Synthesis, Cytotoxicity and Antileishmanial Activity of Aza-stilbene derivatives

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Abstract: Stilbenes are compounds found in numerous medicinal plants and food products with some known biological and even antileishmanial activity. This paper describes the preparation of Aza-stilbene derivatives and their in vitro biological activities against Leishmania species. Most of the compounds with hydroxyl groups (2a, 2b, 2d, 2e and 2f) showed interesting results against three Leishmania species tested. Compound 2f showed the best activity against intracellular forms of L. amazonensis, with IC₅₀ of 7.48 µM, very similar when compared to reference drug Miltefosine. It not possible associate NO production with leishmanicidal activity for all aza-stilbene derivatives. It is noteworthy that none of compounds tested showed cytotoxicity against macrophages.

Keywords: Aza-stilbene derivatives; stilbenes; antileishmanial activity; amastigote forms.

Introduction

Infectious and parasitic diseases are very common in tropical countries and affect the poor population, often below the poverty line. They account for 11% of diseases worldwide, according to WHO data; these diseases are the cause of death of more than 14 million people a year, affecting more than one billion, which represents about one sixth of world population. Among these diseases, of the largest numbers of deaths, morbidity, and cause greater socio-economic impact for developing countries, that’s including leishmaniasis ¹⁻³.

Leishmaniasis is caused by more than 20 protozoa species that belong genus Leishmania, transmitted by about 30 phlebotominae sandfly species. This disease is included as Neglected Tropical Diseases (NTDs), causing large political, economic and social impact³. According to WHO estimations, leishmaniasis is endemic in 98 countries with incidence of 2 million new cases per year and 350 million people living in areas at risk worldwide³⁻⁴. Depending on the parasite species, clinical manifestations may comprises a wide disease spectrum ranging

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localized cutaneous lesions to mucocutaneous form can lead to disfigurement beyond the visceral form which can be fatal if left untreated\(^3\).

Current treatment of leishmaniasis is based on pentavalent antimonials, particularly the pentavalent compound (sodium stibogluconate and meglumine antimoniate), and play a fundamental role in the therapy world for over 70 years\(^1,4,5\). However, they are toxic and currently have known strong resistance, especially in India. There are other alternatives such as amphotericin B, pentamidine, but both have also high toxicity, parenteral route of administration, developing drug resistance and inadequate efficacy\(^4,6\). Miltefosine, the first oral drug, was initially used for the treatment the cancer and have been used in treatment of leishmaniasis, but has high cost, is teratogenic and appearance of resistance strain, thus not recommended for pregnant women\(^4-6\).

Natural stilbenes such as resveratrol, piceatannol, pinosilvin, pterostilbene, pinosilvin monoethyl ether and astringin (Figure 1) are compounds found in many plants (vines and pines) and also in various foods (peanuts and red grapes)\(^7\). The most important natural stilbene is resveratrol (3,5,4’-trihydroxystilbene). This compound is a secondary metabolite produced by roughly 70 species of plants and was found in many natural foods such as grapes, red wine and grape juice\(^8\). Resveratrol was first isolated from the roots of White hellebore (Veratrum album) in 1940 in Japan, and later was found in medicinal plants traditionally used in China and Japan\(^9\). Initially characterized as a phytoalexin, has achieved renown in the scientific literature in 1992 when it was postulated as responsible for the cardiac protective effects of wine (an effect called "French paradox")\(^10,11\). Furthermore, several studies have shown that resveratrol can prevent or inhibit the progression of a variety of diseases such as cancer, cardiovascular disease, ischemic damage and other\(^8,9,12\). Recent studies suggest that resveratrol has parasitic activities, including effect against protozoan as Leishmania sp and Trichomonas vaginalis\(^13,14\).

Figure 1. Structures of natural stilbenes occurrence in higher plants. Gly = β-D-glucopyranoside.

Despite advances in research and development in the use of stilbenes, some studies have been showed that stilbenes being well absorbed by the body, these molecules have low bioavailability, which is a limiting factor for their widespread use as medicine\(^8,9\). This fact
justifies the importance of searching for molecules similar to stilbenes that retain activity, but with better bioavailability. In recent work our research group proposes the use of bioisoster\textsuperscript{15} (Figure 2) to obtain analogues of stilbenes, more specifically aza-stilbenes obtained by the classical reaction of formation of imines\textsuperscript{16,17}.

