

1,2,5-Oxadiazole *N*-oxide derivatives as potential anti-cancer agents: synthesis and biological evaluation. Part IV

Mariana Boiani^a, Hugo Cerecetto^{a,*}, Mercedes González^{a,*}, Mariela Risso^a, Claudio Olea-Azar^b, Oscar E. Piro^c, Eduardo E. Castellano^d, Adela López de Ceráin^e, Olga Ezpeleta^e, Antonio Monge-Vega^e

^aDepartamento de Química Orgánica, Facultad de Química y Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

^bDepartamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile

^cUniversidad Nacional de La Plata e Instituto IFLP(CONICET), Departamento de Física, Facultad de Ciencias Exactas, La Plata, Argentina

^dInstituto de Física de São Carlos, Universidade de São Paulo, Sao Carlos, SP, Brazil

^eCentro de Investigaciones en Farmacobiología Aplicada, Facultad de Farmacia, Universidad de Navarra, Pamplona, Spain

Received 19 March 2001; revised 17 July 2001; accepted 31 July 2001

Abstract – Several new 1,2,5-oxadiazole *N*-oxide derivatives and some deoxygenated analogues were synthesized to be tested as potential selective hypoxic cell cytotoxins. Compounds prepared were designed in order to gain insight into the mechanism of action of this kind of cytotoxin. Compounds were tested in oxia and hypoxia and they proved to be non-selective. 3-Cyano-*N*₂-oxide-4-phenyl-1,2,5-oxadiazole showed the best cytotoxic activity in oxia. The cytotoxicity observed for these derivatives could be explained in terms of the electronic characteristics of the 1,2,5-oxadiazole substituents. Electrochemical and ESR studies were performed on the more cytotoxic derivative. © 2001 Éditions scientifiques et médicales Elsevier SAS

1,2,5-oxadiazole *N*-oxide / anti-cancer agents / structure–activity relationships

1. Introduction

Hypoxic cells present in human tumours give rise to clinical problems in the chemotherapy of solid tumours because they limit the efficiency of fractionated radiotherapy and are also resistant to conventional chemotherapeutic drugs [1–3]. Bioreductive prodrugs, which are cytotoxic maximally in the absence of oxygen, have attracted considerable attention as selective cytotoxins towards hypoxic cells [4]. Several types of compounds that are toxic only under hypoxic conditions are known. These agents belong to the classes of nitro derivatives [5–7], quinone derivatives [8], nitro-

gen mustard derivatives [9–11] and *N*-oxide derivatives [12]. Taking the last as pharmacophore antecedent, our group is undertaking the synthesis and biological characterisation of new potential *N*-oxide-containing prodrugs [13–18]. We have studied the bioactivity of derivatives of 1,2,5-oxadiazole *N*-oxide (e.g. **1–6**, *figure 1*), which could be seen as misonidazole (a nitro derivative) and tirapazamine (an *N*-oxide) related compounds [16–18]. Unfortunately the compounds were totally unselective under hypoxia conditions, although derivatives **1** and **2** showed appreciable activity in oxia (*figure 1*), displaying excellent V79 cell-growth inhibition at the lowest concentration assayed (1 μM) [19].

From the well-known *N*-oxides mechanism of action [21] and the observed activity and the structural characteristics of derivatives developed, we conclude

* Correspondence and reprints

E-mail addresses: hcerecet@bilbo.edu.uy (H. Cerecetto), megonzal@bilbo.edu.uy (M. González).

that the 1,2,5-oxadiazole *N*-oxides probably act as is shown in *figure 1*, where the first step, electronation process, is not reverted by oxygen. In order to explore the structure–activity relationships, we have studied the cytotoxicity of new derivatives of 1,2,5-oxadiazole *N*-oxide. In this paper we report the synthesis and the biological and physicochemical characterisation of 17 1,2,5-oxadiazole *N*-oxide derivatives.

2. Chemistry

Trying to confirm the proposed mechanism of action, we varied the structure of parent compounds in two ways. On the one hand, we designed compounds that change the electrophilic characteristic of the *benzylic*-like position, and on the other hand, we designed deoxygenated analogues of characteristic products (derivatives **1**, **2**, **4** and **5**).

Firstly, were prepared compounds **7–10** (see *figure 2*) as electron-withdrawing substituent derivatives of parent compounds **1** and **6**. The oximes **7** and **8** were obtained by the traditional method [22] and then converted into the nitriles **9** and **10** by dehydration with thionyl chloride as reported by Gasco and coworkers [23]. According to NOE experiments oxime **7** was obtained as the *Z*-isomer and oxime **8** as the

E-isomer. The differential stereochemistry of these oximes was, probably, responsible for the yields obtained in the following dehydration process (preparation of **9** and **10**). To change the electrophilic aldehyde centre, compounds **1** and **6** were transformed into derivatives **11** and **12** using condensation processes. The Wittig procedure [24, 25] produced the desired compounds in good yields. Attempts to obtain the corresponding nitro alkenes, **15** and **16**, through nitroaldol condensation [26], were unsuccessful. Long-time heating in basic medium (butylamine) of the reaction mixture, conducted to a total decomposition of the nitro alcohols intermediate **13** and **14**. These were isolated and biologically evaluated before decomposition started.

We have synthesized a series of *benzylic*-like substituted derivatives of compounds **2** and **5**, since substituents at this position are expected to have an important electronic effects on the displayed activity (according to the proposed mechanism). We choose a series of sulphur derivatives with well-known electronic effects (seen the corresponding *F* coefficients of Swain–Lupton [27]) and different steric parameters. These derivatives, **17–20**, were prepared by the methods outlined in *figure 3*. The chloride **2** was transformed into the corresponding thioether derivatives (**17** and **20**) using S_N conditions [28]. The phenylthio

Compound	R ¹	R ²	Fraction of survival in oxia (hypoxia) (%) ^{a,b}	R ^c
1	Ph	CHO	0 (0)	0.13
2	Ph	CH ₂ Cl	0 (2)	0.03
3	Ph	CH=NNHC(S)NH ₂	53 (54)	-0.02
4	Ph		50 (59)	- ^d
5	Ph	CH ₂ OH	55 (63)	0.00
6	CHO	CH ₃	82 (78)	-

(a) Using V79 cells, at 20 μM. (b) From reference [17]. (c) R values for R² substituents, from R¹=Ph derivatives, were taken from ref. [20]. (d) Not available.

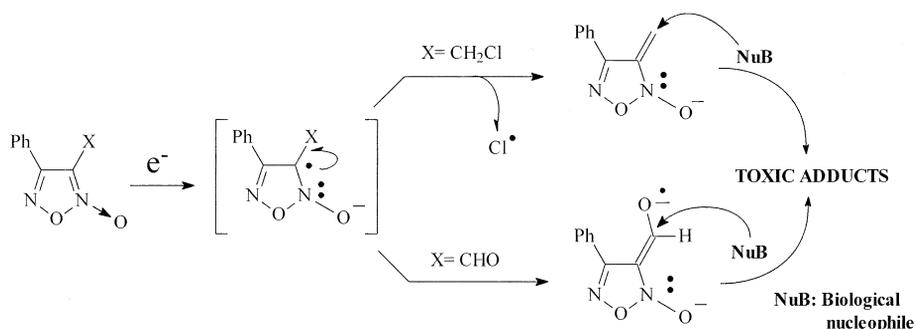


Figure 1. Parent compounds and mechanism of action proposed.

