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## Diglycosyl diselenides alter redox homeostasis and glucose consumption of infective African trypanosomes



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### ABSTRACT

With the aim to develop compounds able to target multiple metabolic pathways and, thus, to lower the chances of drug resistance, we investigated the anti-trypanosomal activity and selectivity of a series of symmetric diglycosyl diselenides and disulfides. Of 18 compounds tested the fully acetylated forms of di- $\beta$ -D-glucopyranosyl and di- $\beta$ -D-galactopyranosyl diselenides (**13** and **15**, respectively) displayed strong growth inhibition against the bloodstream stage of African trypanosomes (EC<sub>50</sub> 0.54  $\mu$ M for **13** and 1.49  $\mu$ M for **15**) although with rather low selectivity (SI < 10 assayed with murine macrophages). Nonacetylated versions of the same sugar diselenides proved to be, however, much less efficient or completely inactive to suppress trypanosome growth. Significantly, the galactosyl (**15**), and to a minor extent the glucosyl (**13**), derivative inhibited glucose catabolism but not its uptake. Both compounds induced redox imbalance in the pathogen. *In vitro* NMR analysis indicated that diglycosyl diselenides react with glutathione, under physiological conditions, via formation of selenenylsulfide bonds. Our results suggest that non-specific cellular targets as well as actors of the glucose and the redox metabolism of the parasite may be affected. These molecules are therefore promising leads for the development of novel multitarget antitrypanosomal agents.

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### 1. Introduction

Chemotherapy represents, definitely, the first and most important line of defense to treat (sub)tropical diseases, like sleeping sickness or Chagas disease, caused by pathogenic trypanosomes. Molecules endowed with antitrypanosomal activity have been known since the beginning of the 20th century; almost all of them being produced by synthetic organic chemistry. This point is noticeable because lead molecules or precursors with therapeutic promise are often obtained from natural sources like plants or microorganisms in other areas of medicinal chemistry research. Chemotherapeutic approaches for African trypanosomiasis have

been amply covered in recent reviews (Lüscher et al., 2007; Bacchi, 2009a; Steverding, 2010; Simarro et al., 2012; Babokhov et al., 2013) and will, therefore, only be briefly outlined here.

Emerging from German dyestuff industry research, naphthalenesulfonic acid derivative suramin (Bayer 205) was one of the first hits. Arsenobenzene derivatives of melamine: melarsene, melarsene oxide and melarsoprol have also long been known and are still being used against human African trypanosomiasis (HAT; sleeping sickness). The third group of anti-HAT structures comprises aromatic bis(amidines) like pentamidine or DB289 (Thuita et al., 2015). Nifurtimox has long been in use for the treatment of *Trypanosoma cruzi*-induced Chagas disease but it has recently been found effective against sleeping sickness, particularly in combination with eflornithine, itself an anti-HAT drug. Several molecules are being tested as new drug candidates; those which have entered Phase I clinical trials at least are reviewed by (Babokhov et al., 2013). The chemical structures of these trypanocidal molecules together with some others (see below) that did not reach yet the

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clinical phase are shown in Fig. 1.

Almost all of the molecules in Fig. 1 contain aromatic/heteroaromatic ring systems and the common heteroatoms oxygen and nitrogen. The occurrence of other V/VI column heteroatoms are much sparser: arsenic in the three melarsen derivatives, sulfur in suramine, melarsoprol, nifurtimox and ebsulfur (Lu et al., 2013), and selenium in ebselen (Joyce et al., 2013) only. Although carbohydrates, natural and synthetic derivatives alike, constitute one of the largest and most varied group of biologically active compounds, these moieties occur only in some nucleoside derivatives with trypanocidal activity, such as Genzyme 644131/MDL-738 (Bacchi et al., 2009b) or cordycepin and analogs (Vodnala et al., 2013). Antitrypanosomal activities were recently recorded for auranofin (see below), a gold-containing thiosugar (Ilari et al., 2012) and for some *N*-acyl-glucosamine derivatives (D'Antonio et al., 2015). Particularly, carbohydrate metabolism deserves attention as a potential target for the inhibition of trypanosomes because these pathogens depend on glucose as a preferred source of energy (Yorke et al., 1929; Bringaud et al., 2006) and as building block of several metabolic and structural macromolecules (Creek et al., 2015). Worth noting, glucose is the only source of energy for the bloodstream form of African trypanosomes.

Glycosyl disulfide derivatives, a novel class of carbohydrate structures (for a review see (Szilágyi and Varela, 2006)), gained importance recently as various biological activities were recorded for them such as binding to lectins (André et al., 2006; Murthy et al., 2009; Martín-Santamaría et al., 2011; André et al., 2015), enzyme

inhibition (Kim et al., 2007) or anti-tumor activity (André et al., 2015). Moreover, some derivatives featuring monosaccharide sugar moieties attached to aromatic cores by disulfide linkages were found to inhibit the growth of *T. cruzi* at low  $\mu\text{M}$  range (Gutiérrez et al., 2013).

Encouraged by these results and by the fact that the bloodstream stage of the African trypanosomes is dependent on glucose consumption for survival we have set up a panel of carbohydrate structures exposing mono- or disaccharide moieties mounted on aromatic scaffolds by linker motifs containing sulfur or selenium atoms (1–12, Fig. 2). In a separate study these compounds were found to bind to lectins and have activity against tumor cell lines (Kaltner et al., 2017).

Organoselenium compounds and, particularly, symmetric diorganyl diselenides are known to have multiple biological activities and are relatively nontoxic to higher organisms (Shaaban et al., 2015). Some diaryl diselenide derivatives displayed anti-proliferative activity towards the intracellular form of *Leishmania infantum* (Plano et al., 2011; Baquedano et al., 2016). Some of them proved to inhibit trypanothione reductase (TR), the major reductase of trypanosomatids that contributes to redox homeostasis (Krauth-Siegel and Comini, 2008), suggesting that its mode of action involves interference with the intracellular redox balance. Worth noting, trypanothione metabolism has been involved in conferring trypanosomatids with resistance to different clinical drugs (Mäser et al., 2003; Maya et al., 2004; Walker et al., 2012; Alsford et al., 2012). To our knowledge, potential

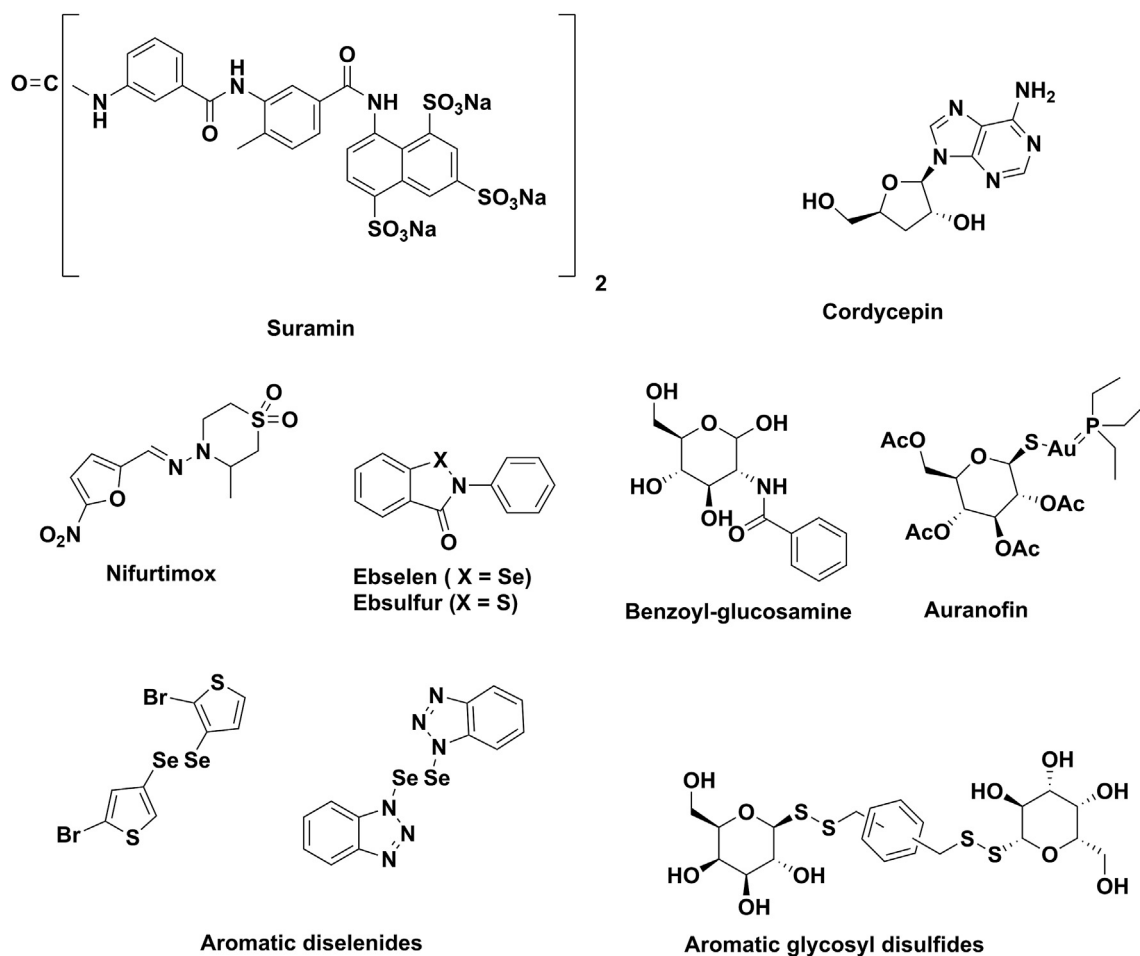


Fig. 1. Chemical structures of selected anti-trypanosomatid molecules containing sulfur, selenium atoms and/or carbohydrate moieties.

