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Full Length Research Paper

Formulation of Basic Compounds as Arginase Inhibitors with Assessment Against *Leishmania amazonensis*

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In this work, we synthesized substances and evaluated the potential leishmanicidal activity. Thirty-four (34) compounds from different chemical classes were selected for a simple synthesis in overall good yields, eight of which are not described in the literature. From the 34 compounds evaluated for preliminary leishmanicidal activity, three can be considered as prototypes for the development of leishmanicidal agents. Computational chemistry calculations, including comparative modeling of the enzyme arginase from *Leishmania amazonensis* and a molecular docking simulation of the synthesized molecules into the active site of the model were carried out.

Key words: Leishmaniasis, arginase, molecular docking, thiourea, bisguanidine, amidine, biological evaluation, comparative modeling.

INTRODUCTION

Leishmaniasis is a disease that is closely related to poverty, especially in some developing countries, due to poor living conditions and sanitation problems in some areas. Over 90% of the cases of the disease are reported in different parts of India, Nepal, Sudan, Bangladesh and Brazil (Dujardin et al., 2008). However, the migration of population associated with globalization and climate change has led to reported cases in European countries, including France, Portugal and Spain (Dujardin et al., 2008). The World Health Organization (WHO) estimates that the disease threatens approximately 350 million individuals in 88 countries worldwide (WHO, 2010).

There are many species of the protozoa that can infect humans and are responsible for the following main forms of leishmaniasis (Ashford, 2000; Avlonitis et al., 2003; Croft et al., 2003): cutaneous, mucocutaneous, diffuse cutaneous and visceral leishmaniasis. Cutaneous leishmaniasis (CL) is caused by *Leishmania major* and mucocutaneous leishmaniasis (ML) is caused by *Leishmania braziliensis* and generates sores on the skin. Diffuse cutaneous leishmaniasis (DCL) is caused by *Leishmania amazonensis* and generates chronic ulcera-

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tions on the skin that are difficult to treat. Visceral leishmaniasis (VL) can be caused by *Leishmania donovani*, *Leishmania infantum* and *Leishmania chagasi* and is the most severe form of the disease, which can be fatal if not treated.

Leishmaniasis, especially VL, has been recognized as an opportunistic infection in patients with weakened immune systems such as acquired immunodeficiency syndrome (AIDS) (Alvar et al., 2008).

The first-line drugs for treating leishmaniasis are pentavalent antimonials, sodium stibogluconate (Pentostam®) and meglumine antimoniate (Glucantime®). However, they require high doses and patient hospitalization for three to four weeks for parenteral administration and are highly toxic (Guerin et al., 2002). Moreover, cases of resistance to antimonials have been reported, mainly in India (Alavi-Naini, 2008). A second line of treatment is used when an inadequate response to the first line exists.

Examples of drugs used in this situation are amphotericin B, pentamidine, miltefosine and paromomycin. These drugs are also very toxic and administered parenterally (Avlonitis et al., 2003; Croft et al., 2003; Guerin et al., 2002). Due to the increasing number of published reports on drug resistance and toxicity, as well as the occurrence of *Leishmania*/HIV co-infection, there is an urgent need to search for alternative therapies.

The use of simple molecules which are cheap and easy to obtain is an interesting approach for a neglected tropical disease. An important approach for the rational development of new drug candidates is the selection of a specific target that can interact with the studied compounds.

The arginase enzyme of *Leishmania* has shown to be a promising target (Iniesta et al., 2001; Wanasen et al., 2008) and simple molecules have shown to be good inhibitors for arginase. The activity of arginase in trypanosomatids has proven to be specific to the genus and has been used as a tool for classification and identification.

Leishmania is a genus in the family in which arginase is believed to have a role in the pro-duction of *L*-ornithine (Silva et al., 2002). *L*-ornithine is used in the synthesis of polyamines that have important roles in cellular processes such as growth (Iniesta et al., 2001; Roberts et al., 2004).

Thus, the objective of this work was to synthesize molecules, which were based on simple structures of known arginase inhibitors and evaluate them against *L. amazonensis*. We used easy and inexpensive methods for the synthesis of 34 molecules which were evaluated against *L. amazonensis*. Moreover, in order to test the hypothesis that those molecules would work as arginase inhibitors, computational chemistry calculations were carried out, including comparative modeling of the enzymes, followed by docking simulations of each molecule with that model.

MATERIALS AND METHODS

Synthesis

As leishmaniasis is a neglected disease that is related to poverty, we have chosen to synthesize molecules that can be inexpensively obtained and could originate in different chemical classes. Of the 34 compounds that were synthesized, 8 of them had not been reported in the literature (4, 14, 16, 20, 23, 27 to 29) (Figure 1). The synthetic routes used to obtain the compounds in this work are described in the results and discussion sections.

Despite the fact that all the methods used for the preparation of the investigated compounds are already present in literature, we described the methodologies for the synthesis of the eight new substances.

Preparation of the compounds 36-37

It is as described in the literature for the same compounds by Pierce and coworkers (1998).

Preparation of the compounds 01-04

It is as described in the literature for the same compounds by Urbanski and coworkers (1967). For the compound 04, the method is described thus: A mixture of 4.0 mmols of the amine (35), 1 mL of concentrated HCl and 4.0 mmols of cyanoguanidine in 10 mL of water was kept under reflux with magnetic stirring for 3 h. The reaction mixture was concentrated to half its volume, and left in a refrigerator until the next day. The precipitate formed was filtered under vacuum and washed with an ice mixture of EtOH : EtOAc (1:2). Aminourea hydrochloride was obtained as a white solid in 75% yield.

m.p.= 199-202 ^{0}C; ^{1}H NMR (500 MHz, DMSO-d_6, δ): 1.32 (t, J= 7Hz, 3H, CH_3); 4.30 (q, J= 7Hz, 2H, CH_2); 7.60 (d, J= 9Hz, 2H, H-2); 7.95 (d, J= 9Hz, 2H, H-3); 8.38 (sl, 4H-C(=NH_2^+)NH_2); 10.46 (sl, 1H-C(O)NHC(NH_2^+)); 10.76 (sl, 1H-ArNH); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 14.28 (CH_3); 60.33 (CH_2); 118.75 (C-2); 124.87 (C-4); 130.47 (C-3); 142.17 (C-1); 151.21 (C=O); 155.08 (C=NH_2^+); 165.35 (CO_2Et); IV (KBr, cm-1): 3427-3122 (ν_{assim} NH2); 3076 (νC$_{ar}$ H); 2987-2877 (νC$_{sp^3}$ H); 2362 (νN^+H); 1734 (νC=O$_{ester}$); 1686 (νC=O$_{urea}$); 1606 (νC=NH); 1522 (νNH); 1286 (νC-O).

Preparation of the compounds 05-06

It is as described in the literature for the same compounds by Rabjohn and coworkers (1963).

Preparation of the compounds 07-08

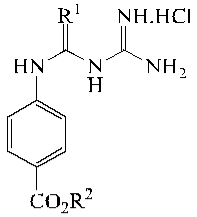
It is as described in the literature for the same compounds by Lange and Reed (1926).

Preparation of the compounds 09-10

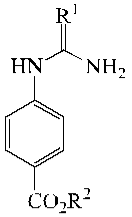
It is as described in the literature for the same compounds by Nakayama and coworkers (1993).

