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Synthesis of Chiral 3-[[(Aryl) methyl] amino]- and 3-[[(Heteroaryl)-methyl]-amino]-quinuclidines with High Biological Activity against Intracellular *Trypansoma cruzi* Amastigotes

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A B S T R A C T

Inhibition of the sterol biosynthesis is a highly attractive strategy for chemotherapy of Trypanosoma cruzi, because this protozoa mainly depends on endogenous ergosterol. Various racemic, but only a few chiral quinuclidines were proven to be strong inhibitors of ergosterol biosynthesis in protozoa and fungi. In this study we describe the preparation of chiral 3-[[(aryl-) methyl] amino]- and 3-[[(heteroaryl)methyl]-amino]quinuclidines and their evaluation as inhibitors of intracellular Trypanosoma cruzi amastigotes with the aim to explore their potential for the development of new drugs against the Chagas disease. Main objective was to study the influence of the absolute configuration of the stereo center at C-3 on the biological activity on Trypanosoma cruzi. Most compounds showed a high biological activity against growth of intracellular Trypanosoma cruzi amastigotes. This is the first study comparing the biological activity of enantiomers of selected chiral 3-[[(aryl) methyl] amino]- and 3-[[(heteroaryl)methyl]amino]quinuclidines on Trypanosoma cruzi intracellular amastigotes. (S)-enantiomers exhibited a higher biological activity against this pathogenic protozoa as (R)-enantiomers. All compounds were highly selective towards the parasite and showed low cytotoxicity to host cells.

Keywords: Chiral Arylquinuclidines, Trypanosoma cruzi, Intracellular Amastigotes.

A R T I C L E I N F O

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1. Introduction

Chagas disease is caused by the pathogenic protozoa *Trypanosoma cruzi*. The disease causes high rates of mortality and morbidity, especially in tropical regions of Latin America. The current drugs available to treat the disease suffer from limited clinical efficacy and toxic side effects[1]. The enzymes of the sterol pathway are attractive targets for the specific treatment of Chagas disease, because the aethiologic agent of this disease requires endogenous ergosterol and other 24-alkylated sterols for growth and survival and are unable to use the abundant supply of cholesterol present in mammalian host [5]. The ergosterol biosynthesis of *Trypanosoma cruzi* and its importance for current drug design and development of novel drugs against the Chagas disease has been recently reviewed by Julio Urbina[2].The *squalene synthase* (SQS), which catalyzes the condensation of two moles of farnesyl pyrophosphate to squalene (Figure 1), the first committed step of the sterol pathway, became an attractive target[3,4] in Medicinal Chemistry, because SQS-inhibitors were expected to lower cholesterol levels in man without causing the same side effects observed for the well known HMG-CoA-reductase inhibitors [3,4]. Briefly later various aryl- and heteroaryl quinuclidines were found to be strong inhibitors of protozoal *squalene synthase* (SQS) and growth of pathogenic tropical protozoa[5-9] *Leishmania sp.*, *Trypanosoma sp.* and fungi *Candida albicans*, *Candida tropicalis* and *Candida parapsilopsis*[10]. Most ergosterol biosynthesis inhibitors exhibit a high biological activity and low cytotoxicity. Julio Urbina demonstrated, that two known *human* SQS inhibitors with chiral heteroaryl quinuclidine structure exhibit a high antitrypanosomal activity *in vitro* and have a high curative effect on Chagas disease in a small animal model [11]. Only a few examples of chiral aryl- and hetero aryl quinuclidines with biological activity on pathogenic protozoa were reported by Julio Urbina and us [8, 11]. G. R. Brown [4] described only one example of a *(rac)*-3-[[(aryl) methyl] amino]-quinuclidine with biological activity on the human enzyme (SQS), but it has never been tested for biological activity on tropical parasites. Therefore we planned to prepare a small library of chiral 3-[[(aryl) methyl] amino]-quinuclidines & 3-[[(heteroaryl)-methyl]-amino]-quinuclidines and test their activity on *T. cruzi* in order to get some structure activity relationships (SAR) with main focus on stereochemistry at C-3.

