International Journal for Parasitology: Drugs and Drug Resistance 24 (2024) 100529

Contents lists available at ScienceDirect

International Journal for Parasitology: Drugs and Drug Resistance

journal homepage: www.elsevier.com/locate/ijpddr

Selenosugars targeting the infective stage of *Trypanosoma brucei* with high selectivity

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ARTICLE INFO

Keywords:
Selenoglycosides
Bloodstream trypanosoma
Macrophage
Redox biosensor
Oxidative stress

ABSTRACT

Earlier evidences showed that diglycosyl diselenides are active against the infective stage of African trypanosomes (top hits IC_{50} 0.5 and 1.5 μM) but poorly selective (selectivity index <10). Here we extended the study to 33 new seleno-glycoconjugates with the aim to improve potency and selectivity. Three selenoglycosides and three glycosyl selenenylsulfides displayed IC_{50} against bloodstream *Trypanosoma brucei* in the sub-μM range (IC_{50} 0.35–0.77 μM) and four of them showed an improved selectivity (selectivity index >38-folds *vs.* murine and human macrohages). For the glycosyl selenylsulfides, the anti-trypanosomal activity was not significantly influenced by the nature of the moiety attached to the sulfur atom. Except for a quinoline-, and to a minor extent a nitro-derivative, the most selective hits induced a rapid (within 60 min) and marked perturbation of the LMWT-redox homeostasis. The formation of selenenylsulfide glycoconjugates with free thiols has been identified as a potential mechanism involved in this process.

1. Introduction

Satisfactory medications to tropical diseases such as sleeping sickness, Chagas disease or leishmaniasis, caused by trypanosomatid parasites, are still missing despite continuing worldwide efforts (Field et al., 2017; Pérez-Molina and Molina, 2018; Burza et al., 2018). In this regard, neglected by the big pharma, the discovery of new molecular entities targeting pathogenic trypanosomatids still continue being a major task of academic laboratories. Furthermore, several trypanosomatids species infect and produce debilitating and mortal diseases to live-stock, which impairs the economic development of the areas and countries affected (Yaro et al., 2016).

Carbohydrate metabolism is essential for the functioning and survival of trypanosomatids in multiple ways such as for the energetic metabolism (McNae et al., 2021; Nowicki et al., 2008) and/or assembly of the glycoproteins providing a glycan coat on the surface of the parasites (Valente et al., 2019; Moreno et al., 2019; Morotti et al., 2019). Therefore small-molecule glycoconjugates have often been tested for anti-trypanosomal activities (for a recent review, see Campo et al., 2018). Organoselenium compounds have attracted much interest not just as important tools in synthetic chemistry (Baldassari and Lüdtke, 2021) but also because of their multiple biological activities (Rocha et al., 2017), in particular as agents against oxidative stress (Shaaban et al., 2019). Furthermore, it is of special note that antileishmanial effects were recorded for various selenoamide-type molecules (de Sousa Luis et al., 2019; Al-Tamimi et al., 2019).

We have previously demonstrated that glycosyl disulfide derivatives exert trypanocidal activities against trypomastigotes, the extracellular infective stage, of *Trypanosoma cruzi* from different strains (Gutiérrez et al., 2013).

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[1] This paper is dedicated to the memory of Katalin E. Kövér who, sadly, passed away during the preparation of the manuscript.
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https://doi.org/10.1016/j.ijpddr.2024.100529
Received 3 January 2024; Received in revised form 16 February 2024; Accepted 27 February 2024
Available online 2 March 2024

