4-Cyano-2-oxo-1,2,4-oxadiazolo[2,3-*a*]quinoxaline 5-*N*-oxides. New Synthetic Method and Reaction with Alcohols. Potential Cytotoxic Activity

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Several quinoxaline 1,4-di-*N*-oxides have been shown to be efficient and selective cytotoxins for hypoxic cells. We present now a series of 4-cyano-2-oxo-1,2,4-oxadiazolo[2,3-*a*]quinoxaline 5-*N*-oxides **2a-2k**. They were prepared starting from 3-amino-2-quinoxalinecarbonitrile 1,4-di-*N*-oxides **1a-1k** and 2-chloroethyl isocyanate in dry dioxane at 100-110°. A reaction mechanism is proposed. The treatment of **1a** with phenyl isocyanate afforded **2a**. Reaction of **2c** with silica gel yielded **1c**. Compounds **2a-2g** were heated in the presence of ethanol and 2-propanol giving the corresponding carbamates **3a-3g** and **4a-4g**. Compound **2d** was already obtained by heating a mixture of **1d** and ethyl chloroformiate. Compound **2b** was prepared when the carbamate **3b** was heated at 150°. Quinoxalines were tested as cytotoxic agents both in oxic and hypoxic cells. The most interesting compounds were **3g** and **4g**.

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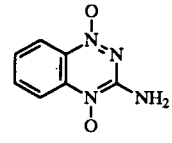
Many human tumors contain hypoxic cells [1] and the radioresistence of these cells can be a limiting factor in radiotherapy [2,3]. Preclinical studies suggest that hypoxic cells may also be refractory to certain chemotherapeutic drugs [4,5]. Accordingly, anti-tumor agents can be made selective by virtue of high activity under hypoxic conditions [6,7]. Benzotriazine and quinoxaline 1,4-di-*N*-oxides have proved to be useful hypoxia-selective therapeutic agents. The benzotriazine Tirapazamine (Figure 1) was the first drug to be introduced into the clinic purely as a bioreductive agent; this compound has shown significant toxicity for hypoxic mammalian cells both *in vitro* and *in vivo* [7-10].



Tirapazamine

Figure 1.

We demonstrated that several quinoxaline 1,4-di-*N*-oxides were more potent and selective than Tirapazamine when they were assayed on V79 cells [11-13]. Preliminary studies of structure-activity relationships suggested the importance of the cyano group in the 2 position; also, mild electron-withdrawing substituents in 6(7) position, *e.g.* Cl, F, CF_3, increased potency under hypoxic conditions. We describe now the preparation and the cytotoxicities of 4-cyano-2-oxo-1,2,4-oxadiazolo[2,3-*a*]quinoxaline 5-*N*-oxides **2a-2k** (Table I) and 3-alkoxycarbonylamino-2-quinoxalinecarbonitrile 1,4-di-*N*-oxides **3a-3g** and **4a-4g** (Table II).

Table I

7,8-Substituted 4-cyano-2-oxo-1,2,4-oxadiazolo[2,3-*a*]quinoxaline 5-*N*-Oxides

Compound	R_7	R_8	mp (°C)	Yield%	Reaction Time (h)	CO Absortion (cm^{-1})	Formula
2a	H	H	189-190	31	24	1815	$C_{10}H_4N_4O_3$
2b	CH_3	H	166-167	60	40	1805	$C_{11}H_6N_4O_3$
2c	CH_3	CH_3	241-242	18	48	1804	$C_{12}H_8N_4O_3$
2d	Cl	H	186-187	60	18	1814	$C_{10}H_8ClN_4O_3$
2e	Cl	CH_3O	220-221	23	30	1831	$C_{11}H_5ClN_4O_4$
2f	F	H	178-179	6	14	1805	$C_{10}H_3FN_4O_3$
2g	Cl	Cl	268-269	22	24	1805	$C_{10}H_2Cl_2N_4O_3$
2h	CH_3O	H	216-217	23	30	1807	$C_{11}H_6N_4O_4$
2i	F	F	180-181	57	30	1802	$C_{10}H_2F_2N_4O_3$
2j	Cl	F	208-209	15	72	1809	$C_{10}H_2FClN_4O_3$
2k	CF_3O	H	210-211	6	18	1809	$C_{11}H_3F_3N_4O_4$

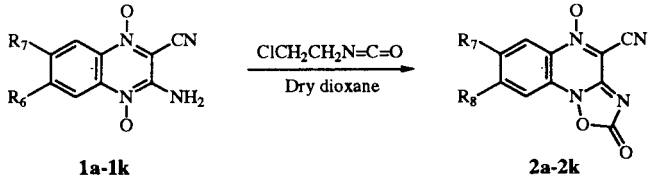
Table II

6,7-Substituted 3-alkoxycarbonylamino-2-quinoxalinecarbonitrile 1,4-Di-*N*-oxides

Compound	R_6	R_7	mp (°C)	Yield %	Reaction Time (h)	CO Absortion (cm^{-1})	Formula
3a	H	H	154-155	61	1.5	1727	$C_{12}H_{10}N_4O_4$
3b	H	CH_3	152-153	46	2.5	1721	$C_{13}H_{12}N_4O_4$
3c	CH_3	CH_3	212(d)	66	2	1742	$C_{14}H_{14}N_4O_4$
3d	H	Cl	179-180	17	2	1724	$C_{12}H_9ClN_4O_4$
3e	CH_3O	Cl	163(d)	31	1.5	1747	$C_{13}H_{11}ClN_4O_5$
3g	Cl	Cl	171(d)	57	2	1727	$C_{12}H_8Cl_2N_4O_4$
4a	H	H	158-159	74	2	1724	$C_{13}H_{12}N_4O_4$
4b	H	CH_3	154-155	56	2.5	1718	$C_{14}H_{14}N_4O_4$
4c	CH_3	CH_3	188-189	62	4	1733	$C_{15}H_{16}N_4O_4$
4d	H	Cl	180-181	67	2	1720	$C_{13}H_{11}ClN_4O_4$
4e	CH_3O	Cl	163(d)	46	3	1761	$C_{14}H_{13}ClN_4O_5$
4g	Cl	Cl	194(d)	43	1	1768	$C_{13}H_{10}Cl_2N_4O_4$

