Med Chem Res (2012) 21:1439–1444
DOI 10.1007/s00044-011-9660-y

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Effect of complexation of 3-aminoquinoxaline-2-carbonitrile 1,4-dioxides with palladium and copper on their anti-*T. cruzi* activity

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Received: 8 May 2010 / Accepted: 4 May 2011 / Published online: 20 May 2011

Abstract Pd(II) and Cu(II) complexes of 3-aminoquinoxaline-2-carbonitrile 1,4-dioxides were prepared in order to improve the anti-*Trypanosoma cruzi* activity of these ligands. The in vitro evaluations demonstrated that the metal complexation modified the activity of the ligands in different manners. Except for one compound, complexation with palladium increased the trypanosomicidal activity 20–80-times. Besides, copper also modified favorably the activity, however, the copper compounds resulted less active than the palladium ones, at the studied doses.

Keywords Palladium complexes · Copper complexes · Chagas disease · 3-Aminoquinoxaline-2-carbonitrile 1 · 4-dioxide · *Trypanosoma cruzi*

Introduction

Chagas' disease is the third largest neglected disease in Latin America after malaria and schistosomiasis, affecting at least 15 million people with more than 25 million at the risk of infection (http://www.who.int/tdr.). Its infectious agent is the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*), with symptoms progressing from mild swelling to intestinal disease and ultimately heart failure. The current drugs for this disease are two nitroaromatic compounds, Nifurtimox (Nfx, Lampit®) and Benznidazole (Bnz, Rochagan®) (Rodrigues Coura and Castro 2002; Schofield *et al.*, 2006). This chemotherapy is insufficient, as it exhibits unacceptable side effects and often do not completely eliminate the parasite despite chronic administration (Cerecetto and González, 2007, 2010; Castro *et al.*, 2006). Moreover, resistance to these agents has emerged (http://www.who.int/tdr/diseases/chagas/direction.htm). For these reasons the development of more safe and efficient drugs against Chagas' disease is urgent (Nwaka and Ridley, 2003). Several types of compounds have been described as anti-*T. cruzi* agents acting on different biological targets (Rivera *et al.*, 2009). Some quinoxaline derivatives were carefully selected from Dr. Antonio Monge chemical-library in order to evaluate them against *T. cruzi* being 3-aminosubstituted-2-carbonitrile derivatives (i.e., **1** and **2**, Fig. 1) good in vitro *T. cruzi* growth inhibitors (Aguirre *et al.*, 2004; Ancizu *et al.*, 2009) while the 3-amino-2-carbonitrile derivatives were inactive (i.e. **3–7**, Fig. 1). However, QSAR study predicts derivatives **3–7** to be more active than experimentally found, i.e., percentage of growth inhibition for derivative **7** at 25 μM would be 60% but experimentally this compound inhibited 25% at this concentration. The low activities displayed by these derivatives could be the result of their low solubilities in the physiological media (Zamalloa *et al.*, 1997a, b). Another possibility, which was not identified in the previous QSAR studies (Aguirre *et al.*, 2004), is that anti-*T. cruzi* agents must comply with specific structural requirements, i.e., volume, in the 3-amino moiety like derivatives **1** and **2** (Fig. 1). Recently, the authors have reported the biological characterization as anti-*T. cruzi*

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agents of a series of vanadyl complexes of the type $[V^{IV}O(L)_2]$ where L are the bidentate 3-aminoquinoxaline-2-carbonitrile 1,4-dioxides ligands (Urquiola *et al.*, 2006) (**8–11**, Fig. 1). Complexation to vanadium of the quinoxaline ligands led to excellent antiprotozoal activity similar to that of the reference drugs Nfx and Bnz and higher than that of the corresponding free ligands. The anti-trypanosomal activity of $[V^{IV}O(L)_2]$ complexes could be explained on the basis of their lipophilicities and the electronic characteristics of the quinoxaline substituents (Urquiola *et al.*, 2006). On the other hand, the use of multitarget-directed drugs (MTDDs) emerges as a new strategy for the development of new Chagas' disease agents (Cavalli and Bolognesi, 2009). This approach, based in the combination of two or more pharmacophores into a new chemical entity, defined as hybrid-drugs, is still in its early stage for this illness. One of the recent focuses has been the development of MTDDs as anti-*T. cruzi* agents using DNA-interacting moieties linked to free-radical releasing- or fumarate reductase inhibitor-pharmacophores where the first one was represented by palladium, platinum or gold metals and the second one by the 5-nitrofuryl scaffold (Vietes *et al.*, 2008a, b, 2009; Otero *et al.*, 2006a).

