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Synthesis and *in vitro* activity of limonene derivatives against *Leishmania* and *Trypanosoma*

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ABSTRACT

The synthesis and *in vitro* activity of *R*(+)-Limonene derivatives against *Leishmania* and *Trypanosoma cruzi* strains are reported. Seven compounds have shown better *in vitro* activity against *Leishmania (V.)braziliensis* than the standard drug pentamidine. Additionally, we have identified two promising new anti-*T. cruzi* limonene derivatives.

1. Introduction

Parasitic diseases are the cause of much suffering and deaths throughout the world, mainly in developing areas like Africa, Asia and Latin America. In these regions the economic and social impact caused by these illnesses are very high. Protozoan parasites of the *Trypanosoma* and *Leishmania* genera are amongst the main morbidity and mortality causes in Latin America. Leishmaniasis is a parasitic disease affecting around 12 million people in 80 countries of the world, endangering about 350 million people living in endemic areas, particularly in the African continent, India, Middle East and Latin America [1]. Clinical manifestations include the cutaneous, mucocutaneous and visceral forms, the latter leading to a very high incidence of fatalities if the disease is left untreated. On the other hand, Chagas' disease, a chronic systemic parasitic infection caused by the protozoan parasite *Trypanosoma cruzi*, is endemic in South and Central America, Mexico and the southern United States, being considered one of the most serious parasite diseases in tropical regions [1,2]. There are currently 8 to 10 million people infected and a further 28 million, mainly in Latin America, are at risk of infection [3,4]. Typically, 30–40% of the chagasic individuals show clinical symptoms of the chronic phase associated with neuronal, cardiac and digestive dysfunctions, and at least 12,500 die every year [4,5].

In spite of their clinical importance, the therapeutic arsenal available to treat these illnesses is deficient, with most of the drugs presenting several side effects. Antimonial compounds such as meglumine antimoniate or sodium stibogluconate are still the first-choice drugs for Leishmaniasis. Second line drugs include amphotericin B and pentamidine. All these drugs share a high incidence of undesirable and potentially serious side effects. For the treatment of Chagas' disease only nifurtimox and benznidazole, both developed about 30 years ago, are available. Both of them are active against the acute stage of illness and present strong side effects.

Unfortunately, all of these treatments have significant drawbacks in terms of route of administration, length of treatment,

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toxicity and cost, which limit their use in endemic areas. For all these reasons, the search for new leishmanicidal and trypanosomicidal agents are a priority [6].

Limonene (**1**, Scheme 1) is a monoterpene and a main constituent of the essential oil of citrical plants, available in both enantiomeric forms: *R*(+) (**1**) and *S*(−)-limonene. This terpene has been used as a building block for the organic synthesis of several compounds [7–9] as well as a chiral auxiliary in asymmetric syntheses [10]. It is well known that essential oils containing this terpene can present anti-microbial [11], anti-fungal [12], anti-malarial [13] and anti-tumoral action [14]. The action mechanisms involved are unclear, but for *P. falciparum* it was associated with inhibition of dolichol and ubiquinone biosynthesis and reduction in protein isoprenylation [13]. Limonene has two double bounds and one of them or even the two insaturations can be responsible for the interaction with receptor. In a recent work, Arruda et al. [15] showed that limonene was effective in *in vitro* and *in vivo* assays against *Leishmania* species with an IC_{50} of 185 μM in the MTT test. Ferrarini et al. have stereoselectively synthesized several limonene β-aminoalcohol derivatives at the cyclic double bond and evaluated its leishmanicidal activity against isolated parasites. They observed that the inhibitory action against promastigotes is greater than of the standard drug pentamidine, and two of the synthesized compounds were found to be 100-fold more potent. In this test, limonene has not shown any activity [16]. This same library showed a good activity against both larvae and eggs of *Rhipicephalus (Boophilus) microplus* [17], indicating the high potentiality of these compounds. These results have encouraged new investigations involving limonene derivatives with an amino group at isoprene (exocyclic double bond) group. Our group has recently published the synthesis of some limonene derivatives from one-pot tandem hydroformylation/reductive amination (also known as hydroaminomethylation) [18]. The hydroaminomethylation is a versatile reaction presenting a very high atom economy. In this process three reactions (hydroformylation, imine/enamine formation and its reduction) occur in a tandem form, where the reduction (hydrogenation) step is catalyzed by the hydroformylation catalyst. In our previous work [18] we have obtained the amine derivatives **2**–**8** (Scheme 1) in about 10–29 h of reaction time, depending of the amine used as substrate. In this present work, we synthesized seven new compounds in order to evaluate if the structural modifications at isoprenyl chain contribute to the pharmacological effect; so, we examined this series searching for anti-leishmania and anti-trypanosoma activities.