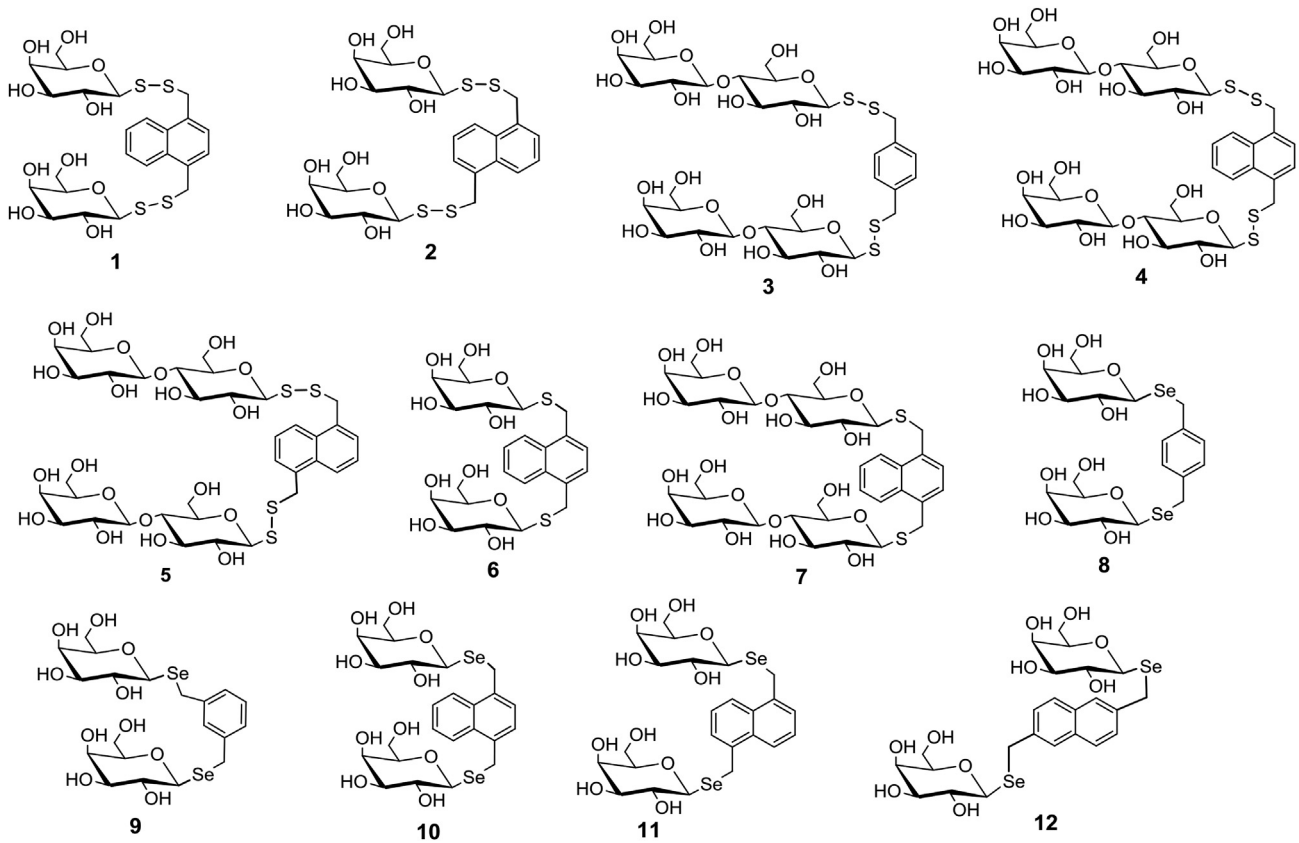


Fig. 2. Chemical structures of sugar derivatives with aromatic cores tested against *Trypanosoma brucei*.

antitrypanosome activities of sugar diselenides have not yet been investigated. We have therefore added compounds 13–18 (Fig. 3) to our testing panel.

The rationale behind this approach was to target simultaneously two major metabolic pathways for these organisms: glycolysis and redox homeostasis with the major aim to increase compound

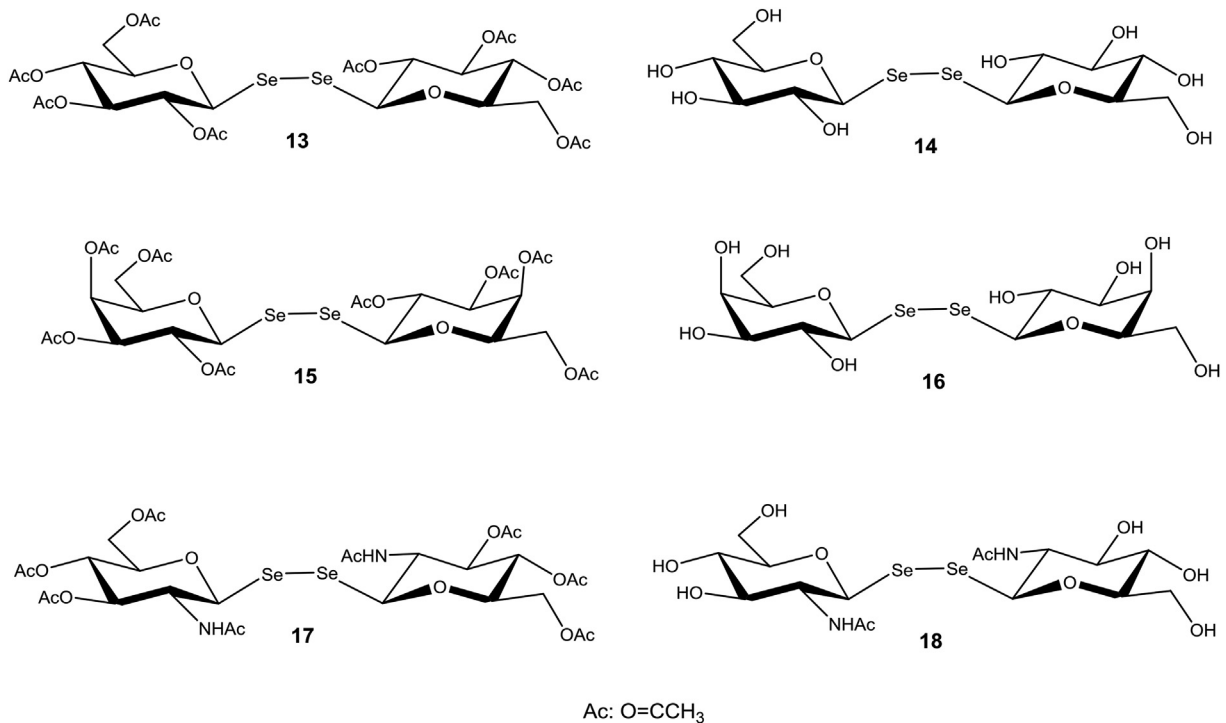


Fig. 3. Chemical structures of diglycosyl diselenides tested against *Trypanosoma brucei*.

efficacy and lower the possibilities for emergence of drug resistance. The biological activity of the new derivatives was tested against the infective form of *Trypanosoma brucei brucei*, causative agent of Nagana cattle disease and model organism of the subspecies pathogenic to humans. The potential mode of action of the most active derivatives was investigated.

## 2. Material and methods

### 2.1. Chemistry

Of the compounds listed in Fig. 2, **1–5** are disulfide glycoside analogs of those previously tested against *T. cruzi* (Gutiérrez et al., 2013). The sugar moiety is either galactose (**1, 2**) or lactose (**3–5**) bound by  $\beta$ -glycosidic linkage to a benzene- (**3**) or a naphthalene (**1, 2, 4** and **5**) central core. **6** and **7** are analogs of **1** and **4**, respectively, containing one sulfur atom less in the linker chains. **8–12** are analogous selenoglycosides with benzene- (**8, 9**) or naphthalene (**10–12**) aromatic cores. The chemical syntheses of **1–12** were recently published (Kaltner et al., 2017). Compounds **13** (Wagner and Nuhn, 1964), **15** (Kawai et al., 2005) and **17** (Illyés et al., 2016) are diselenides of *O*- and/or *N*-acetylated glucose, galactose and glucosamine, respectively, whereas **14** (Wagner and Nuhn, 1964), **16** (André et al., 2015) and **18** (Boutureira et al., 2012) are the corresponding non-acetylated versions (Fig. 3).

