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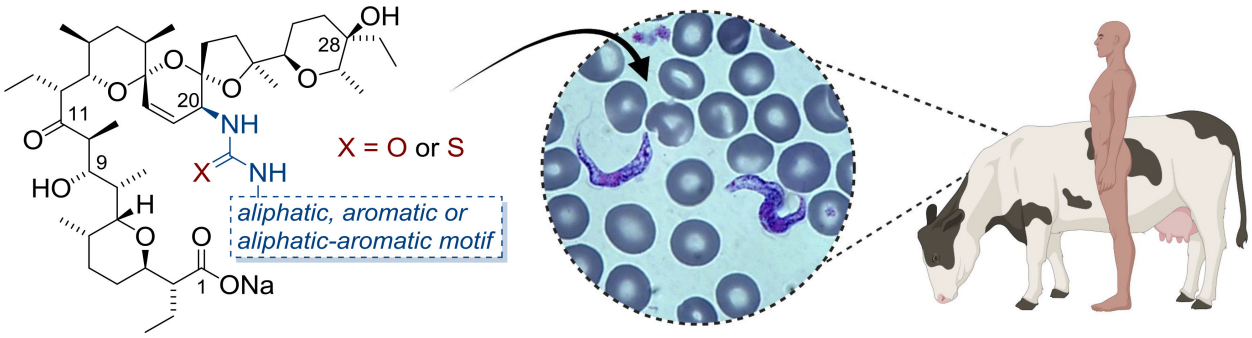

European Journal of Medicinal Chemistry

Synthesis of urea and thiourea derivatives of C20-*epi*-aminosalinomycin and their activity against *Trypanosoma brucei*

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Abstract: Salinomycin (**SAL**) is a natural polyether ionophore that exhibits a very broad spectrum of biological effects, ranging from anticancer to antiparasitic activities. Our recent studies have shown that the chemical modification of the **SAL** biomolecule is a fruitful strategy for generating lead compounds for the development of novel antitrypanosomal agents. As a continuation of our program to develop trypanocidal active lead structures, we synthesized a series of 14 novel urea and thiourea analogs of C20-*epi*-aminosalinomycin (compound **2b**). The trypanocidal and cytotoxic activities of the derivatives were assessed with the mammalian life cycle stage of *Trypanosoma brucei* and human leukemic HL-60 cells, respectively. The most antitrypanosomal compounds were the two thiourea derivatives **4b** (C20-*n*-butylthiourea) and **4d** (C20-phenylthiourea) with 50% growth inhibition (GI_{50}) values of 0.18 and 0.22 μM and selectivity indices of 47 and 41, respectively. As potent **SAL** derivatives have been shown to induce strong cell swelling in bloodstream forms of *T. brucei*, the effect of compounds **4b** and **4d** to increase the cell volume of the parasite was also investigated. Interestingly, both derivatives were capable to induce faster cell swelling in bloodstream-form trypanosomes than the reference compound **SAL**. These findings support the suggestion that C20-*epi*-aminosalinomycin derivatives are suitable leads in the rational development of new and improved trypanocidal drugs.

1. Introduction

African trypanosomiasis is a neglected tropical disease (NTD) caused by protozoan parasites of the genus *Trypanosoma.* The disease affects both people and livestock in sub-Saharan regions of Africa and is known as sleeping sickness in humans or nagana disease in animals [1]. The parasites are transmitted to their mammalian host by both male and female tsetse flies during blood feeding. African trypanosomiasis causes severe illness in humans and livestock and, if left untreated, is fatal in most cases. While the introduction of Fexinidazole as an oral trypanocide has improved the treatment of sleeping sickness, the currently used therapeutic strategies are limited due to their ineffectiveness and some serious side effects [2,3]. Furthermore, the phenomenon of drug resistance in African trypanosomiasis is a growing problem, especially in nagana disease [2,4,5]. Although recently, the number of reported cases of sleeping sickness has significantly fallen due to sustained control efforts in affected regions [6], nagana disease still remains a major problem in sub-Saharan Africa with annual economic costs of around US$4.5 billion [7]. In addition, there is increasing evidence that livestock animals may serve as a reservoir of human-infective trypanosomes and, therefore, should also be treated to prevent zoonotic transmission of the parasite [8,9]. However, one of the main problems in animal trypanosomiasis is that the available treatment options are getting increasingly ineffective [2] and thus, the development of new drug candidates is of top current priority.

Natural polyether ionophores have been shown to display trypanocidal activity against several protozoan parasites, including African trypanosomes [10]. Particularly promising in this context is salinomycin (**SAL**, **1**, Figure 1), a monocarboxylic polyether ionophore, and its semi-synthetic analogs, as these compounds have been shown to exhibit strong antiproliferative activity against *Trypanosoma brucei in vitro* [11–14]. As the orientation of the C20 hydroxyl of

SAL may affect its stability, efficacy, and/or selectivity, we previously used a synthetic strategy for the diastereoselective inversion of the absolute configuration at the C20 position. This led to the discovery of compounds with promising trypanocidal activity (Figure 1) [14]. Considering these previous findings, we were now interested to see whether the presence of an amine at the C20 position with inversed stereochemistry (Figure 1) could also increase the ionophoretic properties of those derivatives, and concomitantly, their antiparasitic activity. Knowing that urea and thiourea represent well-established privileged structures in medicinal chemistry, also in the design of promising antiparasitic agents [15], we decided to synthesize a new series of (thio)urea derivatives of **SAL** (Figure 1), and evaluate their antitrypanosomal effects, to gain further insight into the structure-activity relationship (SAR) of this group of ionophoretic active compounds. The synthesis of (thio)ureas of **SAL** was also rationally justified by the promising bioactivity of structurally similar amide and carbamate derivatives, reported previously by other authors [16].

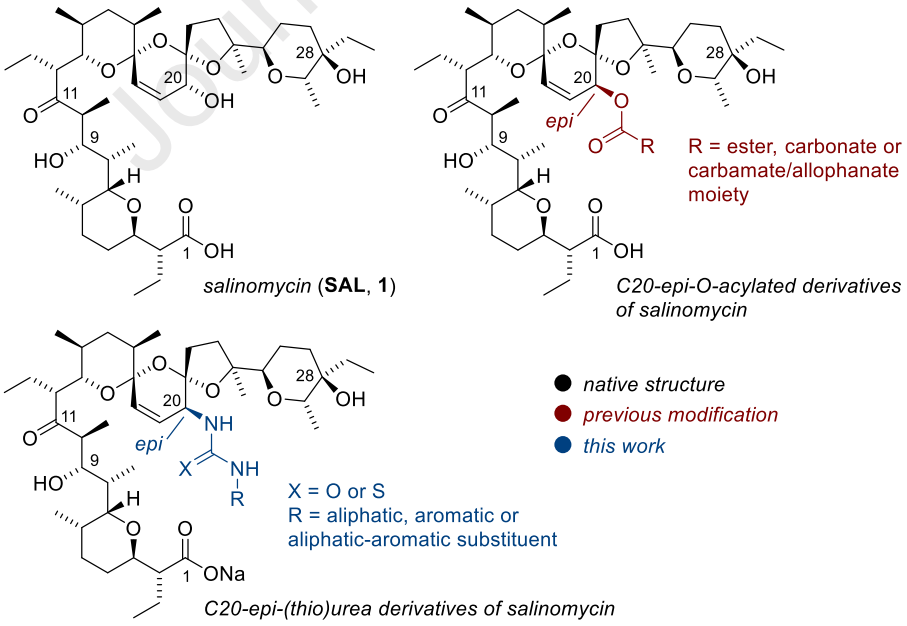


Figure 1. Molecular structures of the parent compound salinomycin, C20-*epi*-salinomycin derivatives previously reported, and novel C20-*epi*-(thio)urea salinomycin derivatives.

In this paper, we describe the access to a series of 14 newly synthesized urea and thiourea analogs of C20-*epi*-aminosalinomycin obtained through a cascade of selective transformations of the C20 hydroxyl of **SAL** (Scheme 1). We also report on the trypanocidal and cytotoxic activity of these compounds using the bloodstream forms of *T. brucei* and human myeloid HL-60 cells, respectively. Moreover, the effectiveness of the most promising analogs to induce cell swelling in trypanosomes was also investigated.