2. Chemistry

Using the same protocol from the previous report [18], extending only the hydrogenation step time (24–60 h, depending on the amine used as substrate) we were able to synthesize new aromatic derivatives (**9**–**14**, see Scheme 1). For this purpose, we have selected aniline derivatives, furfurylamine and 2-aminopyridine as amine substrates. All synthesized compounds (including the previously synthesized ones **2**–**8**) were obtained as a 50:50 mixture of diastereomers, according to the performed GC-FID and NMR analyses [18].

Ethanolamine derivative **16** was obtained by the use of a classic reductive amination protocol, employing the aldehyde derivative **15**, ethanolamine and $NaCNBH_3$ as reducing agent. The sulphonamide **17** was obtained from a direct tosylation [19] of *n*-propyl derivative **2** (Scheme 2).

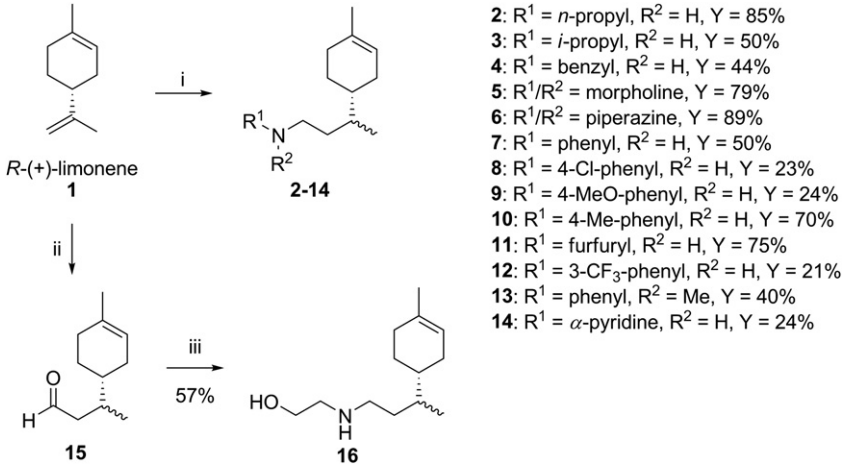
3. Results and discussion

3.1. L. (V.) braziliensis (promastigote) assay

In order to determine the leishmanicidal activity of the synthesized compounds, they were tested against *in vitro* cultures of the promastigotes of *Leishmania (Viannia) braziliensis* [20] employing pentamidine as a standard drug. The results are gathered in Table 1 and they show that, except for compound **5**, the introduction of amine groups in the limonene (**1**) scaffold have led to more active compounds. Seven of the fifteen compounds (**2**, **7**, **10**, **11**, **14**, **16** and **17**) tested in this assay presented a lower IC_{50} value than pentamidine. Compounds **3**, **4** and **6** showed low activity while **1** was found to be inactive against the parasite, as well as the morpholine derivative **5**.

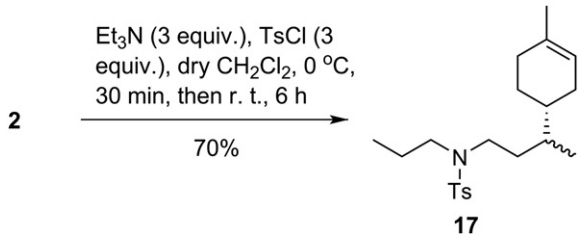
Comparing **2** with **3**, it seems that increasing the volume of the alkyl group R decreases the activity of the compound. The good activity of the aminoalcohol **16** indicates that polarity is not a relevant factor to the activity of the compounds. Six-member cycles like the ones in **5** (morpholine) and **6** (piperazine) are not good for the activity of the compounds, probably due to the same steric factors affecting the activity of **3**.