2. Experimental

Chemistry

Compounds were purified by flash column chromatography or normal column chromatography on silica gel $F_{256,}$ Merck Darmstadt, 60 - 230 Mesh or 230 – 400 Mesh. Solvents were purchased by *Synth* (Productos para laboratory Ltd., São Paulo) or by Sigma- Aldrich in analytical grade quality and used without further purification. For thin layer chromatography TLC Silica gel 60 plates with fluorescence indicator F_{245} of Merck (Darmstadt) were utilized. Compounds with no UV absorbtion were detected by potassium permanganate/potassium carbonate reagent (2 % $KMnO_4$, 2 % K_2CO_3 in H_2O). Biphenyl carboxaldehyde **5a** was purchased from Sigma-Aldrich. Compounds (R,S)-**3** [12], (S,R)-**3**[12], (R)-**4**[12], (S)-**4**[12] and **5 b**[14] were prepared according to known procedures. ^{1}H-NMR- and ^{13}C-NMR- and DEPT-spectroscopy was performed on Bruker advanced 400, 500 and 600 MHz-NMR-spectrometers. IR-spectroscopy was performed on a FT-IR-spectrometer ABB Bomem 100. UV-spectroscopy was performed on an Agilent 8453 UV-spectrometer. The specific optical rotation was determined on a Perkin-Elmer Model 341 polarimeter. Melting points were determined on a melting-point-apparatus Fisher Scientific IA 9000. Mass spectroscopy and High Resolution Mass Spectroscopy was performed on a Leco Citius LC-HRT Mass spectrometer by Thompson Mass Spectroscopy Laboratory of IQ-UNICAMP. Anhydrous solvents were prepared by drying commercial solvents utilizing known standard methods (toluene was dried by distillation over Na, methylene chloride by distillation over CaH_2, methanol was dried by molecular sieve MS 4 Å).

(R)- and (S)-**4** were prepared by a known method[12] of asymmetric synthesis reported by Michel Langlois[12] starting from quinuclidine-3-one **1** and (R)- or (S)-phenyl ethylamine. (R)- and (S)-**6 a, b** were prepared by reductive amination[13] of aryl- or heteroaryl carboxaldehydes **5a, b** with (R)- or (S)-3-aminoquinuclidine (R)- and (S)-**4**[12] according to known methods [13]. In some cases a two step procedure has been applied. At first the imines were prepared by heating **5 b** [14] with (R)- or (S)-**4** [12] for five hours in toluene on a Dean Stark apparatus (azeotropic removal of water). Removal of the toluene and subsequent reduction of the crude imine with excess $NaBH_4$ in methanol at room temperature for 5 h furnished (R)- and (S)-**6 b** (Scheme 1).

(S)&(R)-3-[[(Biphenyl-4-yl)methyl]amino]-quinuclidine: (S)-**6a** and (R)-**6a:** To a solution of (S)- or (R)-3-aminoquinuclidine dihydrochloride[12] (80 mg, 0,4 mmol) in 4 mL ethanol is added anhydrous sodium carbonate (424 mg, 4 mmol). The mixture is stirred for one hour at room temperature and then filtered through a small layer of celite. To the filtrate is added 4-biphenylcarboxaldehyde (55 mg, 0.3 mmol) and the reaction mixture is stirred for two hours with molecular sieve 4 Å (100 mg). Then, sodium boranate (12 mg, 0,21mmol) is added and the reaction mixture is stirred for 24 hours at room temperature. The solvent is removed by evaporation under reduced pressure and the

residue is purified by chromatography on silica gel with chloroform : methanol : ammonia = 90: 9 : 1 as an eluent. The product is obtained as a beige colored solid.