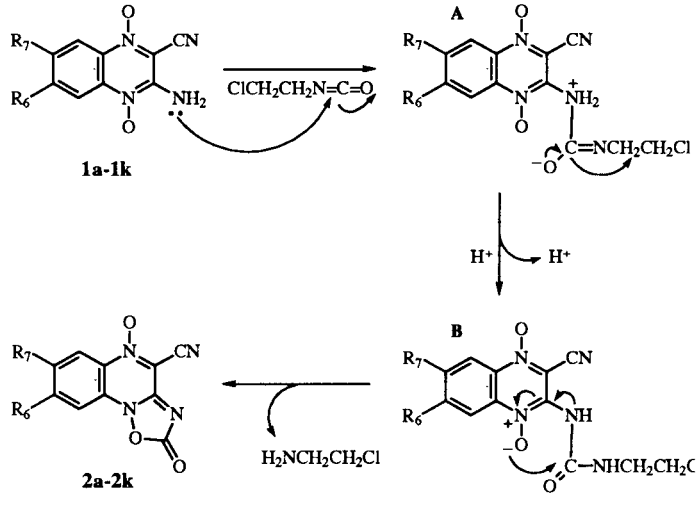
The oxadiazolo[2,3-*a*]quinoxaline 5-*N*-oxides **2a-2k** were obtained as described in Scheme 1, starting from 6,7-substituted 3-amino-2-quinoxalinecarbonitrile 1,4-di-*N*-oxides **1a-1k** and 2-chloroethyl isocyanate in dry toluene at 100-110°. Some of these compounds were previously prepared by reaction with phosgene in chlorobenzene at 90° [14]. The reaction mechanism for this cyclization can therefore be assumed to proceed as follows; it would consist on the attack of the carbonyl group of the isocyanate to the 3-amino group of **1a-1k** affording the intermediate **A**, which would suffer an internal reorganization giving the corresponding urea derivative **B**. The carbonyl group of **B** could react with the nearest *N*-oxide moiety yielding the final oxadiazole ring by leaving a molecule of 2-chloroethylamine (Scheme 2).

Scheme 1



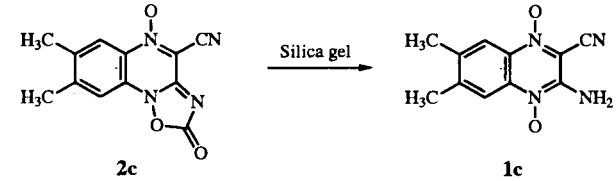
1a-1k **2a-2k**

Scheme 2



The optimal temperature for the reaction was 100-110° and the reaction time was among 14 and 72 hours (Table II). The reaction was monitored by tlc and a new yellow spot appeared showing higher R_f than the original orange-red spot of the starting material. The tlc were scanned under daylight and the new yellow spot darkened slowly. To study this behaviour an experiment was done: a mixture of **2c** and excess silica gel in chloroform was stirred at room temperature while the initial yellow colour changed to orange. After stirring for nine days only one red compound was observed and identified as the 3-aminoquinoxaline **1c** (Scheme 3) [15].

Scheme 3



2c **1c**

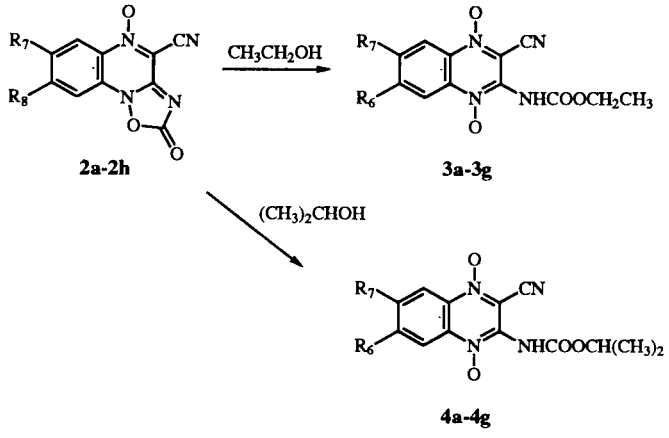
The ir spectra of compounds **2a-2k** presented a very strong absorption at approximately 1800 cm^{-1} corresponding to the carbonyl group (Table I).

Reaction of **1a** with phenyl isocyanate afforded **2a**, suggesting a similar mechanism to that described in Scheme 2. The reaction of **1** with isotiocyanates, *e.g.* phenyl isotiocyanate gave impure materials, which could not be purified.

The carbamates were readily prepared from their corresponding 4-cyano-2-oxo-1,2,4-oxadiazolo[2,3-*a*]quinoxaline 5-*N*-oxides **2a-2g** and alcohols, demonstrating their reactivity with weak nucleophiles. When ethanol was used 3-ethoxycarbonylamino-2-quinoxalinecarbonitrile 1,4-di-*N*-oxides **3a-3g** were obtained (Table II); with 2-propanol 3-isopropoxycarbonylamino-2-quinoxalinecarbonitrile 1,4-di-*N*-oxides **4a-4g** were isolated (Table II), as illustrated

in Scheme 4. We have selected electronwithdrawing groups (chlorine preferably) and electron-donating groups in order to establish structure-activity relationships.

