Eur. J. Med. Chem. 35 (2000) 21–30

S0223523400001124/FLA


**Original article**

# New quinoxalinecarbonitrile 1,4-di-N-oxide derivatives as hypoxic-cytotoxic agents

Miguel Ángel Ortega[a], María José Morancho[a], Francisco Javier Martínez-Crespo[a], Yolanda Sainz[a], María Elena Montoya[c], Adela López de Ceráin[b], Antonio Monge[a]*

[a]Department of Medicinal Chemistry, CIFA, Universidad de Navarra, 31080 Pamplona, Spain
[b]Department of Toxicology, CIFA, Universidad de Navarra, 31080 Pamplona, Spain
[c]Faculty of Pharmacy and Biochemistry, Universidad Nacional Mayor de San Marcos, Lima, Peru

Received 21 January 1999; accepted 31 March 1999

**Abstract** – We report the synthesis and biological in vitro activities of 16 new 2-quinoxalinecarbonitrile 1,4-di-N-oxides. These compounds present new basic lateral chains (piperazines and anilines) in the 3 position as well as different substituents in the 6 and/or 7 positions of the quinoxaline ring. Among piperazine derivatives, **4b** (a 7-chloro-3-(4-methylpiperazin-1-yl) derivative) was the most potent ($P = 0.5 \times 10^{-6}$ M). In general, aniline derivatives were more potent and selective than the former, compound **12b** (with a 4-(methylphenyl)amino moiety in the 3 position and a chlorine atom in the 7 position) being the best one ($P = 3 \times 10^{-6}$ M and HCR > 16). © 2000 Éditions scientifiques et médicales Elsevier SAS

**hypoxia-cytotoxicity / quinoxaline 1,4-di-N-oxide / solid tumour / bioreductive activation**

## 1. Introduction

It has been proposed that the hypoxic cells in solid tumours play a negative role in the success of some human antitumour therapies because of their resistance to radiotherapy and conventional chemotherapeutic agents. The presence of hypoxic cells in solid tumours has been demonstrated both in clinical human tumours and human tumour xenografts in rodents by several techniques [1–3]. The hypoxic cellular population resistant to chemotherapy can firstly be explained because the location of these cells in poorly vascularized regions of the tumour renders the access of drugs difficult [4]. Also, many antineoplastic agents have a cytotoxicity dependent on oxygen [5]. Moreover, the hypoxic cells do not follow a normal cellular cycle and, therefore, a low response to cell cycle-specific agents can be expected. On the other hand, the microenvironment surrounding these hypoxic regions (low oxygen pressure, pH and nutrients) may favour the appearance of drug resistance through mutagenic mechanisms [6].

*Correspondence and reprints: amonge@unav.es

In 1972, Sartorelli et al. introduced the concept of bioreductive alkylation: the hypoxic cells in solid tumours, which are living in an environment more inclined towards reductive reactions than those which are well oxygenated, could transform some drugs into cytotoxic species capable of alkylating DNA. These prodrugs would be activated through a reductive mechanism in the absence of oxygen and, for this reason, be more toxic for hypoxic cells than for well-oxygenated ones [7]. Since then, a great number of hypoxic cell cytotoxins have been designed in order to take a therapeutic advantage of the hypoxia.

It has been well established that these selective cytotoxins for hypoxic cells, the so called bioreductive agents, undergo reductive activation by enzyme-catalysed reactions, which are inhibited or reverted by oxygen. Several reductases can mediate these reactions, including the NADPH microsomal enzymes cytochrome P450 reductase and cytochrome P450, as well as the cytosolic enzymes xanthine-oxidase, aldehyde oxidase and DT-diaphorase [8]. The ultimate cell cytotoxin is usually a DNA alkylating agent or, in the case of aromatic N-oxides, a hydrogen-abstracting radical.

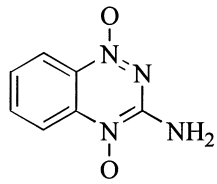
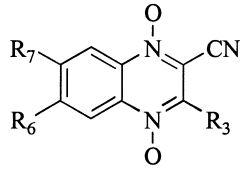


**Figure 1.** WIN 59075 (Tirapazamine).

Several kinds of compounds that are activated under hypoxic conditions are at various stages of development: nitroderivatives, including nitroimidazoles [9], 9-alkylamino-1-nitroacridines [10] and nitroquinolines [11], quinone derivatives [12] (the mitomycin C derivative, E09), and agents derived from 1,2,4-benzotriazine-1,4-di-N-oxide [13] and quinoxaline-1,4-di-N-oxide [14]. Taking the benzotriazine-3-amino-1,2,4-benzotriazine-1,4-dioxide (WIN 59075; Tirapazamine) as structural antecedent, our group is involved in the synthesis and biological evaluation of new agents derived from quinoxaline-1,4-di-N-oxide and related compounds that have proved to be efficient cytotoxic agents for hypoxic cells of solid tumours [14–17]. The structure of WIN 59075 is represented in *figure 1*.

In order to advance in the knowledge of structure–activity relationships, we have explored different amino substituents in position 3 of the quinoxaline ring together with electron-donating and electron-withdrawing groups in position 6 and/or 7. In this paper we report the synthesis and biological studies of 16 new compounds derived from 2-quinoxalinecarbonitrile-1,4-di-N-oxides with selective cytotoxic activity under hypoxic conditions. They are presented with their biological data in *table I*.

