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Original article

Synthesis and biological evaluation against *Mycobacterium tuberculosis* and *Leishmania amazonensis* of a series of diaminated terpenoids

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ABSTRACT

We report the synthesis of a series of diaminated terpenoids containing, as side-chain of the diamine core, the "head-to-tail" prenyl derivatives, with amino amino spacers of variable length. *In vitro* biological activity of these compounds was evaluated against *Mycobacterium tuberculosis* and *Leishmania amazonensis*, and the structure-activity relationships are discussed. Different biological results were observed depending on the terpenic side-chain length. The best results were obtained for *trans,trans*-farnesol derivatives. Moreover, these results demonstrated that the stereochemistry of the double bond could play an important role in determining antitubercular and antileishmanial activities of these compounds.

1. Introduction

Tuberculosis (TB) and leishmaniasis caused by *Mycobacterium tuberculosis* and *Leishmania spp.*, respectively are diseases marked by low efficacy treatments having several side-effects with a resulting great impact on human health around the world. According to the 2016 global tuberculosis report of the World Health Organization (WHO) it is estimated that about 10.4 million new cases of TB occurred globally with 1.8 million deaths. More worrying is the growing number of Multi-Drug Resistant-TB (MDR-TB) cases (about 480,000) of which it is estimated that 9.5% are Extensively-Drug Resistant-TB (XDR-TB) [1]. For leishmaniasis, there is no effective and completely safe medication for any of the clinical forms [2] and 1.3 million new cases with 20,000 to 30,000 deaths occur annually [3]. Therefore, these two diseases are a public health problem and the development of new drugs with reduced time treatment, low side effects and mainly active against MDR-TB and XDR-TB is needed. In the last decades, due to their biological activities, natural polyamines and their analogues or conjugates have become of great interest in different research areas [4]. Among the polyamines, the diamine compounds have shown activity against both TB and several species of *Leishmania* [5–9]. Based on these previous results we decided to synthesize a new series of diaminated terpenoids and evaluate the influence of prenyl and head-to-tail prenyl derivatives linked to cyclic and acyclic diamine groups with different lengths of a carbon spacer between *N,N* atoms. *In vitro* antileishmanial and antitubercular activities as well as the structure-activity relationships were also evaluated.

2. Results and discussion

2.1. Chemistry

Sixteen terpenic diamines were synthesized using minor modifications of synthetic routes reported in the literature [10,11] (Scheme 1). Initially, the terpenic alcohols: 3-methylbut-2-en-1-ol, (*E* and *Z*)-3,7-dimethylocta-2,6-dien-1-ol and (2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol were converted into their respective bromides by treatment with phosphorus tribromide (PBr_3) using adequate solvents. Each allyl bromide (compounds **5–8**) was then reacted with an excess of the corresponding diamine (ethane-1,2-diamine, propane-1,3-diamine, butane-1,4-diamine or 1,4-diazacyclohexane) in dichloromethane at −18 °C for 24 h to provide the desired compounds **9–24** in poor to good yields in the range of 21–89%. The successful synthesis of compounds **9–24** was

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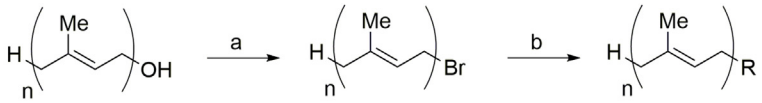
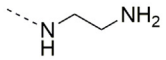
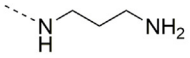
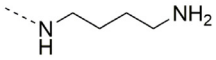
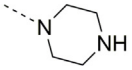


1: n = 1
2: n = 2 (E)
3: n = 2 (Z)
4: n = 3 (E, E)

5: n = 1
6: n = 2 (E)
7: n = 2 (Z)
8: n = 3 (E, E)

R	n	Compound	Yield (%)
	1	**9**	89
	2 (*E*)	**10**	56
	2 (*Z*)	**11**	63
	3 (*E, E*)	**12**	31
	1	**13**	22
	2 (*E*)	**14**	54
	2 (*Z*)	**15**	37
	3 (*E, E*)	**16**	21
	1	**17**	40
	2 (*E*)	**18**	31
	2 (*Z*)	**19**	38
	3 (*E, E*)	**20**	30
	1	**21**	51
	2 (*E*)	**22**	82
	2 (*Z*)	**23**	46
	3 (*E, E*)	**24**	40

Scheme 1. Reagents and conditions: (a) **1**, PBr3, ethyl ether, −18 °C, 40 min; **2**, **3** or **4**, PBr_3, THF, −18 °C, 40 min; (b) 5, 6, 7 or 8, CH_2Cl_2, diamine (ethane-1,2-diamine, propane-1,3-diamine, butane-1,4-diamine or 1,4-diazacyclohexane) −18 °C, 24 h.

confirmed using ^{1}H NMR, ^{13}C NMR and ESI-HRMS for the novel compounds **9**, **15**, **18** and **19**.

2.2. Biological assays

2.2.1. In vitro *activity against* Mycobacterium tuberculosis

Initially, the synthesized terpenic diamines were screened against *M. tuberculosis* H37Rv ATCC 27294 by agar microdilution method to evaluate their biological activity in terms of Minimal Inhibition Concentration (MIC) values, which is defined as the lowest drug concentration that will inhibit the visible growth of an organism after the incubation period [12]. The LogP values of the compounds **9–24** and rifampicin (standard drug) were obtained using free online services of the MolSoft software. The LogP values and MIC of these compounds in this series and rifampicin are summarized in Table 1.

According to Table 1, only compounds **12**, **16**, **18**, **20** and **24** showed anti-TB activity. Compound **18** (MIC: 450 μM), the (*E*) isomer of compound **19** (inactive), showed activity against *M. tuberculosis* H37Rv strain, suggesting that the stereochemistry of the double bond plays a role in anti-TB activity.

Compounds **12**, **16**, **20** and **24** have the same (2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl (or *trans,trans*-farnesyl) substituent on the nitrogen atom of the diamino group. Thus, these results allow to infer that the number of spacer carbon atoms between the amino groups could also influence the activity of these compounds.