Scheme 4

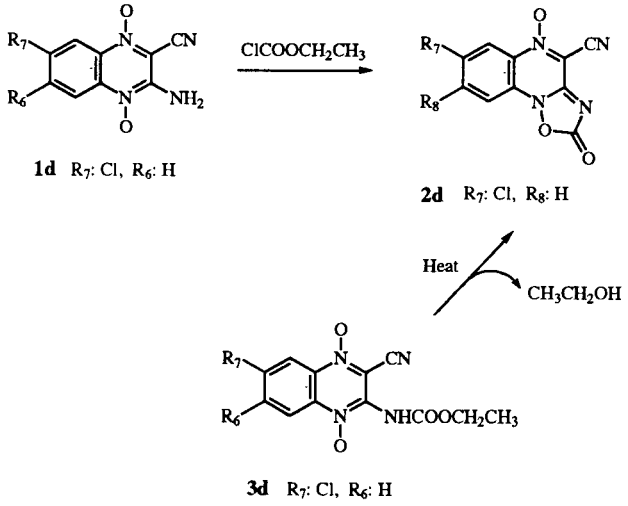


2a-2h **3a-3g** **4a-4g**

The oxadiazolo[2,3-*a*]quinoxaline 5-*N*-oxides **2a-2g** were dissolved in the corresponding alcohol and the mixture was heated under reflux for 1-4 hours. Each reaction was monitored by tlc showing a new yellow spot. The mixture was allowed to stand at room temperature and the precipitate was collected giving the corresponding compound **3a-3g** and **4a-4g**.

We tried to prepare 3-ethoxycarbonylamino-7-chloro-2-quinoxaline carbonitrile 1,4-di-*N*-oxide **3d** by heating under reflux a mixture of 3-amino-7-chloro-2-quinoxalinecarbonitrile 1,4-di-*N*-oxide **1d** and ethyl chloroformiate. However we obtained the unexpected 7-chloro-4-cyano-2-oxo-1,2,4-oxadiazolo[2,3-*a*]quinoxaline 5-*N*-oxide **2d**, as illustrated in Scheme 5. Also **2d** was isolated when the carbamate **3d** was heated at 150° by loss of a molecule of ethanol (Scheme 5). It has been reported that

Scheme 5



1d R_7: Cl, R_6: H

2d R_7: Cl, R_8: H

3d R_7: Cl, R_6: H

2-aminoquinoxaline 1,4-di-*N*-oxide reacted with ethyl chloroformiate at 20° giving the corresponding carbamate [16], while 2-aminoquinoline 1-*N*-oxide afforded the oxadiazolo system [17].

The ir spectra of the carbamates showed a very strong absortion at 1718-1768 cm^{-1} corresponding to carbonyl group.

Compounds were subjected to preliminary cytotoxic evaluation in V79 cells in hypoxic and aerobic conditions

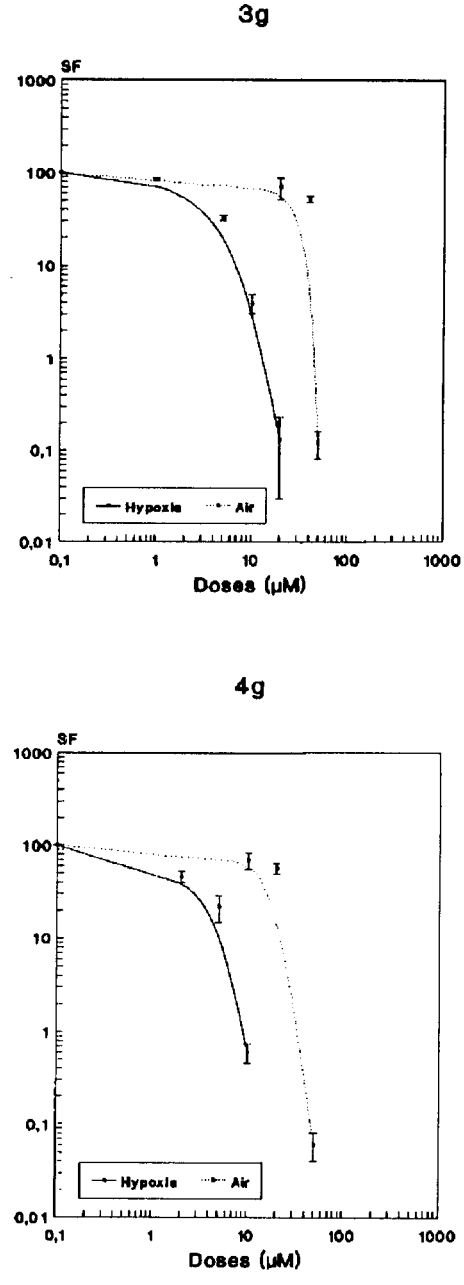


Figure 2. Potency: concentrated in micromolar that gives 1% clonogenic cell survival in hypoxia; hypoxic cyctoxicity ratio is the dose in air/dose in hypoxia giving 99% cell killing.

at 20, 5 and 1 μ*M* determining the survival fraction. The most interesting compound in the oxadiazolo series was **2g**, which showed a potency lower than 5 μ*M*, although it was not selective. We can conclude that the lack of one of the *N*-oxides decreased the hypoxia selectivity . This also was observed in tirapazamine mono-*N*-oxides (SR 4317) and reduced benzotriazines (SR 4330) [18] and in mono-*N*-oxides or reduced quinoxalines [19].

Electron-withdrawing substituents increased the potency and the selectivity of the quinoxalines. Thus, compounds bearing two chlorine atoms in the aromatic ring demonstrated the best *in vitro* profile, such as **3g** and **4g**. The ethyl carbamate **3g** showed a potency of 10 μ*M* and hypoxia cytotoxicity ratio = 4 (Figure 2). The isopropyl carbamate **4g** exhibited similar features than its analogue **3g**, demonstrating a potency of 9 μ*M* and a hypoxia cytotoxicity ratio = 3 (Figure 2, see Footnotes).