**Table I.** New synthesized compounds and biological results.



| Compound | $R_3$ | $R_6$ | $R_7$ | Molecular formula | P[a] | HCR[b] |
|---|---|---|---|---|---|---|
| **4b** | 4-methylpiperazin-1-yl | H | Cl | $C_{14}H_{14}ClN_5O_2 \cdot HCl \cdot 2\ H_2O$ | 0.5 | 8 |
| **5b** | 4-(4-chlorophenyl)piperazin-1-yl | H | Cl | $C_{19}H_{15}Cl_2N_5O_2$ | 8 | 3 |
| **6b** | 4-(2-methoxyphenyl)piperazin-1-yl | H | Cl | $C_{20}H_{18}ClN_5O_3$ | 6 | 2 |
| **6c** | 4-(2-methoxyphenyl)piperazin-1-yl | Cl | H | $C_{20}H_{18}ClN_5O_3$ | L.Y.[c] | L.Y. |
| **7a** | 4-(4-nitrophenyl)piperazin-1-yl | H | H | $C_{19}H_{16}N_6O_4$ | 6 | 1.6 |
| **7b** | 4-(4-nitrophenyl)piperazin-1-yl | H | Cl | $C_{19}H_{15}ClN_6O_4$ | 4 | 5 |
| **7d** | 4-(4-nitrophenyl)piperazin-1-yl | $CH_3$ | $CH_3$ | $C_{21}H_{20}N_6O_4$ | > 20 | N.T[d]. |
| **7e** | 4-(4-nitrophenyl)piperazin-1-yl | $OCH_3$ | Cl | $C_{20}H_{17}ClN_6O_5$ | 2 | > 50 |
| **7f** | 4-(4-nitrophenyl)piperazin-1-yl | H | $OCH_3$ | $C_{20}H_{18}N_6O_5$ | 10 | 7 |
| **8b** | 4-piperonylpiperazin-1-yl | H | Cl | $C_{21}H_{18}ClN_5O_4$ | 6 | 1.5 |
| **8c** | 4-piperonylpiperazin-1-yl | Cl | H | $C_{21}H_{18}ClN_5O_4$ | L.Y. | L.Y. |
| **9b** | 4-(3-trifluoromethylphenyl)piperazin-1-yl | H | Cl | $C_{20}H_{15}ClF_3N_5O_2$ | 7 | 3 |
| **10b** | phenylamino | H | Cl | $C_{15}H_9ClN_4O_2$ | 4 | 10 |
| **11b** | 4-(n-butylphenyl)amino | H | Cl | $C_{19}H_{17}ClN_4O_2$ | 10 | > 10 |
| **12b** | 4-(methylphenyl)amino | H | Cl | $C_{16}H_{11}ClN_4O_2$ | 3 | > 16 |
| **13d** | 3-dimethylamino-1-propylamino | $CH_3$ | $CH_3$ | $C_{16}H_{21}N_5O_2 \cdot HCl \cdot 0{,}3\ H_2O$ | 10 | > 50 |
| **14**[e] | H | H | H | $C_9H_5N_3O_2$ | 5 | > 100 |
| **15**[e] | $NH_2$ | H | H | $C_9H_6N_4O_2$ | 30 | 80 |
| **16**[e] | 3-dimethylamino-1-propylamino | H | H | $C_{14}H_{17}N_5O_2 \cdot HCl$ | 1 | 300 |
| **17**[e] | 2-diethylamino-1-ethylamino | H | H | $C_{15}H_{19}N_5O_2 \cdot HCl \cdot 0.25H_2O$ | 0.9 | 120 |
| **18**[e] | 3-dimethylamino-1-propylamino | H | $CH_3$ | $C_{15}H_{19}N_5O_2 \cdot HCl$ | 1 | 300 |
| WIN 59075[e] | | | | $C_7H_6N_4O_2$ | 30 | 75 |

[a]Potency = dose in micromolar which gives 1% of control cell survival in hypoxia. [b]HCR (hypoxic cytotoxicity ratio) = the dose in air divided by the dose in hypoxia giving 1% of control cell survival. [c]Low Yield = a sufficient amount was not obtained for the biological assays, only for their identification. [d]N.T. = not tested; 1% cell survival under hypoxic conditions was not reached in the screening assay (at 20 μM). [e]See reference [14].

## 2. Chemistry

Several ideas have been obtained from previous studies of structure–activity relationships: the cyano moiety in the 2 position seems to be necessary for the cytotoxic activity; the presence of an electron-withdrawing substituent in the 7(6) position, e.g. Cl, $CF_3$ or F, increases the potency under hypoxic conditions of all of the series studied [17] and electron-donating substituents, such as $CH_3$ and $OCH_3$ in the 6 and/or 7 position decreased, in general, the potency but had a good effect on selectivity of deaminated compounds [15]. With respect to the amine in the 3 position, although it is not necessary for hypoxic cytotoxicity, it has been demonstrated that the nature of this amine can exert a very strong influence on the potency and HCR parameters [15, 17].

According to these conclusions, we intended to study the modulation of the activity through the introduction of different piperazines in the 3 position of the 2-quinoxalinecarbonitrile-1,4-di-N-oxide ring. The introduction of aromatic amines in this position had not been studied to date, so we decided to also employ aniline derivatives with the aim of studying the modification of the cytotoxic activity. We found difficulties in preparing the 7(6)-$CF_3$ derivatives, so we chose the Cl substituent for the mentioned position. Electron-donating substituents such as $CH_3$ and $OCH_3$ were also chosen for the 6 and 7 positions. On the other hand, **13d**, a 6,7-dimethyl derivative with a 3-dimethylamino-1-propylamino chain at the 3 position, was prepared because other quinoxaline 1,4-di-N-oxides with this substituent have been shown to be interesting in in vitro profiles [15].

In this paper, a total of 16 new compounds are reported. The synthesis was carried out as shown in *figure 2*. Compounds **1a–1f** were formed from the corresponding commercial substituted anilines or 2-nitroanilines [14, 16]. Reaction of **1a–1f** with malononitrile in the presence of triethylamine as condensing base [18–21] in DMF at 0 °C afforded the **2a–2f** derivatives.

In order to obtain compounds **3a–3f**, attempts to replace the amino group in **2a–2f** by chlorine using the conventional Sandmeyer reaction were unsuccessful, probably due to the basicity of that group being decreased and because of the low solubility of the compounds. However, 3-amino-2-quinoxalinecarbonitrile without N-oxides were readily converted into the 3-chloro derivatives [22]. So compounds **3a–3f** were synthesized by reaction of the corresponding 3-amino-2-quinoxalinecarbonitrile 1,4-di-N-oxide derivative with anhydrous cooper(II) chloride and tert-butyl nitrite in dry acetonitrile, heating at 80–85 °C under nitrogen atmosphere for 2–3h [15, 23]. As noted, **3a–3f** have all been reported before by this method [15].

Amines **4b–9b** were obtained by reaction of the corresponding 3-chloroquinoxaline derivative with the appropriate piperazine in dry chloroform or dichloromethane, in the presence of $K_2CO_3$ in order to facilitate the reaction. The nucleophilic substitution of the chlorine in an aryl halide, by a nucleophilic reagent such as amine, may occur if the former is activated: the chlorine or fluorine in the 6 and/or 7 position in the quinoxaline ring has never been replaced by a nucleophilic reagent, while the same group in the 3 position is readily replaced due to the presence of the contiguous N-oxide and cyano moieties.