Among the different trypanosomatid species that are pathogenic to mammals, the bloodstream form of African trypanosomes (e.g. *Trypanosoma brucei*) dwells in extracellular environments (blood, lymph, cerebrospinal fluid and interstitial space) and is strictly dependent on carbohydrate metabolism. These organisms present a very efficient glycolytic rate and turnover of surface glycoproteins (Opperdoes, 1987; Garrison et al., 2021), which supply the high demand of ATP for growth and of antigenic variation to escape immune recognition by the host. On this basis, we speculated that Se-functionalized glycosyl-compounds should be active against African trypanosomes. This hypothesis was confirmed by observing strong growth inhibition effects (in the low μM range) of symmetric diglycopyranosyl diselenides on the bloodstream stage of *T. brucei* (Franco et al., 2017). It was furthermore found that diglycosyl diselenides may interfere with glucose consumption and redox homeostasis, the last by reacting with low molecular weight thiols, under physiological conditions, *via* formation of selenenylsulfide bonds (Franco et al., 2017). However, most of these bioactive compounds displayed a marginal capacity to discriminate the host and pathogen cell in their killing mechanism (selectivity indexes ≤10). Improvement of this property, by lowering mammalian cytotoxicity and/or by increasing the potency of the seleno-compounds towards the parasite, is a must to render them suitable candidates for further drug development. Moreover, carbohydrate-based compounds attracted our attention as privileged carrier-scaffold that may favor the active transport of the drug through the blood-brain barrier. This is of upmost clinical relevance since, during the second stage of the disease, African trypanosomes colonize the host central nervous system and most pharmaceutical available do not permeate into this space.

Bearing this in mind, we have extended testing of further seleno-glycoconjugates that fall into three groups regarding their chemical structures; selenoglycosides (**Series I**), seleno-disaccharides (**Series II**) and glycosyl-selenenylsulfides (**Series III**; Fig. 1). Furthermore, depending on the group linked to the selenium atom, **Series I** can be divided into the following subgroups: alkyl- (**1**–**4**) or aralkyl- (**5**–**14**), and aryl glycosylselenides (**20**–**24**), and selenoesters (**15**–**19**). Considering that peracetylation of monosaccharides is well known to improve cell permeability by increasing hydrophobicity, most compounds tested here were acetylated and a few of them not (**11**, **13**, **14** and **22**).

The syntheses of compounds belonging to **Series I** (**1**–**10**, **15**–**24**) and from **Series II** (**25**–**27**) have been published in (Kumar et al., 2012; André et al., 2015; Raics et al., 2022), while those of **Series III** (**28**–**33**) in (Illyés et al., 2016). For **11**–**14** see Supporting **information**.

2. Results and discussion

The activity of the seleno-glycoconjugates against the bloodstream stage of *T. b. brucei* was evaluated in a preliminary screening performed at fixed compound concentrations (Table 1). This revealed six derivatives from **Series I** (**8**, **12**, **14**, **16**, **18** and **21**), one from **Series II** (**26**) and four from **Series III** (**28**, **30**, **31** and **33**) that lowered parasite

