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PAPER

## New parasite inhibitors encompassing novel conformationally-locked 5'-acyl sulfamoyl adenosines†‡

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We describe the design, synthesis and biological evaluation of conformationally-locked 5'-acyl sulfamoyl adenosine derivatives as new parasitic inhibitors against *Trypanosoma* and *Leishmania*. The conformationally-locked (3'-endo, North-type) nucleosides have been synthesized by covalently attaching a 4'-CH<sub>2</sub>-O-2' bridge (Fig. 2) across C2'-C4' of adenosine in order to reduce the conformational flexibility of the pentose ring. This is designed to decrease the entropic penalty for complex formation with the target protein, which may improve free-energy of stabilization of the complex leading to improved potency. Conformationally-locked 5'-acyl sulfamoyl adenosine derivatives (**16**–**22**) were tested against parasitic protozoans for the first time in this work, and showed potent inhibition of *Trypanosoma cruzi*, *Trypanosoma brucei*, *Trypanosoma rhodesiense* and *Leishmania infantum* with IC<sub>50</sub> = 0.25–0.51 μM. In particular, the potent 5'-pentanyl acyl sulfamoyl adenosine derivative **17** (IC<sub>50</sub> = 0.25 μM) against intracellular *L. infantum* amastigotes and *Trypanosoma* subspecies is interesting in view of its almost insignificant cytotoxicity in murine macrophage host cells (CC<sub>50</sub> >4 μM) and in diploid human fibroblasts MRC-5 cell lines (CC<sub>50</sub> 4 μM). This work also suggests that variable alkyl chain length of the acyl group on the acylsulfamoyl side chain at 5' can modulate the toxicity of 5'-O-sulfamoylnucleoside analogues. This conformationally-locked sulfamoyl adenosine scaffold presents some interesting possibilities for further drug design and lead optimization.

### 1. Introduction

Chagas' disease or American trypanosomiasis is caused by the parasitic protozoan agent *Trypanosoma cruzi* (*T. cruzi*) and is a serious health problem in Central and South American continents, affecting about 17 million people living in poor rural areas.<sup>1</sup> Depending on physiological state and age of the patient it has a mortality rate of 8–12%.<sup>2,3</sup> Chagas' disease is now spreading to other continents due to immigration, which was once confined to the Latin American region.<sup>4</sup> *Trypanosoma brucei* (*T.b. brucei*) and *Trypanosoma rhodesiense* (*T.b. rhod.*) are causative agents for human African trypanosomiasis.<sup>5,6</sup> It is mainly transmitted by the infected faeces of blood-sucking triatomine bugs, infection through blood transfusion, from infected mother to child or organ donation.<sup>2,4</sup> To date no vaccine is available against Chagas' disease.<sup>4</sup> The disease can be controlled by chemotherapy of benznidazole (**5**) or nifurtimox (**6**) if

administered in its initial stage. The longer the person is infected the potency of these drugs decreases. The parasites mostly hide in muscles of the heart and digestive system in the chronic phase, due to which patients suffer from cardiac and digestive disorders.<sup>4</sup> Benznidazole or nifurtimox are not effective in the chronic phase of infection, and are also associated with high toxicity and side effects.<sup>2,4,7–13</sup>

Leishmaniasis is a group of diseases ranging from self-healing cutaneous ulcers to severe visceral diseases which even may cause death. It is caused by a protozoan parasite of species of the genus *Leishmania*.<sup>14,15</sup> These are digenetic organisms present in a flagellated promastigote in the gut of the sandfly vectors and an intracellular amastigote in the mammalian host.<sup>14,16</sup> Leishmaniasis is transmitted to humans by some species of *phlebotomine* sandfly. This infection is endemic in 88 countries from tropical and sub-tropical regions affecting 12 million people, especially those living in poor rural areas, with 2 million new cases annually.<sup>17–19</sup> The standard chemotherapy treatment includes antimony-based drugs, amphotericin-B, paromomycin, and oral miltefosine.<sup>20–27</sup>

Although significant information on the biochemistry and physiology of these parasites is available, current treatments are effective only in the acute stage, having several limitations associated with toxic side effects, prolonged treatment duration and parasite resistance.<sup>8–10,19,28–32</sup> This high rate of therapeutic