Preparation of the compounds 44-49

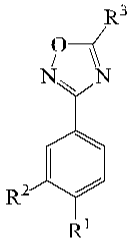
It is as described in the literature for the same compounds by Wang



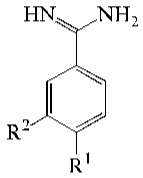
(1) R^1=NH;R^2=H
(2) R^1=NH;R^2=Et
(3) R^1=O;R^2=H
(4) R^1=O;R^2=Et



(5) R^1=O;R^2=H
(6) R^1=O;R^2=Et
(7) R^1=S;R^2=H
(8) R^1=S;R^2=Et
(9) R^1=$NH_2^+Cl^-$;R^2=H
(10) R^1=$NH_2^+Cl^-$;R^2=Et



(11) R^1=CO_2Me; R^2=H; R^3=CH_2SCN
(12) R^1=CO_2H; R^2=H; R^3=CH_2SCN
(13) R^1=NO_2; R^2=H; R^3=CH_2SCN
(14) R^1=$NHCO_2CH_2SCN$; R^2=H; R^3=CH_2SCN
(15) R^1=Cl; R^2=H; R^3=CH_2SCN
(16) R^1-R^2= -OCH_2O;R^3=CH_2SCN
(17) R^1=CO_2Me;R^2=H;R^3=Me
(18) R^1=CO_2H;R^2=H;R^3=Me
(19) R^1=NO_2;R^2=H;R^3=Me
(20) R^1=NHCOMe;R^2=H;R^3=Me
(21) R^1=NH_2;R^2=H;R^3=Me
(22) R^1=Cl;R^2=H;R^3=Me
(23) R^1-R^2= -OCH_2O;R^3=Me
(24) R^1=CO_2Me;R^2=H;R^3=CF_3
(25) R^1=CO_2H;R^2=H;R^3=CF_3
(26) R^1=NO_2;R^2=H;R^3=CF_3
(27) R^1=$NHCOCF_3$;R^2=H;R^3=CF_3
(28) R^1=Cl;R^2=H;R^3=CF_3
(29) R^1-R^2= -OCH_2O;R^3=CF_3



(30) R^1=CO_2CH_3;R^2=H
(31) R^1=NO_2;R^2=H
(32) R^1=$NHCOCH_3$;R^2=H
(33) R^1=Cl;R^2=H
(34) R^1-R^2= -OCH_2O

Figure 1. Synthesized compounds (1-34).

and coworkers (1998).

Preparation of the compounds 50-55

It is as described in the literature for the same compounds by Judkins and coworkers (1996).

Preparation of the compounds 56-61 and 62-67

It is as described in the literature for the same compounds by Palazzo and coworkers (1961).

Preparation of the compounds 11-16

It is as described in the literature for the same compounds by Palazzo and coworkers (1961). For the compounds 14 and 16, the method is described thus: A solution of chloro derivative (1.0 mmol), 2.40 mg (3.15 mmols) of NH_4SCN, 5 mL of DMSO was heated in water bath for 1 h. At the end of this time, 20 mL of water was added and the precipitate formed was filtered under vacuum and washed with water.

Compound 14. m.p.= 230°C (dec.); ^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ): 3.91 (s, 3H, CH_3); 4.89 (s, 2H, H-9); 8.17 (m, 4H, H-2,3,5,6); ^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ): 26.91 (C-9); 37.27 (CH_2SCN); 111.93 (C-10); 119.47 (C-3,5); 120.72 (C-1); 128.02 (C-2,6); 141.35 (C-4); 165.05 (CO); 167.55 (C-7); 175.36 (C-8); IV (KBr, cm^{-1}): 3340 e 3290 (νNH); 3016 (νC$_{ar}$ H); 2953 e 2933 (νC$_{sp^3}$ H); 2160 (νC≡N); 1687 (νC=O + δNH); 1525 (νC-N + δNH).

Compound 16. m.p.= 114-117°C; ^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ): 4.93 (s, 2H, H-9); 6.16 (s, 2H, OCH_2O); 7.12 (d, *J*= 8 Hz, 1H, H-5); 7.46 (d, *J*= 2 Hz, 1H, H-2); 7.59 (dd, *J*= 8 Hz e 2Hz, 1H, H-6); ^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ): 26.88 (C-9); 101.91 (OCH_2O); 111.95 (C-10); 108.98 (C-5); 106.40 (C-2); 119.22 (C-1); 122.17 (C-6); 148.03 (C-3); 150.22 (C-4); 167.62 (C-7); 175.26 (C-8); IV (KBr, cm^{-1}): 3099 e 3014 (νC$_{ar}$ H); 2962 e 2923 (νC$_{sp^3}$ H); 2786 (νC$_{sp^3}$ H-OCH_2O); 2157 (νC≡N); 929 (νO-C-O).

Preparation of the compounds 17-23

It is as described in the literature for the same compounds by Bergmann and coworkers (1953). For the compounds 20 and 23, the method is described thus: To a solution of 1.0 mmol of amidoxime in 10 mL of glacial acetic acid under argon atmosphere was added 0.2 mL (2.00 mmols, d = 1.082 mL/cm^3) of acetic anhydride. The mixture was kept under reflux for one hour. After the reaction mixture was achieved at the room temperature, water was added. The product was filtered under vacuum and taken with water.

Compound 20. m.p.= 156-159°C; ^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ): 2.09 (s, 3H, $COCH_3$); 2.65 (s, 3H, H-9); 7.76 (d, *J*= 9Hz, 2H, H-3,5); 7.93 (d, *J*= 9Hz, 2H, H-2,6); 10.23 (sl, 1H-NH).

Compound 23. m.p.= 112-114°C; ^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ): 2.64 (s, 3H, H-9); 6.14 (s, 2H, OCH_2O); 7.08 (d, *J*= 8Hz, 1H, H-5); 7.42 (s, 1H, H-2); 7.55 (d, *J*= 8Hz); ^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ): 11.91 (C-9); 101.79 (OCH_2O); 108.85 (C-5); 106.45 (C-2); 119.98 (C-1); 147.93 (C-3); 149.86 (C-6); 149.86 (C-4); 167.22 (C-7); 177.08 (C-8).

Preparation of the compounds 24-29

It is as described in the literature for the same compounds by Cottrell and coworkers (2004). For the compounds 27-29 the method is described thus: To a solution of amidoxime (1.00 mmol) and 13.8 mg (1.00 mmol) of K_2CO_3 in 10 mL of anhydrous chloroform was added 0.2 mL (1.20 mmols, d = 1.490 mL/cm^3) of trifluoroacetic anhydride. The mixture was stirred at room temperature for two hours. The solvent was removed on rotary evaporator and the residue was dissolved in ethyl acetate. The organic solution was washed with water (3 x 20 mL), dried with Na_2SO_4 and evaporated in rotavapor, providing the product in 95% yield.

Compound 27. m.p.= colorless oil; ^{1}H NMR (500 MHz, DMSO-*d*$_6$, δ): 7.93-8.12 (m, 4H, H-2,3,5,6); 11.62 (sl, 1H, NH); ^{13}C NMR (125 MHz, DMSO-*d*$_6$, δ): 115.54 (q, $J_{C,F}$= 286 Hz, CF_3); 115.72 (q, $J_{C,F}$= 273 Hz, C-9); 121.11 (C-1); 121.34 (C-3,5); 128.35 (C-2,6); 140.03 (C-4); 154.74 (q, $J_{C,F}$= 37 Hz, CO); 164.96 (q, $J_{C,F}$= 37 Hz, C-8); 167.81 (C-7); ^{19}F NMR (376 MHz, DMSO-*d*$_6$, δ): -65.32; -74.48 ($CONHCF_3$).

Compound 28. m.p.= 150-152°C; ^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ): 7.70-8.10 (m, 4H, H-2,3,5,6); ^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ): 115.66 (q, $J_{C,F}$= 273 Hz, C-9); 123.27 (C-1); 127.14 (C-2,6); 129.70 (C-3,5); 137.33 (C-4); 165.10 (q, $J_{C,F}$= 44 Hz, C-8); 167.24 (C-7); ^{19}F NMR (376 MHz, DMSO-*d*$_6$, δ): -65.28.

Compound 29. m.p.= 35-37°C; ^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ): 6.18 (s, 2H, OCH_2O); 7.14 (q, *J*= 8 Hz, 1H, H-5); 7.50 (d, *J*= 2 Hz, 1H, H-2); 7.55 (dd, *J*= 8 Hz, *J*= 2 Hz, 1H, H-6); ^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ): 26.88 (C-9); 101.90 (OCH_2O); 106.62 (C-2); 109.14 (C-5); 115.72 (q, $J_{C,F}$= 273 Hz, C-9); 119.79 (C-1); 122.83 (C-6); 148.23 (C-3); 150.89 (C-4); 164.73 (q, $J_{C,F}$= 44 Hz, C-8); 168.07 (C-7); ^{19}F NMR (376 MHz, DMSO-*d*$_6$, δ): -65.36.

Preparation of the compounds 68-72 and 30-34

It is as described in the literature for the same compounds by Judkins and coworkers (1996).