As is well known, in principle, isomer-position mixtures of the quinoxaline-1,4-di-N-oxide derivatives can be formed from unsymmetrically substituted benzofuroxanes, with the 7-isomer being more abundant than the 6 one [19]. In practice, the workup and purification by flash chromatography allows isolation of the former, while the latter one is usually discarded. In this work, the minor isomer was obtained only with compounds **6** and **8**. We were able to prove that the proportion of the minor isomer was certainly smaller than that of the major one: 18.2% for **6** and 6.2% for **8**. In the case of compound **8**, the quantity of **8c** obtained was not enough to carry out elemental analyses, so we report infrared and $^1$H-NMR spectroscopy results for this 6-isomer. On the other hand, in order to assign the greater fraction to the 7-isomer and the minor to the 6-isomer, $^1$H-NMR spectral data was studied. The electron-donating character of the piperazine may influence the intensity of the nuclear magnetic resonance field and consequently the corresponding chemical shifts of the hydrogens in the 6 and 7 positions. The hydrogen in the 7 position generated a signal which appeared at higher field than that of the hydrogen in the 6 position; in this way the major fraction obtained from the chromatographic column was related to the 7-isomer, and the minor one with the 6-isomer. Sufficient quantities of both 6-isomers could be obtained for their identification, but not for the biological assays. Compounds in this group of piperazine derivatives were obtained as free bases, and only **4b** was prepared as a hydrochloride salt by dissolving the free base in acetone and adding a few drops of concentrated hydrochloric acid. All of them were obtained in low yields: from 24% for the compound **4b** which contains the more activated piperazine (4-methylpiperazine) to 1% for the compound **8c** which is bearing a 4-piperonylpiperazine in the 3 position of the quinoxaline ring. All of these piperazine derivatives were found to be hygroscopic and very photosensitive (much more than the other ones described in this paper) and

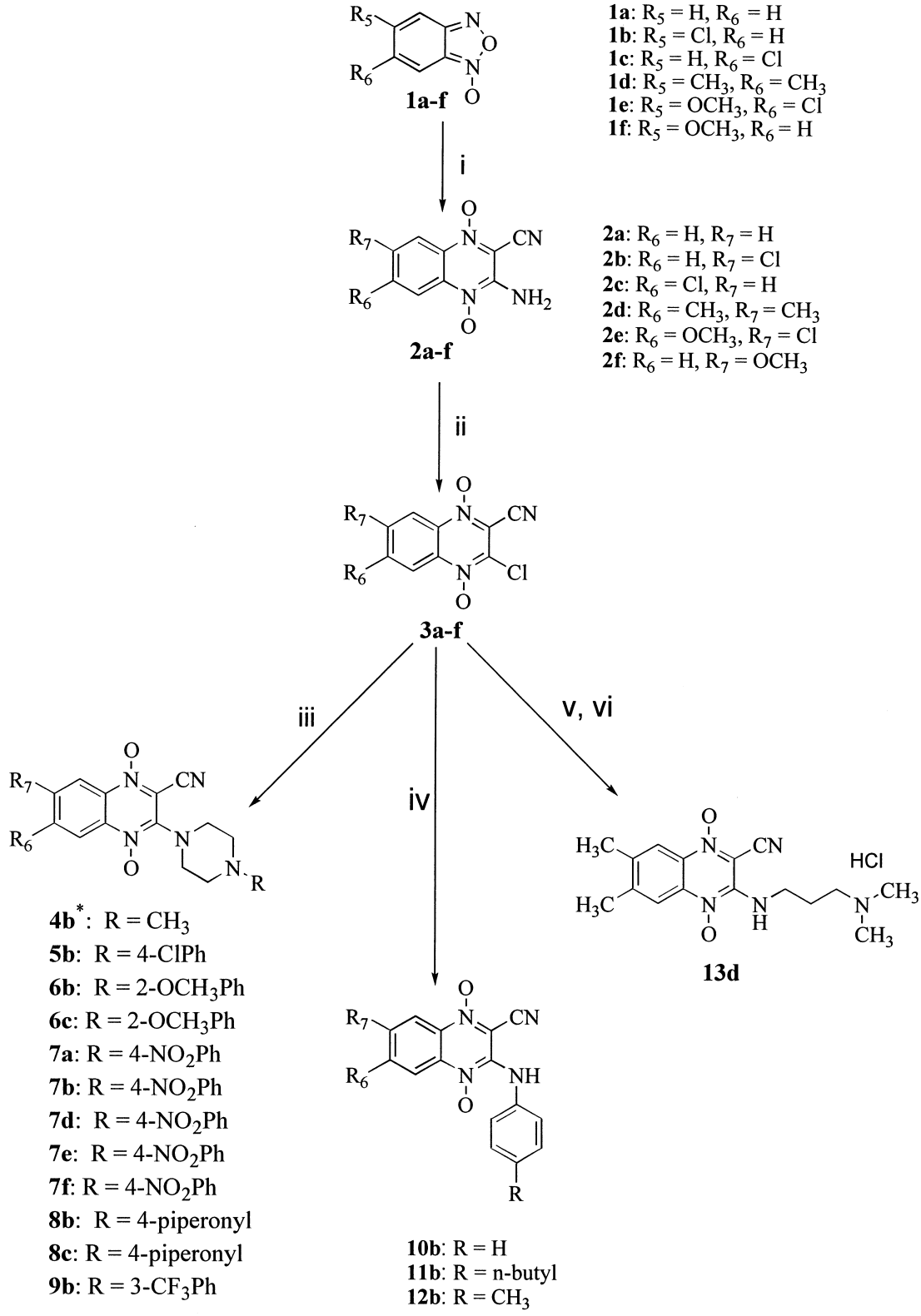



**Figure 2.** Synthetic route for compounds **4b–13d.** Conditions: (i) malononitrile, triethylamine, DMF, 0 °C; (ii) tert-butylnitrite, acetonitrile, $CuCl_2$, nitrogen atmosphere, 80–85 °C; (iii) 4-substituted piperazine, $K_2CO_3$, chloroform or dichlorometane (according to each compound), room temperature; (iv) 4-substituted aniline, $K_2CO_3$, chloroform, room temperature; (v) 3-dimethylamino-1-propylamine, $K_2CO_3$, chloroform, room temperature; (vi) concentrated HCl, dry acetone. *As hydrochloride salt (see vi).

therefore must be kept from moisture and sunlight. For example, we observed how, in a few hours, a solution of compound **7b** turned from intense red to greenish yellow under light exposure. This fact is in agreement with some results regarding photoreactivity of heteroaromatic N-oxides, referred to in some studies [21, 24, 25].

The preparation of compound **13d** was carried out from 3-chloro-6,7-dimethyl-2-quinoxalinecarbonitrile-1,4-di-N-oxide **3d**, which was reacted in darkness with 3-dimethylamino-1-propylamine in dry chloroform. $K_2CO_3$ was added in order to facilitate the aromatic nucleophilic substitution.

Anilines were less reactive than aliphatic amines in this reaction. As previously reported [26], reaction with aniline itself and analogues *para*-substituted with activating methyl or n-butyl groups gave compounds **10b–12b**, whereas anilines bearing deactivating electron-withdrawing groups (*para*-nitro, *para*-chloro and *para*-phenyl) did not react.