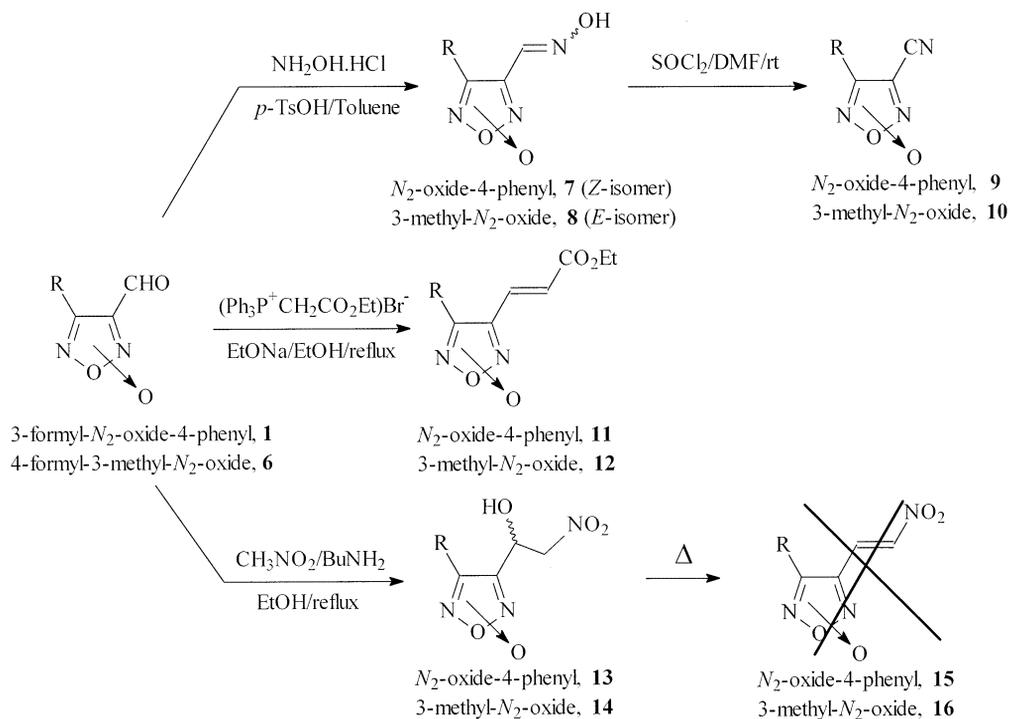


Figure 2. Electrophilic centre modifications.

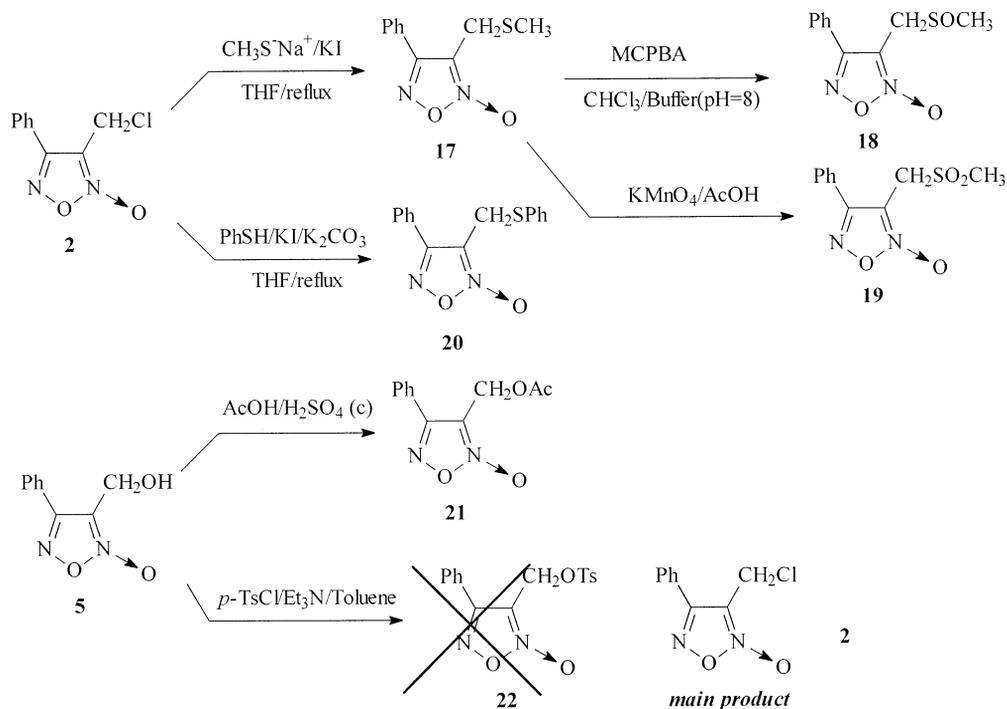


Figure 3. Benzylic-like centre modifications.

derivative (**20**) was obtained in a low yield with an extensive auto-oxidation of thiophenol. Compound **17** was transformed into derivatives **18** and **19** using an adequate oxidising reagent [28, 29]. Derivative **5** was transformed into the corresponding ester **21** as shown in *figure 3*. Attempts to obtain the corresponding tosylate **22** were unsuccessful, in all cases compound **2** was isolated probably as a chloride tosylate-displacement in the reaction medium [30].

Finally, in an attempt to demonstrate the importance of the *N*-oxide moiety in the mechanism of action, the deoxy derivatives, **23–26**, were prepared (see *figure 4*). When triphenylphosphine was used as deoxygenating agent [31, 32] unfavourable results were obtained. The use of Zn in NH_4Cl solution led to the deoxy derivatives **23** and **24** in moderate yields [33].

The reduction was clearly observed through HETCOR experiments (HMOC and HMBC). The molecular structure of derivative **23** has been determined by X-ray diffraction methods. *Figure 5* displays the ORTEP drawing [34], showing the labelling of the non-H atoms and their displacement ellipsoids at 30% probability level. When we tried to reduce derivative **2**, under the same conditions, compound **27** was generated as a reductive dimerisation Zn-prompted process. This desired deoxy analogue, **25**, and **26** were prepared from alcohol **23** through classical procedures [17].

3. Pharmacology

The 1,2,5-oxadiazole *N*-oxide derivatives were tested in a cloning assay using V79 cells. Suspension

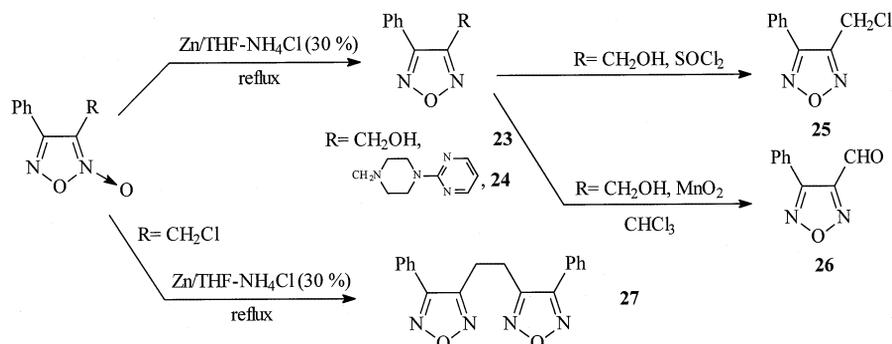
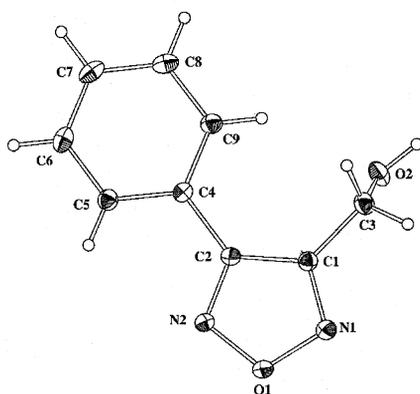


Figure 4. Deoxygenated derivatives synthesis.