Compound **12** (LogP: 3.95) has the pharmacophore group ethane-1,2-diamine as in the first-line antitubercular drug ethambutol and displayed a MIC value of 95 μM. Compound **16** (LogP: 4.43) is more lipophilic than compound **12** due to the presence of three spacer carbon atoms between the amino groups, and it is also more active against H37Rv strain, with a MIC value of 90 μM. In contrast, compound **20**, derived from butane-1,4-diamine, is more lipophilic than compounds **12** and **16** with a LogP value of 4.92, but showed less activity against the H37Rv strain, with a MIC value of 171 μM.

Replacement of the acyclic diamine moiety by the cyclic diamine 1,4-diazacyclohexane (piperazine) contributed to increasing the activity against *M. tuberculosis* H37Rv strain since this structural modification led to an increase of overall molecular lipophilicity without significantly altering the distance between the amino groups when compared to the pharmacophore group ethane-1,2-diamine. Another point is the resulting structural rigidification that could also be responsible for improving antitubercular activity.

For the three diamino systems, ethane-1,2-diamine, propane-1,3-diamine and 1,4-diazabicyclohexane each containing four different terpenes, only the *trans,trans*-farnesyl derivatives were active. For the butane-1,4-diamine system, the geranyl derivative was also active but less so than the *N-trans,trans*-farnesyl analogue. This fact may be simply associated with the higher lipophilicity of this compound (LogP ≥ 3.95). However, it is not possible to affirm only that the higher lipophilicity of compounds could increase their antitubercular activity because small structural modifications are capable of altering the physical chemistry properties as well as steric and conformational factors thus changing the degree of affinity and specificity of the binding between the bioactive molecule and its bioreceptor. The study of the structure-activity relationships of the terpenic diamines against H37Rv strain is summarized in Fig. 1.

2.2.2. In vitro *activity against* Leishmania amazonensis *and selectivity index*

The synthesized compounds **9-24** also were screened against *L. amazonensis* promastigotes (MHOM/BR/77/LOT0016). After 48 h of incubation the toxic effect was evaluated by microscopic observation followed by the colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method [13]. By

Table 1
Structure, Molecular Weight (MW), LogP values and *in vitro* activity against *M. tuberculosis* H37Rv of compounds **9–24**.

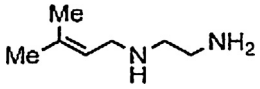
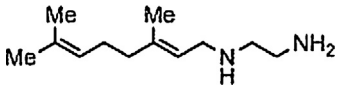
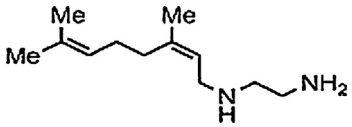
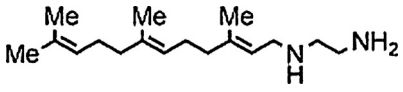
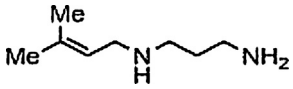
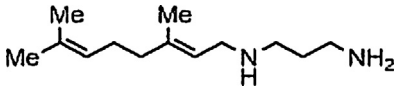
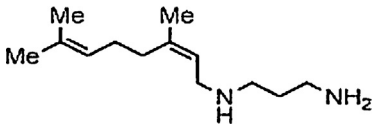
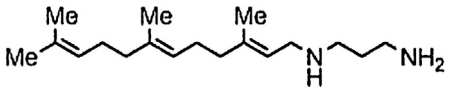
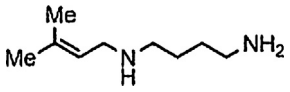
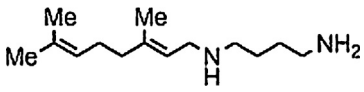
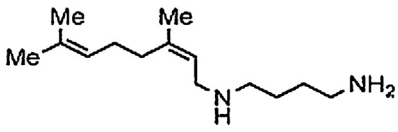
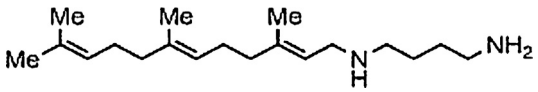
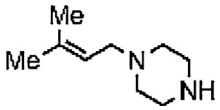
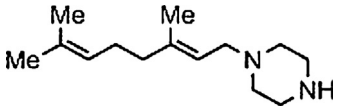
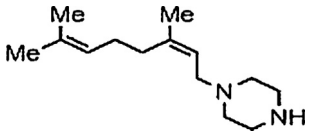
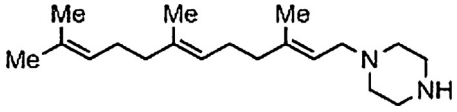
Compound	Structure	MW (g/mol)	LogP*	MIC (μM)
9		128.13	−0.04	NA
10		196.19	1.96	NA
11		196.19	1.96	NA
12		264.26	3.95	95.0
13		142.15	0.45	NA
14		210.21	2.44	NA
15		210.21	2.44	NA
16		278.27	4.43	90.0
17		156.16	0.93	NA
18		224.23	2.92	450.0
19		224.23	2.92	NA
20		292.29	4.92	171.0
21		154.15	1.00	NA

Table 1 (*Continued*)

Compound	Structure	MW (g/mol)	LogP*	MIC (μM)
				
22		222.21	2.99	NA
23		222.21	2.99	NA
24		290.27	4.99	86.0
Rifampicin	–	822.94	3.19	1.21

NA: Not Active.
* The LogP values were calculated employing the MolSoft software.

linear interpolation these results were described as half maximal inhibitory concentration (IC_{50}). The LogP of the compounds **9-24** and amphotericin B (standard drug) was also obtained using free online services of the MolSoft software. All results are summarized in Table 2.

Compounds **9**, **13**, **17** and **21** showed no activity against *L. amazonensis* promastigotes, suggesting that the prenyl group does not contribute to the anti-leishmanial activity of these compounds. This fact can be associated with low lipophilicity of these molecules ($LogP \leq 1.0$) which can result in a weak interaction with the lipophilic membrane of the parasite or hinder membrane penetration of the compound.

