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ScienceDirect
European Journal of Medicinal Chemistry 44 (2009) 1745–1750
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Short communication

Comparative study of the trypanocidal activity of the methyl 1-nitrophenyl-1,2,3,4-9*H*-tetrahydro-β-carboline-3-carboxylate derivatives and benznidazole using theoretical calculations and cyclic voltammetry

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Received 25 November 2007; received in revised form 17 March 2008; accepted 20 March 2008
Available online 10 April 2008

Abstract

The *cis* and *trans* isomers of methyl 1-(*m*-nitro)phenyl and 1-(*p*-nitro)phenyl-1,2,3,4-tetrahydro-9*H*-β-carboline-3-carboxylates (compounds **3a**,**b**, **4a** and **b**) were synthesized and evaluated *in vitro* against epimastigote forms of *Trypanosoma cruzi*. Among all of the evaluated tetrahydro-β-carboline derivatives, the compound *trans*-methyl 1-(*m*-nitro)phenyl-1,2,3,4-9*H*-tetrahydro-β-carboline-3-carboxylate (**3b**) was found to exhibit significant trypanocidal activity ($IC_{50} = 22.2$ μM). Theoretical studies of molecular conformations and electronic properties for the synthesized compounds and benznidazole, as well as, the cyclic voltammetric (CV) behaviors' determination were performed. A comparative study of the trypanocidal activity of the nitrophenyl-tetrahydro-β-carbolines derivatives and benznidazole, using the results of theoretical calculations and of the cyclic voltammetry experiments, is presented.
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Keywords: Tetrahydro-β-carbolines; *Trypanosoma cruzi*; Cyclic voltammetry; Theoretical calculations

1. Introduction

Trypanosomiases are a widespread group of parasitic diseases that affect over several 100 millions of people, especially in the tropical and subtropical countries [1]. The parasite *Trypanosoma cruzi* is the causative agent of Chagas disease, an endemic infection that affects mainly the Central and South America, where 18 million people are infected and approximately 400,000 deaths per year [2].

Nifurtimox (nfx) and benznidazole (bnz), the drugs used in the treatment of this pathology, are effective only during the acute phase of the disease. Moreover, these compounds cause serious side effects, such as peripheral neuropathy, anorexia, vomiting, allergy, and cardiac and renal toxicities [3].

Natural and synthetic β-carbolines and tetrahydro-β-carbolines alkaloids are well-known compounds that possess a variety of biological properties, such as anticonvulsive, ansiolytic, sedative, antimicrobial, antithrombotic, anti-HIV, antiproliferative, insecticidal, and parasiticidal [4–6]. In the recent years, this class of compounds has been tested in trypanocidal assays against *T. cruzi* [7–9].

The importance of the search for new effective chemotherapy agents for the treatment of Chagas disease together with the trypanocidal activity reported for tetrahydro-β-carboline alkaloids, lead us to study this class of compounds.

In this work we report the *in vitro* trypanocidal activities of *cis* and *trans* isomers of 1-(*m*-nitro)phenyl and 1-(*p*-nitro)phenyl-1,2,3,4-tetrahydro-9*H*-β-carboline-3-carboxylates (compounds

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doi:10.1016/j.ejmech.2008.03.044

3a,**b**, **4a** and **b**). In order to establish a possible relationship between electrochemical properties and trypanocidal activities we performed electrochemical measurements by cyclic voltammetry for the nitrophenyl-tetrahydro-β-carbolines synthesized. The electronic distribution around the molecular surface, charge centers, electrostatic potential maps (EPMs), atomic charges, dipole moments and Mulliken charges were also investigated for the compounds synthesized and for benznidazole. A comparative study of the trypanocidal activity of the nitrophenyl-tetrahydro-β-carbolines derivatives and benznidazole, using the results of theoretical calculations and of the cyclic voltammetry experiments, was performed.

2. Results and discussion

2.1. Chemistry

Methyl tetrahydro-β-carboline-3-carboxylates **3**a,**b**, **4a** and **b** were prepared from the Pictet–Spengler reaction of L-tryptophan with an appropriated aldehyde [10] (Scheme 1). The synthesis of these compounds was previously described in the literature [11–13]. The compounds were characterized by spectral data (^{1}H and ^{13}C NMR, MS and IV). The stereochemistry of the *cis* and *trans* isomers was assigned on the basis of ^{13}C NMR data [14].