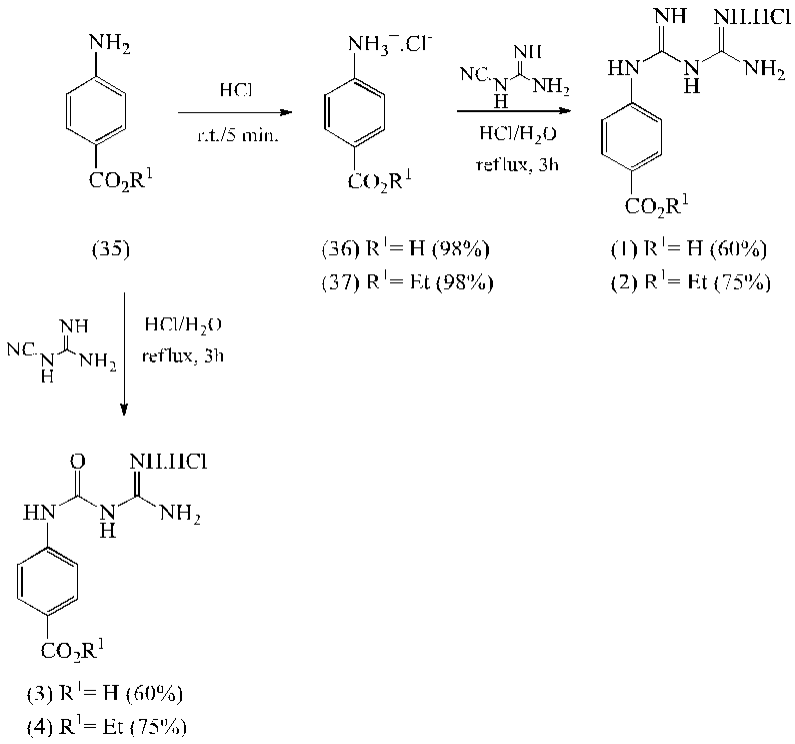
Biological evaluation

The main objective of this work was to identify compounds with potential leishmanicidal activity. Thus, 34 compounds in four different chemical classes were synthesized and evaluated for their *in vitro* activity against *L. amazonensis*. The *in vitro* effectiveness of the synthesized compounds was analyzed in the promastigote form of *L. amazonensis* (MHOM/BR/77/LTB0016). Parasites were maintained by animal passage and cryopreserved in liquid nitrogen. Promastigotes were cultured at 25°C in Schneider's Insect Medium of pH 7.2 containing 10% (v/v) of heat-inactivated fetal bovine serum (Cysne-Finkelstein et al., 1998). For the activity assay, the parasite culture was diluted with the fresh medium to a final concentration of 5 x 10^6 parasites/mL. The substances were dissolved in dimethylsulfoxide (DMSO) except for amidines 32-34, which were dissolved in water and tested within a concentration range of 0.16 to 320 μg mL^{-1} (0.74-1493.33 μM). Drugs were added to a 96-well microtitre plate (up to 1.6% final DMSO concentration) and incubated at 26°C for 24 h with the parasites in their metacyclic phase (Canto-Cavalheiro et al., 1997). Pentamidine was used as the reference drug. Results represent the mean values of three experiments and were expressed as IC_{50}, that is, the concentration of a compound that caused a 50% reduction in survival/viability in comparison with identical cultures without the compounds. The procedure used to observe the drug effects was the MTT reduction, using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT, Sigma, MO, USA) and the result was read on an ELISA reader at 490 nm (Denizot et al., 1986).

Molecular modeling

The synthesized compounds were modeled using the Sybyl 8.0 molecular modeling package (Tripos, 2012). The hydrogen atoms were added to the structures and 1,000 steps of energy minimization were performed by the steepest descent method, using Gasteiger-Hückel charges and a dielectric constant of 80, in the Tripos force field. The structures were further optimized using the conjugated gradient method.

Ligand-enzyme docking simulations were performed with the molecular docking algorithm MolDock (Molegro, 2012) using the Molegro Virtual Docker 4.2.0 package. MolDock uses a heuristic search algorithm (termed guided differential evolution), which is a combination of differential evolution and a cavity-prediction algorithm. The docking scoring function is an extension of the piecewise linear potential (PLP) (Molegro, 2012). After the ligands and the protein coordinates were imported, all structural parameters, including bond type, hybridization, explicit hydrogen, charges, and flexible torsions, were assigned using the automatic preparation function in Molegro Virtual Docker software. For each compound, 100 docking runs were performed with the initial



Scheme 1. Synthetic route used to obtain the compounds 1-4.

population of 50 individuals. After each compound had been docked, they were energy-minimized into the active site of the enzyme.

RESULTS AND DISCUSSION

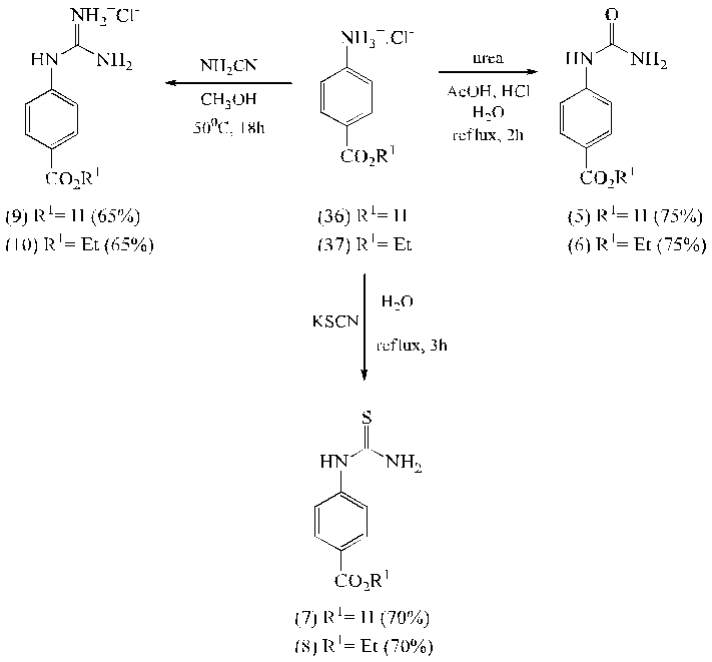
All the substances synthesized in this work were proposed based in prototypes that showed high affinity for the arginase enzyme (Han et al., 2002). The guanidine function (9-10) was modified biososterically, and the urea (5-6) and thiourea (7-8) functions were obtained. As the catalytic site of the arginase have a great number of hydrophilic aminoacids (Cox et al., 2001) bisguanidines (1-2) and amidineureas (3- 4) were selected for searching new possible sites of interaction with the enzyme and, as far, greater affinity. The compounds 11-29 have the 1,2,4-oxadiazole ring in their structure that can act as a pro-drug for the amidine group (Kitamura et al., 2001) and is also described as important for the leishmanicidal activity in compounds such as pentamidine.

Synthesis

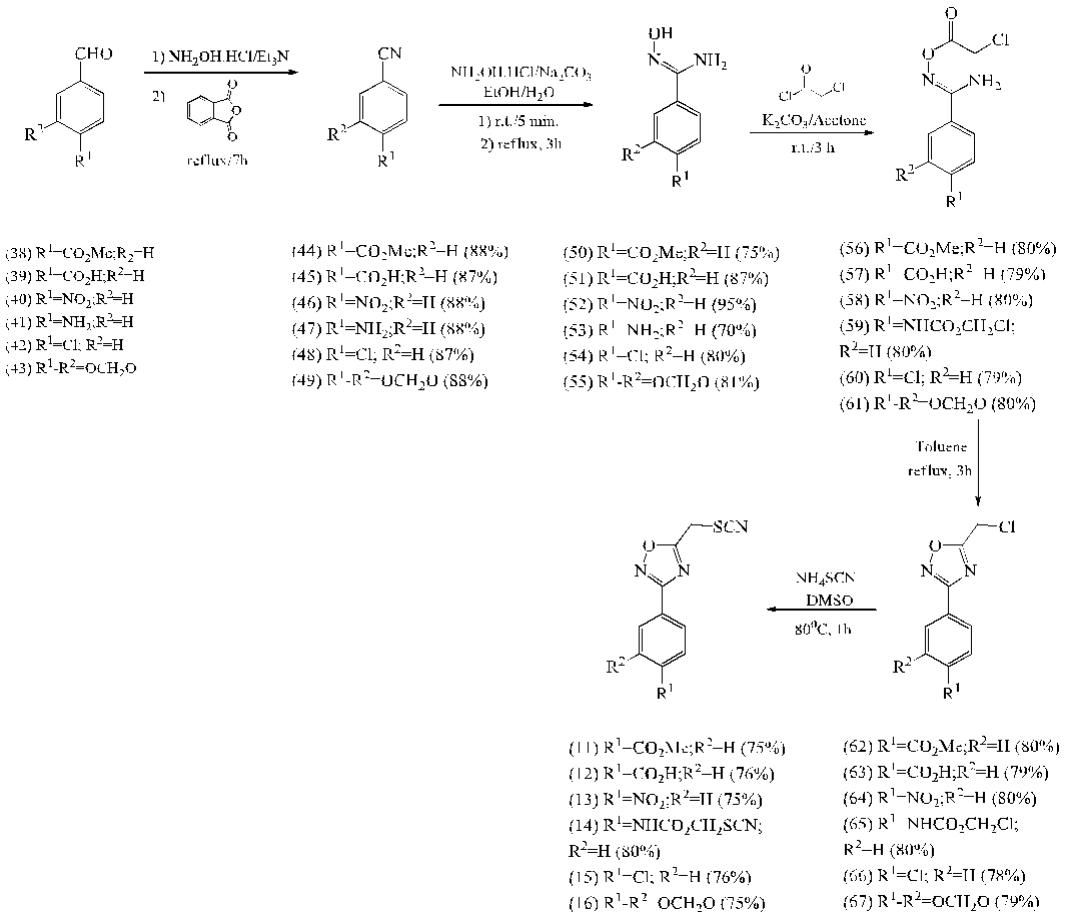
The bisguanidine compounds (1-2) were obtained by the reaction of salts of 4-aminobenzoic acid or the respective esters (Pierce et al., 1998) with cyanoguanidine in water under reflux (Urbanski et al., 1967). Amidineurea derivatives (3-4) were also obtained as hydrochloride salts from the reaction with the corresponding amines (35) (Pierce et al., 1998; Urbanski et al., 1967) (Scheme 1).