Having this in mind, the authors have studied the biological behavior, against *T. cruzi*, of new hybrid-drugs obtained by the complexation of 3-amino-2-carbonitrile-quinoxaline 1,4-dioxides with palladium and copper as an effort to develop novel MTDD trypanosomicidal agents by changing the volume at the 3-amino level and by improving bioavailability of the organic ligands. To the best of the knowledge, this report constitutes the first of its kind in using Pd and Cu complexes of quinoxaline 1,4-dioxides in the design of targeted antitrypanosomal agents.

Materials and methods

Chemistry

All common laboratory chemicals were purchased from commercial sources and used without further purification. The ligands, 3-aminoquinoxaline-2-carbonitrile 1,4-dioxides, were synthesized by reaction of the corresponding benzofuroxan and malonitrile as previously described (González *et al.*, 2007). The benzo-substituted quinoxalines were obtained as mixtures of 6- and 7-substituted isomers (González *et al.*, 2007). The palladium complexes, **12–15**, and the copper complexes, **16–21** (Scheme 1) were synthesized and characterized following previously established methodologies (Urquiola *et al.*, 2009, 2008). In particular, palladium complexes **12**·H_2O, **13**·H_2O, **14**·$3H_2O$, and **15**·$2CH_3OH$ were synthesized by ligand substitution onto $[PdCl_4]^{-2}$ under reflux in methanol. Thermogravimetric, FAB-MS and ^{1}H-NMR results were exhaustively discussed and were in agreement with the proposed analytical and structural formula (Urquiola *et al.*, 2009).

On the other hand, copper complexes **16–21** were synthesised sonicating each ligand in dried ethanol until total dissolution. To the resulting solution 20.0 mL of an ethanolic solution of $CuSO_4·5H_2O$ (0.075 mmol) were added. After stirring for half an hour, a purple precipitate was formed, then filtered, washed with small portions of ethanol, and dried at room temperature.

The complex **22**, [Cu(**3**-H)(ala-H)], where **3**-H and ala-H are the deprotonated quinoxaline **3** (Fig. 1) and alanine, was synthesized through a solid state technique by mixing $CuCO_3Cu(OH)_2$ (11 mg), alanine (9 mg), and **3** (20.0 mg), adding some drops of water and heating the mixture at