As we have previously observed for aminoalcohols limonene derivatives [16], in the present work the aromatic derivative **7** is the most active compound of the series. Variations at the benzene ring of this compound have lead to **8**, **9**, **10**, **12**, **13** and **14** with lower activities. The more bulky benzyl derivative **4** is about 22 fold less



2: R^1 = *n*-propyl, R^2 = H, Y = 85%
3: R^1 = *i*-propyl, R^2 = H, Y = 50%
4: R^1 = benzyl, R^2 = H, Y = 44%
5: R^1/R^2 = morpholine, Y = 79%
6: R^1/R^2 = piperazine, Y = 89%
7: R^1 = phenyl, R^2 = H, Y = 50%
8: R^1 = 4-Cl-phenyl, R^2 = H, Y = 23%
9: R^1 = 4-MeO-phenyl, R^2 = H, Y = 24%
10: R^1 = 4-Me-phenyl, R^2 = H, Y = 70%
11: R^1 = furfuryl, R^2 = H, Y = 75%
12: R^1 = 3-CF_3-phenyl, R^2 = H, Y = 21%
13: R^1 = phenyl, R^2 = Me, Y = 40%
14: R^1 = α-pyridine, R^2 = H, Y = 24%

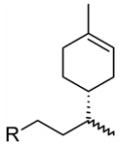
Scheme 1. Conditions: (i) $HRhCO(PPh_3)_3$ (cat.), CO (20 bar), H_2 (20 bar), amine substrate, THF, 100 °C, 5–24 h, then H_2 (40 bar), 5–48 h; (ii) $HRhCO(PPh_3)_3$ (cat.), CO (20 bar), H_2 (20 bar), THF, 100 °C, 5 h; (iii) ethanolamine (4 equiv.), AcOH (cat.), CH_2Cl_2, r. t., 5 h, then $NaCNBH_3$ (3 equiv), 24 h, r. t.



Scheme 2. Synthesis of derivative **17**.

active than **7**, indicating that the volume of the compounds is important to the activity, although replacing the phenyl ring of **4** for a furane ring in **11** results in a more active compound. The sulphonamide **17** seems to be an exception to this volume/activity "rule", presenting an activity similar to **7**.

Table 1
L. braziliensis IC_{50} of the synthesized products **1**–**14**, **16** and **17**.



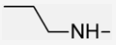
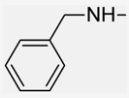
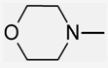
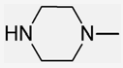
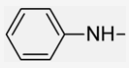
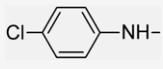
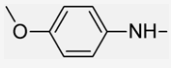
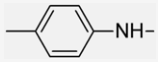
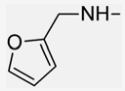
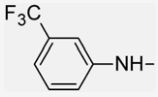
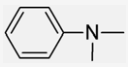
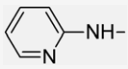
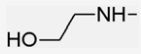
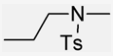
Product	R	IC_{50} (μM)[a]
1 (limonene)	–	876.2 ± 216
2		17.2 ± 0.9
3		253.6 ± 33.9
4		257.9 ± 22.5
5		1027 ± 138.6
6		269.5 ± 38.9
7		11.5 ± 0.8
8		58.4 ± 5.1
9		57.6 ± 7.3

Table 1 (*continued*)

Product	R	IC_{50} (μM)[a]
10		28.0 ± 1.2
11		35.6 ± 1.6
12		84.6 ± 2.6
13		54.5 ± 14.8
14		27.0 ± 2.9
16		23.2 ± 1.9
17		12.1 ± 0.3
Pentamidine	–	48.5 ± 28.7

[a] IC_{50} Values are means ± standard deviation of three experiments.

