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Selective hypoxia-cytotoxins based on vanadyl complexes with 3-aminoquinoxaline-2-carbonitrile-N^1,N^4-dioxide derivatives

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Abstract

A new vanadyl complex with the formula $VO(L1)_2$, where L1 = 3-amino-6(7)-chloroquinoxaline-2-carbonitrile N^1, N^4-dioxide, has been synthesized and characterized by elemental analyses, conductometry, fast atom bombardment mass spectroscopy (FAB-MS) and electronic, Fourier transform infrared (FTIR), Raman, nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) spectroscopies. Results were compared with those previously reported for analogous vanadium complexes with other 3-aminoquinoxaline-2-carbonitrile N^1,N^4-dioxide derivatives as ligands. As an effort to develop novel metal-based selective hypoxia-cytotoxins and to improve bioavailability and pharmacological and toxicological properties of aminoquinoxaline carbonitrile *N*-dioxides bioreductive prodrugs, the new complex and $VO(L)_2$ complexes, with L = 3-amino-6(7)-bromoquinoxaline-2-carbonitrile N^1,N^4-dioxide (L2) and 3-amino-6(7)-methylquinoxaline-2-carbonitrile N^1,N^4-dioxide (L3), were subjected to cytotoxic evaluation in V79 cells in hypoxic and aerobic conditions. The complexes resulted in vitro more potent cytotoxins than the free ligands (i.e. potencies $P_{VO(L1)2}$ = 3.0, P_{L1} = 9.0 μM) and Tirapazamine (P = 30.0 μM) and showed excellent selective cytotoxicity in hypoxia, being no cytotoxic in oxia. In addition, the solubility in hydrophilic solvents resulted significantly higher for the vanadyl complexes than for the free ligands. These results could be indicative that complexation of the quinoxaline-2-carbonitrile N^1,N^4-dioxide derivatives with vanadium could improve their bioavailability. In addition, a new aspect of the series has been investigated. A detailed comparison of the electrochemical behavior of the free ligands and the complexes has been performed searching for a correlation between reduction potentials of the complexes and their activities and hypoxia selectivities.

Keywords: Vanadium; Quinoxaline *N*-dioxides; N,O ligands; Anti-tumor agents; Hypoxia selective cytotoxins

1. Introduction

Cancerous cells can become relatively isolated from the blood supply due to their rapid growth, turning increasingly difficult the diffusion of oxygen and resulting, frequently, in hypoxia. Environmental difference between these hypoxic solid tumor cells and normal tissue might

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be exploitable in the design of novel drugs [1]. Compounds able to be selectively bioactivated in the absence of oxygen by their metabolism to active cytotoxic species have been developed as selective anti-tumor agents [2,3]. Having the hypoxic-cytotoxin 3-amino-1,2,4-benzotriazine-1,4-dioxide (Tirapazamine) as structural antecedent [4], the capacity of a series of quinoxaline N^1,N^4-dioxide derivatives to act as bioreductive drugs has been previously described by us [2,5–7]. Although excellent in vitro biological results have been obtained with some 3-amino-2-carbonitrile-quinoxaline N^1,N^4-dioxide derivatives, they were not useful for therapy owing to too short in vivo half lives and low solubility in physiological media [8–11].

As an effort to develop novel selective hypoxia-cytotoxins and to improve bioavailability and pharmacological and toxicological properties of quinoxaline N^1,N^4-dioxide derivatives, we focused our current research on the development of metal complexes of this kind of ligands. The proper selection of the metal centre is of fundamental importance towards selectively targeting transition metal complexes to hypoxic cells. The complexes may release in vivo the ligand, i.e. the bioactive drug, by direct ligand substitution or reduction by cellular reductases and subsequent substitution. For this purpose the complexes may have reduction potentials in the appropriate range [1,3,12,13]. In a previous work novel copper complexes with 3-amino-2-carbonitrile-quinoxaline N^1,N^4-dioxide derivatives were synthesized, characterized and in vitro biologically evaluated. Although the complexes showed excellent selective cytotoxicity in hypoxia, they still showed poor solubility in physiological media [14]. Complexes with other metals are currently under development. Although medicinal applications of vanadium compounds have focused on their in vitro and in vivo activity in the treatment of diabetes, anti-carcinogenic activity, tumor growth inhibition and prophylaxis against carcinogenesis due to selected vanadium compounds are well known [15–19]. Having this in mind, we have studied the complexation of 3-amino-2-carbonitrile-quinoxaline N^1,N^4-dioxide derivatives with vanadium [20]. In the present work a new V(IV) complex, $VO(L1)_2$ was synthesized and characterized by elemental analyses, conductometry, fast atom bombardment mass spectrometry (FAB-MS) and electronic, Fourier transform infrared (FTIR), Raman, nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) spectroscopies. Characterization results were compared with those previously reported for analogous vanadium complexes of this series [20]. Three $VO(L)_2$ complexes of the series (L shown in Fig. 1) were subjected to cytotoxic evaluation in V79 cells in hypoxic and aerobic conditions. In addition, a detailed comparison of the electrochemical behavior of the free ligands and the complexes has been performed searching for a correlation between reduction potentials of the complexes and their activities and hypoxia selectivities.