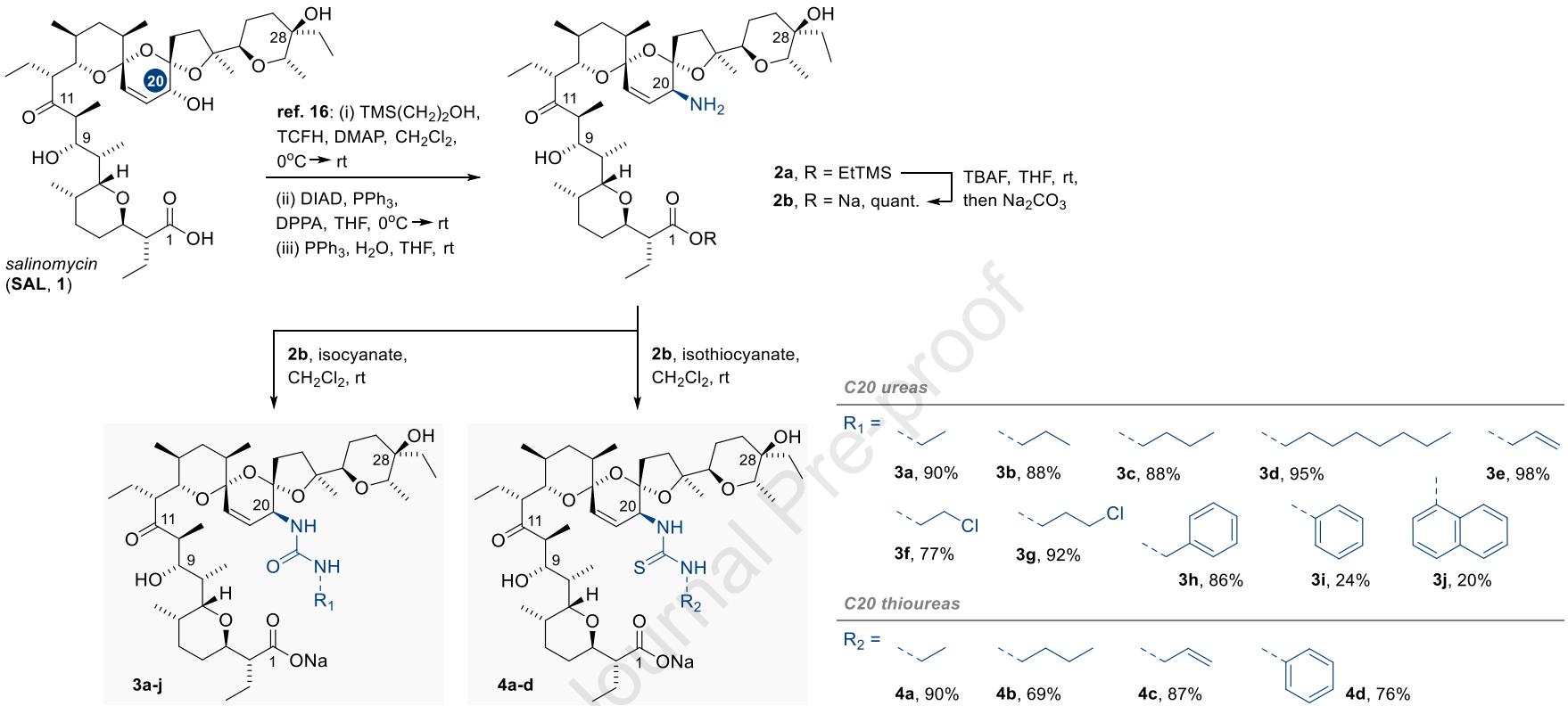
2. Results and discussion

2.1. Design and synthesis of analogs

C20-*epi*-aminosalinomycin (**2b**, Scheme 1) was synthesized in a three-step procedure according to the protocol previously reported by Jiang and co-workers [16]. Very briefly, with respect to the unfavorable conformation of the **SAL** biomolecule for the Mitsunobu reaction, it was crucial to mask the carboxyl group of **SAL** at the C1 position by esterification to give a 2-(trimethylsilyl)ethyl ester [16]. Next, considering the much greater reactivity of the allylic C20 hydroxyl compared to that of the C9 and C28 hydroxyl groups, the Mitsunobu reaction was selectively and smoothly accomplished. Specifically, the reaction between C1-masked **SAL** and DPPA led exclusively to the formation of the C20-*epi*-azidosalinomycin with the inversion of the absolute configuration at the C20 position [16]. With this compound in hand, the azido group was then reduced to an amino group using the Staudinger reaction conditions [16], providing compound **2a**. The NMR data of **2a** were in good agreement with those found in the reference literature [16]. Subsequently, the C1 functionality of **2a** was quantitatively deprotected from its orthogonal blocking group with TBAF to give the key intermediate product **2b** (Scheme 1).

The reaction of the amino group with isocyanates or isothiocyanates resulted in the corresponding urea and thiourea analogs **3a-j** and **4a-d**, respectively (Scheme 1). Of note is that full selectivity towards the C20 amino group was observed without isolation of any C9 and/or C28 (thio)urethane side products. With respect to the urea products, 10 commercially available isocyanates were used to give the semi-synthetic products with structurally divergent motifs, including short saturated (compounds **3a–c**) and unsaturated (compound **3e**) aliphatic chains, long aliphatic chains (compound **3d**), aliphatic chains with additional halogen atoms (compounds **3f–g**), and aromatic substituents (compounds **3h–j**). To further expand the structural diversity at the C20 position and to facilitate SAR studies, thiourea derivatives (compounds **4a–d**) were designed to contain moieties corresponding to some of the products from the urea series.

We used spectroscopic (NMR, FT-IR) and spectrometric (ESI MS) methods to confirm the homogeneity and structure of the newly semi-synthesized products (Supplementary material, Figures S1–S45). Very briefly, in the ^{13}C NMR spectra, the signal of the highest analytical significance was assigned to the newly introduced carbonyl group of the urea or thiourea moiety at the C20 position, which is observed in the range of 155.4–157.8 ppm and 181.3–182.5 ppm, respectively. On the other hand, in the ^{1}H NMR spectra of **3a–j** and **4a–d**, the NH protons of the C20 urea and thiourea groups are generally observed as two separated broad singlets with a maximum at about 5.33 ppm and 4.97 ppm, whose shift strongly depended on the type of the urea/thiourea substituent.



Scheme 1. Synthesis of urea and thiourea derivatives of C20-*epi*-aminosalinomycin.

2.2. Trypanocidal activity

SAL, C20-*epi*-aminosalinomycin **2b**, and its urea and thiourea derivatives **3a-j** and **4a-d**, respectively, were evaluated for their antiproliferative activity against bloodstream forms of *T. brucei* and human myeloid HL-60 cells *in vitro*. HL-60 cells were used for determining the cytotoxic activity of the compounds because, like bloodstream-form trypanosomes, they are fast-proliferating cells and grow in suspension. In addition, the sensitivity of HL-60 cells for approved trypanocides and **SAL** derivatives is well established [12–14,17,18]. The trypanocidal and cytotoxic activities of the compounds were assessed using the resazurin cell viability test [12–14] and expressed as minimum inhibitory concentration (MIC) values and 50% growth inhibition (GI_{50}) values (the definition of MIC and GI_{50} values can be found in section 4.4.1). GI_{50} values were determined by linear interpolation according to the methods described in [19].

The first point to note is that C20-*epi*-aminosalinomycin **2b** displayed about 10 times less trypanocidal activity than **SAL** (Table 1). A similar observation was previously made with C20-*epi*-salinomycin [14], suggesting that the absolute configuration at the C20 position seems to be critical for the trypanocidal activity. To prove this suggestion, the trypanocidal activity of C20-aminosalinomycin would be needed to be determined. On the other hand, the cytotoxic activity of **2b** evaluated with HL-60 cells was only 4 times lower than that of **SAL** (Table 1). In contrast, no difference in cytotoxicity was previously found between **SAL** and C20-*epi*-salinomycin [14].

All urea and thiourea derivatives of C20-*epi*-aminosalinomycin **2b** showed a concentration-dependent effect on the proliferation of bloodstream forms of *T. brucei*. Most urea derivatives displayed similar antitrypanosomal activity as the parent compound **2b** (Table 1). Only compounds **3c** and **3d** exhibited 10-fold stronger trypanocidal activity than **2b** (Table 1). In contrast, all four thiourea derivatives **4a–d** displayed 10-fold more potent trypanocidal activity

than the starting substance **2b** (Table 1). Based on GI_{50} values, compounds **4b** and **4d** were even slightly more trypanocidal than **SAL** (Table 1). This latter finding indicates that it is possible to synthesize **SAL** derivatives with better trypanocidal activity. Of interest to note is also, that of the corresponding urea and thiourea pairs with the same substituent (ethyl: **3a** and **4a**; *n*-butyl: **3c** and **4b**; allyl: **3e** and **4c**; and phenyl: **3i** and **4d**), only the compound pair containing the *n*-butyl chain shared GI_{50} values in the sub-micromolar range (Table 1). For all other pairs, the urea derivatives were 10 times less potent in inhibiting the growth of bloodstream-form trypanosome *in vitro* than the thiourea compounds (Table 1).

