Anti-Amoebic Activity of Acyclic and Cyclic-Samarium Complexes on *Acanthamoeba*

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ABSTRACT

This work investigated the anti-amoebic activity of two samarium complexes, the acyclic complex [bis(picrato)(pentaethylene glycol)samarium(III)] picrate – referred to as $[Sm(Pic)_2(EO5)](Pic)$ and the cyclic complex [bis(picrato)(18-crown-6)samarium(III)] picrate – referred to as $[Sm(Pic)_2(18C6)](Pic)$. Both Sm complexes caused morphological transformation of the protozoa *Acanthamoeba* from its native trophozoite form carrying a spine-like structure called acanthopodia, to round-shaped cells with loss of the acanthopodia structure, a trademark response to environmental stress. Further investigation however, revealed that the two forms of the Sm complexes exerted unique cytotoxicity characteristics. Firstly, the IC_{50} of the acyclic complex (0.7 μg/mL) was ~10-fold lower than IC_{50} of the cyclic Sm complex (6.5 μg/mL). Secondly, treatment of the *Acanthamoeba* with the acyclic complex caused apoptosis of the treated cells, while the treatment with the cyclic complex caused necrosis evident by the release of cellular materials. Both treatments induced DNA damage in *Acanthamoeba*. Finally, a molecular docking simulation revealed the potential capability of the acyclic complex to form hydrogen bonds with profilin – a membrane protein present in eukaryotes, including *Acanthamoeba*, that plays important roles in the formation and degradation of actin cytoskeleton. Not found for the cyclic complex, such potential interactions could be the underlying reason, at least in part, for the much higher cytotoxicity of the acyclic complex and also possibly, for the observed differences in the cytotoxicity traits. Nonetheless, with IC_{50} values of <10 μg/mL, both the acyclic and cyclic Sm complexes feature a promising potential as cytotoxic agents to fight amoebic infections.

Keywords: *Acyclic and cyclic structures; Antiamoebic; Apoptosis; Necrosis; Profilin; Samarium complexes*

1. INTRODUCTION

The ubiquitously present free-living amoebic protozoa belonging to the genus *Acanthamoeba* are the etiological agents of prominent diseases like amoebic keratitis (infection of the eye) and granulomatous amoebic encephalitis (fatal disease of the central nervous system) (Marciano-Cabral and Cabral 2003). Equally alarming is the increasing number of disseminated infections caused by this pathogen in individuals with AIDS (Marciano-Cabral and Cabral 2003). The life cycle of *Acanthamoeba* consists of two developmental stages – an actively feeding trophozoite stage and a dormant cyst stage (Ibrahim et al. 2007). Trophozoites are known to be susceptible to most of the amoebicidal agents but the cysticidal activities of these agents are limited (Ortillés et al. 2017). These challenges, along with the risk factors that are associated with *Acanthamoeba* being present worldwide (Marciano-Cabral and Cabral 2003), demand for an urgent need for the development of new anti-amoebic drugs with improved efficacy against this pathogen.

To address this, the present work seeks to investigate the anti-amoebic activity of lanthanides, that is, samarium complexes with acyclic and cyclic structures. Lanthanides have found applications as diagnostic and prognostic probes in clinical laboratories (Misra et al. 2004). Their use as anticancer agents is also rising (Misra et al. 2004). These applications are, at least in part, due to the lanthanide cations and their complexes having unique molecular structures, enabling interaction with many chiral biological substrates (Tsukube and Shinoda 2002). The unique structures of the lanthanide compounds also permit fine tuning of their configurations as well as electronic properties, which can be adapted as per specific amino acid in biological substrates (Tsukube and Shinoda 2002). Lanthanides have also been reported to exert antimicrobial activity against Gram-negative bacteria (Shiju et al. 2013). Recently, the terbium trinitrate.trihydrate.18-crown ether-6, $Tb(NO_3)_3(OH_2)_3$.(18C6) complex has been reported as anti-amoebic agent (Kusrini et al., 2016).

To further expand the antimicrobial spectrum of lanthanides, this study reports the *in vitro* anti-amoebic activity of samarium complexes with acyclic (pentaethylene glycol, EO5) and cyclic (18-crown-6, 18C6) structures. Two samarium complexes of acyclic $[Sm(Pic)_2(EO5)](Pic)$ and cyclic $[Sm(Pic)_2(18C6)](Pic)$ structures (Fig. 1) were investigated in this study for their activity against a clinical isolate of *Acanthamoeba*. Their unique 'open' and 'closed' molecular structures, respectively, as revealed herein, gave rise to distinct characteristics of anti-amoebic activity. The anti-amoebic activity of the complexes was assessed on the basis of cytotoxicity and genotoxicity parameters, as well as on the changes in cellular morphology. Finally, an *in silico* molecular docking simulation was performed to identify the potential biological target of the acyclic and cyclic Sm complexes.

2. MATERIALS AND METHODS

2.1 Preparation of Sm complexes and cultivation of Acanthamoeba

The acyclic $[Sm(Pic)_2(EO5)](Pic)$ and cyclic $[Sm(Pic)_2(18C6)](Pic)$ samarium (Sm) complexes were synthesized according to Saleh et al. (Saleh et al., 2007; Saleh et al., 2008). One mg of each compound was dissolved in 60 µL dimethyl sulfoxide (DMSO). Protease-yeast glucose (PYG) culture medium was added into the solution to make the final volume to 1000 µL. Next, the solution was homogenized by vortexing prior to storage at 4°C.

The *in vitro* anti-amoebic activity of the acyclic and cyclic samarium complexes was tested on a pathogenic strain of *Acanthamoeba*, isolated from the corneal scrapings of a keratitis patient (Kamel and Norazah 1995). The amoebae was sub-cultured and maintained in an axenic medium at the Biochemical Laboratory in the School of Fundamental Science, Universiti Malaysia Terengganu, Malaysia. For the work with the samarium complexes, the *Acanthamoeba* was cultivated in PYG medium, prepared by mixing 6.5 g protease, 6.5 g yeast, 15 g D+ glucose and making up to 1000 ml final volume using Page Amoeba Solution (PAS). The media was autoclaved for 2 h and thereafter stored for further use. *Acanthamoeba* cultivation was performed at 30˚C with sub-culturing every 3 days.

2.2 Determination of IC_{50} values of acyclic and cyclic Sm complexes by MTT assay on Acanthamoeba

A series of increasing concentrations of the acyclic and cyclic complexes were tested to determine their IC_{50} value, the concentration that reduces the mean cell viability of the treated *Acanthamoeba* to 50% relative to the healthy (untreated) control. IC_{50} allows for the comparison of concentrations of chemicals necessary to inhibit any measurable biological parameters, such as cell proliferation, protein or DNA synthesis. In a 6-well plate, the *Acanthamoeba* at 10^4 cells/ml were exposed to the Sm complexes at 30˚C for 24 h. The highest Sm complexes concentration used in this study was 30 µg/mL with double series dilution in accordance to the OECD guideline for toxicity assessment (2007). Duplicate toxicity assays were carried out and the standard error of means were determined. Independent sample ANOVA and Duncan test from SPSS v11.5 Windows statistical package were used at 95% confidence interval (CI) to validate the significant difference between treated samples to those of the control samples.

For the cell viability assay, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was prepared by mixing 5 mg MTT in 1 mL of sterile phosphate buffered saline (PBS). Following the 24 h exposure to Sm complexes, the cells were harvested and subjected to centrifugation at 3000 rpm for 15 min. The pellets were washed with PBS and further centrifuged at 1000 rpm for 5 min to discard the supernatant. Into the

cell pellet, 10 µL MTT stock solution and 100 µL PBS solution were added and the resulting cell suspension was incubated at 30˚C for 4 h. After the incubation, 100 µL DMSO was added into the suspension (to dissolve the formed formazan crystal) and subjected to absorbance reading (570 nm, Dynatech MR580 MicroElisa).

2.3 Microscopy study of Acanthamoeba treated with acyclic and cyclic Sm complexes

The microscopy study was carried out on *Acanthamoeba* culture that was treated with 50% IC_{50} concentration of the samarium complexes at 30˚C for 24 h. The morphological structure of the treated cells was analyzed with light microscopy and was compared to the untreated cell control. The treated *Acanthamoeba* culture was also subjected to acridine orange (AO)/propidium iodide (PI) staining to identify the mode of cell death. For this double staining, the *Acanthamoeba* cell pellet, as previously prepared, was re-suspended in 100 µL AO/PI staining solution, which was prepared by adding 2 µL AO (1 mg/mL) and 2 µL PI (1 mg/mL) to 996 µL PBS. The resulting cell suspension was incubated in the dark for 10 min. Thereafter, the cells were viewed with Leica DMire fluorescence microscope (Germany).

2.4 Analysis of DNA damage by alkaline comet assay

The *Acanthamoeba* cells were exposed to the samarium complexes at their IC_{25} concentration at 30˚C for 2 h. The cells were then harvested and subjected to centrifugation at 1000 rpm for 5 min. The pellet was mixed with 80 µL 0.7% low melting agarose (LMA) and the mixture was spread above the first layer of 0.6% normal melting agarose (NMA) that was prepared earlier. Cover slip was placed on the second layer of agarose. Then, 200 µL 0.5% LMA was placed on top of the second layer of agarose. The three-layered slide was incubated in alkaline lysis buffer at 4°C for 1 h, then submerged in cold electrophoresis buffer (pH > 13) for 1 h, followed by electrophoresis for 5 min at 1 V/cm, 300 mA. The slide was then neutralised by three times treatment with 400 nM Tris-HCl (pH 7.5) and stained with ethidium bromide (EtBr). The slide was left overnight prior to the microscopy analysis with Leica Dmire fluorescence microscope at 590 nm excitation filter setting.

The combination of DNA gel electrophoresis with fluorescence microscopy helped picture the passage of DNA strands from an individual agarose-embedded cell. If the negatively charged DNA contains any breakage, DNA supercoils get relaxed and the broken ends are able to move towards the anode during the electrophoresis process (Olive and Banáth 2006). Later, comets obtained were scored in the range of 0-4 as described by Collins 2004. For detecting the DNA damage in individual cells, different scores were given based on the proportion of DNA at the tail. Five classes of comets from 0 (no tail) to 4 (almost all DNAs in tail) was adequate for comet

grouping when visual scoring was conducted. Type 0 indicates rounded or intact DNA with no tail, type 1 indicates the presence of 25% of DNA at tail, type 2 indicates about 25-50% DNA at tail, type 3 comet indicates 50 to 75% of DNA at tail and type 4 indicates more than 75% of DNA at tail (Mat Amin 2012).

2.5 In silico molecular docking simulation of the acylic and cyclic Sm complexes to Acanthamoeba profilin protein

Autodock version 4 with the latest Lamarckian Genetic Algorithm (LGA) was employed to visualize the interactions between the Sm complexes with a target protein. For the docking simulations and the clustering of results, Autogrid was used to visualize conformations, to look for conformational similarity and to visualize affinity potentials. The docking simulation first identified areas of high positive potential on the surfaces of two *Acanthamoeba* isoforms and these areas mapped to the actin binding sites of profilin protein. Autodock was run several times to provide the docked conformations of the Sm complexes to the profilin protein.

3. RESULTS AND DISCUSSION

3.1 The effect of the acyclic and cyclic Sm complexes on Acanthamoeba's viability

Exposure of *Acanthamoeba* to 0 to 30 µg/mL acyclic $[Sm(Pic)_2(EO5)](Pic)$ and cyclic $[Sm(Pic)_2(18C6)](Pic)$ complexes resulted in a dose-dependent anti-amoebic activity, assessed based on the extent of cell death of the treated samples relative to the untreated control. MTT assay, which measures the activity of mitochondrial oxidoreductase enzymes, was employed to determine the number of viable cells remained following the exposure. As shown in Fig. 2a, presence of 0.7 µg/mL (IC_{50}) of the acyclic Sm complex already killed ~50% of the *Acanthamoeba*, while the 30 µg/mL exposure almost completely eradicated the cells. Exposure to the cyclic Sm complex resulted in less extent of the anti-amoebic activity, with ~50% non-viable cells detected at 6.5 µg/mL (IC_{50}), while further increasing the treatment dosage to 15 µg/mL saw ~85% non-viable cells (Fig. 2b). Note that for protozoan parasites, compounds with IC_{50} of 10 to 50 µg/mL are considered with moderate toxicity, while those with IC_{50} of > 50 µg/mL are designated as non-toxic (Gessler et al. 1994). More recent classification has lowered the IC_{50} to <5 µg/mL for cytotoxic activity, 5 to 10 µg/mL for moderate toxicity and >10 µg/mL as non-toxic (Deharo et al. 2001). Regardless, the findings herein indicate the potential for the acyclic and cyclic Sm complexes as anti-amoebic agents, both with IC_{50} <10 µg/mL. Further detailed studies on the cellular responses of *Acanthamoeba* to the samarium complexes as well as the induced DNA damage, will provide insights into the distinct characteristics of anti-amoebic activity of the acyclic and cyclic forms of the lanthanide complexes.

3.2. Morphological changes and modes of cell death of the Sm complexes-treated Acanthamoeba

Here, *Acanthamoeba* was exposed to 50% IC_{50} concentration of the acyclic (0.35 µg/mL) and cyclic (3.3 µg/mL) Sm complexes for 24 h. Under the light microscope, untreated cells showed the presence of *Acanthamoeba* trophozoites or vegetative cells with the characteristic presence of spine-like structures called acanthopodia on their surface (Fig. 3a). Exposure to both acyclic and cyclic Sm complexes resulted in the loss of the acanthopodia structure, transforming the *Acanthamoeba* into round-shaped cells (Fig. 3b, c). Such transformation is a trait of the encystment process, an innate defence mechanism that converts vegetative cells to cyst or dormant form of *Acanthamoeba* under environmental stress (Khan 2006). The double-walled cyst acts as a shell, hence protecting the parasite in hostile conditions. Interestingly, unlike those treated with the acyclic complex, cells with visibly ruptured membrane were observed upon treatment to the cyclic complexes (Fig. 3b, c) and this phenomenon is in fact a trademark for a specific cell death mode, as revealed by the fluorescence double staining of the *Acanthamoeba*, as follows.

The investigations into the modes of cell death in *Acanthamoeba* were according to Darzynkewick et al. 1997 (Darzynkiewicz et al. 1997), which differentiate apoptotic from necrotic cells upon exposure to Sm complexes based on membrane integrity and visible changes in cytoplasmic components. Apoptosis is a programmed cell death (PCD) of unwanted cells, whereas necrosis is an un-programmed premature cell death caused by external stresses, including exposure to cytotoxic agents (Darzynkiewicz et al. 1997). Apoptosis is characterized by cell shrinkage, blebbing of the plasma membrane with loss of permeability, condensation of the chromatins and in some cases, fragmentation of the nucleus, while still retaining the integrity of the organelles (Kerr et al. 1972). A single cell can undergo apoptosis with no apparent effect on neighbouring cells. Necrosis is characterised by cytoplasm swelling, destruction of organelles and disruption of the plasma membrane, leading to the release of intracellular contents (Darzynkiewicz et al. 1997). Following incubation of the *Acanthamoeba* with the acyclic and cyclic Sm complexes at their 50% IC_{50} concentrations, the double cell staining with acridine orange (AO) and propidium iodide (PI) enabled identification of the types of cell death. AO is a membrane permeable cationic dye that selectively binds to DNA or RNA of viable cells and emits green fluorescence, whereas PI only enters cells with damaged membranes and emits orange fluorescence upon binding to nucleic acid. Early apoptotic *Acanthamoeba* cells are indicated by the presence of condensed chromatin, in this case visible as bright green condensed nuclei. Also note that green cells are viable cells with intact membrane, while orange cells are dead cells with compromised membrane.

Exposure of the *Acanthamoeba* to acyclic Sm complexes at 50% IC_{50} dosage resulted in plasma membrane disruption in minor fraction of the cell population, visibly indicated by the occurrence of orange fluorescence entities in these cells (with the majority of the cells still with intact membrane emitting green fluorescence) (Fig. 3e). This is in contrast to the untreated cells, all visible as green fluorescence cells and therefore, indicating healthy cells with intact plasma membrane (Fig. 3d). Detailed assessments of the microscopy images revealed the presence of cells with green and orange fluorescence nuclei acid entities following treatment with the acyclic complexes. The smaller size of these nucleic acid entities when compared to those in the untreated cells suggest the condensation of the chromatins (Fig. 3d, e). Indeed, unlike the healthy cells, there were also visible presence of what thought to be nucleic acid fragments throughout the cytoplasm, suggesting disintegration of the nuclear envelope followed by nuclear fragmentation (Fig. 3e, enlarged cell shown in the panel; note that the nucleus could not be detected in these cells). These fragments are thought to result from the activity of Ca^{2+}- and Mg^{2+}-dependent nuclear endonucleases that cleave DNA between nucleosomal units (linker DNA) (Arends et al. 1990). As part of a major regulatory step in apoptotic pathway, nuclear endonucleases are activated by the death receptor caspase 3, leading to DNA fragmentation (Jänicke et al. 1998), as also detected with further DNA damage assay in the present study, as later discussed. Taken together, these observations suggest early stage apoptosis of the *Acanthamoeba*.

Exposure of the *Acanthamoeba* to the cyclic Sm complexes at 50% IC_{50} dosage resulted in not only plasma membrane disruption (as visibly indicated by occurrence of intracellular red fluorescence entities), but also the release of cellular content, suggesting necrotic type of cell death (Fig. 3c, f). We also observed swelling of the *Acanthamoeba* organelles (enlarged cell shown in the panel, Fig. 3f), which is commonly reported with necrotic cells (Murakami et al. 2011). Further, we observed the presence of 0.1 to 0.2 µm yellow orange granules in the *Acanthamoeba* for both the acyclic and cyclic Sm complexes treatments (enlarged cells in panels in Fig. 3e, f). These fluorescence granules are thought to be the lysosomes, taking up the AO stain through the known sequestration and digestion process of cytoplasmic macromolecules, called the autophagy. More specifically, research inquiries have indicated the uptake of AO by the lysosome in its uncharged form, becoming protonated and thus entrapped in the organelle (Darzynkiewicz et al. 1997). The non-existence of these granules in the untreated cells suggested the absence of such autophagy process in the healthy amoeba (Fig. 3d).

We hypothesize that the distinct types of cell damage are due to, at least in part, the different types of Sm^{3+} ion bonded with the chelating agents of acyclic (EO5) and cyclic (18-crown-6) ligands that constitute in the acyclic and cyclic Sm complexes. The early *Acanthamoeba* apoptosis observed with the acyclic Sm complex exposure is thought to result from the acyclic structure of EO5 with two terminal alcohol (OH) groups, facilitating interactions

with profilin, a membrane protein that has important role in the growth of cytoskeleton, as later shown in detail with a molecular docking simulation. Studies have indeed reported apoptosis trigger by metals with acyclic ligand (Mukherjee et al. 2011), while cyclic compounds could initiate necrotic type of cell death (Kumar et al. 2016). In agreement, the cyclic Sm complex herein appears to disrupt the *Acanthamoeba* membrane and release of cellular materials, a trademark of necrosis. The changes in membrane integrity is thought to relate to Ca^{2+} influx among the many possible mechanisms, including reactive oxygen species (ROS) generation and DNA damage (Galluzzi et al. 2014). It is also possible that the negatively charged surface proteins of *Acanthamoeba* interact with the picrate (Pic) structure of the cyclic Sm complexes, inducing structural and permeability changes on the membrane, leading to the observed leakage of cytoplasmic components and thus the *Acanthamoeba* death. Note that the molecular docking simulation indicates absence of compatible region in the cyclic Sm complex to interact with the profilin protein structure.

3.3 The acyclic and cyclic Sm complexes-induced DNA damage

The potential of the acyclic and cyclic Sm complexes to cause DNA damage on *Acanthamoeba* were studied with the alkaline comet assay. The versatile assay is considered sensitive for assessing single- and double-strand DNA breaks in cells (Tice et al. 2000; Collins et al. 2008). Herein, the assay was performed on the *Acanthamoeba* upon their exposure to the Sm complexes at IC_{25} concentrations. The dosage was to avoid the possible false positive results at higher dosage (Prego-Faraldo et al. 2015). The assay involves lysis of cells in agarose and subsequent electrophoresis of the lysed cells with visible migration of the cells' damaged DNA, forming a 'comet' trail. Different scores for DNA damage are assigned based on the proportion of the formed comet trail relative to the total DNA. Degrees of DNA damage of the Sm complexes-treated *Acanthamoeba* were herein determined according to Collins 2004, whereby five scores for the formed comet from 0 (no trail, intact DNA) to 4 (almost all DNA forms trail, severe DNA breaks) were assigned.

As shown in Fig. 4 and Table 1, exposure of the *Acanthamoeba* to IC_{25} concentration of the acyclic Sm complex displayed ~30% manifestation of type 1 comets, which corresponds to 25% DNA damage of the cells ($p > 0.05$, Kruskall-Wallis test). Less appearance of type 2 and 3 (25-75% DNA damage) comets were observed, while type 4 comet (>75% DNA damage) was the least detected (~2% appearance). Comparable observations were also found for the IC_{25} treatment of the *Acanthamoeba* with the cyclic complex ($p > 0.05$). These findings of DNA strand breaks are consistent with the earlier mentioned early apoptotic DNA fragmentation (with the AO/PI double staining) observed with the acyclic complex-treated samples (Fig. 3e). As with the cyclic Sm complex, it appears

that the induced necrosis cell death (Fig. 3f) was also associated with DNA strand breaks. Some studies in eukaryotes have observed cytoplasmic changes being associated with DNA disruptions (Liu and Wilson 2010) and indeed, we observed the swelling of cellular organelles in the cyclic complexes-treated *Acanthamoeba* samples (Fig. 3f, enlarged cell in panel). Up to this stage, we have observed the different toxicity levels and characteristics of the Sm complexes, and the following molecular docking simulation may reveal the cause, at least in part, of such differences.