The ***E*** stereoisomer, compound **18** (derived from geranyl group), was more active (IC_{50}: 72.66 ± 12.61 μM) than the corresponding ***Z*** stereoisomer compound **19** (IC_{50}: 95.53 ± 3.53 μM; derived from neryl group), suggesting that the stereochemistry of the double bond could be important for activity against L. *amazonensis* BR/77 strain. Curiously, these results corroborate those previously observed for *M. tuberculosis*: considering the butane-1,4-diamine core, the compound derived from neryl group was inactive while

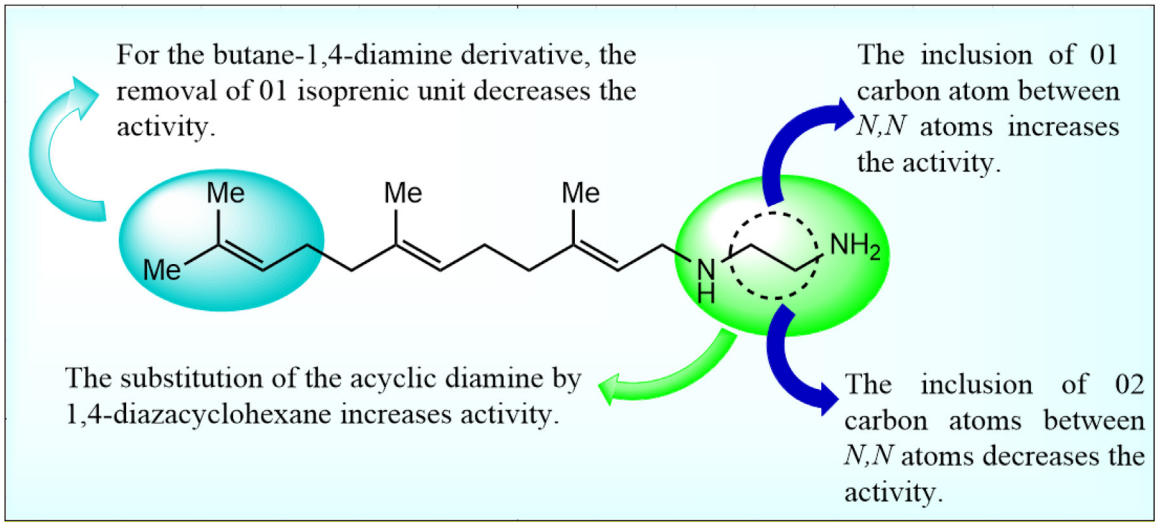


Fig. 1. Structure-activity relationships of terpenic diamines against *Mycobacterium tuberculosis* H37Rv strain.

Table 2
Structure, LogP values, *in vitro Leishmania amazonensis* inhibition (IC_{50}), fibroblasts 3T3 cytotoxicity (CC_{50}) and selectivity index (SI).

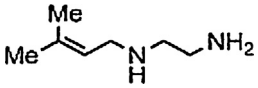
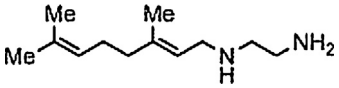
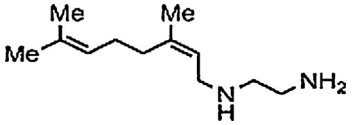
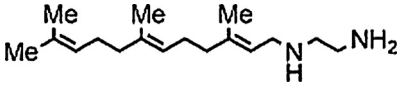
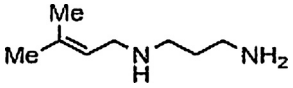
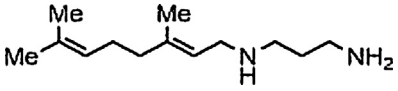
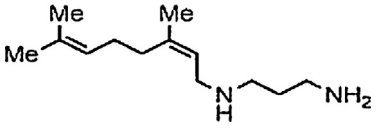
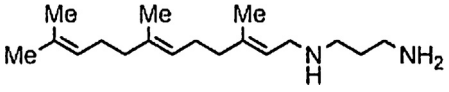
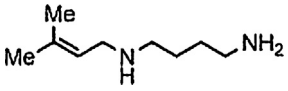
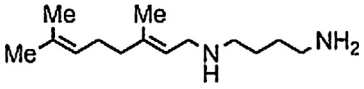
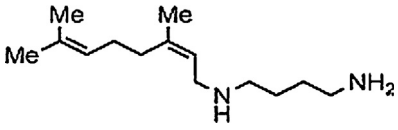
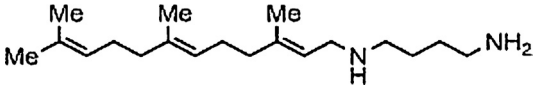
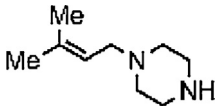
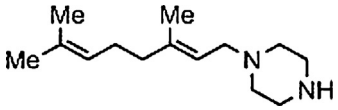
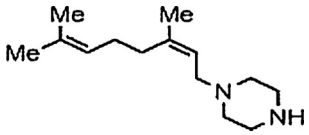
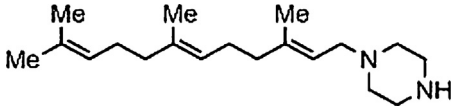
Compound	Structure	LogP*	IC_{50} (μM)	CC_{50} (μM)	SI**
9		−0.04	NA	ND	ND
10		1.96	26.7 ±1.51	108.74 ± 5.45	4.07
11		1.96	21.51 ±2.81	83.18 ± 5.16	3.87
12		3.95	16.83 ±2.35	<12.5	<0.74
13		0.45	NA	ND	ND
14		2.44	35.95 ±3.44	79.38 ± 16.70	2.21
15		2.44	35.81 ±0.61	38.78 ± 10.11	1.08
16		4.43	4.91 ±0.77	<12.5	<2.55
17		0.93	NA	ND	ND
18		2.92	72.66 ±12.61	52.71 ± 9.02	0.73
19		2.92	95.53 ± 3.53	72.94 ± 19,86	0.76
20		4.92	15.01 ± 0.98	<12.5	<0.83
21		1.00	NA	ND	ND

Table 2 (*Continued*)

Compound	Structure	LogP*	IC_{50} (μM)	CC_{50} (μM)	SI**
					
22		2.99	NA	ND	ND
23		2.99	NA	ND	ND
24		4.99	12.99 ±0.70	<12.5	<0.96
Amphotericin B		−2.43	0.1	>10	>100

NA: No Active; ND: Not Determined.