All newly synthesized compounds exhibited cytotoxic activity against HL-60 cells (Table 1). However, the cytotoxicity of the compounds was generally lower than their trypanocidal activity. There was a strong correlation between the trypanocidal and cytotoxic activity of the derivatives (Supplementary material, Figure S46), i.e., compounds with potent trypanocidal activity displayed also higher cytotoxic activity. Overall, urea derivatives were somewhat less cytotoxic than the thiourea compounds. Nevertheless, the selectivity indices (MIC and GI_{50} ratios of cytotoxicity to antitrypanosomal activity) of the thiourea derivatives were 2- to 4-fold greater than those of the urea compounds (Table 1). The only exception was the urea compound **3c** with a GI_{50} ratio of 55 (Table 1).

To assess the efficacy of the newly synthesized derivatives, their trypanocidal and cytotoxic activities were compared with those of suramin and ethidium bromide, two drugs used in the treatment of sleeping sickness and nagana disease, respectively. The most trypanocidal compounds **3c**, **3d**, and **4a–d** were 5 to 10 times less potent than suramin (Table 1). As suramin is nontoxic to HL-60 cells, the anti-sleeping sickness drug is 10 to 100 times more effective in killing bloodstream-form trypanosomes *in vitro* than the six urea and thiourea compounds. On

the other hand, the MIC value of the six derivatives was 10 times lower than that of ethidium bromide, while their GI_{50} values were only 2- to 4 times higher than the GI_{50} value of the anti-nagana drug (Table 1). Thus, based on the MIC ratios, the six derivatives are 10 to 100 times more effective in killing the parasite *in vitro* than the animal trypanocide ethidium bromide. It should be emphasized that MIC values provide a more robust measure of antitrypanosomal effects than GI_{50} values, especially when the dose-response curve of a drug is flat like in the case of ethidium bromide indicated by the great difference between the MIC value (10 μM) and the GI_{50} value (0.087 μM).

Table 1. MIC and GI_{50} values[a)] and ratios of urea and thiourea derivatives of C20-*epi*-aminosalinomycin.

Compound	*T. brucei*		HL-60		Selectivity	
	MIC (μM)	**GI_{50} (μM)**	**MIC (μM)**	**GI_{50} (μM)**	**MIC ratio**	**GI_{50} ratio**
1[b)]	1	0.27 ± 0.01	100	20.0 ± 5.6	100	74.1
2b	10	3.3 ± 0.30	100	83.2 ± 18.0	10	25.2
3a	10	3.2 ± 0.15	100	42.0 ± 5.2	10	13.1
3b	10	2.9 ± 0.10	100	36.7 ± 6.7	10	12.7
3c	1	0.36 ± 0.12	100	19.8 ± 3.8	100	55.0
3d	1	0.27 ± 0.03	10	6.3 ± 1.3	10	23.3
3e	10	3.2 ± 0.10	100	38.3 ± 3.6	10	12.0
3f	10	3.2 ± 0.20	100	38.5 ± 2.8	10	12.0
3g	10	2.3 ± 0.40	100	18.1 ± 3.4	10	7.9
3h	10	1.5 ± 0.50	100	16.7 ± 1.0	10	11.1
3i	10	2.9 ± 0.10	100	44.9 ± 4.7	10	15.5
3j	10	1.9 ± 0.40	100	13.5 ± 0.30	10	7.1
4a	1	0.33 ± 0.01	100	18.5 ± 4.6	100	56.1
4b	1	0.18 ± 0.04	10	8.4 ± 1.0	10	46.7
4c	1	0.31 ± 0.02	100	15.1 ± 5.7	100	48.7
4d	1	0.22 ± 0.04	10	8.9 ± 4.4	10	40.5
suramin	0.1	0.039 ± 0.007	>100	>100	>1000	>2564
ethidium bromide	10	0.087 ± 0.012	10	6.4 ± 0.30	1	73.6

[a)]For GI_{50} values, mean values and standard deviations of three independent experiments are shown. As the compounds were tested at 10-fold dilutions starting from 100 μM, the MIC can only take values of 100 μM, 10 μM, 1 μM, 100 nM, 10 nM, and 1 nM. The same MIC value was determined for each compound in three independent experiments.
[b)]Salinomycin sodium salt was used as a reference compound.

2.3. Cell swelling activity

The mode of antitrypanosomal action of **SAL** and its derivatives is due to their ionophoretic activity inducing an influx of sodium cations followed by osmotic water uptake, leading to massive swelling of the trypanosomes [11–14]. Moreover, it has been shown that the trypanocidal activity of **SAL** derivatives correlates with their cell swelling activity, i.e., the stronger the trypanocidal activity the stronger the cell swelling activity [12−14]. To see whether this is also true for urea and thiourea derivatives of **2b**, the effect of three compounds, **3c**, **4b**, and **4d**, in comparison with **2b** and **SAL**, on the cell volume of bloodstream-form trypanosomes was determined. The swelling of trypanosomes can be easily determined by light scattering, whereby a decrease in absorbance corresponds to an increase in cell volume [12−14,20]. Like C20-*epi*-salinomycin [14], C20-*epi*-aminosalinomycin **2b** was found not to induce any significant swelling in trypanosomes (Figure 2). This finding is in agreement with previous observations that **SAL** derivatives with low trypanocidal activity (GI_{50} > 3 μM) generally cause weak swelling in bloodstream forms of *T. brucei* [12,14]. On the other hand, compounds **4b** and **4d**, the only two derivatives with a lower GI_{50} value than that of **SAL**, induced much stronger cell swelling in trypanosomes (Figure 2). This result is also in accordance with previous findings that compounds with stronger *in vitro* trypanocidal activity than **SAL** display increased cell swelling activity [12,14]. Compound **3c** fits this trend as it has a slightly greater GI_{50} value and lower swelling capability than **SAL** (Figure 2).

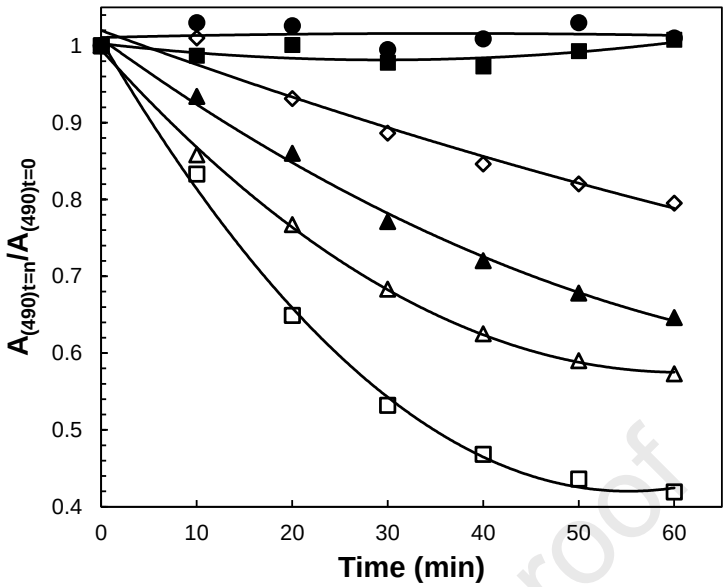


Figure 2. Effect of selected urea and thiourea derivatives of C20-*epi*-aminosalinomycin on cell swelling of *T. brucei* bloodstream forms. Trypanosomes (5×10^7 mL^{-1}) were incubated with 100 mM **SAL** (sodium salt, closed triangles), **2b** (closed squares), **3c** (open diamonds), **4b** (open triangles), and **4d** (open squares) in Baltz medium in the presence of 0.9% DMSO. Controls (closed circles) were incubated with 0.9% DMSO alone. The absorbance of the cultures at 490 nm was measured every 10 min for 60 min. It should be noted that a decrease in absorbance corresponds to an increase in cell volume. For clarity, only the mean values of three independent experiments are shown. The standard deviations ranged between 0.003 to 0.084 AU.

2.4. Structure-activity relationship (SAR) implications

As the C20 hydroxyl group of **SAL** does not take part in the complexation of metal ions [21–23], modifications at this position do usually not affect the binding of ions directly. This is supported by the fact that C20-deoxysalinomycin, a compound related to **SAL** that lacks its C20 hydroxyl group, was found to be a promising trypanocidal agent and almost an order of magnitude more effective than **SAL** [24]. On the contrary, modifications at the C20 position can lead to derivatives with better inhibitory activity compared to **SAL** [14,25]. This may be due to an increase in lipophilicity and/or permeability of **SAL** derivatives that may facilitate their uptake into the lipophilic environment of biological membranes.