2.2. Biology

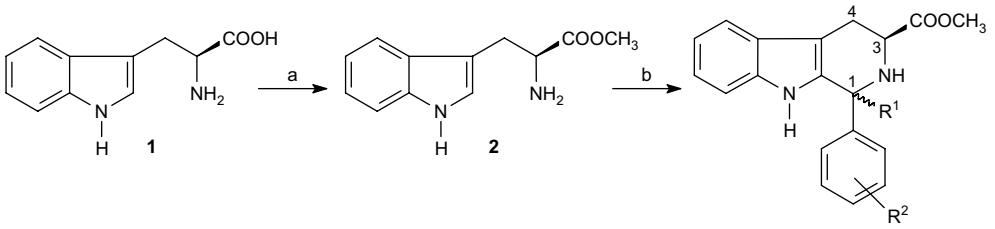
The compounds **3a**,**b**, **4a** and **b** were tested *in vitro* against epimastigote forms of *T. cruzi*. Benznidazole was used as the reference trypanocidal drug. The IC_{50} values for the trypanocidal activity of the tetrahydro-β-carbolines derivatives are presented in Table 1. The results showed that among compounds tested, *trans*-methyl 1-(*m*-nitro)phenyl-1,2,3,4-9*H*-tetrahydro-β-carboline-3-carboxylate (**3b**) was the most active with IC_{50} of 22.2 μM. Moreover, the isomer *trans*-methyl 1-(*p*-nitro)phenyl-1,2,3,4-9*H*-tetrahydro-β-carboline-3-carboxylate (**4b**) was inactive ($IC_{50} > 100$ μM), indicating that the activity depends on both stereochemistry at C-1 and position of the nitro group in the aromatic ring. The toxicity for LLC-MK_2 and J774G8 macrophage cells and the activity against the protozoa were compared by using the selectivity index (SI) ratio (CC_{50} for LLC-MK_2 or J774G8 cells/IC_{50} for protozoa). Compound **3b**, which demonstrated better trypanocidal activity, was also more selective against the parasites than the mammalian cells, with a selectivity index ratio of SI 25.6 and 35.7 in LLC-MK_2 and macrophages cells, respectively.

2.3. Conformational analysis

The literature presents several studies correlating the trypanocidal activity with structural parameters, as well as, with the redox potential of the active compounds [15,16]. Thus, an investigation on these properties could be useful to understand the action mechanism against the protozoan parasite. Based on this prediction, we investigated the electronic distribution around the molecular surface, charge centers, EPMs (electrostatic potential maps), atomic charges and dipole moments for compounds **3a**,**b**, **4a** and **b** and benznidazole (Table 2). The calculations were carried out on the most stable conformer of each isomer. The conformer structures resulting from the nitrogen inversion for *cis* and *trans* isomers were optimized at 6-311 + G(d) basis set. The spatial orientation for the substituent groups was analyzed by the rotation of N–C3–C–O and N–C1–C1′–C2′ dihedral angles. Benznidazole was submitted to the same computational treatment, in order to compare its conformational preference with the tetrahydro-β-carboline derivatives synthesized.

Through the electrostatic potential maps showed in Fig. 1, we can observe negative potential sites that include electronegative atoms and their neighborhoods and positive potential sites, mainly around hydrogen atoms. The electronic density is concentrated on nitro and carbonyl groups. In the EPM of benznidazole (Fig. 2) we can observe the same electronic concentration on carbonyl and nitro groups. Table 2 contains the electronic parameter values for all compounds and for benznidazole.

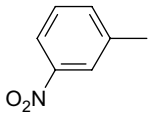
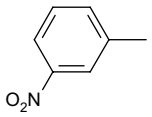
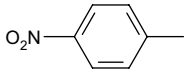
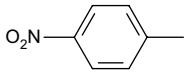
The atomic charge values (Table 2) on the carbon atom of carbonyl group for the nitrophenyl-tetrahydro-β-carboline derivatives synthesized were all negatives and with the same largeness, whereas for benznidazole was positive (+0.158). For the oxygen atom of the carbonyl group and for the nitrogen atom of the nitro group the values showed no significant difference. The atomic charge values for the oxygen atoms in the nitro group of the benznidazole was +0.075 au; for



3a: R^1 = Hα; R^2 = 3-NO_2
3b: R^1 = Hβ; R^2 = 3-NO_2
4a: R^1 = Hα; R^2 = 4-NO_2
4b: R^1 = Hβ; R^2 = 4-NO_2

Scheme 1. (a) H_3COH, H_2SO_4, reflux, 12 h; (b) aldehyde (R^1COH), CH_2Cl_2, TFA (2.2 mol equiv.), rt, 48 h.