* The LogP values were calculated employing the MolSoft software.

** SI, calculated based on CC_{50}/IC_{50} ratios.

the geranyl derivative showed a MIC value of 450 μM. Moreover, it is important to note that the compounds derived from neryl and geranyl groups are more active when they have only two spacer carbon atoms between the amino groups and increasing the carbon chain length results in a decrease in activity of these compounds. Compounds **22** and **23** are also stereoisomers and have the heterocyclic 1,4-diazacyclohexane as diamine core. These compounds, in turn, exhibited no activity against the parasite.

The terpenic diamines derived from *trans,trans*-farnesyl group (**12**, **16**, **20** and **24**) are the most lipophilic compounds in our series, with LogP values between 3.95 and 4.99. These diamines were the most active against *L. amazonensis* promastigote forms, with IC_{50} values between 4.91 ± 0.77 and 16.83 ± 2.35 μM, again suggesting that lipophilicity could play an important role in determining the biological activity of this class of compounds. It is important to emphasize that compound **16** (IC_{50}: 4.91 ± 0.77), having three spacer carbon atoms between the amino groups, displayed the best anti-leishmanial activity of this series. These results also allow us to say that the replacement of the acyclic diamine by 1,4-diazacyclohexane contributes to decreased activity against the parasite.

The larger number of compounds active against *L. amazonensis* (10/16) compared to *M. tuberculosis* (5/16) can be explained by the inhibitory concentrations being expressed by IC_{50} for *L. amazonensis* and IC_{90} for *M. tuberculosis*. An additional point concerns the different structural characteristics of these microorganisms: *L. amazonensis* is eukaryotic while *M. tuberculosis* is prokaryotic, and consequently these microorganisms have differing metabolism and membrane structures that can affect the activities of these compounds.

The *in vitro* cytotoxicity of the active leishmanial compounds was also evaluated by MTT assay against uninfected 3T3 fibroblast cell line and the results were expressed as the 50% cytotoxic concentration (CC_{50}) value. Thus, these data allowed the Selectivity Index (SI) of the compounds to be determined by calculating the CC_{50}/IC_{50} ratio. All data are summarized in Table 2.

In our view, the most interesting results were obtained for compounds derived from *trans,trans*-farnesol. These compounds displayed the best anti-leishmanial activity and better selectivity.

3. Conclusion

In this study, we report the synthesis of a series of 16 head-to-tail prenyl diamino derivatives and evaluation of their biological activity. *Trans,trans*-farnesol derivatives, the more lipophilic compounds, showed the best results against both *M. tuberculosis* and *L. amazonensis*. These results suggest the importance of lipophilicity in developing new drugs against *M. tuberculosis* as a consequence of improved membrane penetration thus increasing the compound's chances of reaching the intracellularly-located parasite. The higher microbicidal activity of *trans,trans*-farnesol derivatives may not only be related to improved lipophilicity but can also be related to specific features of the *trans,trans*-farnesyl substituent. On comparing the microbicidal activity of diaminated terpenoids containing ten carbon atoms as side-chain substituents, the stereochemistry of the double bond appears to play an important role.

4. Experimental

4.1. Chemistry: general comments

All reagents and solvents were reagent grade and were used without prior purification. All reactions were monitored by thin layer chromatography (TLC, Sigma-Aldrich® 60). The allyl bromides (compounds **5–8**) were purified by liquid–liquid extraction and compounds **9–24** were purified by liquid–liquid extraction followed by flash chromatography on Sigma-Aldrich® silica gel 60

(230–400 mesh) using CH_2Cl_2:CH_3OH:NH_4OH (80:18:2) as eluent. The IR spectra were acquired on a Perkin Elmer Spectrum 100 FTIR spectrophotometer with an Attenuated Total Reflectance (ATR) attachment and only significant peaks were recorded. ^{1}H NMR (300 or 500 MHz) and ^{13}C NMR (75 or 125 MHz) spectra of the final compounds (**9-24**) were recorded in $CDCl_3$ on a Bruker Avance ACX300 (300 MHz) or on a Bruker Avance III HD spectrometer (500 MHz). The chemical shifts (δ) are quoted in parts per million (ppm) downfield from the internal reference standard, tetramethylsilane (TMS), and the coupling constants (*J*) were recorded in Hertz. Splitting pattern abbreviations are as follows: s = singlet, brs = broad singlet, d = doublet, t = triplet and m = multiplet. ESI-HRMS mass spectra were carried out on a Bruker MicroTOF spectrometer. The LogP values were calculated using the free online services of the MolSoft Software.

4.1.1. General procedure for synthesis of the allyl bromides

The terpenic alcohols: 3-methylbut-2-en-1-ol, (*E* and *Z*)-3,7-dimethylocta-2,6-dien-1-ol and (2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol were used as starting materials to prepare the intermediate bromides (Scheme 1). The allylic alcohol (10 mmol) was solubilized in THF (10 mL) and a solution of PBr_3 (3.3 mmol) in THF (5 mL) was added dropwise. The reaction mixture was stirring for 40 min at −18 °C. At the end of the reaction period the solution was concentrated *in vacuo* and the residual oil was dissolved in diethyl ether/hexane (20 mL; 1:1 v/v) and washed sequentially with aqueous $NaHCO_3$ (2 × 10 mL; 5% m/v) and distilled water (2 × 10 mL). The organic phase was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure.

The above procedure was applied to allylic alcohol **1**, 3-methylbut-2-en-1-ol (10 mmol), using diethyl ether as solvent providing compound **5**.

4.1.2. General procedure for the synthesis of N-*isoprenyl diamines (9–24)*

Compounds **5, 6, 7** or **8** (0.5 mmol) were dissolved in dichloromethane and added dropwise to a vigorously stirred solution of ethane-1,2-diamine (5.0 mmol) in dichloromethane at −18 °C over 3 h. The reaction mixture was allowed to come to room temperature with stirring over 24 h. The mixture was then concentrated *in vacuo* and the residual oil was dissolved in dichloromethane (20 mL). The solution was washed with water (3 × 5 mL) to remove excess diamine. The organic phase was dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified by flash chromatography on silica gel using CH_2Cl_2: CH_3OH:NH_4OH (80:18:2) as eluent.

4.1.2.1. N^1-(3-methylbut-2-en-1-yl)ethane-1,2-diamine (9). The obtained product was a yellow oil (yield: 0.057 g, 89%). **FTIR** (ATR, cm^{-1}): 3351.89 (υ_{as}, N—H), 3287.35 (υ_s, N—H), 1671.34 (υ, C=C), 1572.45 (δ, NH_2), 1102.91 (υ, C—N), 1122.75 (υ, C—N). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ (ppm): 1.58 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 2.60 (t, 2H, CH_2, *J* = 6.0 Hz), 2.74 (t, 2H, CH_2, *J* = 6 Hz), 3.15 (d, 2H, CH_2, *J* = 7 Hz), 5.18 (t, 1H, CH, *J* = 6.5 Hz). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ_C (ppm): 18.02 (CH_3), 25.87 (CH_3), 41.89 (CH_2), 47.25 (CH_2), 52.15 (CH_2), 123.08 (CH), 134.42 (C). **ESI-HRMS**: *m*/*z* calculated for $C_7H_{17}N_2$ $[M+H]^+$ 129.1386, found 129.1388.

Compounds **10-24** were synthesized according to the methodology described above and structural characterization data are given below.

4.1.2.2. (E)-N^1-(3,7-dimethylocta-2,6-dien-1-yl)ethane-1,2-diamine (10). Yellow oil (yield: 1.1517 g, 58%). **FTIR** (ATR, cm^{-1}): 3357.0 (υ_{as}, N—H), 3291.0 (υ_s, N—H), 1666 (υ, C=C), 1583.0 (δ, NH_2), 1108 (υ, C—N). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ (ppm): 1.56 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 1.64 (s, 3H, CH_3), 1.97 (t, 2H, CH_2, *J* = 7.0 Hz), 2.03-2.07 (m, 2H, CH_2), 2.63 (t, 2H, CH_2, *J* = 6.0 Hz), 2.77 (t, 2H, CH_2, *J* = 6.0 Hz), 3.19 (d, 2H, CH_2, *J* = 6,5 Hz), 5.05 (t, 1H, CH, *J* = 6.0 Hz), 5.22 (t, 1H, CH, *J* = 6.0 Hz). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ (ppm): 16.47 (CH_3), 17.86 (CH_3), 25.87 (CH_3), 26.69 (CH_2), 39.81 (CH_2), 42.01 (CH_2), 47.27 (CH_2), 52.27 (CH_2), 122.97 (CH), 124.29 (CH), 131.69 (C), 137.93 (C).

4.1.2.3. (Z)- N^1-(3,7-dimethylocta-2,6-dien-1-yl)ethane-1,2-diamine (11). Yellow oil (yield: 1.01 g, 63%). **FTIR** (ATR, cm^{-1}): 3356.96 (υ_{as}, N—H), 3281.01 (υ_s, N—H), 1663.76 (υ, C=C), 1568.33 (δ, NH_2), 1107.46 (υ, C—N). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ (ppm): 1.55 (s, 3H, CH_3), 1.63 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 2.00 (m, 4H, 2×CH_2), 2.17 (brs, 3H, NH and NH_2), 2.63 (t, 2H, CH_2 *J* = 6.0 Hz), 2.77 (t, 2H, CH_2, *J* = 6.0 Hz), 3.17 (d, 2H, CH_2, *J* = 7.0 Hz), 5.05 (m, 1H, CH), 5.22 (t, 1H, CH, *J* = 6.5 Hz). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ (ppm): 17.80 (CH_3), 23.56 (CH_3), 25.86 (CH_3), 26.74 (CH_2), 32.27 (CH_2), 41.73 (CH_2), 46.91 (CH_2), 51.91 (CH_2), 123.56 (CH), 124.11 (CH), 132.02 (C), 138.34 (C).

4.1.2.4. N^1-[(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]ethane-1,2-diamine (12). Yellow oil (yield: 0.310 g, 31%). **FTIR** (ATR, cm^{-1}): 3356.96 (υ_{as}, N—H), 3286.07 (υ_s, N—H), 1666.29 (υ, C=C), 1567.48 (δ, NH_2), 1108.80 (υ, C—N). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ (ppm): 1.57 (s, 6H, 2xCH_3), 1.62 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 2.00 (m, 8H, 4xCH_2), 2.65 (t, 2H, CH_2, *J* = 6.0 Hz), 2.79 (t, 2H, CH_2, *J* = 6.0 Hz), 3.21 (d, 2H, CH_2, *J* = 6.5 Hz), 5.07 (m, 2H, 2xCH), 5.24 (m, 1H, CH). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ (ppm): 16.21 (CH_3), 16.54 (CH_3), 17.89 (CH_3), 25.90 (CH_3), 26.64 (CH_2), 26.95 (CH_2), 39.84 (CH_2), 39.92 (CH_2), 41.89 (CH_2), 47.21 (CH_2), 52.14 (CH_2), 122.67 (CH), 124.15 (CH), 124.53 (CH), 131.50 (C), 135.38 (C), 138.30 (C).