To assess whether lipophilicity and/or permeability of the C20-*epi*-(thio)urea derivatives of **SAL** correlate with their trypanocidal activity, predicted Log *P* and Log P_{app} values were calculated. The predicted Log *P* values of chemically unmodified **SAL**, **2b** and C20-*epi*-(thio)urea derivatives of **SAL** ranged between 3.24 and 7.26 (Supplementary material, Table S1) indicating that the lipophilicity of the compounds varies by four orders of magnitude. In the case of **SAL**, the predicted Log *P* value was in close agreement with the measured Log *P* value (>6.2 [26]) suggesting that the predicted Log *P* values of **SAL** derivatives may also correspond well with measured data. The correlation between predicted Log *P* values of **SAL**, **2b** and C20-*epi*-(thio)urea derivatives of **SAL** with their GI_{50} values showed a moderate negative association (r = −0.62; r range for moderate negative correlation: −0.40 to −0.69 [27]) (Supplementary material, Figure S47A). This finding indicates that the lipophilicity of ionophoretic active compounds seems to be somewhat important in determining their trypanocidal activity. On the other hand, the range of the predicted Log P_{app} values of **SAL**, **2b** and C20-*epi*-(thio)urea derivatives of **SAL** was within one order of magnitude (−0.192 to 0.346; Supplementary material, Table S1).

However, the relationship between the predicted Log P_{app} values of the compounds and their GI_{50} values was less pronounced. The association between these two parameters showed a weak positive correlation (r = 0.21; r range for weak positive correlation: 0.10 to 0.39 [27]) (Supplementary material, Figure S47B). It should also be mentioned that the predicted P_{app} values of **SAL**, **2b** and C20-*epi*-(thio)urea derivatives of **SAL** (Supplementary material, Table S1) indicate that they all can be classified as low-permeable compounds (P_{app} $<5 \times 10^{-6}$ cm s^{-1} [28]). Thus, the permeability of ionophoretic active compounds seems to be less crucial for their antitrypanosomal action. This interpretation is supported by the fact that the unmodified parent

compound **SAL** has the lowest P_{app} values (0.6×10^{-6} cm s^{-1}) of all compounds tested (Supplementary material, Table S1) but is one of the most trypanocidal agents (Table 1).

To see whether the cell swelling activity of ionophoretic active compounds is dependent on lipophilicity and/or permeability, correlation analyses between the rate constant *k* of the decrease in absorbance of the cell swelling assay by **SAL**, **3c**, **4b**, and **4d**, and their Log *P* and Log P_{app} values, respectively, were conducted. It was found that the correlation between *k* and Log *P* values showed only a weak positive association (r = 0.28; r range for weak positive correlation: 0.10 to 0.39 [27]) (Supplementary material, Figure S48A). On the other hand, correlation analysis between *k* and log P_{app} values revealed a moderate negative association between the two parameters (r = −0.57; r range for moderate negative correlation: −0.40 to −0.69 [27]) (Supplementary material, Figure S48B). This finding seems plausible because the swelling activity of ionophoretic compounds should be dependent on their permeability rather than on their lipophilicity. However, it must be pointed out that the significance of the correlations between the rate constant *k* and Log *P* and Log P_{app} values, respectively, is rather limited as they are only based on four data points.

3. Conclusions

In summary, a series of 14 urea and thiourea derivatives of C20-*epi*-aminosalinomycin (**2b**) with inversed stereochemistry at the C20 position was obtained. All compounds were evaluated for their trypanocidal activity and selectivity. Most derivatives were found to display similar antitrypanosomal activity to the parent compound **2b**. In addition, the selectivity of all compounds was found to be only moderate, implying that cytotoxicity could be an issue for further drug candidate development. However, it should be mentioned that a cancer cell line was

used for determining the selectivity and that, compared with non-malignant cells, the cytotoxicity of the derivatives may therefore be overestimated.

Nevertheless, six analogs (**3c**, **3d**, and **4a–d**) were found to display 10 times stronger trypanocidal activity than the parent compound **2b**, two of which (**4b** and **4d**) were also slightly more potent than salinomycin (**SAL**). With GI_{50} values of 0.1597 μg mL^{-1} and 0.1996 μg mL^{-1} (0.18 μM and 0.22 μM), respectively, the derivatives **4b** and **4d** also match the GI_{50} activity criteria for *T. brucei* hit compounds, which is GI_{50} <0.20 μg mL^{-1} [29]. However, their GI_{50} ratios (46.7 and 40.5, respectively) fall short of the selectivity criteria for *T. brucei* hit compounds, which is >100 [29]. It should be possible to further improve the trypanocidal activity and/or selectivity of these hit compounds through modification of their C1 carboxyl moiety [14]. Specifically, while the introduction of a simple amide or ester group at the C1 position may contribute rather to the reduction of the antitrypanosomal potential of the C20-*epi*-(thio)urea derivatives of **SAL**, the conjugation of these compounds with hydroxamic acids or other ion-binding structural motifs could lead to the improvement of antiproliferative activity against *T. brucei*. As SAR correlation analyses have shown, further improvement in trypanocidal and cell swelling activity of C20-*epi*-(thio)urea derivatives of **SAL** may be also achievable by increasing the lipophilicity and permeability of the compounds, respectively. Finally, the two most trypanocidal compounds **4b** and **4d** displayed faster cell swelling activity than **SAL**, which could be advantageous as the parasite is killed more quickly.

4. Experimental

4.1. General procedures

All commercially available reagents and solvents were purchased from two sources, Merck or Trimen Chemicals S.A., and used without further purification. A detailed description of the general procedures, equipment, measurement parameters, and software can be found in the Supplementary material.

*4.2. Synthesis of compounds **2a** and **2b***

SAL in its sodium salt form was isolated in gram quantities from the commercially available veterinary premix SACOX®, according to previous protocols [30,31], and was further transformed into its acid form by extraction with aqueous sulphuric acid (pH = 1.0) [32]. Intermediate product **2a** was resynthesized following the protocol published by Jiang and co-workers [16], while **2b** was obtained by removing the orthogonal blocking group with TBAF, as previously shown for **SAL** by Strand and co-workers [33]. The ^{1}H NMR and ^{13}C NMR spectra, together with the ESI MS data of **2b** are included in the Supplementary material (Figures S1–S3).

*4.3. General procedure for the preparation of urea and thiourea analogs of C20-epi-aminosalinomycin (**3a–j** and **4a–d**)*

To a solution of C20-*epi*-aminosalinomycin **2b** (1.0 equiv.) in anhydrous CH_2Cl_2, the respective isocyanate (3.0 equiv.) or isothiocyanate (3.0 equiv.) was introduced dropwise under stirring at ambient temperature. The resulting solutions were stirred overnight, then washed with Na_2CO_3 (0.1 M aq.), and the collected organic layers were subsequently concentrated *in vacuo*. Purification on silica gel using the CombiFlash system (0→40% acetone/chloroform) gave the pure urea compounds **3a**–**j** (20–98% yield) and thiourea compounds **4a**–**d** (69–90% yield) as

clear oils. After thrice evaporation to dryness with *n*-pentane, the oily products were completely converted into white amorphous solids. The ^{1}H NMR and ^{13}C NMR spectra, together with the ESI MS data of compounds **3a–j** and **4a–d** are included in the Supplementary material (Figures S4–S45).

*C20-ethylurea of C20-epi-aminosalinomycin **3a**:* Yield: 90 mg, 90%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC; R_f: 0.47 in 50% acetone/CH_2Cl_2. Strains green with PMA; ^{1}H NMR (400 MHz, CD_2Cl_2) δ 6.16 (d, *J* = 10.6 Hz, 1H), 5.98 (dd, *J* = 10.5, 5.8 Hz, 1H), 5.18 (br, s, 1H), 4.67 (br, s, 1H), 4.28–4.14 (m, 2H), 4.06 (d, *J* = 10.4 Hz, 1H), 3.94 (d, *J* = 9.4 Hz, 1H), 3.71 (dd, *J* = 11.0, 4.7 Hz, 1H), 3.57 (dd, *J* = 10.1, 1.2 Hz, 1H), 3.53 (d, *J* = 10.1 Hz, 1H), 3.33 (dd, *J* = 12.0, 1.8 Hz, 1H), 3.16–2.98 (m, 2H), 2.75–2.52 (m, 3H), 2.15 (ddd, *J* = 25.4, 19.4, 11.4 Hz, 1H), 2.00−0.50 (m, 57H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 218.0, 184.3 (C1 carboxylate), 157.3 (carbamide group), 126.8, 124.8, 109.3, 99.2, 90.0, 76.3, 76.1, 75.4, 74.6, 71.5, 70.2, 67.5, 55.7, 51.3, 50.5, 47.5, 40.3, 38.9, 36.33, 36.26, 35.7, 33.0, 32.8, 32.6, 29.1, 28.3, 27.9, 27.1, 24.1, 20.9, 20.3, 17.6, 17.2, 16.2, 15.5, 14.8, 13.3, 12.7, 12.2, 10.8, 6.8, 6.5 ppm; FT-IR (KBr tablet): 3488 (br, s), 3365 (br, s), 3321 (br, s), 2963 (s), 2933 (s), 2875 (s), 1714 (s), 1664 (s), 1653 (s), 1566 (s), 1496 (m), 1459 (s), 1405 (s), 1380 (s), 1361 (m), 1339 (m), 1326 (m), 1301 (s) cm^{-1}; ESI MS (m/z): $[M+H]^+$ Calcd for $C_{45}H_{76}N_2NaO_{11}^+$ 843.5; Found 844; HRMS (ESI^+) *m/z*: $[M+H]^+$ Calcd for $C_{45}H_{76}N_2NaO_{11}^+$ 843.5347; Found 843.5341.