![Figure 2. Bioisosterism tool utilized for development of new derivates.](image)

**Experimental Section**

**Antileishmanial activity**

Antipromastigote activity: three *Leishmania* species were used: *L. amazonensis* (IFLA/Br/67/PH8), *L. major* (MRHO/SU/59P) and *L. braziliensis* (MHOM/Br/75/M2903). Antileishmanial activity of compounds was initially established in promastigotes. Briefly, log-phase promastigotes of *Leishmania* were incubated with or without compounds at various concentrations in 96-well tissue culture plates for 72 h at 24°C and cell viability was assessed using MTT colorimetric method, as described previously\textsuperscript{18,19}. Miltefosine was used as positive control. Controls containing 0.5% DMSO and medium alone were also included. Results were expressed as the concentration inhibited 50% of cell growth (IC\textsubscript{50}).

Antiamastigote activity: peritoneal macrophages were obtained from BALB/c mice previously inoculated with 3% thioglycolate medium. Adherent macrophages (2x10\textsuperscript{5} cells per well) were infected with stationary-phase promastigotes *Leishmania* and incubated for 3 h at 33°C in 5% CO\textsubscript{2}. Non-phagocytosed promastigotes were removed by washing and infected macrophages were incubated with compounds (100.0, 50.0, 25.0, 12.5 and 6.25 μM) for 72 h at 33°C in 5% CO\textsubscript{2}. Cells were fixed and stained with Giemsa for parasite counting and examined microscopically for intracellular amastigotes. Results were expressed the concentration inhibited 50% of cell growth (IC\textsubscript{50}). Index of infection was obtained by multiplying the percentage of infected cells by number of amastigotes and dividing by number total cells (infected and non-infected). Miltefosine was used as the reference drug.

**Cytotoxicity assay against mammalian cells**

Peritoneal macrophages from mice BALB/c were used for cytotoxicity assay. The cells were incubated in different concentrations of the compounds and cell viability was determined with the MTT assay and was confirmed by comparing the control group morphology via light microscopy.

**Nitric oxide (NO) production**

NO production was determined in an aliquota (50 μL) of the supernatants of *Leishmania*-macrophages after 48 h in the presence of the compounds. Assay was performed as described by Green et al. (1982)\textsuperscript{20} and as described previously\textsuperscript{19}. Absorbance was measured at 540 nm
using a microplate reader (Multiskan MS microplate reader, LabSystems Oy, Helsinki, Finland). Nitrite content was quantified by extrapolation from sodium nitrite standard curve in each experiment. All the assays were carried out in duplicate. Positive control: 10 μg/ml of lipopolysaccharide (LPS) from Escherichia coli J5 (Sigma-Aldrich).

**Statistical analysis**

For promastigote forms of Leishmania assay and cytotoxicity on macrophages, the IC\textsubscript{50} values were carried out at 5% significance level (\( p < 0.05 \), CI 95%), calculated using a nonlinear regression curve, by using GraFit Version 5 software (Erithacus Software, Horley, U.K). For amastigote Leishmania assays, the IC\textsubscript{50} values were calculated from the program Probit using methods of Lichtfield and Wilcoxon and the statistical analysis was performed with the program GraphPad Prism 4 (GraphPad Software, San Diego, CA). One-way ANOVA was applied to compare all the groups. To compare the control with each compound, concentration was applied Dunnett post-test. Differences were regarded as significant when \( p < 0.0001 \) (*** and \( p < 0.001 \) (**).

**Results and Discussion**

**Chemistry**

Formation of similar reaction was performed by condensation of aromatic amines (aniline, 4-hydroxyaniline, 1,2-phenylenediamine and 4-aminosalicylic acid) with aromatic aldehydes (benzaldehyde, \( p \)-anisaldehyde, \( p \)-hydroxybenzaldehyde, vanillin, salicylaldehyde, 3,4,5-trimethoxybenzaldehyde, 4-dimethylaminobenzaldehyde and 4-nitrobenzaldehyde), using EtOH or MeOH as solvent at room temperature as shown in Scheme 1.