To obtain the derivatives 5-6, the reaction was carried out using the corresponding amine salts with urea, acetic acid and hydrochloric acid in water under reflux for two hours (Rabjohn, 1963) (Scheme 2). Compounds 7-8 were obtained from the reaction of the corresponding amine salts with potassium thiocyanate in water, leading to products in good yields (Lange et al., 1926). The synthesis of hydrochloride guanidine derivatives 9-10 were performed by reaction of amine salts formed in the previous step (36, 37), with cyanamide in methanol at 50°C (Nakayama et al., 1993) (Scheme 2).

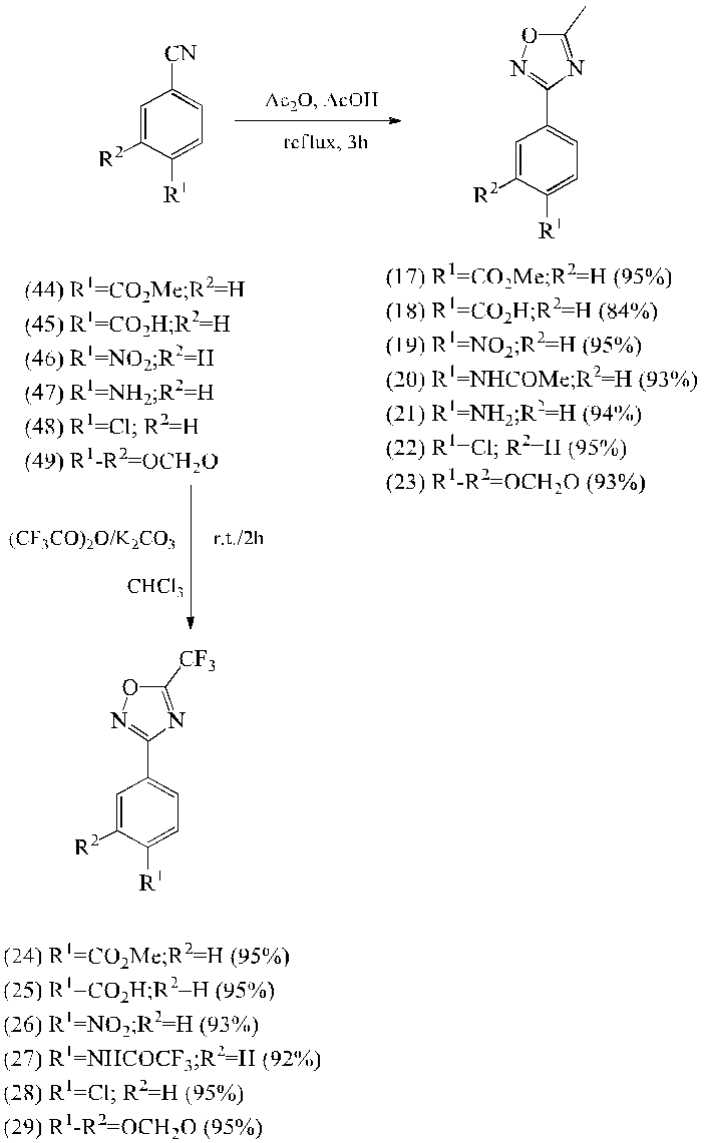
The series of compounds 11-16 comprises substances that contain the 1,2,4-oxadiazole nucleus. This heterocyclic nucleus has been extensively described as a bioisostere of ester and amide functional groups (Quan et al., 2004; Poulain et al., 2001). Additionally, this heterocyclic acts as a pro-drug of the amidine group (Kitamura et al., 2001). The synthesis of these compounds is shown in Scheme 3. The first reaction step is the formation of nitriles using the methodology described by Wang and coworkers (Wang et al., 1998). The next step produces the amidoxime (50-55) (Judkins



Scheme 2. Synthetic route used to obtain the compounds 5-10.



Scheme 3. Synthetic route used for obtaining compounds 11-16.



Scheme 4. Synthetic route used to obtain the compounds 17-29.

et al., 1996), followed by *O*-acylation and cyclization, to obtain the 1,2,4-oxadiazole ring (62-67) (Palazzo et al., 1961). Thiocyanate derivatives were obtained from the chlorides by a nucleophilic substitution reaction (Haugwitz et al., 1985) (Scheme 3).

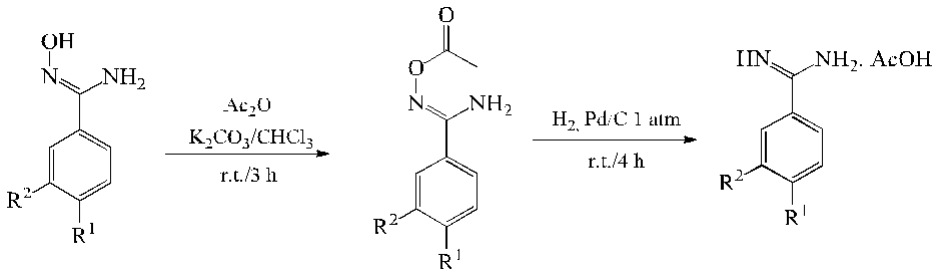
Although compounds 17-29 were derived from 1,2,4-oxadiazoles, the methodology employed for their synthesis was an one-pot acylation and cyclization, which is faster and more efficient than conventional methods, and gives products in high yields and purities (Bergmann et al., 1953; Cottrell et al., 2004) (Scheme 4).

The synthesis of amidine compounds 30-34 was accomplished by the reduction of *O*-acetylated derivatives 68-72 in two steps, using a methodology developed by Judkins and coworkers (1980). This methodology allows the obtention of the amidines in the salt form and uses conditions milder than the direct reduction of the amidoxime; once the N-O bound is easier to be hydrogenated when oxygen is connected to an electron withdrawing group (Scheme 5).

It is noteworthy that despite the many methodologies to obtain the 34 simple compounds that are already described in the literature, we obtained eight new compounds (4, 14, 16, 20, 23, 27-29).

Biological evaluation

The results of the biological evaluation are summarized in Table 1. Pentamidine was used as a standard. The



(50) R^1−CO_2Me;R^2−H (68) R^1−CO_2Me;R^2−H (92%) (30) R^1−CO_2Me;R^2−H (75%)
(52) R^1=NO_2;R^2=H (69) R^1=NO_2;R^2=H (95%) (31) R^1=NO_2;R^2=H (75%)
(53) R^1=NH_2;R^2=H (70) R^1=NH_2;R^2=H (95%) (32) R^1=NH_2;R^2=H (74%)
(54) R^1=Cl; R^2=H (71) R^1=Cl; R^2=H (93%) (33) R^1=Cl; R^2=H (74%)
(55) R^1-R^2=OCH_2O (72) R^1-R^2=OCH_2O (94%) (34) R^1-R^2=OCH_2O (75%)

Scheme 5. Synthetic route for obtaining compounds 30-34.

