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Selective inactivation of triosephosphate isomerase from *Trypanosoma cruzi* by brevifolin carboxylate derivatives isolated from *Geranium bellum* Rose

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ABSTRACT

In the search of molecules that can serve as leads in the design of a new drug for the treatment of Chagas' disease, we found that some brevifolin carboxylate derivatives isolated from *Geranium bellum* Rose, inactivate triosephosphate isomerase from *Trypanosoma cruzi* (TcTIM) in a species-specific manner. After spectroscopic characterization, these compounds were identified as methylbrevifolin carboxylate (**1**), ethylbrevifolin carboxylate (**2**), butylbrevifolin carboxylate (**3**) and the methylated derivate methyl tri-*O*-methylbrevifolin carboxylate (**4**). The concentrations required to inactivate fifty percent the activity of TcTIM were 6.5, 8 and 14 μM of **1**, **2** and **3**, respectively, while compound **4** had no inhibitory effect. Molecular docking simulations of **1** on the structure of TcTIM showed that residues of both monomers interact with the compound. These compounds are very selective with respect to the parasite enzyme, since they showed no effect on the activity of human TIM at concentrations as high as 1 mM. In conclusion, the brevifolin carboxylate derivatives described here are excellent leads in the search of a new chemotherapy for the treatment of this disease.

Trypanosomatids are the causative agents of a number of compromising diseases affecting not only humans but also domestic animals. Chagas' disease caused by the parasite *Trypanosoma cruzi* is widely disseminated in Central and South America, representing an endemic disease in 21 countries of this geographic region. It has been estimated that this disease affects 9.8–11 million people, and 60 million are at risk.[1,2]

The nitrofuran, nifurtimox and the nitroimidazole, Benznidazole are the current drugs employed for treatment of Chagas' disease. However, not only the adverse effects promoted by both drugs but also the failure to clearance parasites result in a frequent interruption of treatment.[3] On the other hand, Gentian Violet constitutes the only alternative for eliminating *T. cruzi* from blood during transfusions.[4] Therefore, there is an urgent need to develop new and efficient drugs against *T. cruzi*.

An important characteristic in the metabolism of trypanosomatids is their dependence on glycolysis as an energy source for cellular work. Thus, enzymes of this pathway represent excellent targets for the search of selective molecules to inhibit them and affect their metabolic function.

In this context, several groups have proposed triosephosphate isomerase (TIM) as a target for drug design against parasitic diseases.[5–7] TIM catalyzes the isomerization of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate in the fifth step of the glycolytic pathway. Structurally, most of the known TIMs are homodimers, each monomer consisting of eight parallel β-strands forming a barrel, surrounded by eight α-helices. The interface between monomers occupies a significant portion of the molecular surface area of each monomer, around 1496 Å in TIM from *T. cruzi* (TcTIM).[8] Interestingly, TIM is active only in its dimeric form;[9,10] therefore, the targeting of small molecules to the interface of TIM may potentially induce structural modifications and alter the dimer integrity may provoke enzyme inactivation.

Products isolated from nature represent an important and vast source of new leads for drug design. In fact, around 40% of all new chemical entities approved from 1981 to 2006 had a nature origin; in reference to antiparasite drugs, this figure is close to 50%.[11] Of particular interest for this study, it has been reported that several plant derived compounds posses anti-trypanosomal activity.[12,13]

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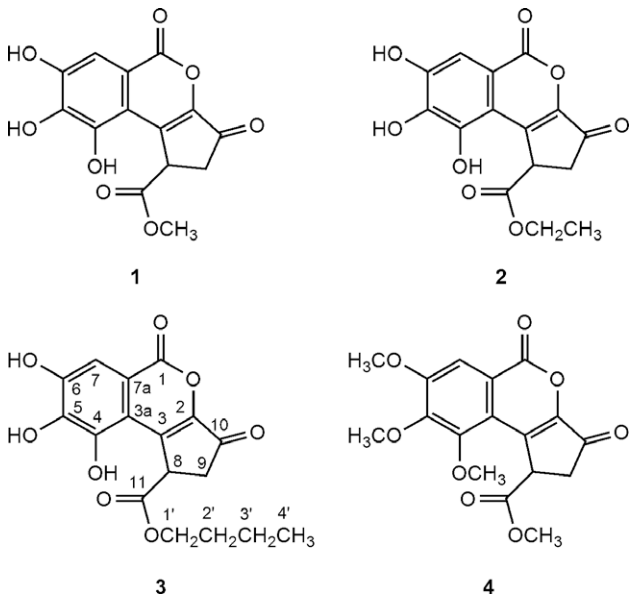