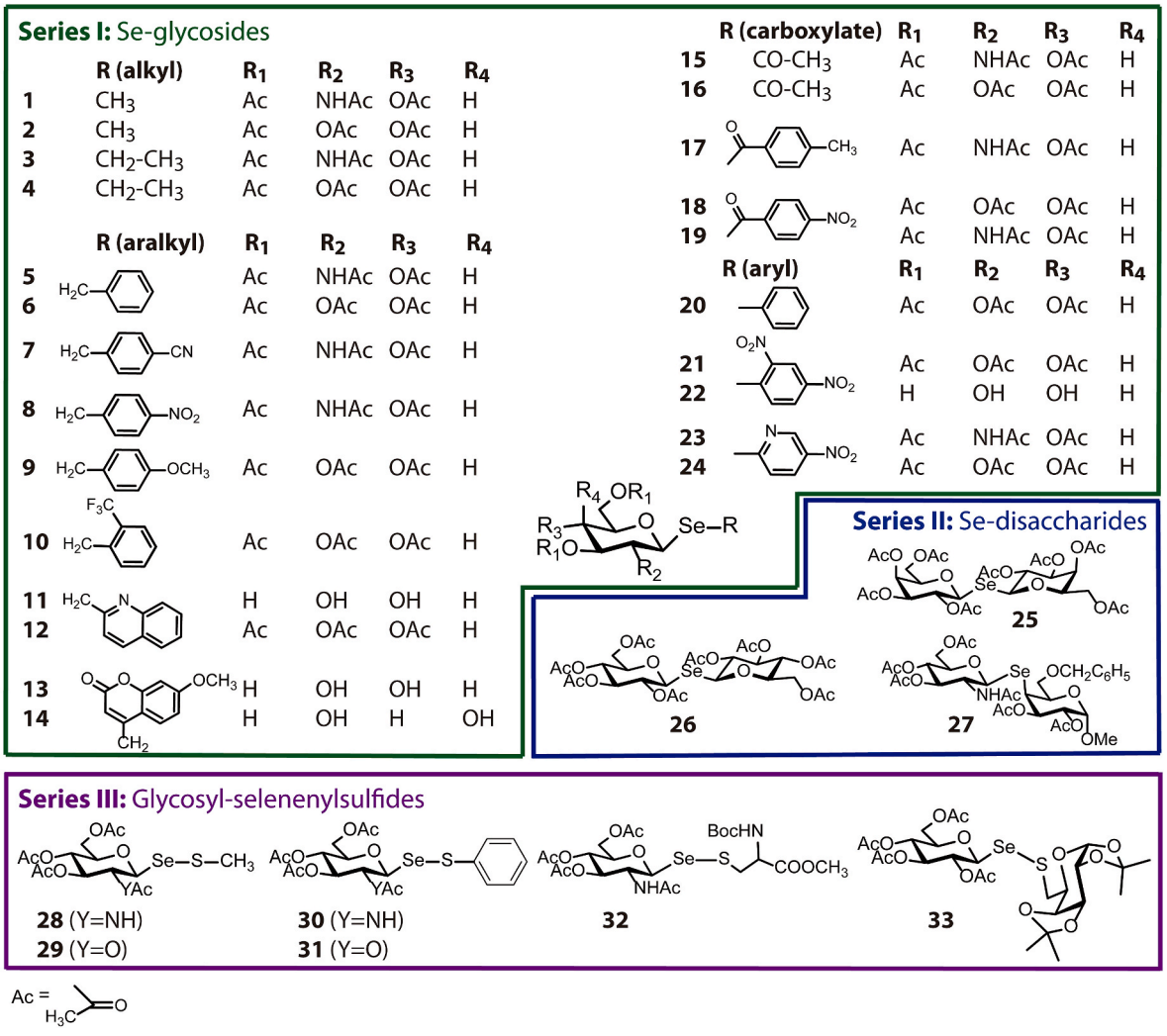


Fig. 1. Seleno-glycoconjugates investigated in this study.

Table 1
Biological activity of selenoglycoconjugates.

Entry		*T. b. brucei*		Murine macrophages	
	Se bound	Viability (%)[a]	EC_{50} (μM)	CC_{50} (μM)	SI
1	Alkyl/aralkyl	96.4 ± 13.4			
2		91.9 ± 14.8			
3		98.3 ± 20.7			
4		92.8 ± 19.5			
5		88.1 ± 17.5			
6		94.2 ± 23.5			
7		93.8 ± 21.5			
8		46.9 ± 23.9	7.69 ± 0.63	8.6 ± 0.8	1
9		93.1 ± 9.3			
10		92.5 ± 10.3			
11		57.5 ± 3.8			
12		4.6 ± 0.8	1.2 ± 0.1	26.6 ± 1.2	22
13		57.3 ± 0.4			
14		43.6 ± 3.1	4.1 ± 1.0	>100	>24
15	ester	96.4 ± 13.4			
16		0.9 ± 0.0	0.43 ± 0.01	22.8 ± 2.8	53
17		90.4 ± 20.2			
18		0.9 ± 0.1	0.37 ± 0.02	14.8 ± 0.4	40
19		86.1 ± 4.7			
20	aryl	96.4 ± 13.4			
21		4.4 ± 0.5	0.39 ± 0.02	19.3 ± 1.6	49
22		69.4 ± 5.1			
23		93.9 ± 22.6			
24		73.6 ± 2.7			
25	saccharide	96.4 ± 17.7			
26		50.7 ± 6.9	3.64 ± 0.11	10.8 ± 2.4	3
27		97.9 ± 22.0			
28	selenosulfide	0.9 ± 0.1	0.37 ± 0.02	2.8 ± 1.0	8
29		82.8 ± 12.1			
30		45.6 ± 5.0	4.74 ± 0.16	28.7 ± 2.1	6
31		1.9 ± 0.2	0.77 ± 0.02	5.1 ± 0.3	7
32		120.0 ± 6.4			
33		1.0 ± 0.1	0.35 ± 0.02	13.5 ± 1.0	38
Nfx[b]			6.0 ± 0.4	140.0 ± 2.0	23
Sur[c]			0.36 ± 0.03	ND[e]	ND
Ber[d]			0.43 ± 0.07	ND	ND