Interatomic bond distances (Å) and angles (°)

Bond angles		Bond distances	
N(1)-O(1)-N(2)	110.20(9)	O(1)-N(1)	1.380(2)
C(1)-N(1)-O(1)	106.2(1)	O(1)-N(2)	1.383(1)
C(2)-N(2)-O(1)	106.6(1)	O(2)-C(3)	1.428(2)
N(1)-C(1)-C(2)	108.9(1)	N(1)-C(1)	1.304(2)
N(1)-C(1)-C(3)	120.0(1)	N(2)-C(2)	1.306(2)
C(2)-C(1)-C(3)	131.1(1)	C(1)-C(2)	1.438(2)
N(2)-C(2)-C(1)	108.1(1)	C(1)-C(3)	1.493(2)
N(2)-C(2)-C(4)	121.1(1)	C(2)-C(4)	1.474(2)
C(1)-C(2)-C(4)	130.8(1)	C(4)-C(9)	1.396(2)
O(2)-C(3)-C(1)	108.0(1)	C(4)-C(5)	1.403(2)
C(9)-C(4)-C(5)	119.4(1)	C(5)-C(6)	1.386(2)
C(9)-C(4)-C(2)	120.8(1)	C(6)-C(7)	1.387(2)
C(5)-C(4)-C(2)	119.8(1)	C(7)-C(8)	1.385(2)
C(6)-C(5)-C(4)	120.0(1)	C(8)-C(9)	1.393(2)
C(5)-C(6)-C(7)	120.4(2)		
C(8)-C(7)-C(6)	120.0(1)		
C(7)-C(8)-C(9)	120.3(1)		
C(8)-C(9)-C(4)	120.0(1)		

Figure 5. Molecular plot of derivative **23** at 120 K (showing the labelling of the non-H atoms and their displacement ellipsoids at 30% probability level) and interatomic bond distances (Å) and angles (°).

Table I. Physical and biological properties of 1,2,5-oxadiazole *N*-oxide derivatives.

Compound	M.p. (°C) (crystallisation solvent)	ν_{\max} (cm ⁻¹)	Biological evaluation			
			Conc. (M)	<i>SFair</i> (%) ^{a b}	<i>SFhipox</i> (%) ^{b c}	<i>R</i> ^d
7	147.4–149.0 (toluene)	3322, 1599	20	35 ± 1	70 ± 12	-0.13
8	116.0–117.0, CC ^e	3312, 1605	20	33 ± 6	78 ± 23	–
9	73.0–74.0, CC ^e	2244, 1611	20	0 ± 0	0 ± 0	0.19
			10	37 ± 20	100 ± 0	
			5	93 ± 8	90 ± 13	
10	Oil, CC ^e	2357, 1634	20	35 ± 10	64 ± 14	–
11	51.5–51.9, CC ^e	1719, 1590	20	72 ± 11	96 ± 6	-0.19
12	61.8–62.7, CC ^e	1707, 1605	20	84 ± 20	91 ± 13	–
13	Oil, CC ^e	3480, 1570	20	100 ± 0 ^f	89 ± 15	- ^g
14	Oil, CC ^e	3500, 1575	20	87 ± 11	88 ± 17	–
17	Oil, CC ^e	2870, 1430	20	79 ± 29	98 ± 3	- ^g
18	94.2–95.4 (petroleum ether–EtOAc)	1595, 1038	20	67 ± 5	47 ± 5	- ^g
19	131.7–133.0 (petroleum ether–EtOAc)	1321, 1148	20	100 ± 0 ^f	100 ± 0 ^f	- ^g
20	Oil, CC ^e	2860, 1598	20	15 ± 3	100 ± 0 ^f	- ^g
21	Oil, CC ^e	1740, 1235	20	100 ± 0 ^f	100 ± 0 ^f	- ^g
23	63.4–64.6, CC ^e	3520, 1595	20	96 ± 5	96 ± 6	0.00
24	87.5–90.0, CC ^e	1592, 1449	20	100 ± 0 ^f	77 ± 2	- ^g
25	Oil, CC ^e	1590, 1450	20	68 ± 16	68 ± 23	0.03
26	Oil, CC ^e	1700, 1595	20	94 ± 8	98 ± 2	0.13
Reference ^h	–	–	5	100 ± 0	0 ± 0	–

^a Survival fraction in air.

^b The tests were carried out in duplicate.

^c Survival fraction in hypoxia.

^d *R* values for R² substituents, from R¹ = Ph derivatives, were taken from Ref. [20].

^e Purification by chromatographic column.

^f Highest concentration tested: 20 μM.

^g Not available.

^h Reference: 7-Chloro-3-(3-dimethylaminopropylamino)quinoxaline-2-carbonitrile *N*₁,*N*₄-dioxide, hydrochloride.

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 the compounds were tested firstly at 20 μM employing duplicate flasks in both air and nitrogen and the survival fraction under both conditions (*SFair* and *SFhipox*) (see Section 6) was determined [17]. When compounds became active at this concentration, they were assayed at lower doses. Derivative **9**, which was potent at 20 μM, was also tested at 10 and 5 μM. The results obtained are summarised in *table I*.

4. Results

4.1. Electrochemical studies

To relate the electrochemical properties to the observed cytotoxicity, the most characteristic derivatives

assayed and their methyl analogues, **9** and **10**, were analysed by cyclic voltammetry in deaerated dimethyl sulphoxide (DMSO) solution at a mercury dropping working electrode [35, 36]. Two typical reduction peaks were observed during forward cathodic scan, which are linked to the corresponding oxidation peak on the reverse anodic scan (*figure 6a*). Only the first reduction step, a one-electron process, was studied as a function of voltage sweep rate and switching potential. *Table II* summarises the electrochemical parameters obtained at a sweep rate of 1.0 V s⁻¹ of derivatives **9** and **10**. The free radical of compound **9** generated at the first reduction process was investigated using ESR spectroscopy. This radical was generated by electrolytical reduction *in situ* at room temperature in DMSO, applying the same cathodic potential obtained from the cyclic voltammetry experiments [16, 37]. The hyperfine patterns indicate that the unpaired electron is delocalised as far as the heterocyclic system (*figure 6b*). A double-triplet reso-

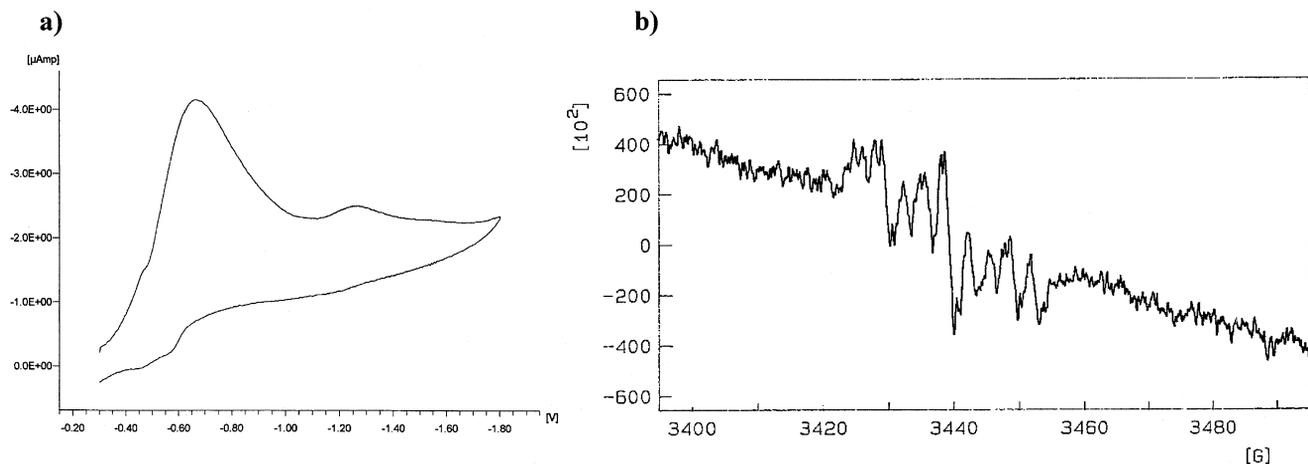


Figure 6. (a) Cyclic voltammogram of derivative **9** at 298 K in DMSO; sweep rate 1.0 V s^{-1} . (b) ESR spectrum of derivative **9** at 298 K in DMSO; electrolytical generation.

nance band suggests that two nitrogens (*N*-heterocyclic and *N*-nitrile) are coupling with the free electron.