*C20-n-propylurea of C20-epi-aminosalinomycin **3b**:* Yield: 91 mg, 88%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC; R_f: 0.27 in 33% acetone/$CHCl_3$. Strains green with PMA; ^{1}H NMR (401 MHz, CD_2Cl_2) δ 6.22 (dd, *J* = 10.7, 0.5 Hz, 1H), 6.04 (dd, *J* = 10.6, 5.8 Hz, 1H), 5.24 (br, s, 1H), 4.91 (br, s, 1H), 4.32–4.22 (m, 2H),

4.09 (dd, *J* = 17.9, 10.0 Hz, 2H), 3.77 (dd, *J* = 11.0, 4.7 Hz, 1H), 3.67–3.56 (m, 2H), 3.38 (dd, *J* = 12.0, 2.2 Hz, 1H), 3.14– 2.96 (m, 2H), 2.81–2.57 (m, 3H), 2.31–2.08 (m, 1H), 2.05−0.50 (m, 59H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 218.3, 184.4 (C1 carboxylate), 157.7 (carbamide group), 127.1, 125.0, 109.6, 99.5, 90.2, 76.6, 76.3, 75.7, 74.8, 71.7, 70.4, 67.8, 56.0, 51.6, 50.7, 47.8, 42.9, 40.5, 39.2, 36.53, 36.48, 33.2, 33.1, 32.9, 29.3, 28.6, 28.2, 27.4, 24.3, 24.0, 21.2, 20.6, 17.8, 17.5, 16.5, 15.0, 13.5, 12.9, 12.4, 11.7, 11.1, 7.0, 6.8 ppm; FT-IR (KBr tablet): 3488 (br, s), 3372 (br, s), 3310 (br, s), 2963 (s), 2934 (s), 2875 (s), 1714 (s), 1663 (s), 1651 (s), 1566 (s), 1499 (m), 1460 (s), 1405 (s), 1381 (s), 1358 (m), 1339 (m), 1327 (m), 1300 (s) cm^{-1}; ESI MS (m/z): $[M+H]^+$ Calcd for $C_{46}H_{78}N_2NaO_{11}^+$ 857.5; Found 858; HRMS (ESI^+) *m/z*: $[M+H]^+$ Calcd for $C_{46}H_{78}N_2NaO_{11}^+$ 857.5503; Found 857.5486.

C20-n-butylurea of C20-epi-aminosalinomycin ***3c****:* Yield: 93 mg, 88%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC; R_f: 0.29 in 33% acetone/$CHCl_3$. Strains green with PMA; ^{1}H NMR (401 MHz, CD_2Cl_2) δ 6.21 (dd, *J* = 10.6, 0.5 Hz, 1H), 6.03 (dd, *J* = 10.5, 5.8 Hz, 1H), 5.23 (br, s, 1H), 4.99 (br, s, 1H), 4.32–4.21 (m, 2H), 4.11 (dd, *J* = 9.8, 2.1 Hz, 2H), 3.77 (dd, *J* = 11.0, 4.7 Hz, 1H), 3.63 (dd, *J* = 10.1, 2.0 Hz, 1H), 3.59 (d, *J* = 10.1 Hz, 1H), 3.38 (dd, *J* = 12.0, 2.1 Hz, 1H), 3.17–2.98 (m, 2H), 2.80–2.58 (m, 3H), 2.28–2.09 (m, 1H), 2.05−0.50 (m, 61H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 218.3, 184.4 (C1 carboxylate), 157.8 (carbamide group), 127.1, 125.0, 109.6, 99.5, 90.2, 76.6, 76.3, 75.7, 74.8, 71.7, 70.5, 67.8, 56.0, 51.6, 50.7, 47.8, 40.9, 40.5, 39.2, 36.55, 36.49, 33.2, 33.1, 32.9 (2C), 29.3, 28.6, 28.2, 27.4, 24.3, 21.2, 20.6 (2C), 17.8, 17.5, 16.5, 15.0, 14.2, 13.5, 12.9, 12.4, 11.1, 7.0, 6.8 ppm; FT-IR (KBr tablet): 3492 (br, s), 3366 (br, s), 3315 (br, s), 2962 (s), 2934 (s), 2874 (s), 1714 (s), 1664 (s), 1649 (s), 1565 (s), 1499 (m), 1460 (s), 1405 (s), 1381 (s), 1358 (m), 1339 (m),

1326 (m), 1301 (s) cm^{-1}; ESI MS (m/z): $[M+H]^+$ Calcd for $C_{47}H_{80}N_2NaO_{11}^+$ 871.6; Found 872; HRMS (ESI^+) *m/z*: $[M+H]^+$ Calcd for $C_{47}H_{80}N_2NaO_{11}^+$ 871.5660; Found 871.5651.

C20-n-octylurea of C20-epi-aminosalinomycin ***3d:*** Yield: 160 mg, 95%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC; R_f: 0.43 in 33% acetone/$CHCl_3$. Strains green with PMA; ^1H NMR (400 MHz, CD_2Cl_2) δ 6.23 (d, *J* = 10.7 Hz, 1H), 6.05 (dd, *J* = 10.6, 5.9 Hz, 1H), 5.18 (br, s, 1H), 4.86 (br, s, 1H), 4.34–4.23 (m, 2H), 4.12 (dd, *J* = 10.4, 3.8 Hz, 1H), 4.01 (d, *J* = 9.6 Hz, 1H), 3.78 (dd, *J* = 11.0, 4.8 Hz, 1H), 3.64 (dd, *J* = 10.1, 2.1 Hz, 1H), 3.59 (d, *J* = 10.1 Hz, 1H), 3.39 (dd, *J* = 12.0, 2.2 Hz, 1H), 3.16–3.00 (m, 2H), 2.81–2.74 (m, 1H), 2.72 (dd, *J* = 11.1, 2.5 Hz, 1H), 2.69–2.59 (m, 1H), 2.30–2.12 (m, 2H), 2.05–0.50 (m, 68H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 218.0, 184.4 (C1 carboxylate), 157.4 (carbamide group), 126.8, 124.8, 109.3, 99.2, 90.0, 76.3, 76.0, 75.5, 74.6, 71.5, 70.3, 67.5, 55.7, 51.3, 50.4, 47.6, 41.0, 40.3, 38.9, 36.4, 36.3, 33.0, 32.8, 32.6, 32.2, 30.5, 29.7, 29.6, 29.1, 28.3, 28.0, 27.2, 27.1, 24.1, 23.0, 21.0, 20.3, 17.6, 17.3, 16.2, 14.8, 14.2, 13.2, 12.7, 12.2, 10.8, 6.8, 6.5 ppm; FT-IR (KBr tablet): 3483 (br, s), 3362 (br, s), 3314 (br, s), 2961 (s), 2930 (s), 2873 (s), 1714 (s), 1662 (s), 1647 (s), 1567 (s), 1499 (m), 1460 (s), 1405 (s), 1380 (s), 1358 (m), 1339 (m), 1326 (m), 1301 (s) cm^{-1}; ESI MS (m/z): $[M+H]^+$ Calcd for $C_{51}H_{88}N_2NaO_{11}^+$ 927.6; Found 928; HRMS (ESI^+) *m/z*: $[M+H]^+$ Calcd for $C_{51}H_{88}N_2NaO_{11}^+$ 927.6286; Found 927.6277.