(S)3-[[(Biphenyl-4-yl)methyl]amino]-quinuclidine(S)-6a: Yield : 50 mg (43 %),**[~]^{D} (20 ° C) =** -35 ° (c = 1, $CHCl_3$), **M.P.:** 96-98 °C, **^{1}H-NMR ($CDCl_3$, 500 MHz):** δ-Z-1,35-1,44 (m, 1H); 1,46-1,54 (m, 1H); 1,67-1,76 (m, 1H); 1,86-2,02 (m, 2H); 2,46-2,56 (m, 1H); 2,73-2,89 (m, 4H); 2,90-3,00 (m, 1H); 3,13-3,21 (m, 1H); 3,74-3,86 (m, 2H); 7,30-7,38 (m, 1H); 7,39-7,49 (m, 4 H), 7,54-7,64 (m, 4 H) ppm.**^{13}C-NMR ($CDCl_3$, 500 MHz):**δ = 19,9 (CH_2); 25,1 (CH); 26,0 (CH_2); 47,0 (CH_2); 47,8 (CH_2); 51,2 (CH_2); 54,4 (CH); 56,7 (CH_2); 127,08 (2 CH); 127,21 (2 CH); 128,6 (3 CH); 128,8 (2 CH); 139,7 (C); 139,9 (C); 141 (C) ppm.**IR (KBr):**ν = 3432; 2919; 1486; 1384; 824; 760; 697 cm^{-1}.**MS (ESI) m/z (%):** 293,3 (13); 167,1 (100).**HRMS (ESI, M+1):** found = 293,20140; calculated = 293,20177.

(R)-3-[[(Biphenyl-4-yl)methyl]amino]-quinuclidine(R)6a Yield : 40 mg (35 %), **[~]^{D} (20 ° C) =** +34 ° (c = 1, $CHCl_3$), **M.P. =** 96-98 °C, **^{1}H-NMR ($CDCl_3$, 500 MHz):** δ Z-1,35-1,43 (m, 1H); 1,45-1,53 (m, 1H); 1,65-1,75 (m, 1H); 1,87-1,99 (m, 2H); 2,46-2,54 (m, 1H); 2,72-2,88 (m, 4H); 2,89-2,97 (m, 1H); 3,13-3,20 (m, 1H); 3,73-3,85 (m, 2H); 7,29-7,37 (m, 1H); 7,40-7,48 (m, 4H); 7,55-7,65 (m, 4H) ppm, **^{13}C-NMR ($CDCl_3$, 500 MHz):**δ = 20,2 (CH_2); 25,0 (CH); 26,0 (CH_2); 47,0 (CH_2); 47,7 (CH_2); 51,3 (CH_2); 54,3 (CH); 56,7 (CH_2); 127,08 (2 CH); 127,22 (2 CH); 128,56 (3 CH); 128,77 (2 CH); 139,68 (C); 139,98 (C); 140,99 (C) ppm.**IR (KBr):**ν = 3443; 2913; 1637; 1486; 1384; 837; 759; 690 cm^{-1}.**MS (ESI) :**m/z (%) = 293,3 (15); 167,1 (100).**HRMS (ESI, M+1):** found = 293,20150; calculated = 293,20177.

(R)&(S)-3-[[[4-(2-Benzoxazolyl) phenyl] methyl] amino] - quinuclidine: (S)-and (R)-**6 b**

A mixture of 60 mg (0,3mmol) (S)- or (R)-3-aminoquinuclidine dihydrochloride[12] (is added to a concentrated solution of sodium hydroxide (50%; 500 mg, 8,91 mmol) in water. The mixture is extracted with chloroform (20 x 2 mL). The organic phases are dried with anhydrous sodium sulfate, filtered through a small layer of celite , and evaporated in vacuo. The residue is dissolved together with 30 mg (0,1mmol) 4-(benzoxazolyl)-benzaldehyde [14] in 50 mL of toluene and heated on a Dean-Stark trap for 5 h, water is removed continuously by azeotropic distillation. Excess toluene is removed in vacuo and the residue is dissolved in 20 ml of dry methanol. To the solution is added 30 mg (0,7 mmol) sodium borohydride at 0°C under stirring. The mixture is stirred at 0 ° C for 1 h and at 20 ° C for 14 h. Then the solvent is removed by evaporation under reduced pressure and the residue is purified by column chromatography on SiO_2 with chloroform: methanol: ammonium hydroxide = 9: 1: 0.1.

