e**) and 1.12 mmol of $CsHCO_3$ in 8 mL of anhydrous DMF, was stirred at 70 °C for 2.5 h. were added and the stirring was continued for 24 h. On cooling at room temperature, the reaction mixture was then diluted with water (80 mL) and the crude precipitate obtained was collected by filtration, washed and crystallized (acetone) to afford the desired **6e** in 40% of yield. M. p. 101-102 °C; TLC (light petroleum-ethyl acetate 7:3): R_f 0.26; UV (EtOH): λ_{max} 208, 232, 286, 356 nm; ^{1}H NMR ($CDCl_3$): δ 7.53 (1H, s, H-5), 7.41 (2H, d, *J* = 8.8 Hz, H-3' + H-5'), 7.30 (1H, dd, *J* = 8.4 and 1.8 Hz, H-7), 7.19 (1H, d, *J* = 8.4 Hz, H-8), 6.80 (2H, d, *J* = 8.8 Hz, H-2' + H-6'), 4.25 (2H, s, CH_2), 3.78 (3H, s, O-CH_3), 3.71 (3H, s, N-CH_3), 2.45 (3H, s, C_6-CH_3). LC/MS: 327 (M+H); *Anal. Calcd. For* $C_{18}H_{18}N_2O_2S$ (C,H,N): C, 66.23; H, 5.56; N, 8.58. *Found* C, 66.62; H, 5.24; N, 8.88.

General Procedure for Preparation of 3-arylthiomethyl-1-benzyl-7-trifluoromethylquinoxalin-2-ones (8a-g) and 2-arylthiomethyl-3-benzyloxy-6-trifluoromethylquinoxalines (10a,b,e-h)

To a mechanically vigorously stirred mixture of equimolar amounts of 1-benzyl-3-bromomethyl-7-trifluoromethylquinoxalin-2-one (**7**) and the appropriate thiophenol (**5a-f**) (0.75 mmol), or of 2-benzyloxy-3-bromomethyl-7-trifluromethylquinoxaline (**9**) with **5a**,**b**,**e-h**, in chloroform (10 mL), a solution of 1.12 mmol of NaOH and 0.075 mmol of benzyltriethylammonium chloride in 10 mL of water was added dropwise. The reaction mixture was then heated to 50 °C and the stirring continued for 8-22 h, as reported below. On cooling at room temperature, the chloroformic component was separated and the aqueous solution was extracted with chloroform (3 X 20 mL). The chloroform extracts were combined and washed with a saturated aqueous solution of NaCl and dried on anhydrous sodium sulfate. On evaporation of liquor mothers, the crude solid obtained was purified by chromatography on silica gel column or by crystallization (methanol). Reaction times, melting points, yields, analytical and spectroscopical data are reported below.

1-benzyl-7-trifluoromethyl-3-((naphtalen-2-ylthio)methyl)quinoxalin-2-one (8a) was obtained in 79% yield by chromatography (eluent: 9:1 mixture of light petroleum/ethyl acetate) after 12 h under reflux; m. p. 129-130 °C (methanol); TLC (light petroleum/ethyl acetate 9:1): R_f 0.28; UV (EtOH): λ_{max} 219, 250, 281, 344 nm; ^{1}H NMR ($CDCl_3$): δ 7.98 (1H, s, H-1'), 7.87-7.70 (2H, m, H-5 + H-8), 7.56-7.38 (2H, m, H-6 + 1 benzyl-H), 7.32-7.22 (10 aromatic H), 5.51 (2H, s, N-CH_2), 4.53 (2H, s, S-CH_2). LC/MS: 477 (M+H); *Anal. Calcd. For* $C_{27}H_{19}F_3N_2OS$ + 1 H_2O (C,H,N): C, 65.58; H, 4.28; N, 5.66. *Found* C, 65.48; H, 4.24; N, 5.49.

1-benzyl-7-trifluoromethyl-3-((pyrimidin-2-ylthio)methyl)quinoxalin-2-one (8b) was obtained in 79% yield after 12 h under reflux; m. p. 183-184 °C (methanol); TLC (chloroform/methanol 95:5): R_f 0.81; UV (EtOH): λ_{max} 208, 234, 279, 341 nm; ^{1}H NMR ($CDCl_3$): δ 8.54 (2H, m, H-4' + H-6'), 7.95 (1H, d, *J* = 8.2 Hz, H-5), 7.53 (1H, s, H-8), 7.50 (1H, d, *J* = 8.2 Hz, H-6), 7.34-25 (5H, m, 5 benzyl-H), 6.98 (1H, m, H-5'), 5.53 (2H, s, N-CH_2), 4.82 (2H, s, S-CH_2). LC/MS: 429 (M+H); *Anal. Calcd. For* $C_{21}H_{15}F_3N_4OS$ (C,H,N): C, 58.87; H, 3.53; N, 13.08. *Found* C, 59.20; H, 3.41; N, 13.32.