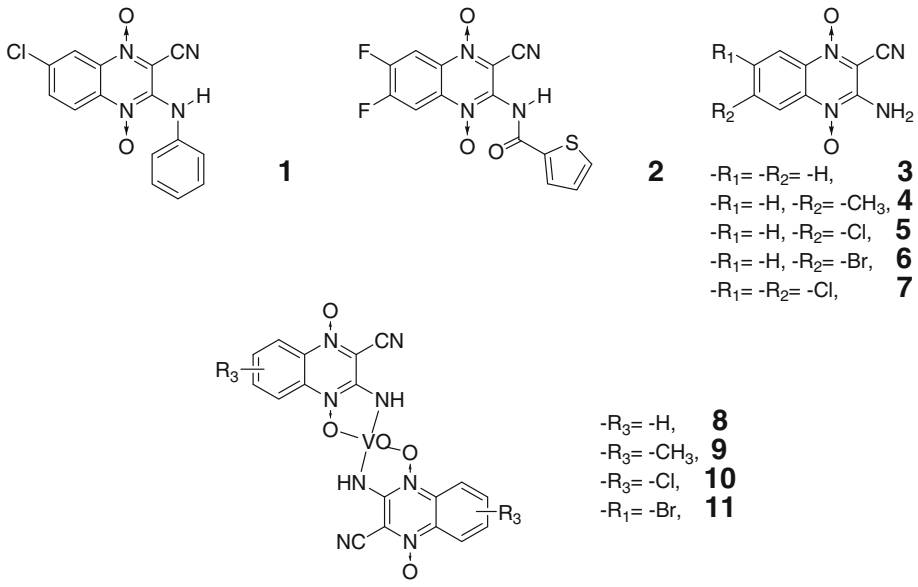
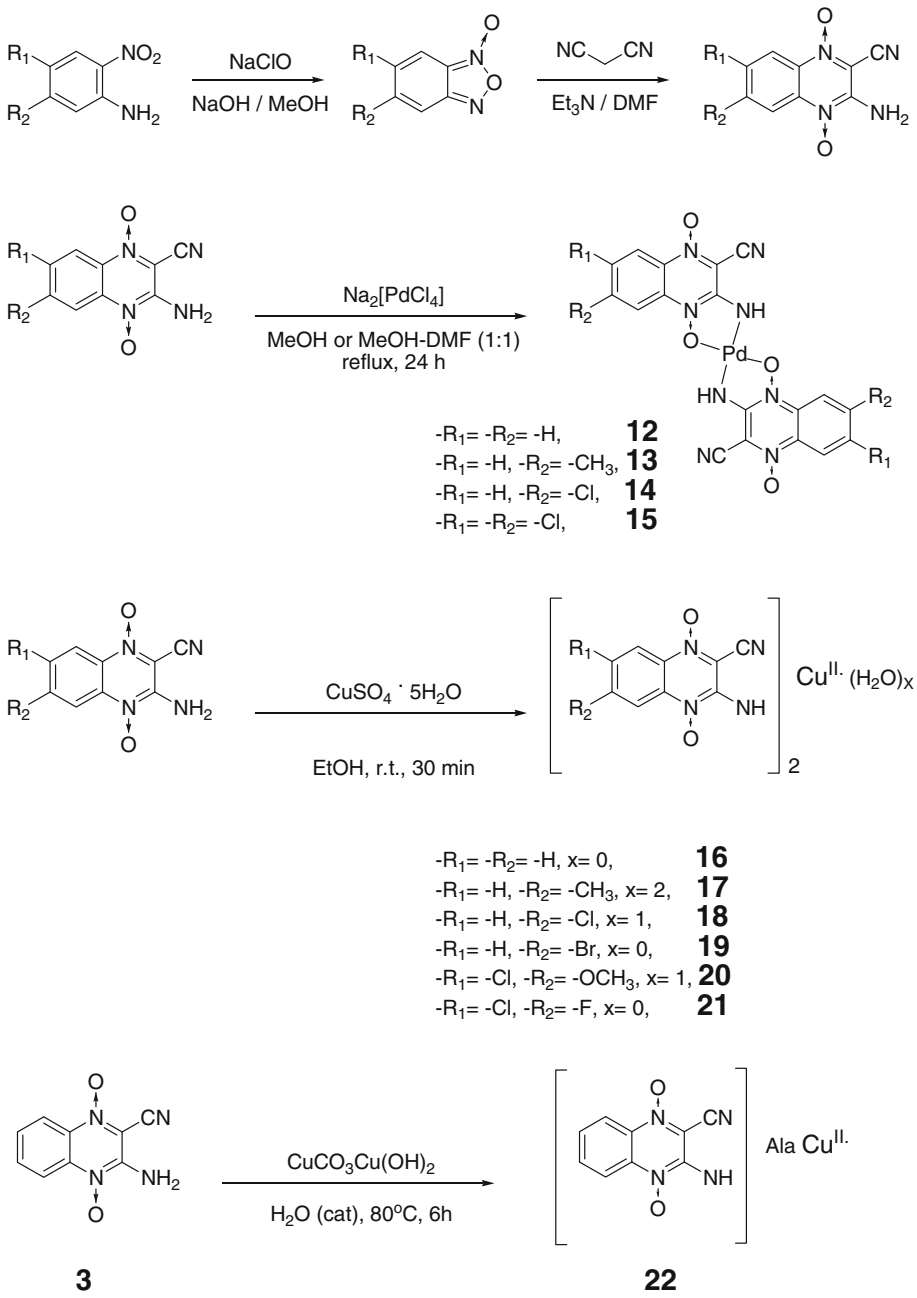