5. Discussion

With the aim to obtain hypoxic-selective cytotoxins and to analyse the structural requirements in the cytotoxic activity of 1,2,5-oxadiazole *N*-oxides, 17 1,2,5-oxadiazole derivatives were prepared and evaluated under hypoxic and aerobic conditions. The compounds proved to be non-selective, compound **9** being the most potent derivative at $20 \mu\text{M}$. But at $5 \mu\text{M}$ concentration the reference drug (7-chloro-3-(3-dimethylaminopropylamino)quinoxaline-2-carbonitrile *N*₁,*N*₄-dioxide) shows hypoxic-selectivity, while compound **9** shows no cytotoxic results.

The lack of hypoxic-selectivity could be explained for the redox properties of derivatives. All derivatives previously described [16] and the compounds studied in this paper showed more negative potential than that expected for anaerobic selectivity [38–40].

Compound **9** bears a 3-substituent (3-CN) with an *R* parameter positive, as the more cytotoxic parent compounds (derivatives **1** and **2** with 3-formyl and 3-chloromethyl moieties) (see *figure 1* and *table I*). In the series of phenyl analogues, clearly, the electronic effect (evaluated as *R* descriptor) of the substituent in 3-position and the presence/absence of *N*-oxide moiety showed that they play an important role in the biological activity displayed. The mesomeric electron

withdrawing effects of 3-substituents are in accordance with the proposed mechanism shown in *figure 1*.

The methyl analogue, **10**, showed some degree of cytotoxicity in oxia at $20 \mu\text{M}$. The activity of derivatives **9** and **10** is in accordance with the reduction potential determined by voltammetry. The less negative potential for compound **9** than compound **10** indicates the greater facility to mono-electronation of the former. The ESR spectra show that the extended delocalisation of derivative **9** free radical is in concordance with the reduction potential, as is with the observed activity.

The *benzylic*-like substituted derivatives **17**, **19**, and **21** showed low cytotoxic activity, derivative **18** displayed moderate degree of selectivity for hypoxic conditions and compound **20** showed cytotoxic under aerobic conditions. The activity of these derivatives could not be related to the electronic effects of the substituents, considering the *F* parameters of Swain–Lupton, though compounds with substituents of similar *F* values showed very different biological activities (compare **2** and **21**, $F_{\text{Cl}} = F_{\text{OAc}} = 0.41$; or **18** and **19**, $F_{\text{SOMe}} = 0.52$ and $F_{\text{SO}_2\text{Me}} = 0.54$). The activity could be

Table II. Electrochemical properties of derivatives **9** and **10**.

Compound	E_{pc1} (V) vs. SCE	$E_{\text{pc1}} - E_{\text{pa1}}$ (V)	$i_{\text{pc1}}/i_{\text{pa1}}$
9	−0.67	0.08	1.8
10	−0.90	0.07	2.0

related to the capacity of good leaving group as Cl substituent. This fact and the decrease of activity of the deoxy analogues, **23–26**, are totally in agreement with the mechanism of action proposed. The chemical production of compound **27**, in a reductive medium, indicates that the *benzylic*-like position in compound **2**, could be participating in the electron delocalisation more than the corresponding position in compounds **4** and **5**, though they produced chemically the deoxy derivatives under reductive conditions. This fact is also in agreement with the proposed mechanism, compounds **4** and **5** show less cytotoxicity [41].

Compared to quinoxaline N_1, N_4 -dioxide (reference compounds) [13, 42] these derivatives showed better solubility in the biological medium. In spite of the lack of selectivity of compounds **1**, **2** and **9**, this kind of physicochemical property transforms them into good leaders that could be chemically modified in order to obtain the desired bioactivity.

Also, the well-known NO-release properties of derivative **9** and related compounds [43–45] make it a potential vasodilator at the tumour zone with the corresponding blood-flux increment. Combining the solubility, NO-release properties and cytotoxicity proved in this paper, compound **9** could be a good target for cancer therapy.

Biological evaluation in vivo of derivative **9** is currently in progress.

6. Experimental protocols

6.1. Chemistry

6.1.1. General

All starting materials were commercially available research-grade chemicals and used without further purification. Compounds **1**, **2**, **4–6**, and **9** were prepared according to literature [17, 23] methods. All solvents were dried and distilled prior to use. All the reactions were carried out in a nitrogen atmosphere. The typical work-up included washing the organic layer with brine, drying with sodium sulphate and evaporating in vacuo. Melting points were determined using a Leitz Microscope Heating Stage Model 350 apparatus and are uncorrected. Elemental analyses were obtained from vacuum-dried samples (over phosphorous pentoxide at room temperature) and performed on a Fisons EA 1108 CHNS-O analyser, and were within 0.4% of theoretical values. Infrared spectra were recorded on a Perkin–

Elmer 1310 apparatus, using potassium bromide tablets for solid and oil products. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra were recorded on a Bruker DPX-400 (at 400 MHz and 100 MHz) instrument, with tetramethylsilane as the internal reference and in the indicated solvent; the chemical shifts are reported in ppm. NOE, COSY and HETCOR experiments were performed so as to obtain a correct characterisation of some derivatives. Mass spectra were recorded on a Shimadzu GC–MS QP 1100 EX instrument at 70 eV.

6.1.2. Synthesis procedures

6.1.2.1. General procedure for preparation of derivatives **7** and **8**

A mixture of aldehyde (**1** or **6**, 1 equiv.), hydroxylamine hydrochloride (1 equiv.) and *p*-TsOH (catalytic amounts) in ethanol as solvent was heated at reflux until the aldehyde was not present (SiO_2 , petroleum ether–AcOEt, 8:2). The solvent was evaporated in vacuo and the residue was dissolved with EtOAc. After the work-up procedure the residue was purified as indicated in table I.

6.1.2.2. 3-Formyl- N_2 -oxide-4-phenyl-1,2,5-oxadiazole *Z*-oxime (**7**)

Yield 40%. $^1\text{H-NMR}$ (NOE experiment) ($\text{DMSO-}d_6$) 7.61 (m, 3H, H–Ar), 7.78 (m, 2H, H–Ar), 7.93 (s, 1H, CH=N), 12.45 (s, 1H, OH). $^{13}\text{C-NMR}$ (HMOC and HMBC experiments) ($\text{DMSO-}d_6$) 111.51 ($\text{C}=\text{N}^+\text{O}^-$), 127.00–132.00 (C–Ar), 136.05 (C=NOH), 156.42 (C=N). MS (EI, 70 eV); m/z (percentage of relative abundance): 205 (50.5, $[\text{M}^+]$), 189 (9.3), 175 (16.5). Anal. ($\text{C}_9\text{H}_7\text{N}_3\text{O}_3$) C, H, N.

6.1.2.3. 4-Formyl-3-methyl- N_2 -oxide-1,2,5-oxadiazole *E*-oxime (**8**)

Column chromatography (SiO_2 , petroleum ether–EtOAc (0–50%)). Yield 74%. $^1\text{H-NMR}$ (NOE experiment) ($\text{DMSO-}d_6$) 2.27 (s, 3H, CH_3), 8.27 (s, 1H, CH=N), 12.49 (s, 1H, OH). $^{13}\text{C-NMR}$ (HMOC and HMBC experiments) ($\text{DMSO-}d_6$) 10.03 (CH_3), 112.46 ($\text{C}=\text{N}^+\text{O}^-$), 139.89 (C=NOH), 153.61 (C=N). MS (EI, 70 eV); m/z (percentage of relative abundance): 143 (23.2, $[\text{M}^+]$), 113 (28.0), 83 (31.3). Anal. ($\text{C}_4\text{H}_5\text{N}_3\text{O}_3$) C, H, N.