1-benzyl-7-trifluoromethyl-3-((3,4-dichlorophenylthio)methyl)quinoxalin-2-one (8c) was obtained in 34% yield after 20 h under reflux; m. p. 75-76 °C (diethyl ether); TLC (light petroleum-ethyl acetate 9:1): R_f 0.27; UV (EtOH): λ_{max} 206, 232, 272, 344 nm; ^{1}H NMR ($CDCl_3$): δ 7.92 (1H, d, *J* = 8.2 Hz, H-5), 7.65-7.58 (2H, m, 2 aromatic-H), 7.53 (1H, s, H-8), 7.40-7.15 (7H, m, H-6 + 6 aromatic-H), 5.52 (2H, s, N-CH_2), 4.41 (2H, s, S-CH_2). LC/MS: 495, 497 and 499 (M+H), 517, 519 and 521 (M+Na), 533, 535 and 537 (M+K+H); *Anal. Calcd. For* $C_{23}H_{15}Cl_2F_3N_2OS$ (C,H,Cl,N):

C, 55.77; H, 3.05; Cl, 14.31; N, 5.66. *Found* C, 55.40; H, 3.21; Cl, 14.60 N, 5.41.

1-benzyl-7-trifluoromethyl-3-((3,4-dimethoxyphenylthio)methyl)quinoxalin-2-one (8d) was obtained in 58% yield after 10 h under reflux; m. p. 131-132 °C (methanol); TLC (light petroleum-ethyl acetate 9:1): R_f 0.06; UV (EtOH): λ_{max} 208, 230, 281, 345 nm; ^{1}H NMR ($CDCl_3$): δ 7.83 (1H, d, *J* = 8.0 Hz, H-5), 7.53 (1H, s, H-8), 7.50 (1H, d, *J* = 8.0 Hz, H-6), 7.40-7.15 (5H, m, 5 benzyl-H), 7.06 (1H, d, *J* = 8.2 Hz, H-6'), 7.01(1H, s, H-2'), 6.75 (1H, d, *J* = 8.2 Hz, H-5'),5.52 (2H, s, N-CH_2), 4.33 (2H, s, S-CH_2), 3.85 (3H, s, O-CH_3), 3.76 (3H, s, O-CH_3). LC/MS: 487 (M+H); *Anal. Calcd. For* $C_{25}H_{21}F_3N_2O_3S$ (C,H,N): C, 61.72; H, 4.35; N, 5.76. *Found* C, 61.70; H, 4.41; N, 5.83.

1-benzyl-7-trifluoromethyl-3-((4-methoxyphenylthio)methyl)quinoxalin-2-one (8e) was obtained in 52% yield after 9 h under reflux; m. p. 114-115 °C (acetone); TLC (light petroleum-ethyl acetate 9:1): R_f 0.17; UV (EtOH): λ_{max} 204, 229, 280, 346 nm; ^{1}H NMR ($CDCl_3$): δ 7.82 (1H, d, *J* = 8.4 Hz, H-5), 7.51 (1H, s, H-8), 7.49 (1H, d, *J* = 8.4 Hz, H-6), 7.41-7.23 (7H, m, 7 aromatic-H), 6.80 (2H, d, *J* = 8.0 Hz, H-3' + H-5'), 5.51 (2H, s, N-CH_2), 4.29 (2H, s, S-CH_2), 3.77 (3H, s, O-CH_3). LC/MS: 457 (M+H); *Anal. Calcd. For* $C_{24}H_{19}F_3N_2O_2S$ (C,H,N): C, 63.15; H, 4.20; N, 6.14. *Found* C, 61.40; H, 4.38; N, 5.89.