Scheme 1. Synthetic pathway for Aza-stilbene derivatives.
Methods performed to characterize the synthesized compounds were $^1$H NMR, $^{13}$C, IR and melting points. These data are presented in Table 1 and are in agreement with the literature.\(^{21-34}\)

**Table 1.** Spectral data of aza-stilbene derivatives.

| Compound | $\delta$ C-H=N | $\delta$ C=N | $\bar{\nu}$ C=N | Melting Point | Yield (%) |
|----------|----------------|-------------|----------------|--------------|-----------|
| la       | 8.44           | 160.0       | 1602           | 89.2-90.7 (89.2-90.7)\(^{24}\) | 74.0      |
| lb       | 8.96           | 163.5       | 1614           | 50.7-51.4 (50.0-50.5)\(^{30}\) | 63.0      |
| lc       | 8.51           | 159.8       | 1602           | 61.4-62.1 (62.7-63.4)\(^{24}\) | 65.0      |
| ld       | 8.43           | 160.2       | 1622           | 53.1-54.2 (52.0-53.0)\(^{31}\) | 63.0      |
| le       | 7.58           | 159.9       | 1629           | 133.8-134.4 (135.0)\(^{32}\) | 87.0      |
| lf       | 8.39           | 159.9       | 1600           | 96.8-97.3 (97.0-98.0)\(^{33}\) | 72.0      |
| lg       | 8.80           | 158.8       | 1600           | 89.6-90.7 (90.0-90.5)\(^{24}\) | 75.0      |
| 2a       | 8.38           | 156.9       | 1610           | 182.7 (182.0-184.0)\(^{34}\) | 63.0      |
| 2b       | 8.43           | 160.2       | 1607           | 203.7 (203.0-205.0)\(^{34}\) | 62.0      |
| 2c       | 8.89           | 160.2       | 1616           | 141.4 (140.0)\(^{35}\) | 55.0      |
| 2d       | 8.51           | 161.5       | 1609           | 189.0 (187.0)\(^{36}\) | 67.0      |
| 2e       | 8.42           | 157.2       | 1606           | 197.0 (198.0)\(^{37}\) | 60.0      |
| 2f       | 8.51           | 157.0       | 1624           | 185.4         | 68.0      |
| 2g       | 8.38           | 156.9       | 1610           | 182.7 (182-184)\(^{38}\) | 79.0      |
| 2h       | 8.73           | 157.3       | 1624           | 172.1 (168.5)\(^{39}\) | 76.0      |
| 3a       | 9.78           | 172.2       | 1602           | 182.4 (175.0-180.0)\(^{40}\) | 57.0      |
| 3b       | 10.26          | 172.0       | 1606           | 158.6 (156.0-158.0)\(^{41}\) | 73.0      |
| 3c       | 9.66           | 172.0       | 1605           | 190.0-191.5 (189.0-190.0)\(^{42}\) | 59.0      |

*NMR experiments were performed at 300 MHz for $^1$H and 75 MHz for $^{13}$C in DMSO-$d_6$ (ppm) and I.R. experiments was performed at KBr support (cm$^{-1}$). M.P. Data (°C) were compared to literature data which are given in parentheses.

Signals between $\delta$ 7.58-10.26 ppm ($^1$H NMR) refer to C-H imine it’s an indication of imine formation for the aza-stilbene derivative. Additionally the analysis of $^{13}$C NMR specter showed a signal at $\delta$ 157.0-172.2 ppm refferent at carbon C=N of imine.

**Biological**

In a preliminary screening, eighteen aza-stilbene derivatives were assayed against promastigote forms of *L. amazonensis, L. braziliensis* and *L. major*. Parasites were incubated with the compounds for 72 hours at 24°C in different concentrations. Results were expressed as the concentration inhibiting parasite growth by 50% (IC$_{50}$). Among the compounds tested, only compounds 2a, 2b, 2d, 2e and 2f showed activity against promastigote forms of *Leishmania* with IC$_{50}$ values ranging from 14.5 to 50.46 µM (Table 2).
Table 2. *In vitro* activities of the compounds against promastigote forms of *Leishmania* species and murine macrophages.