6.1.2.4. General procedure for preparation of derivatives **9** and **10**

To a mixture of oxime (**7** or **8**, 1 mmol) and DMF as solvent (2.0 mL) was added SOCl_2 (0.3 mL). The mix-

ture was stirred at room temperature until the oxime was not present (SiO₂, petroleum ether–EtOAc 1:2). The excess of SOCl₂ was eliminated with a saturated aqueous solution of NaHCO₃. The nitrile was extracted three times with EtOAc. After the work-up procedure the residue was purified by chromatography.

6.1.2.5. 4-Cyano-3-methyl-N₂-oxide-1,2,5-oxadiazole (10)

Column chromatography (SiO₂, petroleum ether–EtOAc (0–20%)). Yield 3%. ¹H-NMR (DMSO-*d*₆) 2.23 (s, 3H, CH₃). ¹³C-NMR (HMQC and HMBC experiments) (DMSO-*d*₆) 8.52 (CH₃), 109.07 (CN), 114.43 (C=N⁺O⁻), 137.74 (C=N). MS (EI, 70 eV); *m/z* (percentage of relative abundance): 125 (100.0, [M⁺]), 95 (23.5), 65 (7.4). Anal. (C₄H₃N₃O₂) C, H, N.

6.1.2.6. General procedure for preparation of derivatives 11 and 12

To a solution of sodium (1 equiv.) in anhydrous ethanol with intensive stirring a solution of ethyloxycarbonylmethyl triphenyl phosphonium bromide (1 equiv.) in anhydrous ethanol was added rapidly. Stirring was continued for 30 min. Then a solution of aldehyde (**1** or **6**, 1 equiv.) in anhydrous ethanol was added and the resulting mixture was heated at reflux until the aldehyde was not present (SiO₂, petroleum ether–EtOAc, 1:1). The mixture was concentrated in vacuo and treated with EtOAc. After the work-up process the residue was purified by chromatography (SiO₂, petroleum ether–EtOAc (0–30%)).

6.1.2.7. 3-(Ethyloxypropenoyl)-N₂-oxide-4-phenyl-1,2,5-oxadiazole (11)

Yield 71%. ¹H-NMR (CDCl₃) 1.28 (t, 3H, *J* = 7.0 Hz, CH₃), 4.27 (q, *J* = 7.0 Hz, 2H, CH₂O), 7.20 (d, *J* = 14.0 Hz, 1H, =CH), 7.42 (d, *J* = 14.0 Hz, 1H, =CH), 7.58 (m, 5H, H–Ar). MS (EI, 70 eV); *m/z* (percentage of relative abundance): 260 (29.9, [M⁺]), 215 (13.3), 200 (100.0). Anal. (C₁₃H₁₂N₂O₄) C, H, N.

6.1.2.8. 4-(Ethyloxypropenoyl)-3-methyl-N₂-oxide-1,2,5-oxadiazole (12)

Yield 85%. ¹H-NMR (CDCl₃) 1.29 (t, 3H, *J* = 7.2 Hz, CH₃), 2.27 (s, 3H, CH₃), 4.24 (q, *J* = 7.1 Hz, 2H, CH₂O), 6.64 (d, *J* = 16.4 Hz, 1H, =CH), 7.40 (d, *J* = 16.4 Hz, 1H, =CH). ¹³C-NMR (HMQC and HMBC experiments) (CDCl₃) 8.82 (CH₃), 14.46 (CH₃), 61.78 (CH₂), 111.53 (C=N⁺O⁻), 127.32 (C=), 128.67 (C=), 153.31 (C=N), 165.16 (C=O). MS (EI, 70 eV); *m/z*

(percentage of relative abundance): 198 (12.8, [M⁺]), 168 (12.0), 153 (13.9). Anal. (C₈H₁₀N₂O₄) C, H, N.

6.1.2.9. General procedure for preparation of derivatives 13 and 14

A mixture of aldehyde (**1** or **6**, 1 equiv.), nitromethane (1 equiv.) and butylamine (catalytic amounts) in ethanol was heated at reflux until the aldehyde was not present (SiO₂, petroleum ether–EtOAc, 1:1). The solvent was evaporated in vacuo and the residue was dissolved with EtOAc. After the work-up procedure the residue was purified by chromatography (SiO₂, petroleum ether–EtOAc, 4:1).

6.1.2.10. 3-(1-Hydroxy-2-nitroethyl)-N₂-oxide-4-phenyl-1,2,5-oxadiazole (13)

Yield 38%. ¹H-NMR (COSY experiment) (CDCl₃) 4.72 (dd, *J*₁ = 14.1 Hz, *J*₂ = 4.2 Hz, 1H, CHNO₂), 4.78 (bs, 1H, OH), 4.98 (dd, *J*₁ = 14.1 Hz, *J*₂ = 8.7 Hz, 1H, CHNO₂), 5.63 (dd, *J*₁ = 8.7 Hz, *J*₂ = 4.1 Hz, 1H, CHOH), 7.61 (m, 3H, H–Ar), 7.77 (m, 2H, H–Ar). ¹³C-NMR (HMQC and HMBC experiments) (CDCl₃) 62.00 (CHOH), 76.00 (CH₂NO₂), 113.00 (C=N⁺O⁻), 125.00–132.00 (C–Ar), 155.00 (C=N). MS (EI, 70 eV); *m/z* (percentage of relative abundance): 251 (5.0, [M⁺]), 233 (4.2), 217 (0.4), 203 (3.1). Anal. (C₁₀H₉N₃O₅) C, H, N.

6.1.2.11. 4-(1-Hydroxy-2-nitroethyl)-3-methyl-N₂-oxide-1,2,5-oxadiazole (14)

Yield 25%. ¹H-NMR (COSY experiment) (CDCl₃) 2.29 (s, 3H, CH₃), 4.99 (dd, *J*₁ = 13.6 Hz, *J*₂ = 9.1 Hz, 1H, CHNO₂), 5.15 (dd, *J*₁ = 13.6 Hz, *J*₂ = 3.7 Hz, 1H, CHNO₂), 5.72 (dd, *J*₁ = 8.8 Hz, *J*₂ = 3.5 Hz, 1H, CHOH), 5.94 (bs, 1H, OH). ¹³C-NMR (HMQC and HMBC experiments) (CDCl₃) 7.75 (CH₃), 64.00 (CHOH), 78.00 (CH₂NO₂), 112.68 (C=N⁺O⁻), 157.81 (C=N). MS (EI, 70 eV); *m/z* (percentage of relative abundance): 189 (37.7, [M⁺]), 171 (1.1), 155 (0.3), 141 (1.6). Anal. (C₅H₇N₃O₅) C, H, N.

6.1.2.12. 3-Methylthiomethyl-N₂-oxide-4-phenyl-1,2,5-oxadiazole (17)

A mixture of chloride **2** (500 mg, 2.4 mmol), KI (40 mg, 0.24 mmol) and THF (10.0 mL) as solvent was stirred at 0 °C. Then sodium thiomethoxide (168 mg, 2.4 mmol) was added in three portions and the mixture was heated at reflux for 6 h. After filtering through celite and evaporating the solvent in vacuo, the residue was dissolved with EtOAc. After the work-up procedure the

residue was purified by chromatography (SiO₂, petroleum ether–EtOAc (0–5%)). Yield 34%. ¹H-NMR (CDCl₃) 2.19 (s, 3H, CH₃), 3.72 (s, 2H, CH₂), 7.53 (m, 3H, H–Ar), 7.79 (m, 2H, H–Ar). ¹³C-NMR (HMQC and HMBC experiments) (CDCl₃) 16.44 (CH₃), 26.09 (CH₂), 114.66 (C=N⁺O⁻), 126.00–132.00 (C–Ar), 156.90 (C=N). MS (EI, 70 eV); *m/z* (percentage of relative abundance): 222 (3.1, [M⁺]), 205 (2.2), 159 (31.5). Anal. (C₁₀H₁₀N₂O₂S) C, H, N.