1-benzyl-7-trifluoromethyl-3-((2-carboxymethylphenylthio)methyl)quinoxalin-2-one (8f) was obtained in 50% yield by chromatography (eluent: 9:1 mixture of light petroleum/ethyl acetate) after 22 h under reflux; m. p. 49-50 °C (diethyl ether); TLC (light petroleum-ethyl acetate 9:1): R_f 0.14; UV (EtOH): λ_{max} 208, 240, 284, 340 nm; ^{1}H NMR ($CDCl_3$): δ 7.94 (1H, d, *J* = 8.1 Hz, H-5), 7.76 (1H, d, *J* = 8.1 Hz, H-3'), 7.53 (1H, s, H-8), 7.50 (1H, d, *J* = 8.1 Hz, H-6), 7.35-7.17 (8H, m, 8 aromatic-H), 5.52 (2H, s, N-CH_2), 4.51 (2H, s, S-CH_2), 3.90 (3H, s, O-CH_3). LC/MS: 485 (M+H); *Anal. Calcd. For* $C_{25}H_{19}F_3N_2O_3S$ (C,H,N): C, 61.98; H, 3.95; N, 5.78. *Found* C, 61.70; H, 4.08; N, 5.59.

1-benzyl-7-trifluoromethyl-3-((4-isopropylphenylthio)methyl)quinoxalin-2-one (8g) was obtained in 57% yield after 8 h under reflux; m. p. 102-103 °C (methanol); TLC (light petroleum-ethyl acetate 9:1): R_f 0.33; UV (EtOH): λ_{max} 207, 228, 249, 280, 346 nm; ^{1}H NMR ($CDCl_3$): δ 7.84 (1H, d, *J* = 8.2 Hz, H-5), 7.52 (1H, s, H-8), 7.49 (1H, d, *J* = 8.2 Hz, H-6), 7.40 (2H, d, *J* = 8.4 Hz, H-2' + H-6'), 7.36-7.26 (5H, m, 5 benzyl-H), 7.13 (2H, d, *J* = 8.4 Hz, H-3' + H-5'), 5.52 (2H, s, N-CH_2), 4.38 (2H, s, S-CH_2), 2.86 (1H, m, C*H*$(CH_3)_2$), 1.21 (6H, d, *J* = 6.8 Hz, CH(CH_3)$_2$). LC/MS: 469 (M+H); *Anal. Calcd. For* $C_{26}H_{23}F_3N_2OS$ + 1 H_2O (C,H,N): C, 64.18; H, 5.18; N, 5.76. *Found* C, 64.09; H, 5.21; N, 5.71.

3-benzyloxy-6-trifluoromethyl-2-((naphtalen-2-ylthio)methyl)quinoxaline (10a) was obtained in 50% yield by chromatography (eluent: 95:5 mixture of light petroleum/ethyl acetate) after 13 h under reflux; m. p. 99-100 °C (diethyl ether); TLC (light petroleum/ethyl acetate 9:1): R_f 0.51; UV (EtOH): λ_{max} 208, 223, 244, 281, 333 nm; ^{1}H NMR ($CDCl_3$): *δ* 8.13 (1H, s, H-1'), 7.98 (1H, d, *J* = 8.6 Hz, H-8), 7.90 (1H, s, H-5), 7.80-7.36 (12H, m, H-7 + 11 aromatic-H), 5.56 (2H, s, O-CH_2), 4.52 (2H, s, S-CH_2). LC/MS: 477 (M+H); *Anal. Calcd. For* $C_{27}H_{19}F_3N_2OS$ (C,H,N): C, 68.05; H, 4.02; N, 5.88. *Found* C, 68.36; H, 3.99; N, 5.96.

3-benzyloxy-6-trifluoromethyl-2-((pyrimidin-2-ylthio)methyl)quinoxaline (10b) was obtained in 95% yield after 14 h under reflux; m. p. 134-135 °C (methanol); TLC (chloroform/methanol 95:5): R_f 0.85; UV (EtOH): λ_{max} 208, 245, 279, 325 nm; ^{1}H NMR ($CDCl_3$): *δ* 8.46 (2H, m, H-4' + H-6'), 8.14 (1H, s, H-5), 8.08 (1H, d, *J* = 8.4 Hz, H-8), 7.72 (1H, d, *J* = 8.4 Hz, H-7), 7.50-7.36 (5H, m, 5 benzyl-H), 6.93 (1H, m, H-5'), 5.59 (2H, s, O-CH_2), 4.83 (2H, s, S-CH_2). LC/MS: 429 (M+H); *Anal. Calcd. For* $C_{21}H_{15}F_3N_4OS$ (C,H,N): C, 58.87; H, 3.53; N, 13.08. *Found* C, 58.80; H, 3.61; N, 13.31.