Table 1
IC_{50} values against *T. cruzi* and cathodic potential values for the tetrahydro-β-carbolines **3a**,**b** and **4a**,**b**

Compound	Isomer	C-1 substituent	IC_{50} (μM)	E_{pc} (V)
3a	*cis*		93.5	−1.113
3b	*trans*		22.2	−1.098 (peak I) −1.205 (peak II)
4a	*cis*		42.7	−1.096
4b	*trans*		>100	−1.118
Benznidazole				−1.044

the derivatives **3a**,**b**, **4a** and **b** the values were +0.004, +0.003, −0.004 and −0.010 au, respectively.

The observed potential values are all positives for carbon and negatives for oxygen atoms of the carbonyl group, and negatives for the aromatic ring. A great difference was observed for the electrostatic potential values of the nitro group. The most active compounds (**3b** and **4a**) presented more negative values for the electrostatic potential of the nitro group when compared to those of the inactive compounds (**3a** and **4b**). The potential values on the oxygen and nitrogen atoms were both negatives for the actives compounds **3b** and **4a**, being −105.0 and −89.7 kcal/mol, for oxygen, and −18.0 and −52.1 kcal/mol, for the nitrogen, respectively. The inactive compounds (**3a** and **4b**) exhibit minor potentials on the oxygen atom (−80.3 and −36.4 kcal/mol) and positive potential values on the nitrogen atom (+5.30 and +1.90 kcal/mol). The potential values for the benznidazole were −23.8 and −99.1 kcal/mol, for the nitrogen and oxygen atoms, respectively. The difference in the activity between **3b** and **4a** is probably due to the major atomic charge concentration in the nitrogen atom of the nitro group, being −0.218 in **3b** and −0.204 in **4a**.

In order to verify if there is correlation between the charge on the carbon carrying the nitrophenyl groups and the trypanocidal activities of **3a**,**b** and **4a**,**b** the Mulliken charges on C-1 were calculated. Analyzing the data showed in Tables 1 and 2, we can observe the same order for activity and Mulliken charges values: **3b** > **4a** > **3a** > **4b**. Thus, the most active compound for each series (*m*- or *p*-nitrophenyl) is also that one presenting higher charge on C-1.

In summary, our results showed that the electrostatic potential and the atomic charges values on nitro group, as well as, the Mulliken charges values on C-1, are useful parameters for the analysis of trypanocidal activity of compounds **3a**,**b** and **4a**,**b**.

2.4. Electrochemical measurements

Studies have demonstrated that the redox potential of the nitro group has influence on the trypanocidal activity. The main intermediate products responsible for the cytotoxic action of the nitrocompounds are the nitro-radical anion (R-$NO_2^{\bullet -}$) and hydroxylamine derivative (R-NHOH), which are produced in the reduction of the nitro group [17,18].

Cyclic voltammograms and the values of the cathodic peak potentials (E_{pc}) of compounds **3a**,**b**, **4a**,**b** and benznidazole are shown in Fig. 3 and Table 1, respectively. The voltammograms of compounds **3a**, **4a**,**b** and benznidazole (Fig. 3) show only one peak, which correspond to the nitro/nitro-radical anion couple (R-NO_2/R-$NO_2^{\bullet -}$). Our data for the benznidazole were similar to those previously reported [19]. On the other hand, the cyclic voltammogram for the most active compound **3b** shows two signals, the first one due to the nitro/nitro-radical anion couple (peak I) and the second, due to the nitro-radical anion reduction to form the hydroxylamine derivative (peak II). The results presented in Table 1 show that the facility of the nitro group reduction to nitro-radical anion for the active compounds **3b** ($E_{pc} = -1.098$ V for peak I) and **4a** ($E_{pc} = -1.096$ V) are very similar. These data led us to suggest that the nitro-radical anion reduction to form the hydroxylamine derivative (peak II) for **3b** is responsible for its higher activity in relation to **4a**.

From the E_{pc} values for the first cathodic peak (Table 1) we can observe the following order for the reduction: **3b** > **3a** and **4a** > **4b**. Therefore, the most active compound for each series (*m*- or *p*-nitrophenyl) is also that one presenting higher easiness of reduction.