C20-allylurea of C20-epi-aminosalinomycin ***3e:*** Yield: 124 mg, 98%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC; R_f: 0.30 in 33% acetone/$CHCl_3$. Strains green with PMA; ^1H NMR (401 MHz, CD_2Cl_2) δ 6.23 (d, *J* = 10.7 Hz, 1H), 6.04 (dd, *J* = 10.6, 5.8 Hz, 1H), 5.83 (ddt, *J* = 17.2, 10.5, 5.3 Hz, 1H), 5.24 (br, s, 1H), 5.15 (dq, *J* = 17.2, 1.7 Hz, 1H), 5.08 (dq, *J* = 10.3, 1.5 Hz, 1H), 4.92 (br, s, 1H), 4.27 (dt, *J* = 9.4, 6.0 Hz, 2H), 4.14–4.08 (m, 2H), 3.82–3.69 (m, 4H), 3.63 (dd, *J* = 10.1, 2.1 Hz, 1H), 3.59 (d, *J* = 10.1 Hz, 1H), 3.39

(dd, *J* = 12.0, 2.2 Hz, 1H), 2.80–2.59 (m, 4H), 2.28–2.14 (m, 1H), 2.07−0.50 (m, 52H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 218.3, 184.4 (C1 carboxylate), 157.5 (carbamide group), 136.1, 127.0, 125.1, 115.8, 109.5, 99.5, 90.3, 76.6, 76.3, 75.7, 74.9, 71.7, 70.4, 67.8, 56.0, 51.5, 50.7, 47.8, 43.6, 40.5, 39.2, 36.6, 36.5, 33.2, 33.1, 32.9, 29.3, 28.6, 28.2, 27.4, 24.3, 21.2, 20.6, 17.8, 17.5, 16.5, 15.0, 13.5, 12.9, 12.4, 11.0, 7.0, 6.8 ppm; FT-IR (KBr tablet): 3481 (br, s), 3366 (br, s), 3310 (br, s), 2963 (s), 2937 (s), 2875 (s), 1714 (s), 1665 (s), 1644 (s), 1567 (s), 1499 (m), 1460 (s), 1405 (s), 1380 (s), 1359 (m), 1339 (m), 1326 (m), 1301 (s) cm^{-1}; ESI MS (m/z): $[M+H]^+$ Calcd for $C_{46}H_{76}N_2NaO_{11}^+$ 855.5; Found 856; HRMS (ESI^+) *m/z*: $[M+H]^+$ Calcd for $C_{46}H_{76}N_2NaO_{11}^+$ 855.5347; Found 855.5346.

C20-2-chloroethylurea of C20-epi-aminosalinomycin ***3f****:* Yield: 82 mg, 77%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC; R_f: 0.43 in 33% acetone/$CHCl_3$. Strains green with PMA; ^{1}H NMR (401 MHz, CD_2Cl_2) δ 6.22 (d, *J* = 10.6 Hz, 1H), 5.93 (ddd, *J* = 138.3, 70.5, 1.8 Hz, 1H), 5.66 (br, s, 1H), 5.09 (br, s, 1H), 4.30–4.22 (m, 3H), 4.11 (d, *J* = 10.1 Hz, 1H), 3.76 (dd, *J* = 11.0, 4.5 Hz, 1H), 3.65–3.45 (m, 7H), 3.44–3.33 (m, 3H), 2.80–2.67 (m, 2H), 2.67–2.61 (m, 1H), 2.59 (s, 1H), 2.31–2.03 (m, 2H), 2.00−0.50 (m, 49H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 218.1, 184.4 (C1 carboxylate), 157.4 (carbamide group), 126.6, 125.0, 109.3, 99.3, 90.0, 76.5, 76.1, 75.6, 74.7, 71.6, 70.4, 67.7, 55.8, 54.2, 51.4, 50.3, 47.8, 45.0, 42.7, 40.3, 39.0, 36.4, 36.3, 33.0, 32.9, 32.6, 29.5, 29.1, 28.4, 28.0, 27.2, 24.1, 21.0, 17.6, 17.3, 16.2, 14.8, 13.3, 12.7, 12.3, 6.8, 6.6 ppm; FT-IR (KBr tablet): 3506 (br, s), 3386 (br, s), 3324 (br, s), 2965 (s), 2936 (s), 2876 (s), 1714 (s), 1667 (s), 1650 (s), 1564 (s), 1501 (m), 1460 (s), 1404 (s), 1382 (s), 1358 (m), 1338 (m), 1326 (m), 1301 (s) cm^{-1}; ESI MS (m/z): $[M+H]^+$ Calcd for $C_{45}H_{75}ClN_2NaO_{11}^+$ 877.5; Found 878; HRMS (ESI^+) *m/z*: $[M+H]^+$ Calcd for $C_{45}H_{75}ClN_2NaO_{11}^+$ 877.4957; Found 877.4953.

*C20-3-chloropropylurea of C20-epi-aminosalinomycin **3g**:* Yield: 99 mg, 92%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC; R_f: 0.46 in 33% acetone/$CHCl_3$. Strains green with PMA; ^{1}H NMR (400 MHz, CD_2Cl_2) δ 6.17 (d, *J* = 10.6 Hz, 1H), 5.98 (dd, *J* = 10.6, 5.8 Hz, 1H), 5.10 (br, s, 1H), 5.00 (br, s, 1H), 4.26–4.16 (m, 2H), 4.06 (d, *J* = 10.4 Hz, 1H), 3.98 (d, *J* = 9.5 Hz, 1H), 3.71 (dd, *J* = 10.9, 4.7 Hz, 1H), 3.57 (dd, *J* = 10.1, 2.0 Hz, 1H), 3.54–3.49 (m, 2H), 3.33 (dd, *J* = 12.0, 2.1 Hz, 1H), 3.21 (dtt, *J* = 13.6, 12.7, 6.5 Hz, 2H), 2.77–2.52 (m, 4H), 2.21–2.07 (m, 2H), 2.00−0.50 (m, 55H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 218.0, 184.3 (C1 carboxylate), 157.4 (carbamide group), 126.6, 124.9, 109.2, 99.2, 90.0, 76.4, 76.0, 75.5, 74.6, 71.5, 70.3, 67.5, 55.7, 51.3, 50.4, 47.7, 43.1, 40.2, 38.9, 38.1, 36.4, 36.2, 33.2, 33.0, 32.8, 32.6, 29.1, 28.3, 27.9, 27.1, 24.1, 21.0, 20.3, 17.6, 17.3, 16.2, 14.8, 13.2, 12.7, 12.2, 10.8, 6.8, 6.5 ppm; FT-IR (KBr tablet): 3477 (br, s), 3375 (br, s), 3308 (br, s), 2963 (s), 2934 (s), 2875 (s), 1714 (s), 1667 (s), 1648 (s), 1566 (s), 1501 (m), 1459 (s), 1404 (s), 1381 (s), 1358 (m), 1339 (m), 1326 (m), 1300 (s) cm^{-1}; ESI MS (m/z): $[M+H]^+$ Calcd for $C_{46}H_{77}ClN_2NaO_{11}^+$ 891.5; Found 892; HRMS (ESI^+) *m/z*: $[M+H]^+$ Calcd for $C_{46}H_{77}ClN_2NaO_{11}^+$ 891.5114; Found 891.5120.

*C20-benzylurea of C20-epi-aminosalinomycin **3h**:* Yield: 142 mg, 86%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC; R_f: 0.54 in 33% acetone/$CHCl_3$. UV-active and strains green with PMA; ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.37–7.19 (m, 5H), 6.22 (d, *J* = 10.7 Hz, 1H), 6.05 (dd, *J* = 10.6, 5.8 Hz, 1H), 5.57 (br, s, 1H), 5.05 (br, s, 1H), 4.41–4.21 (m, 4H), 4.20–4.08 (m, 2H), 3.77 (dd, *J* = 11.0, 4.7 Hz, 1H), 3.62 (dd, *J* = 10.1, 2.0 Hz, 1H), 3.56 (d, *J* = 10.2 Hz, 1H), 3.38 (dd, *J* = 12.0, 2.1 Hz, 1H), 2.84–2.60 (m, 5H), 2.23–2.11 (m, 2H), 2.05−0.50 (m, 51H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 218.0, 184.6 (C1 carboxylate), 157.5 (carbamide group), 140.0, 128.8 (2C), 127.6 (2C), 127.4, 126.5, 124.9,

109.2, 99.2, 90.0, 76.3, 76.0, 75.5, 74.6, 71.5, 70.5, 67.6, 55.7, 51.3, 50.3, 47.7, 44.6, 40.2, 38.9, 36.5, 36.3, 33.0, 32.8, 32.6, 28.9, 28.3, 28.0, 27.1, 24.2, 21.0, 20.3, 17.6, 17.2, 16.2, 14.8, 13.2, 12.6, 12.2, 10.8, 6.8, 6.5 ppm; FT-IR (KBr tablet): 3475 (br, s), 3431 (br, s), 3364 (br, s), 3306 (br, s), 3089 (m), 3062 (m), 3030 (m), 2962 (s), 2935 (s), 2875 (s), 1714 (s), 1664 (s), 1648 (s), 1603 (m), 1565 (s), 1496 (m), 1458 (s), 1405 (s), 1381 (s), 1359 (m), 1339 (m), 1326 (m), 1301 (s) cm^{-1}; ESI MS (m/z): $[M+H]^+$ Calcd for $C_{50}H_{78}N_2NaO_{11}^+$ 905.5; Found 906; HRMS (ESI^+) *m/z*: $[M+H]^+$ Calcd for $C_{50}H_{78}N_2NaO_{11}^+$ 905.5503; Found 905.5514.