3-benzyloxy-6-trifluoromethyl-2-((4-methoxyphenylthio)methyl)quinoxaline (10e) was obtained in 96% yield after 10 h under reflux; m. p. 104-105 °C (acetone); TLC (light petroleum-ethyl acetate 9:1): R_f 0.47; UV (EtOH): λ_{max} 205, 228, 245, 330 nm; ^{1}H NMR ($CDCl_3$): δ 8.13 (1H, s, H-5), 7.97 (1H, d, *J* = 8.4 Hz, H-8), 7.71 (1H, d, *J* = 8.4 Hz, H-7), 7.53-7.22 (7H, m, 7 aromatic-H), 6.72 (2H, d, *J* = 8.6 Hz, H-3' + H-5'), 5.56 (2H, s, O-CH_2), 4.29 (2H, s, S-CH_2), 3.75 (3H, s, O-CH_3). LC/MS: 457 (M+H); *Anal. Calcd. For* $C_{24}H_{19}F_3N_2O_2S$ (C,H,N): C, 63.15; H, 4.20; N, 6.14. *Found* C, 63.50; H, 3.98; N, 6.02.

3-benzyloxy-6-trifluoromethyl-2-((2-carboxymethylphenylthio)methyl)quinoxaline (10f) was obtained in 92% yield after 10 h under reflux; m. p. 113-114 °C (acetone); TLC (light petroleum-ethyl acetate 9:1): R_f 0.38; UV (EtOH): λ_{max} 208, 224, 246, 328 nm; ^{1}H NMR ($CDCl_3$): δ 8.13 (1H, s, H-5), 8.06 (1H, d, *J* = 8.1 Hz, H-8), 7.91 (1H, d, *J* = 8.1 Hz, H-7), 7.75 (3H, m, 3 aromatic-H), 7.51-7.14 (6H, m, 6 aromatic-H), 5.61 (2H, s, O-CH_2), 4.50 (2H, s, S-CH_2), 3.88 (3H, s, O-CH_3). LC/MS: 485 (M+H); *Anal. Calcd. For* $C_{25}H_{19}F_3N_2O_3S$ (C,H,N): C, 61.98; H, 3.95; N, 5.78. *Found* C, 62.11; H, 3.90; N, 5.70.

3-benzyloxy-6-trifluoromethyl-2-((4-isopropylphenylthio)methyl)quinoxaline (10g) was obtained in 96% yield by chromatography (mixture of light petroleum ethyl acetate 9:1) after 12 h under reflux; m. p. 60-61 °C (diethyl ether); TLC (light petroleum-ethyl acetate 9:1): R_f 0.63; UV (EtOH): λ_{max} 205, 245, 335 nm; ^{1}H NMR ($CDCl_3$): δ 8.13 (1H, s, H-5), 7.98 (1H, d, *J* = 8.6 Hz, H-8), 7.70 (1H, d, *J* = 8.6 Hz, H-7), 7.55-7.25 (7H, m, H-2' + H-6' + 5 benzyl-H), 7.07 (2H, d, *J* = 8.4 Hz, H-3' + H-5'), 5.56 (2H, s, O-CH_2), 4.38 (2H, s, S-CH_2), 2.86 (1H, m, C*H*$(CH_3)_2$), 1.20 (6H, d, *J* = 6.8 Hz, CH(CH_3)$_2$). LC/MS: 469 (M+H); *Anal. Calcd. For* $C_{26}H_{23}F_3N_2O_2S$ (C,H,N): C, 66.65; H, 4.95; N, 5.98. *Found* C, 66.83; H, 5.07; N, 5.74.

3-benzyloxy-6-trifluoromethyl-2-((3-trifluoromethylphenylthio)methyl)quinoxaline (10h) was obtained in 76% yield after 8 h under reflux; m. p. 61-62 °C (methanol); TLC (light petroleum-ethyl acetate 9:1): R_f 0.40; UV (EtOH): λ_{max} 207, 246, 327 nm; ^{1}H NMR ($CDCl_3$): δ 8.13 (1H, s, H-5), 8.01 (1H, d, *J* = 8.6 Hz, H-8), 7.79 (1H, s, H-2'), 7.73 (1H, d, *J* = 8.6 Hz, H-7), 7.58-7.25 (8H, m, + H-4' H-5' + H-6' + 5 benzyl-H), 5.60 (2H, s, O-CH_2), 4.46 (2H, s, S-CH_2). LC/MS: 495 (M+H); *Anal. Calcd. For* $C_{24}H_{16}F_6N_2OS$ + 1 H_2O (C,H,N): C, 56.25; H, 3.54; N, 5.47. *Found* C, 56.18; H, 3.58; N, 5.36.