## 3. Pharmacology

The quinoxaline derivatives were tested in a cloning assay using V79 cells. Suspension cultures were established from exponentially growing cells and gassed with pure air or nitrogen for 30 min before dosing with the compounds. Treatment lasted 2 h and gassing was continuous during this time. All of the compounds were tested at 20 μM in duplicate flasks in both air and nitrogen. The compounds that were more toxic in hypoxia than in air were tested at different doses to obtain a dose–response curve in air and hypoxia. The potency was defined as the dose which gives 1% of control cell survival in hypoxia. The hypoxic cytotoxicity ratio (HCR) was calculated by dividing the dose in air by the dose in hypoxia giving 1% of control cell survival.

In a previous work we reported some 2-quinoxalinecarbonitrile-1,4-di-N-oxides bearing a 3-dimethylamino-1-propylamino lateral chain to be very potent and selective cytotoxins [15]. They were toxic for hypoxic cells at $10^{-6}$ M and between 170-fold and 300-fold higher doses were needed to have the same effect in well-oxygenated cells. Both potency and selectivity had been largely improved with respect to the 3-amino **15** and the deaminated **14** analogues (*table I*). In an attempt to explore other basic substituents at the 3 position of the quinoxaline ring some new derivatives have been synthesized and tested.

Among the 3-[4-(4-nitrophenyl)piperazin-1-yl] derivatives **7a–7f**, compound **7a**, with no substituent in positions 6 and 7, is more potent ($P = 6 \times 10^{-6}$ M) than the corresponding 3-amino analogue **15** ($P = 30 \times 10^{-6}$ M), but less potent than the deaminated analogue **14** ($P = 5 \times 10^{-6}$ M) and the 3-(3-dimethylamino-1-propylamino) **16** ($P = 1 \times 10^{-6}$ M) and 3-(2-diethylamino-1-ethylamino) **17** ($P = 0.9 \times 10^{-6}$ M) derivatives; besides, its selectivity is very low (HCR = 1.6). The introduction of a chlorine atom in position 7 (**7b**) slightly improves the selectivity (HCR = 5), and the introduction of an electron-donating group, such as $CH_3$ (**7d**) or $OCH_3$ (**7f**) notably decreases hypoxic cytotoxicity. In this series, the best in vitro profile is achieved with compound **7e**, with a Cl in position 7 and an $OCH_3$ in position 6, being the most potent and selective compound ($P = 2 \times 10^{-6}$ M, HCR > 50).

Among the other piperazine derivatives, all of them with a chlorine in position 7 (**4b**, **5b**, **6b**, **8b** and **9b**), compound **4b** shows the best in vitro profile: $P = 0.5 \times 10^{-6}$ M, HCR = 8. Aniline derivatives **10b–12b** are less potent (between 3 and $10 \times 10^{-6}$ M), but more selective than the former (HCR = 10). Selectivity of compounds **11b** and **12b** could not be accurately determined because at doses higher than 20 μM these two compounds precipitated in the culture medium. Finally, compound **13d**, with two methyl groups in positions 6 and 7, is less potent ($P = 10 \times 10^{-6}$ M) than the monosubstituted $CH_3$ analogue **18** ($P = 1 \times 10^{-6}$ M), thus confirming the hypothesis that the presence of a same electron-donating group both in 6 and 7 positions decreases the hypoxic toxicity of these compounds more than the presence of only one substituent.

## 4. Results and discussion

With the aim of improving the hypoxia-selective cytotoxicity of previous 2-quinoxalinecarbonitrile-1,4-di-N-oxides, 16 new quinoxaline derivatives have been prepared bearing 9 new amines (which had not been used to date) in the 3 position of the aromatic ring. Among the piperazine derivatives, compound **7e** bearing 4-(4-nitrophenyl)piperazin-1-yl, a methyl group and a chlorine atom at the 3, 6 and 7 positions, respectively, showed the best in vitro profile: Potency = $2 \times 10^{-6}$ M and HCR = 50 (only compound **4b** bearing 4-methylpiperazin-1-yl at the 3 position and a chlorine in the 7 position, had a potency better than that of the former ($0.5 \times 10^{-6}$ M). Derivatives **7a–7f** bearing the same amine (1-(4-nitrophenyl)piperazine) exhibit different potency values depending on the substituents at 6 and/or 7 position (thus, compounds **7b** and **7e** were the most potent, and **7d** and **7f** the less potent. This is in agreement with what has been said about the influence of the substituents nature on the potency: mild electron-withdrawing groups in the 6(7)

position, e.g. Cl (**7b** and **7e**) increase potency under hypoxic conditions, while electron-donating substituents, e.g. $CH_3$ (**7d**) and $OCH_3$ (**7f**), decrease it.

With respect to aniline derivatives, compounds **10b** and **12b** showed a similar potency and both of them were better than that of compound **11b**, suggesting that aliphatic groups greater than $CH_3$ in the '*para*' position of the aniline, decrease the potency.

Compound **13d** showed a potency lower than its monomethyl analogue **18**, thus confirming, once again, that electron-donating groups at the 6(7) position decrease the hypoxic cytotoxicity.

In short, we can assert that the new compounds are more potent than tirapazamine but less selective than the previously synthesized **14**, **15**, **16**, **17** and **18** derivatives.

## 5. Conclusions

With these results and the previously published in vitro profiles from many other quinoxalines [14, 15] we can conclude that these new 2-quinoxalinecarbonitrile-1,4-di-N-oxides are relatively potent but not as selective as they were expected to be. The low selectivity is thought to be due to the presence of aromatic rigid moieties (anilines and arylpiperazines) at the 3 position of the quinoxaline ring. This type of amine is quite different from the aliphatic (N,N-dialkylamino)alkylamino chains borne by the previously synthesized **16**, **17** and **18** derivatives that exhibited the best P and HCR values.

Preparation of new 2-quinoxalinecarbonitrile-1,4-di-N-oxides with other amines, with either aromatic rigid systems or non-rigid moieties at the 3 position of the quinoxaline ring, would be of great interest in order to verify if the low selectivities are due to the presence of aromatic rigid moieties.

## 6. Experimental protocols

### *6.1. Chemistry*

#### *6.1.1. General*

Melting points were determined using a Mettler FP82+FP80 apparatus and are uncorrected. Elemental analyses were obtained from vacuum-dried samples (over phosphorus pentoxide at 3–4 mm Hg, 24 h, at ca. 80–100 °C). Infrared spectra were recorded on a Perkin-Elmer 681 apparatus, using potassium bromide tablets for the preparation of the samples; the frequencies are expressed in $cm^{-1}$. The $^1$H-NMR spectra were obtained on a Brucker AC-200E (200 MHz) instrument, using tetramethylsilane as the internal reference, at a concentration of ca. 0.1 g/mL and with dimethylsulfoxide-$d_6$ (DMSO-$d_6$) as the solvent; the chemical shifts are reported in parts per million (ppm) of tetramethylsilane in δ units, and the *J* values are given in hertz (Hz). The mass spectra were recorded on a Hewlett-Packard 5988-A instrument at 70 eV.