Fig. 1 Trypanosomicidal 3-aminoquinoxaline-2-carbonitrile 1,4-dioxides, **1** and **2**, inactive ones, **3–7**, and their corresponding vanadium complexes, **8–11**

Scheme 1 Synthetic procedures for the preparation of ligands, palladium, and cooper complexes



80°C during 6 h (Tarallo *et al.*, 2009). A red complex was obtained.

The results of the elemental analysis (%) obtained with a Carlo Erba EA 1108 analyzer, the yields and the IR spectra performed with a Bomen M102 instrument (4000–400 cm^{-1}) were:

12, Found/Calc.(%): C, 41.41/41.20; H, 2.52/2.30; N, 21.34/21.35. Yield: 52%. IR (KBr) (ν/cm^{-1}): 3400 (N–H), 1561 and 1618 (C=N→O), 2235 (C≡N).

13, Found/Calc.(%): C, 43.23/43.30; H, 2.95/2.91; N, 20.04/20.20. Yield: 61%. IR (KBr) (ν/cm^{-1}): 3406 (N–H), 1561 and 1618 (C=N→O), 2230 (C≡N).

14, Found/Calc.(%): C, 34.36/34.23; H, 2.20/2.23; N, 17.65/17.74. Yield: 45%. IR (KBr) (ν/cm^{-1}): 3396 (N–H), 1614 and 1664 (C=N→O), 2242 (C≡N).

15, Found/Calc.(%): C, 33.75/33.80; H, 2.06/1.99; N, 15.67/15.77. Yield: 67%. IR (KBr) (ν/cm^{-1}): 3347 (N–H), 1561 and 1579 (C=N→O), 2232 (C≡N).

16, Found/Calc.(%): C, 46.17/46.41; N, 23.94/24.05; H, 2.49/2.16. Yield: 53%. IR (KBr) (ν/cm^{-1}): 3386 (N–H), 1575 and 1593 (C=N→O), 2221 (C≡N).

17, Found/Calc.(%): C 45.47/45.47; N 21.17/21.17; H 3.13/3.17. Yield 55%. IR (KBr) (ν/cm^{-1}): 3356 (N–H), 1610, 1592 and 1593 (C=N→O), 2225 (C≡N).

18, Found/Calc. (%): C 39.78/39.09; N 20.49/20.27; H 2.31/1.81. Yield 48%. IR (KBr) (ν/cm^{-1}): 3358 (N–H), 1589 and 1561 (C=N→O), 2225 (C≡N).

19, Found/Calc. (%): C 34.15/34.54; N 17.25/17.91; H 2.17/1.26. Yield 54%. IR (KBr) (ν/cm^{-1}): 3364 (N–H), 1586 and 1561 (C=N→O), 2221 (C≡N).

20, Found/Calc.(%): C, 39.62/39.18; N, 17.92/18.28; H, 1.96/2.29. Yield 39%. IR (KBr) (ν/cm^{-1}): 3386 (N–H), 1562 and 1584 (C=N→O), 2228 (C≡N), 1340 (N→O).

21, Found/Calc.(%): C, 37.98/37.88; N, 19.35/19.63; H, 1.08/1.06. Yield 41%. IR (KBr) (ν/cm^{-1}): 3362 (N–H), 1573 and 1595 (C=N→O), 2231 (C≡N).

22, Found/Calc. (%): C, 40.97/40.48; N, 19.91/;19.74, H, 3.15/3.05. Yield: 95%. IR (KBr) (ν/cm^{-1}): 3385 (NH); 2226 (C≡N), 1395 (COO$^-$).