EXPERIMENTAL

Melting points were determined using a Mettler FP82+FP80 apparatus and are uncorrected. Elemental analyses were obtained from vacuum-dried samples (over phosphorous pentoxide at 3-4 mm Hg, 24 hours at about 80-100°). Elemental analyses were performed on a Carlo-Erba Strumentazione 1106 Analyzer and were within ±0.4% of the theoretical values except where otherwise stated. Infrared spectra were recorded on a Perkin-Elmer 681 apparatus using potassium bromide tablets; the frequencies are expressed in cm^{-1}. The ^1H nmr spectra were obtained on a Bruker AC-200E (200 MHz) instrument, with tetramethylsilane as the internal reference, at a concentration of about 0.1 g/ml and with dimethyl sulfoxide-d_6 as the solvent; the chemical shifts are reported in ppm of tetramethylsilane in δ units and coupling constants (J) 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 with silica gel 60 Merck (HF, 254-266, Merck or DSF-5, Cammaga) with ethyl acetate and the plates were scanned under daylight and ultraviolet light at 254 and 366 nm. Commercially available reagents were used without further purification and were purchased from the suppliers: Aldrich, Lonza, Maybridge and Scharlau.

6(7)-Substituted-3-amino-2-quinoxalinecarbonitrile 1,4-Di-*N*-oxides **1a-1k**.

General Procedure.

Powdered 5(6)-substituted benzofuroxane (10.00 mmoles) [12] and malononitrile (0.70 g, 10.60 mmoles) were dissolved in dry dimethylformamide (4 ml). The resulting solution was stirred at 0° in an ice-bath. Another solution of triethylamine (5 drops) in dry dimethylformamide (3 ml) was added dropwise. The mixture was stirred at room temperature for 24 hours. The resulting red solid was filtered and washed with diethyl ether giving the corresponding compound **1a-1k**, which was used without further purification.

7(8)-Substituted-4-cyano-2-oxo-1,2,4-oxadiazolo[2,3-*a*]quinoxaline 5-*N*-Oxides **2a-2k**.

General Procedure.

A mixture of the 6(7)-substituted-3-amino-2-quinoxalinecarbonitrile 1,4-di-*N*-oxide **1a-1k** (4.90 mmoles), 2-chloroethyl isocyanate (30.12 mmoles) and dry dioxane (20 ml) was stirred and heated at 100-110° for 18-72 hours. The mixture was allowed to stand at room temperature. The resulting precipitate was collected and recrystallized from toluene giving the corresponding compound **2a-2k**.

4-Cyano-2-oxo-1,2,4-oxadiazolo[2,3-*a*]quinoxaline 5-*N*-Oxide (**2a**).

This compound had ir (potassium bromide): 2237 (CN), 1815 (CO), 1548 (N-CO), 1378 (NO) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 7.86 (t, 1H, H_8, J_{7-8} = 7.5 Hz), 8.11 (d, 1H, H_9), 8.16 (t, 1H, H_7), 8.47 (d, 1H, H_6, J_{6-7} = 8.6 Hz); ms: m/z 228 (M^+, 100), 212 (6), 186 (31), 170 (4).

Anal. Calcd. for $C_{10}H_4N_4O_3$: C, 52.63; H, 1.75; N, 24.56. Found: C, 52.99; H, 1.72; N, 24.76.

4-Cyano-7-methyl-2-oxo-1,2,4-oxadiazolo[2,3-*a*]quinoxaline 5-*N*-Oxide (**2b**).

This compound had ir (potassium bromide): 2237 (CN), 1805 (CO), 1552 (N-CO), 1390 (NO) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 2.50 (s, 3H, CH_3), 8.01 (d, 2H, H_8+H_9), 8.30 (s, 1H, H_6); ms: m/z 242 (M^+, 45), 200 (100), 184 (21).

Anal. Calcd. for $C_{11}H_6N_4O_3$: C, 54.54; H, 2.48; N, 22.76. Found: C, 54.16; H, 2.82; N, 22.40.

4-Cyano-7,8-dimethyl-2-oxo-1,2,4-oxadiazolo[2,3-*a*]quinoxaline 5-*N*-Oxide (**2c**).

This compound had ir (potassium bromide): 2236 (CN), 1804 (CO), 1542 (N-CO), 1380 (NO) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 2.50 (s, 6H, CH_3), 7.93 (s, 1H, H_9), 8.23 (s, 1H, H_6); ms: m/z 256 (M^+, 1), 214 (100), 198 (21).

Anal. Calcd. for $C_{12}H_8N_4O_3$: C, 56.25; H, 3.12; N, 21.87. Found: C, 56.35; H, 3.48; N, 21.89.

4-Cyano-7-chloro-2-oxo-1,2,4-oxadiazolo[2,3-*a*]quinoxaline 5-*N*-Oxide (**2d**).

This compound had ir (potassium bromide): 2234 (CN), 1814 (CO), 1544 (N-CO), 1395 (NO) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 8.16 (d, 1H, H_9, J_{8-9} = 8.9 Hz), 8.25 (dd, 1H, H_8, J_{8-9} = 9.0 Hz, J_{6-8} = 1.3 Hz), 8.54 (d, 1H, H_6, J_{6-8} = 1.3 Hz); ms: m/z 262 (M^+, 100), 246 (5), 220 (13), 204 (3).

Anal. Calcd. for $C_{10}H_3ClN_4O_3$: C, 45.71; H, 1.14; N, 21.33. Found: C, 46.06; H, 1.32; N, 21.41.