Thin-layer chromatography (TLC) was carried out on silica gel (DSF-5, Cammaga 0.3 mm thickness), with the following solvents: dichloromethane for compounds **4b–9b** and ethyl acetate for compounds **10b–12b**; the plates were scanned under ultraviolet light at 254 and 366 nm. Column chromatography was carried out with Merck silica gel 60 (70–230 mesh ASTM). Elemental analyses were performed on a Carlo-Erba 1106 Instrument and are within the calculated values ± 0.4%, except where otherwise stated.

Compounds **1a–1f** and **2a–2f** were previously synthesized and published [14, 16], and also compounds **3a–3f** [15]. In the same way, compounds **10b–12b** have been previously synthesized and published as antituberculosis agents [26].

#### *6.1.2. Synthesis procedures*

##### *6.1.2.1. 7-Chloro-3-(4-methylpiperazin-1-yl)-2-quinoxalinecarbonitrile-1,4-di-N-oxide hydrochloride* **4b**

A mixture of **3b** and **3c** (0.53 g, 2.07 mmol), $K_2CO_3$ (0.30 g, 2.17 mmol) and 1-methylpiperazine (0.21 g, 2.10 mmol) in dry dichloromethane was stirred at room temperature, in darkness, for 4 days. After filtering off to separate inorganic salts and evaporating under reduced pressure, the residue was purified by chromatography, eluting with a dichloromethane/ethyl acetate/methanol gradient. First, the 6-isomer appeared and then the 7-isomer. The 6-isomer was not obtained in enough quantity for its identification. The 7-isomer was dissolved in dry acetone. Addition of a few drops of concentrated HCl afforded a red precipitate which was recrystallized from ethyl acetate/methanol: 24% yield; m.p. 187–189 °C; IR (KBr) 3 097, 2 576, 2 237, 1 328 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) δ 2.51 (s, 3H, $CH_3$), 2.84 (s, 4H, $2CH_2$ piperazine), 3.80 (s, 4H, $2CH_2$ piperazine), 8.13 (d, 1H, $H_6$), 8.43–8.48 (m, 2H, $H_5$, $H_8$); 11.45 (b.s., 1H, HCl). Ms (EI, 70 eV); m/z (percentage of relative abundance): 302 (50.49), 285 (23.51), 70 (100.00). Anal. ($C_{14}H_{14}ClN_5O_2 \cdot HCl \cdot 2H_2O$) C, H, N; N: calcd., 17.85; found, 17.43.

##### *6.1.2.2. 7-Chloro-3-[4-(4-chlorophenyl)piperazin-1-yl]-2-quinoxalinecarbonitrile-1,4-di-N-oxide* **5b**

A mixture of **3b** (0.19 g, 0.74 mmol), $K_2CO_3$ (0.17 g, 1.26 mmol) and 1-(4-chlorophenyl)piperazine (0.17 g, 0.89 mmol) in dry chloroform was stirred at room tem-

perature, in darkness, for 4 weeks. After filtering off to separate inorganic salts and washing with dichloromethane, the liquid was evaporated to dryness, and the residue was purified by flash chromatography, eluting with a dichloromethane/ethyl acetate gradient. The obtained fractions were concentrated under reduced pressure and recrystallized from dry acetone: 8% yield; m.p. 186–187 °C; IR (KBr) 3 070, 2 230, 1 324 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) δ 3.32 (s, 4H, $2CH_2$ piperazine), 3.61 (s, 4H, $2CH_2$ piperazine), 7.02 (d, $J$ = 8.8 Hz, 2H, $H_{2'}$, $H_{6'}$), 7.25 (d, $J$ = 8.7 Hz, 2H, $H_{3'}$, $H_{5'}$), 8.04 (dd, $J_1$ = 9.1 Hz, $J_2$ = 1.6 Hz, 1H, $H_6$), 8.39–8.43 (m, 2H, $H_5$, $H_8$). Ms (EI, 70 eV); m/z (percentage of relative abundance): 399 (7.54), 382 (19.58), 243 (18.62). Anal. ($C_{19}H_{15}Cl_2N_5O_2$) C, H, N; C: calcd., 54.81; found, 55.26.

*6.1.2.3. 7(6)-Chloro-3-[4-(2-methoxyphenyl)piperazin-1-yl]-2-quinoxalinecarbonitrile-1,4-di-N-oxide* **6b** *and* **6c**

A mixture of **3b** and **3c** (1.22 g, 4.76 mmol), $K_2CO_3$ (0.99 g, 7.17 mmol) and 1-(2-methoxyphenyl)piperazine (0.96 g, 5 mmol) in dry chloroform was stirred at room temperature, in darkness, for 4 weeks. After filtering off to separate inorganic salts and washing with dry chloroform, the liquid was evaporated and the obtained residue was purified by flash chromatography eluting with distilled chloroform. First, the 6-isomer **6c** appeared and then the 7-isomer **6b**, which was the most abundant. After concentrating to dryness, the reaction products were obtained. The 7-isomer was recrystallized from acetone/methanol. **6b**: 11% yield; m.p. 195 °C; IR (KBr) 3 100, 2 250, 1 238 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) δ 3.16 (s, 4H, $2CH_2$ piperazine), 3.65 (s, 4H, $2CH_2$ piperazine), 3.83 (s, 3H, $OCH_3$), 6.96–6.99 (m, 4H, Ar), 8.07 (d, $J$ = 9.6 Hz, 1H, $H_6$), 8.41 (s, 1H, $H_8$), 8.45 (d, $J$ = 9.6 Hz, 1H, $H_5$). Ms (EI, 70 eV); m/z (percentage of relative abundance): 395 (9.82), 394 (14.18), 377 (19.41), 190 (7.41), 162 (100.00). Anal. ($C_{20}H_{18}ClN_5O_3$) C, H, N. **6c**: 2% yield; m.p. 146–147 °C; IR (KBr) 3 100, 2 250, 1 238 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) δ 3.16 (s, 4H, $2CH_2$ piperazine), 3.66 (s, 4H, $2CH_2$ piperazine), 3.82 (s, 3H, $OCH_3$), 6.95–6.97 (m, 4H, Ar), 7.91 (dd, $J_1$ = 9.4 Hz, $J_2$ = 1.4 Hz, 1H, $H_7$), 8.38 (d, $J$ = 9.4 Hz, 1H, $H_8$), 8.43 (d, $J$ = 1.3 Hz, 1H, $H_5$). Ms (EI, 70 eV); m/z (percentage of relative abundance): spectrum not available. Anal. ($C_{20}H_{18}ClN_5O_3$) C, H, N; H: calcd., 4.37; found, 4.98; N: calcd, 17.01; found, 15.62.