3.2. *L. amazonensis* and cytotoxicity assay

The four most active compounds against *L. braziliensis* promastigotes (**2**, **7**, **16** and **17**) were also tested against *L. amazonensis* promastigotes and their cytotoxicity was assayed against the Rhesus monkey kidney cell line (LLCMK2) [21]. These results are detailed in Table 2 and show that derivative **2** was the most active compound against *L. amazonensis*. Additionally, all of the derivatives studied presented low toxicity against LLCMK2 cell line (at least 3 times greater IC_{50} values for mammalian cells compared to *L. amazonensis*, see selectivity indexes in Table 2) and 10–11 times greater than *L. (V.) braziliensis* IC_{50} values. When compared to the anti-*T. cruzi* activity, the selectivity indexes, specially for derivative **7** (SI = 10.4) were also high.

3.3. Anti-Trypanosoma cruzi assay

Selected compounds, **2**, **4**–**7**, **11**, **13** and **16**, were also tested against *in vitro* cultures of *T. cruzi* epimastigotes (Tulahuen 2 strain) [22]. The results (Table 3) show that, compounds **6** and **7** are excellent hits as anti-*T. cruzi* agents for further structural

Table 2
In vitro L. amazonensis (promastigote form) and LLCMK2 IC_{50} assay results.

Product	*L. amazonensis* IC_{50} 48 h (μM)	LLCMK2 IC_{50} 48 h (μM)	SI[a]
2	53.54 ± 6.88	192.06 ± 72.99	3.6
7	89.75 ± 22.32	312.43 ± 134.98	3.5 (10.4[b])
16	173.97 ± 33.02	586.86 ± 341.84	3.4
17	195.75 ± 51.73	>1012	>5.2

[a] SI: selectivity index: $IC_{50,LLCMK2}/IC_{50,L.\ amazonensis}$
[b] $IC_{50,LLCMK2}/IC_{50,T.\ cruzi}$.

Table 3
In vitro activity of limonene derivatives against *T. cruzi* Tulahuen 2 strain.

Product	GI (%)[a,b]	IC_{50} (μM)[b]
1 (limonene)	0.0	>50.0
2	21.0	>50.0
4	53.5	50.0
5	20.7	>50.0
6	82.4	19.2
7	70.5	29.5
11	51.4	50.0
13	0.0	>50.0
16	41.8	>50.0
Nifurtimox	>100.0	7.7

[a] Growth inhibition (%) of the given parasite at 50 μM.
[b] Values are means ± standard deviation of three experiments.

modifications, showing IC_{50} values in the order of the standard drug Nifurtimox. Contrasting to the anti-*L. braziliensis* assay findings, compounds **2** and **11**–**16** are inactive against *T. cruzi*, being **6** the most active of them. In a structural point of view, chemical changes on the parent compound conducted to active compounds (comparing activities of parent compound **1** with the synthesized derivatives). Unfortunately, we were unable to establish a structure–activity relationship with this library.

4. Conclusion

In conclusion, we have synthesized seven new compounds with good *in vitro* activity against *L. (V.) braziliensis*, in an easy, fast and scalable one-pot three step synthetic protocol, using a natural and easily available starting material. The four most active compounds in this assay also presented activity against the promastigote form of *L. amazonensis* and low toxicity against LLCMK2 cell line. Moreover, new anti-*T. cruzi* limonene derivatives with adequate selectivity indexes (compounds **6** and **7**) have been identified.

New assays aiming at elucidating these compounds anti-parasitic mechanisms of action are being prepared and will be reported elsewhere.