4-Cyano-7-chloro-8-methoxy-2-oxo-1,2,4-oxadiazolo[2,3-*a*]-quinoxaline 5-*N*-Oxide (**2e**).

This compound had ir (potassium bromide): 2234 (CN), 1831 (CO), 1546 (N-CO), 1369 (NO) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 4.10 (s, 3H, CH_3O), 7.93 (s, 1H, H_9), 8.51 (s, 1H, H_6); ms: m/z 292 (M^+, 7), 250 (100), 235 (69).

Anal. Calcd. for $C_{11}H_5ClN_4O_4$: C, 45.13; H, 1.71; N, 19.14. Found: C, 45.43; H, 1.94; N, 19.31.

4-Cyano-7-fluoro-2-oxo-1,2,4-oxadiazolo[2,3-*a*]quinoxaline 5-*N*-Oxide (**2f**).

This compound had ir (potassium bromide): 2234 (CN), 1805 (CO), 1549 (N-CO), 1380 (NO) cm^{-1}; ^1H nmr (dimethyl sulfox-

ide-d_6): δ 8.16-8.25 (m, 2H, H_7+H_9), 8.41 (dd, 1H, H_6, J_{6-7} = 8.7 Hz); ms: m/z 246 (M^+, 100), 230 (6), 204 (22), 188 (3).

Anal. Calcd. for $C_{10}H_3FN_4O_3$: C, 48.78; H, 1.22; N, 22.76. Found: C, 48.44; H, 1.25; N, 22.38.

4-Cyano-7,8-dichloro-2-oxo-1,2,4-oxadiazolo[2,3-*a*]quinoxaline 5-*N*-Oxide (**2g**).

This compound had ir (potassium bromide): 2232 (CN), 1805 (CO), 1550 (N-CO), 1380 (NO) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 8.68 (s, 1H, H_9), 8.78 (s, 1H, H_6); ms: m/z 296 (M^+, 18), 280 (8), 254 (100), 238 (15).

Anal. Calcd. for $C_{10}H_2Cl_2N_4O_3$: C, 40.40; H, 0.68; N, 18.85. Found: C, 40.24; H, 0.68; N, 18.82.

4-Cyano-7-methoxy-2-oxo-1,2,4-oxadiazolo[2,3-*a*]quinoxaline 5-*N*-Oxide (**2h**).

This compound had ir (potassium bromide): 2237 (CN), 1807 (CO), 1551 (N-CO), 1376 (NO) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 3.99 (s, 3H, CH_3O), 7.80-7.84 (m, 2H, H_8+H_9), 8.10 (d, 1H, H_6, J_{6-7} = 9.6 Hz); ms: m/z 258 (M^+, 21), 242 (2), 216 (100), 200 (14).

Anal. Calcd. for $C_{11}H_6N_4O_4$: C, 51.16; H, 2.32; N, 21.70. Found: C, 51.28; H, 2.38; N, 21.73.

4-Cyano-7,8-difluoro-2-oxo-1,2,4-oxadiazolo[2,3-*a*]quinoxaline 5-*N*-Oxide (**2i**).

This compound had ir (potassium bromide): 2237 (CN), 1802 (CO), 1548 (N-CO), 1384 (NO) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 8.55 (dd, 1H, H_9, J_{8-9} = 9.6 Hz, J_{7-9}= 6.7 Hz), 8.71 (dd, 1H, H_6, J_{6-7} = 10.0 Hz, J_{6-8} = 7.5 Hz); ms: m/z 264 (M^+, 31), 222 (100), 206 (14).

Anal. Calcd. for $C_{10}H_2F_2N_4O_3$: C, 45.45; H, 0.76; N, 21.21. Found: C, 45.84; H, 1.02; N, 21.46.

4-Cyano-7-chloro-8-fluoro-2-oxo-1,2,4-oxadiazolo[2,3-*a*]-quinoxaline 5-*N*Oxide (**2j**).

This compound had ir (potassium bromide): 2235 (CN), 1809 (CO), 1551 (N-CO), 1398 (NO) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 8.65 (d, 1H, H_9, J_{8-9} = 9.1 Hz), 8.69 (d, 1H, H_6, J_{6-8} = 6.4 Hz); ms: m/z 280 (M^+, 27), 238 (100), 222 (18).

Anal. Calcd. for $C_{10}H_2FClN_4O_3$: C, 42.78; H, 0.71; N, 19.96. Found: C, 43.07; H, 0.88; N, 19.80.

4-Cyano-7-trifluoromethoxy-2-oxo-1,2,4-oxadiazolo[2,3-*a*]-quinoxaline 5-*N*-Oxide (**2k**).

This compound had ir (potassium bromide): 2237 (CN), 1809 (CO), 1559 (N-CO), 1394 (NO) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 8.21-8.40 (m, 2H, H_8+H_9), 8.52 (s, 1H, H_6); ms: m/z 312 (M^+, 15), 296 (1), 270 (99), 254 (8), 69 (100).

Anal. Calcd. for $C_{11}H_3F_3N_4O_4$: C, 42.31; H, 0.96; N, 17.95. Found: C, 42.20; H, 0.96; N, 17.61.

6(7)-Substituted-3-ethoxycarbonylamino-2-quinoxalinecarbonitrile 1,4-Di-*N*-oxides **3a-3g**.

A mixture of the corresponding 7(8)-substituted-4-cyano-2-oxo-1,2,4-oxadiazolo[2,3-*a*]quinoxaline 5-*N*-oxide **2a-2g** (0.25 g) and ethanol (25 ml) was heated under reflux for 1.5-2.5 hours. The mixture was allowed to stand at room temperature and the resulting precipitate was collected and washed with diethyl ether giving the carbamate **3a-3g**.