Table 2
Electronic parameters, dipole moments and Mulliken charges at C-1 for **3a**,**b**, **4a**,**b** and benznidazole

Compounds	Electrostatic potential values[a]			Atomic charges[b]		Dipole[c]	Mulliken charges at C-1
	C=O (C/O)	Ring	NO_2 (N/O)	C=O (C/O)	NO_2 (N/O)		
3a	+79.0/−80.3	−52.1	+5.30/−80.3	−0.183/−0.260	−0.223/+0.004	2.59	−0.5944
3b	+78.4/−76.0	−37.0	−18.0/−105.0	−0.163/−0.225	−0.218/+0.003	5.09	−0.8850
4a	+78.4/−70.9	−33.3	−52.1/−89.7	−0.203/−0.260	−0.204/−0.004	4.38	−0.6212
4b	+78.0/−74.0	−36.4	+1.90/−36.4	−0.198/−0.225	−0.208/−0.010	5.90	−0.3045
Benznidazole	+18.8/−88.5	−43.9	−23.8/−99.1	+0.158/−0.292	−0.394/+0.075	7.22	–

[a] In kcal/mol.
[b] In au.
[c] In Debye.

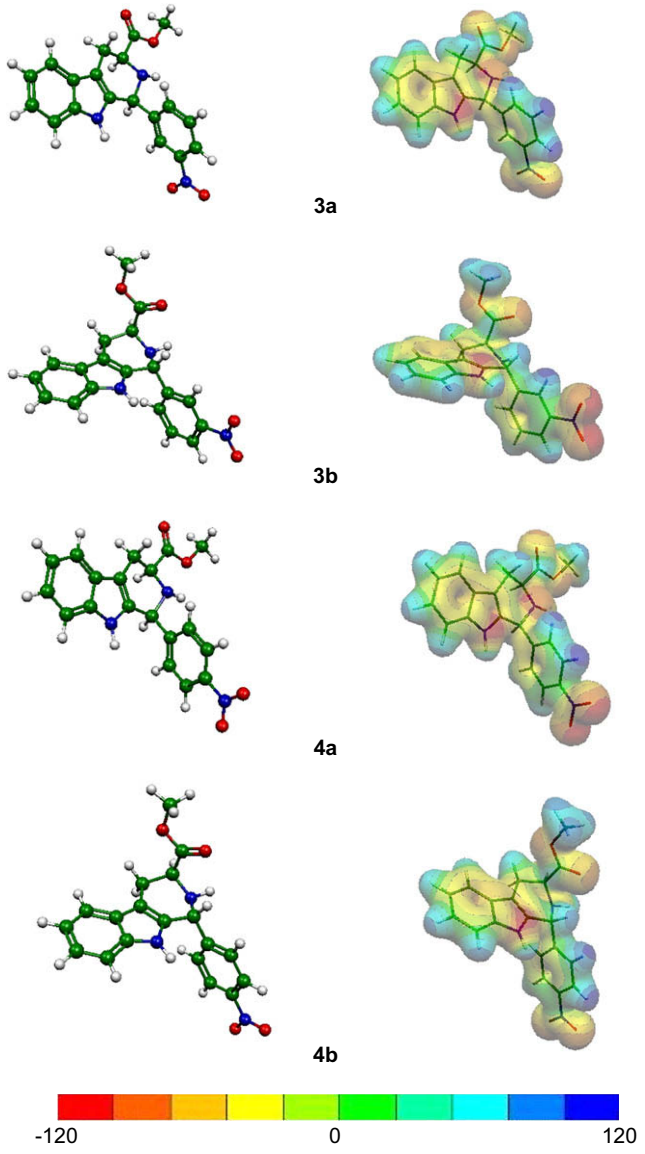


Fig. 1. Calculated electrostatic potentials on the molecular surface for the most stable conformers for the compounds **3a**,**b** and **4a**,**b** (colour ranges in kcal/mol).

3. Conclusions

We reported the evaluation of the trypanocidal activity against epimastigote forms of *T. cruzi* of the *cis* and *trans* isomers of the tetrahydro-β-carbolines containing an *m*- or *p*-nitrophenyl group at C-1. The results of the biological assays demonstrated that the isomer *trans* of the *m*-nitrophenyl-tetrahydro-β-carboline (compound **3b**) was the most active and less cytotoxic among all tested derivatives. Our results showed that the electronic potential on nitro group and the Mulliken charges on C-1 are adequate parameters to explain the difference in the activity of the synthesized compounds. Based on the theoretical calculations and on the cyclic voltammograms data it was reasonable to conclude that the difference in the trypanocidal activity of the compounds **3a**,**b**, **4a** and **b** depends, mainly, on the easiness reduction of nitro group. The higher activity of **3b** in relation to **4a** is probably due to the nitro-radical anion reduction to form the hydroxylamine derivative, as observed in the voltammogram of **3b**.

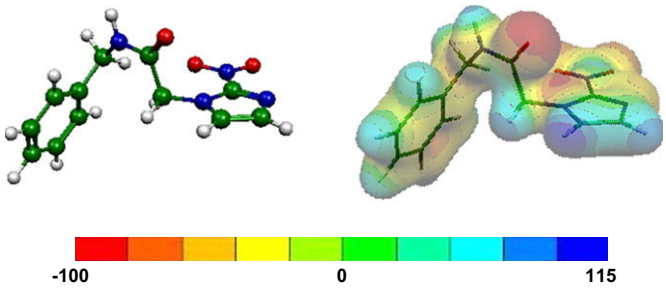


Fig. 2. Calculated electrostatic potentials on the molecular surface for benznidazole.

4. Experimental protocols

4.1. Biology

Epimastigote forms of *T. cruzi* Y strain were grown in liver infusion tryptose (LIT) medium supplemented with 10% inactivated fetal bovine serum (Gibco Invitrogen Corporation, New York, USA) and assayed with synthetic chemical compounds (5, 10, 50, and 100 μg/mL). The compounds were dissolved in 1% DMSO at the concentration which did not affect the growth of the parasites. Next, 1×10^6 protozoa/mL was introduced into 24-well plate each containing 1 mL of diluted compounds. Cell growth was determined by counting the parasites with a Neubauer haemocytometer after incubation for 96 h at 28 °C. Assays were performed in duplicate on separate occasions.

4.2. Computational methods

Molecular modeling studies were performed on the *cis* and *trans*-methyl-1-(3-nitrophenyl)-1,2,3,4-tetrahydro-9*H*-β-carboline-3-carboxylates **3a** and **b**, *cis* and *trans*-methyl-1-(4-nitrophenyl)-1,2,3,4-tetrahydro-9*H*-β-carboline-3-carboxylates **4a** and **b** and the benznidazole, in order to calculate the stereoelectronic properties of electronic surface density, including electrostatic potential maps. A detailed conformational search for each of the molecules was performed, using DFT methods, to find out the minimum energy point, beginning with potential energy surface (PES) obtained through pre-determined dihedral turns (scan) and then, fully geometry optimization of the minimum point. The PES was built using the semi-empirical method (AM1); the geometry optimization was carried out using the hybrid method B3LYP [20] along the 6-311 + G(d) basis set. In each case, frequency calculations were employed to confirm the structures as minimum point. The electrostatic potential maps (EPMs) were calculated at HF/6-31 G level of theory and the maps were plotted using the MOLEKEL package [21]. The Gaussian 03 package [22] was used to perform this set of calculations.

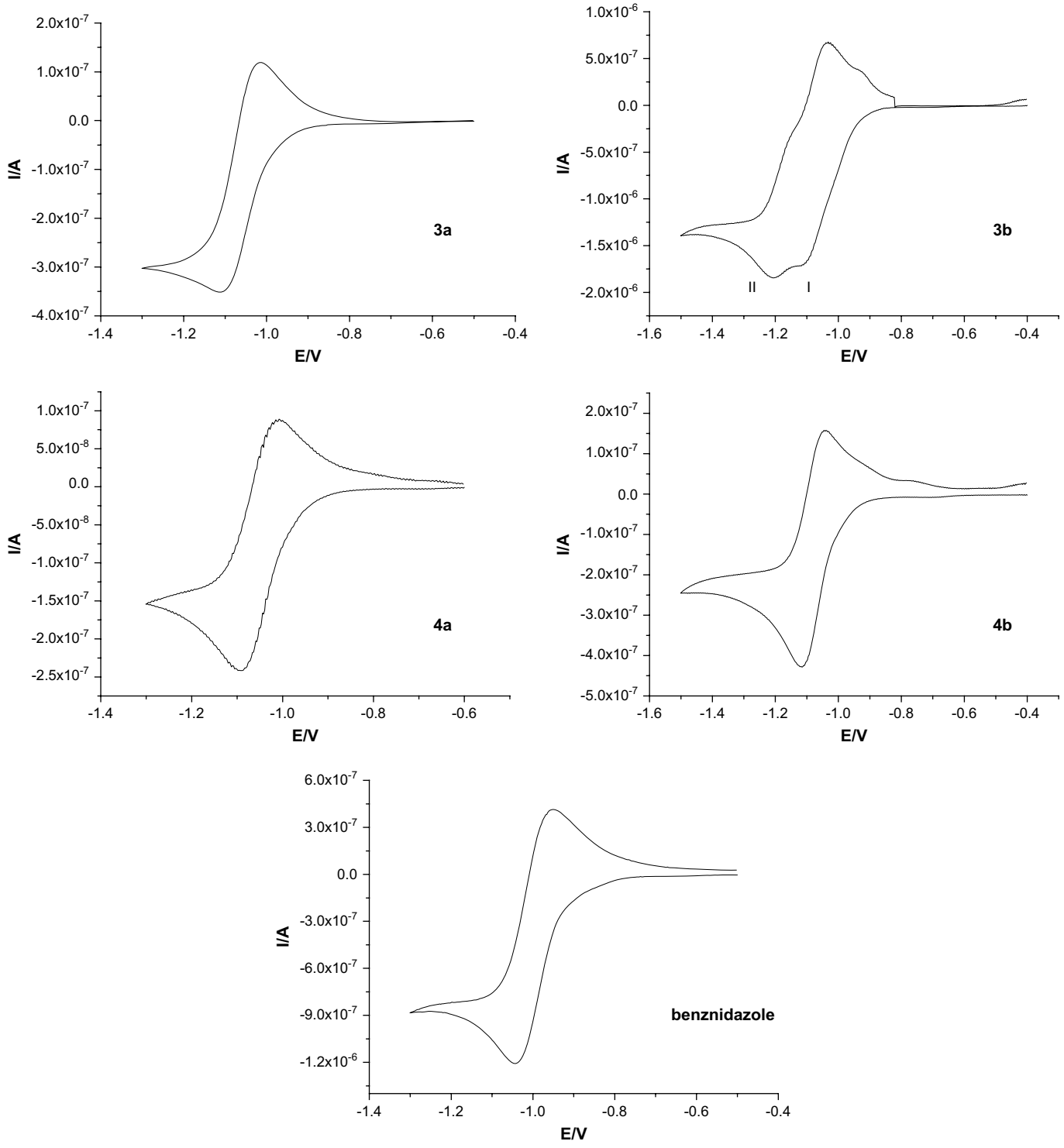


Fig. 3. Cyclic voltamograms at 1 mM in DMF of the compounds **3a**,**b**, **4a**,**b** and benznidazole.

4.3. Cyclic voltammetry

Cyclic voltammetry (CV) was performed using an Eco Chemie Autolab potentiostat/galvanostat equipped with a 663 VA stand Metrohm. All experiments were carried out using an electrochemical cell of 20 mL with a three-electrode system. A drop mercury electrode (DME) was used as the working electrode, a glassy carbon as counter-electrode and a calomel electrode as the reference electrode. The scan rate used in all experiments was 0.100 V/s. Each sample was prepared at a concentration of 1.0 mM in a 10 mL of a 0.10 M supporting electrolyte solution of tetrabutylammonium nitrate (Aldrich) in DMF. For all experiments, the solutions were degassed with nitrogen for 5 min prior to analysis.

4.4. Chemistry

4.4.1. General procedure for compounds **3a**,**b**, **4a** and **b**

Methyl tetrahydro-β-carboline-3-carboxylates **3a**,**b**, **4a** and **b** were prepared using the Pictet–Spengler reaction, accordingly to the procedure previously reported [10].

A mixture of L-tryptophan methyl ester (480 mg, 2.2 mmol), 3-nitrobenzaldehyde or 4-nitrobenzaldehyde (2.2 mmol) and trifluoroacetic acid (4.8 mmol) in CH_2Cl_2 (20 mL) was stirred at room temperature for 48 h followed by evaporation of the solvent. Treatment of the crude product with 10% Na_2CO_3, extraction with EtOAc (3 × 20 mL), dreading of organic layer under anhydrous Na_2SO_4, filtration and solvent evaporation afforded a residue which was purified on chromatographic column (silica flash; hexane-EtOAc 20%) to give the pure *cis* and *trans* products.

Acknowledgements

The authors thank CNPq for financial support and CAPES for fellowship to Lilian T. Düsman Tonin.

References

[1] http://www.who.int/tdr/diseases/chagas/, acessed in November 05, 2007.
[2] A. Moncayo, Tropical Disease Research Progress 1991–1992, WHO, Geneva, 1993.
[3] J.D. Maya, B.K. Cassels, P. Iturriaga-Vásquez, J. Ferreira, M. Faúndez, N. Galanti, A. Ferreira, A. Morello, Comp. Biochem. Physiol., A Mol. Integr. Physiol. 146 (2007) 601–620.
[4] N. Lin, M. Zhao, C. Wang, S. Peng, Bioorg. Med. Chem. Lett. 12 (2002) 585–587.
[5] X. Yu, W. Lin, J. Li, M. Yang, Bioorg. Med. Chem. Lett. 14 (2004) 3127–3130.
[6] R. Cao, W. Peng, H. Chen, X. Hou, H. Guan, Q. Chen, Y. Ma, A. Xu, Eur. J. Med. Chem. 40 (2005) 249–257.
[7] P. Rivas, B.K. Cassels, A. Morello, Y. Repetto, Comp. Biochem. Physiol. C, Toxicol. Pharmacol. 122 (1999) 27–31.
[8] J.C. Cavin, S.M. Krassner, E. Rodriguez, J. Ethnopharmacol. 19 (1987) 89–94.
[9] M.E. Ferreira, H. Nakayama, A.R. de Arias, A. Schinini, N.V. de Bilbao, E. Serna, D. Lagoutte, F. Soriano-Agatón, E. Poupon, R. Hocquemiller, A. Fournet, J. Ethnopharmacol. 109 (2007) 258–263.
[10] P.D. Bailey, S.P. Hollinshead, N.R. McLay, Tetrahedron Lett. 28 (1987) 5177–5180.
[11] M. Muthukrishnan, S.V. More, D.R. Garud, C.V. Ramana, R.R. Joshi, R.A. Joshi, J. Heterocycl. Chem. 43 (2006) 767–772.
[12] K. Singh, P.K. Deb, P. Venugopalan, Tetrahedron 57 (2001) 1939.
[13] B. Saha, S. Sharma, D. Sawant, B. Kundu, Tetrahedron Lett. 48 (2007) 1379.
[14] L.T. Dusman, C.C. Bocca, E.A. Basso, M.H. Sarragiotto, J. Mol. Struct. 754 (2005) 45–50.
[15] V.F. Ferreira, A. Jorqueira, A.M.T. Souza, M.N. da Silva, M.C.B.V. de Souza, R.M. Gouvêa, C.R. Rodrigues, A.V. Pinto, H.C. Castro, D.O. Santos, H.P. Araújo, S.C. Bourguignon, Bioorg. Med. Chem. 14 (2006) 5459–5466.
[16] G. Aguirre, L. Boiani, H. Cerecetto, M. Fernández, M. González, A. Denicola, L. Otero, D. Gambino, C. Rigol, C. Olea-Azar, M. Faundez, Bioorg. Med. Chem. 12 (2004) 4885–4893.
[17] C. Viodé, C. de Albuquerque, G. Chauvière, C. Houée-Lévin, J. Perie, New J. Chem. 21 (1997) 1331–1338.
[18] A. Guissani, Y. Henry, N. Lougmani, B. Hickel, Free Radic. Biol. Med. 8 (1990) 173–189.
[19] S. Bollo, L.J. Núñez-Vergara, M. Bontá, G. Chauviere, J. Périé, J.A. Squella, J. Electroanal. Chem. 511 (2001) 46–54.
[20] C. Lee, W. Yang, R.G. Parr, Phys. Rev. B Condens. Matter Mater. Phys. 37 (1988) 785–789.
[21] P.H.P. Flükiger, S. Lüthi, J. Portmann, Weber, MOLEKEL 4.3, Swiss Center for Scientic Computing, Manno, Switzerland, 2000–2002.
[22] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery, T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople, Gaussian 03, Revision B.04, Gaussian, Inc., Pittsburgh, 2003.