5. Experimental protocols

5.1. General experimental procedures

^1H and ^{13}C NMR spectra were obtained in a INOVA-300 spectrophotometer with standard pulse sequences operating at 300 MHz in ^1H NMR and 75 MHz in ^{13}C NMR, using $CDCl_3$ as solvent. Chemical shifts are reported as δ values (ppm) relative to TMS (0.0 ppm). Gas chromatography was performed in a Shimadzu model GC-17A instrument with FID detector and equipped with a DB-5 (30 m × 0.25 mm) column. The carrier gas was hydrogen with a flux of 1.1 mL/min. The following method was used: sample injection = 0.5 μL; initial column temperature = 50 °C; heating rate = 10 °C/min; final temperature = 250 °C; final temperature hold = 10 min (total method time = 30 min). Mass spectrometry was performed in a Shimadzu model CGMS-QP5050 with resolution range from 45 to 400 Da, in SCAN mode (70 eV), coupled with a Shimadzu model GC-17A gas chromatograph equipped with a DB-17MS (30 m × 0.25 mm) column. The carrier gas was Helium with a flux of 1.4 mL/min. The method was the same as detailed in the gas chromatography. The mass spectrometry results are reported as the *m*/*z* ratio with the respective relative abundance in parenthesis. The M^+ symbol indicates that the peak is the molecular ion of the compound.

5.2. Synthetic protocols

The procedure for the synthesis of compounds **2**–**7** was reported on ref. 18. Protocols for compounds **8**–**17** are reported below.

5.2.1. General method for the synthesis of amines *8*–*14*

A stainless steel autoclave reactor was charged with a mixture of limonene **1** (1.0 mL, 1.19 g, 8.73 mmol), $HRh(CO)(PPh_3)_3$ (11.48 mg, 0.0125 mmol, 0.143 mol%),THF (10 mL) and amine substrate under argon atmosphere. The reactor was purged and charged with 20 bar of H_2 and 20 bar of CO (*CAUTION! Carbon monoxide is a very toxic gas and its handling must be done in a well-ventilated hood*) and heated over a heating plate with magnetic stirring and a silicon oil bath at 100 °C for 5 h. After this time the reactor was cooled, depressurized, purged and pressurized with 40 bar of H_2, and heated in the same conditions as above for some time (required time for each amine is detailed below). The reactor was cooled, depressurized and the reaction mixture was eluted in a tiny silica-gel column for the remotion of the catalyst. The solvent and the lightweight components of the mixture (limonene, isomerizated products) were removed under reduced pressure.

5.2.2. 4-Chloro-N-[3-(4-methylcyclohexen-3-yl)-butyl]-aniline *8*

Amine substrate: 4-chloroaniline (1.114 g, 8.73 mmol). Hydrogenation step time: 20 h. The product was isolated by frac. distillation. Yield: 23%. Mass spectrometry: 277.20 (M^+, 8.27). ^1H NMR (300 MHz, $CDCl_3$): 0.9 (3H, m), 1.3 (4H, m), 1.65 (3H, s), 1.75 (3H, m), 1.98 (3H, m), 3.08 (2H), 5.38 (1H, s), 6.49 (2H, d, $J = 8.6$ Hz), 7.09 (2H, d, $J = 8.6$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 15.7, 16.2, 23.4, 25.2, 26.9, 27.3, 29.2, 30.7, 30.8, 35.0, 35.1, 38.1, 38.3, 42.8, 113.6, 120.7, 120.8, 128.9, 133.9, 147.

5.2.3. 4-Methoxy-N-[3-(4-methylcyclohexen-3-yl)-butyl]-aniline *9*

Amine substrate: anisidine (1.183 g, 9.6 mmol). Hydrogenation step time: 24 h. The product was isolated by frac. distillation followed by acid-base extraction. Yield: 24%. Mass spectrometry: 273.40 (M^+, 9.12). ^1H NMR: (300 MHz, $CDCl_3$): 0.9 (3H, m), 1.3 (4H, m), 1.65 (3H, s), 1.75 (3H), 1.98 (3H, m), 3.08 (2H, m), 3.65 (3H, s), 5.38 (1H, s), 6.5 (2H, d, $J = 8.8$ Hz), 6.73 (2H, d, $J = 8.8$ Hz). ^{13}C NMR: (75 MHz, $CDCl_3$): 15.7, 16.2, 23.4, 25.2, 26.9, 27.3, 29.2, 30.7, 30.8, 35.0, 35.1, 38.1, 38.3, 42.8, 48.0, 114.9, 115.0, 115.5, 115.8, 119.8, 134.0, 142.8, 152.0.