The electronic spectra recorded on a Shimadzu UV-1603 spectrophotometer in DMF solution of all the copper complexes show a d-d broad band in the 500–594 nm range characteristic of Cu(II) in a distorted octahedral geometry (Lever, 1984).

Conductometric measurements of complexes were performed at 25°C in 10^{-3}–10^{-4} M DMF and DMSO solutions using a Conductivity Meter 4310 Jenway (Geary, 1971). The measurements showed no significant change in the conductivity in comparison with that of the pure solvents. This behavior is in agreement with the neutrality of the complexes, according to the assigned formula. In addition, the stability of the complexes in DMSO solutions during the time involved in the biological tests was stated by this technique.

Low-temperature (120 K) EPR experiments of DMSO solution of complexes **16–21** were performed using a Varian E109 spectrometer equipped with a rectangular cavity and 100 kHz field modulation. The g-values were obtained by means of spectral simulation using the software package EasySpin (Stoll and Schweiger, 2006). An exhaustive EPR spectrum analysis of these copper compounds was previously reported (Torre *et al.*, 2005; Urquiola *et al.*, 2008).

Biology

Trypanosoma cruzi epimastigotes (Tulahuen 2 strain) were grown at 28°C in an axenic medium (BHI-Tryptose) as previously described (Urquiola *et al.*, 2006; Otero *et al.*, 2006a, b), complemented with 5% foetal calf serum. Cells were harvested in the late log phase, re-suspended in fresh medium, counted in Neubauer's chamber and placed in 24-well plates (2×10^6/ml). Cell growth was measured as the absorbance of the culture at 590 nm, which was proved to be proportional to the number of cells present. Before inoculation, the media were supplemented with the indicated amount of the studied compound from a stock solution in DMSO. The final concentration of DMSO in the culture media never exceeded 1% and the control was run in the presence of 1% DMSO and in the absence of any compound. No effect on epimastigotes growth was observed by the presence of up to 1% DMSO in the culture media. Nfx was used as the reference trypanosomicidal drugs. The percentage of growth inhibition was calculated as follows $\{1-[(Ap-A0p)/(Ac-A0c)]\} \times 100$, where $Ap = A_{590}$ of the culture containing the studied compound at day 5; $A0p = A_{590}$ of the culture containing the studied compound right after addition of the inocula (day 0); $Ac = A_{590}$ of the culture in the absence of any compound (control) at day 5; $A0c = A_{590}$ in the absence of the compound at day 0.

Results and discussion

The synthesis of the quinoxaline ligands (Scheme 1) were achieved using previously described methods yielding the desired intermediates in excellent yields and high purities. The previously described synthetic approaches for the palladium, **12–15**, and the copper complexes, **16–21** (Scheme 1), were performed with excellent yields and high purities. Complex **22** was designed as analog of copper complex **16** in order to evaluate the effect of the inclusion of a hydrosoluble co-ligand, alanine, in the anti-*T. cruzi* activity. Cooper complex **22** was prepared using a novel methodology in solvent-free conditions using as cooper reagent $CuCO_3{\cdot}Cu(OH)_2$. Analytical data and conductometric, thermogravimetric and FAB-MS results were in agreement with the proposed analytical and structural formula. Conductometric measurements showed that the complexes behave as non electrolytes, in agreement with the non charged formula proposed. In addition, it was demonstrated by conductometry that the compounds are

Table 1 Percentage of *Trypanosoma cruzi* growth inhibitions (PGI, %) for the studied compounds

Ligand	PGI	V-complex	PGI	Pd-complex	PGI	Cu-complex	PGI
3	25.0	**8**	59.5	**12**	40.8	**16**	62.7
4	5.0	**9**	47.5	**13**	53.9	**17**	14.0
5	0.0	**10**	95.0	**14**	78.1	**18**	4.0
6	31.5	**11**	91.5			**19**	0.0
7	25.0			**15**	4.8	**21**	0.0
						22	3.9
						23	35.0
Nfx	100.0						

PGI percentage of growth inhibition (%) of Tulahuen 2 epimastigotes at 25 μM. The results are the mean values of three different experiments with a SD less than 10% in all cases