4.1.2.5. N^1-(3-methylbut-2-en-1-yl)propane-1,3-diamine (13). Yellow oil (yield: 0.7535 g, 22%). **FTIR** (ATR, cm^{-1}): 3356.96 (υ_{as}, N—H), 3286.11 (υ_s, N—H), 1666.29 (υ, C=C), 1566.77 (δ, NH_2), 1095.45 (υ, C—N). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ (ppm): 1.59 (m, 5H, CH_2 and CH_3), 1.66 (s, 3H, CH_3), 2.33 (brs, 3H, NH and NH_2), 2.62 (t, 2H, CH_2, *J* = 7.0 Hz), 2.72 (t, 2H, CH_2, *J* = 7.0 Hz), 3.15 (d, 2H, CH_2, *J* = 6.5 Hz), 5.19 (m, 1H, CH). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ (ppm): 18.05 (CH_3), 25.89 (CH_3), 33.63 (CH_2), 40.59 (CH_2), 47.44 (2xCH_2), 122.94 (CH), 134.52 (C).

4.1.2.6. (E)-N^1-(3,7-dimethylocta-2,6-dien-1-yl)propane-1,3-diamine (14). Yellow oil (yield: 0.90 g, 54%). **FTIR** (ATR, cm^{-1}): 3356.96 (υ_{as}, N—H), 3284.42 (υ_s, N—H), 1666.29 (υ, C=C), 1567.77 (δ, NH_2), 1106.85 (υ, C—N). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm): 1.57 (s, 3H, CH_3), 1.61 (s, CH_3), 1.65 (m, 5H, CH2 and CH_3), 1.98-2.06 (m, 7H, 2xCH_2, NH and NH_2), 2.66 (t, 2H, CH_2, *J* = 6.9 Hz), 2.76 (t, 2H, CH_2, *J* =6.9 Hz), 3.20 (d, 2H, CH_2, *J* = 6.9 Hz), 5.06 (t, 1H, CH, *J* = 6.3 Hz), 5.22 (t, 1H, CH, *J* = 6.3 Hz). **^{13}C NMR** ($CDCl_3$, 75 MHz) δ (ppm): 16.52 (CH_3), 17.89 (CH_3), 25.89 (CH_3), 26.73 (CH_2), 33.72 (CH_2), 39.85 (CH_2), 40.81 (CH_2), 47.42 (CH_2), 47.58 (CH_2), 122.69 (CH), 124.32 (CH), 131.76 (C), 138.27 (C).

4.1.2.7. (Z)-N^1-(3,7-dimethylocta-2,6-dien-1-yl)propane-1,3-diamine (15). Yellow oil (yield: 0.6588 g, 37%). **FTIR** (ATR, cm^{-1}): 3351.89 (υ_{as}, N—H), 3281.41 (υ_s, N—H), 1666.29 (υ, C=C), 1567.36 (δ, NH_2), 1106.68 (υ, C—N). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ (ppm): 1.56 (s, 3H, CH_3), 1.62 (m, 2H, CH_2), 1.64 (s, 3H, CH_3), 1.68 (s, 3H, CH_3), 2.02 (s, 4H, 2×CH_2), 2.16 (brs, 3H, NH and NH_2), 2.64 (m, 2H, CH_2), 2.74 (m, 2H, CH_2), 3.16 (d, 2H, CH_2, *J* = 6.5 Hz), 5.05 (m, 1H, CH), 5.23 (m, 1H, CH). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ (ppm): 17.84 (CH_3), 23.59 (CH_3), 25.91 (CH_3), 26.77 (CH_2), 32.29 (CH_2), 33.60 (CH_2), 40.72 (CH_2), 47.20 (CH_2), 47.59 (CH_2), 123.59 (CH), 124.14 (CH), 132.06 (C), 138.32 (C). **ESI-HRMS**: *m*/*z* calculated for $C_{13}H_{27}N_2$. $[M+H]^+$ 211.2169, found 211.2166.

4.1.2.8. N^1-[(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl] propane-1,3-diamine (16). Yellow oil (yield: 0.214 g, 21%). **FTIR** (ATR, cm^{-1}): 3372 (υ_{as}, N—H), 3270 (υ_s, N—H), 1663 (υ, C=C), 1107 (υ, C—N). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ (ppm): 1.56 (s, 6H, 2xCH_3), 1.61 (s, 3H, CH_3), 1.64-1.66 (m, 5H, CH_2 and CH_3), 1.92-2.10 (m, 11H, 4×CH_2, NH and NH_2), 2.66 (t, 2H, CH_2, *J* = 6.9 Hz), 2.76 (t, 2H, CH_2, *J* = 6.9 Hz), 3.21 (d, 2H, CH_2, *J* = 6.8 Hz), 5.04-5.08 (m, 2H, 2×CH), 5.23 (t, 1H, CH, *J* = 6.7 Hz). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ (ppm): 16.20 (CH_3), 16.54 (CH_3), 17.88 (CH_3), 25.89 (CH_3), 26.62 (CH_2), 26.94 (CH_2), 33.38 (CH_2), 39.82 (CH_2), 39.90 (CH_2), 40.79 (CH_2), 47.32 (CH_2), 47.57 (CH_2), 122.37 (CH), 124.11 (CH), 124.51 (CH), 131.49 (C), 135.39 (C), 138.52 (C).

4.1.2.9. N^1-(3-methylbut-2-en-1-yl)butane-1,4-diamine (17). Yellow oil (yield: 0.4819 g, 40%). **FTIR** (ATR, cm^{-1}): 3356 (υ_{as}, N—H), 3270 (υ_s, N—H), 1668 (υ, C=C), 1585.0 (δ, NH_2), 1097 (υ, C—N). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ (ppm): 1.45 (m, 4H, 2xCH_2), 1.60 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 2.57 (t, 2H, CH_2, *J* = 6.5 Hz), 2.66 (t, 2H, CH_2, *J* = 6.5 Hz), 3.16 (d, 2H, CH_2, *J* = 7.0 Hz), 5.21 (m, 1H, CH). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ (ppm): 18.07 (CH_3), 25.92 (CH_3), 27.66 (CH_2), 31.76 (CH_2), 42.28 (CH_2), 47.43 (CH_2), 49.51 (CH_2), 123.12 (CH), 134.40 (C).