(S)-3-[[[4-(2-Benzoxazolyl)phenyl]methyl]amino]-quinuclidine :(S)-**6 b**

Yield : 25 mg (62 %), **[~]^{D} (20 ° C) =** -29 ° (c = 1, $CHCl_3$), **M.P. :** 152-153 °C, **^{1}H-NMR ($CDCl_3$, 500 MHz):**δ = 1,37-1,45 (m, 1H); 1,46-1,55 (m, 1H); 1,68-1,76 (m, 1H); 1,87-2,07 (m, 2H); 2,49-2,56 (m, 1H); 2,72-2,90 (m, 4H); 2,91-3,00 (m, 1H); 3,15-3,22 (m, 1H); 3,81-3,90 (m, 2H); 7,34-7,38 (m, 2H); 7,51 (d, J= 8Hz, 2H); 7,58-7,61 (m, 1H); 7,76-7,80 (m, 1H); 8,23 (d, J= 8 Hz, 2H) ppm.**^{13}C-NMR ($CDCl_3$, 500 MHz):**δ = 19,6 (CH_2); 24,9 (CH); 25,8 (CH_2); 46,7 (CH_2); 47,4 (CH_2); 51,3 (CH_2); 54,2 (CH); 56,7 (CH_2); 110,3 (CH); 119,8 (CH); 124,3 (CH); 124,9 (CH); 125,8 (C); 127,6 (2 CH); 128,3 (2 CH); 142 (C); 144,3 (C); 150,4 (C); 162,9 (C) ppm.**IR (KBr):**ν = 3448; 3212; 2924; 2861, 1620, 1555, 1499, 1453, 1384, 1243, 1053, 1017, 827, 761, 746 cm^{-1}.**MS (ESI)** : m/z (%) = 334 (3); 317 (6), 289 (3), 208 (100).**HRMS (ESI, M+1):** found = 334,19154; calculated = 334,19194.

(R)-3-[[[4-(2-Benzoxazolyl)phenyl]methyl]amino]-quinuclidine :(R)-**6 b**

Yield : 30 mg (74 %), **[~]^{D} (20 ° C) =** +28 ° (c = 1, $CHCl_3$), **M.P.:** 152-153 °C, **^{1}H-NMR ($CDCl_3$, 500 MHz):** δ =1,35-1,44 (m, 1H); 1,45-1,55 (m, 1H); 1,67-1,77 (m, 1H); 1,86-2,00 (m, 2H); 2,47-2,55 (m, 1H); 2,71-2,89 (m, 4H); 2,91-2,99 (m, 1H); 3,14-3,20 (m, 1H); 3,79-3,90 (m, 2H); 7,34-7,38 (m, 2H); 7,51 (d, J= 8Hz, 2H); 7,57-7,61 (m, 1H); 7,76-7,80 (m, 1H); 8,23 (d, J= 8 Hz, 2H) ppm, **^{13}C-NMR ($CDCl_3$, 500 MHz):** δ =19,9 (CH_2); 25,2 (CH); 25,9 (CH_2); 47,0 (CH_2); 47,6 (CH_2); 51,3 (CH_2); 54,5 (CH); 56,6 (CH_2); 110,5 (CH); 119,9 (CH); 124,6 (CH); 125,0 (CH); 126,0 (C); 127,8 (2 CH); 128,3 (2 CH); 142,1 (C); 144,6 (C); 150,8 (C); 163,0 (C) ppm.**IR (KBr):**ν = 3442; 3212; 2923; 2861, 1620, 1555, 1499, 1453, 1384, 1242, 1053, 1017, 827, 761, 745 cm^{-1}.**MS (ESI)** : m/z (%) = 334 (3); 317 (6), 289 (3), 208 (100).

HRMS (ESI, M+1): found = 334, 19163; calculated = 334, 19194.