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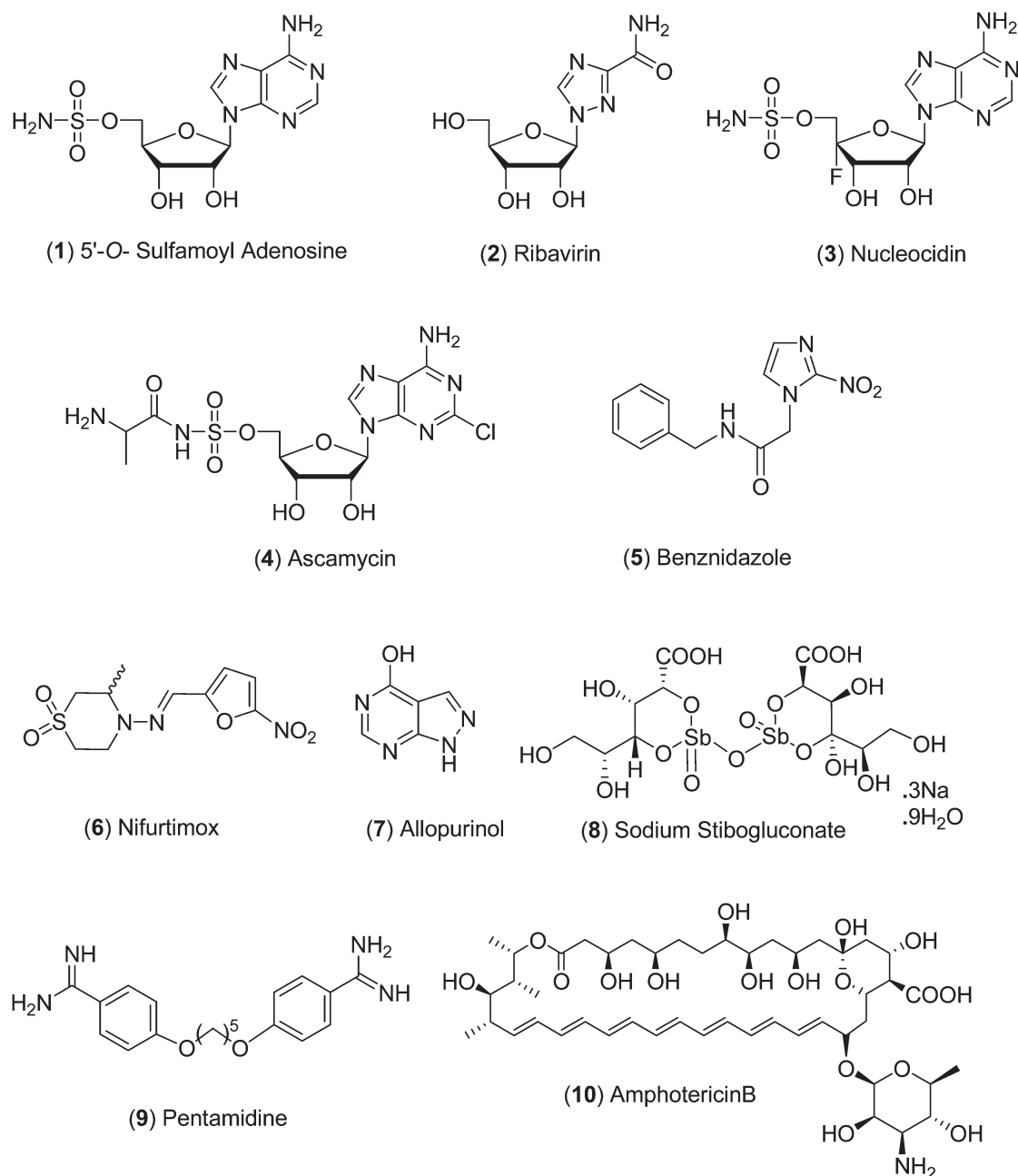
† This article is part of the *Organic & Biomolecular Chemistry* 10th Anniversary issue.

‡ Electronic supplementary information available: Spectral data (<sup>1</sup>H, <sup>13</sup>C NMR, DEPT, HSQC, LCMS, Mass and HPLC) for all new compounds are included. See DOI: 10.1039/c2ob25879j

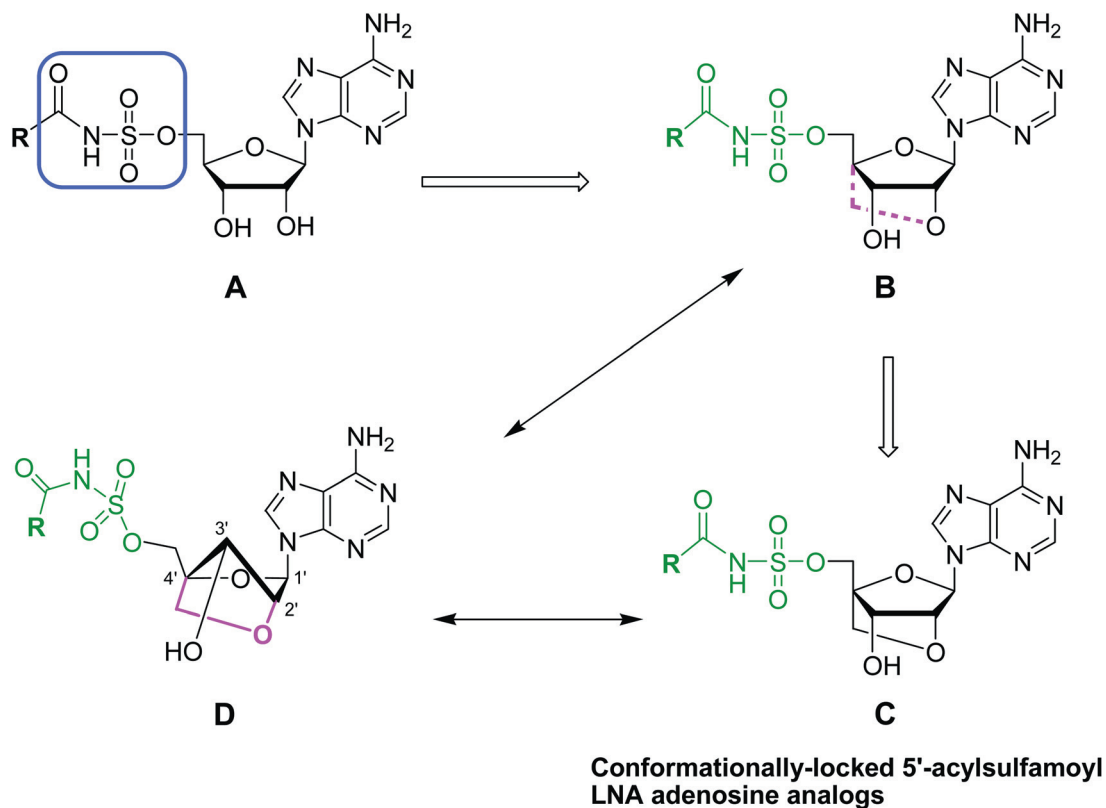
failure calls for new rational approaches to develop alternative drugs.