C20-phenylurea of C20-epi-aminosalinomycin ***3i****:* Yield: 26 mg, 24%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC; R_f: 0.62 in 33% acetone/$CHCl_3$. UV-active and strains green with PMA; ^1H NMR (600 MHz, CD_2Cl_2) δ 7.33 (d, *J* = 6.8 Hz, 2H), 7.28–7.22 (m, 2H), 7.07–6.97 (m, 1H), 6.24 (d, *J* = 10.6 Hz, 1H), 6.09 (dd, *J* = 10.5, 5.7 Hz, 1H), 5.26 (br, s, 1H), 5.11 (br, s, 1H), 4.62 (br, s, 1H), 4.43 (s, 1H), 4.29 (dd, *J* = 13.2, 6.5 Hz, 1H), 4.14 (d, *J* = 10.3 Hz, 1H), 3.79 (dd, *J* = 11.0, 4.6 Hz, 1H), 3.64 (dd, *J* = 10.1, 1.9 Hz, 1H), 3.58 (d, *J* = 10.1 Hz, 1H), 3.39 (dd, *J* = 12.1, 2.1 Hz, 1H), 3.02–2.60 (m, 7H), 2.34–2.14 (m, 2H), 2.03–0.50 (m, 49H) ppm; ^{13}C NMR (151 MHz, CD_2Cl_2) δ 218.2, 184.6 (C1 carboxylate), 155.4 (carbamide group), 139.3, 129.3 (2C), 126.4, 125.2, 123.8, 121.2 (2C), 109.1, 99.3, 90.1, 76.4, 76.1, 75.6, 74.6, 71.6, 70.6, 67.7, 55.8, 51.4, 50.3, 40.3, 38.9, 36.6, 36.3, 33.0, 32.8, 32.6, 29.4, 29.0, 28.4, 27.9, 27.2, 24.2, 21.0, 20.4, 17.6, 17.1, 16.2, 14.8, 13.3, 12.7, 12.3, 10.9, 6.8, 6.5 ppm; FT-IR (KBr tablet): 3473 (br, s), 3385 (br, s), 3321 (br, s), 3087 (m), 3056 (m), 3039 (m), 2963 (s), 2935 (s), 2875 (s), 1714 (s), 1680 (br, s), 1616 (m), 1600 (s), 1558 (br, s), 1516 (s), 1500 (s), 1460 (s), 1443 (s), 1404 (s), 1381 (s), 1357 (m), 1339 (m), 1325 (m), 1315 (s), 1301 (m) cm^{-1}; ESI MS (m/z): $[M+H]^+$ Calcd for $C_{49}H_{76}N_2NaO_{11}^+$ 891.5; Found 892; HRMS (ESI^+) *m/z*: $[M+H]^+$ Calcd for $C_{49}H_{76}N_2NaO_{11}^+$ 891.5347; Found 891.5338.

*C20-1-naphthylurea of C20-epi-aminosalinomycin **3j**:* Yield: 23 mg, 20%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC; R_f: 0.52 in 33% EtOAc/*n*-hexane. UV-active and strains green with PMA; ^{1}H NMR (401 MHz, CD_2Cl_2) δ 8.07–8.02 (m, 1H), 7.88–7.84 (m, 1H), 7.75 (d, *J* = 8.1 Hz, 1H), 7.53 (dqd, *J* = 8.4, 6.8, 1.6 Hz, 2H), 7.49–7.41 (m, 2H), 6.94 (br, s, 1H), 6.02 (dt, *J* = 10.5, 8.0 Hz, 2H), 5.20 (br, s, 1H), 4.45–4.24 (m, 3H), 4.09 (d, *J* = 10.3 Hz, 1H), 3.77 (dd, *J* = 11.1, 4.5 Hz, 1H), 3.61 (dd, *J* = 10.3, 1.5 Hz, 1H), 3.48 (d, *J* = 10.1 Hz, 1H), 3.36 (dd, *J* = 12.0, 2.1 Hz, 1H), 2.75 (td, *J* = 10.9, 3.1 Hz, 1H), 2.71–2.54 (m, 3H), 2.34–2.11 (m, 1H), 2.08−0.50 (m, 53H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 218.3, 184.4 (C1 carboxylate), 156.7 (carbamide group), 135.1, 133.5, 130.5, 128.9, 127.8, 127.3, 127.1, 126.6, 126.3, 125.2, 124.9, 123.0, 109.1, 99.4, 90.4, 76.6, 76.3, 75.5, 74.8, 71.8, 70.4, 67.8, 55.9, 51.5, 50.7, 47.4, 40.2, 38.9, 36.7, 36.5, 33.1, 33.0, 32.8, 30.3, 29.3, 28.6, 28.1, 27.4, 24.3, 21.1, 20.6, 17.7, 16.4, 16.2, 15.0, 13.4, 12.9, 12.4, 11.0, 7.0, 6.8 ppm; FT-IR (KBr tablet): 3471 (br, s), 3429 (s), 3349 (br, s), 3272 (br, s), 3092 (m), 3052 (m), 2962 (s), 2929 (s), 2873 (s), 1714 (s), 1674 (s), 1663 (s), 1631 (m), 1596 (m), 1565 (s), 1551 (s), 1521 (s), 1460 (s), 1405 (s), 1380 (s), 1358 (m), 1342 (s), 1326 (m), 1301 (s) cm^{-1}; ESI MS (m/z): $[M+H]^+$ Calcd for $C_{53}H_{78}N_2NaO_{11}^+$ 941.5; Found 942; HRMS (ESI^+) *m/z*: $[M+H]^+$ Calcd for $C_{53}H_{78}N_2NaO_{11}^+$ 941.5503; Found 941.5509.

*C20-ethylthiourea of C20-epi-aminosalinomycin **4a**:* Yield: 94 mg, 90%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC; R_f: 0.72 in 33% acetone/$CHCl_3$. Strains green with PMA; ^{1}H NMR (401 MHz, CD_2Cl_2) δ 6.28 (d, *J* = 10.6 Hz, 1H), 6.06 (dd, *J* = 10.0, 5.0 Hz, 1H), 5.35 (br, s, 1H), 4.90 (br, s, 1H), 4.26 (q, *J* = 6.7 Hz, 1H), 4.11 (d, *J* = 10.4 Hz, 1H), 3.78 (dd, *J* = 11.0, 4.6 Hz, 1H), 3.72–3.52 (m, 3H), 3.39 (dt, *J* = 13.5, 6.7 Hz, 3H), 2.70 (dddd, *J* = 30.5, 27.3, 15.5, 7.9 Hz, 3H), 2.40−0.50 (m, 59H) ppm; ^{13}C NMR

(101 MHz, CD_2Cl_2) δ 218.5, 184.6 (C1 carboxylate), 182.1 (thiocarbamide group), 125.9, 125.6, 109.0, 99.5, 90.5, 76.8, 76.3, 75.9, 74.7, 71.7, 70.6, 68.0, 56.0, 51.6, 51.1, 50.4, 40.4, 39.1, 36.9, 36.4, 33.14, 33.06, 32.8, 29.7, 29.5, 28.6, 27.9, 27.4, 24.2, 21.3, 20.6, 17.8, 17.4, 16.3, 15.0, 14.5, 13.4, 12.9, 12.5, 11.1, 7.0, 6.7 ppm; FT-IR (KBr tablet): 3466 (br, s), 3423 (s), 3364 (br, s), 3287 (br, s), 3058 (m), 2964 (s), 2934 (s), 2875 (s), 1714 (s), 1668 (m), 1638 (m), 1564 (s), 1535 (s), 1497 (m), 1459 (s), 1404 (s), 1381 (s), 1359 (s), 1339 (s), 1327 (s), 1300 (s) cm^{-1}; ESI MS (m/z): $[M+H]^+$ Calcd for $C_{45}H_{76}N_2NaO_{10}S^+$ 859.5; Found 860; HRMS (ESI^+) *m/z*: $[M+H]^+$ Calcd for $C_{45}H_{76}N_2NaO_{10}S^+$ 859.5118; Found 859.5123.