Biology

The assay was performed as described by Moraes and collaborators [17]. Briefly, on day 1, U2OS cells were seeded in black µClear 384-well tissue culture treated polystyrene plates (Greiner Bio-One) and incubated for 24 h at 37° C and 5% CO_2. On day 2, *T. cruzi* Y strain trypomastigotes were harvested from the supernatant of LLC-MK_2 cells infected monolayers and added to the microplates. On day 3, compounds were two-fold serially diluted in 100% DMSO to 15 dilution points in a polypropylene (PP) 384 well plate (Greiner BioOne), followed by an intermediate dilution performed by transferring 6 µl of the compound solutions from stock plates onto high volume Master Block plate (Greiner BioOne) containing 94 µl of sterile DPBS/well, diluting compounds 16.7-fold. Finally, 10µl of compound solution were transferred from the intermediate plates onto assay plates containing *Trypanosoma cruzi*-infected U2OS cells. Final well volume in the assay plate is 60 µl, and final DMSO concentration is 1%. The highest compound concentration tested was 200 µM at the assay plate, except for benznidazole, which started at 400 µM. Each compound concentration was tested in doublets (i.e.. two wells per plate), and each experiment was performed in duplicate (i.e.. two independent experiments). Plates were fixed with 4% para formaldehyde at 96 h after compound addition, followed three washes in PBS and staining with 5 µM Draq 5. Plates were imaged in the High Content Analysis System Operetta (Perkin Elmer) with a 20x WD objective. The acquired images were analyzed with the

High Content Analysis (HCA) software Harmony (Perkin Elmer) for identification, segmentation and quantitation of host cell nucleus, cytoplasm and intracellular parasite. Infection ratio (IR) was defined as the ratio between the total number of infected cells and the total number of cells. The raw data for IR values was normalized to negative (infected cells, DMSO-mock treated) and positive (non-infected cells) controls to determine the normalized antiparasitic activity. Normalized Activity values were processed with the Graphpad Prism software, version 6, for generation of sigmoidal dose-response (variable slope) nonlinear curve fitting and determination of EC_{50} and CC_{50} values by interpolation.

3. Results and Discussion

Chemistry: Compounds (S)-**6 a**, (R)-**6 a**, (S)-**6 b**, (R)-**6 b**, (S, R)-**3**and (R, S)-**3**were prepared according to scheme 1, by application of the method of Michel Langlois *et al.*[12], standard methods of reductive amination[13]ormodified procedures. Yields, optical activities and spectroscopic data are given in the experimental part.

Biology

For the bioassay a phenotypic approach has been applied. When compared with classical target-based assays, the phenotypic approaches present a significant advantage: it precludes the demand of validated targets, which speeds up the drug discovery process considerably[15, 16]. The high content biological assay was performed utilizing *Trypanosoma cruzi* Y strain infected U2OS human host cells, following the protocol previously described[17]. Briefly, 24 h after U2OS *T. cruzi* infection, the compounds were serially diluted, starting at 200 µM and followed with 14 points of dilution by a factor of 2-fold, and transferred to assay plates. The final DMSO concentration at assay wells was 1%. Benznidazole was used as positive control. Following 96 h of continuous compound exposure, plates were fixed and stained for further imaging and analysing through an automated high content microscope system. The images were then processed to provide quantifiable output data (such as infection ratio, number of parasites or host cell number), which is used to determine tested compounds activity. Data is normalized to negative (DMSO-mock treated) and blank (not infected cells) controls to define the antiparasitic activity and host cell toxicity.

Medicinal Chemistry

All compounds (S)- and (R)-**6 a, b**, (S,R)-**3** and (R,S)-**3**, exhibit a remarkably high biological activity against intracellular *Trypanosoma cruzi*a mastigotes (Table 1). The (S)-enantiomer of **6 a** is with EC_{50} = 2.74 µM significantly more active than the (R)-enantiomer of **6 a**, which exhibits a lower biological activity with EC_{50} = 5.09 µM. Beyond this, (S)-**6 a** presented a relative high maximum activity of 104.4 %, while (R)-**6 a** did not reach 100 % (maximum activity of (R)-**6** a= 95 %).The benzoxazolyl phenyl-derivative (S)-**6 b** exhibits a relatively high antitrypanosomal activity with EC_{50} = 4.39 µM, less potent than (S)-**6 a**, but with the highest maximum activity value observed for all tested compounds. With the exception of (R)-**6 a**, all tested compounds presented satisfactory selectivity (S.I.>10), which according to Keiser and Burri[18] are selective enough to progress in following stages. (S)-**6 b** is significantly more potent than (R)-**6 b**, giving evidence to a possible general rule, which implies, that (S)-enantiomers are more active than (R)-enantiomers in this compounds class. (R)-**6 b** presented a significantly lower selectivity index (SI = 17.2) when compared with (S)-**6 b** (SI>45). The compound potency of (S, R)-**3** against *Trypanosoma cruzi* (EC_{50} = 11.4 µM) is significantly lower than those observed for most chiral quinuclidines utilized in this study, as well as the maximum biological activity (75.1 %). Preliminary structure activity relationships [3-8] of non-chiral aryl- and heteroarylquinuclidines[3-8] suggest that the smaller aryl-substituent may explain this finding. (R, S)-**3** exhibits a significantly lower potency against *Trypanosoma cruzi* (EC_{50} = 15.10 µM). The selectivity index of (R, S)-**3** is slightly lower (S.I.>=13.24).