Modified adenosine derivatives are known to possess anti-protozoal activity.<sup>33–38</sup> Adenosine derivatives display antitrypanosomal activity by inhibiting glyceraldehyde-3-phosphate dehydrogenase (GAPDH),<sup>34–36,38,39</sup> or polyamine syntheses.<sup>33,40</sup> The strategy used so far to develop new inhibitors of these enzymes involves base subunit modification and/or carbohydrate modification (at 2' and/or 5') of adenosine. An important way of discovering new antitrypanosomal and antileishmanial agents is exploring new scaffolds, studying their SAR and structurally optimizing validated lead compounds.

In view of the antiparasitic activity displayed by 2',3'-bis-hydroxy sulfamoyl adenosine (**1**, Fig. 1) and aminoacylsulfamoyl adenosine (ascamycin<sup>41</sup> **4**, Fig. 1), we became interested in exploring acyl-sulfamoyl derivatives of adenosines with a modified locked-sugar backbone (**C**, Fig. 2) to see its effect against *Trypanosoma* subsp. and *Leishmania*. Further retrosynthetic analysis to synthesize sugar-locked 2'-*O*,4'-*C*-methylene-linked bicyclic ribonucleosides (**C**, Fig. 2) of acylsulfamoyl adenosine (**A**) is given in Fig. 2. The main feature of the synthesis is how the constituent pentose-sugar containing a –CH<sub>2</sub>– bridge, connecting the 2'-*O* of ribose with the 4'-*C* (**C**, Fig. 2) has locked the flexible pentose-sugar to the fixed 3'-*endo*



**Fig. 1** Known inhibitors of *Trypanosoma* and *Leishmania*: compounds **1–4** are known to possess anti-parasitic activity. Compounds **5–10** are the standard front-line drugs: against *Trypanosoma* spp. (**5** & **6**); *Leishmania* (**7**, **8**, **9** & **10**).



**Fig. 2** 2'-*O*, 4'-*C*-Methylene-linked bicyclic ribonucleoside bond is shown with a pink dotted line in the conformationally-constrained core **B**. **D** is the general bicyclic structure according to IUPAC rule. General formula **C** represents the series of compounds synthesized in this work.

conformation. Because of the reduction in the conformational flexibility of the pentose sugar, we expect that there will be a decrease of the entropic penalty for the complex formation with the target protein, which will in turn give improved free-energy of stabilization of the complex, and hence increased potency.

Here we describe the results of studies involving our novel antiprotozoal conformationally-locked sulfamoyl derivatives of adenosine with new modifications at the 2', 4' and 5' centers in the sugar moiety. Most of these compounds **16–22**<sup>42</sup> are extremely potent against Trypanosomiasis and Leishmaniasis, with  $IC_{50}$  of 0.25–0.51  $\mu\text{M}$ . In particular, compound **17** (*in vitro* activity,  $IC_{50}$  = 0.25  $\mu\text{M}$ ), which is potent against intracellular *L. infantum* amastigotes and *Trypanosoma* subspecies, is interesting in view of its almost insignificant cytotoxicity in murine macrophage host cells ( $CC_{50}$  >4  $\mu\text{M}$ ) and in diploid human fibroblasts MRC-5 cell line against mammalian cells ( $CC_{50}$  4  $\mu\text{M}$ ). Growth inhibition assays were performed on compounds **16–22** (Table 1) against protozoans including epimastigote form of *T. cruzi*, *T. brucei* subsp. *brucei*, *T. brucei* subsp. *rhodesiense* and *L. infantum*.

## 2. Results

### 2.1. Chemistry

Locked nucleic acid (LNA), compound **14**<sup>43</sup> (Scheme 1) served as the starting material for the synthesis of compounds **16–22**. Compound **14**, on sulfamoylation reaction with sulfamoyl chloride in dry DMF and  $\text{Et}_3\text{N}$ , gave sulfonamide **15** (31%).

Debenzylation of sulfonamide **15** using  $\text{Pd}(\text{OH})_2/\text{C}$  and  $\text{HCO}_2\text{NH}_4$  furnished alcohol **16** in good yield (87%). The target compounds **17–22** were synthesized by treating sulfonamide **16** with the corresponding *N*-hydroxy succinimide aliphatic ester in dry DMF and  $\text{Cs}_2\text{CO}_3$  to furnish the synthesis of target compounds **17–22** in 15–34% yield.

### 2.2. Antiprotozoal activity

Growth inhibition assays against protozoans including epimastigote form of *T. cruzi* (Tulahuen 2 strain), *T. brucei* subsp. *brucei* 427, *T. brucei* subsp. *rhodesiense* STIB900 and *L. infantum* (MHOM/MA (BE)/67) were performed on compounds **16–22** (Table 1). Parasites were grown in the presence of the compounds and the percentage growth inhibition was determined against control (no drug added to the medium). A cytotoxicity assay<sup>44</sup> involving diploid human lung fibroblasts (MRC-5) was performed to assess the toxicity of compounds against mammalian cells. The results are expressed as percent reduction in cell viability compared to untreated control.