3-Ethoxycarbonylamino-2-quinoxalinecarbonitrile 1,4-Di-*N*-oxide (**3a**).

This compound had ir (potassium bromide): 3223 (NH), 2989 (CH), 2236 (CN), 1727 (C=O), 1537 (N-CO), 1331 (NO), 1255 (C-O) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 1.28 (t, 3H, CH_3, J = 7.0 Hz), 4.22 (c, 2H, CH_2, J = 6.9 Hz), 7.96-8.12 (m, 2H, H_6+H_7), 8.42-8.51 (m, 2H, H_5+H_8), 10.65-11.05 (bs, 1H, NH); ms: m/z 274 (M^+, 14), 258 (5), 228 (48), 202 (100), 186 (80).

Anal. Calcd. for $C_{12}H_{10}N_4O_4$: C, 52.55; H, 3.65; N, 20.44. Found: C, 52.79; H, 3.79; N, 20.52.

3-Ethoxycarbonylamino-7-methyl-2-quinoxalinecarbonitrile 1,4-Di-*N*-oxide (**3b**).

This compound had ir (potassium bromide): 3238 (NH), 2992 (CH), 2236 (CN), 1721 (C=O), 1532 (N-CO), 1327 (NO), 1284 (C-O) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 1.26 (t, 3H, CH_3, J = 6.9 Hz), 2.59 (s, 3H, CH_3 aromatic), 4.21 (c, 2H, CH_2, J = 7.0 Hz), 7.92 (d, 1H, H_6, J_{5-6} = 8.8 Hz), 8.26 (s, 1H, H_8), 8.38 (d, 1H, H_5, J_{5-6} = 8.7 Hz), 10.60-10.95 (bs, 1H, NH); ms: m/z 288 (M^+, 18), 272 (3), 242 (35), 216 (100).

Anal. Calcd. for $C_{13}H_{12}N_4O_4$: C, 54.16; H, 4.17; N, 19.44. Found: C, 54.06; H, 4.26; N, 19.67.

3-Ethoxycarbonylamino-6,7-dimethyl-2-quinoxalinecarbonitrile 1,4-Di-*N*-oxide (**3c**).

This compound had ir (potassium bromide): 3180 (NH), 2988 (CH), 2237 (CN), 1742 (C=O), 1520 (N-CO), 1329 (NO), 1246 (C-O) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 1.27 (t, 3H, CH_3, J = 7.0 Hz), 2.09 (s, 6H, CH_3 aromatic), 4.21 (c, 2H, CH_2, J = 6.9 Hz), 8.23 (s, 1H, H_5 or H_8), 8.28 (s, 1H, H_5 or H_8), 10.70-10.85 (bs, 1H, NH); ms: m/z 302 (M^+, 11), 286 (3), 256 (86), 230 (61), 214 (100).

Anal. Calcd. for $C_{14}H_{14}N_4O_4$: C, 55.63; H, 4.63; N, 18.54. Found: C, 55.53; H, 4.40; N, 18.91.

7-Chloro-3-ethoxycarbonylamino-2-quinoxalinecarbonitrile 1,4-Di-*N*-oxide (**3d**).

This compound had ir (potassium bromide): 3240 (NH), 2989 (CH), 2237 (CN), 1724 (C=O), 1531 (N-CO), 1322 (NO), 1249 (C-O) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 1.27 (t, 3H, CH_3, J = 7.0 Hz), 4.22 (c, 2H, CH_2, J = 7.0 Hz), 8.09 (dd, 1H, H_6, J_{5-6} = 9.1 Hz, J_{6-8} = 2.1 Hz), 8.44 (d, 1H, H_8), 8.47 (s, 1H, H_5, J_{5-6} = 9.3 Hz), 10.90-10.95 (bs, 1H, NH); ms: m/z 308 (M^+, 3), 292 (5), 246 (12), 220 (100).

Anal. Calcd. for $C_{12}H_9ClN_4O_4$: C, 46.68; H, 2.92; N, 18.15. Found: C, 46.55; H, 2.88; N, 17.98.

7-Chloro-3-ethoxycarbonylamino-6-methoxy-2-quinoxalinecarbonitrile 1,4-Di-*N*-oxide (**3e**).

This compound had ir (potassium bromide): 3109 (NH), 2988 (CH), 2236 (CN), 1747 (C=O), 1539 (N-CO), 1322 (NO), 1236 (C-O) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 1.27 (t, 3H, CH_3), 4.11 (s, 3H, CH_3O), 4.23 (c, 2H, CH_2), 7.84 (s, 1H, H_5), 8.51 (s, 1H, H_8), 10.72-11.00 (bs, 1H, NH); ms: m/z 338 (M^+, 4), 322 (7), 250 (100).

Anal. Calcd. for $C_{13}H_{11}ClN_4O_5$: C, 46.08; H, 3.25; N, 16.54. Found: C, 45.96; H, 3.37; N, 16.69.

6,7-Dichloro-3-ethoxycarbonylamino-2-quinoxalinecarbonitrile 1,4-Di-*N*-oxide (**3g**).

This compound had ir (potassium bromide): 3112 (NH), 2986 (CH), 2236 (CN), 1727 (C=O), 1528 (N-CO), 1318 (NO), 1241 (C-O) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 1.27 (t, 3H, CH_3), 4.23 (c, 2H, CH_2), 8.65 (s, 1H, H_5 or H_8), 8.67 (s, 1H, H_5

or H_8), 10.92-11.20 (bs, 1H, NH); ms: m/z 342 (M^+, 10), 326 (4), 280 (13), 254 (100).

Anal. Calcd. for $C_{12}H_8Cl_2N_4O_4$: C, 41.98; H, 2.33; N, 16.33. Found: C, 42.11; H, 2.42; N, 16.38.