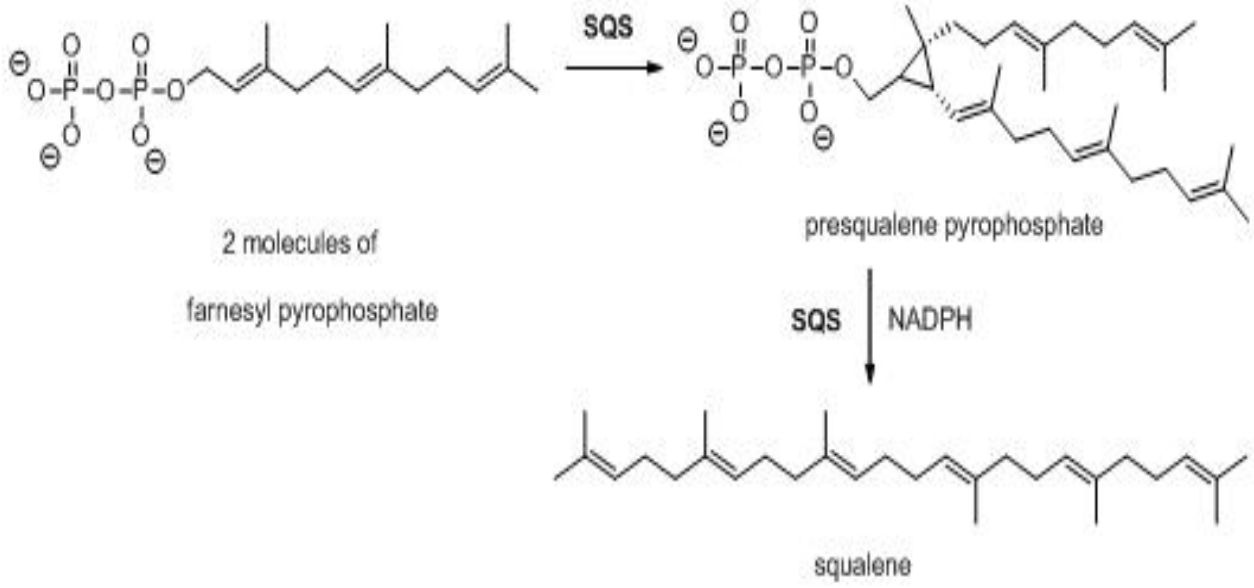
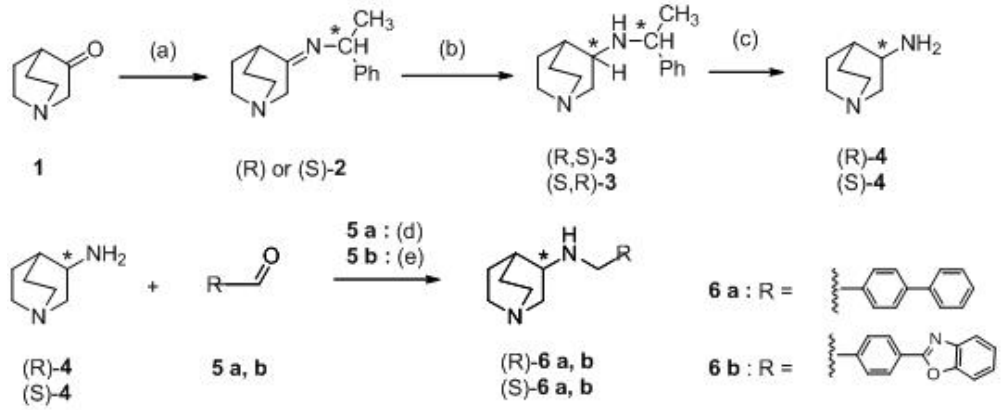