6.1.2.13. Methyl[(*N*₂-oxide-4-phenyl-1,2,5-oxadiazole-3-yl)methyl]sulfoxide (**18**)

A heterogeneous mixture of thioether **17** (100 mg, 0.45 mmol), *meta*-chloroperbenzoic acid (78 mg, 0.45 mmol), CHCl₃ (15.0 mL) and buffer phosphate (pH 8) (10.0 mL) was stirred at room temperature for 24 h. After the work-up procedure the residue was purified by chromatography (SiO₂, petroleum ether–EtOAc (0–100%)) and then the fraction corresponding to the product was crystallised as indicated in *table I*. Yield 76%. ¹H-NMR (CDCl₃) 2.79 (s, 3H, CH₃), 4.21 (d, *J* = 14.2 Hz, 1H, CH), 4.27 (d, *J* = 14.2 Hz, 1H, CH), 7.61 (m, 3H, H–Ar), 7.97 (m, 2H, H–Ar). ¹³C-NMR (HMQC and HMBC experiments) (CDCl₃) 39.69 (CH₃), 47.41 (CH₂), 110.57 (C=N⁺O⁻), 126.00–132.00 (C–Ar), 158.61 (C=N). MS (EI, 70 eV); *m/z* (percentage of relative abundance): 238 (4.0, [M⁺]), 175 (74.4), 159 (4.1). Anal. (C₁₀H₁₀N₂O₃S) C, H, N.

6.1.2.14. Methyl[(*N*₂-oxide-4-phenyl-1,2,5-oxadiazole-3-yl)methyl]sulfone (**19**)

A mixture of thioether **17** (100 mg, 0.45 mmol) and acetic acid (5.0 mL) as solvent was stirred at 0 °C. Then KMnO₄ (142 mg, 0.90 mmol) was added in three portions and the mixture was stirred at room temperature for 24 h. The acetic acid was evaporated in vacuo and the residue was dissolved in EtOAc. After the work-up procedure the residue was purified by chromatography (SiO₂, petroleum ether–EtOAc (0–20%)) and then the fraction corresponding to the product was crystallised as indicated in *table I*. Yield 55%. ¹H-NMR (CDCl₃) 3.13 (s, 3H, CH₃), 4.68 (s, 2H, CH₂), 7.61 (m, 3H, H–Ar), 7.93 (m, 2H, H–Ar). ¹³C-NMR (HMQC and HMBC experiments) (CDCl₃) 44.73 (CH₃), 49.84 (CH₂), 108.94 (C=N⁺O⁻), 125.00–133.00 (C–Ar), 157.91 (C=N). MS (EI, 70 eV); *m/z* (percentage of relative abundance): 254 (3.7, [M⁺]), 238 (0.7), 224 (3.1), 175 (4.8). Anal. (C₁₀H₁₀N₂O₄S) C, H, N.

6.1.2.15. *N*₂-Oxide-4-phenyl-3-phenylthiomethyl-1,2,5-oxadiazole (**20**)

A mixture of chloride **2** (500 mg, 2.4 mmol), thiophenol (264 mg, 2.4 mmol), K₂CO₃ (332 mg, 2.4 mmol), KI (40 mg, 0.24 mmol) and THF (10.0 mL) as solvent was heated at reflux for 24 h. After filtering through celite and evaporating the solvent in vacuo, the residue was dissolved with EtOAc. After the work-up procedure the residue was purified by chromatography (SiO₂, petroleum ether–EtOAc (0–5%)). Yield 12%. ¹H-NMR (CDCl₃) 4.11 (s, 2H, CH₂), 7.20–7.76 (m, 10H, H–Ar). ¹³C-NMR (HMQC and HMBC experiments) (CDCl₃) 28.47 (CH₂), 113.72 (C=N⁺O⁻), 125.00–135.00 (C–Ar), 156.89 (C=N). MS (EI, 70 eV); *m/z* (percentage of relative abundance): 284 (6.3, [M⁺]), 267 (49.8), 223 (16.4). Anal. (C₁₅H₁₂N₂O₂S) C, H, N.

6.1.2.16. 3-Acetyloxymethyl-*N*₂-oxide-4-phenyl-1,2,5-oxadiazole (**21**)

A mixture of alcohol **5** (500 mg, 2.6 mmol), H₂SO₄ conc. (0.14 mL, 2.6 mmol) and acetic acid (5.0 mL) as solvent was stirred at room temperature for 24 h. The acetic acid was evaporated in vacuo and the residue was dissolved in EtOAc and treated with NaHCO₃ saturated solution three times. After the work-up procedure the residue was purified by chromatography (SiO₂, petroleum ether–EtOAc (0–10%)). Yield 24%. ¹H-NMR (CDCl₃) 2.10 (s, 3H, CH₃), 5.14 (s, 2H, CH₂), 7.54 (m, 3H, H–Ar), 7.72 (m, 2H, H–Ar). ¹³C-NMR (HMQC and HMBC experiments) (CDCl₃) 20.75 (CH₃), 54.47 (CH₂), 111.68 (C=N⁺O⁻), 126.00–132.00 (C–Ar), 157.11 (C=N), 170.31 (C=O). MS (EI, 70 eV); *m/z* (percentage of relative abundance): 234 (2.7, [M⁺]), 218 (0.5), 204 (5.1). Anal. (C₁₁H₁₀N₂O₄) C, H, N.

6.1.2.17. General procedure for preparation of derivatives **23** and **24**

A mixture of *N*-oxide (**4** or **5**, 0.27 mmol), Zn dust (1.3 mmol), NH₄Cl solution (30%) (4.7 mL) and THF (5.0 mL) was heated at reflux for 3 h. After filtering through celite the solution was extracted three times with Et₂O (10 mL). After the work-up process the residue was purified by chromatography.

6.1.2.18. 3-Hydroxymethyl-4-phenyl-1,2,5-oxadiazole (**23**)

Column chromatography (SiO₂, petroleum ether–EtOAc (0–50%)). Yield 45% yield. ¹H-NMR (CDCl₃) 2.54 (bs, 1H, OH), 4.99 (s, 2H, CH₂O), 7.54 (m, 3H, H–Ar), 7.89 (m, 2H, H–Ar). ¹³C-NMR (HMQC and

HMBC experiments) (CDCl_3) 54.69 (CH_2OH), 125.00–132.00 (C-Ar), 152.94 (C=N), 154.23 (C=N). MS (EI, 70 eV); m/z (percentage of relative abundance): 176 (100.0, $[\text{M}^{*+}]$), 146 (9.9), 131 (20.1). Anal. ($\text{C}_9\text{H}_8\text{N}_2\text{O}_2$) C, H, N.

6.1.2.19. 4-Phenyl-3-[4-(pyrimidine-2-yl)piperazine-1-ylmethyl]-1,2,5-oxadiazole (24)

Column chromatography (SiO_2 , petroleum ether–EtOAc (0–30%)). Yield 58%. $^1\text{H-NMR}$ (CDCl_3) 2.66 (m, 4H), 3.86 (s, 2H), 3.88 (m, 4H), 6.52 (t, $J = 4.7$ Hz, 1H), 7.52 (m, 3H, H–Ar), 8.03 (m, 2H, H–Ar), 8.35 (d, $J = 4.7$ Hz, 2H). $^{13}\text{C-NMR}$ (HMQC and HMBC experiments) (CDCl_3) 44.99 ($\text{CH}_2\text{-N}$), 50.87 (Ar– $\text{CH}_2\text{-N}$), 53.11 (CH_2N), 110.49 ($\text{C}_{\text{Pyrimidinyl}}$), 126.00–131.00 (C-Ar), 150.63 (C=N), 155.50 (C=N), 158.12 ($\text{C}_{\text{Pyrimidinyl}}$), 162.07 ($\text{C}_{\text{Pyrimidinyl}}$). MS (EI, 70 eV); m/z (percentage of relative abundance): 322 (12.7, $[\text{M}^{*+}]$), 292 (0.2), 260 (1.1). Anal. ($\text{C}_{17}\text{H}_{18}\text{N}_6\text{O}$) C, H, N.