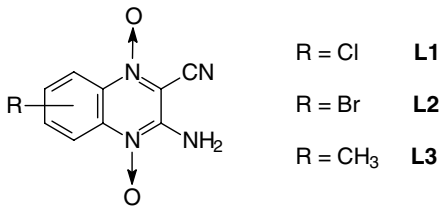

Fig. 1. Selected ligands L: L1 = 3-amino-6(7)-chloroquinoxaline-2-carbonitrile N^1, N^4-dioxide, L2 = 3-amino-6(7)-bromoquinoxaline-2-carbonitrile N^1,N^4-dioxide and L3 = 3-amino-6(7)-methylquinoxaline-2-carbonitrile N^1, N^4-dioxide.

2. Experimental

2.1. Materials

All common laboratory chemicals were purchased from commercial sources and used without further purification. The ligands, 3-amino-6(7)-chloroquinoxaline-2-carbonitrile N^1,N^4-dioxide (L1), 3-amino-6(7)-bromoquinoxaline-2-carbonitrile N^1,N^4-dioxide (L2) and 3-amino-6(7)-methylquinoxaline-2-carbonitrile N^1,N^4-dioxide (L3) were synthesized as a mixture of 6- and 7-substituted isomers by reaction of the corresponding benzofuroxan and malonitrile and characterized, as previously described [14]. $V^{IV}O(acac)_2$ (where acac = acetylacetonate) was prepared according to a well established literature procedure [21].

2.2. Syntheses of the complexes

$V^{IV}O(L2)_2$ and $V^{IV}O(L3)_2$ were prepared by the procedure previously reported, refluxing $V^{IV}O(acac)_2$ (60 mg, 0.227 mmol) with L2 or L3 (0.454 mmol) in methanol (18 mL) during 24–30 h [20]. The new complex $V^{IV}O(L1)_2$ was synthesized by a similar procedure by refluxing $V^{IV}O(acac)_2$ (60 mg, 0.227 mmol) with L1 (107 mg, 0.454 mmol) in methanol (18 mL) during 30 h. The vanadyl complex was filtered off from the hot solution as a red solid.

[V^{IV} O(3-amino-6(7)-chloroquinoxaline-2-carbonitrile N^1, N^4-dioxide)$_2$], $V^{IV}O(L1)_2$: Yield: 49 mg, 40%. Anal (%) Calc. for $VO(C_9H_4N_4O_2Cl)_2$: C, 40.17; H, 1.50; N, 20.82. Found: C, 40.35; H, 1.31; N, 20.80. λ_{max} (DMF): 517 nm, $\varepsilon = 8.6 \times 10^3$ $M^{-1}\,cm^{-1}$. FAB^+ MS *m/z* (assignment): 537/538/539/540/541 ($M^{+\cdot}$), 520/521/522/523/524 ($M^{+\cdot}-OH^{\cdot}$), 498/499/500 ($M^{+\cdot}-O^{\cdot}-CN^{\cdot}+3H^{\cdot}$) and $[L+H^{\cdot}-O^{\cdot}]^+$: 221/223.

The complexes are soluble in acetonitrile, dichloromethane, dimethyl sulfoxide (DMSO) and dimethylformamide (DMF) and partially soluble in methanol and ethanol. The solubility in hydrophilic solvents, like light alcohols, resulted significantly higher for the vanadyl complexes than for the free ligands.

2.3. Physicochemical characterization

C, H and N analyses were performed with a Carlo Erba Model EA1108 elemental analyzer. Routine FAB+ spectra