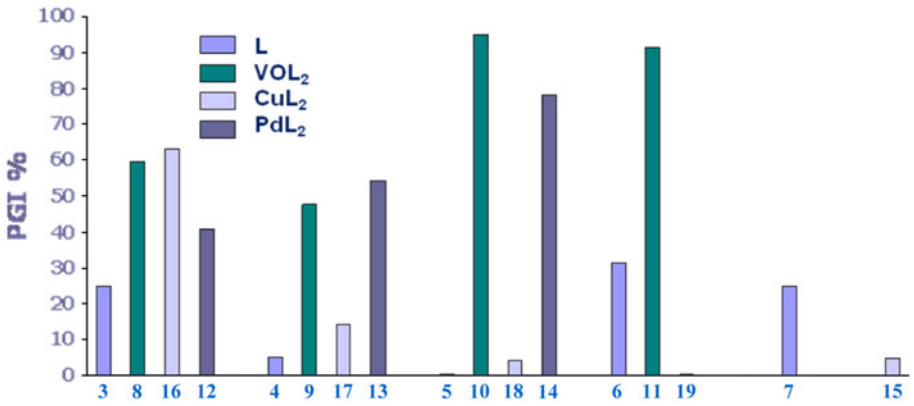


Fig. 2 Comparative trypanosomicidal activities (*PGI T. cruzi* percentages of growth inhibition at 25.0 μM)

stable in DMF solution for at least 6 days at 30°C and in DMSO for at least 24 h. Thermogravimetic measurements showed the presence of crystallization water molecules in the cases of palladium complexes and coordinated water molecules for the copper ones. Electronic, IR, ^{1}H NMR (for Pd complexes), and ESR (for Cu complexes) spectroscopies allow us to complete the structural characterizations.

The results of anti-*T. cruzi* behavior of the studied complexes, together with the ligands and vanadium parent complexes activities, are given in Table 1 and Fig. 2. Except for one complex, **15**, the palladium derivatization modified positively the trypanosomicidal activities increasing the ligands behaviors from 20- to 80-times. On the other hand, the copper derivatization also modified positively the studied activity, however, more poorly active complexes, at the studied doses, with this metal were obtained. From the Pd (II)-complexes series the most relevant derivative was the chloro-derivative **14** while in the case of Cu (II)-complexes series was the un-substituted-derivative **16** (Table 1). No clear structure-activity relationship was observed when the authors analyzed together both series of complexes. However, when it was analyzed each series independently some facts could be achieved. For the palladium-derivatized quinoxaline 1,4-dioxides, like the vanadium-complex parent compounds (Urquiola *et al.*, 2006), the electronic effect of the substituent seem to be playing a role in the anti-*T. cruzi* behaviors, i.e., the inductive electron-withdrawing substituent capability increase the activity of the studied compound (compare activities of **12** and **14**, Table 1). Derivative **15** ruled out from this proposal, maybe the inadequate solubility of this compound in the biological milieu could uncover the actual result. On the other hand, for the copper-derivatized quinoxaline 1,4-dioxides the steric effect of the substituent seem to be playing in the anti-*T. cruzi* behaviors and the substituents electronic effect seem to be the opposite of the Pd (II) series, compare the activity of the unsubstituted derivative, **16**, the most active derivative of this series, and the activities of the methyl-substituted-, **17**, or the chloro-substituted derivative, **18** (Table 1).

In general, the derivatization of 3-aminoquinoxaline-2-carbonitrile 1,4-dioxides with palladium and copper produced improvement of the ligands activity, compare anti-*T. cruzi* activities of ligands **3**, **4**, and **5** and the corresponding activities of Pd (II) complexes **12**, **13**, and **14**, or the corresponding of Cu (II) complexes **16**, or the mix-complex **22**, **17**, and **18** (Table 1). The inclusion of alanine as co-ligand in the mix-Cu (II) complex **22** conducted to a decrease in the anti-*T. cruzi* activity indicating, like the vanadium-complex parent compounds (Urquiola *et al.*, 2006), that a more lipophilic entity, as result of a second quinoxaline 1,4-dioxide ligand, produces more trypanosomicidal agents.