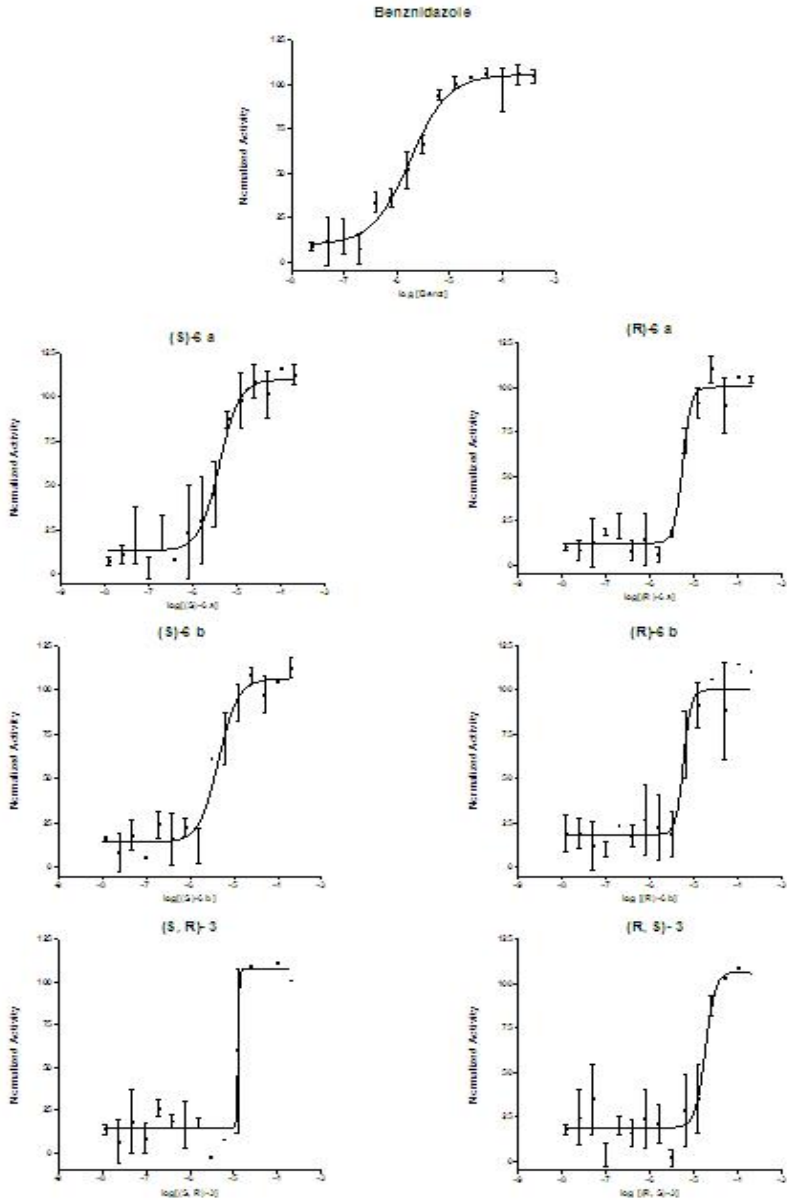