### 2.2. Biology

#### 2.2.1. Viability assays for trypanosomes and murine macrophages

Bloodstream *T. b. brucei* (strain 427) cell line 449 expressing an ectopic copy of the redox biosensor hGrx-roGFP2 (Gutscher et al., 2008) was grown in HMI-9 medium complemented with 10% (v/v) Fetal Bovine Serum Tetracycline-free (FBS; GIBCO®) in a humidified incubator with 5% CO<sub>2</sub> and at 37 °C. Phleomycin (0.2 µg/mL) and hygromycin (5 µg/mL) were added to select for the constitutive expression of the tetracycline repressor protein and for the hGrx-roGFP2 gene, respectively, whereas the expression of the last was induced by supplementing the medium with 1 µg/mL oxytetracycline for 24 h. Exponentially growing parasites were resuspended in fresh medium at a density of  $5 \times 10^5$  cells/mL. 200 µL of this cell suspension was seeded per well (96-well culture microplate). Next, 2 µL of the different compounds (final concentration of 5 µM) or DMSO (1% v/v) were added in triplicates. After 24 h incubation, 100 µL from each well were transferred to a tube containing 200 µL of sterile PBS with glucose 1% (w/v). Prior to analysis by flow cytometry, propidium iodide (PI) was added at a final concentration of 2 µg/mL and used as a viability marker. Samples were analyzed with a C6Accuri flow cytometer (BD) using a 488 nm laser and the following filters  $\lambda_{em} = 530/40$  nm and  $\lambda_{em} = 613/30$  nm for GFP and PI signal, respectively. The data were processed and analyzed with the C6Accuri software.

The murine macrophages (cell line J774) were cultivated in DMEM medium supplemented with 10% (v/v) FBS (GIBCO®), 10 U/mL penicillin and 10 µg/mL streptomycin, under a humidified 5% CO<sub>2</sub>/95% air atmosphere at 37 °C. Cell viability was assessed using the WST-1 reagent.

For the selected compounds, EC<sub>50</sub> was determined using a 7-point inhibition plot with each concentration tested in triplicate as described in (Maiwald et al., 2014) for *T. brucei* and in (Demoro et al., 2012) for murine macrophages. EC<sub>50</sub> values were obtained from dose/response curves fitted to a sigmoidal Hill equation (errors calculated using errors propagation) or extrapolated from non-linear fitting plots. The error is expressed as S.D and estimated as  $\sigma$  (n-1). For all assays, cell viability was calculated as follows: viability (%) = 100 x (number of cells for compound Y at concentration X/

number of cells in the DMSO-treated control).

#### 2.2.2. Assays involving *T. brucei* redox reporter cell line

Bloodstream *T. b. brucei* expressing the redox biosensor hGrx-roGFP2 was grown as described above. Two million parasites/mL were seeded per well in a 24-well plate and incubated (5% CO<sub>2</sub> and 37 °C) with 0.54 µM **13**, 1.49 µM **15**, 25 µM menadione, 78 nM suramin or 1% v/v DMSO for 4 h. Next, 100 µL from each well were transferred to a tube containing 200 µL of sterile PBS with glucose 1% (w/v). For each condition tested, a second sample was incubated with 1 mM DTT for 15 min prior to analysis by flow cytometry, PI was added at a final concentration of 2 µg/mL. All samples were analyzed with a C6Accuri flow cytometer (BD) as described above. GFP fluorescence (filter  $\lambda_{em} = 530/40$  nm) was measured only for viable cells (PI negative). The data were processed and analyzed with the C6Accuri software. Samples were analyzed by triplicate and the error is expressed as S.D.

#### 2.2.3. In vitro assays with recombinant redox biosensor

Recombinant hGrx-roGFP2 was expressed with an N-terminal His-tag from *Escherichia coli* strain BL21 (DE3) grown in TB medium supplemented with ampicillin. At an optical density (600 nm) of 0.8, IPTG (0.5 mM) was added to the culture and incubation resumed for 16 h at 20 °C. Cells were then harvested by centrifugation at 5000g for 20 min at 4 °C and the pellet resuspended in Buffer A (50 mM sodium phosphate pH 8.0, 300 mM NaCl, with protease inhibitors and lysozyme). Cells were further lysed by three cycles of sonication (45% power, 2 s pulse on/off, for 1 min) and debris removed by centrifugation at 16,000g for 1 h at 4 °C. Protein purification was done using a 1 mL HisTrap column (GE Healthcare) pre-equilibrated with Buffer A. hGrx-roGFP2 was eluted by a step gradient from 0 to 500 mM imidazole. The purity of recombinant hGrx-roGFP2 was of 95% as judged by Coomassie-stained SDS-PAGE.

For the redox assays, hGrx-roGFP2 was pre-reduced with DTT 20 mM in PBS containing 1 mM EDTA for 1 h at room temperature. The excess of reducing agent was removed by gel filtration on a Sephadex G25 (PD10 column, GE-Healthcare) equilibrated with PBS (pH 7.4) 1 mM EDTA. Protein concentration was measured at 280 nm, where  $\epsilon_{280} = 23.290 \text{ M}^{-1} \text{ cm}^{-1}$  for hGrx-roGFP2.

The reduced biosensor (1 µM) was treated with **13** or **15** at their respective EC<sub>50</sub> concentrations for 1 min and 1 h at room temperature, and then the fluorescence spectra at  $\lambda_{ex} = 380\text{--}510$  nm were recorded on a Cary Eclipse equipment. Controls included incubation of the biosensor with 0.2 mM glutathione disulfide (GSSG), 1 mM DTT or glutathione (GSH) added at equimolar concentration with respect to the compound tested.

#### 2.2.4. Glucose measurement

Bloodstream *T. b. brucei* grown as described above were plated at  $5.3\text{--}5.8 \times 10^6$  parasites/mL per well in a 24-well plate and added of 0.54 µM **13**, 1.49 µM **15**, 78 nM suramin, 5 µM ebselen, 0.31 µM tri-thiazol (compound **10b** from Franco et al., 2017) or 1% v/v DMSO. After 4 h incubation, viable parasites were quantified by light microscopy counting on a Neubauer chamber, then centrifuged at 2000 g for 10 min at room temperature. The supernatant was collected and glucose concentration was measured as described below.

Inhibition of glucose uptake was studied using a modified method from that described in (Seyfang and Duzsenko, 1991). Bloodstream parasites in mid exponential growth phase were centrifuged at 2000 g for 10 min at 20 °C, washed with cold PBS 1X and resuspended at a final density of  $6.1 \times 10^7$  cells/mL in cold and fresh complete culture medium. Parasites were kept at 4 °C for 5 min, then incubated for 30 min at 37 °C with DMSO 1% (v/v) or the

following compounds added at 5X their EC<sub>50</sub>: phloretin (500 μM), NFX (50 μM), **13** (2.7 μM) or **15** (7.45 μM). The experiment was stopped by boiling the samples for 5 min in a water bath. Cell debris were pelleted by centrifugation at 13000g for 10 min at 4 °C and glucose concentration was determined in the supernatant using a Bioprofile Basic 2 analyzer (Nova Biomedicals).

For both experiments, the samples were analyzed by triplicate and the error is expressed as S.D.

### 2.3. NMR spectroscopy

NMR samples for the <sup>1</sup>H,<sup>1</sup>H-NOESY, <sup>1</sup>H,<sup>13</sup>C-HSQC and the <sup>1</sup>H,<sup>77</sup>Se-CPMG-HSQMBC (Williamson et al., 2000; Kövér et al., 2006) correlation measurements contained ca. 25 mM diselenide derivatives **13** or **15** with three- to four times excess of glutathione in a solvent consisting of 1:1 DMSO-*d*<sub>6</sub>: phosphate buffer (100 mM in D<sub>2</sub>O or H<sub>2</sub>O:D<sub>2</sub>O 9:1, pH 7.0). NMR experiments were performed at 298 K (NOESY, <sup>1</sup>H,<sup>13</sup>C-HSQC) and at 313 K (HSQMBC), respectively, on a Bruker Avance II NMR spectrometer operating at 500.13 MHz for <sup>1</sup>H equipped with a 5 mm inverse BBI-Z probe. The <sup>1</sup>H resonances were assigned using standard homonuclear and heteronuclear correlation experiments. <sup>1</sup>H,<sup>1</sup>H-NOESY spectra were recorded using a mixing time of 200 ms and with 32 scans per t<sub>1</sub> increment. <sup>1</sup>H,<sup>77</sup>Se CPMG-HSQMBC correlation experiments were performed using a heteronuclear long-range coupling evolution time of 35 ms. The number of scans per t<sub>1</sub> increment varied between 320 and 640 depending on the concentration/solubility of the reaction product.

## 3. Results and discussion

### 3.1. Biological evaluation

The anti-trypanosomal activity of the symmetric diglycosyl diselenides and disulfides (compounds **1–18**) was tested against the bloodstream stage of *T. b. brucei*, including nifurtimox (NFX) as control drug. Added at a concentration of 5 μM, only a subset of disaccharides harboring a diselenide bond and lacking an aromatic linker (compounds **13**, **14**, **15** and **17**) displayed medium to strong anti-proliferative activity (37–97% growth inhibition, Table 1). Interestingly, acetylation of the sugar hydroxyl groups (in **13**, **15** and **17**) appears to be an important determinant of biological activity. In fact, the corresponding non-acetylated derivatives displayed lower (e.g., 43% for **14** compared to 97% for **13**) to null (compare **15** vs. **16** and **17** vs. **18**) growth inhibition.

Of all the molecules tested, **13** and **15** emerged as the most potent derivatives with EC<sub>50</sub> of 0.54 and 1.49 μM (Fig. 4A), respectively, followed by **14** and **17** with EC<sub>50</sub> ~5 μM (Table 1), all of them exceeding the activity of the control drug nifurtimox (EC<sub>50</sub> ~10 μM, under our assay conditions). The selectivity index of the most active compounds determined against murine macrophages was of 9.1 for **13** and 4.6 for **15** (Fig. 4B), these values being close to that of nifurtimox (SI ~10).

### 3.2. Potential mode of action of diglycosyl diselenides

The chemical nature of the disaccharide diselenides suggests that their anti-trypanosomal activity may be a consequence of some interference with the glucose- and/or redox metabolism of the parasite. In order to infer the potential mode of action of the most active compounds, namely **13** and **15**, we first investigated the remaining level of glucose in the culture supernatant of parasites treated for 4 h with **13**, **15**, the antitypanosomal drug suramin (a compound with multi-target activity; Willson et al., 1993), ebselen (an inhibitor of hexokinase 1 and trypanothione reductase from

**Table 1**  
Antiproliferative activity of disaccharide disulfides and –diselenides against infective African trypanosomes.

Compound <sup>a</sup>	Acetylated sugar	Parasite survival <sup>b</sup> (%)
<b>1</b>	N	88.4 ± 2.9
<b>2</b>	N	91.9 ± 1.8
<b>3</b>	N	93.4 ± 2.2
<b>4</b>	N	91.9 ± 1.8
<b>5</b>	N	93.5 ± 1.3
<b>6</b>	N	96.9 ± 0.3
<b>7</b>	N	91.1 ± 6.8
<b>8</b>	N	100.4 ± 1.8
<b>9</b>	N	99.6 ± 1.8
<b>10</b>	N	94.5 ± 6.5
<b>11</b>	N	96.0 ± 6.8
<b>12</b>	N	94.1 ± 5.0
<b>13</b>	Y	2.7 ± 0.4
<b>14</b>	N	57.1 ± 3.0
<b>15</b>	Y	3.3 ± 0.3
<b>16</b>	N	96.4 ± 14.7
<b>17</b>	Y	63.3 ± 3.0
<b>18</b>	N	120.5 ± 5.3
<b>NFX</b>	n.a.	28.2 ± 5.5

N = No.

Y = Yes.

n.a. = not applicable.

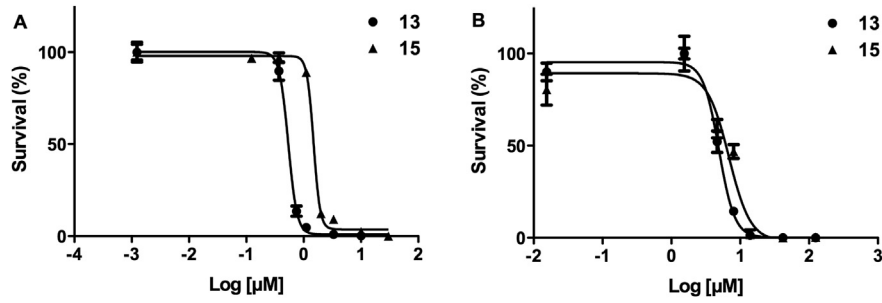
<sup>a</sup> Bloodstream stage *T. b. brucei* were exposed during 24 h to 5 μM diglycosyl compounds (**1–18**) or 15 μM of the control drug nifurtimox (NFX).

<sup>b</sup> Cell viability was assessed by flow cytometry and is expressed as % survival ± standard deviation (n = 3) relative to non-treated parasites.

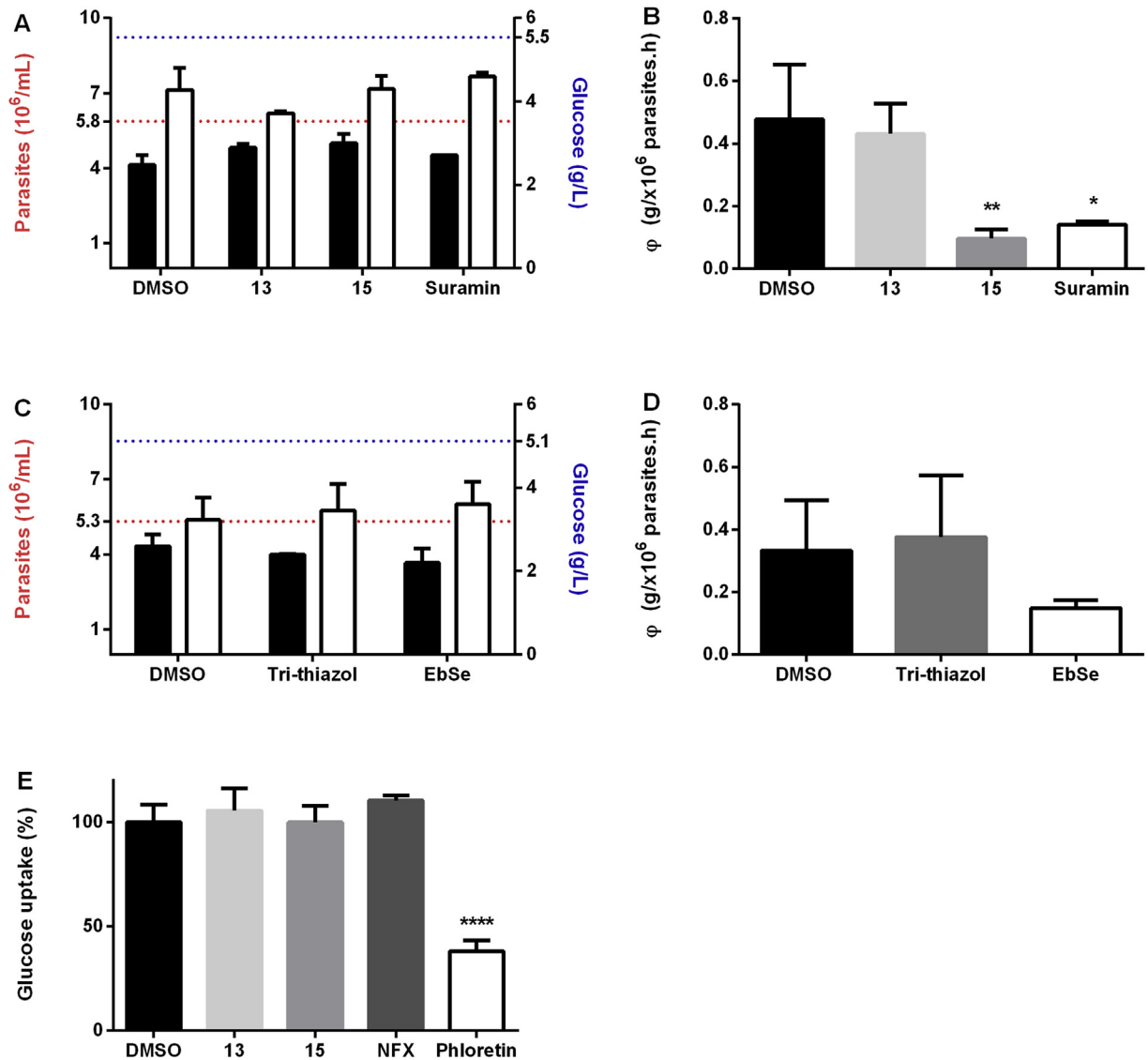
*T. brucei*; Joice et al., 2013; | Lu et al., 2013) and a tri-thiazol that affects the integrity of the parasite lysosome (Franco et al., 2017). All compounds were tested at their corresponding EC<sub>50</sub> or with the vehicle alone (1% v/v DMSO) (Fig. 5A–D). Under these experimental conditions only the tri-thiazol and ebselen displayed some anti-proliferative activity with respect to the DMSO control, albeit not statistically significant. After 4 h incubation, the content of residual glucose in the medium supernatant was almost halved for all cultures with respect to the initial content (>5 g/L, Fig. 5A and C). However, comparison of the rate of glucose consumption (φ = g/L glucose consumed per million parasites in 1 h) showed that parasites treated with **15** (φ = 0.096) or with suramin (φ = 0.141) presented a significantly lower capacity (<30%) to metabolize glucose compared to the control condition (φ = 0.478 for DMSO) (Fig. 5B). Although compound **13** reduced nearly 10% glucose consumption (φ = 0.431) vs. DMSO, this difference was not statistically significant. Under similar experimental conditions, the hexokinase 1 inhibitor ebselen (φ = 0.184), but not a highly cytotoxic and non-mechanistically related tri-thiazol (φ = 0.764), reduced by 44% glucose consumption compared to the vehicle (φ = 0.332) (Fig. 5D).

An additional experiment was conducted to address whether the metabolic phenotype induced by compounds **13** and **15** in bloodstream trypanosomes involves the inhibition of glucose uptake. Measurement of glucose level in the culture medium upon a short exposure (30 min) of parasites to the diglycosyl diselenides added to 5X their EC<sub>50</sub> did not reveal differences in glucose uptake compared to vehicle treated cells (Fig. 5E). A similar outcome was obtained for nifurtimox (50 μM), a compound that does not directly affect glucose uptake but impair redox homeostasis (Hall and Wilkinson, 2012; Alsford et al., 2012) whereas phloretin (500 μM), a known competitive inhibitor of hexose transport in *T. brucei* (Seyfang and Duszko, 1991), reduced glucose uptake by 63% (Fig. 5E). Taken together, our data show that at low micromolar concentrations the diglycosyl diselenides do not interfere with glucose uptake and that the galactosyl derivative **15** affects significantly glucose catabolism.

Interestingly, while **15** exerted a higher depletion of glucose



**Fig. 4.** Dose-response curves for the most active diglycosyl diselenides. **A)** Bloodstream *T. b. brucei* and **B)** murine macrophages (cell line J774) were treated with different concentrations of **13** and **15**. After 24 h incubation, cell viability was assessed by flow cytometry (Accuri BD) using propidium iodide as exclusion dye. The data were fitted to the Hill equation with  $R^2$  values  $> 0.96$ . The  $EC_{50}$  against *T. b. brucei* is  $0.54 \pm 0.05 \mu\text{M}$  for **13** and  $1.49 \pm 0.01 \mu\text{M}$  for **15**, contrasted to the values of  $4.9 \pm 0.45 \mu\text{M}$  for **13** and  $6.9 \pm 0.54 \mu\text{M}$  for **15** against macrophages.



**Fig. 5. Glucose catabolism and uptake studies** in bloodstream *T. b. brucei*. **A)** and **C)** Residual glucose and parasite proliferation. Cell density (black bars) and residual glucose in culture supernatant (white bars). The red and blue dotted lines depict the initial cell density ( $5.8 \times 10^6$  cells/mL and  $5.3 \times 10^6$  cells/mL) and glucose concentration (5.5 g/L and 5.1 g/L) in the culture medium of the corresponding experiments. **B)** and **D)** Glucose consumption. Parasites were treated with vehicle (1% v/v DMSO), **13** (0.54  $\mu\text{M}$ ), **15** (1.49  $\mu\text{M}$ ), suramin (78 nM), ebselen (5  $\mu\text{M}$ ) and tri-thiazol (0.31  $\mu\text{M}$ ) for 4 h and glucose consumption ( $\phi$ ) is expressed as g/L glucose consumed per million cells in 1 h. **E)** Glucose uptake. Parasites were treated with vehicle (1% v/v DMSO), **13** (2.7  $\mu\text{M}$ ), **15** (7.45  $\mu\text{M}$ ), nifurtimox (50  $\mu\text{M}$ ) or the control drug phloretin (500  $\mu\text{M}$ ) for 30 min. All values are expressed as average  $\pm$  SD ( $n = 3$ ). Statistical analysis was performed applying One Way ANOVA test followed by Dunnet's multiple comparisons posttest. \*\*\*\*, \*\* and \* denote differences with  $p$  values  $< 0.0001$ ,  $< 0.01$  and  $< 0.05$ , respectively, vs. DMSO control. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

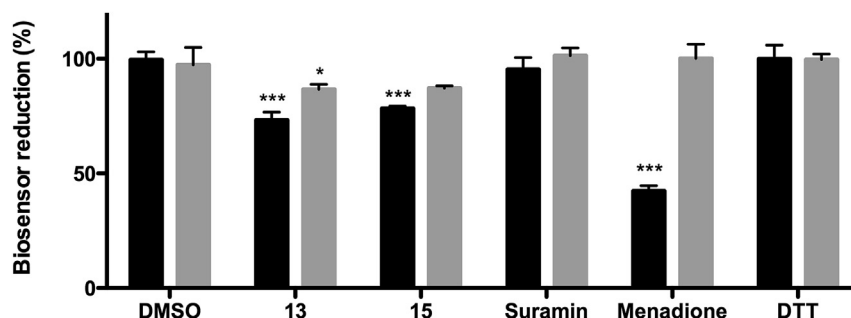
consumption than **13**, the last one produced a comparatively stronger cytotoxic effect on cells (Fig. 4A). Next we were interested to gain insight into a potential interference of the compounds with the cell redox homeostasis using parasites that express a fluorescent redox biosensor in their cytosol. The biosensor hGrx-roGFP2 allows quantifying the ratio of reduced and oxidized glutathione in a non-invasive manner (Gutscher et al., 2008). Though trypanosomes lack glutathione reductase, the redox state of glutathione is in equilibrium with that of trypanothione, the major redox cofactor of these parasites, via a class I glutaredoxin (Comini et al., 2013; Musunda et al., 2015). Thus, this tool provides a rationale measure of the overall intracellular redox state of the parasite. As shown in Fig. 6, bloodstream *T. b. brucei* treated with the diglycosyl diselenides at their EC<sub>50</sub> for 4 h displayed a marked oxidation of the biosensor (30% for **13** and 25% for **15**) that was only partially reverted upon 15 min incubation with the membrane permeable reducing agent dithiothreitol (DTT). As expected, treatment with the vehicle or suramin did not induce redox changes while the redox cyler drug menadione produced a significant oxidation (60%) of the biosensor that was fully reversible after exposure to 1 mM DTT for 15 min.

Despite hGrx-roGFP2 has been shown to be highly selective and sensitive (nM concentration) towards glutathione disulfide (GSSG) (Gutscher et al., 2008), the diselenides **13** and **15** may, in principle, act as direct oxidants of the cysteines present in the biosensor. This hypothesis was ruled out upon treatment of recombinant hGrx-roGFP2, purified from transformed bacteria, with **13** and **15** at their EC<sub>50</sub>. No changes in the fluorescence spectra of hGrx-roGFP2 were observed after 1 min or 1 h incubation with either of these compounds, while the biosensor was rapidly ( $\leq 1$  min) oxidized by

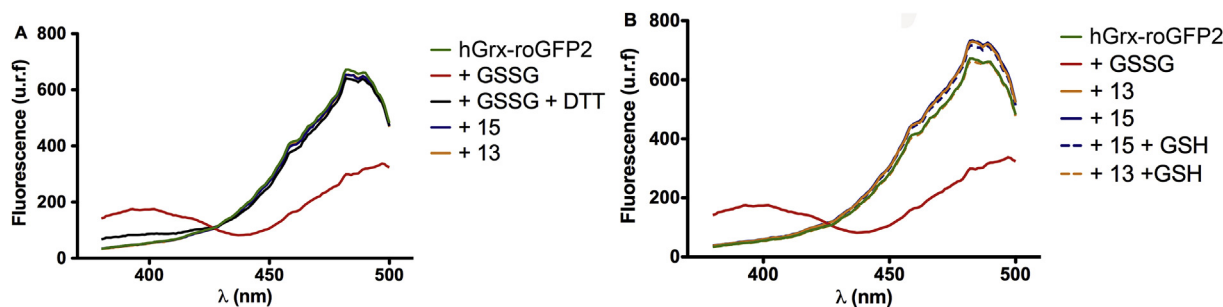
200 nM GSSG and reduced following addition of 1 mM DTT. In addition, co-incubation of **13** or **15** with an equimolar concentration of glutathione for 1 h did not trigger redox changes in hGrx-roGFP2, suggesting that **13** or **15** are neither able to oxidize the low molecular weight thiol to its disulfide form (Fig. 7).

The oxidative shift in the intracellular redox milieu of the parasite may well originate from a compromised supply of ATP to synthesize low molecular weight thiols (e.g. glutathione, trypanothione) that cannot counteract the endogenous metabolic demand for reducing power (Krauth-Siegel and Comini, 2008). Moreover, it was suggested that nucleophilic attack on the Se-Se bond in diaryl-diselenides by cysteine residues in proteins/enzymes, such as TR, might also influence redox balance via putative selenol derivatives (Font et al., 2015). However, the chemical nature of diglycosyl diselenides prompts us to hypothesize that they may also react directly with components of the redox metabolism, in particular with low molecular mass thiols.

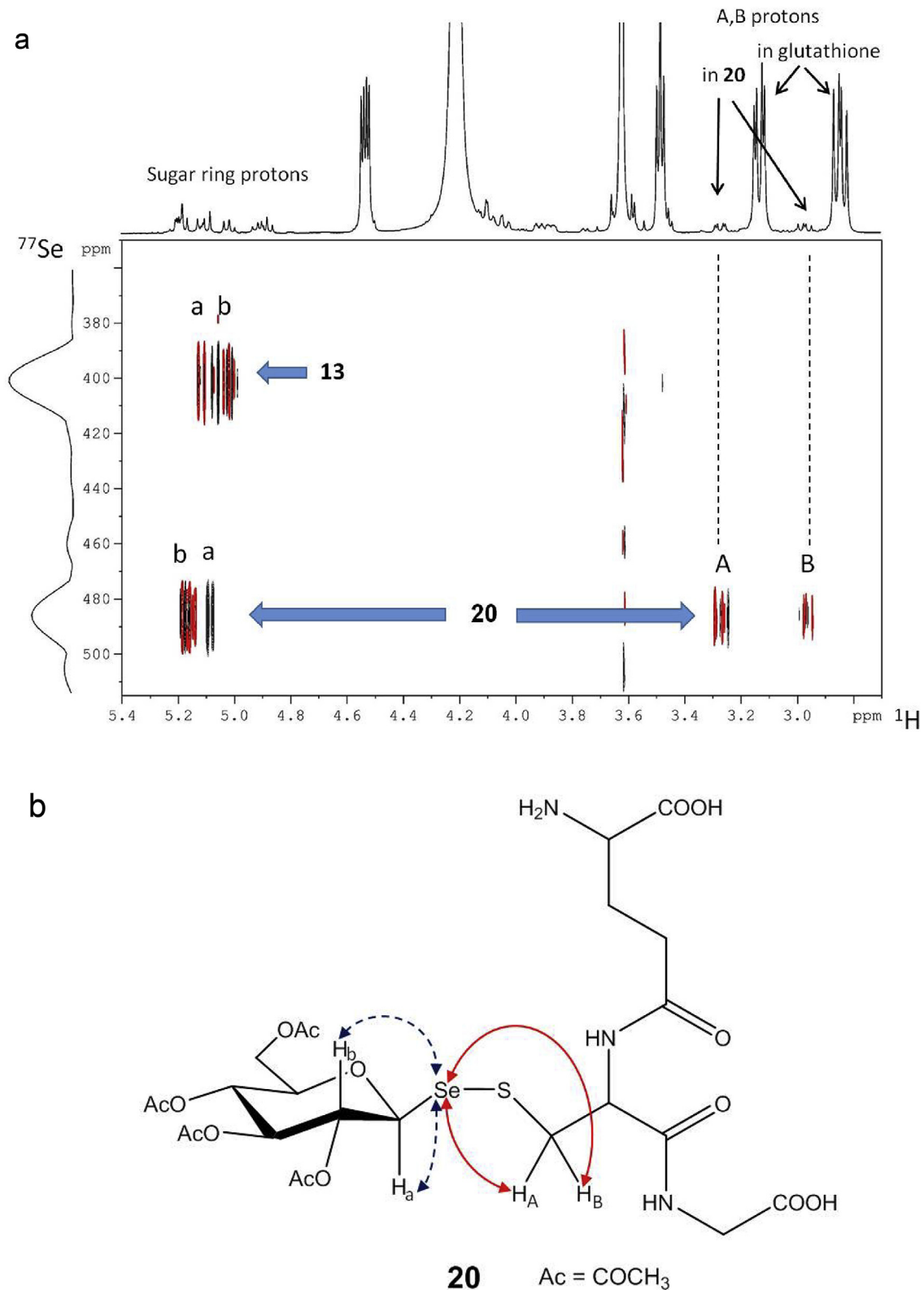
To address this question, we performed NMR spectroscopic measurements with mixtures made up of the two most active diselenides (**13** and **15**) and excess glutathione under near physiological conditions. In the <sup>1</sup>H NMR spectrum of **13** + glutathione a second set of resonances for the Cys-βCH<sub>2</sub> protons (A, B) of glutathione appears (Fig. 8a), which indicates the formation of a new molecular species. <sup>1</sup>H,<sup>77</sup>Se-heteronuclear correlation experiments (Williamson et al., 2000; Kövér et al., 2006; Boros and Kövér, 2011) performed with the above sample clearly revealed long-range couplings between the <sup>77</sup>Se nucleus and the Cys-βCH<sub>2</sub> protons (A, B) of glutathione (Fig. 8a). These results can be explained by the formation of a molecular species containing the sugar moiety attached to the cysteine residue of glutathione via a



**Fig. 6.** Intracellular redox changes induced by **13** and **15** on bloodstream *T. b. brucei*. Two million parasites were exposed to 0.54  $\mu$ M **13**, 1.49  $\mu$ M **15**, 78 nM suramin, 25  $\mu$ M menadione, 1 mM DTT or left untreated (vehicle, 1% v/v DMSO) for 4 h (black bars) and then treated for 15 min with 1 mM DTT (grey bars). The values are expressed as percentage ( $\pm$ SD) reduction of the biosensor relative to the control condition with DMSO without added DTT ( $n = 3$ ). Statistical analysis was performed applying One Way ANOVA test followed by Dunnet's multiple comparisons posttest. \*\*\*, \*\* and \* denote differences with  $p$  values  $< 0.001$ ,  $< 0.01$  and  $< 0.5$ , respectively, vs DMSO control.



**Fig. 7.** Compounds **13** and **15** do not induce oxidation (to disulfide form) of the redox biosensor or glutathione *in vitro*. Fluorescence spectra of the recombinant redox biosensor (hGrx-roGFP2, 1  $\mu$ M, green line) incubated with compound **13** (orange line) or **15** (blue line) at their EC<sub>50</sub> values, and added or not of 0.2  $\mu$ M oxidized glutathione (GSSG, red line) and 1 mM DTT (black line), or an equimolar concentration of reduced glutathione, dashed orange line or dashed blue for **13** or **15**, respectively). Data obtained upon (A) immediate reaction ( $\leq 1$  min) and (B) 1 h incubation and expressed as relative fluorescence units, u.r.f., vs. excitation wavelength. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 8.** a)  $^1\text{H},^{77}\text{Se}$  multiple-bond CPMG-HSQMBC correlation spectrum of a solution of **13** in 1:1 DMSO- $d_6$ : phosphate buffer (100 mM in  $\text{D}_2\text{O}$ , pH 7.0) and glutathione in 1:4 M ratio reveals  $^1\text{H},^{77}\text{Se}$  coupling connectivities between selenium and the cysteine  $\beta$ -protons (A, B) of glutathione. b) Structure of selenenylsulfide **20** highlighting crucial  $^1\text{H},^{77}\text{Se}$  coupling connectivities. Shown above the 2D map is the  $^1\text{H}$  NMR spectrum with indications of the relevant protons in b). The  $^{77}\text{Se}$  spectrum (on the left of the 2D map) provides further confirmation for the structure of **20** via a distinctly different  $^{77}\text{Se}$  chemical shift with respect to that in **13** ( $\delta_{\text{Se}}$  483 ppm in **20** vs. 400 ppm in **13**).



selenenylsulfide bond (**20**, Fig. 8b).

Similar results were obtained for the reactions of **15** with glutathione or with *N*-acetylcysteine indicating the formation of selenenylsulfides **19** and **22**, respectively (Supplementary Figs. S1 and S2). Further support for the structure of **19** was obtained by 2D  $^1\text{H},^1\text{H}$ -NOESY (Supplementary Fig. S3) as well as by  $^1\text{H},^{13}\text{C}$ -correlated 2D HSQC spectra (Supplementary Fig. S4). At this point we got intrigued to inquire about the behavior of **16**, the deacetylated form of **15**, in the presence of glutathione in view of the fact that **16** was found to be practically inactive in the growth inhibition test (Table 1). The  $^1\text{H},^{77}\text{Se}$  correlation experiment run under identical conditions indicated the formation of a selenenylsulfide derivative, **21** (Supplementary Fig. S5), in complete analogy to what has been observed for **15**. This result shows that, while selenenylsulfide formation is likely to take place in these systems, such reactions alone cannot account for the striking difference in trypanosome inhibition efficiency (Table 1) between the acetylated (such as **15**) and non-acetylated (such as **16**) forms of diglycosyl diselenides. It is of note that the chemical synthesis of analogous glycosylselenenyl-glutathione derivatives has been reported using a similar protocol which has, furthermore, been found useful for the glycosylation *via* SeS-linkages of cysteine residues in proteins like subtilisin (Boutureira et al., 2012). This further supports the propensity of the glycosyl diselenides to react with glutathione or similar thiol compounds. Worth noting, formation of glutathione disulfide was also observed in a control sample containing the reduced form of glutathione and lacking **13** and **15**, which suggests that this species is not a product of the reaction between the mixed selenenylsulfide with a second glutathione molecule but rather originates upon spontaneous oxidation. This conclusion is in line with the results from the *in vitro* assays with the highly sensitive redox biosensor that, in a 1 h time-window, did not show formation of glutathione disulfide in a sample containing glutathione and **13** or **15** (Fig. 7). Diglycosyl diselenides like **13** or **15** may, on the other hand, react with critical cysteine residues in glycolytic or redox enzymes that will become glycosylated *via* SeS-linkages similarly to what has been reported for subtilisin (Boutureira et al., 2012) or act as TR pseudo-substrates (Baquedano et al., 2016).

It is also possible that glycosylselenenyl glutathione derivatives like **20** act as recognition scaffolds for trypanothione-dependent enzymes such as TR (Krieger et al., 2002) and trypanredoxin (Comini et al., 2007) which are indispensable for parasite survival. At this point is worth to mention that a low resolution crystal structure of a TR-aurofin, a gold complex of 1-thio-2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranose and triethylphosphine revealed that the carbohydrate moiety of the drug occupies the binding site of trypanothione (Ilari et al., 2012). Thus, it is tempting to speculate a similar binding mode for the sugar scaffold of **19** (Supplementary Fig. S1) or **20** to TR.

#### 4. Conclusion

We have investigated a panel of 18 carbohydrate derivatives for potential antitrypanosomal activity. The chemical structures of these molecules are characterized by the attachment of two glycosyl units either by various divalent aromatic linkers incorporating sulfur or selenium atoms or simply connected by diselenide linkages through their anomeric carbons. Among these structures four acetylated diglycosyl diselenides presented remarkable potency towards killing of bloodstream stage African trypanosomes. The two most active compounds (**13**,  $\text{EC}_{50}$  0.54  $\mu\text{M}$  and **15**,  $\text{EC}_{50}$  1.49  $\mu\text{M}$ ) generated an oxidizing environment in the parasites. In addition, only **15** repressed glucose catabolism, but not its uptake, to a significant level that mirrored that exerted by the hexokinase 1 inhibitor ebselen or suramin, compounds that inhibit glycolysis

among other cellular processes.

Unfortunately, our data do not allow to dissect whether the glycolytic enzymes are a primary target of the digalactosyl diselenide **15** or if the observed metabolic phenotype is consequence of a more pleiotropic effect of its cytotoxicity. In this regard, given the chemical nature of **15** it is also possible that this compound interfere with the galactose metabolism, which has been shown to be indispensable for African trypanosomes and is directly linked to glucose metabolism *via* an epimerase that converts UDP-glucose to UDP-galactose (Roper et al., 2002; Urbaniak et al., 2006). Cross-regulation between these pathways is not fully understood and it is tempting to speculate that inhibition of galactose metabolism may entail a negative regulation of glucose catabolism.

The selectivity indices of these derivatives were, however, rather low (SI 4.6 to 9). Assays with a redox-reporter cell line indicated that diglycosyl diselenides decrease the GSH/GSSG ratio probably by depletion of reduced glutathione and/or by interfering with redox processes. NMR studies of the interaction between **13** and **15** and glutathione or *N*Ac-cysteine indicated that glycosylation of thiol groups in these molecules takes place under mild, quasi physiological conditions *via* formation of SeS-linkages between the reactants. The resulting selenenylsulfide derivatives may potentially act as inhibitors of enzymes in the glucose and redox metabolism of the parasites. The low selectivity of the most active compounds indicates that these molecules may share molecular targets in the pathogen and in the host as well. Nonetheless, the enhanced cytotoxic effect of the compounds towards parasites can be explained by the fact that glutathione concentration in trypanosomes is at least one to two-orders of magnitude lower than in mammalian cells and, additionally, due to the higher dependency of infective African trypanosomes on glucose catabolism for obtaining energy, reducing power and as precursor of several important macromolecules (Creek et al., 2015). Future studies should address the potential of selenosugar derivatives towards trypanosome inhibition as well as a quest for potential targets in the parasite and mammalian cells.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ijpddr.2017.08.001>.

## References

- Alsford, S., Eckert, S., Baker, N., Glover, L., Sanchez-Flores, A., Leung, K.F., Turner, D.J., Field, M.C., Berriman, M., Horn, D., 2012. High-throughput decoding of anti-trypanosomal drug efficacy and resistance. *Nature* 482, 232–236.
- André, S., Kövér, K.E., Gabius, H.J., Szilágyi, L., 2015. Thio- and selenoglycosides as ligands for biomedically relevant lectins: valency–activity correlations for benzene-based dithiogalactoside clusters and first assessment for (di) seleno-digalactosides. *Bioorg. Med. Chem. Lett.* 25, 931–935.
- André, S., Pei, Z., Siebert, H.C., Ramström, O., Gabius, H.J., 2006. Glycosyldisulfides from dynamic combinatorial libraries as O-glycoside mimetics for plant and endogenous lectins: their reactivities in solid-phase and cell assays and conformational analysis by molecular dynamics simulations. *Bioorg. Med. Chem.* 14, 6314–6326.
- Babokhov, P., Sanyaolu, A.O., Oyibo, W.A., Fagbenro-Beyioku, A.F., Iriemenam, N.C., 2013. A current analysis of chemotherapy strategies for the treatment of human African trypanosomiasis. *Pathog. Glob. Health* 107, 242–252.
- Bacchi, C.J., 2009a. Chemotherapy of human African trypanosomiasis. *Interdiscip. Perspect. Infect. Dis.* 195040–195045.
- Bacchi, C.J., Barker Jr., R.H., Rodriguez, A., Hirth, B., Rattendi, D., Yarlett, N., Hendrick, C.L., Sybertz, E., 2009b. Trypanocidal activity of 8-methyl-5-[[[(Z)-4-aminobut-2-enyl]- (methylamino)]adenosine (Genz-644131), an adenosylmethionine decarboxylase inhibitor. *Antimicrob. Agents Chemother.* 53, 3269–3272.
- Baquedano, Y., Alcolea, V., Toro, M.A., Gutiérrez, K.J., Nguewa, P., Font, M., Moreno, E., Espuelas, S., Jiménez-Ruiz, A., Palop, J.A., Plano, D., Sanmartín, C., 2016. Novel heteroaryl selenocyanates and diselenides as potent anti-leishmanial agents. *Antimicrob. Agents Chemother.* 60, 3802–3812.
- Boros, S., Kövér, K.E., 2011. Low-power composite CPMG HMQMBC experiment for accurate measurement of long-range heteronuclear coupling constants. *Magn. Reson. Chem.* 49, 106.
- Boutureira, O., Bernardes, G.J.L., Fernández-González, M., Anthony, D.C., Davis, B.G., 2012. Selenenylsulfide-linked homogeneous glycopeptides and glycoproteins: synthesis of human “hepatic Se metabolite A”. *Angew. Chem. Int. Ed. Engl.* 51, 1432–1436.
- Bringaud, F., Rivière, L., Coustou, V., 2006. Energy metabolism of trypanosomatids: adaptation to available carbon sources. *Mol. Biochem. Parasitol.* 149, 1–9.
- Comini, M.A., Krauth-Siegel, R.L., Flohé, L., 2007. Depletion of the thioredoxin homologue trypanredoxin impairs antioxidative defence in African trypanosomes. *Biochem. J.* 402, 43–49.
- Comini, M.A., Krauth-Siegel, R.L., Bellanda, M., 2013. Mono- and dithiol glutaredoxins in the trypanothione-based redox metabolism of pathogenic trypanosomes. *Antioxid. Redox Signal* 19, 708–722.
- Creek, D.J., Mazet, M., Achcar, F., Anderson, J., Kim, D.H., Kamour, R., Morand, P., Millieroux, Y., Biran, M., Kerkhoven, E.J., Chokkathukalam, A., Weidt, S.K., Burgess, K.E., Breiting, R., Watson, D.G., Bringaud, F., Barrett, M.P., 2015. Probing the metabolic network in bloodstream-form *Trypanosoma brucei* using untargeted metabolomics with stable isotope labelled glucose. *PLoS Pathog.* 11, e1004689.
- D’Antonio, E.L., Deinema, M.S., Kearns, S.P., Frey, T.A., Tanghe, S., Perry, K., Roy, T.A., Gracz, H.S., Rodriguez, A., D’Antonio, J., 2015. Structure-based approach to the identification of a novel group of selective glucosamine analogue inhibitors of *Trypanosoma cruzi* glucokinase. *Mol. Biochem. Parasitol.* 204, 64–76.
- Demoro, B., Sarniguet, C., Sánchez-Delgado, R., Rossi, M., Liebowitz, D., Caruso, F., Olea-Azar, C., Moreno, V., Medeiros, A., Comini, M.A., Otero, L., Gambino, D., 2012. New organoruthenium complexes with bioactive thiosemicarbazones as co-ligands: potential anti-trypanosomal agents. *Dalton Trans.* 41, 1534–1543.
- Font, M., Baquedano, Y., Plano, D., Moreno, E., Espuelas, S., Sanmartín, C., Palop, J.A., 2015. Molecular descriptors calculation as a tool in the analysis of the anti-leishmanial activity achieved by two series of diselenide derivatives. An insight into its potential action mechanism. *J. Mol. Graph. Model* 60, 63–78.
- Franco, J., Medeiros, A., Benítez, D., Perelmutter, K., Serra, G., Comini, M.A., Scarone, L., 2017. In vitro activity and mode of action of Distamycin analogues against African trypanosomes. *Eur. J. Med. Chem.* 126, 776–778.
- Gutiérrez, B., Muñoz, C., Osorio, L., Fehér, K., Illyés, T.Z., Papp, Z., Kumar, A.A., Kövér, K.E., Sagua, H., Araya, J.E., Morales, P., Szilágyi, L., González, J., 2013. Aromatic glycosyl disulfide derivatives: evaluation of their inhibitory activities against *Trypanosoma cruzi*. *Bioorg. Med. Chem. Lett.* 23, 3576–3579.
- Gutscher, M., Pauleau, A.L., Marty, L., Brach, T., Wabnitz, G.H., Samstag, Y., Meyer, A.J., Dick, T.P., 2008. Real-time imaging of the intracellular glutathione redox potential. *Nat. Meth.* 5, 553–559.
- Hall, B.S., Wilkinson, S.R., 2012. Activation of benzimidazole by trypanosomal type I nitroreductases results in glyoxal formation. *Antimicrob. Agents Chemother.* 56, 115–123.
- Ilari, A., Baiocco, P., Messori, L., Fiorillo, A., Boffi, A., Gramiccia, M., Di Muccio, T., Colotti, G., 2012. A gold-containing drug against parasitic polyamine metabolism: the X-ray structure of trypanothione reductase from *Leishmania infantum* in complex with aurano-fin reveals a dual mechanism of enzyme inhibition. *Amino Acids* 42, 803–811.
- Illyés, T.Z., Balla, S., Bényei, A., Kumar, A.A., Timári, I., Kövér, K.E., Szilágyi, L., 2016. Exploring the syntheses of novel glycomimetics. Carbohydrate derivatives with Se-S- or Se-Se- glycosidic linkages. *Chem. Sel.* 1, 2383–2388.
- Joice, A.C., Harris, M.T., Kahney, E.W., Dodson, H.C., Maselli, A.G., Whitehead, D.C., Morris, J.C., 2013. Exploring the mode of action of ebselen in *Trypanosoma brucei* hexokinase inhibition. *Int. J. Parasitol. Drugs Drug Resist* 3, 154–160.
- Kaltner, H., Szabó, T., Fehér, K., André, S., Balla, S., Manning, J.C., Szilágyi, L., Gabius, H.J., 2017. Bivalent O-glycoside mimetics with *S/disulfide/Se* substitutions and aromatic core: synthesis, molecular modeling and inhibitory activity on biomedically relevant lectins in assays of increasing physiological relevance. *Bioorg. Med. Chem.* 25, 3158–3170.
- Kawai, Y., Ando, H., Ozeki, H., Koketsu, M., Ishihara, H., 2005. A facile method for  $\beta$ -selenoglycoside synthesis using  $\beta$ -p-methylbenzoyl selenoglycoside as the selenating unit. *Org. Lett.* 7, 4653–4656.
- Kim, E.J., Amorelli, B., Abdo, M., Thomas, C.J., Love, D.C., Knapp, S., Hanover, J.A., 2007. Distinctive inhibition of O-GlcNAcase isoforms by an  $\alpha$ -GlcNAc thiolsulfonate. *J. Am. Chem. Soc.* 129, 14854–14855.
- Kövér, K.E., Batta, G., Fehér, K., 2006. Accurate measurement of long-range heteronuclear coupling constants from undistorted multiplets of an enhanced CPMG-HSQMBC experiment. *J. Magn. Reson.* 181, 89–97.
- Krauth-Siegel, R.L., Comini, M.A., 2008. Redox control in trypanosomatids, parasitic protozoa with trypanothione-based thiol metabolism. *Biochim. Biophys. Acta* 1780, 1236–1248.
- Krieger, S., Schwarz, W., Ariyanayagam, M.R., Fairlamb, A.H., Krauth-Siegel, R.L., Clayton, C., 2002. Trypanosomes lacking trypanothione reductase are avirulent and show increased sensitivity to oxidative stress. *Mol. Microbiol.* 35, 542–552.
- Lu, J., Vodnala, S.K., Gustavsson, A.L., Gustafsson, T.N., Sjöberg, B., Johansson, H.A., Kumar, S., Tjernberg, A., Engman, L., Rottenberg, M.E., Holmgren, A., 2013. Ebsulfur is a benzisothiazolone cytotoxic inhibitor targeting the trypanothione reductase of *Trypanosoma brucei*. *J. Biol. Chem.* 288, 27456–27468.
- Lüscher, A., de Koning, H.P., Mäser, P., 2007. Chemotherapeutic strategies against *Trypanosoma brucei*: drug targets vs. drug targeting. *Curr. Pharm. Des.* 13, 555–567.
- Mäser, P., Lüscher, A., Kaminsky, R., 2003. Drug transport and drug resistance in African trypanosomes. *Drug resist. Updat* 6, 281–290.
- Maiwald, F., Benítez, D., Charquero, D., Dar, M.A., Erdmann, H., Preu, L., Koch, O., Hölscher, C., Loaëc, N., Meijer, L., Comini, M.A., Kunick, C., 2014. 9- and 11-substituted 4-azapallones are potent and selective inhibitors of African trypanosoma. *Eur. J. Med. Chem.* 83, 274–283.
- Martin-Santamaría, S., André, S., Buzamet, E., Caraballo, R., Fernández-Cureses, G., Morando, M., Ribeiro, J.P., Ramírez-Gualito, K., de Pascual-Teresa, B., Cañada, F.J., Menéndez, M., Ramström, O., Jiménez-Barbero, J., Solís, D., Gabius, H.J., 2011. Symmetric dithiodigalactoside: strategic combination of binding studies and detection of selectivity between a plant toxin and human lectins. *Org. Biomol. Chem.* 9, 5445–5455.
- Maya, J.D., Rodríguez, A., Pino, L., Pabón, A., Ferreira, J., Pavani, M., Repetto, Y., Morello, A., 2004. Effects of buthionine sulfoximine nifurtimox and benznidazole upon trypanothione and metallothionein proteins in *Trypanosoma cruzi*. *Biol. Res.* 37, 61–69.
- Murthy, B.N., Sinha, S., Suroli, A., Jayaraman, N., Szilágyi, L., Szabó, I., Kövér, K.E., 2009. Interactions of aromatic mannosyl disulfide derivatives with Concanavalin A: synthesis, thermodynamic and NMR spectroscopy studies. *Carbohydr. Res.* 344, 1758–1763.
- Musunda, B., Benítez, D., Dirdjaja, N., Comini, M.A., Krauth-Siegel, R.L., 2015. Glutaredoxin-deficiency confers bloodstream *Trypanosoma brucei* with improved thermotolerance. *Mol. Biochem. Parasitol.* 204, 93–105.
- Plano, D., Baquedano, Y., Moreno-Mateos, D., Font, M., Jiménez-Ruiz, A., Palop, J.A., Sanmartín, C., 2011. Selenocyanates and diselenides: a new class of potent anti-leishmanial agents. *Eur. J. Med. Chem.* 46, 3315–3323.
- Roper, J.R., Guther, M.L., Milne, K.G., Ferguson, M.A., 2002. Galactose metabolism is essential for the African sleeping sickness parasite *Trypanosoma brucei*. *Proc. Natl. Acad. Sci. U. S. A.* 99, 5884–5889.
- Shaaban, S., Negm, A., Sobh, M.A., Wessjohann, L.A., 2015. Organoselenocyanates and symmetrical diselenides redox modulators: design, synthesis and biological evaluation. *Eur. J. Med. Chem.* 97, 190–201.
- Seyfang, A., Duszynski, M., 1991. Specificity of glucose transport in *Trypanosoma brucei*. Effective inhibition by phloretin and cytochalasin B. *Eur. J. Biochem.* 202, 191–196.
- Simarro, P.P., Franco, J., Diarra, A., Postigo, J.A., Jannin, J., 2012. Update on field use of the available drugs for the chemotherapy of human African trypanosomiasis. *Parasitology* 139, 842–846.
- Steverding, D., 2010. The development of drugs for treatment of sleeping sickness: a historical review. *Parasit. Vectors* 3, 15–23.
- Szilágyi, L., Varela, O., 2006. Non-conventional glycosidic linkages: syntheses and structures of thiooligosaccharides and carbohydrates with three-bond glycosidic connections. *Curr. Org. Chem.* 10, 1745–1770.
- Thuita, J.K., Wolf, K.K., Murilla, G.A., Bridges, A.S., Boykin, D.W., Mutuku, J.N., Liu, Q., Jones, S.K., Gem, C.O., Ching, S., Tidwell, R.R., Wang, M.Z., Paine, M.F., Brun, R., 2015. Chemotherapy of second stage human African trypanosomiasis: comparison between the parenteral diamidine DB829 and its oral prodrug DB868 in Vervet monkeys. *PLoS Negl. Trop. Dis.* 9, e000340.
- Urbaniak, M.D., Turnock, D.C., Ferguson, M.A., 2006. Galactose starvation in a bloodstream form *Trypanosoma brucei* UDP-glucose 4'-epimerase conditional null mutant. *Eukaryot. Cell.* 5, 1906–1913.
- Vodnala, S.K., Lundbäck, T., Yeheskieli, E., Sjöberg, B., Gustavsson, A.L., Svensson, R., Olivera, G.C., Eze, A.A., de Koning, H.P., Hammarström, L.G., Rottenberg, M.E., 2013. Structure–activity relationships of synthetic cordycepin analogues as experimental therapeutics for African trypanosomiasis. *J. Med. Chem.* 56, 9861–9873.
- Wagner, G., Nuhn, P., 1964. Synthese von Selenoglykosiden mit Acetyl-glykosyl-

- isoselenuronium-bromiden, 4. Mitt. Über „Selenoglykoside“ Arch. Pharm. Weinh. 297, 461–473.
- Walker, J., Gongora, R., Vasquez, J.J., Drummelsmith, J., Burchmore, R., Roy, G., Ouellette, M., Gomez, M.A., Saravia, N.G., 2012. Discovery of factors linked to antimony resistance in *Leishmania panamensis* through differential proteome analysis. *Mol. Biochem. Parasitol.* 183, 166–176.
- Williamson, R.T., Marquez, B.L., Gerwick, W.H., Kóvér, K.E., 2000. Survey of NMR experiments for the determination of  $^n\text{J}(\text{C,H})$  heteronuclear coupling constants in small molecules. *Magn. Reson. Chem.* 38, 265–273.
- Willson, M., Callens, M., Kuntz, D.A., Perié, J., Opperdoes, F.R., 1993. Synthesis and activity of inhibitors highly specific for the glycolytic enzymes from *Trypanosoma brucei*. *Mol. Biochem. Parasitol.* 59, 201–210.
- Yorke, W., Adams, A.R.D., Murgatroyd, M.F., 1929. Studies in chemotherapy. I. A method for maintaining pathogenic trypanosomes alive in vitro at 37°C. for 24 hours. *Ann. Trop. Med. Parasitol.* 23, 501–518.