| Compounds | L. amazonensis | L. braziliensis | L. major | Macrophages |
|-----------|----------------|----------------|----------|-------------|
| 1a        | > 100.00       | > 100.00       | > 100.00 | > 100.00    |
| 1b        | > 100.00       | > 100.00       | 31.49±2.76 | > 100.00   |
| 1c        | > 100.00       | > 100.00       | > 100.00 | > 100.00    |
| 1d        | > 100.00       | > 100.00       | > 100.00 | > 100.00    |
| 1e        | > 100.00       | > 100.00       | > 100.00 | > 100.00    |
| 1f        | > 100.00       | > 100.00       | > 100.00 | > 100.00    |
| 1g        | > 100.00       | > 100.00       | > 100.00 | > 100.00    |
| 2a        | 16.77±0.47     | 17.22±0.15     | > 87.00  | > 100.00    |
| 2b        | 36.98±0.73     | > 87.00        | 20.27±2.98 | > 100.00   |
| 2c        | > 87.00        | > 87.00        | > 87.00  | > 100.00    |
| 2d        | 20.69±0.33     | 43.37±3.90     | 16.32±1.19 | > 100.00   |
| 2e        | 16.95±1.32     | 50.46±0.59     | 14.5±0.19 | > 100.00    |
| 2f        | 35.81±2.57     | 30.71±2.36     | > 87.00  | > 100.00    |
| 2g        | > 87.00        | > 87.00        | > 87.00  | > 100.00    |
| 2h        | > 87.00        | > 87.00        | > 87.00  | > 100.00    |
| 3a        | > 87.00        | > 87.00        | > 87.00  | > 100.00    |
| 3b        | > 87.00        | > 87.00        | > 87.00  | > 100.00    |
| 3c        | > 87.00        | > 87.00        | > 87.00  | > 100.00    |
| Miltefosine | 21.39±1.18     | 28.07±0.47     | 20.00±0.51 | > 100.00   |

*Data are IC$_{50}$ values in µM ± standard deviation. These data represents the average of 3 independent experiments.*

*Miltefosine was used as reference drug.*

In this work aza-stilbene derivatives were assayed against three main *Leishmania* species which affect humans and related to cutaneous manifestations: *L. braziliensis* and *L. amazonensis* are reported in Latin America and *L. major* is found in several countries of the Old World. In general, the compounds tested showed varying activity against all *Leishmania* species tested. This fact is not uncommon and has been reported by several authors$^{19, 35}$. However, it is interesting when a compound shows activity against several *Leishmania* species since this reinforces their leishmanicidal property. Regarding this, the compounds 2d and 2e which have methoxy group and methoxy group with hydroxyl in ortho-position, respectively, were active in all *Leishmania* species tested. Compound 2e showed also the best leishmanicidal activity with IC$_{50}$ of 14.5 µM against promastigotes of *L. major*. Furthermore, several compounds showed more active than to miltefosine, which was used as reference drug.

With respect the cytotoxicity of aza-stilbene derivatives on peritoneal macrophages, no compounds showed toxicity against these mammalian cells (Table 2). As can be observed all compounds tested showed IC$_{50}$ values > 100 µM. These results are very interesting because data from our laboratory has indicated that peritoneal macrophages are cells with strong sensibility for *in vitro* assays in comparing to immortal cell lines, as J744A1 macrophages$^{19}$. In view of the good results of the aza-stilbene against promatigotes, we decided to choose the series 2a, 2b, 2d, 2e and 2f to test in amastigotes, which is an intracellular parasite and are responsible for clinical manifestations in humans$^{36, 37}$. Table 3 shows the effect of these compounds against intracellular forms of *Leishmania* species. Compounds containing
hydroxyl grouping \(2a, 2b, 2d, 2e\) and \(2f\) showed a significant activity against amastigotes of \(L. amazonensis\) with IC\(_{50}\) values below of 25.0 µM. These results are consistent with the literature, since the some authors demonstrated leishmanicidal activity of stilbene analogs\(^{13}\). These stilbene analogs are poly-hydroxylated compounds, have C-4 hydroxyl in aromatic ring on their structures and all active compounds tested against \(Leishmania\) amastigotes showed superior results when compared to resveratrol\(^{13}\). Furthermore, analogs of resveratrol also exhibited antitumor, antioxidant, and anti-inflammatory activities\(^{38}\).