Figure 1: Condensation of two molecules of farnesyl pyrophosphate to squalene[5]



Scheme 1: Reagents and conditions: (a) (R)- or (S)-phenyl ethylamine, TosOH, toluene, Dean Stark trap, reflux, 12 h, 80 % (b) $NaBH_4$, MeOH, 0ºC..r.t, 4 hr, 82 %. (c) 20 % $Pd(OH)_2$, 1 atm H_2, 60 ºC, 5 h, 71 %. (d) $NaBH_4$, MeOH, r.t. 5 h, (S)-**6 a** : 43 %, (R)-**6 a** : 35 %. (e) i: toluene, Dean Stark, reflux, 5 h, ii : $NaBH_4$, MeOH, r.t. 5 h (S)-**6 b** : 62 %, (R)-**6 b** : 74 %.



Scheme 2: Anti-*Trypanosoma. Cruzi* activity of compounds benznidazole, **(S)-6 a, (R)-6 a, (S)-6 b, (R)-6 b, (S,R)-3** and **(R, S)-3**. Y-axis: Normalized Activity values (black dots and curves); X-axis: Log of compound concentration. Mean values (dots and squares) and standard deviation (bars) are shown. Data obtained from two independent experiments.

Table 1: Inhibition of *Trypanosoma cruzia intracellular* amastigotes and cytotoxicity to UO2S host cells.

Compound	*Trypanosoma cruzi* EC_{50} («M)	Maximum Activity (%)	U2OS cells CC_{50} («M)	Selectivity Index S.I.
Benznidazole	1.31 ± 0.13	108.2 ± 10.9	N.D.	> 305
(S)-**6 a**	2.74 ± 1.86	104.4±17.2	135	49.3
(R)-**6 a**	5.53 ± 0.30	98.4± 8.1	52.2	9.44
(S)-**6 b**	4.39 ± 2.50	108.6± 4.7	N.D.	> 45
(R)-**6 b**	5.09 ± 1.64	95.0± 22.9	87.6	17.2
(S, R)-**3**	11.40 ± 1.2	75.1±45.7	N.D.	> 17.5
(R, S)-**3**	15.1± 0.68	77.2±44.6	N.D.	> 13.2

N.D. indicates that the mean value could not be calculated. S.I. selectivity index, a ratio between CC_{50} and EC_{50} whenever CC_{50}could not be calculated, S.I. is determined as a ratio between the highest compound concentration tested and EC_{50}. $n = 2$ independent experiments

4. Conclusion

In this study a small library of chiral 3-[[(aryl) methyl]amino]-quinuclidines and 3-[[(heteroaryl) methyl]-amino]-quinuclidines was prepared by a known method of asymmetric synthesis starting from quinuclidine-3-one utilizing (R)- and (S)-phenylethylamine as a chiral auxiliary for the preparation of the chiral (R)- and (S)- 3-aminoquinuclidine building blocks and standard methods of reductive amination for coupling of the latter with aryl- and heteroaryl carboxaldehydes. The chiral 3-[[(aryl) methyl] amino]-quinuclidines and 3-[[(heteroaryl) methyl] amino]-quinuclidines were tested for biological activity against *Trypanosoma cruzi*intracellular amastigotes in a phenotypic cell-based assay. Most compounds showed a high activity against *Trypanosoma cruzi.* All compounds with (S)-configuration at C-3 were significantly more potent than the (R)-enantiomers. Biphenyl derivative (S)-**6 a** exhibited the most potent activity (EC_{50} = 2.74 μM). Benzoxazolyl phenyl derivative (S)-**6 b** exhibited a lower biological activity (EC_{50} = 4.39 μM), but a high selectivity towards the parasite. This is the first study on structure activity relationships (SAR) of chiral 3-[[(aryl) methyl]amino]-quinuclidines and 3-[[(heteroaryl)-methyl]amino]-quinuclidines and the growth of *Trypanosoma cruzi*, which attributes clearly the absolute stereochemistry of three pairs of enantiomers to their biological activity against intracellular amastigotes of *Trypanosoma cruzi*. This finding is of high potential to give strong impetus on research on the area of ergosterol biosynthesis inhibitors (EBI) and antitrypanosomal drug design. Although we strongly assume inhibition of squalene synthase (SQS) to be responsible for the biological activity of all compounds in this study, detailed investigations on the mode of action are currently done in collaboration with our partner groups in Campinas (LNBio) and Rio de Janeiro (UFRJ).

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