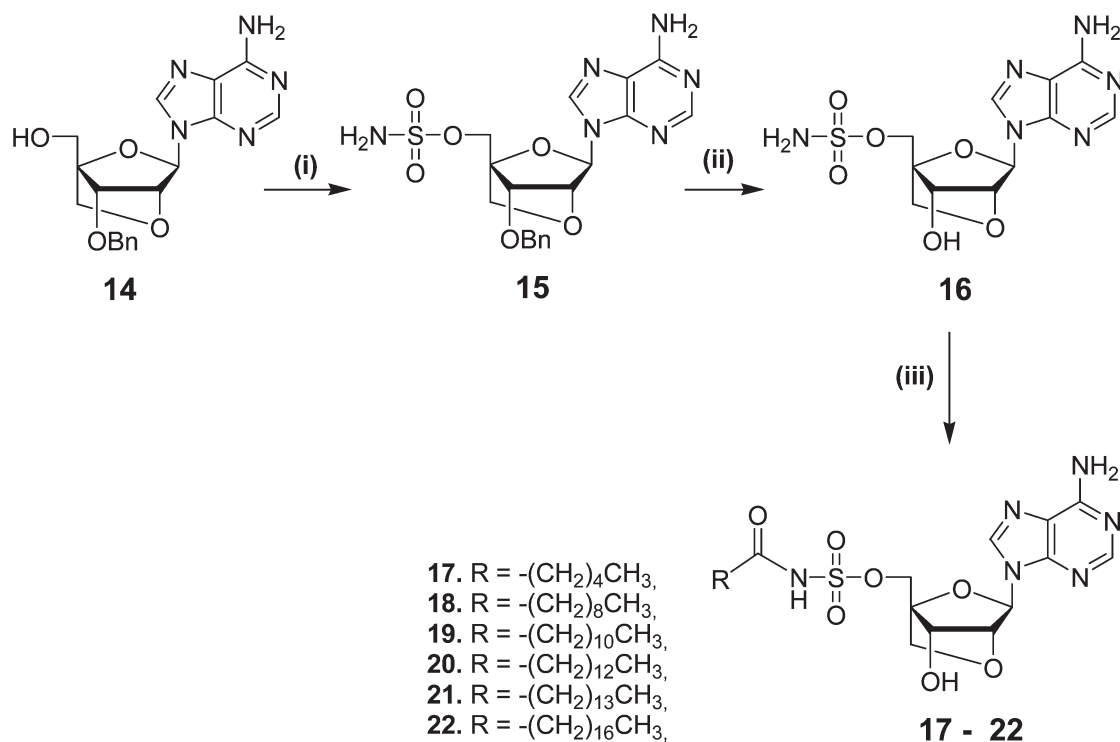
## 3. Discussion

All compounds (**16–22**) reported here were found to be very active *in vitro* against protozoans including epimastigote form of *T. cruzi*, *T. brucei*, *T. rhodesiense* and *L. infantum*, having  $IC_{50}$ s in the range of 0.25–0.49  $\mu\text{M}$ ; 0.25  $\mu\text{M}$ ; 0.25  $\mu\text{M}$  and 0.25–0.51  $\mu\text{M}$  respectively. These compounds were tested for their cytotoxicity in murine macrophage host cells (Cytotoxic

**Table 1** *In vitro* activity ( $IC_{50}^a$ ,  $\mu M$ ) of compounds against *T. cruzi*, *T. brucei brucei* (*T.b. brucei*), *T. brucei rhodesiense* (*T.b. rhod.*) and *Leishmania infantum* (*L. inf.*)

Compd. no.	R	<i>T. cruzi</i> ( $IC_{50}$ , $\mu M$ )	<i>T.b. brucei</i> ( $IC_{50}$ , $\mu M$ )	<i>T.b. rhod.</i> ( $IC_{50}$ , $\mu M$ )	<i>L. inf.</i> ( $IC_{50}$ , $\mu M$ )	Cytotoxicity ( $\mu M$ )
<b>16</b>	—	0.25	0.25	0.25	0.25	0.25
<b>17</b>	—(CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub>	0.25	0.25	0.25	0.40	4
<b>18</b>	—(CH <sub>2</sub> ) <sub>8</sub> CH <sub>3</sub>	0.25	0.25	0.25	0.25	1
<b>19</b>	—(CH <sub>2</sub> ) <sub>10</sub> CH <sub>3</sub>	0.25	0.25	0.25	0.25	1
<b>20</b>	—(CH <sub>2</sub> ) <sub>12</sub> CH <sub>3</sub>	0.25	0.25	0.25	0.32	1
<b>21</b>	—(CH <sub>2</sub> ) <sub>13</sub> CH <sub>3</sub>	0.25	0.25	0.25	0.25	1
<b>22</b>	—(CH <sub>2</sub> ) <sub>16</sub> CH <sub>3</sub>	0.49	0.25	0.25	0.51	1
<b>5<sup>b</sup></b>	Benznidazole	3.66	—	—	—	ND <sup>c</sup>
<b>6<sup>b</sup></b>	Nifurtimox	1.80	—	—	—	ND <sup>c</sup>
<b>7<sup>b</sup></b>	Allopurinol	—	—	—	8.8	>32
<b>8<sup>b</sup></b>	Sodium stibogluconate	—	—	—	5.6	>32
<b>9<sup>b</sup></b>	Pentamidine	—	—	—	>32	>32
<b>10<sup>b</sup></b>	Amphotericin B	—	—	—	0.1	>32

Data are displayed as mean  $\pm$  SD, as obtained from quadruplicate wells. Cytotoxicity in murine macrophage host cells (Cytotoxic concentration;  $CC_{50}$  > 4  $\mu M$ ) and ( $CC_{50}$  0.25–4  $\mu M$ ) in human fibroblasts MRC-5 cell line.<sup>a</sup>  $IC_{50}$  = compound concentration ( $\mu M$ ) required to reduce parasite viability by 50%.<sup>b</sup> Compounds **5–10** are standard control drugs used in respective experiments, *Trypanosoma* spp. (**5** & **6**), *Leishmania* (**7**, **8**, **9** & **10**);<sup>c</sup> ND = not determined.



**Scheme 1** Synthesis of conformationally-locked 5'-acylsulfamoyl LNA adenosine analogs (**16–22**). *Reagents and conditions:* (i) dry DMF, dry Et<sub>3</sub>N, H<sub>2</sub>NSO<sub>2</sub>Cl (in dry DCM), 1 h; 31% (ii) dry EtOH, 20% Pd(OH)<sub>2</sub>/C, HCO<sub>2</sub>NH<sub>4</sub>, 80 °C, 15 h; 87% (iii) dry DMF, Cs<sub>2</sub>CO<sub>3</sub>, corresponding *N*-hydroxy succinimide ester, rt, 1–2 h, **17** (20%), **18** (27%), **19** (22%), **20** (25%), **21** (34%) and **22** (15%).



concentration;  $CC_{50} >4 \mu\text{M}$ ) and ( $CC_{50} 0.25\text{--}4 \mu\text{M}$ ) in human fibroblasts MRC-5 cell lines.

Compound **16** seems to be more toxic to MRC-5 cell lines whereas other molecules (**17–22**) were not found to be toxic up to  $1\text{--}4 \mu\text{M}$  concentration. These cytotoxicity results further suggest the importance of the alkyl chain (R group, Table 1), since compound **16** which is devoid of an acyl chain was found to be toxic at the level of its  $IC_{50}$  concentration ( $0.25 \mu\text{M}$ ), whereas compound **17** ( $IC_{50} 4.0 \mu\text{M}$ ) with a pentanyl chain is non-toxic up to  $4 \mu\text{M}$  concentration. Increasing the chain length from (C-9 to C-17) in compounds **18–22** resulted in increased cytotoxicity ( $CC_{50}$  of  $1 \mu\text{M}$ ). Compounds **16–22** are analogues of adenosine and this class of molecules is known to possess antitrypanosomal and antileishmanial activity.<sup>45,46</sup> 5'-O-acyl sulfamoyl adenosine (**1**) derivatives have previously been reported ( $IC_{50} = 0.5\text{--}50 \mu\text{M}$ ) for their antiparasitic activity.<sup>47</sup> The wide activity of these compounds acting against *Trypanosoma* and *Leishmania* can be explained based on earlier studies which suggests that *Trypanosoma* (*T. Cruzi*) enzymes are structurally and functionally similar to *Leishmania* enzymes.<sup>48</sup> Both organisms have a high degree of sequence similarity (64%) in ergosta-type steroids which is a main membrane steroid.<sup>48</sup> Therefore it is quite reasonable to suggest that the compounds that inhibit the growth of *Trypanosoma* may also have an inhibitory effect on *Leishmania*. Apart from this, purine analogs are metabolized by these parasites to nucleotides and aminated to the analogs of adenine nucleotides.<sup>49</sup> This halts protein synthesis and causes the breakdown of RNA. The metabolic pathways for purines in protozoans differ significantly from the corresponding pathways in human beings. As an example, allopurinol (**7**, Fig. 1) is nontoxic to human beings and is aminated to adenine nucleotide analogs by parasites.<sup>49</sup>

### 3.1. Antiparasitic structure–activity comparison amongst adenosine analogs, 1–4 with 16–22

The structural and functional design of previously synthesized 5'-O-sulfamoyl adenosine analogues (**1–4**, Fig. 1)<sup>41,47,50,51</sup> and our present compounds (**16–22**, Table 1) are all inspired by naturally-occurring adenosine 5'-phosphate (AMP), wherein the

phosphate moiety is mimicked by the isosteric sulfamate, which readily crosses cellular membranes due to the nonionic nature of the sulfamoyl ester group. However, the compounds (**1–4**, Fig. 1) from the literature were found to be extremely toxic.<sup>52–54</sup> Therefore, we became interested in designing a new class of molecules in which 9-adeninyl and the 5'-sulfamate moieties were retained as active pharmacophores. We also introduced conformationally-locked pentose-sugar as a unique sugar moiety that is devoid of any intrinsic sugar flexibility. We argued that the resulting adenosine derivatives **16–22** are likely to be biologically active against parasites without enhancing the toxicity of the molecules, because it is known that the sugar conformation seems to play an important role<sup>47</sup> in dictating the antiparasitic activity of adenosine sulfamoyl analogues;<sup>47</sup> the C3'-endo sugar (North-type) conformation of ribose sugar in ribavirin derivative **11** [1-(5'-O-sulfamoyl- $\beta$ -D-ribofuranosyl)-1,2,4-triazole-3-carboxamide] gives an  $ED_{50}$  of  $0.03 \mu\text{g mL}^{-1}$ , whereas a C2'-endo sugar (South-type) conformation of ribose sugar in compound **12** [1-(5'-O-sulfamoyl- $\beta$ -D-ribofuranosyl)-1,2,4-triazole-3-thiocarboxamide] and compound **13** [1-(5'-O-sulfamoyl- $\beta$ -D-ribofuranosyl)-1,2,4-triazole-3-carbonitrile] was found to be relatively less active, with  $ED_{50}$  of  $2.5 \mu\text{g mL}^{-1}$  and  $5.0 \mu\text{g mL}^{-1}$ , respectively (Fig. 3).

### 3.2. Role of the sugar conformation

The role of the pentose-sugar conformation seems to play an important role in determining the antiparasitic activity of adenosine sulfamoyl analogues. The crystal structures of compounds **11–13** (Fig. 3) clearly showed the role of the sugar conformation in that compound **11** ( $ED_{50} = 0.03 \mu\text{g mL}^{-1}$ ), which displayed the best activity against *L. donovani*, is locked in C3'-endo (North-type) conformation.<sup>47</sup> On the other hand, compounds **12** & **13** (Fig. 3) are locked in C3'-exo (South-type) conformation, and found to be poorly active with  $ED_{50} = 2.5 \mu\text{g mL}^{-1}$  and  $5.0 \mu\text{g mL}^{-1}$ , respectively.<sup>47</sup> Despite the conformational differences in the sugar ring in these compounds **11–13** (Fig. 3), the 5'-sulfamoyl moiety is similarly oriented in these molecules with respect to the ribose ring, with the  $\text{NH}_2$  group positioning itself over the sugar-ring.<sup>47</sup> This finding supports the orientation of the

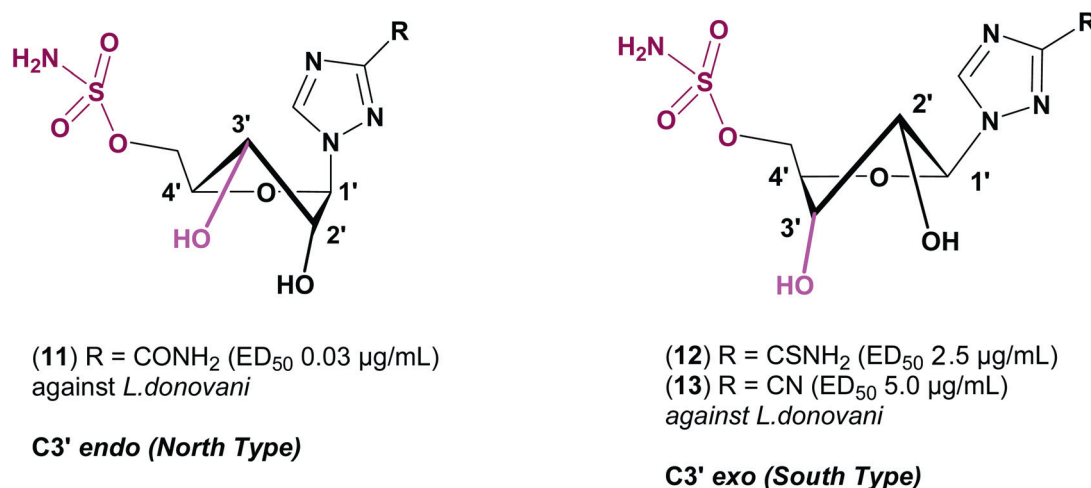


Fig. 3 Sulfamoyl derivatives of ribavirin in North and South type conformations.

5'-sulfamoyl group being relatively less important than the sugar conformation, and further supports the crucial role of the sugar conformation in determining the antiparasitic activity.<sup>47</sup> Therefore, based on this rationale, we synthesized compounds (**16–22** in Scheme 1) by covalently attaching a 4'-CH<sub>2</sub>-O-2' bridge (Fig. 2) across C2'-C4' of adenosine, which conformationally-locked (3'-endo, North-type) the nucleosides and led to excellent anti-parasitic activity (IC<sub>50</sub> = 0.25–0.51 μM).

## 4. Conclusion

In conclusion, this study provides valuable data that 3'-endo-locked 5'-acyl sulfamoylnucleosides **16–22** exhibit interesting antileishmanial & antitrypanosomal properties with insignificant toxicity to mammalian cells. Their unique and potent activity profile represents new important directions in the search for novel antiparasitic agents as a "proof-of-concept" that bicyclic 5'-acyl sulfamoyl adenosines possess strong antitrypanosomal and antileishmania activity. This class of molecules should be explored further to bridge the gap of unmet need for new antiparasitic agents to combat neglected diseases. This work also suggests that variable chain length of the 5'-acyl group can modulate the toxicity of 5'-O-sulfamoylnucleoside analogues. Additional studies with compounds **16–22** on selected parasitic species, pharmacological profiling of the individual compound, mechanism-of-action studies involving enzymatic assays, and dose response toxicity evaluations are currently under investigation.

## 5. Implication

The uniqueness of the sugar-locked 2'-O,4'-C-methylene-linked bicyclic ribonucleosides of acylsulfamoyl adenosine is that their 3'-endo-locked conformation has successfully inhibited the growth of protozoan parasites with insignificant cytotoxicity in murine macrophage host cells (CC<sub>50</sub> >4 μM) and in diploid human fibroblasts MRC-5 cell line against mammalian cells (CC<sub>50</sub> 4 μM). The notable chemical feature of the 3'-endo locked compounds is their pendant 2'-O, 4'-C-methylene fused bridge on the pentofuranosylribose unit, which virtually restricts the conformational flexibility of the pentose-sugar ring, hence reducing entropic contribution to the binding free-energy to the target protein. The activity of these molecules in parasite infected primary mouse peritoneal macrophages is now followed by a search for the target enzyme in the parasites. This would allow us to determine the inhibition constant of the target enzyme as well as the pharmacokinetic properties of the 3'-endo locked compounds, which we believe would lead us to determine the crystal structure of the complex to advance the structure-activity relationship of these new bicyclic ribonucleosides of acylsulfamoyl adenosine molecules.

## 6. Experimental section

### 6.1. Chemistry – general experimental methods

All chemicals and reagents used were of reagent grade. Purification and drying of reagents and solvents was carried out according to literature procedure.<sup>55</sup> Thin layer chromatographic

analyses were performed on E-Merck 60 F 254 precoated aluminium thin layer chromatographic plates. All air-sensitive reactions were carried out under a nitrogen atmosphere. Melting points were determined on a Büchi melting point B-540 instrument and are uncorrected. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker Biospin 400 MHz spectrometer in the indicated solvents (TMS as an internal standard). The values of chemical shifts are expressed in ppm and the coupling constants (*J*) in hertz (Hz). Mass spectra were recorded in API 2000 LC/MS/MS system spectrometer and the IR spectra were recorded on Perkin Elmer FT-IR spectrometer.

**(1S,3R,4R,7S)-7-Benzoyloxy-1-methanesulfonamide-3-(adenine-7-yl)-2,5-dioxabicyclo [2.2.1]heptane (15).** To a solution of sulfamoyl chloride (6.8 mmol) in dry DCM was added ((1S,3R,4S,7R)-3-(6-amino-9H-purin-9-yl)-7-(benzyloxy)-2,5-dioxabicyclo-[2.2.1]heptan-1-yl)methanol, **14**<sup>43</sup> (1.0 g, 2.71 mmol) in dry DMF (25 mL) and dry Et<sub>3</sub>N (0.49 mL, 3.54 mmol) at 0 °C. Reaction mixture was stirred at rt for 1 h. Reaction was quenched with water (60 mL) and extracted with ethyl acetate (3 × 50 mL). Aqueous layer was basified to pH = 8 by 2 M aq. NaOH solution. Aqueous layer was extracted with ethyl acetate (3 × 30 mL). Combined organic layer was washed with water (2 × 30 mL) and then with brine (40 mL). Organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under vacuum to give crude product. Crude product was purified by column chromatography (silica gel 100–200 mesh, eluent: 4% MeOH in DCM) to give pure compound **15** (0.400 g, 31%). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 3.92 (d, *J* = 8.0 Hz, 1 H, H6''), 4.04 (d, *J* = 8.0 Hz, 1 H, H6'), 4.41 (d, *J* = 11.6 Hz, 1 H, H5''), 4.54 (s, 1 H, H3'), 4.57 (d, *J* = 11.6 Hz, 1 H, H5'), 4.67 (d, *J* = 2.44 Hz, 2 H, CH<sub>2</sub>Ph), 4.85 (s, 1 H, H2'), 6.02 (s, 1 H, H1'), 7.26–7.40 (m, 7 H, 2 H D<sub>2</sub>O exchangeable, NH<sub>2</sub>-SO<sub>3</sub><sup>-</sup>, OCH<sub>2</sub>Ph), 7.70 (s, 2 H, D<sub>2</sub>O exchangeable, C6-NH<sub>2</sub>), 8.14 (s, 1 H, H2), 8.20 (s, 1 H, H8). <sup>13</sup>C NMR (100.6 MHz, DMSO-d<sub>6</sub>): δ 64.9 (C5'), 71.2 (CH<sub>2</sub>Ph), 71.8 (C6'), 76.9 (C2'), 77.8 (C3'), 84.5 (C4'), 85.3 (C1'), 118.8 (C9), 127.5 (Ar-C), 127.7 (Ar-C), 128.2 (Ar-C), 137.6 (Ar-C), 138.1 (C2), 148.6 (Ar-C), 152.7 (C8), 156.0 (C6). *m/z* calculated for [M]<sup>+</sup> C<sub>18</sub>H<sub>20</sub>N<sub>6</sub>O<sub>6</sub>S, 448.45; found, 448.70.

**(1S,3R,4R,7S)-7-Hydroxy-1-methanesulfonamide-3-(adenine-7-yl)-2,5-dioxabicyclo [2.2.1]heptane (16).** To a solution of **15** (5.0 g, 13.95 mmol) in EtOH (100 mL) was added 20% Pd(OH)<sub>2</sub>/C (3.0 g) and ammonium formate (52.79 g, 837 mmol) and heated to reflux. The same amount of ammonium formate and 20% Pd(OH)<sub>2</sub>/C was added after 5 h and reaction was further refluxed for 10 h. The reaction mixture was filtered over celite and organic phase was evaporated to give crude product. Crude product was purified by column chromatography (silica gel 100–200 mesh, eluent: 8–10% MeOH in DCM) to give pure compound **16** (3.5 g, 87%). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 3.87 (d, *J* = 7.9 Hz, 1 H, H6''), 4.02 (d, *J* = 7.9 Hz, 1 H, H6'), 4.34–4.46 (m, 2 H, H5'', H2'), 4.50 (s, 1 H, H3'), 4.54–4.64 (m, 1 H, H5'), 5.95 (s, 1 H, H1'), 6.02 (d, *J* = 4.0 Hz, 1 H, D<sub>2</sub>O exchangeable, OH), 7.36 (s, 2 H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>-SO<sub>3</sub><sup>-</sup>), 7.67 (s, 2 H, D<sub>2</sub>O exchangeable, C6-NH<sub>2</sub>-), 8.15 (s, 1 H, H2), 8.26 (s, 1 H, H8). <sup>13</sup>C NMR (100.6 MHz, DMSO-d<sub>6</sub>): δ 65.5 (C5'), 70.9 (C2'), 71.3 (C6'), 79.3 (C3'), 85.26 (C1'),

85.31 (C4'), 118.9 (Ar-C), 138.1 (C8), 148.6 (Ar-C), 152.8 (C2), 156.0 (C6).  $m/z$  calculated for  $[M + H]^+$  C<sub>11</sub>H<sub>14</sub>N<sub>6</sub>O<sub>6</sub>S, 358.33; found, 359.30.

#### General procedure A, for *N*-hydroxy succinimide esters

Lauric acid (0.502 g, 2.51 mmol) was dissolved in dry DCM (3 mL), DIC (0.38 mL, 2.51 mmol) and *N*-hydroxy succinimide (0.289 g, 2.51 mmol) was added. Reaction was stirred at room temperature under nitrogen atmosphere for 15 min, filtered through cotton bed and filtrate was concentrated under vacuum to give pure lauric *N*-hydroxy succinimide ester.