Conclusion

The results show that the antitrypanosomal activity of the quinoxalines is clearly modified after derivatization. The parent ligands having poor trypanosomicidal activity became, in the majority of the cases, active on complexation with palladium and, in some cases, with copper. These observations suggest that these metal-based active agents possess a great potential as therapeutic agents.

Acknowledgments DB wishes to thank ANII for scholarships. Financial support from RIDIMEDCHAG-CYTED is acknowledged.

References

Aguirre G, Cerecetto H, Di Maio R, González M, Montoya Alfaro ME, Jaso A, Zarranz B, Ortega MA, Aldana I, Monge-Vega A (2004) Quinoxaline N, N′-dioxide derivatives and related compounds as growth inhibitors of *Trypanosoma cruzi* structure-activity relationships. Bioorg Med Chem Lett 14: 3835–3839

Ancizu S, Moreno E, Torres E, Burguete A, Pérez-Silanes S, Benítez D, Villar R, Solano B, Marín A, Aldana I, Cerecetto H, González

M, Monge A (2009) Heterocyclic-2-carboxylic acid (3-cyano-1, 4-di-N-oxidequinoxalin-2-yl)amide derivatives as hits for the development of neglected disease drugs. Molecules 14:2256–2272

Castro JA, de Meca MM, Bartel LC (2006) Toxic side effects of drugs used to treat Chagas' disease (American trypanosomiasis). Hum Exp Toxicol 25:471–479

Cavalli A, Bolognesi ML (2009) Neglected tropical diseases: multi-target-directed ligands in the search for novel lead candidates against *Trypanosoma* and *Leishmania*. J Med Chem 52: 7339–7359

Cerecetto H, González M (2007) Benzofuroxan and Furoxan. Chemistry and biology. In: Khan MTH (ed) Topics in heterocyclic chemistry. Bioactive heterocycles V, vol 10. Springer, Berlin

Cerecetto H, González M (2010) Synthetic medicinal chemistry in Chagas' disease: compounds at the final stage of "Hit-to-Lead" phase. Pharmaceuticals 2. doi:10.3390/ph20x000x

Geary WJ (1971) The use of conductivity measurements in organic solvents for the characterisation of coordination compounds. Coord Chem Rev 7:81–122

González M, Cerecetto H, Monge A (2007) Quinoxaline 1,4-dioxide and phenazine 5,10-dioxide. Chemistry and biology. In: Khan MTH (ed) Topics in Heterocyclic Chemistry. Bioactive heterocycles V, vol.11 Springer, Berlin

Lever ABP (1984) Inorganic electronic spectroscopy. Elsevier, Amsterdam

Nwaka S, Ridley RG (2003) Virtual drug discovery and development for neglected diseases through public-private partnerships. Nat Rev Drug Disc 2:919–928

Otero L, Vieites M, Boiani L, Denicola A, Rigol C, Opazo L, Olea-Azar C, Maya JD, Morello A, Krauth-Siegel RL, Piro OE, Castellano E, González M, Gambino D, Cerecetto H (2006a) Novel antitrypanosomal agents based on palladium nitrofurylthiosemicarbazone complexes: DNA and redox metabolism as potential therapeutic targets. J Med Chem 49:3322–3331

Otero L, Aguirre G, Boiani L, Denicola A, Rigol C, Olea-Azar C, Maya JD, Morello A, González M, Gambino D, Cerecetto H (2006b) Nitrofurylsemicarbazone rhenium and ruthenium complexes as anti-trypanosomal agents. Eur J Med Chem 41:1231–1239

Rivera G, Bocanegra-García V, Ordaz-Pichardo C, Nogueda-Torres B, Monge A (2009) New therapeutic targets for drug design against *Trypanosoma cruzi*, advances and perspectives. Curr Med Chem 16:3286–3293

Rodrigues Coura J, Castro SL (2002) A critical review on Chagas disease chemotherapy. Mem Inst Oswaldo Cruz 97:3–24

Schofield CJ, Jannin J, Salvatella R (2006) The future of Chagas disease control. Trends Parasitol 22:583–588

Stoll S, Schweiger A (2006) EasySpin, a comprehensive software package for spectral simulation and analysis in EPR. J Magn Reson 178:42–55

Tarallo MB, Costa-Filho AJ, Vieira ED, Monge A, Leite CQ, Pavan FR, Borthagaray G, Gambino D, Torre MH (2009) Research of new mixed-chelate copper complexes with quinoxaline N1, N4-dioxide derivatives and alanine as ligands, potential antimycobacterial agents. J Argentine Chem Soc 97:80–89

Torre MH, Gambino D, Araujo J, Cerecetto H, González M, Lavaggi ML, Azqueta A, López de Cerain A, Monge Vega A, Abram U, Costa-Filho AJ (2005) Novel Cu(II) Quinoxaline N1, N4–dioxide complexes as selective hypoxic cytotoxins. Eur J Med Chem 40(5):473–480

Urquiola C, Vieites M, Aguirre G, Marín A, Solano B, Arrambide G, Noblía P, Lavaggi ML, Torre MH, González M, Monge A, Gambino D, Cerecetto H (2006) Improving anti-trypanosomal activity of 3-aminoquinoxaline-2-carbonitrile N1, N4-dioxide derivatives by complexation with vanadium. Bioorg Med Chem 15:5503–5509

Urquiola C, Gambino D, Cabrera M, Lavaggi ML, Cerecetto H, González M, López de Ceráin A, Monge A, Costa-Filho AJ, Torre MH (2008) New copper-based complexes with quinoxaline N(1), N(4)-dioxide derivatives, potential antitumoral agents. J Inorg Biochem 102:119–126

Urquiola C, Vieites M, Torre MH, Cabrera M, Lavaggi L, Cerecetto H, González M, López de Ceráin A, Monge A, Smircich P, Garat B, Gambino D (2009) Cytotoxic palladium complexes of bioreductive quinoxaline N1, N4-dioxide prodrugs. Bioorg Med Chem 17:1623–1629

Vieites M, Otero L, Santos D, Toloza J, Figueroa R, Norambuena E, Olea-Azar C, Aguirre G, Cerecetto H, González M, Morello A, Maya JD, Garat B, Gambino D (2008a) Platinum(II) metal complexes as potential anti-*Trypanosoma cruzi* agents. J Inorg Biochem 102:1033–1043

Vieites M, Smircich P, Parajon-Costa B, Rodríguez J, Galaz V, Olea-Azar C, Otero L, Aguirre G, Cerecetto H, González M, Gómez-Barrio A, Garat B, Gambino D (2008b) Potent in vitro anti-*Trypanosoma cruzi* activity of pyridine-2-thiol *N*-oxide metal complexes having an inhibitory effect on parasite-specific fumarate reductase. J Biol Inorg Chem 13:723–735

Vieites M, Otero L, Santos D, Olea-Azar C, Norambuena E, Aguirre G, Cerecetto H, González M, Kemmerling U, Morello A, Maya DJ, Gambino D (2009) Platinum-based complexes of bioactive 3-(5-nitrofuryl)acroleine thiosemicarbazones showing anti-*Trypanosoma cruzi* activity. J Inorg Biochem 103:411–418

Zamalloa E, Aldana I, Bachiller CM, Monge A (1997a) Physicochemical properties and stability of the new hypoxia-selective agent 7-chloro-3-[[N,N-dimethylamino)propyl]amino]-2-quinoxalinecarbonitrile 1,4-di-N-oxide hydrochloride. Arzneimittelforsch 47:873–878

Zamalloa E, Dios-Viéitez C, González-Peña E, Monge A (1997b) Determination of a new hypoxia selective agent from 2-quinoxalinecarbonitrile 1,4-di-N-oxides in plasma by high performance liquid chromatography. Arzneimittelforsch 47:1044–1046