General Procedure for Preparation of 2,3-bis(aryloxymethyl)-6-trifluoromethylquinoxalines (13a-c)

To a mechanically vigorously stirred mixture of 2,3-bis(bromomethyl)-6-trifluoromethylquinoxaline (**11**) (1.0 mmol) and the appropriate phenol (**12a-c**) (4.0 mmol) in chloroform (15 mL), a solution of 4 mmol of NaOH and 0.2 mmol of benzyltriethylammonium chloride in 15 mL of water was added dropwise. The reaction mixture was then heated to 70 °C and the stirring continued for 12 h. On cooling at room temperature the crude solid, if present, was collected by filtration, washed with water dried and crystallized (diethyl ether or methanol). The chloroformic component was separated and the aqueous solution was extracted with chloroform (3 X 20 mL). The chloroform extracts were combined and washed with a saturated aqueous solution of NaCl and dried on anhydrous sodium sulfate. On evaporation of liquor mothers, the crude solid obtained was purified by crystallization. Melting points, yields, analytical and spectroscopical data are reported below.

2,3-bis(phenoxymethyl)-6-trifluoromethylquinoxaline (13a) was obtained in 70% yield; m. p. 40-42 °C (diethyl ether); TLC (light petroleum-ethyl acetate 9:1): R_f 0.35; UV (EtOH): λ_{max} 210, 268, 301 nm; ^{1}H NMR ($CDCl_3$): δ 8.45 (1H, s, H-5), 8.25 (1H, d, *J* = 8.8 Hz, H-8), 7.96 (1H, d, *J* = 8.8 Hz, H-7), 7.30-7.23 (4H, m, 4 aromatic-H), 7.03-.93 (6H, m, 6 aromatic-H), 5.58 (4H, s, 2 CH_2). LC/MS: 411 (M+H); *Anal. Calcd. For* $C_{23}H_{17}F_3N_2O_2$ (C,H,N): C, 67.31; H, 4.18; N, 6.83. *Found* C, 67.50; H, 4.09; N, 6.70.

2,3-bis(4-methoxyphenoxymethyl)-6-trifluoromethylquinoxaline (13b) was obtained in 77% yield; m. p. 93-94 °C (diethyl ether); TLC (light petroleum-ethyl acetate 9:1): R_f 0.28; UV (EtOH): λ_{max} 219, 271, 304 nm; ^{1}H NMR ($CDCl_3$): δ 8.46 (1H, s, H-5), 8.26 (1H, d, *J* = 8.8 Hz, H-8), 7.96 (1H, d, *J* = 8.8 Hz, H-7), 6.95 (4H, dd, *J* = 7.0 and 2.4 Hz, H-2' + H-6' + H-2'' + H-6'' +), 6.80 (4H, dd, *J* = 7.0 and 2.4 Hz, H-3' + H-5' + H-3'' + H-5'' +), 5.52 (4H, s, 2 CH_2), 3.76 (6H, s, 2 CH_3). LC/MS: 471 (M+H); *Anal. Calcd. For* $C_{25}H_{21}F_3N_2O_4$ (C,H,N): C, 63.83; H, 4.50; N, 5.95. *Found* C, 64.02; H, 4.44; N, 6.04.

2,3-bis(2-benzamidephenoxymethyl)-6-trifluoromethylquinoxaline (13c) was obtained in 67% yield; m. p. 208-209 °C (methanol).; TLC (chloroform/methanol 98:2): R_f 0.79; UV (EtOH): λ_{max} 204, 236, 272 nm; ^{1}H NMR ($CDCl_3$): δ 10.00 (2H, s, 2 NH), 8.20 (3H, m, H-5 + 2 aromatic-H), 8.01 (1H, d, *J* = 9.0 Hz, H-8), 7.88 (1H, d, *J* = 9.0 Hz, H-7), 7.55-7.08 (16H, m, 16 aromatic-H), 5.75 (2H, s, CH_2O), 5.73 (2H, s, CH_2O). LC/MS: 649 (M+H); *Anal. Calcd. For* $C_{37}H_{27}F_3N_4O_4$ (C,H,N): C, 68.51; H, 4.20; N, 8.64. *Found* C, 68.38; H, 4.24; N, 6.81.

BIOLOGICAL ASSAYS

Compounds

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

Cell Lines

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-tumour-derived cell lines 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 [29].

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