4.1.2.10. (E)-N^1-(3,7-dimethylocta-2,6-dien-1-yl)butane-1,4-diamine (18). Yellow oil (yield: 0.2082 g, 31%). **FTIR** (ATR, cm^{-1}): 3351 (υ_{as}, N—H), 3281 (υ_s, N—H), 1666 (υ, C=C), 1572.0 (δ, NH_2), 1107 (υ, C—N). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ (ppm): 1.46 (m, 4H, 2xCH_2), 1.55 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 1.63 (s, 3H, CH_3), 1.96 (m, 2H, CH_2), 2.03 (m, 2H, CH_2), 2.57 (t, 2H, CH_2, *J* = 6.0 Hz), 2.66 (t, 2H, CH_2, *J* = 6.0 Hz), 3.18 (d, 2H, CH_2, *J* = 6.0 Hz), 5.04 (t, 1H, CH, *J* = 7.0 Hz), 5.21 (t, 1H, CH, *J* = 7.0 Hz). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ (ppm): 16.46 (CH_3), 17.85 (CH_3), 25.87 (CH_3), 26.67 (CH_2), 27.66 (CH_2), 31.77 (CH_2), 39.80 (CH_2), 42.28 (CH_2), 47.36 (CH_2), 49.47 (CH_2), 122.91 (CH), 124,29 (CH), 131.67 (C), 137.67 (C). **ESI-HRMS**: *m*/*z* calculated for $C_{14}H_{29}N_2$. $[M+H]^+$ 225.2325, found 225.2333.

4.1.2.11. (Z)-N^1-(3,7-dimethylocta-2,6-dien-1-yl)butane-1,4-diamine (19). Yellow oil (yield: 0.616 g, 38%). **FTIR** (ATR, cm^{-1}): 3367.08 (υ_{as}, N—H), 3284.31 (υ_s, N—H), 1663.76 (υ, C=C), 1567.54 (δ, NH_2), 1107.20 (υ, C—N). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ_H (ppm): 1.46 (m, 4H, 2xCH_2), 1.56 (s, 3H, CH_3), 1.64 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 1.82 (s, 3H, 1NH and 1NH_2), 2.01 (m, 4H, 2xCH_2), 2.57 (t, 2H, CH_2, *J* = 6.5 Hz), 2.66 (t, 2H, CH_2, *J* = 6.5 Hz), 3.15 (d, 2H, CH_2, *J* = 6.5 Hz), 5.05 (t, 1H, CH, *J* = 7.0 Hz), 5.22 (t, 1H, CH, *J* = 7.0 Hz). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ_C (ppm): 17.81 (CH_3), 23.57 (CH_3), 25.88 (CH_3), 26.76 (CH_2), 27.65 (CH_2), 31.67 (CH_2), 32.26 (CH_2), 42.21 (CH_2), 47.12 (CH_2), 49.48 (CH_2), 123.76 (CH), 124.15 (CH), 132.01 (C), 138.09 (C). **ESI-HRMS**: *m*/*z* calculated for $C_{14}H_{29}N_2$. $[M+H]^+$ 225.2325, found 225.2328.

4.1.2.12. N^1-[(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl] butane-1,4-diamine (20). Yellow oil (yield: 0.157 g, 30%). **FTIR** (ATR, cm^{-1}): 3356.96 (υ_{as}, N—H), 3281.01 (υ_s, N—H), 1663.76 (υ, C=C), 1565.16 (δ, NH_2), 1108.60 (υ, C—N). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ (ppm): 1.49 (m, 4H, 2 x CH_2), 1.55 (s, 6H, 2xCH_3), 1.60 (s, 3H, CH_3), 1.63 (s, 3H, CH_3), 1.92 (t, 2H, CH_2), 1.96-2.07 (m, 6H, 3×CH_2), 2.60 (t, 2H, CH_2, *J* = 7.0 Hz), 2.69 (brs, 5H, CH_2, NH and NH_2), 3.21 (d, 2H, CH_2, *J* = 7.0 Hz), 5.05 (m, 2H, 2xCH), 5.22 (t, 1H, CH, *J* = 7.0 Hz). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ (ppm): 16.18 (CH_3), 16.52 (CH_3), 17.85 (CH_3), 25.86 (CH_2), 26.60 (CH_3), 26.91 (CH_2), 27.41 (CH_2), 31.19 (CH_2), 39.80 (CH_2), 39.88 (CH_2), 41.91 (CH_2), 46.98 (CH_2), 49.01 (CH_2), 121.90 (CH), 124.06 (CH), 124.48 (CH), 131.45 (C), 135.38 (C), 138.82 (C).

4.1.2.13. 1-(3-methylbut-2-en-yl)piperazine (21). Yellow oil (yield: 0.7903 g, 51%). **FTIR** (ATR, cm^{-1}): 3274.89 (υ, N—H), 1675.08 (υ, C=C), 1112 (υ, C—N). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ (ppm): 1.56 (s, 3H, CH_3), 1.64 (s, 3H, CH_3), 2.35 (s, 4H, 2×CH_2), 2.63 (1H, NH), 2.83 (t, 4H, 2xCH_2, *J* = 4.0 Hz), 2.85 (d, 2H, CH_2, *J* = 7.0 Hz), 5.16 (t, 1H, CH, *J* = 6.5 Hz). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ (ppm): 18.12 (CH_3), 26.00 (CH_3), 46.00 (2xCH_2), 54.24 (2xCH_2), 56.71 (CH_2), 120.76 (CH), 135.55 (C).

4.1.2.14. (E)-1-(3,7-dimethylocta-2,6-dien-1-yl)piperazine (22). Yellow oil (yield: 0.1833 g, 82%). **FTIR** (ATR, cm^{-1}): 3265.82 (υ, N—H), 1666.29 (υ, C=C), 1112.12 (υ, C—N). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ (ppm): 1.54 (s, 3H, CH_3), 1.58 (s, 3H, CH_3), 1.62 (s, 3H, CH_3), 1.98 (m, 2H, CH_2), 2.04 (m, 2H, CH_2), 2.39 (s, 4H, 2xCH_2), 2.58 (s, 1H, NH), 2.87 (t, 4H, 2xCH_2, *J* = 3.5 Hz), 2.91 (d, 2H, CH_2, *J* = 6.5 Hz), 5.03 (t, 1H, CH, *J* = 7), 5.20 (t, 1H, CH, *J* = 6.5). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ (ppm): 16.56 (CH_3), 17.84 (CH_3), 25.85 (CH_3), 26.57 (CH_2), 39.95 (CH_2), 46.09 (2xCH_2), 54.29 (2xCH_2), 56.73 (CH_2), 120.76 (CH), 124.29 (CH), 131.66 (C), 139.11 (C).