*6.1.2.4. 3-[4-(4-Nitrophenyl)piperazin-1-yl]-2-quinoxalinecarbonitrile-1,4-di-N-oxide* **7a**

A mixture of **3a** (0.7 g, 3.16 mmol), $K_2CO_3$ (0.64 g, 4.7 mmol) and 1-(4-nitrophenyl)piperazine (1.4 g, 6.76 mmol) in chloroform was stirred at room temperature (the appearance of an orange colour was observed in a few minutes), in darkness, for 12 days. After filtering off to separate inorganic salts and washing with chloroform, the liquid was evaporated to dryness and the obtained residue was chromatographed, eluting with a chloroform/ethyl acetate gradient. When solvents were eliminated under reduced pressure, a new solid was obtained. Recrystallization from ethyl acetate/dichloromethane afforded the target compound **7a**: 14% yield; m.p. 217–219 °C; IR (KBr) 3 412, 1 525, 1 376, 1 312 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) δ 3.65 (s, 4H, $2CH_2$ piperazine), 3.70 (s, 4H, $2CH_2$ piperazine), 7.13 (d, $J$ = 9.3 Hz, 2H, $H_{2'}$, $H_{6'}$), 7.90 (t, $J$ = 7.8 Hz, 1H, $H_7$), 8.04 (t, $J$ = 8.4 Hz, 1H, $H_6$), 8.10 (d, $J$ = 9.1 Hz, 2H, $H_{3'}$, $H_{5'}$), 8.38–8.47 (m, 2H, $H_5$, $H_8$). Ms (EI, 70 eV); m/z (percentage of relative abundance): 376 (12.12), 177 (100.00). Anal. ($C_{19}H_{16}N_6O_4$) C, H, N.

*6.1.2.5. 7-Chloro-3-[4-(4-nitrophenyl)piperazin-1-yl]-2-quinoxalinecarbonitrile-1,4-di-N-oxide* **7b**

A mixture of **3b** (0.88 g, 3.43 mmol), $K_2CO_3$ (0.5 g, 3.62 mmol) and 1-(4-nitrophenyl)piperazine (0.7 g, 3.38 mmol) in dry chloroform was stirred at room temperature, in darkness, for 4 weeks. After filtering off to separate inorganic salts and washing with chloroform, the obtained orange-red solution was evaporated to dryness and the residue was chromatographed, eluting with a chloroform/ethyl acetate gradient. The new solid (very hygroscopic) was dried under reduced pressure at 50 °C in the presence of $P_2O_5$. Recrystallization from dioxane afforded a dark garnet solid **7b**: 8% yield; m.p. 170 °C; IR (KBr) 3 100, 1 599, 1 324, 818 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) δ 3.63 (s, 4H, $2CH_2$ piperazine), 3.70 (s, 4H, $2CH_2$ piperazine), 7.14 (d, $J$ = 9.0 Hz, 2H, $H_{2'}$, $H_{6'}$), 8.08–8.12 (m, 3H, $H_{3'}$, $H_{5'}$, $H_6$), 8.42–8.47 (m, 2H, $H_5$, $H_8$). Ms (EI, 70 eV); m/z (percentage of relative abundance): 410 (3.32), 393 (12.61), 177 (100.00). Anal. ($C_{19}H_{15}ClN_6O_4$) C, H, N; N: calcd., 19.69; found, 19.27.

*6.1.2.6. 6,7-Dimethyl-3-[4-(4-nitrophenyl)piperazin-1-yl]-2-quinoxalinecarbonitrile-1,4-di-N-oxide* **7d**

A mixture of **3d** (0.3 g, 1.20 mmol), $K_2CO_3$ (0.49, 3.6 mmol) and 1-(4-nitrophenyl)piperazine (0.9 g, 4.34 mmol) in chloroform was stirred at room temperature, in darkness, for 12 days. After filtering off to separate inorganic salts, the remaining liquid was concentrated under reduced pressure and the residue was purified by flash chromatography, eluting with a dichloromethane/ethyl acetate gradient. All the fractions were monitored by TLC and those of them which resulted of interest were concentrated giving a new residue.

Recrystallization from dichloromethane afforded a dark orange solid **7d**: 19% yield; m.p. 232–235 °C; IR (KBr) 3 423, 2 226, 1 520, 1 354, 1 317 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) δ 3.34 (s, 6H, $2CH_3$), 3.60 (s, 4H, $2CH_2$ piperazine), 3.68 (s, 4H, $2CH_2$ piperazine), 7.12 (d, $J$ = 8.5 Hz, 2H, $H_{2'}$, $H_{6'}$), 8.09 (d, $J$ = 8.9 Hz, 2H, $H_{3'}$, $H_{5'}$), 8.17 (s, 1H, $H_5$), 8.22 (s, 1H, $H_8$). Ms (EI, 70 eV); m/z (percentage of relative abundance): 404 (26.30), 403 (45.96), 388 (13.63), 206 (6.42), 177 (100.00). Anal. ($C_{21}H_{20}N_6O_4$) C, H, N; N: calcd., 20.00; found, 18.35.

*6.1.2.7. 7-Chloro-6-methoxy-3-[4-(4-nitrophenyl)piperazin-1-yl]-2-quinoxalinecarbonitrile-1,4-di-N-oxide* **7e**

A mixture of **3e** (0.46 g, 1.60 mmol), $K_2CO_3$ (0.55 g, 3.97 mmol) and 1-(4-nitrophenyl)piperazine (1.00 g, 4.83 mmol) in chloroform was stirred at room temperature, in darkness, for 1 month. After filtering off the reaction mixture to separate inorganic salts and washing with plenty of chloroform, the solvent was eliminated under low pressure; then, the obtained residue was purified by flash chromatography, eluting with a chloroform/ethyl acetate gradient. The fractions were concentrated to dryness and recrystallized from dioxane, affording a precipitate **7e**: 15% yield; m.p. 232–235 °C; IR (KBr) 3 423, 3 081, 2 226, 1 386, 1 322 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) δ 3.56–3.62 (m, 4H, $2CH_2$ piperazine), 3.68–3.70 (m, 4H, $2CH_2$ piperazine), 4.09 (s, 3H, $OCH_3$), 7.10 (d, $J$ = 9.4 Hz, 2H, $H_{2'}$, $H_{6'}$), 7.79 (s, 1H, $H_8$), 8.07 (d, $J$ = 9.3 Hz, 2H, $H_{3'}$, $H_{5'}$), 8.45 (s, 1H, $H_5$). Ms (EI, 70 eV); m/z (percentage of relative abundance): 439 (20.09), 423 (32.62), 247 (16.94), 177 (100.00). Anal. ($C_{20}H_{17}ClN_6O_5$) C, H, N.