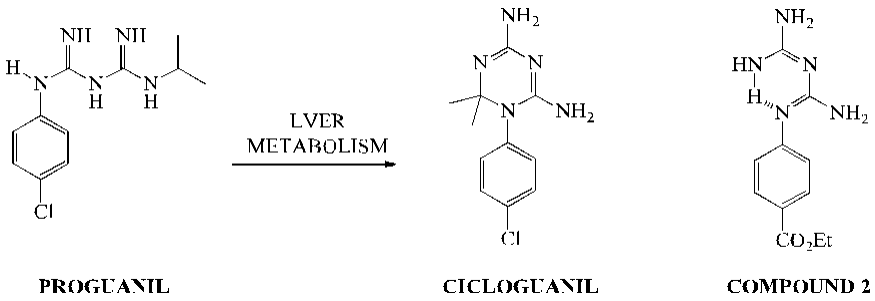
Table 1. Results of *in vitro* activity against *L.amazonensis.*

Compound	IC_{50} (µM)	Compound	IC_{50} (µM)
Pentamidine	0.46	18	784
1	156	19	1561
2	70	20	1474
3	155	21	1828
4	1119	22	1649
5	889	23	1569
6	769	24	1176
7	408	25	1240
8	45	26	ND
9	186	27	985
10	165	28	1290
11	1164	29	1240
12	1226	30	1798
13	1221	31	30
14	922	32	113
15	ND	33	519
16	1226	34	244
17	1468		

method applied in this evaluation has already been successfully used in other studies previously described (Ferreira et al., 2007).

From analysis of the results, all compounds are less active than pentamidine. However, three of them have reasonable activity. Compound 8, 2 and 31 showed IC_{50} of 45, 70 and 30 µM, respectively. Compound 2 is a structural analogue of proguanil, a drug used to treat malaria (Lalloo et al., 2007). Proguanil suffers metabolic bioactivation and its active metabolite cicloguanil is related to compound 2. Although there is no possibility that compound 2 will generate a second cyclized compound, it may present a similar conformation and tautomeric forms of the structure cicloguanil, as pointed out by Bharatam et al. (2005) (Scheme 6).

The amine 31 was the substance that showed the most potent activity against *Leishmania*. The nitro group in 31 seems to have a crucial role in the activity because the other compounds were less active (32-34) or inactive (30). The literature reports a number of compounds that are structurally similar to amidine 31 and have high levels of arginase inhibition (Ferreira, 2004; Ferreira et al., 2007). Thus, there is the possibility that compound 31 inhibits arginase of the parasite; however, *in vitro* argi-



Scheme 6. Structural similarity between the cicloguanil and biguanidine 2.

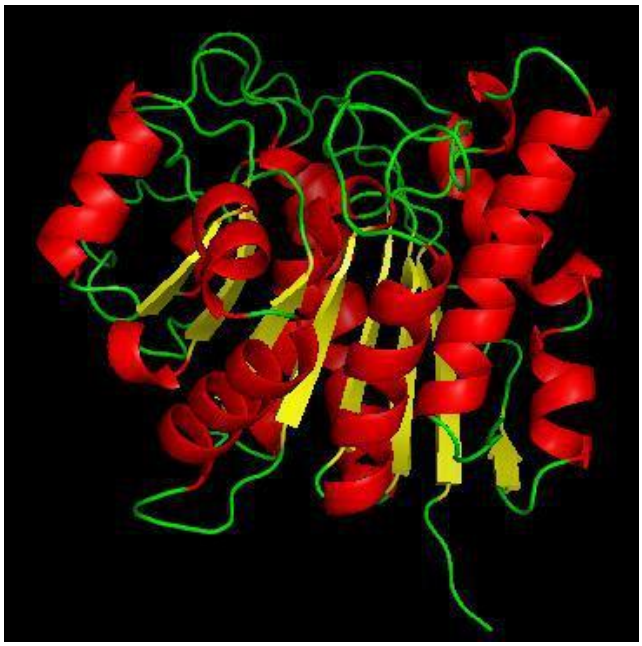


Figure 2. The constructed model for the arginase of *L. amazonensis.*

nase enzyme evaluation is necessary to ensure this possibility.

In general, the 5-aryl-3-substituted-1,2,4-oxadiazole compounds were less active or inactive independently on the substituent in position 3.

Compounds 2, 8 and 31 can be considered prototypes even though they are less active of pentamidine. Thus, their molecular structures can be modified through the introduction of other substituents in the aromatic ring that enable an improvement in the biological activity.

Molecular modeling

Comparative modeling of *arginase* of L. *amazonensis*

Structure-based approaches using the target 'receptor' structure are helpful in designing and developing more selective lead compounds. However, to date, no crystallographic structure of *Leishmania* spp*.* arginase exists (Boeckmann et al., 2003). Thus, to further investigate the possibility of this compounds to target the arginase enzyme, we constructed a theoretical model of *L. amazonensis* arginase (*La*_arginase) by using the comparative modeling paradigm (Berman et al., 2000; Marti-Renom et al., 2000). This enzyme model is shown in Figure 2. The main structural elements of the model include eight strand-parallel β-sheets, eleven α-helices and several extended loops. Because disruptions are found within several helices, some of them are not continuous.

The target sequence (*L. amazonensis* arginase) was obtained from the TrEMBL data base (Santos-Filho et al., 2003) (code TrEMBL*:* 096394), and the template

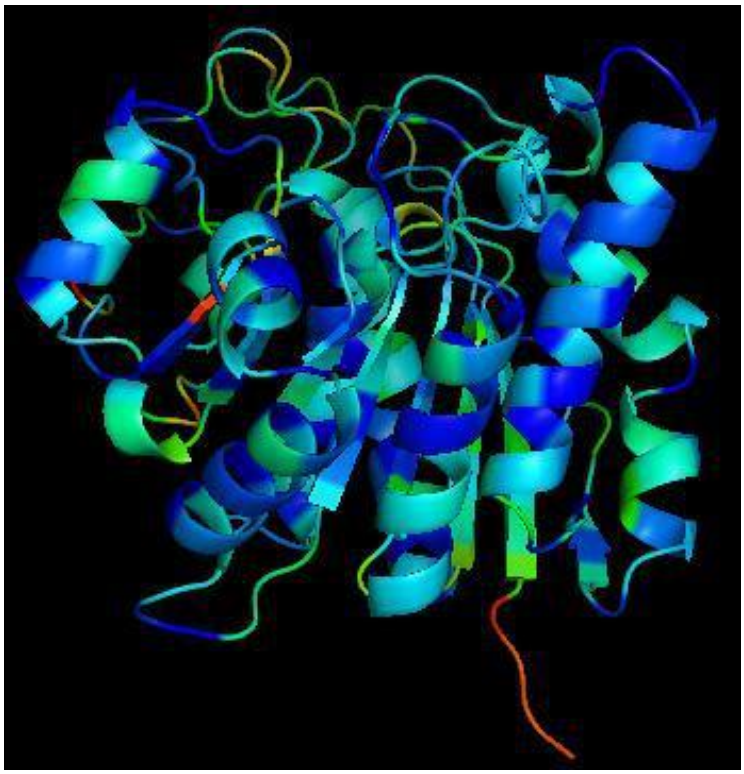


Figure 3. B-factor constructed model for the arginase of *L. amazonensis.*

enzymes used for modeling its 3D-structure were obtained from Protein Data Bank (PDB) (Berman et al., 2000). After submitting that sequence to the basic local alignment search tool (BLAST) (Altschul et al., 1990), six enzymes were selected from PDB, with a similarity toward the target enzyme ranging from 49.6 to 50.7%. The corresponding PDB codes of the enzymes are 3E6V, 1WVA, 1T4S, 1HQX, 1RLA and 2RLA (Cama et al., 2004; Di Costanzo et al., 2005; Kanyo et al., 1996; Lavulo et al., 2001; Scolnick et al., 1997; Shishova et al., 2009) with similarity to the target sequence of 53.4, 52.8, 50.7, 50.7, 50.4 and 49.6%, respectively.

In order to check the quality of the constructed enzyme model, it was submitted to validation analysis. First, the dynamics of the model were verified. Thus, the B-factor (atomic displacement parameter) (Debye, 1914; Waller et al., 1923), which reflects the fluctuation of the system on its average position, was calculated (Figure 3). From the B-factor analysis, one can infer that the model for arginase of *L. amazonensis* is thermally stable because its structure shows “cold-temperature” (blue) color deviation.

The enzyme model was stereochemically evaluated by submitting it to the PROCHECK analysis (Laskowski et al., 1993). Figures 4 and 5 show the Ramachandran plot and the main-chain parameters for the model, respectively. The Ramachandran plot of the constructed enzyme model shows that 97.8% of the amino acid residues are in favorable regions. For the main-chain parameter (Figure 5), no problems were found in relation to poor contact, C_α tetrahedron distortion, any hydrogen-bond and energy-bond. Moreover, the average G-factor (Laskowski et al., 1993), which provides the normality degree of the protein’s properties is inside the permitted value. It is noteworthy that the structural parameters of the active site residues are in statistically favorable regions. Moreover, none of them showed geometric, conformational or energetic problems.

Docking calculation

Table 2 shows docking score values for all synthesized compounds in kcal/mol, and Figure 6 shows details of the docked compounds into the binding pocket of arginase of *L. amazonensis*.

According to Table 2 and Figure 6, the synthesized molecules could work as promising inhibitors for *L. amazonensis* arginase. The molecules showed that they can be very well accommodated into the active site of the enzyme. However, is important to mention that no strong correlation between the bioassays and the docking scores were observed in this study. This is because the biological evaluations were conducted in the parasite and molecular docking simulations in the enzyme arginase. Thus, the molecules are sent to the bioassays in the

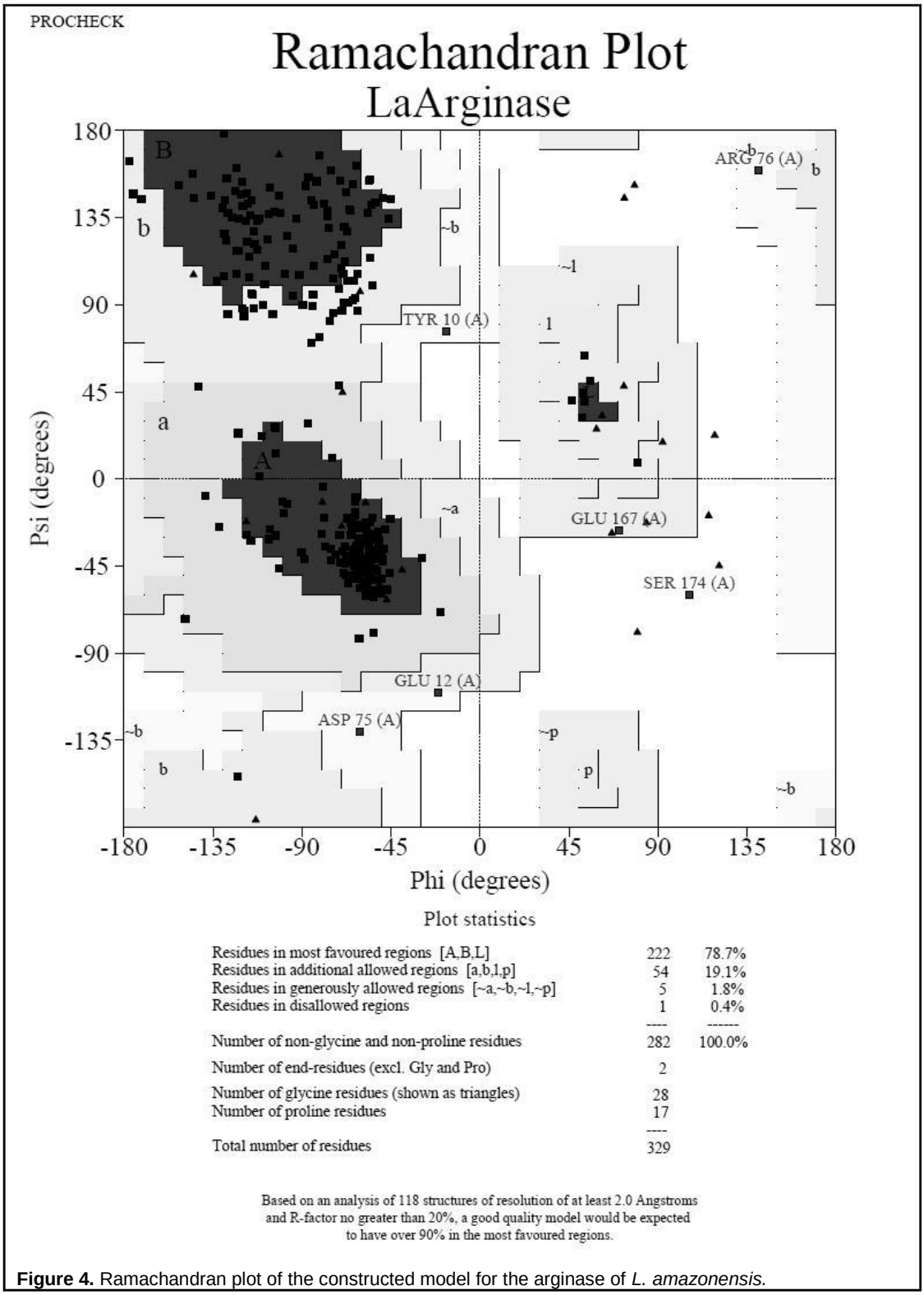


Figure 4. Ramachandran plot of the constructed model for the arginase of *L. amazonensis.*

arginase enzyme in order to establish a correlation between biological assays and molecular modeling. This correlation is crucial for structural changes in molecules that may be proposed to improve their leishmanicidal profile. In conclusion, we are proposing the investigation in subsequent works for the use of those molecules as

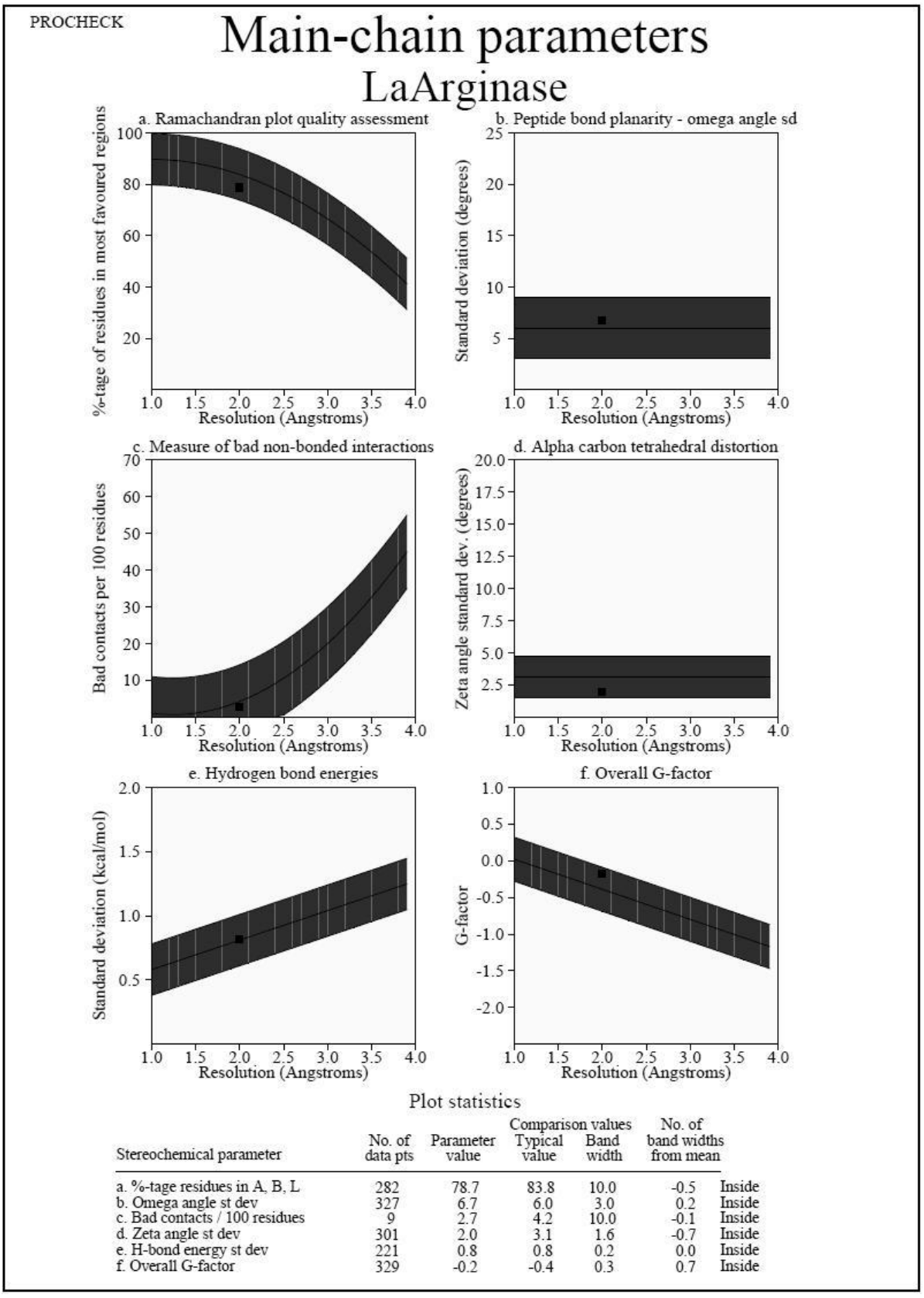


Figure 5. Main-chain parameters for the constructed model for the arginase of *L. amazonensis.* The black square represents the modeled enzyme.

Table 2. Docking score values for all synthesized compounds in kcal/mol.

Compound	Docking score (kcal/mol)	Compound	Docking Score (kcal/mol)	Compound	Docking score (kcal/mol)
4	-98.07	14	-86.71	21	-73.07
16	-94.52	28	-85.60	5	-71.24
29	-93.17	6	-85.27	19	-70.77
25	-92.07	8	-84.71	9	-69.53
26	-91.16	20	-84.36	22	-68.04
3	-90.70	23	-83.99	34	-66.77
1	-90.20	18	-79.65	32	-63.29
2	-89.13	17	-79.23	31	-58.01
12	-88.73	10	-78.57	33	-54.96
24	-87.33	11	-78.44		
27	-87.22	7	-73.47		

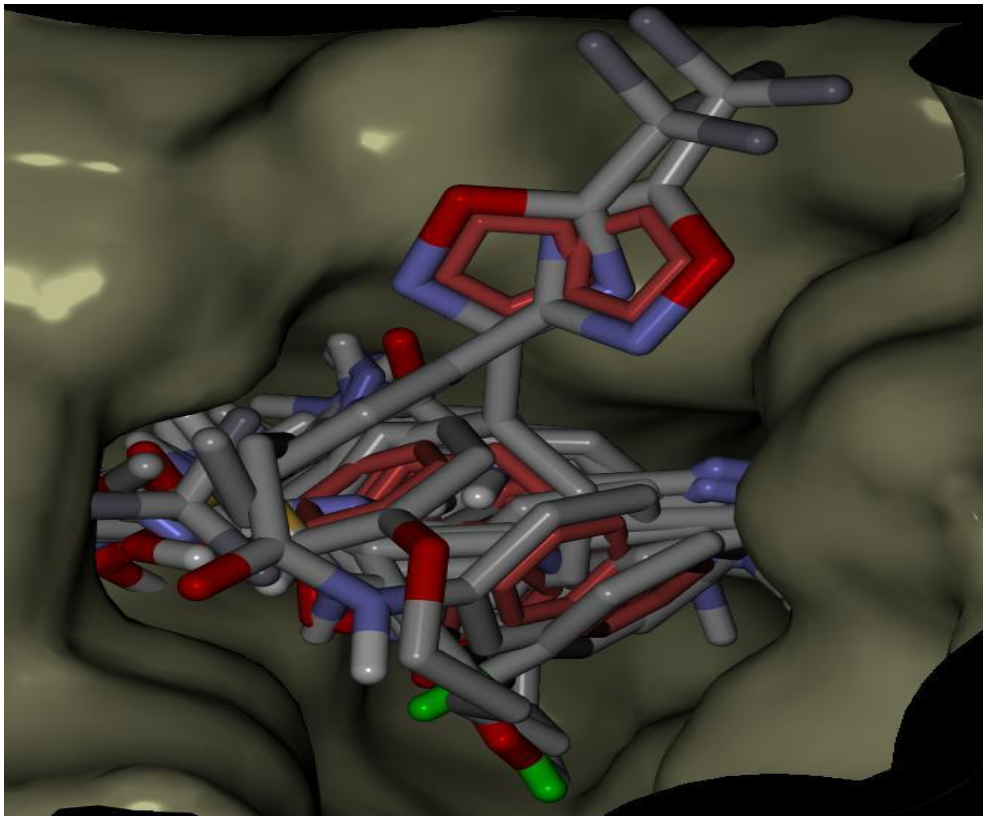


Figure 6. Detail of the docked compounds into the binding pocket of *L. amazonensis* arginase. Green, red and blue/orange residues are the hydrophobic, charged and polar ones.

potential new lead for designing new inhibitors of arginase from *Leishmania* spp.

Conclusions

Leishmaniasis is considered a neglected disease by the World Health Organization (WHO) and was recognized as one of the most neglected tropical diseases in the 60's. The search for new targets for its therapy led us to the synthesis of simple molecules of different chemical classes. The selection of these substances was supported by the literature descriptions that indicated probable leishmanicidal activity as arginase inhibitors. Additionally, the obtained molecules were prepared in a

simple synthetic methodology and, consequently, may be economically viable for use in poor or developing countries. Thirty-four (34) compounds from different chemical classes were synthesized, eight of which had no report in the literature (4, 14, 16, 20, 23 and 27-29). The biological evaluation of the compounds showed that among the 34 compounds synthesized, 19 were active, but only three presented moderate leishmanicidal activity. However, all were less actives than pentamidine. Thus, we can conclude that thiourea 8, bisguanidine 2 and amidine 31 can be considered as future prototypes for the development of more active compounds.

From their structures, we can propose rational molecular changes. The synthesized compounds in Figure 6 that were shown to be potential good inhibitors for the enzyme arginase of *L. amazonensis* were docked into the active site of the constructed model for *L. amazonensis* arginase study. The molecular docking approach was then applied, and the results are shown in Figure 6. Although no correlation was found between the docking, biological evaluation of the compounds can act to inhibit arginase because it settle well in the catalytic site of the enzyme.

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REFERENCES

Alavi-Naini R (2008). Topical morphine for the treatment of cutaneous leishmaniasis Med. Hyp. 70:81-84.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990). Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Alvar J, Aparicio P, Aseffa A, Boer MD, Cañavate C, Dedet JP, Gradoni L, Horst RT, López-Vélez R, Moreno J (2008). The relationship between Leishmaniasis and AIDS: the second 10 years. Clin. Microbiol. Rev. 21:334-359.

Ashford R W (2000). The leishmaniases as emerging and reemerging zoonoses. Int. J. Parasitol. 30:1269-1281.

Avlonitis MR, Lekka E, Detsi A, Koufaki M, Calogeropoulou T, Scoulica E, Siapi E, Kyrikou I, Mavromoustakos T, Tsotins A, Grdadolnik SG, Makriyannis A (2003). Antileishmanial Ring-Substituted Ether Phospholipids. J. Med. Chem. 46:755-767.

Bergmann ED, Bendas H, D' Avilla U (1953). New substances of possible chemotherapeutical value. J. Org. Chem. 18:64-69.

Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissing IN, Shindyalov PE (2000). The Protein Data Bank. Nucleic acids. Res. 28:235-242. (www.pdb.org).

Bharatam PV, Patel DS, Iqbal P (2005). Pharmacophoric features of biguanidine derivatives: an electronic and structural analysis. J. Med. Chem. 48:7615-7622.

Boeckmann B, Bairoch A, Apweiler R, Blatter MC, Estreicher A, Gasteiger E, Martin MJ, Michoud K, O'Donovan C, Phan I, Pilbout S, Schneider M (2003). The SWISS-PROT protein knowledgebase and its supplement TrEMBL in 2003. Nucleic Acids Res. 31:365-370.

Cama E, Pethe S, Boucher JL, Han S, Emig FA, Ash DE, Viola RE, Mansuy D, Christianson, DW (2004). Inhibitor coordination interactions in the binuclear manganese cluster of arginase. Biochem. 43:8987-8999.

Canto-Cavalheiro MM, Echevarria A, Araújo CA, Bravo MF, Santos LH, Jansen AM, Leon LL (1997). The potential effects of new synthetic drugs against *Leishmania amazonensis* and *Trypanosoma cruzi.* Microbios. 90:51-60.

Cottrell D, Capers J, Salem M, Deluca-Fradley D, Croft S, Werbovetz S (2004). Antikinetoplastid activity of 3-aryl-5-thiocyanatomethyl-1,2,4-oxadiazoles. Bioorg. Med. Chem. 12:2815-2824.

Cox JD, Cama E, Collelouri DM, Pethe S, Boucher JL, Mansuy D, Ash DE, Christianson DW (2001). Mechanistic and metabolic interferences from the binding of substrates analogues and products to arginase. Biochem. 40:2689-2701.

Croft SL, Coombs GH (2003). Leishmaniasis- current chemotherapy and recent advances in the search for novel drugs. Trends Parasitol. 19:502-508.

Cysne-Finkelstein L, Aguiar-Alves F, Temporal RM, Leon LL (1998). *Leishmania amazonensis*: long-term cultivation of axenic amastigotes is associated to metacyclogenesis of promastigotas. Exp. Parasitol. 89:58-62.

Debye P (1914). Interference of R.ovrddot.ontgen rays and heat motion. Ann. Phys. 43:49-95.

Denizot F, Lang R (1986). The potential effects of new synthetic drugs against Leishmania amazonensis and Trypanosoma cruzi. J. Immunol. Methods 89:271-277.

Di Costanzo L, Sabio G, Mora A, Rodriguez PC, Ochoa AC, Centeno F, Christianson DW (2005). Crystal structure of human arginase I at 1.29-Å resolution and exploration of inhibition in the immune response. Proc. Natl. Acad. Sci. 102:13058-13063.

Dujardin JC, Campino L, Canavate C, Jean-Pierre D, Luigi G, Ketty S, Apostolos M, Yusuf O, Marleen B (2008). Spread of vector-borne diseases and neglect of Leishmaniasis, Europe. Emerg. Infect. Dis. 14:1013-1018.

Ferreira SB (2004). Síntese de novos *Gem*-difluormetil imidazóis com potencial atividade anti-tripanossomatídeos. Tese de Mestrado. Instituto de Química, Universidade Federal do Rio de Janeiro.

Ferreira SB, Costa MS, Boechat N, Bezerra RJS, Genestra MS, Canto-Cavalheiro MM, Kover WB, Ferreira VF (2007). Synthesis and evaluation of new difluoromethyl azoles as antileishmanial agents. Eur. J. Med. Chem. 42:1388-1395.

Guerin PJ, Olliaro P, Sundar S, Boelaert M, Croft SL, Desjeux P, Wasunna MK, Bryceson ADM (2002). Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda. Lancet Infect. Dis. 2:494-501.

Han S, Moore RA, Viola RE (2002). Synthesis and evaluation of alternative substrates for arginase. Bioorg. Chem. 30:81-94.

Haugwitz RD, Martinez AJ, Venslavsky J, Angel RG, Maurer BV, Jacobs GA, Narayanan VL, Cruthers LR, Szanto J (1985). Antiparasitic agents. 6. Synthesis and anthelmintic activities of novel isothiocyanatophenyl-1,2,4-oxadiazoles. J. Med. Chem. 28:1234-1241.

Iniesta V, Gómez-Nieto LC, Corraliza I (2001). The inhibition of arginase by N(omega)-hydroxy-l-arginine controls the growth of Leishmania inside macrophages. J. Exp. Med. 193:777-784.

Judkins BD, Allen DG, Cook TA, Evans B, Sardharwala TE (1996). A versatile synthesis of amidines from nitriles via amidoximes. Synth. Commun. 26:4351-4367.

Kanyo ZF, Scolnick LR, Ash DE, Christianson DW (1996). Structure of a unique binuclear manganese cluster in arginase. Nat. 383:554-557.

Kitamura S, Fukushi H, Miyawaki T, Kawamura M, Terashita Z, Naka T (2001). Orally active GPIIb/IIIa antagonists: synthesis and biological activities of masked amidines as prodrugs of 2-[(3S)-4-[(2S)-2-(4-amidinobenzoylamino)-3-(4-methoxyphenyl) propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic acid. Chem. Pharm. Bull. 49:268-277.

Lalloo DG, Shingadia D, Pasvol G, Chiodini PL, Whitty CJ, Beeching NJ, Hill DR, Warrell DA, Bannister BA (2007). Malaria treatment guidelines. J. Infect. 54:111-121.

Lange NA, Reed WR (1926). Some p-phenoxyureas and thioureas derived from p-phenoxyaniline. The effect of the phenoxy group on the taste. J. Am. Chem. Soc. 48:1069-1074.

Laskowski RA, MacArthur MW, Moss DS, Thornthon JM (1993). Procheck: a program to check the stereochemical quality of protein

structures. J. Appl. Cryst. 26:283-291.
Lavulo LT, Sossong Jr TM, Brigham-Burke MR, Doyle ML, Cox JD, Christianson DW, Ash DE (2001). Subunit-subunit interactions in trimeric arginase. Generation of active monomers by mutation of a single amino acid. J. Biol. Chem. 276:14242-14248.
Marti-Renom MA, Stuart AC, Fiser A, Sanchez R, Melo F, Sali A (2000). Comparative protein structure modeling of genes and genomes. Annu. Rev. Biophys. Biomol. Struct. 29:291-325.
Molegro VD (2012). Moellers Alle 8, Building 1110, DK-8000 Aarhus C, Denmark.
Nakayama T, Taira S, Ikeda M, Ashizawa H, Oda M, Arakawa K, Fujii S (1993). Synthesis and structure-activity study of protease inhibitors. V. Chemical modification of 6-amidino-2-naphthyl 4-guanidinobenzoate. Chem. Pharm. Bull. 41:117-125.
Palazzo G, Tavella M, Strain G, Silvestrini B (1961). 1,2,4-Oxadiazoles. IV. Synthesis and pharmacological properties of a series of substituted aminoalkyl-1,2,4-oxadiazoles. J. Med. Pharm. Chem. 4:351-367.
Pierce ME, Parsons JR, Radesca LA, Lo YS, Silverman S, Moore JR, Islam Q, Choudhury A, Fortunak JMD, Nguyen D, Luo C, Morgan SL, Davis WP, Confalone PN, Chen CY, Tiller RD, Frey L, Tan L, Xu F, Zhao D, Thompson AS, Corley EG, Grabowski EJJ, Reamer R, Reider PJ (1998). Practical Asymmetric Synthesis of Efavirenz (DMP 266), an HIV-1 Reverse Transcriptase Inhibitor. J. Org. Chem. 63:8536-8543.
Poulain RF, Tartar AL, Déprez BP (2001). Parallel synthesis of 1,2,4-oxadiazoles from carboxylic acids using an improved, uronium-based, activation. Tetrahedron Lett. 42:1495-1498.
Quan C, Kurth M (2004). Solid-Phase Synthesis of 5-Isoxazol-4-yl-[1,2,4]oxadiazoles. J. Org. Chem. 69:1470-1474.
Rabjohn N (1963). Organic Syntheses Collection. John Wiley & Sons Inc., New York, Vol. 4.
Roberts SC, Tancer MJ, Polinsky MR, Gibson KM, Heby O, Ullman B (2004). Arginase Plays a Pivotal Role in Polyamine Precursor Metabolism in Leishmania: characterization of gene deletion mutants. J. Biol. Chem. 279:23668-23678.
Santos-Filho AO, Alencastro RB (2003). Protein homology modeling. Quim. Nova 26:253-259.
Scolnick LR, Kanyo ZF, Cavalli RC, Ash DE, Christianson DW (1997). Altering the binuclear manganese cluster of arginase diminishes thermostability and catalytic function. Biochem. 36:10558-10565.
Shishova EY, Di Costanzo L, Emig FA, Ash DE, Christianson DW (2009). Probing the specificity determinants of amino acid recognition by arginase. Biochem. 48:121-131.
Silva ER, Castilho TM, Pioker FC, Tomich CHPS, Floeter-Winter LM (2002). Genomic organisation and transcription characterisation of the gene encoding Leishmania (Leishmania) amazonensis arginase and its protein structure prediction. Intern. J. Parasitol. 32:727-737.
Tripos S (2012). 1699 South Hanley Road, St. Louis, MO 63144-2319, USA.
Urbanski T, Serafin B, Zylowski J (1967). Potential antimalarial compounds. IX. Pyrimidine derivatives of urea and guanidine. J. Med. Chem. 10:521-525.
Waller I (1923). The question of the influence of heat motion on the scattering of R.ovrddot.ontgen rays. Zeitschrift für Physik 17:398-408.
Wanasen N, Soong L (2008). L-arginine metabolism and its impact on host immunity, against *Leishmania* infection. Immunol. Res. 41:15-25.
Wang EC, Lin GJ (1998). A new one pot method for the conversion of aldehydes into nitriles using hydroxylamine and phthalic anhydride. Tetrahedron Lett. 39:4047-4050.
World Health Organization (WHO) - http://whqlibdoc.who.int/publications/2010/9789241564090_eng.pdf, accessed in January 2013.