6(7)-Substituted-3-isopropoxycarbonylamino-2-quinoxalinecarbonitrile 1,4-Di-*N*-oxides **4a-4g**.

The title compounds were prepared following the procedure reported for the synthesis of **3a-3g**, using 2-propanol as the reagent.

3-Isopropoxycarbonylamino-2-quinoxalinecarbonitrile 1,4-Di-*N*-oxide (**4a**).

This compound had ir (potassium bromide): 3236 (NH), 2986 (CH), 2235 (CN), 1724 (C=O), 1536 (N-CO), 1333 (NO), 1259 (C-O) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 1.29 (d, 6H, CH_3), 4.95 (m, 1H, -CH =, J = 6.1 Hz), 7.95-8.12 (m, 2H, H_6+H_7), 8.42-8.50 (m, 2H, H_5+H_8), 10.73-10.99 (bs, 1H, NH); ms: m/z 288 (M^+, 6), 272 (1), 228 (32), 212 (9), 202 (100).

Anal. Calcd. for $C_{13}H_{12}N_4O_4$: C, 54.17; H, 4.17; N, 19.44. Found: C, 53.81; H, 4.21; N, 19.39.

3-Isopropoxycarbonylamino-7-methyl-2-quinoxalinecarbonitrile 1,4-Di-*N*-oxide (**4b**).

This compound had ir (potassium bromide): 3238 (NH), 2988 (CH), 2236 (CN), 1718 (C=O), 1531 (N-CO), 1328 (NO), 1257 (C-O) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 1.28 (d, 6H, CH_3, J = 6.1 Hz), 4.90-4.97 (m, 1H, CH=, J = 6.2 Hz), 7.92 (d, 1H, H_6, J_{5-6} = 8.9 Hz), 8.26 (s, 1H, H_8), 8.38 (d, 1H, H_5, J_{5-6} = 8.7 Hz), 10.61-11.00 (bs, 1H, NH); ms: m/z 302 (M^+, 7), 242 (33), 216 (100), 200 (34).

Anal. Calcd. for $C_{14}H_{14}N_4O_4$: C, 55.63; H, 4.63; N, 18.54. Found: C, 55.75; H, 4.72; N, 18.75.

3-Isopropoxycarbonylamino-6,7-dimethyl-2-quinoxalinecarbonitrile 1,4-Di-*N*-oxide (**4c**).

This compound had ir (potassium bromide): 3187 (NH), 2987 (CH), 2235 (CN), 1733 (C=O), 1519 (N-CO), 1328 (NO), 1251 (C-O) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 1.24 (d, 6H, CH_3, J = 6.2 Hz), 2.47 (s, 6H, CH_3 aromatic), 4.86-4.92 (m, 1H, -CH=, J = 6.2 Hz), 8.17 (s, 1H, H_5 or H_8), 8.21 (s, 1H, H_5 or H_8), 10.32-10.71 (bs, 1H, NH); ms: m/z 316 (M^+, 7), 300 (3), 257 (9), 230 (100).

Anal. Calcd. for $C_{15}H_{16}N_4O_4$: C, 56.96; H, 5.06; N, 17.72. Found: C, 56.86; H, 5.18; N, 17.93.

7-Chloro-3-isopropoxycarbonylamino-2-quinoxalinecarbonitrile 1,4-Di-*N*-oxide (**4d**).

This compound had ir (potassium bromide): 3238 (NH), 2988 (CH), 2234 (CN), 1720 (C=O), 1531 (N-CO), 1323 (NO), 1257 (C-O) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 1.30 (d, 6H, CH_3, J = 6.2 Hz), 4.92-4.98 (m, 1H, -CH=, J = 6.2 Hz), 8.10 (dd, 1H, H_6, J_{5-6} = 9.1 Hz, J_{6-8} = 2.2 Hz), 8.45 (s, 1H, H_8), 8.47 (s, 1H, H_5, J_{5-6} = 9.4 Hz), 10.62-11.00 (bs, 1H, NH); ms: m/z 322 (M^+, 5), 306 (3), 263 (9), 247 (7), 236 (86), 220 (100).

Anal. Calcd. for $C_{13}H_{11}ClN_4O_4$: C, 48.37; H, 3.41; N, 17.36. Found: C, 48.22; H, 3.53; N, 17.35.

7-Chloro-3-isopropoxycarbonylamino-6-methoxy-2-quinoxalinecarbonitrile 1,4-Di-*N*-oxide (**4e**).

This compound had ir (potassium bromide): 3261 (NH), 2991 (CH), 2236 (CN), 1761 (C=O), 1531 (N-CO), 1324 (NO), 1237 (C-O) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 1.29 (d, 6H, CH_3, J = 6.2 Hz), 4.12 (s, 1H, CH_3O), 4.91-4.97 (m, 1H, -CH=, J = 6.2 Hz), 8.01 (s, 1H, H_5), 8.52 (s, 1H, H_8), 10.61-10.89 (bs, 1H, NH); ms: m/z 292 (38), 266 (35), 250 (100).

Anal. Calcd. for $C_{14}H_{13}ClN_4O_5$: C, 47.66; H, 3.69; N, 15.89. Found: C, 47.42; H, 3.81; N, 16.26.

6,7-Dichloro-3-isopropoxycarbonylamino-2-quinoxalinecarbonitrile 1,4-Di-*N*-oxide (**4g**).

This compound had ir (potassium bromide): 3259 (NH), 2994 (CH), 2234 (CN), 1768 (C=O), 1530 (N-CO), 1346 (NO), 1236 (C-O) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 1.28 (d, 6H, CH_3, J = 6.2 Hz), 4.92-4.96 (m, 1H, -CH=), 8.65 (s, 2H, H_5+H_8), 10.73-11.20 (bs, 1H, NH); ms: m/z 356 (M^+, 5), 297 (13), 270 (100).