[a] Viability determined upon a 24 h incubation of bloodstream parasites with compounds tested at 5 μM.
[b] Nfx: Nifurtimox.
[c] Sur: Suramine.
[d] Ber: Berenil.
[e] ND: not determined.

viability to ≤50% at 5 μM. For these compounds, the EC_{50} was determined from concentration-response plots. Compounds **16**, **18**, **21**, **28**, **31** and **33**, have sub-μM potencies (773–350 nM) whereas **8**, **12**, **14**, **26** and **30** display one digit μM EC_{50} (1.2–7.7 μM). African trypanosomes are extracellular pathogens and macrophages play an important role as the host front line defense that contribute to control parasite infection (Stijlemans et al., 2007). Therefore, the cytotoxicity of the hit compounds was tested against murine (cell line J774; Table 1) and human macrophages (cell line THP-1; Table S1). Overall, the mammalian cells resulted less sensitive against the hits than *T. brucei* (Table 1; CC_{50} from 2.8 to >100 μM). Despite the differences in the initial cell density used in the assays, the selected hits displayed an identical trend of cytotoxicity towards both macrophage cell lines, with **14** and **28** being the less and the most cytotoxic, respectively, and **16** being the most selective.

The largest subgroup (alkyl/aralkylglycosyl-selenides, compounds **1–14**), showed three active compounds (**8**, **12** and **14**) towards *T. brucei* with an EC_{50} of 7.7, 1.2 and 4.1 μM, respectively. Interestingly, the analogues substituted with a methoxycoumarin (**14**) and a quinoline (**12**) moiety proved far more selective (SI ≥ 22) than the cytotoxic *p*-nitrobenzene derivative (**8**, SI = 1). Coumarin and quinolone are privileged bioactive scaffolds, with several derivatives being reported trypanocidal against bloodstream *T. brucei* (Balogun et al., 2019; Koester et al., 2022). Therefore, it is tempting to speculate that these moieties contribute by themselves to the anti-trypanosomal activity observed in **12** and **14**, and in the corresponding glucosyl analogues **11** and **13** (EC_{50} ~5 μM). Within the group of glycosyl selenoesters (**15–19**), two compounds (**16** and **18**) showed activity towards *T. brucei* in the nM range, and high selectivity (SI > 40).

It is interesting to note that the active compounds are mostly glucose or galactose (**14**) derivatives while the inactive ones are glucosamine derivatives, which would allow us to think that the presence of this NHAc group is detrimental for the activity. The only exception to this rule is **8**, for which the *p*-nitrobenzene group is clearly conferring a non-selective bioactivity. On the other hand, the inclusion of the selenoester functional group can be important when it comes to activity, since it provides another electrophilic position with respect to the alkylglycosyl-selenides. This can be evidenced comparing the difference of potency and cytotoxicity of compounds **16** and **4**. Finally, in the arylglycosides group, an active candidate was found, **21**, with an nM IC_{50} (0.39 ± 0.02 μM) and a very good selectivity (SI = 49). An important element for the activity turned out to be the nitro substituents since compound **20**, with an unmodified aromatic ring, was inactive. This may be due to their interaction in the target active site, or to the electron withdrawing effect of the nitro group, which allows, for example, the stabilization of a negative charge if their activity could be due to the loss of arylglycosyl-selenides. Replacement of the phenyl group by a pyridine heterocycle (**23** and **24**) also was detrimental for activity.

With respect to the few selenosaccharides tested (**Series II**), only the acetylated diglucosyl derivative **26** proved active against *T. b. brucei* (EC_{50} = 3.6 μM) but similarly cytotoxic to murine macrophages (SI = 3). In contrast, the di-galactosyl derivative **25** and N-acetylglucopyranosyl-galactopyranoside (**27**) lacked anti-trypanosomal activity. This is in line with the statement above on the inactivity of NHAc derivatives.

For **Series III**, several acetylated forms of β-D-glucopyranosyl selenenylsulfide proved highly active against bloodstream parasites. For the S-methylated derivatives, the glucosamine (**28**, EC_{50} = 0.37 μM) but not the glucose (**29**, 83% viability at 5 μM) core contributed to activity. The opposite behavior was observed when the S-substituent was a phenyl group: the glycosyl derivative **31** (EC_{50} = 0.77 μM) was 6-folds more potent than the glucosamine derivative (**30**, EC_{50} = 4.74 μM). The selenide-sulfide bond appears to be a major contributor of bioactivity as revealed by the sub-μM activity of **28** and **31**, and the lack of it in the corresponding analogues **1** and **20** (both inactive at 5 μM). Sulfur conjugation to a protected alanine (**32**) did not confer the compound anti-trypanosomal activity. In contrast, linking the acetylated form of the β-D-glucopyranosyl moiety through a selenenylsulfide bond to di-O-isopropylidene α-D-galactopyranose yielded the most active compound of this series (**33**, EC_{50} = 0.35 μM). The potency of **33** is similar to that reported previously for a related compound, an acetylated form of a di-β-D-glucopyranosyl diselenide, against *T. b. brucei* (EC_{50} = 0.54 μM; Franco et al., 2017), but the selectivity was remarkably enhanced, **33** being four times more selective.

Restricting the comparative analysis to compounds with a non-acetylated counterpart (**11** *vs* **12** and **22** *vs* **21**), it seems that the protection of the sugar's OH groups is beneficial for bioactivity (compare viability % or EC_{50} values in Table 1). Probably due to the favored passive diffusion of the peracetylated sugars.

Taking into account the redox chemistry of Se and previous evidence obtained with diselenyl-diglycosides (Franco et al., 2017), the effect of the most selective (SI ≥ 8) hits from each group (**12**, **14**, **16**, **18**, **21**, **28** and **33**) on the thiol-redox metabolism of the parasite was investigated. The experiments were conducted with a redox-reporter cell line of bloodstream parasites that express a redox-sensitive version of the Green Fluorescent Protein (roGFP2) whose fluorescence spectrum shows a ratiometric variation according to the intracellular level of oxidized vs. reduced low molecular weight thiols (Gutscher et al., 2008). Worth noting, a short exposure time of the cells to the compounds was chosen because it allows performing an early diagnosis of the intracellular redox

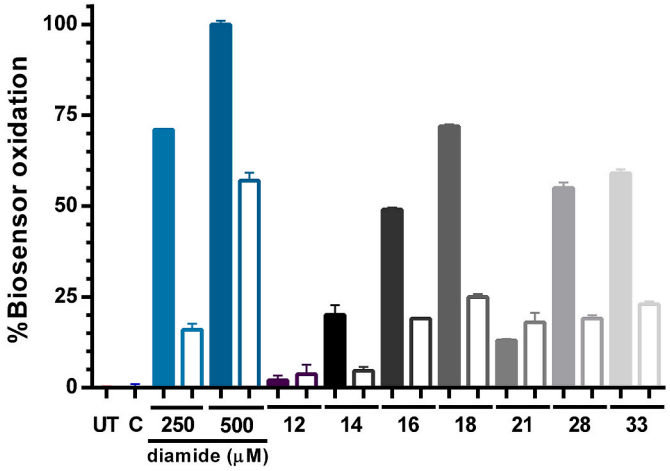


Fig. 2. Intracellular redox changes induced in bloodstream *T. brucei* by the most selective hits. Redox reporter parasites in the exponential phase were exposed for 1 h to the corresponding EC_{50} of each compound or for 20 min to the thiol-oxidant agent diamide (solid bars). Thereafter, the samples were incubated for 20 min with 1 mM DTT (empty bars). All samples were analyzed by flow cytometry and the % oxidation of the biosensor is referred to conditions yielding maximum (diamide 500 μM) and minimum (UT) biosensor oxidation. UT, untreated cells; C, cells treated with vehicle (1% v/v DMSO).

state of low molecular weight thiols and avoids the miss-assignment of low fluorescence intensity signals to potential secondary (e.g. impaired expression, spontaneous oxidation or low chromophore maturation of the biosensor) but not to the primary effects of the selenoglycosydes (e.g. thiol oxidation, inhibition of redox enzymes, etc).

Except for the quinolino Se-glycoside **12**, all other hits assayed exerted a significant ($p < 0.001$ compared to DMSO-treated parasites) oxidation of the redox biosensor when added to the parasites at their corresponding EC_{50} for 1 h (Fig. 2). According to their oxidative potency, the compounds can be ordered as follows: **18** (72% oxidation) > **33** ≈ **28** (59-55% oxidation) > **16** (49% oxidation) > **14** (20% oxidation) > **21** (13% oxidation). With the exception of **21**, treatment with DTT reverted the oxidation of the biosensor induced by all compounds, supporting the redox basis of their mode of action. The rapid onset (1 h) of the marked redox unbalance triggered by **16**, **18**, **28** and **33** at sublethal concentrations suggests that this is the main mechanism by which these hits cause parasite damage.

With respect to the chemical basis behind the redox activity of these compounds, for **16** and **18** is tempting to speculate that the presence of the carbonyl group is important for the activity, the carbon of the carbonyl group being the preferred electrophile. The selenoester bonds in **16** and **18** are highly polarizable resulting in the formation of symmetric diselenides under mild conditions such as in the presence of a weak base (Kawai et al., 2005; Nanami et al., 2007). We have now observed formation of symmetric diselenide **A** when **16** was kept in aqueous phosphate buffer of pH 7.38 (Scheme 1 and Fig. 3).

We have previously obtained NMR evidence that reaction of **A** with

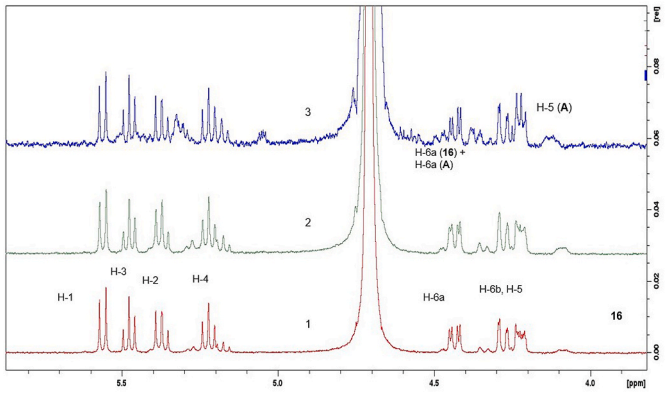


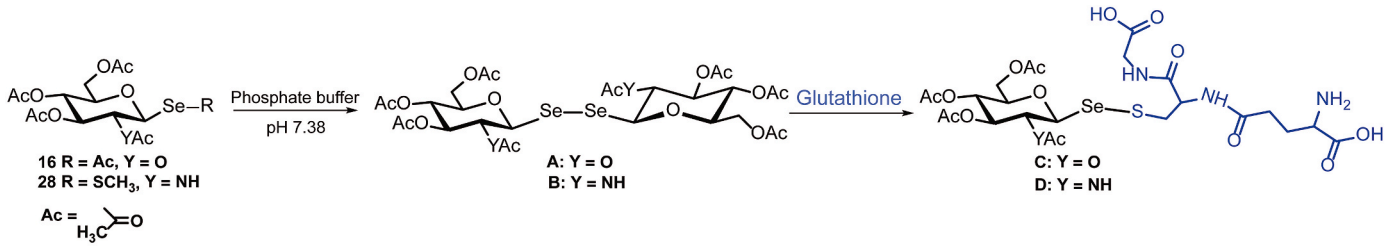
Fig. 3. ^{1}H NMR spectra of **16** in phosphate buffer in D_2O-DMSO-d_6 (9:1), pH 7.38, T = 37 °C. (1) at 0 min; (2) after 30 min; (3) after 24 h. The increase in the intensity of the distinct H-6a (4.32–4.42 ppm) and H-5 (4.1 ppm) signals of **A** (superposed on those of **16**) confirms the formation of the symmetric diselenide **A**.

glutathione, as a model of low molecular weight thiols, resulted in the formation of glutathionyl selenenylsulfide **C** under similar conditions (Franco et al., 2017). We have now found further evidence for this mechanism using mass spectrometry: the peak at m/z 718.2 in the LCMS chromatogram which agrees with $[M+H]^+$ of structure of **C** (from **16**) shown on Scheme 1 (Figs. S1 and S2). Similar behavior was observed for the selenosulfide **28** i. e, formation of GSH conjugate **D** through the symmetric diglycosyl diselenide **B** (Scheme 1, Fig. S3). It is therefore suggested that the route depicted in Scheme 1 may be a mechanism for depletion of biological thiols by glycosyl selenoesters or selenenylsulfides under mild conditions. The quinolin-2-ylmethyl- **12**, the methoxycoumarin- **14** and the dinitrophenyl- **21** selenenylglycosides, on the other hand, do not contain a polarizable Se-(CO)-R (such as in **16**) or Se–S (such as in **28**) bond, hence, the lack of, or reduced activity in the redox biosensor assay is in line with the suggested molecular mechanism. To this point the stability of **12**, under conditions listed to Fig. 3, serves as an example of negative control (Fig. S4).

Furthermore, the presence of the coumarin group in **14** may be responsible for ameliorating the oxidant effect of the Se-glycoside moiety. This is supported by the fact that coumarins are well known for their antioxidant action by direct scavenging of radicals (i.e. *via* electron donation from the ortho-hydroxyl group) and by inhibiting transition metal-induced reactive species generation (i.e. *via* bidentate metal chelation; Payá et al., 1992).

3. Conclusions

From 33 selenium-containing glycoconjugates (alkyl- and aralkyl Se-glycosides, Se-carboxylates, Se-sulfides and Se-disaccharides), four derivatives have improved potency ($EC_{50} < 0.5$ μM) and selectivity (SI > 38 with murine macrophages as mammalian cell model) with respect to previously characterized di-β-D-gluco- and galactospyranosyl



Scheme 1. Proposed reaction mechanism of glycosyl selenoesters (e.g. **16**) and glycosyl selenosulfides (e.g. **28**) in physiological media based on experimental evidence.

diselenides (EC_{50} = 0.5–1.5 μM, SI < 10) (Franco et al., 2017). Two of these hits are selenocarboxylates (**16** and **18**). Although several monoglycosyl-selenenylsulfides were active towards the pathogen, most of them (3 out of 4 hits) displayed marginal selectivity (SI ≤ 8), suggesting that the selenenylsulfide function may confer the compounds a non-specific mode of action. As pointed above, acetylated disaccharides bearing a diselenyl moiety proved cytotoxic to macrophages. This property was marginally improved in the selenenylsulfide disaccharide **33**. However, the compound displayed an almost two-fold higher anti-parasitic potency, giving as a result a good SI. Except for the quinoline and the dinitrophenyl glycosides **12** and **21**, the other five hits (**14**, **16**, **18**, **28** and **33**) were able to alter the LMWT-redox homeostasis of the pathogen to a significant extent in a short time window (60 min). *In vitro*, these compounds proved reactive against a physiological LMWT (GSH), which led to the formation of a selenenylsulfide and suggest that free cellular thiols may be molecular targets. Except that **14**, **16**, **18**, **28** and **33** accumulate rapidly to concentrations approaching those of LMWT (amount of free thiols contributed by LMWT in bloodstream *T. b. brucei* ≥1 mM; Krauth-Siegel and Comini, 2008), depletion of LMWT by formation of selenenylsulfides is unlikely to be the primary cause of parasite death. Instead, the rapid onset of the intracellular oxidative milieu they induced at low or sub-μM concentrations (EC50 < 0.5 μM) suggest a mode of action involving the inhibition of key enzymes linked to the thiol-redox metabolism of the parasite. In this respect, highly nucleophilic cysteine residues in redox enzymes qualify as suited candidates for reacting and forming covalent complexes with the selenenylglycosides. Future studies will aim to identify the potential macromolecular targets of the most interesting hits as well as their *in vivo* performance (therapeutic efficacy and pharmaco-kinetics and -dynamics profile).

Author contributions

All authors have given approval to the final version of the manuscript.

Funding sources

This study was supported by FOCEM (Fondo para la Convergencia Estructural del Mercosur, Grant Number COF 03/11) in Uruguay and NKFI (National Research, Development and Innovation Office) grant no. NN-128368, in Hungary.

Notes

The authors declare no competing financial interest.

Declaration of competing interest

The authors declare no competing financial interest.

Acknowledgements

ED, AM and MAC belong to the Uruguayan National System of Researchers (SNI) from ANII (Agencia Nacional de Investigación e Innovación), Uruguay. Thanks are due to Dr. Attila Gáspár, Department of Inorganic and Analytical Chemistry, Faculty of Science and Technology, University of Debrecen, Hungary, for the ESI HRMS spectra.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpddr.2024.100529.

Abbreviations

GSH	glutathione
LMWT	low molecular weight thiols
roGFP2	redox-sensitive Green Fluorescence Protein 2
T. b. brucei	*Trypanosoma brucei*
T. cruzi	*Trypanosoma cruzi*

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