*6.1.2.8. 7-Methoxy-3-[4-(4-nitrophenyl)piperazin-1-yl]-2-quinoxalinecarbonitrile-1,4-di-N-oxide* **7f**

A mixture of **3f** (0.17 g, 0.67 mmol), $K_2CO_3$ (0.12, 0.93 mmol) and 1-(4-nitrophenyl)piperazine (0.42 g, 2.02 mmol) in chloroform was stirred at room temperature, in darkness, for 22 days. After filtering off to separate inorganic salts, the remaining liquid was evaporated under low pressure and the residue was chromatographed, eluting with a toluene/ethyl acetate gradient. The fractions were concentrated and recrystallized from dioxane, affording the target compound **7f**: 17% yield; m.p. 218–220 °C; IR (KBr) 3 448, 3 112, 1 508, 1 395, 1 333 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) δ 3.56–3.57 (m, 4H, $2CH_2$ piperazine), 3.66–3.69 (m, 4H, $2CH_2$ piperazine), 3.98 (s, 3H, $OCH_3$), 7.11 (d, $J$ = 9.2 Hz, 2H, $H_{2'}$, $H_{6'}$), 7.62 (s, 1H, $H_8$), 7.65 (d, $J$ = 8.4 Hz, 1H, $H_6$), 8.08 (d, $J$ = 9.1 Hz, 2H, $H_{3'}$, $H_{5'}$), 8.34 (d, $J$ = 9.0 Hz, 1H, $H_5$). Ms (EI, 70 eV); m/z (percentage of relative abundance): 406 (18.59), 389 (46.30), 213 (58.31), 177 (100.00). Anal. ($C_{20}H_{18}N_6O_5$) C, H, N.

*6.1.2.9. 7(6)-Chloro-3-[(4-piperonil)piperazin-1-yl]-2-quinoxalinecarbonitrile-1,4-di-N-oxide* **8b** *and* **8c**

A mixture of **3b** and **3c** (0.62 g, 2.42 mmol), $K_2CO_3$ (0.4 g, 2.89 mmol) and 1-piperonilpiperazine (0.66 g, 2.99 mmol) in dry chloroform was stirred at room temperature, in darkness, for 20 days. After filtering off to separate inorganic salts, the liquid was evaporated; then, the residue was chromatographed, eluting with a dichloromethane/ethyl acetate gradient. First, the 6-isomer appeared and then the 7-isomer which was the most abundant. After evaporating under low pressure, the 7-isomer was recrystallized from dioxane/diethyl ether. **8b**: 16% yield; m.p. 175 °C; IR (KBr) 1 331, 1 246 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) δ 2.50 (s, 6H, $2CH_2$ piperazine, N–$CH_2$–Ar), 3.47 (s, 4H, $2CH_2$ piperazine), 5.99 (s, 2H, $OCH_2O$), 6.81–6.91 (m, 3H, piperonil), 8.04 (dd, $J$ = 9.2 Hz, 1H, $H_6$), 8.37–8.42 (m, 2H, $H_5$, $H_8$). Ms (EI, 70 eV); m/z (percentage of relative abundance): 423 (1.37), 422 (1.36), 191 (0.85), 135 (100.00). Anal. ($C_{21}H_{18}ClN_5O_4$) C, H, N. **8c**: 1% yield; m.p. 170 °C; IR (KBr) 2 229, 1 331, 1 246 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) δ 2.50 (s, 6H, $2CH_2$ piperazine, N–$CH_2$–Ar), 3.47 (s, 4H, $2CH_2$ piperazine), 6.00 (s, 2H, $OCH_2O$), 6.77–6.91 (m, 3H, piperonil), 7.91 (d, 1H, $H_7$), 8.37 (m, 2H, $H_5$, $H_8$). Ms (EI, 70 eV); m/z (percentage of relative abundance): spectrum not available.

*6.1.2.10. 7-Chloro-3-[4-(3-trifluoromethylphenyl)piperazin-1-yl]-2-quinoxalinecarbonitrile-1,4-di-N-oxide* **9b**

A mixture of **3b** (0.72 g, 2.81 mmol), $K_2CO_3$ (0.5 g, 3.62 mmol) and 1-(3-trifluoromethylphenyl)piperazine hydrochloride (0.8 g, 3.00 mmol) in dry chloroform was stirred at room temperature, in darkness, for 2 weeks. After filtering off to separate inorganic salts the red solution was evaporated and the residue was purified by flash chromatography, eluting with an n-hexane/dichloromethane gradient. After evaporating to dryness, a solid was obtained which was precipitated by adding diethyl ether, affording the target compound **9b**: 8% yield; m.p. 101 °C; IR (KBr) 3 100, 2 230, 1 327, 1 122 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) δ 3.46 (s, 4H, $2CH_2$ piperazine), 3.65 (s, 4H, $2CH_2$ piperazine), 7.12 (d, $J$ = 7.2 Hz, 1H, $H_{6'}$), 7.28–7.33 (m, 2H, $H_{2'}$, $H_{4'}$), 7.46 (t, $J$ = 7.6 Hz, 1H, $H_{5'}$), 8.06 (d, $J$ = 8.6 Hz, 1H, $H_6$), 8.41–8.46 (m, 2H, $H_5$, $H_6$). Ms (EI, 70 eV); m/z (percentage of relative abundance): 433 (11.43), 432 (31.76), 415 (23.08), 228 (19.92). Anal. ($C_{20}H_{15}ClF_3N_5O_2$) C, H, N.

*6.1.2.11. 6,7-Dimethyl-3-[3-(dimethylamino)-1-propylamino]-2-quinoxalinecarbonitrile-1,4-di-N-oxide hydrochloride* **13d**

$K_2CO_3$ (0.10 g, 0.72 mmol) and 3-dimethylamino-1-propylamine (12 drops) were added (the appearance of a strong red colour is immediately observed) to a solution of **3d** (0.60 g, 2.40 mmol) in dry chloroform. The mixture was stirred at room temperature, in darkness, for 5 days. After filtering off to separate inorganic salts and evaporating under reduced pressure, a red oil was obtained which was purified by flash chromatography, eluting with a dichloromethane/ethyl acetate/methanol gradient. All of the fractions were monitored by TLC and the one corresponding to the desired product was concentrated to dryness. The new residue was dissolved in dry acetone and concentrated hydrochloric acid was added (4 drops), whereby a red precipitate appeared which was filtered and recrystallized from acetone/methanol, affording a red scaly solid **13d**: 23% yield; m.p. 197–198 °C; IR (KBr) 3 242, 2 955, 2 598, 1 349 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$) δ 2.09 (m, 2H, C–$CH_2$–C), 2.42 (s, 3H, $CH_3$Ar), 2.47 (s, 3H, $CH_3$Ar), 2.74 (s, 6H, N($CH_3$)$_2$), 3.10–3.16 (m, 2H, –$CH_2$–$N^+$), 3.74–3.77 (m, 2H, NH–$CH_2$–), 8.06 (s, 2H, $H_5$, $H_8$), 8.37 (t, 1H, ArNH), 10.61 (s, 1H, HCl). Ms (EI, 70 eV); m/z (percentage of relative abundance): 315 (0.94, $M^{+\cdot}$), 298 (2.01), 58 (100.00). Anal. ($C_{16}H_{21}N_5O_2$·HCl·0.3$H_2O$) C, H, N.

*6.2. Pharmacology*

*6.2.1. Cells*

V79 cells (Chinese hamster lung fibroblasts) were obtained from ECACC (European Collection of Animal Cell Cultures) and maintained in logarithmic growth as subconfluent monolayers by trypsinization and subculture to 1–2 × $10^4$ cells/$cm^2$ twice weekly. The growth medium was EMEM (Eagle's minimal essential medium), containing 10% (v/v) foetal bovine serum (FBS) and penicillin/streptomycin at 100 U/100 μg/mL.

*6.2.2. Aerobic and hypoxic cytotoxicity: suspension cultures*

Monolayers of V79 cells in exponential growth were trypsinized, and suspension cultures were set up in 50 mL glass flasks: 2 × $10^4$ cells/mL in 30 mL of EMEM containing 10% (v/v) FBS and HEPES (10 mM). The glass flasks were stoppered with rubber caps perforated with two 19G needles to provide gas inlet and outlet. The flasks were submerged and stirred in a water bath at 37 °C where they were gassed with humidified air or pure nitrogen.

*6.2.3. Treatment*

Compound solutions were prepared just before dosing. Stock solutions, 150-fold more concentrated, were prepared in pure dimethyl sulfoxide (DMSO) or sterilized distilled water. Thirty minutes after the start of gassing, 0.2 mL of the stock compound solution was added to each flask, two flasks per dose. In every assay there was one flask with 0.2 mL of DMSO (negative control).

*6.2.4. Cloning*

After 2 h exposure to the compound the cells were centrifuged and resuspended in plating medium (EMEM plus 15% (v/v) FBS and penicillin/streptomycin). Cell numbers were determined with a haemocytometer and $10^2$–$10^3$ cells were plated in 6-well plates to give a final volume of 2 mL/30 mm of well. Plates were incubated at 37 °C in 5% $CO_2$ for 7 days and then stained with aqueous crystal violet. Colonies with more than 64 cells were counted. The plating efficiency (PE) was calculated by dividing the number of colonies by the number of cells seeded. The percent of control cell survival for the compound-treated cultures was calculated as PE (treated)/PE (control) × 100.

**Acknowledgements**

The authors are grateful to Gobierno de Navarra for a predoctoral grant for Miguel Ángel Ortega, to I.C.I. (Instituto de Cooperación Iberoamericana) for a predoctoral grant for María Elena Montoya, and to Proyecto X-2 of CYTED (Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo). The excellent technical assistance of Ms. Elena Menéndez and Ms. Marta Ruiz is also gratefully acknowledged.

**References**

[1] Moulder J.E., Rockwell S., Int. J. Radiat. Oncol. Biol. Phys. 10 (1984) 695–715.

[2] Urtasun R.C., Chapman J.C., Raleigh J.A., Franko A.J., Koch C.J., Int. J. Radiat. Oncol. Biol. Phys. 12 (1986) 1263–1267.

[3] Vaupel P., Schlenger K., Knoop C., Höckel M., Cancer Res. 51 (1991) 3316–3322.

[4] Vaupel P., Kallinowsky F., Okunieff P., Cancer Res. 49 (1989) 6449–6465.

[5] Teicher B.A., Lazo J.S., Sartorelli A.C., Cancer Res. 41 (1981) 73–81.

[6] Rice G.C., Hoy C., Schimke R.T., Proc. Natl. Acad. Sci. USA (1986) 5978–5982.

[7] Lin A.J., Cosby L.A., Shansky C.W., Sartorelli, A.C., J. Med. Chem. 15 (1972) 1247–1252.

[8] Walton M.I., Wolf C.R., Workman P., Int. J. Radiat. Oncol. Biol. Phys. 6 (1989) 983–986.

[9] Aboagye E.O., Lewis A.D., Tracy M., Workman P., Biochem. Pharmacol. 54 (1997) 1217–1224.

[10] Lee H.H., Wilson W.R., Ferry D.M., van Zilj P., Pullen S.M., Denny W.A., J. Med. Chem. 39 (1996) 2508–2517.

[11] Siim B.G., Atwell G.J., Anderson R.F., Wardman P., Pullen S.M., Wilson W.R., Denny W.A., J. Med. Chem. 40 (1997) 1381–1390.

[12] Naylor M.A., Swann E., Everett S.A., Jaffar M., Nolan J., Robertson N., Lockyer S.D. et al., J. Med. Chem. 41 (1998) 2720–2731.

[13] Brown J.M., Br. J. Cancer 67 (1993) 1163–1170.

[14] Monge A., Palop J.A., López de Ceráin A., Senador V., Martínez-Crespo F.J., Sainz Y. et al., J. Med. Chem. 38 (1995) 1786–1792.

[15] Monge A., Martínez-Crespo F.J., López de Ceráin A., Palop J.A., Narro S., Senador V. et al., J. Med. Chem. 38 (1995) 4488–4494.

[16] Monge A., Palop J.A., González M., Martínez-Crespo F.J., López de Ceráin A., Sainz Y. et al., J. Heterocycl. Chem. 32 (1995) 1213–1217.

[17] Martínez-Crespo F.J., Palop J.A., Sainz Y., Narro S., Senador V., González M. et al., J. Heterocycl. Chem. 33 (1996) 1671–1677.

[18] Ley K., Seng F., Synthesis (1975) 415–422.

[19] Cheeseman G.W.H., Cookson R.F., in: Condensed Pyrazines, J. Wiley and sons, New York, 1979, pp. 35–38.

[20] Albini A., Pietra S., in: Heterocyclic N-oxides, CRC Press, Florida, 1991, pp. 187–189.

[21] Haddadin M.J., Issidorides C.H., Heterocycles 35 (1993) 1503–1525.

[22] Monge A., Palop J.A., Piñol A., Martínez-Crespo F.J., Narro S., González M. et al., J. Heterocycl. Chem. 31 (1994) 1135–1139.

[23] Doyle M.P., Siegfried B., Dellaria Jr. J.F., J. Org. Chem. 42 (1977) 2426–2431.

[24] Kurasawa Y., Takada A., Kim H.S., J. Heterocycl. Chem. 32 (1995) 1085–1114.

[25] Jarrar A.A., J. Heterocycl. Chem. 15 (1978) 177–179.

[26] Ortega M.A., Sainz Y., Montoya M.E., López de Ceráin A., Monge A., Die Pharmazie. 54 (1999) 24–25.