Anal. Calcd. for $C_{13}H_{10}Cl_2N_4O_4$: C, 43.70; H, 2.80; N, 15.69. Found: C, 43.83; H, 2.81; N, 15.75.

Biological Methods.

In vitro selective cytotoxicity in hypoxia was evaluated by a clonogenic assay after 2 hours of treatment of V79 suspension cultures gassed with air or nitrogen.

Cells.

V79 cells (Chinese hamster lung fibroblasts) [18] were obtained from the European Collection of Animal Cell Cultures, and maintained in logarithmic-phase growth as subconfluent monolayers by trypsinization and subculture to 1-2•10^4 cells/cm^2 twice weekly. The growth medium was EMEM containing 10% v/v foetal bovine serum (FBS) and nitracrina 0.06 μM.

Aerobic and Hypoxic Cytotoxicity.

Suspension Cultures.

Monolayers of V79 cells in exponential growth were trypsinized and suspension cultures were set up in 50 ml erlenmeyers: 2•10^4 cells/ml in 30 ml of EMEM containing 10% v/v FBS and HEPES 10 m*M*. The erlenmeyers were tightly closed with rubber caps which were perforated with two needles of 19G•40 mm to provide gas inlet and outlet. Erlenmeyers were submerged and stirred in a water bath at 37°, where they were gassed with humidified air or nitrogen.

Treatment.

Drug solutions were prepared just before the assay was carried out. Stock solutions, 150-fold more concentrated, were prepared in pure dimethyl sulfoxide. Thirty minutes after starting to gas the suspension cultures, 0.2 ml of the stock solution was added to the 30 ml of total medium. In every assay there was an erlenmeyer with 0.2 ml of dimethyl sulfoxide (Negative control). For screening, treatment lasted two hours during which gassing was continuous.

Cloning.

After treatment cells were centrifuged and resuspensed in plating medium (EMEM supplemented with 15% v/v FBS and nitracrina 0.06 μ*M*). The cell density was determined with a Hemocytometer and 10^2 - 10^5 cells were plated in 30 mm 6-well plates to give a final volume of 2 ml/well. Plates were incubated at 37° in 5% carbon dioxide for 7 days and were stained with aqueous crystal violet. The plating efficiency was calculated by dividing the number of clones by the number of cells seeded. The survival fraction is the percentage of plating efficiency of treated cultures with respect to the control.

Screening Assays.

Compounds were tested at 20, 5 and 1 μ*M* in duplicate flasks both in aerobic and hypoxic conditions.

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REFERENCES AND NOTES

* Correspondence and reprints: FAX. 34-48-105619.

[1] J. D. Chapman, *Radiother. Oncol.*, **20** (suppl), 13 (1991).

[2] R. A. Gatenby, H. B. Kessler, J. S. Rosenblum, L. R. Coia, P. J. Moldofsky and W. H. Hartz, *Int. J. Radiat. Oncol. Biol. Phys.*, **14**, 831 (1988).

[3] J. Overgaad, *Radiother. Oncol.*, **24**, 564 (1992).

[4] K. A. Kennedy, *Anticancer Drug Des.*, **2**, 181 (1987).

[5] A. C. Sartorelli, *Cancer Res.*, **48**, 775 (1988).

[6] S. Rockwell, K. A. Kennedy and A. C. Sartorelli, *Int. J. Radiat. Oncol. Biol. Phys.*, **12**, 1239 (1986).

[7] E. M. Zeman, J. M. Brown, M. J. Lemmon, V. K. Hirst and W. W. Lee, *Int. J. Radiat. Oncol. Biol. Phys.*, **12**, 1239 (1986).

[8] J. Parrick and L. K. Mehta, *J. Heterocyclic Chem.*, **30**, 323 (1993).

[9] J. M. Brown, *Br. J. Cancer*, **67**, 1163 (1993).

[10] S. Khan and P. J. O'Brien, *Br. J. Cancer*, **71**, 780 (1995).

[11] A. Monge, J. A. Palop, A. López de Ceráin, V. Senador, F. J. Martínez-Crespo, Y. Sainz, S. Narro, E. García, C. de Miguel, M. González, E. Hamilton, A. J. Barker, E. D. Clarke and D. T. Greenhow, *J. Med. Chem.*, **38**, 1786 (1995).

[12] A. Monge, J. A. Palop, M. González, F. J. Martínez-Crespo, A. López de Ceráin, Y. Sainz and S. Narro, *J. Heterocyclic Chem.*, **32**, 1213 (1995).

[13] A. Monge, F. J. Martínez-Crespo, A. López de Ceráin, J. A. Palop, S. Narro, V. Senador, A. Marín, Y. Sainz, M. González, E. Hamilton, A. J. Barker, *J. Med. Chem.*, in press (1995).

[14] F. Seng and K. Ley, German Offen. 2,232,468 (1974); *Chem. Abstr.*, **80**, 83067c (1974).

[15] M. Hasegawa and T. Takabatake, *Synthesis*, 938 (1985).

[16] R. A. Burrell, J. M. Cox and E. G. Savins, *J. Chem. Soc., Perkin Trans 1*, 2707 (1973).

[17] M. Hamana and S. Kumadaki, *Chem. Pharm. Bull.*, **22**, 1506 (1974).

[18] E. Robbins and M. D. Scharff, *J. Cell. Biol.*, **34**, 684 (1967).

[19] A. Monge, J. A. Palop, A. Piñol, F. J. Martínez-Crespo, S. Narro, M. González, Y. Sainz and A. López de Ceráin, *J. Heterocyclic Chem.*, **31**, 1135 (1994).