C20-n-butylthiourea of C20-epi-aminosalinomycin ***4b****:* Yield: 74 mg, 69%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC; R_f: 0.93 in 33% acetone/$CHCl_3$. Strains green with PMA; ^1H NMR (401 MHz, CD_2Cl_2) δ 6.28 (d, *J* = 10.6 Hz, 1H), 6.06 (dd, *J* = 10.8, 5.1 Hz, 1H), 5.33 (br, s, 1H), 4.91 (br, s, 1H), 4.27 (q, *J* = 6.3 Hz, 1H), 4.11 (d, *J* = 10.4 Hz, 1H), 3.78 (dd, *J* = 10.9, 4.2 Hz, 1H), 3.62 (dt, *J* = 11.1, 5.5 Hz, 2H), 3.51–3.09 (m, 3H), 2.85–2.57 (m, 3H), 2.40−0.50 (m, 64H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 218.4, 184.8 (C1 carboxylate), 182.1 (thiocarbamide group), 125.9, 125.6, 108.9, 99.6, 90.5, 76.8, 76.3, 76.0, 74.7, 71.7, 70.6, 68.0, 56.0, 51.6, 51.0, 50.4, 40.4, 39.1, 37.0, 36.4, 33.2, 33.1, 32.8, 31.5, 29.4, 28.6, 28.0, 27.4, 24.3, 21.4, 20.6 (2C), 17.8, 17.4, 16.3, 15.0, 14.1, 13.4 (2C), 12.9, 12.5, 11.1, 7.0, 6.7 ppm; FT-IR (KBr tablet): 3466 (br, s), 3423 (s), 3364 (br, s), 3292 (br, s), 3058 (m), 2962 (s), 2934 (s), 2874 (s), 1714 (s), 1668 (m), 1640 (m), 1564 (s), 1533 (s), 1496 (m), 1460 (s), 1404 (s), 1381 (s), 1359 (s), 1340 (s), 1327 (s), 1300 (s) cm^{-1}; ESI MS (m/z): $[M+H]^+$ Calcd for $C_{47}H_{80}N_2NaO_{10}S^+$ 887.5; Found 888; HRMS (ESI^+) *m/z*: $[M+H]^+$ Calcd for $C_{47}H_{80}N_2NaO_{10}S^+$ 887.5431; Found 887.5438.

C20-allylthiourea of C20-epi-aminosalinomycin ***4c****:* Yield: 83 mg, 87%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC; R_f: 0.71 in 20% acetone/CH_2Cl_2. Strains green with PMA; ^{1}H NMR (401 MHz, CD_2Cl_2) δ 6.28 (d, *J* = 10.6 Hz, 1H), 6.05 (dd, *J* = 10.4, 5.6 Hz, 1H), 5.91–5.48 (m, 1H), 5.37 (br, s, 1H), 5.23–5.14 (m, 2H), 4.94 (br, s, 1H), 4.26 (q, *J* = 6.8 Hz, 1H), 4.06 (t, *J* = 29.9 Hz, 3H), 3.78 (dd, *J* = 10.9, 4.4 Hz, 1H), 3.68–3.44 (m, 3H), 3.40 (dd, *J* = 12.0, 2.0 Hz, 1H), 2.80–2.56 (m, 3H), 2.40−0.50 (m, 56H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 218.4, 184.8 (C1 carboxylate), 182.5 (thiocarbamide group), 134.1, 125.7, 125.6, 117.0, 108.9, 99.6, 90.5, 76.8, 76.3, 76.0, 74.7, 71.7, 70.5, 68.0, 56.0, 51.6, 51.0, 50.3, 40.4, 39.1, 37.0, 36.4, 33.2, 33.0, 32.8, 29.7, 29.4, 28.6, 28.0, 27.4, 24.3, 21.4, 20.6, 17.8, 17.5, 16.3, 15.0, 13.4, 12.9, 12.5, 11.1, 7.0, 6.7 ppm; FT-IR (KBr tablet): 3444 (br, s), 3421 (s), 3381 (br, s), 3292 (br, s), 3080 (m), 3058 (m), 2963 (s), 2934 (s), 2875 (s), 1714 (s), 1669 (m), 1643 (m), 1565 (s), 1533 (s), 1494 (m), 1459 (s), 1404 (s), 1381 (s), 1363 (s), 1338 (s), 1328 (s), 1300 (s) cm^{-1}; ESI MS (m/z): $[M+H]^+$ Calcd for $C_{46}H_{76}N_2NaO_{10}S^+$ 871.5; Found 872; HRMS (ESI^+) *m/z*: $[M+H]^+$ Calcd for $C_{46}H_{76}N_2NaO_{10}S^+$ 871.5118; Found 871.5115.

C20-phenylthiourea of C20-epi-aminosalinomycin ***4d****:* Yield: 75 mg, 76%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC; R_f: 0.67 in 20% acetone/CH_2Cl_2. UV-active and strains green with PMA; ^{1}H NMR (401 MHz, CD_2Cl_2) δ 7.42–7.35 (m, 2H), 7.27 (t, *J* = 7.3 Hz, 1H), 7.16 (d, *J* = 7.2 Hz, 2H), 6.20 (d, *J* = 10.4 Hz, 1H), 6.07 (dd, *J* = 10.5, 5.9 Hz, 1H), 5.60 (d, *J* = 9.3 Hz, 1H), 5.52 (br, s, 1H), 5.10 (dd, *J* = 9.0, 6.1 Hz, 1H), 4.26 (q, *J* = 6.5 Hz, 1H), 4.09 (d, *J* = 10.4 Hz, 1H), 3.78 (dd, *J* = 10.9, 4.0 Hz, 1H), 3.62 (dd, *J* = 15.7, 5.9 Hz, 2H), 3.39 (dd, *J* = 12.0, 1.5 Hz, 1H), 2.78–2.42 (m, 4H), 2.40−0.50 (m, 55H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 218.3, 183.9 (C1 carboxylate), 181.3 (thiocarbamide group), 136.0, 130.6 (2C), 128.0, 126.4 (2C), 125.6, 125.5, 108.6, 99.4, 90.5, 76.6, 76.2, 75.4,

74.5, 71.5, 70.0, 67.7, 55.8, 51.8, 51.4, 50.6, 40.1, 38.7, 36.4, 36.2, 32.9, 32.8, 32.7, 29.4, 28.4, 27.7, 27.3, 23.9, 21.0, 20.4, 17.6, 16.6, 16.2, 14.8, 13.3, 12.8, 12.2, 10.9, 6.8, 6.6 ppm; FT-IR (KBr tablet): 3444 (br, s), 3416 (br, s), 3385 (s), 3266 (br, s), 3107 (m), 3057 (m), 2962 (s), 2934 (s), 2874 (s), 1714 (s), 1668 (m), 1633 (m), 1597 (m), 1567 (s), 1519 (s), 1499 (s), 1459 (s), 1404 (s), 1380 (s), 1358 (s), 1341 (s), 1325 (s), 1317 (s), 1299 (s) cm^{-1}; ESI MS (m/z): $[M+H]^+$ Calcd for $C_{49}H_{76}N_2NaO_{10}S^+$ 907.5; Found 908; HRMS (ESI^+) *m/z*: $[M+H]^+$ Calcd for $C_{49}H_{76}N_2NaO_{10}S^+$ 907.5118; Found 907.5106.

4.4. In vitro biological assays

4.4.1. Trypanocidal and cytotoxic assays

For compound screening, bloodstream forms of *T. brucei* 427-221a [34] and human myeloid HL-60 cells [35] were seeded at an initial cell density of 1×10^4 mL^{-1} and 5×10^4 mL^{-1}, respectively, in 96-well plates in a final volume of 200 μL of Baltz medium [36] supplemented with 16.7% heat-inactivated bovine serum and containing various concentrations of test compounds (10-fold dilutions from 100 μM to 100 nM) and 0.9% DMSO. Wells containing only Baltz medium and 0.9% DMSO served as controls. After 24 h incubation at 37 °C in a humidified atmosphere containing 5% CO_2, 20 μL of a sterile filtered 0.5 mM resazurin solution in PBS was added. Incubation was continued for another 48 h, after which the absorbance of the wells was read on a BioTek ELx808 microplate reader using a test wavelength of 570 nm and a reference wavelength of 630 nm. GI_{50} values (the concentration of a compound required to reduce the growth rate of cells by 50% compared to the control) were calculated by linear interpolation according to the method described by Huber and Koella [19], while MIC values

(the concentration of a compound at which all cells were killed) were determined microscopically.

4.4.2. Swelling experiments

Changes in cell volume of bloodstream form of *T. brucei* 427-221a upon exposure to (thio)urea derivatives of **SAL** were measured by the light scattering methods as previously described [12−14]. In brief, in wells of a 96-well plate, 1×10^7 bloodstream-form trypanosomes were incubated in a final volume of 200 µL Baltz medium with 100 µM test compound and 0.9% DMSO. Controls contained only 0.9% DMSO. The absorbance of the cultures was measured at 490 nm on a BioTEk ELx808 microplate reader every 10 min for 60 min.

Dedication

We would like to dedicate this article to Professor Bogumił Brzezinski in honor of his 80th birthday.

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Appendix A. Supplementary data

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

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1. A series of novel (thio)urea derivatives of salinomycin (**SAL**) was synthesized.
2. Six compounds (**3c**, **3d**, and **4a–d**) showed promising antitrypanosomal activities.
3. Several compounds showed a good selectivity of action.
4. Compounds **4b** and **4d** displayed faster cell swelling activity than native **SAL**.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: