Trypanocidal and cell swelling activity of 20-deoxysalinomycin

Dietmar Steverding, Daniel Strand, Adam Huczyński

PII: S0014-4894(22)00208-9

DOI: https://doi.org/10.1016/j.exppara.2022.108414

Reference: YEXPR 108414

To appear in: *Experimental Parasitology*

Received Date: 5 September 2022

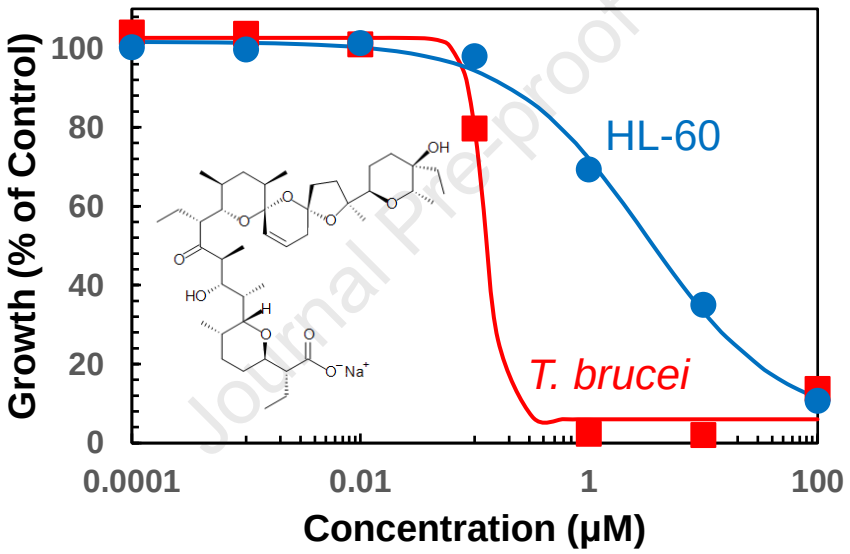
Revised Date: 12 October 2022

Accepted Date: 18 October 2022

Please cite this article as: Steverding, D., Strand, D., Huczyński, A., Trypanocidal and cell swelling activity of 20-deoxysalinomycin, *Experimental Parasitology* (2022), doi: https://doi.org/10.1016/j.exppara.2022.108414.

CRediT author statement

Dietmar Steverding: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – Original draft, Visualization. **Daniel Strand**: Methodology, Validation, Investigation, Recourses, Writing – Review and Editing, Funding acquisition; **Adam Huczyński:** Conceptualization, Resources, Writing – Review and Editing.



Trypanocidal and cell swelling activity of 20-deoxysalinomycin

Dietmar Steverding[a,*], Daniel Strand[b], Adam Huczyński[c]

[a] *Bob Champion Research & Education Building, Norwich Medical School, University of East Anglia, Norwich, U.K.*

[b] *Centre for Analysis and Synthesis, Department of Chemistry, Lund University, Lund, Sweden*

[c] *Department of Medical Chemistry, Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland*

[*] Corresponding authors.

E-mail address: d.steverding@uea.ac.uk

ABSTRACT

The naturally occurring polyether ionophore salinomycin was previously found to display promising anti-proliferative activity against bloodstream forms of *Trypanosoma brucei*. Here, we report the evaluation of 20-deoxysalinomycin, a naturally occurring homolog to salinomycin, for trypanocidal and cell swelling activity. The concentration of 20-deoxysalinomycin required to reduce the growth rate of bloodstream-form trypanosomes by 50% was determined to be 0.12 µM and found to be 8 times more trypanocidal than salinomycin. Moreover, 20-deoxysalinomycin and salinomycin displayed similar cytotoxic activity against human HL-60 cells. Measured as the ratio of cytotoxic to trypanocidal activity, 20-deoxysalinomycin thus exhibit a four-fold higher selectivity compared to salinomycin. The stronger trypanocidal activity of 20-deoxysalinomycin is attributed to an enhanced ability to induce cell swelling in trypanosomes. The findings support 20-deoxysalinomycin as a useful lead in the rational development of new and improved anti-trypanosomal drugs.

Keywords

African trypanosomiasis

Trypanosoma brucei

Polyether ionophores

20-Deoxysalinomycin

1. Introduction

African trypanosomiasis is a parasitic disease affecting both humans and livestock. The disease is known as sleeping sickness in humans and nagana disease in animals and is caused by protozoans belonging to the Salivarian group of the genus *Trypanosoma* (Steverding, 2008). Restricted to sub-Saharan Africa, the geographical distribution of the disease correlates with the spread of tsetse flies, which are the vectors for the parasites (Steverding, 2008). Both male and female tsetse flies transmit trypanosomes to their mammalian host during blood feeding. The disease causes severe illness in humans and livestock and is, without treatment, fatal in most cases. Unfortunately, Only a few therapies are currently available for the treatment of African trypanosomiasis. Most of these have limited efficacy and cause serious side effects (Giordani et al., 2016; Steverding, 2017). Furthermore, drug resistance in African trypanosomiasis is a growing problem, particularly in nagana disease (Delespaux and de Koning, 2007; Giordani et al., 2016; Okello et al., 2022). Although the number of reported cases of sleeping sickness has fallen significantly in recent years due to sustained control efforts in affected regions (WHO, 2019), nagana disease remains a major problem in sub-Saharan Africa with annual economic costs of US$4.5 billion (Mattioli, 2016). One of the main problems in animal trypanosomiasis is that the currently available treatments are becoming increasingly ineffective (Giordani et al., 2016). New drugs for the treatment of this disease are therefore much sought.

Polyether ionophore antibiotics have shown promising anti-proliferative activity against several protozoan parasites including African trypanosomes (Antoszczak et al., 2019a). In particular, the carboxylic polyether ionophore salinomycin and its derivatives have been extensively investigated for their anti-trypanosomal activities (Steverding and Sexton, 2013; Steverding et al., 2016; Antoszczak et al., 2019b; Czerwonka et al., 2021). In the course of these studies, we have found that inverting the relative configuration of the C20 hydroxyl group, situated on the salinomycin C-ring, reduces the trypanocidal activity (Czerwonka et al., 2021). On the other hand, oxidation of the C20 into a keto function leads to an increase in trypanocidal activity compared to the parent compound (Antoszczak et al., 2019b). In light of

these observations, it would be interesting to evaluate the trypanocidal activity of 20-deoxysalinomycin (SY-1), a compound related to salinomycin that lacks its C20 hydroxyl group (Fig. 1). 20-Deoxysalinomycin is an intermediate in the biosynthesis of salinomycin and occurs as a minor product during the fermentation of *Streptomyces albus* ATCC 21838 (Westley et al., 1977; Miyazaki et al., 1978; Yurkovich et al., 2012). Until recently, 20-deoxysalinomycin has not been readily available, but in 2014 a practical semi-synthesis through a regioselective allylic radical-deoxygenation of salinomycin was reported (Huang et al., 2014).

Here, we report on the trypanocidal and cytotoxic activity of 20-deoxysalinomyicn using bloodstream forms of *Trypanosoma brucei* and human myeloid HL-60 cells, respectively. The effectiveness of the 20-deoxysalinomyicn to induce cell swelling in trypanosomes is also described and mechanistic implications are discussed.

2. Materials and methods

2.1. Compounds

20-Deoxysalinomycin sodium salt was prepared as previously described (Huang et al., 2014). Salinomycin sodium salt was isolated from commercially available veterinary premix SACOX® as previously described (Antoszczak et al., 2019b). Suramin sodium salt was purchased from Fluka, Germany.

2.2. Cell culture

Bloodstream forms of *T. brucei* (clone 427-221a; Hirumi et al., 1980) and human myeloid leukaemia HL-60 cells (Collins et al., 1977) were grown in Baltz medium (Baltz et al., 1985) supplemented with 16.7% heat-inactivated bovine serum. The cultures were maintained in an incubator in a humidified atmosphere containing 5% CO_2 at 37 °C.

96 *2.3. In vitro cell toxicity assay*

97

98 The anti-proliferate activity of 20-deoxysalinomycin, salinomycin, and suramin was
99 evaluated as previously described (Merschjohann et al., 2001) with some modifications. In
100 brief, cells (trypanosomes and HL-60 cells) were seeded in 96-well plates to a final volume of
101 200 µl Baltz medium containing various concentrations of test compounds (tenfold dilution
102 from 100 µM to 100 pM) and 0.9% DMSO. Wells containing medium and 0.9% DMSO served
103 as controls. The initial cell densities were 1×10^4/ml for trypanosomes and 5×10^4/ml for HL-
104 60 cells. After incubation for 24 h, 20 µl of a 0.5 mM resazurin solution prepared in sterile PBS
105 was added and the cells were incubated for a further 48 h. Thereafter, the absorbance of wells
106 was read on a BioTek ELx808 microplate reader using a test wavelength of 570 nm and a
107 reference wavelength of 630 nm. The 50% growth inhibition (GI_{50}) value, i.e., the
108 concentration of a compound necessary to reduce the growth rate of cells by 50% compared to
109 the control, was determined using a 4-parameter logistic online curve calculator (AAT
110 Bioquest, 2022).

111

112 *2.4. Cell swelling assay*

113

114 Change in cell volume in flagellated protozoans can be measured by the light scattering
115 technique monitoring the absorbance of cell suspensions between 450 and 550 nm (Park et al.,
116 1977) whereby a decrease in absorbance corresponds to an increase in cell volume. Based on
117 the filter availability of the BioTek ELx808 microplate reader, changes in cell volume of
118 trypanosomes were determined at 490 nm as previously described (Steverding and Sexton,
119 2013). In brief, bloodstream-form trypanosomes were incubated at a density of 5×10^7 cells/ml
120 in 96-well plates in a final volume of 200 µl Baltz medium containing 100 µM ionophore and
121 0.9% DMSO (test) or 0.9% DMSO alone (control). The absorbance of the cultures was
122 measured every 10 min for 1 h. At the end of the experiment, cells were microscopically
123 checked for motility as an indicator of liveliness.

124

3. Results and discussion

20-Deoxysalinomycin showed a dose-dependent inhibitory effect on the growth of bloodstream forms of *T. brucei* with a GI_{50} value of 0.12 μM (Fig. 2a), and was found to be 8.3 times more trypanocidal than the parent compound salinomycin (GI_{50} = 1.00 μM (Fig. 2a)). On the other hand, 20-deoxysalinomycin and salinomycin displayed similar cytotoxic activity toward human HL-60 cells with GI_{50} values of 3.1 μM and 6.2 μM, respectively (Fig. 2b). Thus, while salinomycin showed only moderate selectivity with a GI_{50} ratio of 6 (defined as the cytotoxic to trypanocidal activity ratio), 20-deoxysalinomycin had a four-times higher GI_{50} ratio of 26. Furthermore, 20-deoxysalinomycin was only slightly less trypanocidal than suramin (GI_{50} = 0.044 μM), one of the drugs currently used for the treatment of sleeping sickness (Fig. 2a). However, suramin is nontoxic to human cells (GI_{50} >100 μM). Thus, while the data highlights 20-deoxysalinomycin as having a superior selectivity compared to salinomycin, structural analogues of this compound with stronger trypanocidal activity and further reduced cytotoxicity remain an important pursuit.

The trypanocidal activity of polyether ionophore antibiotics is due to their ability to mediate a sodium ion influx in trypanosomes, which in turn, leads to cell swelling (Steverding and Sexton, 2013; Steverding et al., 2016; Steverding and Huczyński, 2017; Antoszczak et al., 2019b; Czerwonka et al., 2021). In general, salinomycin derivatives with enhanced trypanocidal activity also display increased ionophoretic activity when compared to the unmodified parent compound (Steverding et al., 2016; Antoszczak et al., 2019b; Czerwonka et al., 2021). Exceptions have however been reported (Antoszczak et al., 2019b). For example, salinomycin derivates modified at the C1-position combine enhanced antitrypanosomal properties with reduced ionophoretic activity (Steverding et al., 2016; Antoszczak et al., 2019b).

To gain insight into the factors underlying the activity differences between 20-deoxysalinomycin and salinomycin, we sought to evaluate whether the enhanced trypanocidal activity of 20-deoxysalinomycin was related to increased iontophoresis. Bloodstream forms of *T. brucei* were therefore incubated with 20-deoxysalinomycin at 100 μM concentration. The

resulting swelling of the cells was initially similar to that of parasites treated with salinomycin at the same concentration (Fig. 3). However, after 20 min incubation, the swelling induced by 20-deoxysalinomycin was more pronounced and by the end of the experiment, trypanosomes incubated with 20-deoxysalinomycin had swollen 30% more compared to parasites exposed to the parent compound (Fig. 3). It should be noted that at the end of the experiment, the trypanosomes had lost their normal elongated shape and appeared as round cells; an effect previously described for trypanosomes treated with salinomycin (Steverding and Sexton, 2013). Nevertheless, the trypanosomes were still alive as shown by minor movement. This result indicates that the higher trypanocidal activity of 20-deoxysalinomycin compared to salinomycin is indeed connected to a higher ionophoretic activity. The molecular level origin for this difference is however not obvious. Structurally, the global conformation of salinomycin is conserved in 20-deoxysalinomycin (Huang et al., 2014; Borgström et al., 2017). As for the C20-hydroxyl group of salinomycin, it is involved in intramolecular hydrogen bonding, but not metal coordination (Paulus et al., 1998). We interpret the higher ionophoretic activity of 20-deoxysalinomycin as, at least in part, related to its higher lipophilicity leading to more facile uptake in lipophilic environments like biological membranes (miLogP for 20-deoxysalinomycin = 7.36, miLogP for salinomycin = 6.45; determined using the Molinspiration interactive logP calculator (Molinspiration Cheminformatics, 2022)). However, an additional contributor may be that the absence of a C20-hydroxyl group facilitates ion transport by a faster capture-release mechanism of sodium ions for the deoxygenated structure (Matsumori et al., 2007).

In summary, this study further supports our previous findings that derivatisation of salinomycin is an efficient approach to developing compounds with increased trypanocidal activity (Steverding et al., 2016; Antoszczak et al., 2019b). 20-Deoxysalinomycin, a natural product, structurally related to salinomycin but lacking its hydroxyl group at the C20 position, proved to be a promising trypanocidal compound being almost an order of magnitude more effective and four times more selective than its parent compound when evaluated with bloodstream-form trypanosomes *in vitro*. In addition, 20-deoxysalinomycin matches key criteria for drug candidates against African trypanosomiasis (GI_{50} <0.2 µg/ml, GI_{50} of 20-

deoxysalinomycin = 0.088 µg/ml; SI >100, SI of 20-deoxysalinomycin = 26 (Nkawa and Hudson, 2006)). Going forward, it should be possible to further improve the trypanocidal activity and drug properties of this lead, for instance, through modifications of the C1 carboxylate moiety (Steverding et al., 2016; Antoszczak et al., 2019b). Such studies are underway in our laboratory and will be reported in due course.

CRediT author statement

Dietmar Steverding: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – Original draft, Visualization. **Daniel Strand**: Methodology, Validation, Investigation, Recourses, Writing – Review and Editing, Funding acquisition; **Adam Huczyński:** Conceptualization, Resources, Writing – Review and Editing.

Acknowledgements

Daniel Strand thanks The Swedish Cancer Foundation, The Crafoord Foundation, and the Swedish Research Council for funding.

Declaration of competing interest

The authors declare that they have no conflict of interest.

References

AAT Bioquest, 2022. Quest Graph™ Four Parameter Logistic (4PL) Curve Calculator. https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator (assessed 16 July 2022).

Antoszczak, M., Steverding, D., Huczyński, A., 2019a. Anti-parasitic activity of polyether ionophores. Eur. J. Med. Chem. 166, 32-47.

Antoszczak, M., Steverding, D., Sulik, M., Janczak, J., Huczyński, A., 2019b. Anti-trypanosomal activity of doubly modified salinomycin derivatives. Eur. J. Med. Chem. 173, 90-98.

Baltz, T., Baltz, D., Giroud, C., Crockett, L., 1985. Cultivation in a semi-defined medium of animal infective forms of *Trypanosoma brucei*, *T. equiperdum*, *T. evansi*, *T. rhodesiense* and *T. gambiense*. EMBO J. 4, 1273-1277.

Borgström, B., Huang, X., Hegardt, C., Oredsson, S., Strand, D., 2017. Structure-activity relationships in salinomycin: Cytotoxicity and phenotype selectivity of semi-synthetic derivatives. Chem. Eur. J. 23, 2077-2083.

Collins, S.J., Gallo, R.C., Gallagher, R.E., 1977. Continuous growth and differentiation of human myeloid leukaemic cells in suspension culture. Nature 270, 347-349.

Czerwonka, D., Barcelos, Y., Steverding, D., Cioch, A., Huczyński, A., Antoszczak, M., 2021. Single and double modified analogues of C20-*epi*-salinomycin: A new group of antiparasitic agents against *Trypanosoma brucei*. Eur. J. Med. Chem. 209, 112900.

Delespaux, V., de Koning, H.P., 2007. Drugs and drug resistance in African trypanosomiasis. Drug Resist. Updat. 10, 30-50.

Giordani, F., Morrison, L.J., Rowan, T.G., de Koning, H.P., Barrett, M.P., 2016. The animal trypanosomiases and their chemotherapy: a review. Parasitology 143, 1862-1889.

Hirumi, H., Hirumi, K., Doyle, J.J., Cross, G.A.M., 1980. *In vitro* cloning of animal-infective bloodstream forms of *Trypanosoma brucei*. Parasitology 80, 371-382.

Huang, X., Borgström, B., Månsson, L., Person, L., Oredsson, S., Hegardt, C., Strand, D., 2014. Semisynthesis of SY-1 for investigation of breast cancer stem cell selectivity of C-ring-modified salinomycin analogues. ACS Chem. Biol, 9, 1587-1594.

Matsumori, N., Morooka, A., Murata, M., 2007. Conformation and location of membrane-bound salinomycin-sodium complex deduced from NMR in isotropic bicelles. J. Am. Chem. Soc. 129, 14989-14995.

Mattioli, R.C., Cecchi, G., Paone, M., Herrero, R.A., Simarro, P.P., Priotto, G., Franco, J.R., 2016. The programme against African trypanosomosis. EMPRES-Animal Health 360 46, 9-13.

241 Merschjohann, K., Sporer, F., Steverding, D., Wink, M., 2001. *In vitro* effect of alkaloids on
242 bloodstream forms of *Trypanosoma brucei* and *T. congolense*. Planta Med. 67, 623-
243 627.
244 Miyazaki, Y., Shibata, A., Tsuda, K., Kinashi, H., Ōtake, N., 1978. Isolation, characterization
245 and structure of SY-1 (20-deoxysalinomycin). Agr. Biol. Chem. 42, 2129-2132.
246 Molinspiration Cheminformatics, 2022. Interactive logP calculator.
247 https://www.molinspiration.com/services/logp.html (accessed 3 June 2022).
248 Nwaka, S., Hudson, A. (2006) Innovative lead discovery strategies for tropical diseases. Nat.
249 Rev. Drug Discov. 5, 941-955.
250 Okello, I., Mafie, E., Eastwood, G., Nzalawahe, J., Mboera, L.E.G., 2022. African animal
251 trypanosomiasis: A systematic review on prevalence, risk factors and drug resistance in
252 sub-Saharan Africa. J. Med. Entomol. 59, 1099-1143.
253 Park, J.-H., Schofield, P.J., Edwards, M.R. (1997) *Giardia intestinalis*: volume recovery in
254 response to cell swelling. Exp. Parasitol, 86, 19-28.
255 Paulus, E.F., Kurz, M., Matter, H., Vértesy, L., 1998. Solid-state and solution structure of the
256 salinomycin-sodium complex: Stabilization and different conformers for an ionophore
257 in different environments. J. Am. Chem. Soc. 120, 8209-8221.
258 Steverding, D., 2008. The history of African trypanosomiasis. Parasit. Vectors 1, 3.
259 Steverding, D., 2017. Sleeping sickness and nagana disease caused by *Trypanosoma brucei*,
260 in: Marcondes, C.B. (Ed), Arthropod Borne Diseases. Springer International
261 Publishing, Cham, pp. 277-297.
262 Steverding, D., Huczyński, A., 2017. *Trypanosoma brucei*: trypanocidal and cell swelling
263 activities of lasalocid acid. Parasitol. Res. 116, 3229-3233.
264 Steverding, D., Sexton, D.W., 2013. Trypanocidal activity of salinomycin is due to sodium
265 influx followed by cell swelling. Parasit. Vectors 6, 78.
266 Steverding, D., Antoszczak, M., Huczyńsky, A., 2016. *In vitro* activity of salinomycin and
267 monensin derivatives against *Trypanosoma brucei*. Parasit. Vectors 9, 409.

Westley, J.W., Blount, J.F., Evans, R.H. Jr., Liu, C.-M., 1977. C-17 epimers of deoxy-(O-8)-salinomycin from *Streptomyces albus* (ATCC 21838). J. Antibiot. (Tokyo) 30, 610-612.

WHO, 2019. WHO Interim Guidelines for the Treatment of Gambiense Human African Trypanosomiasis. World Health Organization, Geneva.

Yurkovich, M.E., Tyrakis, P.A., Hong, H., Sun, Y., Samborskyy, M., Kamiya, K., Leadlay, P.F., 2012. Late-stage intermediate in salinomycin biosynthesis is revealed by specific mutation in the biosynthetic gene cluster. ChemBioChem 13, 66-71.

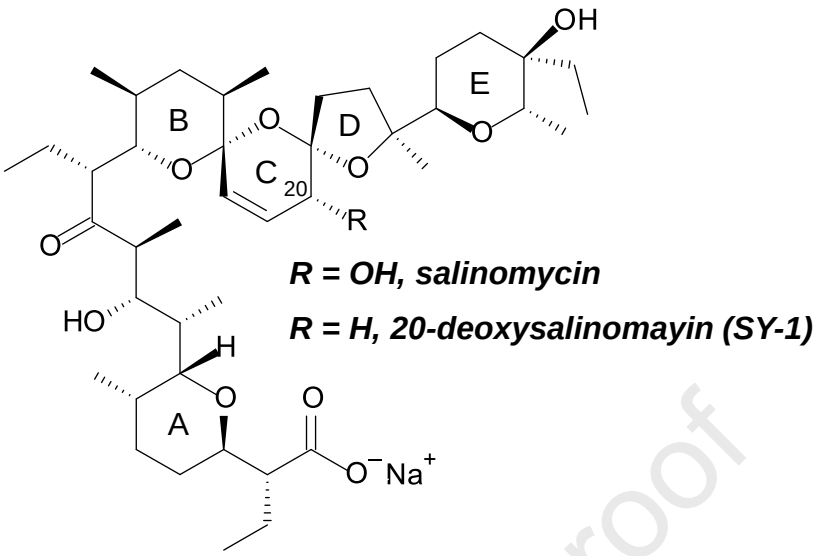
Figure legends

Fig. 1. Structure of salinomycin sodium salt and 20-deoxysalinomycin sodium salt. The PubChem Compound Identifier (CID) for salinomycin sodium salt and 20-deoxysalinomycin sodium salt is 23703990 and 132577037, respectively.

Fig. 2. Effect of 20-deoxysalinomycin, salinomycin and suramin on the growth of bloodstream forms of *T. brucei* and human myeloid leukaemia HL-60 cell. Trypanosomes (**A**) and HL-60 cells (**B**) were incubated with varying concentrations of 20-deoxysalinomy (circles), salinomycin (squares), or suramin (triangles). After 72 h of culture, cell viability and proliferation were determined with the colourimetric dye resazurin. Mean values of the three independent experiments are shown. Dose-response curves were calculated from mean values using the 4-parameter logistic model. For clarity, standard deviations were omitted. The standard deviations ranged between 0.6 and 13.4 percentage points.

Fig. 3. Effect of 20-deoxysalinomycin and salinomycin on the cell volume of bloodstream forms of *T. brucei*. Trypanosomes (5×10^7 cells/ml) were incubated with 100 µM 20-deoxysalinomycin acid (open circles) or salinomycin (open squares) in Baltz medium in the presence of 0.9% DMSO. Controls (closed diamonds) were incubated with 0.9% DMSO. Every 10 min, the absorbance at 490 nm was measured. For clarity, only the mean values of three independent experiments are shown. The standard deviations ranged between 0.035 to 0.105.

299 **Fig. 1**
300

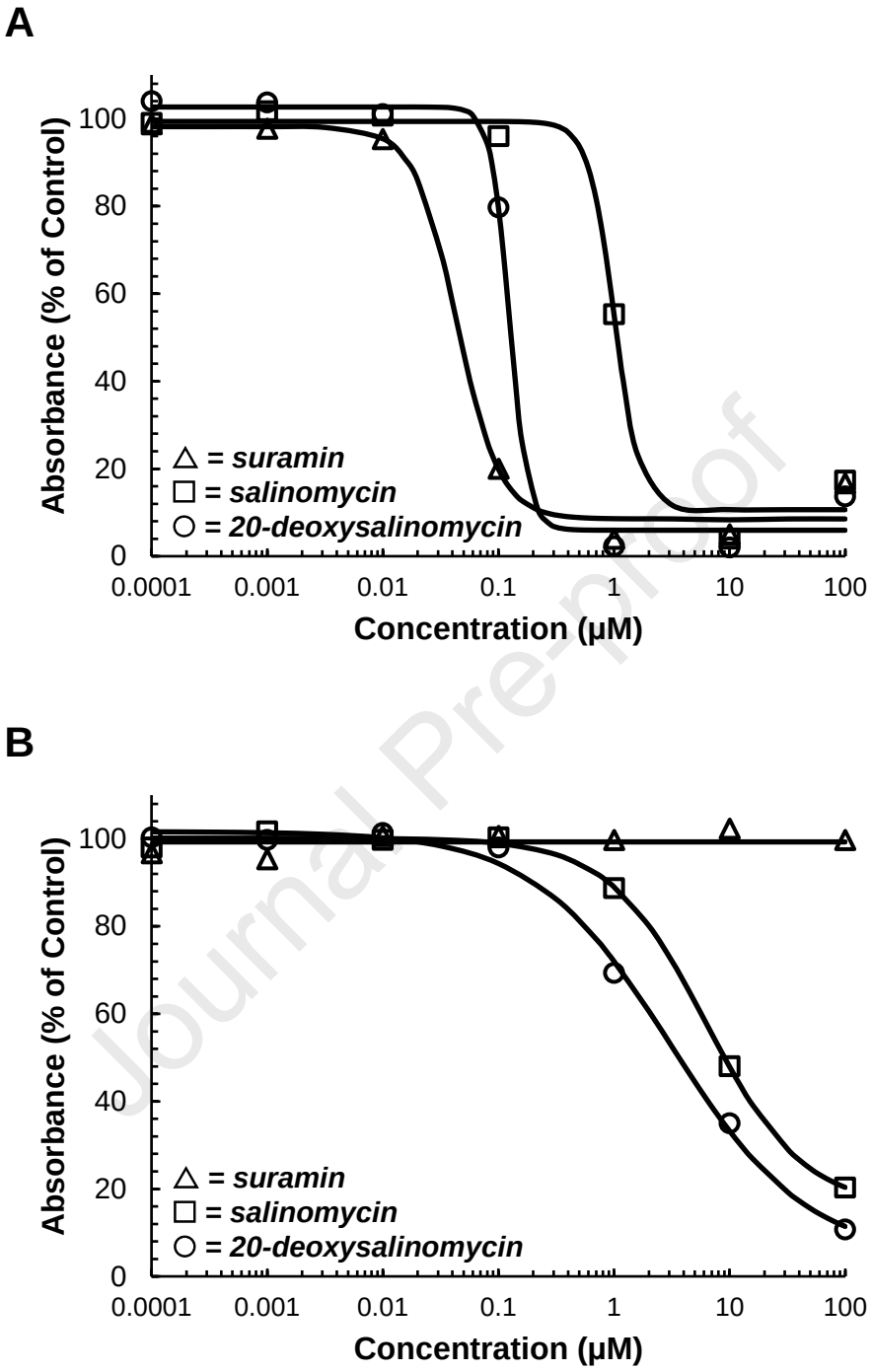


R = OH, salinomycin

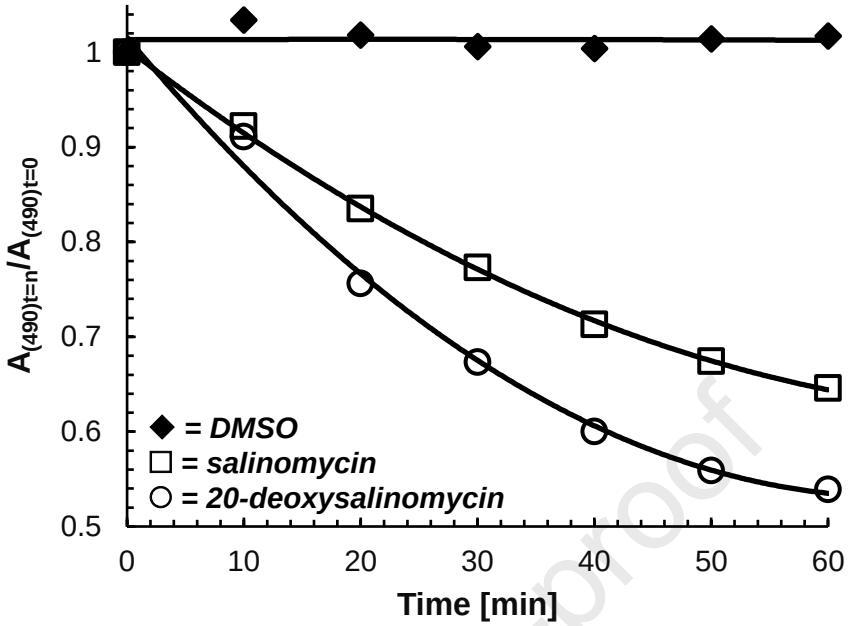
R = H, 20-deoxysalinomayin (SY-1)

301
302

Fig. 2



312 **Fig. 3**
313



314
315

Highlights

- 20-deoxysalinomycin displays stronger trypanocidal activity than salinomycin.
- 20-deoxysalinomycin induces stronger cell swelling in trypanosomes than salinomycin.
- 20-deoxysalinomycin exhibits better selectivity than salinomycin.