5.2.4. 4-Methyl-N-[3-(4-methylcyclohexen-3-yl)-butyl]-aniline *10*

Amine substrate: 4 toluidine (1.029 g, 9.6 mmol). Hydrogenation step time: 48 h. The product was isolated by frac. dstillation. Yield: 70%. Mass spectrometry: 257.30 (M^+, 13.48). ^1H NMR: (300 MHz, $CDCl_3$): 0.9 (3H, m), 1.3 (4H, m), 1.65 (3H, s), 1.75 (3H, m), 1.98 (3H, m), 2.18 (3H, s), 2.42 (1H, s), 3.08 (2H, m), 5.39 (1H, s), 6.45 (2H, d, $J = 7.0$ Hz), 6.9 (2H, d, $J = 7.0$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 15.7, 16.2, 20.3, 23.4, 25.2, 26.9, 27.3, 29.2, 30.7, 30.8, 35.1, 35.2, 38.1, 38.3, 42.8, 113.0, 121.0, 126.0, 129.8, 134.0, 146.4.

5.2.5. 3-(4-Methylcyclohexen-3-yl)-N-furfuryl-1-butanamine *11*

Amine substrate: furfurylamine (0.9 mL, 9.6 mmol). Hydrogenation step time: 22 h. The product was isolated by frac. distillation. Yield: 75%. Mass spectrometry: 247.25 (M^+, 6.28). ^1H NMR (300 MHz, $CDCl_3$): 0.9 (3H, m), 1.3 (4H, m), 1.65 (3H, s), 1.75 (3H, m), 1.98 (3H, m), 2.55 (3H, s), 3.7 (2H, s), 5.39 (1H, s), 6.1 (1H, s), 6.23 (1H, s), 7.25 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$): 15.7, 16.2, 23.4, 25.2, 26.9, 27.3, 29.2, 30.7, 30.8, 35.1, 35.2, 38.1, 38.3, 46.0, 47.5, 108.5, 110.0, 121.0, 134.0, 141.7, 154.1.

5.2.6. 3-Trifluoromethyl-N-[3-(4-methylcyclohexen-3-yl)-butyl]-aniline *12*

Amine substrate: 3-trifluoromethylaniline (1.2 mL, 9.6 mmol). Hydrogenation step time: 48 h. The product was isolated by frac.

distillation. Yield: 21%. Mass spectrometry: 311.20 (M^+, 7.75). ^{1}H NMR (300 MHz, $CDCl_3$): 0.9 (3H, m), 1.3 (4H, m), 1.65 (3H, s), 1.75 (3H, m), 1.98 (3H, m), 3.05 (2H, m), 3.65 (1H, s), 5.38 (1H, s), 6.65 (1H, m), 6.81 (2H, m), 7.18 (1H, m). ^{13}C NMR: (75 MHz, $CDCl_3$): 15.7, 16.2, 23.4, 25.2, 26.9, 27.3, 29.2, 30.7, 30.8, 35.0, 35.1, 38.1, 38.3, 42.0, 108.5, 113.5, 115.8, 120.8, 120.9, 129.9, 132.0, 134.0, 148.5.

5.2.7. N-methyl-N-[3-(4-methylcyclohexen-3-yl)-butyl]-aniline ***13***

Amine substrate: N-methylaniline (1.05 mL, 9.6 mmol). Hydrogenation step time: 22 h. The product was isolated by frac. distillation. Yield: 40%. Mass spectrometry: 257.35 (M^+, 13.67). ^{1}H NMR: (300 MHz, $CDCl_3$): 0.9 (3H, m), 1.3 (4H, m), 1.65 (3H, s), 1.75 (3H, m), 1.98 (3H, m), 2.85 (3H, s), 3.15 (2H, m), 5.38 (1H, s), 6.6 (3H, m), 7.15 (2H, m). ^{13}C NMR: (75 MHz, $CDCl_3$): 15.7, 16.2, 23.4, 25.2, 26.9, 27.3, 29.2, 30.7, 30.8, 35.0, 35.1, 38.1, 38.3, 39.0, 51.5, 112.0, 115.7, 120.9, 129.0, 134.0, 149.2.

