

Synthesis and Antileishmanial Activity of Some Functionalized Peptoids

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We describe the microwave-assisted synthesis of thirteen functionalized peptoids and evaluate their *in vitro* antileishmanial activity against forms of *Leishmania (Leishmania) amazonensis* promastigotes. Synthesis via the Ugi four-component reaction (Ugi 4CR) reaction furnished the compounds of interest in 55-80% yield; reactions were conducted in a microwave reactor and lasted only 10 min. We then screened the antileishmanial activity of the synthesized compounds *in vitro*. To determine the IC₅₀ (inhibitory concentration necessary to inhibit the growth of 50% of parasites) values, we selected the compounds that inhibited *L. (L.) amazonensis* growth by more than 50%. The seven selected compounds displayed IC₅₀ values ranging from 2.6 to 72 μM after incubation for 48 h. Three peptoids gave IC₅₀ values between 2.6 and 7.9 μM and can be considered as bioreactive molecules (hit criteria).

Keywords: peptoid, multicomponent reaction, Ugi reaction, microwave-assisted synthesis, *Leishmania (Leishmania) amazonensis*

Introduction

Protozoan parasites of the *Leishmania* genus cause a group of diseases known as leishmaniasis, a parasitosis that is transmitted by the bite of infected female phlebotomine sandflies in tropical and subtropical countries.¹ These infections affect more than 12 million people, and an estimated 700,000 to 1,000,000 new cases and 20,000 to 30,000 deaths occur annually.¹⁻³ The infection manifests in three main typical ways: cutaneous leishmaniasis (LC), which presents skin lesions, mostly ulcers, on exposed body parts, leading to life-long scars and serious disability; mucocutaneous leishmaniasis (MCL), which partially or totally destroys mucous membranes; and visceral leishmaniasis (LV), which may be lethal if left untreated.¹⁻³ *Leishmania (Leishmania) amazonensis* underlies a diffuse cutaneous form that may also result in visceral leishmaniasis in some cases in Latin America.⁴

Pentavalent antimonials were first used in the clinical setting at the beginning of the last century; they remain the first-choice drugs to treat leishmaniasis. However, these compounds are toxic and poorly tolerated, require daily

injections for up to 28 days, and are becoming ineffective due to proliferation of resistant parasites.⁵ Second-line drugs, like amphotericin B and pentamidine, are options in combined therapy or in cases of antimony treatment failure.^{6,7} Therefore, developing new antileishmanial compounds is imperative.

Peptoids are an emerging class of peptidomimetic molecules that offer an alternative to peptides. Peptoids consist of *N*-substituted glycines where side-chains are located on the amide backbone nitrogen atom rather than the α-carbon in the case of peptides (see Figure 1).⁸ This structural change makes the molecule highly resistant to proteolytic degradation, rendering peptoids a higher stability under physiological conditions and an improved biological lifetime. These non-natural molecules are being increasingly investigated because they are easy to synthesize, and various functionalities can be incorporated into their amide side-chains, which may generate new compounds with several biological activities and interesting pharmaceutical properties.⁹⁻¹²

The Ugi reaction is a multicomponent process that is widely applied in the pharmaceutical industry to prepare libraries of compounds.¹³ This reaction is very useful to produce peptoid-like backbones that occur not only in

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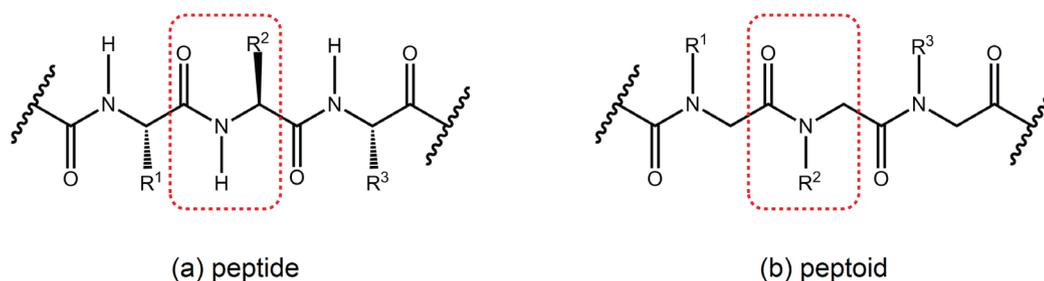


