Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 3555–3558
Bioorganic & Medicinal Chemistry Letters

Toxic effects of natural piperine and its derivatives on epimastigotes and amastigotes of *Trypanosoma cruzi*

Tatiana Santana Ribeiro,[a] Leonardo Freire-de-Lima,[b] José Osvaldo Previato,[b] Lucia Mendonça-Previato,[b] Norton Heise[b] and Marco Edilson Freire de Lima[a,*]

[a]*Universidade Federal Rural do Rio de Janeiro, Instituto de Ciências Exatas, Departamento de Química, Km 7, BR 465 CEP: 23.890-000, Seropédica, RJ Brazil*
[b]*Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho, Centro de Ciências da Saúde. Ilha do Fundão, 21.944-970, Rio de Janeiro, RJ Brazil*

Received 11 February 2004; revised 7 April 2004; accepted 10 April 2004

Abstract—We describe herein an evaluation of trypanocidal effects of the natural alkaloid piperine and twelve synthetic derivatives against epimastigote and amastigote forms of the protozoan parasite *Trypanosoma cruzi*, the causative agent of the incurable human disease, Chagas' disease. The results obtained point to piperine as a suitable template for the development of new drugs with trypanocidal activity.

1. Introduction

The alkaloid piperine **1** (Fig. 1) is the main secondary metabolite in *Piper nigrum*, occurring mainly in the fruits.[1] *Piper nigrum* (popularly known as black pepper) is widely used in folk medicine in India, where it originates.

Piperine **1** is very abundant in the plant, being extracted from the dry fruits with a yield of 3–7%.[2] Various

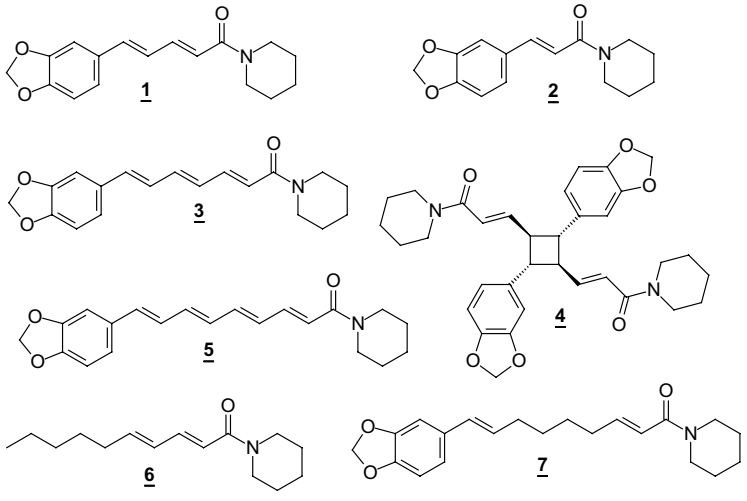


Figure 1. Chemical structures of some natural amides isolated from *Piper* sp.[6,7]

Keywords: Piperine; Piperine analogues; *Piper nigrum*; Chagas' disease; *Trypanosoma cruzi*.
* Corresponding author. Tel./fax: +55-21-2682-2807; e-mail: marco@ufrrj.br

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.04.019

biological activities have been attributed to piperine including insecticidal[3] and nematocidal activity,[4] and inhibition of liver metabolism.[5] Other natural amides have been isolated from *Piper* species. Some are shown in Figure 1.[6,7]

The easy access to plant material (which may be acquired from different sources), the abundance of the natural product as well as the ease of extraction make piperine a useful starting material for the preparation of potentially bioactive compounds.

Kapil described the results of an investigation of piperine activity against promastigote forms of *Leishmania donovani*.[8] More recently, Raay and co-workers described encouraging data obtained in vivo testing hamsters infected with *L. donovani* with piperine intercalated into mannose-coated liposomes.[9] Based on these results, we investigated the activity of this alkaloid and of a series of derivatives on *Trypanosoma cruzi*, another important protozoan parasite and the cause of Chagas' disease (American trypanosomiasis) in humans, an incurable infectious disease responsible for 21,000 deaths and 200,000 new cases annually in 15 Southern Cone countries.[10]

The most common treatment for this disease involves two drugs, nifurtimox **8** and benznidazole **9** (Fig. 2), which are active only during the acute and short-term chronic phase. Benznidazole is now the only drug still available since the production of nifurtimox was stopped. Unfortunately, narrow therapeutic windows, strong side effects, and variable drug susceptibilities among *T. cruzi* strains result in low clinical efficacies for these nitro derivatives.[11] Thus, it is important to study and develop new compounds with antitrypanosomal activity, which may possess enhanced antiparasitic activity associated with low toxicity. Natural products have long been used as templates for the development of new molecules, which may be useful against parasitic diseases (e.g., quinine **10**, Fig. 2, the antimalarial isolated from *Cinchona officinalis*).[12,13]

This work is part of a research program aiming at the use of abundant natural products in the synthesis of new molecules with potential application as antiparasitic drugs. We previously reported the evaluation of antiparasitic activity of curcumin (isolated from *Curcuma longa*) and its derivatives on *Leishmania amazonensis*.[14]

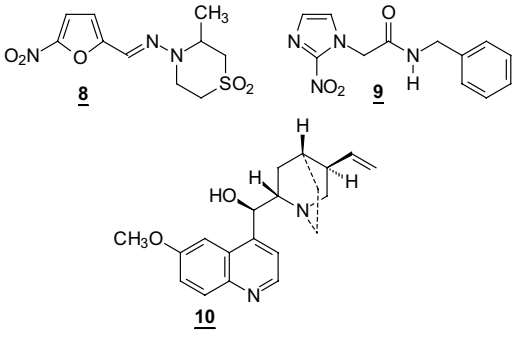


Figure 2. Structures of nifurtimox **8**, benznidazole **9** and quinine **10**.

We began our studies with the isolation of natural piperine **1** from the powdered dry fruits of *Piper nigrum* using the methodology described by Ikan,[2] and obtained the amide in a very pure form in 7% yield. The isolated product showed physical and spectrometric data identical to that reported in the literature.[2,15]

Firstly, piperine was tested against *T. cruzi* epimastigotes. It showed a dose-dependent toxicity, with IC_{50} of 7.36 μM. This preliminary result encouraged us to prepare a series of derivatives in order to determine the chemical features present in piperine responsible for the trypanocidal activity. We have carried out the saturation of the 2,4-diene moiety as well as some chemical transformations on the amide function and have also prepared amides with different length side chains.

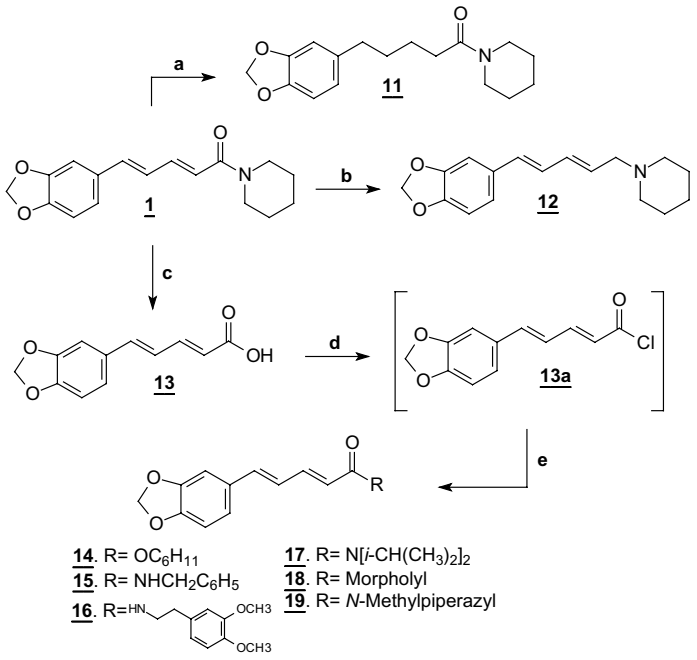
Scheme 1 shows the chemical transformations carried out on **1**, affording derivatives **11**–**20**. The saturated derivative **11** was prepared from **1** by catalytic hydrogenation of the conjugated double bonds.[16] The piperic acid **13** was obtained from **1** in excellent yield through basic hydrolysis.[2] Acid **13** was used as precursor of a series of derivatives, the ester **14** and the amides **15**–**19**, through the intermediacy of the acyl chloride (**13a**) prepared by treatment of **13** with oxalyl chloride,[17] and addition of the relevant alcohol or amine. The allylic amine **12** was obtained as a single product by reduction of **1** with diisobutylaluminum hydride.[18]

The derivatives of the cinnamic series (Scheme 2) were obtained through classical Knoevenagel's methodology employing piperonal **20** as starting material.[19] The cinnamic acid **21** prepared in this way was used to prepare amides **2** and **22**. Piperamide **2** is a natural product isolated by Loder et al. from *Piper novae hollandie*.[20]

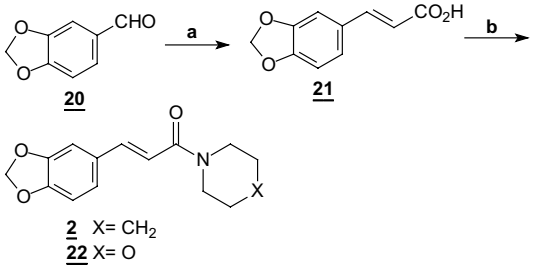
Preparation of amide **3**, an analogue with a seven carbon extended side chain, involved the synthetic route shown in Scheme 3. This amide, named piperettine, is a natural product isolated from *Piper nigrum* and *Piper aurantiacum*.[4,15] Amide **3** was prepared from **13** in five steps, employing an Emmos–Horner reaction of aldehyde **24** with triethylphosphonoacetate,[21] which afforded the ester **25** stereoselectively, in good yield with *E*-configuration at $\Delta^{2,3}$. The reaction sequence from acid **26** to analogue **3** was similar to that employed for other amides (Schemes 1 and 2). This synthetic path afforded piperettine **3** in six steps from piperine **1**, in 26% overall yield.

The structures of all derivatives prepared in this work (Schemes 1–3) were confirmed by IR, MS, ^1H, and ^{13}C NMR spectral data. The assignments are based on 2D NMR experiments and comparison with literature values[2–5] and are consistent with the structures described.

Evaluation of the antiparasitic activity of the natural product and its derivatives was carried by screening



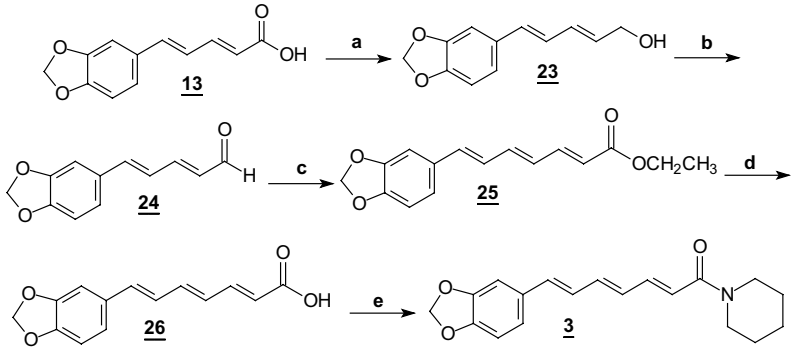
Scheme 1. Chemical transformations on piperine **1**. Reagents and conditions: (a) ethyl acetate, Pd/C, H_2, 2 h (90%); (b) DIBAL-H, toluene, −10 °C, 30 min (80%); (c) KOH, ethanol, reflux, 24 h; then HCl (aq), 0 °C (90%); (d) $(COCl)_2$, 25 °C, 30 min (100%); (e) CH_2Cl_2, alcohol, or amine, 0 °C, 1 h (70–90%).



Scheme 2. Preparation of cinnamic analogues of piperine. Reagents and conditions: (a) malonic acid, pyridine, piperidine (cat.), reflux 3 h; then HCl (aq), 0 °C (85%); (b); (c) $(COCl)_2$, 25 °C, 30 min (100%); then CH_2Cl_2, piperidine, or morpholine, 0 °C, 1 h (90%).

their effects on the proliferation of *T. cruzi* Y-strain epimastigotes grown in axenic culture.[22] The most active compounds were also evaluated for their toxicity against noninfected murine peritoneal macrophages and their ability to reduce the number of intracellular amastigotes in in vitro infected cells.[22] The results are presented in Table 1. The IC_{50} values obtained for the compounds tested were compared with those for benznidazole **9** (Fig. 2), the drug of choice for the treatment of Chagas' disease.

Neither benznidazole nor piperine or any of the synthesized derivatives interfered with the cellular viability of noninfected macrophages up to the concentration of 20 μM as judged by measurements of Trypan blue exclusion and phagocytosis (not shown). As expected, the IC_{50} values for benznidazole are similar to those described previously for epimastigotes and intracellular amastigotes.[26,27] Piperine proved to be a potent *T. cruzi* inhibitor, being more toxic to intracellular amastigotes



Scheme 3. Preparation of piperettine **3** from piperine **1**. Reagents and conditions: (a) DIBAL-H, THF, −10 °C, 1 h (60%); (b) MnO_2, THF, reflux, 3 h (93%); (c) Triethylphosphonoacetate, THF, NaH, −15 °C, 1 h (90%); (d) KOH, CH_3CH_2OH, 25 °C; then HCl (aq), 0 °C (90%); (e) $(COCl)_2$, 25 °C, 30 min; then CH_2Cl_2, piperidine, 0 °C, 1 h (90%).

Table 1. Growth inhibition of *T. cruzi* (epimastigotes and amastigotes) for benznidazole, piperine and its derivatives

Compound	Epimastigotes IC_{50} (μM)	Amastigotes IC_{50} (μM)
1	7.36	4.91
2	>96.52	NT[a]
3	10.67	7.40
9	2.20	2.58
11	19.41	11.52
12	17.49	9.63
13	>114.67	NT
14	>83.33	NT
15	>81.43	NT
16	>65.61	NT
17	14.85	7.77
18	56.13	5.71
19	>83.33	NT
22	>95.78	NT

[a] NT: Not tested.

than epimastigotes. The apparently higher potency on amastigotes may be explained by its preferential uptake by tissue culture cells or by a greater capacity of intracellular amastigotes to fluid-phase pinocytosis.[28] Among the derivatives prepared, acid **13** and ester **14** did not show activity at the maximum dose on epimastigotes, evidence for the need for a nitrogen-containing function for activity. Amides **2**, **15**, **16**, **19**, and **22** were also shown to be inactive against epimastigotes at the dosage tested. Derivatives **2**, **13**–**16**, **19**, and **22** were not evaluated against amastigotes. The loss of toxicity observed for amide **2** clearly demonstrates the importance of the extended carbon side chain in the original molecule, corroborated by the maintenance of toxicity by piperettine **3** against epimastigotes and amastigotes (Table 1). Removal of the double bonds (derivative **11**) did not interfere significantly with activity, suggesting that conjugation is not essential for trypanocidal activity. Surprisingly, changing the piperidine moiety of the natural product for diisopropyl (**17**) or morpholyl groups (**18**) produces loss of activity on epimastigotes but does not interfere significantly with toxicity against intracellular amastigotes. Finally, reduction of the carbonylamide group of piperine gave the allylic amine **12**, which retained significant toxic effects against the parasites, showing that the carbonyl group is not important for the toxic effect. In conclusion, we have synthesized a series of piperine derivatives, which behave as potent inhibitors of the proliferation of *T. cruzi* parasites, and which may be considered suitable template compounds for the design of new and more potent drugs for the treatment of Chagas' disease.

Acknowledgements

Financial support from FAPERJ and fellowships from Capes to T. S. Ribeiro and from CNPq to L. Freire-de-Lima are gratefully acknowledged. We also thank Dr. Christopher Jones (Laboratory for Molecular Structure, NIBSC, Herts, UK) for critical reading of the manuscript.

References and notes

1. Semler, U.; Gross, G. G. *Phytochemistry* **1988**, *27*, 1566.
2. Ikan, R. In *Natural Products: A Laboratory Guide*, 2nd ed.; Academic Press: 1991; pp 233–238.
3. de Paula, V. F.; Barbosa, L. C. D.; Demuner, A. J.; Pilo-Veloso, D.; Picanco, M. C. *Pest Manag. Sci.* **2000**, *56*, 168.
4. Kiuchi, F.; Nakamura, N.; Tsuda, Y.; Kondo, K.; Yoshimura, H. *Chem. Pharm. Bull.* **1988**, *36*, 1452.
5. Koul, S.; Koul, J. L.; Taneja, S. C.; Dhar, K. L.; Jamwal, D. S.; Singh, K.; Reen, R. K.; Sing, J. *Bioorg. Med. Chem.* **2000**, *8*, 251.
6. Parmar, V. S.; Jain, S. C.; Bisht, K. S.; Jain, R.; Taneja, P.; Jha, A.; Tyagi, O. D.; Prasad, A. K.; Wengel, J.; Olsen, C. E.; Boll, P. M. *Phytochemistry* **1997**, *46*, 597.
7. Tsukamoto, S.; Cha, B.-C.; Ohta, T. *Tetrahedron* **2002**, *58*, 1667.
8. Kapil, A. *Planta Med.* **1993**, *59*, 474.
9. Raay, B.; Medda, S.; Mukhopadhyay, S.; Basu, M. K. *Indian J. Biochem. Biophys.* **1999**, *36*, 248.
10. Moncayo, A. *Mem. Inst. Oswaldo Cruz* **2003**, *98*, 577.
11. Coura, J. R.; DeCastro, S. L. *Mem. Inst. Oswaldo Cruz* **2002**, *97*, 3.
12. Cordell, G. A. *Phytochemistry* **2000**, 463.
13. Mann, J. In *Murder, Magic, and Medicine*; Oxford University Press: England, 1992; pp 82–96.
14. Gomes, D. C. F.; Alegrio, L. V.; Lima, M. E. F.; Leon, L. L.; Araújo, C. A. C. *Arzneim.-Forsch./Drug Res.* **2002**, *52*, 120.
15. Araujo-Junior, J. X.; Da-Cunha, E. V. L.; Chaves, M. C. O.; Gray, A. I. *Phytochemistry* **1997**, *44*, 559.
16. Hudlický, M. *Reductions in Organic Chemistry*; John Wiley & Sons: New York, 1986; pp 1–3.
17. Szmuszkovice, J. *J. Org. Chem.* **1964**, *29*, 843.
18. Berardi, F.; Loiodice, F.; Frachiolla, G.; Calabufo, N. A.; Perrone, R.; Tortorella, V. *J. Med. Chem.* **2003**, *46*, 2117.
19. Silva, E. F.; Canto-Cavalheiro, M. M.; Braz, V. R.; Cysne-Finkelstein, L.; Leon, L. L.; Echevarria, A. *Eur. J. Med. Chem.* **2002**, *37*, 979.
20. Loder, J. W.; Moorhous, A.; Russell, G. B. *Austr. J. Chem.* **1969**, *22*, 1531.
21. Marianoff, B. E.; Reitz, A. B. *Chem. Rev.* **1989**, *89*, 863.
22. *Drug screening*. Biological assays on Y-strain epimastigotes (Fundação Oswaldo Cruz, RJ, Brazil) were performed as previously described.[23] Benznidazole **9** (Roche, Rio de Janeiro, Brazil), piperine **1** and its derivatives (Table 1) were stored as 10 mg mL^{-1} stock solutions in dimethylsulfoxide (DMSO), and were serially diluted (1:2) in medium before use (the concentrations of 0.76, 1.56, 3.125, 6.25, 12.5, and 25 μg mL^{-1} were used) in triplicate. Experiments on the intracellular form of the parasite were conducted on *T. cruzi*-infected (Y-strain) macrophages as described before.[23] The 50% inhibitory concentration (IC_{50}) values were determined by linear regression analysis.[24] For cellular viability tests, peritoneal mouse macrophages were treated with the indicated concentrations of compounds for 72 h as described elsewhere.[25]
23. Saraiva, V. B.; Gibaldi, D.; Previato, J. O.; Mendonça-Previato, L.; Bozza, M. T.; Freire-de-Lima, C. G.; Heise, N. *Antimicrob. Agents Chemother.* **2002**, *46*, 3472.
24. Lux, H.; Heise, N.; Klenner, T.; Hart, D. T.; Opperdoes, F. R. *Mol. Biochem. Parasitol.* **2000**, *111*, 1.
25. Delorenzi, J. C.; Freire-de-Lima, L.; Gattass, C. R.; Costa, D. A.; He, L.; Kuehne, M. E.; Saraiva, E. M. B. *Antimicrob. Agents Chemother.* **2002**, *46*, 2111.
26. Neal, R. A.; Bueren, J. *Trans. R. Soc. Trop. Med. Hyg.* **1988**, *82*, 709.
27. Martínez-Díaz, R. A.; Escario, J. A.; Nogal-Ruiz, J. J.; Gómez-Barrio, A. *Mem. Inst. Oswaldo Cruz* **2001**, *96*, 53.
28. Soares, M. J.; de Souza, W. *Parasitol. Res.* **1991**, *77*, 461.