6.1.2.20. 1,2-Bis-(4-phenyl-1,2,5-oxadiazole-3-yl)ethane (27)

Column chromatography (SiO_2 , petroleum ether–EtOAc (0–5%)). M.p. 135.0–135.5 °C; yield 35%. $^1\text{H-NMR}$ (CDCl_3) 2.67 (s, 4H, CH_2), 7.50 (m, 6H, H–Ar), 7.66 (m, 2H, H–Ar). $^{13}\text{C-NMR}$ (HMQC and HMBC experiments) (CDCl_3) 23.03 (CH_2), 127.00–139.00 (C-Ar), 148.20 (C=N), 151.47 (C=N). MS (EI, 70 eV); m/z (percentage of relative abundance): 316 (1.6, $[\text{M}^{*+} - 2\text{H}]$), 314 (4.3), 287 (19.8). Anal. ($\text{C}_{18}\text{H}_{14}\text{N}_4\text{O}_2$) C, H, N.

6.1.2.21. 3-Chloromethyl-4-phenyl-1,2,5-oxadiazole (25)

A mixture of **23** (500 mg, 2.8 mmol) and thionyl chloride (1.24 g, 10.4 mmol) was stirred at room temperature for 24 h. The reaction mixture was treated with ice, NaHCO_3 saturated solution (until basic pH), and extracted three times with EtOAc (20 mL). After the work-up process the residue was purified by chromatography (Al_2O_3 , petroleum ether–EtOAc (0–5%)). Yield 60%. $^1\text{H-NMR}$ (CDCl_3) 4.84 (s, 2H, CH_2Cl), 7.59 (m, 3H, H–Ar), 7.83 (m, 2H, H–Ar). $^{13}\text{C-NMR}$ (HMQC and HMBC experiments) (CDCl_3) 32.99 (C-Cl), 125.00–132.00 (C-Ar), 150.72 (C=N), 153.98 (C=N). MS (EI, 70 eV); m/z (percentage of relative abundance): 194 (60.0, $[\text{M}^{*+}]$), 164 (40.0), 149 (10.0). Anal. ($\text{C}_9\text{H}_7\text{ClN}_2\text{O}$) C, H, N.

6.1.2.22. 3-Formyl-4-phenyl-1,2,5-oxadiazole (26)

A mixture of **23** (500 mg, 2.8 mmol), MnO_2 (1.24 g, 10.4 mmol) and CHCl_3 as solvent was stirred at room temperature for 24 h. The mixture was filtered through

celite and then the solvent was evaporated in vacuo. After the work-up process the residue was purified by chromatography (SiO_2 , petroleum ether–EtOAc (0–30%)). Yield 30% yield. $^1\text{H-NMR}$ (CDCl_3) 7.54 (m, 3H, H–Ar), 7.96 (m, 2H, H–Ar), 10.48 (s, 1H, CHO). $^{13}\text{C-NMR}$ (HMQC and HMBC experiments) (CDCl_3) 124.00–134.00 (C-Ar), 151.58 (C=N), 153.79 (C=N), 182.32 (CHO). MS (EI, 70 eV); m/z (percentage of relative abundance): 174 (100.0, $[\text{M}^{*+}]$), 146 (12.0), 129 (25.0). Anal. ($\text{C}_9\text{H}_6\text{N}_2\text{O}_2$) C, H, N.

6.2. Pharmacology

6.2.1. Cells

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

6.2.2. Aerobic and hypoxic cytotoxicity: suspension cultures

Monolayers of V79 cells in exponential growth were trypsinised, and suspension cultures were set up in 50 mL glass flasks: 2×10^4 cells mL^{-1} 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 DMSO or in sterilised 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). 7-Chloro-3-(3-dimethylaminopropylamino)quinoxaline-2-carbonitrile N_1, N_4 -dioxide, hydrochloride was used as the reference positive drug.

6.2.4. Cloning

After 2 h exposure to the compound the cells were centrifuged and re-suspended 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 per 30 mm of well. Plates were incubated at 37 °C in 5% CO₂ 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.

6.3. Electrochemical method

Cyclic voltammetry experiments were carried out in DMSO (Aldrich, spectroscopy grade) with 0.1 M tetrabutylammonium perchlorate (Fluka) as the supporting electrolyte and purged with nitrogen at room temperature. Typically 10–12 mg of compound was used in a cell volume of ~40 mL. A mercury-dropping electrode was used as the working electrode, a platinum wire as the auxiliary electrode and saturated calomel as the reference electrode. Voltammograms were obtained using a Weenking POS 88 instrument with a Kipp Zenen BD93 recorder. Voltages scan rates ranged from 0.1 to 1.0 V s⁻¹.

6.4. ESR measurement

The radicals were generated by electrolytical reduction in situ at room temperature. ESR spectra were recorded in the X band (9.85 GHz) on a Bruker ECS 106 spectrometer, using a rectangular cavity with a 50 kHz field modulation, in DMSO (Aldrich, spectroscopy grade).

6.5. Crystallography

Suitable single crystals of **23** were obtained by slow evaporation from AcOEt. The X-ray diffraction pattern was collected at 120 K on a Kappa CCD diffractometer employing Mo K α radiation ($\lambda = 0.71073$ Å). Derivative **23** crystallises in the monoclinic $P2_1/n$ space group, with $a = 6.9910(3)$, $b = 6.9180(3)$, $c = 16.8700(8)$ Å, $\beta = 91.762(2)^\circ$ and $Z = 4$. The structure was solved from 1515 reflections with $I > 2\sigma(I)$ and refined to an agreement R_1 -factor of 0.043. The hydrogen atoms were found in a difference Fourier map. However, all H-atoms but the hydroxyl one were positioned stereochemically and refined with the riding model. The hydroxyl

H-atom was refined isotropically. As expected, the heterocycle shows a symmetric bonding structure, with N–O bond lengths equal to within experimental accuracy (average(dispersion) = 1.381(2) Å). C–N bond distances are also experimentally equal to each other (= 1.305(1) Å). The phenyl ring subtends an angle of 19.14(3)° with the heterocycle. The CH₂OH substituent forms a C2C1C3O2 torsion angle of 74.8(2)°, with their hydroxyl H-atom lying nearly on the C1C3O2 plane. The crystal is stabilised by a relatively strong and linear intermolecular H-bond involving the hydroxyl group of a molecule and one of the nitrogen atoms of a neighbour, symmetry related, molecule ($d(\text{H}\cdots\text{N}2') = 1.972$ Å, $\angle(\text{O}2-\text{H}\cdots\text{N}2') = 171^\circ$). The X-ray diffraction intensities were reduced and corrected with DENZO and SCALEPACK programs [46]. The structure was solved by direct and Fourier methods and the final molecular model obtained by anisotropic full-matrix least-squares refinement of the non-H atoms employing SHELXS [47] and SHELXL [48] programs.

7. Supplementary material

Listings of atomic anisotropic displacement parameters, and hydrogen atoms positions and isotropic displacement parameters (two tables) are available from the authors on request.

Acknowledgements

This work was financially supported by Comisión Honoraria de Lucha Contra el Cáncer (Uruguay), Proyecto X-2 of CYTED (Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo), CONICET of Argentina and FAPESP of Brazil. Part of the X-ray diffraction experiments were carried out at the National Diffraction Laboratory (LANADI), La Plata, Argentina.