Figure 1. Representative structures of (a) peptide and (b) peptoid backbone.

peptides, but also in many other biologically important heterocycles.¹⁴ Besides its value in Medicinal Chemistry, the Ugi reaction has gained increased acceptance because it is easily performed in almost any solvent, and it is environmentally friendly.¹⁵

As part of an ongoing program to develop methods to synthesize biologically active compounds,¹⁶ in this study we examine how the classical Ugi reaction (Ugi four-component reaction (Ugi 4CR)) can be employed to synthesize a small library of new peptoids with potential pharmacological activity.¹⁷ Because some peptoids have been shown to display promising activity against *Leishmania*, we also evaluate the antileishmanial activity of these compounds.¹⁸

Experimental

General

High-resolution mass spectrometry (HRMS) were recorded on a Bruker Daltonics micrOTOF II-ESI-TOF (Billerica, MA, USA) operating in the positive ion mode. The samples were diluted in acetonitrile and water (1:1, v/v) at a concentration of 0.02 mg mL⁻¹. Accurate mass measurements were achieved using sodiated trifluoroacetic acid (TFA-Na⁺) as standard for internal calibration on electrospray ionization time of flight (ESI-TOF). Molecular formulas were assigned from accurate mass and exact mass errors lower than 5 ppm. ¹H nuclear magnetic resonance (NMR) spectroscopy was performed on a Bruker DPRX 400 instrument (Bruker, Fällanden, Switzerland) operating at 400 MHz for ¹H and at 100 MHz for ¹³C. Tetramethylsilane (TMS) was used as internal standard. Chemical shifts are reported in ppm (δ); coupling constants (J) are given in hertz (Hz). Signal multiplicities are represented by: s (singlet), d (doublet), dd (double doublet), dq (double quadruplet), and m (multiplet). Infrared (IR) spectra were recorded on a PerkinElmer Spectrum RX IFTIR System (Waltham, MA, USA), in KBr pellets or plates. Unless noted otherwise, all solvents and reagents were commercially available and used without further purification.

General procedure to synthesize peptoids **5a-m**

Preparation of peptoids **5a-m** by the Ugi four-component reaction (Ugi 4CR)

Peptoids **5a-m** were synthesized via one-pot multicomponent methodology; the Ugi four-component reaction (Ugi 4CR) was employed.¹³⁻¹⁵ Reactions were conducted with the corresponding aldehyde **1** (2 mmol), amine **2** (2 mmol), isocyanide **3** (1 mmol), and carboxylic acid **4** (2 mmol) in a 5-mL round bottom flask under argon atmosphere.¹⁰ Anhydrous MgSO₄ (0.05 g) was added to the flask, and the resulting suspension was heated at 60 °C for 10 min in a CEM Discovery® focused microwave oven at 150 W. After that, the reaction mixture was filtered, the solid was washed several times with anhydrous methanol, and this solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with dichloromethane/methanol (95:5, v/v) as eluent, to give the peptoids **5a-m** (55-80% yields).

In vitro antileishmanial assays

The antileishmanial activity was assayed with *L. (L.) amazonensis* promastigotes (MHOM/BR/PH8), which were maintained in culture medium Roswell Park Memorial Institute (RPMI) 1640 (Cultilab, Campinas, SP, Brazil) supplemented with 10% fetal bovine serum (Cultilab, Campinas, SP, Brazil), and 1% antibiotics (10,000 UI mL⁻¹ penicillin and 10 mg mL⁻¹ streptomycin) (Cultilab, Campinas, SP, Brazil), at pH 7.4 at 25 °C.

Five days after the culture was initiated, the antileishmanial activity was screened in 96-well microplates containing 1 × 10⁶ *L. (L.) amazonensis* promastigotes in supplemented RPMI 1640 and 100 μ M of the compounds previously dissolved in dimethyl sulfoxide (DMSO) (Synth, Diadema, SP, Brazil). Cultures were incubated in biological oxygen demand (BOD) incubator (Quimis, Diadema, SP, Brazil) at 25 °C for 24 and 48 h, and the antileishmanial activity was determined by verifying whether the promastigote growth was inhibited, as revealed by counting the total number of live promastigotes in the Neubauer chamber (Global Glass, Porto Alegre, RS, Brazil), on the basis of flagellar motility.¹⁹

The peptoids that inhibited *L. (L.) amazonensis* growth by at least 50% in 48 h were evaluated at final concentrations of 6.25, 12.5, 25.0, 50.0, and 100.0 μM . Bioassays were repeated three times, in triplicate. Amphotericin B (at concentration ranging from 0.6 to 0.038 μM or 1 μM) (Sigma-Aldrich, Saint Louis, MO, USA) was used as positive control, and RPMI 1640 medium with 0.1% DMSO was employed as negative control. Results are expressed as the mean of the percentage of growth inhibition relative to the negative control (0.1% DMSO), and the IC_{50} (inhibitory concentration necessary to inhibit the growth of 50% of parasites) values were calculated by using sigmoid dose-response curves constructed with the GraphPad Prism version 5.0 software for Windows.²⁰

Results and Discussion

Microwave irradiation is a relevant tool for fast and efficient synthesis of compound libraries. Microwave-assisted reactions are advantageous over reactions conducted by conventional heating because they take shorter reaction times and generate fewer by-products.^{21,22}

We obtained the target compounds by one-pot Ugi 4CR from less expensive raw materials (see Scheme 1), by using microwave irradiation as heat source.¹⁰ Table 1 summarizes the results of these reactions.

We confirmed the structures of all the synthesized compounds by ^1H and ^{13}C NMR, Fourier transform IR (FTIR), and high-resolution mass spectral data.

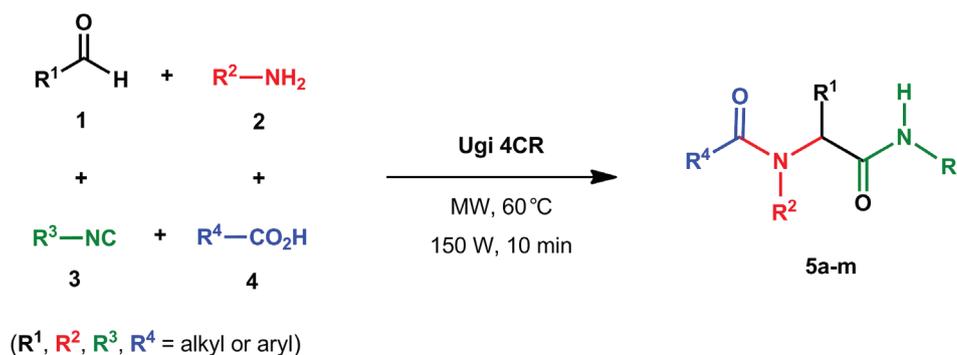
In the biological assays, we tested all the compounds as mixtures of enantiomers or diastereoisomers. Table 2 lists the results of the *in vitro* antileishmanial evaluation of peptoids **5a-m** against *Leishmania (L.) amazonensis* promastigotes (MHOM/BR/PH8).

First, we screened the antileishmanial activity of the synthesized compounds at 100 μM for 24 and 48 h, *in vitro*, as shown in Table 2. Among the evaluated compounds, peptoids **5b**, **5c**, and **5e** inhibited *L. (L.) amazonensis* promastigote growth by approximately 50% within

48 h. Peptoids **5a**, **5d**, **5f**, **5g**, **5j**, and **5l** inhibited *L. (L.) amazonensis* promastigote growth by more than 50% within 48 h (see entries 1, 3, 4, 6, 7, 10 and 12 in Table 2). Peptoid **5j** provided the most promising result: 66.83 and 87.87% *L. (L.) amazonensis* promastigote growth inhibition within 24 and 48 h, respectively. We then selected seven of the tested peptoids to perform future biological studies.

We assessed the seven selected peptoids (**5a**, **5c**, **5d**, **5f**, **5g**, **5j**, and **5l**) at 6.25, 12.5, 25, 50, and 100 μM *in vitro* for 48 h against *L. (L.) amazonensis* promastigotes to determine the IC_{50} values (Table 3). These seven compounds displayed IC_{50} values ranging from 2.6 to 72 μM after incubation for 48 h (Table 3). We used amphotericin B,²³ a polyene antibiotic that binds to specific parasite sterols, as positive control and achieved an IC_{50} value of 0.11 μM at 48 h.

When we compared the percentage of *L. (L.) amazonensis* promastigote growth inhibition by the peptoids **5a-m** (Table 2), we observed that the antileishmanial activity decreased when a benzenoid aromatic ring was replaced by a furan ring, as in the case of compounds **5a** and **5k**. The same occurred when the peptoid acetamido group was replaced by a long chain *n*-alkylamido group, as in the case of compound **5m**. When we related the IC_{50} values to the peptoid structure (Table 3), we verified that the antileishmanial activity was higher for benzamides than for acetamides, as in the case of compounds **5a** and **5d**. However, the presence of an *N*-*sec*-butylacetamido group greatly increased the antileishmanial activity in relation to the *N*-*n*-butylacetamido group, as in the case of compounds **5a** and **5c**. On the other hand, the presence of an *N*-(1-phenylethyl)acetamido group significantly decreased the antileishmanial activity, as in the case of compounds **5f** and **5g**. Apparently, aromatic rings in the peptoid side chain increase the antileishmanial activity if we compare the activities of compounds **5c**, **5e**, **5i**, **5j**, and **5l**. Despite all these considerations, further studies are necessary to investigate how these substituents affect the antileishmanial activity of this class of compounds.



Scheme 1. Preparation of compounds **5a-m** by the Ugi four-component reaction (Ugi 4CR).

Table 1. Yields obtained in the synthesis of compounds **5a-m** produced by Ugi 4CR via Scheme 1^a

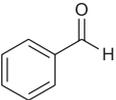
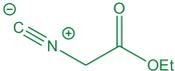
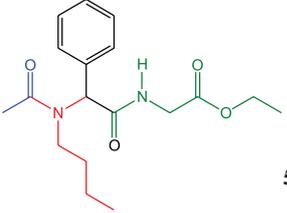
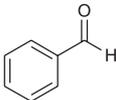
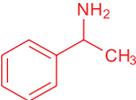
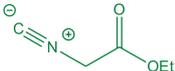
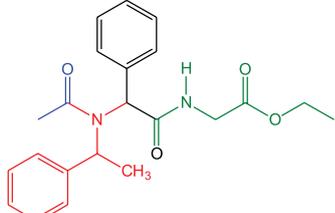
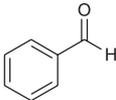
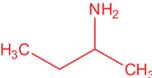
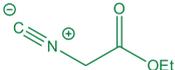
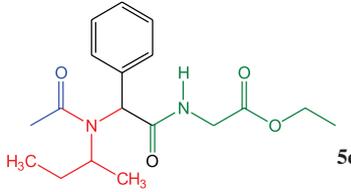
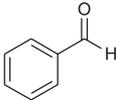
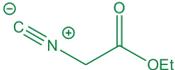
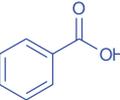
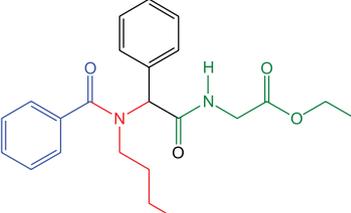
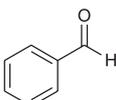
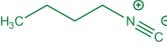
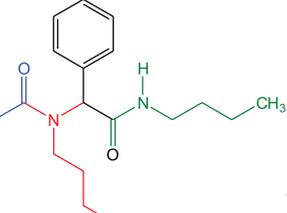
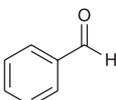
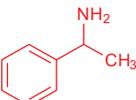
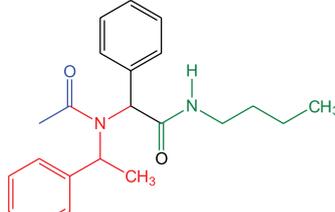
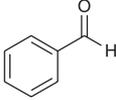
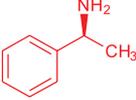
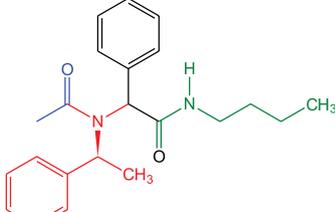
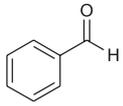
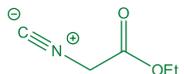
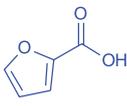
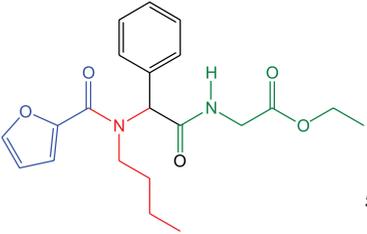
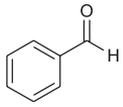
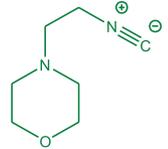
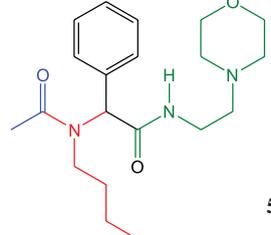
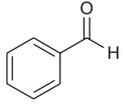
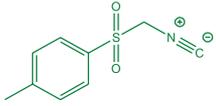
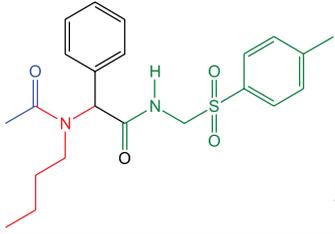
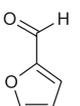
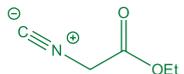
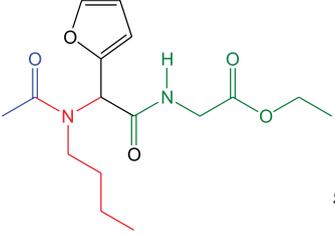
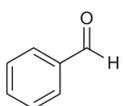
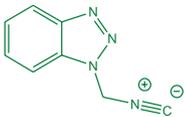
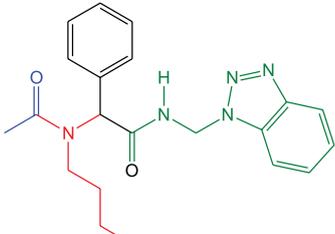
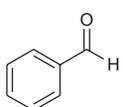
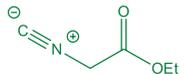
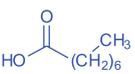
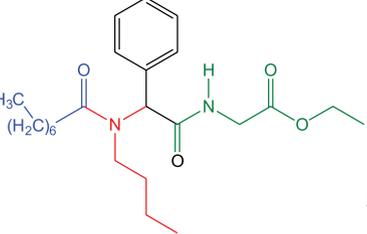
entry	Aldehyde (1)	Amine (2)	Isocyanides (3)	Acid (4)	Product (5)	Yield ^b / %
1						71
2						78
3						58
4						77
5						80
6						77
7						77

Table 1. Yields obtained in the synthesis of compounds **5a-m** produced by Ugi 4CR via Scheme 1^a (cont.)

entry	Aldehyde (1)	Amine (2)	Isocyanides (3)	Acid (4)	Product (5)	Yield ^b / %
8						70
9						55
10						57
11						60
12						68
13						55

^aReactions were conducted with the corresponding aldehyde **1** (2 mmol), amine **2** (2 mmol), isocyanide **3** (1 mmol) and carboxylic acid **4** (2 mmol) in a 5-mL round bottom flask under argon atmosphere. Anhydrous MgSO₄ (0.05 g) was added to the flask, and the resulting suspension was heated at 60 °C for 10 min in a CEM Discovery® focused microwave oven at 150 W. After that, the reaction mixture was filtered, the solid was washed several times with anhydrous methanol, and this solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with dichloromethane/methanol (95:5, v/v) as eluent, to give peptoids **5a-m** (55-80% yields). All the products were analyzed by NMR, FTIR, and high-resolution mass spectral data. The obtained spectra were consistent with the structures of the desired products; ^bisolated yield.

Table 2. *In vitro* antileishmanial activity of peptoids **5a-m** at 100 μM against *L. (L.) amazonensis* promastigotes

entry	Compound	Inhibition \pm SD (24 h) / %	Inhibition \pm SD (48 h) / %
1	5a	11.84 \pm 9.97	54.64 \pm 9.21
2	5b	29.37 \pm 5.3	45.80 \pm 0.66
3	5c	28.24 \pm 2.68	81.33 \pm 1.52
4	5d	31.91 \pm 6.96	81.94 \pm 15.81
5	5e	26.95 \pm 11.42	45.06 \pm 3.54
6	5f	25.98 \pm 11.31	67.41 \pm 9.01
7	5g	20.11 \pm 1.08	55.21 \pm 0.51
8	5h	12.01 \pm 5.41	13.22 \pm 5.97
9	5i	0 \pm 0	1.64 \pm 2.32
10	5j	66.83 \pm 12.19	87.87 \pm 21.36
11	5k	0 \pm 0	25.14 \pm 1.06
12	5l	5.32 \pm 2.40	51.27 \pm 9.95
13	5m	8.72 \pm 3.80	24.05 \pm 10.83
14	amphotericin B	100 \pm 0.0	100 \pm 0.0

Positive control: amphotericin B (1 μM). SD: standard deviation.

According to hit-and-lead criteria,²⁴ the hit is a bioreactive compound, and the lead is a hit compound that has been optimized and has undergone improvements in its pharmacokinetic and pharmacodynamic properties, for example. In addition, for a compound to be considered a hit it must have $\text{IC}_{50} < 10 \mu\text{M}$ against protozoan parasites, which is the inhibitory concentration reached by peptides **5c** (2.8 μM), **5d** (2.6 μM), and **5j** (7.9 μM). Therefore, these three peptoids have potent *in vitro* activity against *L. (L.) amazonensis*, making our results very promising. Furthermore, several peptoids have recently been shown to act against *Leishmania (Leishmania) mexicana* promastigotes with IC_{50} values of $\leq 20 \mu\text{M}$.¹⁸ These results

provide further evidence that peptoids may be a promising new class of anti-infectives and will surely find application as a new class of antileishmanial agents.²⁵

Conclusions

In summary, we have presented an easy and efficient method to obtain some functionalized peptoids by using microwave irradiation as the activating mode of reaction. We conducted the syntheses with inexpensive reagents within shorter reaction times as compared to traditional methods. This method, microwave-assisted synthesis by Ugi 4CR, furnished functionalized peptoids (compounds **5a-m**) in good yields (55-80%). All the compounds were evaluated against *Leishmania (Leishmania) amazonensis* promastigotes *in vitro*. Peptoids **5a-m** displayed variable antileishmanial activity depending on their structures. On the basis of our results, peptoids **5c**, **5d**, and **5j** display the best antileishmanial activity at the assayed concentrations (IC_{50} 2.6-7.9 μM). Now, it is necessary to gain further understanding about their structure-biological activity relationships to expand the application of this kind of peptoids.

Supplementary Information

¹H NMR, ¹³C NMR, FTIR and high-resolution mass spectra of compounds, and the curves used to calculate the IC_{50} values are available free of charge at <http://jbcbs.sbgq.org.br> as PDF file.

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Table 3. *In vitro* antileishmanial activity of *L. (L.) amazonensis* promastigotes and determination of IC_{50} values after incubation for 48 h

Compound	Concentration \pm standard deviation / μM					IC_{50}^a / μM
	Parasite growth inhibition / %					
	100	50	25	12.5	6.25	
5a	56.27 \pm 0.57	45.86 \pm 5.76	42.05 \pm 5.61	34.78 \pm 7.55	29.11 \pm 7.02	60.60 \pm 0.39
5c	81.42 \pm 5.50	74.12 \pm 2.28	68.17 \pm 2.85	63.83 \pm 4.30	58.47 \pm 8.11	2.80 \pm 0.38
5d	84.23 \pm 11.59	78.12 \pm 13.16	69.75 \pm 6.26	64.60 \pm 4.94	61.01 \pm 2.97	2.61 \pm 0.42
5f	58.61 \pm 5.27	45.90 \pm 0.98	38.37 \pm 2.06	30.12 \pm 4.53	23.79 \pm 3.66	59.03 \pm 0.54
5g	52.88 \pm 1.59	49.30 \pm 0.99	34.48 \pm 0.74	28.84 \pm 1.64	25.25 \pm 1.06	72.00 \pm 0.48
5j	75.92 \pm 2.97	67.59 \pm 0.52	62.61 \pm 0.33	53.48 \pm 0.56	48.54 \pm 1.12	7.90 \pm 0.42
5l	69.23 \pm 3.17	63.66 \pm 2.44	55.70 \pm 5.63	48.20 \pm 7.50	43.41 \pm 8.55	13.35 \pm 0.40
Amphotericin B	0.6	0.3	0.15	0.075	0.038	0.11 \pm 0.34
	99.88 \pm 0.60	78.33 \pm 24.43	68.74 \pm 21.97	54.67 \pm 17.11	42.44 \pm 20.97	

^aInhibition concentration 50 (IC_{50}) values were calculated by using a nonlinear regression curve. Negative control: RPMI 1640 medium + 0.1% DMSO.

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