### Table 3. In vitro activities of the compounds against intracellular amastigotes of \(Leishmania\).

| Compostos  | \(L. amazonensis\)     | \(L. braziliensis\)  | \(L. major\)       |
|------------|-------------------------|----------------------|---------------------|
| \(2a\)     | 18.74 (13.55-25.90)     | 75.80 (49.41-116.30) | -                   |
| \(2b\)     | 24.35 (20.19-29.36)     | -                    | 29.18 (20.82-40.91) |
| \(2d\)     | 15.20 (9.67-23.87)      | 59.00 (38.44-90.55)  | 27.15 (20.69-35.63) |
| \(2e\)     | 21.72 (15.88-29.70)     | 70.30 (51.51-95.93)  | 39.11 (26.76-57.16) |
| \(2f\)     | 7.48 (4.36-12.85)       | 61.57 (35.31-107.37) | -                   |
| Miltefosine | 4.15 (2.89-5.96)        | 3.21 (2.26-4.61)     | 7.56 (6.35-8.98)    |

Peritoneal macrophages previously infected with \(Leishmania\) promastigotes in the stationary growth phase were exposed to the compounds for 72h. Results from two assays in duplicate are shown as IC\(_{50}\) values in µM.

\(^{a}\) CI: Confidence interval.

Compound \(2f\) showed the best activity against intracellular forms of \(L. amazonensis\), with IC\(_{50}\) of 7.48 µM (Table 3). This compound presents in its structure the largest degree of substitution on the aromatic rings, tetrasubstituted, three methoxyl groups, that possibly confer a greater cellular penetration. It is interesting to note that compound \(2f\) showed IC\(_{50}\) value near to IC\(_{50}\) of miltefosine (4.15 µM). Miltefosine is recommended to treat leishmaniasis in endemic countries with related resistance to antimonials and has been used for treatment of visceral leishmaniasis in India and Ethiopia, and cutaneous leishmaniasis in Colombia, Bolivia and Guatemala\(^{39}\).

Furthermore, figure 3 also furnishes information about leishmanicidal activity of the compound \(2f\) against amastigotes of \(L. amazonensis\). When the parasites were treated with this compound, a significant dose-dependent decrease of intracellular amastigotes was observed.

**Figure 3:** Effect of the compound \(2f\) on \(L. amazonensis\) interiorized in peritoneal macrophage cells. Statistically significant difference of control: **\(p < 0.001\), *\(p < 0.0001\).
Table 4 shows selectivity and specificity of compounds tested. Regarding this, selectivity furnishes an idea about the toxicity selective action on the compounds on macrophages and it is obtained by dividing IC$_{50}$ of macrophages by IC$_{50}$ of intracellular amastigote forms. In general, all compounds showed good selectivity, being more destructive for intracellular parasite than to the mammalian cells. In a special remark for the compound 2f which showed the best anti-amastigote activity, it was at least 13 times more toxic for amastigote forms of L. amazonensis than to murine macrophages. Specificity provides insight about the behavior of the compounds on the both parasite stages. Muyldier and coworkers$^{40}$ determined a cut-off values to evaluate the specificity of the compounds in amastigote or promastigote form of Leishmania: specificity value >2 define a compound as more active against the intracellular amastigote stage; while a specificity value <0.4 indicate a compound more active against promastigotes; compounds with specificity values between 0.4 and 2 were considered active against both stages$^{40}$. In accordance with this, our results showed that most of the compounds were active in both promastigote and amastigote stages, except the compound 2f which was more active against intracellular forms with specificity value of 4.79. Furthermore, the compound 2f has specificity value near that of miltefosine (4.79 and 5.15, respectively), indicating that the compound 2f and miltefosine exhibit similar activities to intracellular amastigotes of L. amazonensis (Table 4).