5.2.8. 2-{N-[3-(4-methylcyclohexen-3-yl)-butyl]}-aminopyridine ***14***

Amine substrate: 2-aminopyridine (0.904 g, 9.6 mmol). Hydrogenation step time: 24 h. The product was isolated by frac. distillation. Yield: 24%. Mass spectrometry: 244.30 (M^+, 24.52). ^{1}H NMR (300 MHz, $CDCl_3$): 0.9 (3H, m), 1.3 (4H, m), 1.65 (3H, s), 1.75 (3H, m), 1.98 (3H, m), 3.2 (2H, m), 4.45 (1H, s), 5.38 (1H, s), 6.4 (2H, m), 7.35 (1H, m), 8.0 (1H, m). ^{13}C NMR (75 MHz, CDCl3, ppm): 15.7, 16.2, 23.4, 25.2, 26.9, 27.3, 29.2, 30.7, 30.8, 35.0, 35.1, 38.1, 38.3, 40.5, 106.2, 112.3, 121.0, 134.0, 137.7, 150.0, 158.8.

5.2.9. 2-{[3-(4-Methylcyclohexen-3-yl)-butyl]-amino}-ethanol ***16***

A two-neck rounded flask was charged, under argon, with aldehyde **15** (300 mg, 1.804 mmol), ethanolamine (0.17 mL, 2.71 mmol), one drop of conc. sulfuric acid and 5 mL of ethanol. The flask was kept at room temperature, under vigorous stirring, for 4 h, when sodium cyanoborohydride (340 mg, 5.412 mmol) was added. The stirring was maintained for more 22 h. The solvent was removed under reduced pressure and the crude reside was filtered and washed with cold Et_2O. The filtrate was dried over sodium sulfate and the solvent was removed under reduced pressure. The product was isolated by frac. distillation. Yield: 57%. Mass spectrometry: 211.25 (M^+, 1.15). ^{1}H NMR: (300 MHz, $CDCl_3$): 0.9 (3H, m), 1.3 (4H, m), 1.65 (3H, s), 1.75 (3H, m), 1.98 (3H, m), 3.05 (4H, m), 3.8 (2H, m), 4.9 (2H, s), 5.38 (1H, s). ^{13}C NMR: (75 MHz, $CDCl_3$): 15.7, 16.2, 23.4, 25.2, 26.9, 27.3, 29.2, 30.7, 30.8, 35.0, 35.1, 38.1, 38.3, 47.0, 51.0, 58.5, 120.9, 134.0.

5.2.10. N-propyl-N-[3-(4-methylcyclohexen-3-yl)-butyl]-4-metylbenzene-1-sulphonamide ***17***

A two-neck rounded flask was charged, under argon, with 3 mL of CH_2Cl_2, 150 mg of amine **2** (0.717 mmol) and Et_3N (146 mg, 1.434 mmol) in an ice bath, when *p*-toluenesulphonyl chloride (302 mg, 1.58 mmol) was added in small portions, over a 10 min time span. Thirty minutes later, the ice bath was removed and the reaction was carried for additional 4 h. After this time the solvent was removed in a rotatory evaporator and the crude residue was dissolved in ethyl acetate and washed with 12 portions of water (10 mL each). The organic layer was dried over sodium sulfate, filtered and the solvent was removed under reduced pressure. The product was isolated by frac. distillation. Yield: 44%. ^{1}H NMR (300 MHz, $CDCl_3$): 0.85 (3H, m), 0.92 (3H, t, $J = 7.48$ Hz), 1.35 (4H, m), 1.5 (4H, m, $J = 7.5$ Hz), 1.65 (3H, s), 1.95 (4H, s), 2.4 (3H, s), 3.1 (4H, m), 5.38 (1H, s), 7.3 (2H, m), 7.7 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): 11.2, 15.7, 16.1, 21.4, 21.9, 23.4, 25.3, 26.9, 27.4, 29.2, 30.7, 30.8, 34.8, 35.0, 38.1, 38.3, 46.6, 49.8, 120.8, 127.1, 129.5, 134.0, 137.1, 142.0.

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