References

- [1] Rockwell S., Kallman R.F., Radiat. Res. 53 (1973) 281–294.
- [2] Teicher B.A., Lazo J.S., Sartorelli A.C., Cancer Res. 41 (1981) 73–81.
- [3] Teicher B.A., Cancer Metastasis Rev. 13 (1994) 139–168.
- [4] Jaffar M., Stratford I.J., Exp. Opin. Ther. Patents 9 (1999) 1371–1380.

- [5] Lee H.H., Wilson W.R., Ferry D.M., van Zijl P., Pullen S.M., Denny W.A., *J. Med. Chem.* 39 (1996) 2508–2517.
- [6] 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.
- [7] Aboagye E.O., Lewis A.D., Tracy M., Workman P., *Biochem. Pharmacol.* 54 (1997) 1217–1224.
- [8] Naylor M.A., Swann E., Everett S.A., Jaffar M., Nolan J., Robertson N., Lockyer S.D., Patel K.B., Dennis M.F., Stratford M.R., Wardman P., Adams G.E., Moody C.J., Stratford I.J., *J. Med. Chem.* 41 (1998) 2720–2731.
- [9] Palmer B.D., Wilson W.R., Atwell G.J., *J. Med. Chem.* 37 (1994) 2175–2184.
- [10] Wilson W.R., Moselen J.W., Cliffe S., *Int. J. Radiat. Oncol. Biol. Phys.* 29 (1994) 323–327.
- [11] Siim B.G., Denny W.A., Wilson W.R., *Oncol. Res.* 9 (1997) 357–369.
- [12] Brown J.M., *Mol. Med. Today* 6 (2000) 157–162.
- [13] Monge A., Martínez-Crespo F.J., López de Ceráin A., Palop J.A., Narro S., Senador V., Marín A., Sáinz Y., González M., Hamilton E., Barker A.J., *J. Med. Chem.* 38 (1995) 4488–4494.
- [14] Ortega M.A., Morancho M.J., Martínez-Crespo F.J., Sáinz Y., Montoya M.E., López de Ceráin A., Monge A., *Eur. J. Med. Chem.* 35 (2000) 21–30.
- [15] Cerecetto H., González M., Onetto S., Risso M., Saenz P., Seoane G., Bruno A.M., Alarcon J., Olea-Azar C., López de Ceráin A., Ezpeleta O., Monge A., *Med. Chem. Res.* 10 (2001) 328–337.
- [16] Monge A., López de Ceráin A., Ezpeleta O., Cerecetto H., Dias E., Di Maio R., González M., Onetto S., Risso M., Seoane G., Zinola F., Olea-Azar C., *Pharmazie* 53 (1998) 698–702.
- [17] Monge A., López de Ceráin A., Ezpeleta O., Cerecetto H., Dias E., Di Maio R., González M., Onetto S., Seoane G., Suescun L., Marieczurrena R., *Pharmazie* 53 (1998) 758–764.
- [18] Cerecetto H., González M., Risso M., Seoane G., López de Ceráin A., Ezpeleta O., Monge A., Suescun L., Mombrú A., Bruno A.M., *Arch. Pharm.* 333 (2000) 387–393.
- [19] Monge A., López de Ceráin A., Ezpeleta O., unpublished results.
- [20] Hansch C., Leo A., Unger S.H., Kim K.H., Nikaitani D., Lien E.J., *J. Med. Chem.* 16 (1973) 1207–1216.
- [21] Brown J.M., *Br. J. Cancer* 67 (1993) 1163–1170.
- [22] Tietze L.F., Eicher T.H., *Reaction and Synthesis in the Organic Chemistry Laboratory*, University Science Books, Mill Valley, CA, 1989, pp. 118–119.
- [23] Medana C., Ermondi G., Frutero R., Di Stilo A., Ferretti C., Gasco A., *J. Med. Chem.* 37 (1994) 4412–4416.
- [24] Isler O., Gutmann H., Montavon M., Rüegg R., Ryser G., Zeller P., *Helv. Chim. Acta* 40 (1957) 1242–1249.
- [25] Wittig G., Haag W., *Chem. Ber.* 88 (1955) 1654–1666.
- [26] Plenevaux A., Dewey S.L., Fowler J.S., Guillaume M., Wolf A.P., *J. Med. Chem.* 33 (1990) 2015–2019.
- [27] Swain C.G., Lupton E.C., *J. Am. Chem. Soc.* 90 (1968) 4328–4337.
- [28] Monge A., Palop J.A., González M., Martínez-Crespo F.J., López de Ceráin A., Sáinz Y., Narro S., Barker A.J., Hamilton E., *J. Heterocycl. Chem.* 32 (1995) 1213–1217.
- [29] Vogel A.I., *Textbook of Practical Organic Chemistry*, 4th ed., Longman, London, 1978, p. 587.
- [30] Bunton C.A., Hackey D.L., Leresche J.-P., *J. Org. Chem.* 37 (1972) 4036–4039.
- [31] Howard E., Olszewski W.F., *J. Am. Chem. Soc.* 81 (1959) 1483–1484.
- [32] Boyer J.H., Ellzey S.E., *J. Org. Chem.* 26 (1961) 4684–4685.
- [33] Aoyagi Y., Abe T., Ohta A., *Synthesis* 8 (1997) 891–894.
- [34] Johnson C.K., ORTEP-II: A Fortran Thermal-Ellipsoid Plot Program, Report ORNL-5138, Oak Ridge National Laboratory, TN, USA, 1976.
- [35] Olea-Azar C., Atria A.M., Mendizabal F., Di Maio R., Seoane G., Cerecetto H., *Spectrosc. Lett.* 31 (1998) 99–109.
- [36] Olea-Azar C., Atria A.M., Mendizabal F., Di Maio R., Seoane G., Cerecetto H., *Spectrosc. Lett.* 31 (1998) 849–857.
- [37] Cerecetto H., Di Maio R., González M., Risso M., Saenz P., Seoane G., Denicola A., Peluffo G., Quijano C., Olea-Azar C., *J. Med. Chem.* 42 (1999) 1941–1945.
- [38] Brown J.M., Siim B.G., *Semin. Radiat. Oncol.* 6 (1996) 22–36.
- [39] Rauth A.M., Melo T., Misra V., *Int. J. Radiat. Oncol. Biol. Phys.* 42 (1998) 755–762.
- [40] Naylor M.A., Thomson P., *Mini Rev. Med. Chem.* 1 (2001) 17–29.
- [41] Berry J.M., Watson C.Y., Whish W.J.D., Threadgill M.D., *J. Chem. Soc. Perkin Trans.* 1 (1997) 1147–1156.
- [42] Zamilloa E., Aldana I., Bachiller C.M., Monge A., *Arzneim.-Forsch. Drug Res.* 47 (1997) 873–878.
- [43] Di Stilo A., Sorba G., Medana C., Fruttero R., Gasco A., *Farmaco* 52 (1997) 339–341.
- [44] Sorba G., Medana C., Frutero R., Cena C., Di Stilo A., Galli V., Gasco A., *J. Med. Chem.* 40 (1997) 463–469.
- [45] Boschi D., Cena C., Di Stilo A., Fruttero R., Gasco A., *Bioorg. Med. Chem.* 8 (2000) 1727–1732.
- [46] Otwinowski Z., Minor W., in: Carter C.W. Jr., Sweet R.M. (Eds.), *Methods in Enzymology*, Academic Press, New York, 1997, pp. 307–326.
- [47] Sheldrick G.M., SHELXS-97: Program for Crystal Structure Resolution, University of Göttingen, Göttingen, Germany, 1997.
- [48] Sheldrick G.M., SHELXL-97: Program for Crystal Structures Analysis, University of Göttingen, Göttingen, Germany, 1997.