| Compounds | L. amazonensis | | | L. braziliensis | | | L. major | | |
|------------|----------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|            | SE$^a$ | SP$^b$ | SE$^a$ | SP$^b$ | SE$^a$ | SP$^b$ | SE$^a$ | SP$^b$ |
| 2a         | >5.34 | 0.89 | >1.32 | 0.23 | - | - | - | - |
| 2b         | >4.11 | 1.52 | - | - | >3.43 | 0.69 | - | - |
| 2d         | >6.58 | 1.36 | >1.69 | 0.73 | >3.68 | 0.60 | - | - |
| 2e         | >4.60 | 0.78 | >1.42 | 0.72 | >2.56 | 0.37 | - | - |
| 2f         | >13.37 | 4.79 | >1.62 | 0.50 | - | - | - | - |
| Miltefosine | >24.10 | 5.15 | >31.15 | 8.74 | >13.23 | 2.64 | - | - |

$^a$SE (selectivity): IC$_{50}$ of macrophages/IC$_{50}$ of amastigotes of Leishmania.

$^b$SP (specificity): is the ratio between promastigote IC$_{50}$ and intracellular amastigote IC$_{50}$.

According to the results presented in this work, can be observe differences in sensitivity between promastigote and amastigote forms of Leishmania in relation to the aza-stilbene assayed. Several factors can explain this: both parasite stages have different host, promastigotes lives in the gut of the insect vector and amastigotes within mammalian cells; and these forms present biochemistry and morphology differences which can reflect in different targets for the drugs. These differences in sensitivity between both stages of parasite several compounds were also verified by several authors$^{19,35,37,40}$.

Macrophages are the major immunologic route for elimination of Leishmania. Activated macrophages release a variety of cytotoxic molecules which include NO with action against Leishmania$^{41,42}$. So, we tried to investigate if the leishmanicidal effect of the aza-stilbene derivatives could be associated to NO production. This assay was performed in parallel with the antiamastigote assay, collecting the supernatants of Leishmania-macrophages treated with several concentrations of the aza-stilbene derivatives. In general, majority of the derivatives tested induced significant nitrite production in the culture medium only at the maximum concentration tested (100 µM) compared to untreated control (data not shown). However, in
low concentration, mainly close the IC_{50} values of each compound, it was observed variation on NO level production and in fact some even inhibited the spontaneous NO production and it not possible associate NO production with leishmanicidal activity for all aza-stilbene derivatives.

Conclusion

In this paper, we show that some Aza-stilbene derivates with hydroxyl groups exhibit leishmanicidal activity against three *Leishmania* species. Compound 2f was the most active against intracellular forms of *L. amazonensis*, presenting results near the reference drug miltefosine. However, further studies are needed to identify the likely mechanism of action of this drug. Nevertheless, the results indicate the Aza-stilbene derivatives as a promising research line.

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References

1. http://www.who.int/neglected_disease/en/ accessed May 2012.
2. M. den Boer, D. Argaw, J. Jannin, J. Alvar, Clin. Microbiol. Infect., 2011, 17:1471-1477.
3. http://www.who.int/leishmaniasis/en/ accessed May 2012.
4. S. L. Croft, K. Seifert, V. Yardley, Indian J. Med. Res, 2006, 123, 399-410.
5. A. K. Shukla, B. K. Singh, S. Patra, V. K. Dubey, Appl. Biochem. Biotechnol., 2010, 160, 2208-2218.
6. M. Ameen, Clin. Exp. Dermatol., 2010, 35, 699-705.
7. J. Chong, A. Poutaraud, P. Hugueney, Plant Sci., 2009, 177, 143-155.
8. P. Jeandet, B. Delaunois, A. Aziz, D. Donnez, Y. Vasserot, S. Cordelier, E. Courot, J. Biomed. Biotechnol., 2012, doi: 10.1155/2012/579089.
9. J. A. Baur, D. A. Sinclair, Nat. Rev. Drug. Discovery, 2006, 5, 493-506.
10. S. Renaud, M. de Lorgeril, Lancet, 1992, 339, 1523-1526.
11. B. Catalgol, S. Batirel, Y. Taga, N.K. Ozer, Front Pharmacol., 2012, 3, 141-159.
12. S. Bradamante, L. Baregghi, A. Villa, Cardiovasc. Drug Rev., 2004, 22, 169-188.
13. L. Kedzierski, J.M Curtis, M. Kaminska, J. Jodynis-Liebert, M. Murias, Parasitol. Res., 2007, 102, 91-97.
14. S. Ozkoc, S. Tuncay, S. B. Delibas, C. Aksu, Parasitol. Res., 2009, 105,1139-1143.
15. E. J. LaVoie, and G. A. Patani, Chem. Rev., 1996, 96, 3147-3176.
16. N. O. Calil, G. S. G. De Carvalho, D. C. Z. Franco, A. D. Da Silva, N. R. B. Raposo, Lett. Drug Des. Discov., 2012, 9, 8-11.
17. F. R. Pavan, G. S. G. De Carvalho, A. D. Da Silva, C. Q. F. Leite, ScientificWorldJournal, 2011, 11, 1113-1119.
18. J. Mossman, Immunol. Methods, 1983, 65, 55-63.
19. P. A. Machado, F. F. Hilário, L. O. Carvalho, M. L. T. Silveira, R. B. Alves, R. P. Freitas, E. S. Coimbra, Chem. Biol. Drug. Des., 2012, 80, 745–751.
20. L. C. Green, D. A. Wagner, J. Glogwski, P. L. Skipper, J. S. Wishnok, S. R. Tannenbaum, Anal. Biochem., 1982, 126, 131-138.
21. E. H. Cordes, W. P. Jencks, J. Am. Chem. Soc., 1962, 84, 826-831.
22. N. Ebara, Bull. Chem. Soc. Jpn., 1961, 34, 1151-1158.
23. J. B. Stevens, U. K. Pandit, Tetrahedron, 1983, 39, 1395-1400.
24. N. Rani, J. R. Sharma, M. R. Manrao, Pestic. Res. J., 2006, 18, 129-132.
25. M. Sekiya, T. Morimoto, Chem. Pharm. Bull., 1975, 23, 2353-2357.
26. L. X. Cheng, J. J. Tang, H. Luo, X. L. Jin, F. Dai, J. Yang, Y. P. Qian, X. Z. Li, B. Zhou, Bioorg. Med. Chem. Lett., 2010, 20, 2417-2420.
27. W. Manchot, Liebigs Ann., 1912, 388, 103-135.
28. A. Senier, R. B. Forster, J. Chem. Soc. Trans., 1915, 107, 1168-1173.
29. D. M. Ritter, J. Am. Chem. Soc., 1947, 69, 46-50.
30. F. G. Pope, J. Chem. Soc. Trans., 1908, 93, 532-537.
31. D. C. Colinese, J. Chem. Soc., 1971, 5, 864-869.
32. S. J. Wadher, N. A. Karande, S. D. Sonawane, P. G. Yeole, Int. J. Chem. Tech. Res., 2009, 1, 1303-1307.
33. L. D. Rodrigues, Rev. Port. Farm., 1952, 2, 95-98.
34. A. Kocwa, M. Eckstein, Z. Walczak, Dissertationes Pharm., 1951, 3, 149-158.
35. G. S. G. De Carvalho, P. A. Machado, D. T. S. De Paula, E. S. Coimbra, A. D. Silva, ScientificWorldJournal., 2010, 10, 1723-1730.
36. M. Vermeersch, R. I. Luz, K. Tote, J. Timmermans, P. Cos, L. Maes, Antimicrob. Agents Chemother., 2009, 53, 3855-3859.
37. A. G. Tempone, C. M. Oliveira, R. G. S. Berlinck, Planta Med., 2011, 77, 572-585.
38. H. Piotrowska, M. Kucinska, M. Murias, Mutat. Res., 2012, 750, 60-82.
39. P. R. Machado, J. Ampuero, L. H. Guimarães, L. Villas Boas, A. T. Rocha, A. Schriefer, R. S. Sousa, A. Talhari, G. Penna, E. M. Carvalho, PLOS Negl. Trop. Dis., 2010, 4, e912.
40. G. Muylder, K. K. H. Ang, S. Chen, M. R. Arkin, J. C. Engel, J. H. Mckerrow, PLOS Negl. Trop. Dis., 2011, 5, e1253.
41. J. L. Lemesre, D. Sereno, S. Daulouède, B. Veyret, N. Brajon, P. Vincendeau, Exp. Parasitol., 1997, 86: 58-68.
42. R. M. Mukbel, C. P. Jr, K. Gibson, M. Ghosh, C. Petersen, D. E. Jones, Am. J. Trop. Med. Hyg., 2007, 76, 669-675.