Figure 1. Structure of brevifolin carboxylate derivatives. Methylbrevifolin carboxylate (**1**), ethylbrevifolin carboxylate (**2**), buthylbrevifolin carboxylate (**3**) and methyl tri-*O*-methylbrevifolin carboxylate (**4**). Carbon numbering for compound **3** is showed.

Table 1
I_{50} of compounds **1**–**4** on TcTIM and hTIM

Compound	I_{50} (TcTIM)	n	I_{50} (hTIM)
1	6.5 μM	0.81	>1 mM
2	8 μM	0.87	>1 mM
3	14 μM	0.90	>1 mM
4	>1 mM	ND	>1 mM

The I_{50} and n value were obtained from the fitting of concentrations curves to equation $V_i = (V_0 \times I_{50}^n + I^n)$ were V_i indicates activity at a given concentration of the inactivating agent, V_0 is the initial activity, I_{50} the concentration of the compound that induces half-maximal inactivation, I the concentration of the tested agent, and n is a measure of cooperativity.[16] ND no determined.

In this work, we studied the inactivation of TcTIM[14] by four brevifolin carboxylate derivatives (Fig. 1), three of them (compounds **1**, **2** and **3**) were isolated from the plant *Geranium bellum* Rose and one (compound **4**) was obtained by alkylation of **1**.[15] Compounds **1**, **2** and **3** inactivate TcTIM in the low micromolar range (Table 1); these compounds differ on the substituent at the carboxyl group, with methyl, ethyl, and butyl for compounds **1**, **2** and **3**, respectively. The concentration that induces the half-maximal inactivation of the enzyme (I_{50}) as well as the n value for each compound was calculated from concentration curves and fitting the data to equation described in Table 1. Figure 2A shows the concentration curve obtained from compound **1**, for compounds **2** and **3** the curves were similar (data not show). The n value obtained was close to one for the three compounds, suggesting that only one molecule of them is necessary to inactive TcTIM[16] (Table 1). Furthermore, the results indicated that as the carbonated chain is enlarged, inactivation decreases (Table 1). We also explored the role of the three hydroxyl groups in the brevifolin ring; to this end, the protons of the –OH groups in brevifolin ring of compound **1** were replaced by methyl groups to obtain compound **4**. At 1 mM concentration this molecule had no effect on TcTIM (Table 1). This result suggests that at least some of the hydroxyl groups of brevifolin ring play a crucial role in the effect of these molecules.

Inactivation of TcTIM by compounds **1**, **2** and **3** increased with the incubation time. The plots of *ln* of remaining activity versus time were linear, indicating that inactivation was a pseudo-first-order reaction. The first-order rate constants of inactivation at several concentrations of **1**, **2**, and **3** were obtained from the

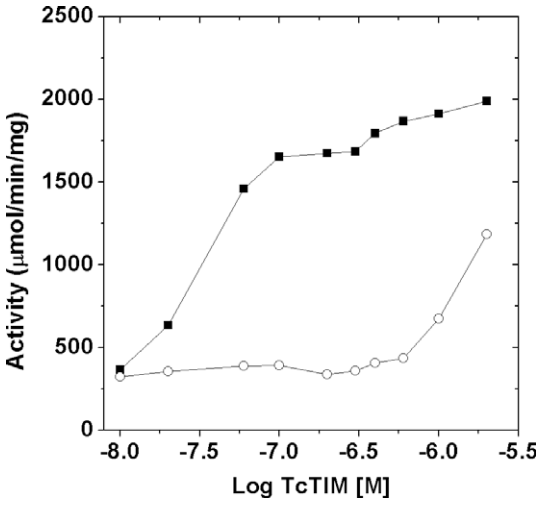

Figure 3. Inactivation of TcTIM at different enzyme concentrations. Specific activity of the enzyme in absence (solid squares) and presence (open circles) of 50 μM of compound **1**. The assay conditions were as described before[14] except that at higher protein concentrations, dilutions were made to measure activity with 5 ng of enzyme.

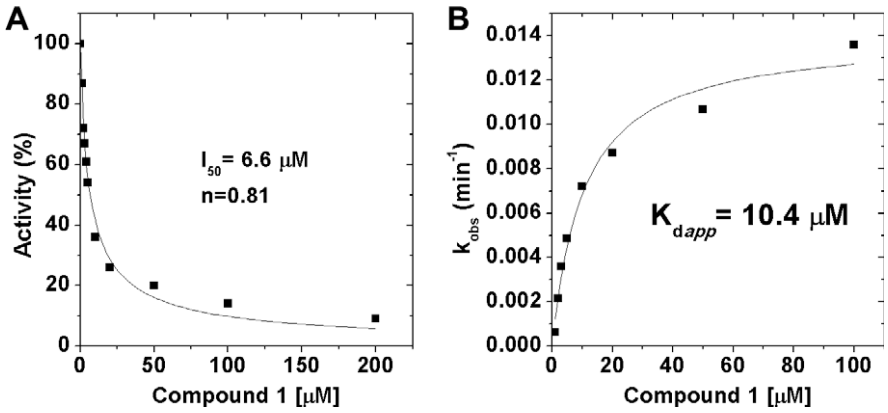

Figure 2. Inactivation of TcTIM by compound **1**. (A) Effect of different concentrations of methylbrevifolin carboxylate on TcTIM activity. (B) Plot of the pseudo-first-order rate constants at different concentrations of **1**; these were determined from measurements of activity at different times of incubation with the indicated compound concentration.

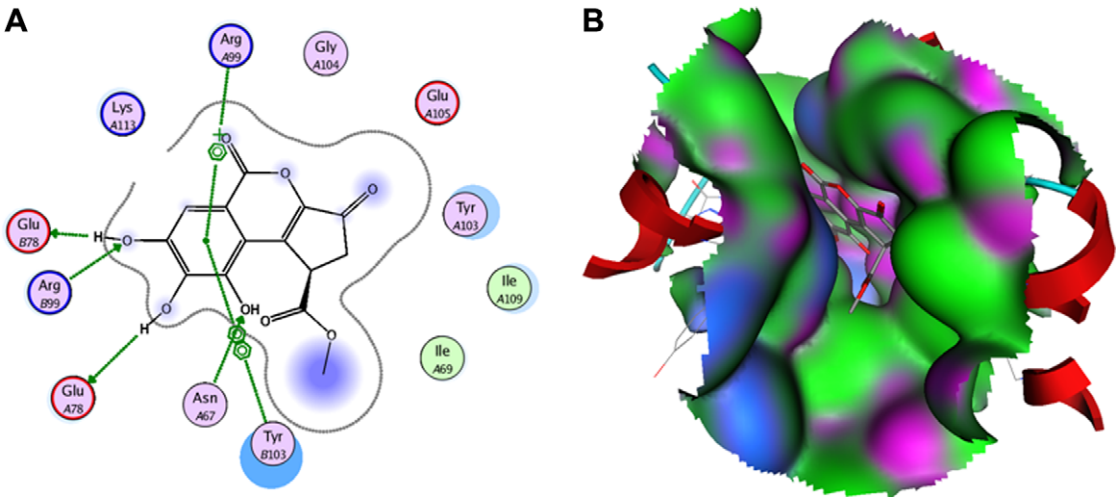

Figure 4. Binding of compound **1** into the TcTIM dimer interface. (A) 2D diagram of interaction between compound **1** and TcTIM. (B) Molecular surface of residues around compound **1** colored by hydrofobicity as follows: acceptors or hydrogen bond donors (purple), mild polar (blue) and hydrofobic (green). The local secondary structure is represented by red ribbons and cyan strands for helices and loops, respectively.

exponential decay of activity; these values were plotted against the compound concentration. The plots were not linear, instead they level off at relatively high concentrations as can be seen for compound **1** (Fig. 2B). This pattern is characteristic of a process in which initial ligand binding is followed by enzyme inactivation in the enzyme–ligand complex.[17] From this plot an apparent dissociation constant and an apparent maximum rate constant for binding and inactivation of 10.4 μM and 0.014 min^{-1} respectively, for compound **1** were obtained (Fig. 2B).