4.1.2.15. (Z)-1-(3,7-dimethylocta-2,6-dien-1-yl)piperazine (23). Yellow oil (yield: 1.2399 g, 46%). **FTIR** (ATR, cm^{-1}): 3265 (υ, N—H), 1666 (υ, C=C), 1029 (υ, C—N). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ_H (ppm): 1.56 (s, 3H, CH_3), 1.63 (s, 3H, CH_3), 1.68 (s, 3H, CH_3), 2.00 (m, 4H, 2xCH_2), 2.41 (s, 4H, 2xCH_2), 2.88 (m, 6H, 3xCH_2), 3.08 (s, 1H, NH), 5.05 (m, 1H, CH), 5–19 (t, 1H, CH, *J* = 7.0 Hz). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ (ppm): 17.82 (CH_3), 23.71 (CH_3), 25.87 (CH_3), 26.66 (CH_2), 32.34 (CH_2), 44.89 (2xCH_2), 54.01 (2xCH_2), 56.45 (CH_2), 121.54 (CH), 124.13 (CH), 131.95 (C), 139.36 (C).

4.1.2.16. 1-[(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl] piperazine (24). Yellow oil (yield: 0.3540 g, 40%). **FTIR** (ATR, cm^{-1}): 3397 (υ, N—H), 1666 (υ, C=C), 1110 (υ, C—N). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ (ppm): 1.54 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 1.63 (s, 3H, CH_3), 1.91 (m, 2H, CH_2), 2.03 (m, 6H, 3xCH_2), 2.43 (s, 4H, 2xCH_2), 2.91 (m, 6H, 3xCH_2), 3.31 (s, 1H, NH), 5.04 (m, 2H, 2xCH), 5.21 (t, 1H, CH, *J* = 6.5 Hz). **^{13}C NMR** ($CDCl_3$, 75 MHz) δ (ppm): 16.20 (CH_3), 16.23 (CH_3), 17.86 (CH_3), 25.87 (CH_3), 26.53 (CH_2), 26.90 (CH_2), 39.88 (CH_2), 39.95 (CH_2), 45.88 (2xCH_2), 53.89 (2xCH_2), 56.65 (CH_2), 120.57 (CH), 124.07 (CH), 124.47 (CH), 131.46 (C), 135.36 (C), 139.38 (C).

4.2. Biological assays

4.2.1. Evaluation of activity against Mycobacterium tuberculosis

All antimycobacterial tests were carried out in a laboratory with level 2 biosafety (BSL2). This level of containment is accepted by World Health Organization according to guidelines described in the "Tuberculosis Laboratory Biosafety Manual" of the WHO [14].

The antimycobacterial activities of the synthesized compounds were assessed against *M. tuberculosis* H37Rv ATCC 27294 [15] using the Micro plate Alamar Blue Assay (MABA) [16]. This methodology is nontoxic, uses thermally-stable reagents and shows good correlation with proportional and BACTEC radiometric methods [17,18]. Briefly, 200 μL of sterile deionized water were added to all outer-perimeter wells of sterile 96-well plates (Falcon, 3072: Becton Dickinson, Lincoln Park NJ) to minimize evaporation of the medium in the test wells during incubation. The 96 plates received 100 μL of the Middlebrook 7H9 broth (Difco Laboratories, Detroit, MI, USA) and a serial dilution of the compounds was made directly on the plate. The final drug concentrations tested were 3.12 to 100.0 μg/mL. Plates were covered and sealed with parafilm and incubated at 37 °C for five days. After this time, 25 μL of a freshly prepared 1:1 mixture of Alamar Blue (Accumed International, Westlake Ohio) reagent and 10% Tween 80 were added to the plate and incubated for 24 h. A blue color in the well was interpreted as absence of bacterial growth and a pink color was scored as growth. The Minimal Inhibition Concentration (MIC) was defined as the lowest drug concentration, which prevented a color change from

blue to pink after six days of incubation. Rifampicin (1.0 μg/mL) was used as the reference drug.

4.2.2. Leishmanicidal activity assay

Culture forms of *Leishmania amazonensis* (MHOM/BR/77/LOT0016) were grown at 26 °C in Schneider's insect medium at pH 7.4, supplemented with 5% heat-inactivated fetal calf serum (FCS) and 2% human urine. Promastigotes were harvested after three days of culture and 5×10^6 parasites/mL were incubated with different concentrations (2.5, 5.0, 10, 20, 40, 80, and 100 μM) of diamines in 96-well plates at 26 °C. Controls with untreated parasites (1% DMSO) or parasites with 0.1 μM Amphotericin B (standard drug) were used. After 48 h the toxic effect was evaluated by microscopic observation followed by the colorimetric MTT method as previously described by da Silva et al. [13]. Quadruplicates were run in the same microplate and the experiments were repeated at least once. The half maximal inhibitory concentration (IC_{50}) was calculated by linear interpolation.

4.2.3. Cell toxicity assay and selectivity index (SI)

3T3 fibroblast cells (ATCC CRL-2752) were seeded on 96-well plates (2×10^4 cell/well) and incubated with different concentrations (12.5, 25, 50, 100, 200, and 300 μM) of diamines in DMEM medium + 10% FCS at 37 °C, 5% CO_2 for 48 h. Cell viability was determined by the colorimetric MTT method as previously described by Sieuwerts et al. [19]. Experiments were performed in quadruplicate and repeated once. The 50% Cytotoxic Concentration (CC_{50}) values were calculated by linear interpolation and the SI was determined by the ratio CC_{50}/IC_{50}.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biopha.2016.10.112.

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