3.4 Molecular docking simulation of the acyclic and cyclic Sm complexes on Acanthamoeba's profilin protein

An *in silico* molecular docking simulation was carried out to investigate the binding affinities of the acyclic and cyclic Sm complexes on an *Acanthamoeba* protein, the profilin 1B (PDB ID: 1ACF). Profilin is an actin-binding membrane protein that involves in the synthesis and degradation of actin microfilaments, the latter are the building blocks for actin cytoskeleton that determine the shape of cells, in this case the *Acanthamoeba* (Vinson et al. 1998). Profilin inhibits the formation of actin microfilaments at high concentration and *vice versa* at low concentration. The docking simulation revealed interactions of the acyclic Sm complex with specific regions in profilin 1B. The interactions were found to occur in hydrophilic pockets of the profilin (Fig. 5a), in the form of hydrogen bonding of the acyclic pentaethylene glycol (EO5) with the embedded presence of Thr35, Ser1, 3, 6 residues, while the Sm^{3+} ion was found only on the surface of the protein (Fig. 5b, c). For the EO5 interactions, it has been known that the sidechain -OH group in amino acids such as, Thr and Ser, are typical oxygen donor atom for hydrogen bonding (Jabeen et al. 2015). Strong hydrogen bonds occurred between the EO5 moiety and the Thr35 and Ser6 residues, with calculated bond lengths of 2.4 to 2.6 Å, while weak hydrogen bond was predicted with the Ser3 residue with bond length of 3.4 Å (Table 2, Fig. 5c). Note that the distance-to-strength correlation of the hydrogen bonds is according to Jeffrey 1997. Further, the calculated free binding energy of the acyclic Sm complex at its potential binding sites in the profilin's hydrophilic pockets was -7.60 kcal (Autodock version 4). The low binding energy indicates strong interactions between the acyclic Sm complex and profilin 1B protein. These interactions however, were not found with the cyclic Sm complex. The docking simulation revealed the inability of the cyclic Sm complex to form hydrogen bond with the amino acid residues due its rigid and cyclic conformation. Taken together, this study provides a novel model that highlights the importance of hydrogen bonds in the interactions of the acyclic Sm complex with the amino acid residues in *Acanthamoeba* profilin 1B. These interactions could disrupt the protein's actin binding capacity and in turn, disturb the control of the *Acanthamoeba*'s shape and its

movement, which is not contradictory to the observed loss of the acanthopodia structure following exposure to the acyclic complex (Fig. 3b).

4. CONCLUSION

In summary, we reported the anti-amoebic activity of Sm complexes, both in the acyclic $[Sm(Pic)_2(EO5)](Pic)$ and cyclic $[Sm(Pic)_2(18C6)](Pic)$ forms against *Acanthamoeba*. Although the two forms were capable of inducing DNA breaks, the acyclic Sm complex however, exhibited higher level of cytotoxicity with IC_{50} of ~0.7 µg/mL compared to the cyclic form with ~10-fold higher IC_{50} concentration. Detailed cellular studies revealed different cytotoxicity characteristics of the Sm complexes. Investigated at their 50% IC_{50} dosage, both acyclic and cyclic Sm complexes induced the loss of the acanthopodia structure, commonly present in healthy *Acanthamoeba*. A more detailed microscopy study, still at the 50% IC_{50} dosage, revealed the occurrence of early apoptotic *Acanthamoeba* cells in the acyclic complexes-treated samples. Assessed at its 50% IC50 dosage, the cyclic Sm complex on the other hand, induced the necrosis phenomenon on the *Acanthamoeba*, with the trademark release of cellular constituents. Such differences in the extent and mechanisms of cytotoxicity could due to, at least in part, the potential capability of the acyclic Sm complex to form hydrogen bonds in the hydrophilic pockets of the membrane protein profilin, unlike the Sm cyclic complex. Revealed by a molecular docking simulation, this interaction could ultimately disrupt the *Acanthamoeba*'s shape and its movement.

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