We further investigated whether the compounds described in this work selectively inactivate TcTIM. To this end, the effect of compounds **1**–**4** on the activity of human TIM (hTIM) was determined. As shown in Table 1, none of the four compounds employed at concentrations as high as 1 mM, affected the activity of hTIM.

In order to investigate the mechanism by which these compounds inactivate TcTIM, a more detailed study of the most effective molecule (i.e., compound **1**) was made. Thus, we first determined if compound **1** acts on the TcTIM dimer, by interfering with the association and dissociation of the monomers. As governed by the dissociation constant between oligomeric proteins, the ratio between monomers and dimers depends on the protein concentration. As shown in Figure 3, the detrimental effect of compound **1** on TcTIM decreased as the concentration of the enzyme increased. This result suggests that compound **1** acts on the interface of the TcTIM dimer interfering with association and dissociation process of the monomers.

To better investigate this notion, a molecular docking simulation of the binding of **1** into TcTIM interface was made.[18] The results of this analysis predict that compound **1** interacts with residues of both TcTIM monomers, establishing hydrogen bonds with Asn67, Glu78 of monomer A and Glu78, Arg99 of monomer B (Fig. 4A). Furthermore, compound **1** had a π–π and a π-cation interactions with Tyr103 of monomer B and Arg99 of monomer A, respectively (Fig. 4A). The amino acids residues that are located at a distance lower than 4.5 Å of the ligand are: Asn67, Ala68, Ile69, Glu78, Arg99, Tyr103, Gly104, Glu105, Ile109 and Lys113 of monomer A; as well as Glu78, Arg99 and Tyr103 of monomer B (Fig. 4A). Thus, it is likely that the hydroxyl groups and the brevifolin carboxylate nucleus are instrumental in the binding of compound **1** to the enzyme and that its effect is modulated by the substituent at the carboxyl group. An interesting observation is that the methyl group of compound **1** binds in a hydrophobic patch at the TcTIM interface (Fig. 4B). This information will prove to be important during search of different substituents for designing new brevifolin carboxylate derivatives.

Although there are previous reports describing pharmacological properties of compound **1**[19–24] there are no currents reports describing the antiparasitic action of this compound. As demonstrated here, compounds **1**, **2** and **3** are highly selective inhibitors of TcTIM; therefore, they are excellent leads for the synthesis of more potent molecules in order to obtain new drugs in the chemotherapy against Chagas' Disease.

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References and notes

1. Moncayo, A. C.; Ortiz Yanine, M. I. *Ann. Trop. Med. Parasitol.* **2006**, *100*, 663.
2. Schofield, C. J.; Jannin, J.; Salvatella, R. *Trends Parasitol.* **2006**, *22*, 583.
3. Urbina, J. A.; Docampo, R. *Trends Parasitol.* **2003**, *19*, 495.
4. Croft, S. L. *Parasitology* **1997**, *114*, S3.
5. Gomez-Puyou, A.; Saavedra-Lira, E.; Becker, I.; Zubillaga, R. A.; Rojo-Dominguez, A.; Perez-Montfort, R. *Chem. Biol.* **1995**, *2*, 847.
6. Velanker, S. S.; Ray, S. S.; Gokhale, R. S.; Balaram, H.; Balaram, P.; Murthy, M. R. N. *Structure* **1997**, *5*, 751.
7. Cortés-Figueroa, A. A.; Pérez-Torres, A.; Salaiza, N.; Cabrera, N.; Escalona-Montaño, A.; Rondán, A.; Aguirre-García, M.; Gómez-Puyou, A.; Pérez-Montfort, R.; Becker, I. *Parasitol Res.* **2008**, *102*, 635.
8. Maldonado, E.; Soriano-García, M.; Moreno, A.; Cabrera, N.; Garza-Ramos, G.; de Gómez-Puyou, M.; Gómez-Puyou, A.; Perez-Montfort, R. *J. Mol Biol.* **1998**, *283*, 193.
9. Waley, S. G. *J. Biochem.* **1973**, *135*, 165.
10. Zabori, S.; Rudolph, R.; Jaenicke, R. *Z. Naturforsch.* **1980**, *35C*, 999.
11. Newman, D. J.; Cragg, G. M.; Snader, K. M. *J. Nat. Prod.* **2007**, *70*, 461.
12. Ercil, D.; Kaloga, M.; Radtke, O.; Sakar, M.; Kiderlen, A.; Kolodziej, H. *Turk. J. Chem.* **2005**, *29*, 437.
13. Salem, M.; Karl, A. W. *Curr. Med. Chem.* **2006**, *13*, 2571.
14. *Activity and inactivation assays*: Activity was assayed in the direction of glyceraldehyde 3-phosphate to dihydroxyacetone phosphate as described elsewhere[5] with 5 ng of the enzymes. For inactivation studies, TIMs were incubated at a concentration of 5 μg/mL in a buffer containing 100 mM triethanolmaine, 10 mM EDTA, pH 7.4 (TE) and 10% of dimethyl sulfoxide (DMSO) at 36 °C. The mixture also contained the compounds at the indicated concentrations. Compounds were dissolved in DMSO. After 2 h, 1 μL was withdrawn an added to 1 mL of reaction mixture for activity assay. None of the molecules tested here affected the activity of α-glycerol phosphate dehydrogenase, the enzyme used for trapping the product. *Enzymes*. Both

TIMs were recombinant products. For the purification of TcTIM and hTIM the methodologies described elsewhere[25,26] were, respectively, followed. *Protein concentration.* This was calculated as reported elsewhere.[27] The molar extinction coefficients for TcTIM and hTIM were 36,440 and 33,460 M^{-1} cm^{-1}, respectively.

15. *Procedure for the preparation of compounds* **1–4**. General: Column Chromatography (CC) was carried out on Merck silica gel 60 (Aldrich, 230–400 ASTM). TLC: silica gel 60 (Aldrich, layer thickness 0.2 mm, 20 × 20 cm with fluorescent indicator F254 nm) plates, NMR measurements were performed at 400 MHz for 1H and 100 MHz for ^{13}C on a Jeol Eclipse 400 spectrometer. *Compound* **1**. Isolation of compound **1** (10 mg) was made as reported elsewhere.[23] R_f = 0.40, in TLC $CHCl_3$–EtOH (8:2). *Compound* **2**. Air-dried aerial parts of *Geranium bellum* Rose (0.5 kg) were extracted with EtOH (4 L) under reflux for 8 h, filtration and evaporation to afforded green viscous oil (55 g), which a portion (20 g) was subjected to CC on silica gel using $CHCl_3$–MeOH (1:0, 10:0.5, 10:1; and 8:2), successive chromatographies under the same conditions afforded compound **2** (10 mg). Their physical and spectroscopic data were identical whit those reported.[28] R_f = 0.48 in TLC $CHCl_3$–EtOH (8:2). *Compound* **3**. Air-dried aerial parts of *G. bellum* (0.5 kg) were extracted with *n*-BuOH (4 L) under reflux for 8 h, filtration and evaporation to afforded green viscous oil, (70 g). This residue was purified by CC on silica gel using $CHCl_3$–MeOH (1:0, 10:0.5, 10:1, and 8:2). Successive chromatographies under the same conditions afforded compound **3** (12 mg). Until our known, this is the first report on this compound. Mp 290–295° decomp. R_f = 0.57, in TLC $CHCl_3$–EtOH (8:2). IR (KBr disk) ν_{max} 3454, 2957, 2926, 1733, 1701, 1664, 1603, 1384 cm^{-1}, Experimental: ^1H NMR (400 MHz acetone-d_6), 7.29 (s, 1, H-7), 4.55 (dd, 1, J = 7.7, 1.8 Hz, H-8), 3.03 (dd, 1, J = 18.7, 7.7 Hz, H-9a), 2.48 (dd, 1, J = 18.7, 1.8 Hz, H-9b), 4.14 (dt, 1, J = 6.4, 10.8 Hz, H-1a′), 4.04 (dt, 1, J = 6.4, 10.8 Hz, H-1b′), 1.58 (m, 2, J = 6.6 Hz, H-2′), 1.29 (m, 2, J = 7 Hz, H-3′), 0.86 (t, 3, J = 7 Hz, H-4′), ^{13}C NMR (100 MHz acetone-d_6), δ 192.4 (C-10), 172.03 (C-11), 160.10 (C-1), 148.84 (C-5), 146.92 (C-2), 142.98 (C-4), 139.37 (C-6), 137.81 (C-3), 115.61 (C-3a), 114.41 (C-7a), 108.68 (C-7), 64.56 (C-1′), 41.06 (C-8), 30.48 (C-9), 37.34 (C-2′), 18.98 (C-3′), 13.35 (C-4′). *Compound* **4**. Methylation of **1** (70 mg) with an excess of ethereal CH_2N_2 gave methyl tri-*O*-methylbrevifolin carboxylate (**4**) which was purified by column chromatographic using silica gel (1 g), and hexane–EtOAc (1:0, 20 mL; 10:1, 22 mL, 5:1, 24 mL; 10:3, 26 mL; 5:2, 28 mL and 2:1, 30 mL) as eluent. Eluates of 5 mL were collected, which fraction 15–27 afforded **4** (30 mg). Their physical and spectroscopic data were identical with those reported.[29]
16. Tellez-Valencia, A.; Avila-Rios, S.; Perez-Montfort, R.; Rodriguez-Romero, A.; Tuena de Gomez-Puyou, M.; Lopez-Calahorra, F.; Gomez-Puyou, A. *Biochem. Biophys. Res. Commun.* **2002**, *295*, 958.
17. Pettigrew, D. W. *Biochemistry* **1986**, *25*, 4711.
18. Molecular docking simulation was made using program MOE (Molecular Operating Environment, www.chemcomp.com). Tridimensional structure coordinates of TcTIM were obtained from Protein Data Bank (www.rcsb.org) with code 1TCD.[8] The region on the interface of TcTIM suitable for docking assays was determined using the alfa-sites tool in the same MOE package. Assignation of partial charges and optimal geometry of compound **1** were carried out with the MMFF94 forcefield. For molecular docking of compound **1** in the interface of TcTIM, 5000 random orientations with variations in position and molecular rotation were assessed, and the score for each one of them were calculated by MOE considering spatial compatibility to the binding site, internal energy of the ligand and protein–ligand interactions.
19. Zhong, Y.; Zuo, C.; Li, F.; Ding, X.; Yao, Q.; Wu, K.; Zhang, Q.; Wang, Z.; Zhou, L. W.; Lan, J.; Wang, X. *Zhongguo Zhong Yao Za Zhi.* **1998**, *23*, 363.
20. Iizuka, T.; Moriyama, H.; Nagai, M. *Biol. Pharm. Bull.* **2006**, *29*, 177.
21. Iizuka, T.; Nagai, M.; Taniguchi, A.; Moriyama, H.; Hoshi, K. *Biol. Pharm. Bull.* **2007**, *30*, 382.
22. Tomczyk, M.; Drozdowska, D.; Bielawska, A.; Bielawski, K.; Gudej, J. *Pharmazie* **2008**, *63*, 389.
23. Camacho-Luis, A.; Gayosso-De-Lucio, J. A.; Torres-Valencia, M. T.; Muñoz-Sánchez, J. L.; Alarcón-Hernández, E.; López, R.; Barrón, B. L. *J. Mex. Chem. Soc.* **2008**, *52*, 103.
24. Fang, S. H.; Rao, Y. K.; Tzeng, Y. M. *J. Ethnopharmacol.* **2008**, *116*, 333.
25. Ostoa-Saloma, P.; Garza-Ramos, G.; Ramirez, J.; Becker, I.; Berzunza, M.; Landa, A.; Gomez-Puyou, A.; de Gomez-Puyou, M.; Perez-Montfort, R. *Eur. J. Biochem.* **1997**, *244*, 700.
26. Mande, S. C.; Mainfroid, V.; Kalk, K. H.; Goraj, K.; Martial, J. A.; Hol, W. G. *Protein Sci.* **1994**, *3*, 810.
27. Pace, C. N.; Vajdos, F.; Fee, L.; Grimsley, G.; Gray, T. *Protein Sci.* **1995**, *4*, 2411.
28. Jia-Sheng, G.; Su-Xian, W.; Xian, L.; Ting-Ru, Z. *Acta Pharm. Sinca* **1987**, *22*, 28.
29. Yoshida, T.; Itoh, H.; Matsunaga, S.; Tanaka, R.; Okuda, T. *Chem. Pharm. Bull.* **1992**, *40*, 53.