#### General procedure B, for compounds 17–22

The respective *N*-hydroxy succinimide aliphatic ester (3 eq) was dissolved in dry DMF (3 mL), sulfonamide **16** (0.300 g, 0.83 mmol, 1 eq) and Cs<sub>2</sub>CO<sub>3</sub> (0.409 g, 1.25 mmol, 1.5 eq) were added to it and stirred at room temperature under nitrogen atmosphere for 1.5 h. Reaction was quenched by water (20 mL), extracted with ethyl acetate (3 × 25 mL), combined organic layer was again washed with water (2 × 15 mL) and then with brine (20 mL). Organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under vacuum to give crude product. Crude product was purified by column chromatography (silica gel 100–200 mesh, eluent: 10% MeOH in DCM) to give pure compound.

**((1*R*,3*R*,4*R*,7*S*)-3-(6-Amino-9*H*-purin-9-yl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptan-1-yl)methyl hexanoylsulfamate (17).** Procedure B, white solid (0.080 g, 20%), mp 121–124 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 0.81 (app t, *J* = 7.0 Hz, 3 H, CH<sub>3</sub>), 1.16–1.27 (m, 4 H, CH<sub>2</sub>), 1.39–1.48 (m, 2 H, CH<sub>2</sub>), 2.02–2.14 (m, 2 H, CH<sub>2</sub>), 3.80 (d, *J* = 8.0 Hz, 1 H, H6''), 3.96 (d, *J* = 8.0 Hz, 1 H, H6'), 4.35 (s, 1 H, H3'), 4.40–4.55 (m, 3 H, H2', H5'', H5'), 5.90 (s, 1 H, H1'), 5.93 (s, 1 H, D<sub>2</sub>O exchangeable, OH), 7.34 (s, 2 H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>), 8.14 (s, 1 H, H2), 8.24 (s, 1 H, H8). <sup>13</sup>C NMR (100.6 MHz, DMSO-*d*<sub>6</sub>): 14.2 (CH<sub>3</sub>), 22.3 (CH<sub>2</sub>), 25.1 (CH<sub>2</sub>), 31.3 (CH<sub>2</sub>), 37.9, (CH<sub>2</sub>), 66.0 (C5'), 71.1 (C2'), 71.6 (C6'), 79.6 (C3'), 85.6 (C1'), 86.0 (C4'), 119.4 (Ar-C), 138.4 (C8), 148.9 (Ar-C), 153.2 (C2), 156.4 (C6), 176.0 (C=O).  $m/z$  calculated for  $[M]^+$  C<sub>17</sub>H<sub>24</sub>N<sub>6</sub>O<sub>7</sub>S, 456.47; found 457.00.

**((1*R*,3*R*,4*R*,7*S*)-3-(6-Amino-9*H*-purin-9-yl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptan-1-yl)methyl decanoylsulfamate (18).** Procedure B, white solid (0.080 g, 27%), mp 115–117 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 0.83 (app t, *J* = 7.0 Hz, 3 H, CH<sub>3</sub>), 1.11–1.32 (m, 12 H, CH<sub>2</sub>), 1.38–1.55 (m, 2 H, CH<sub>2</sub>), 2.08–2.25 (m, 2 H, CH<sub>2</sub>), 3.81 (d, *J* = 8.0 Hz, 1 H, H6''), 3.96 (d, *J* = 8.0 Hz, 1 H, H6'), 4.36 (s, 1 H, H2'), 4.44 (s, 1 H, H3'), 4.48 (d, *J* = 12.0 Hz, 1 H, H5''), 4.60 (d, *J* = 11.1 Hz, 1 H, H5'), 5.91 (s, 1 H, H1'), 5.96 (s, 1 H, D<sub>2</sub>O exchangeable, OH), 7.34 (s, 2 H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>), 8.14 (s, 1 H, H2), 8.24 (s, 1 H, H8), 12.2 (br-s, 1 H, NH). <sup>13</sup>C NMR (100.6 MHz, DMSO-*d*<sub>6</sub>): δ 13.9 (CH<sub>3</sub>), 22.1 (CH<sub>2</sub>), 24.7 (CH<sub>2</sub>), 28.56 (CH<sub>2</sub>), 28.63 (CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 31.2 (CH<sub>2</sub>), 36.8 (CH<sub>2</sub>), 66.4 (C5'), 70.7 (C2'), 71.2 (C6'), 79.2 (C3'), 85.3 (C1'), 85.5 (C4'), 118.9 (Ar-C), 137.9 (C8), 148.4 (Ar-C), 152.7 (C2), 156.0 (C6), 174.3

(C=O).  $m/z$  calculated for  $[M]^+$  C<sub>21</sub>H<sub>32</sub>N<sub>6</sub>O<sub>7</sub>S, 512.58; found, 513.00.

**((1*R*,3*R*,4*R*,7*S*)-3-(6-Amino-9*H*-purin-9-yl)-7-hydroxy-2-oxabicyclo[2.2.1]heptan-1-yl)methyl dodecanoylsulfamate (19).** Procedure B, White solid (0.100 g, 22%), mp 131–133 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 0.84 (app t, *J* = 7.0 Hz, 3 H, CH<sub>3</sub>), 1.14–1.27 (m, 16 H, CH<sub>2</sub>), 1.38–1.54 (m, 2 H, CH<sub>2</sub>), 2.14 (t, *J* = 7.24 Hz, 2 H, CH<sub>2</sub>), 3.81 (d, *J* = 8.56 Hz, 1 H, H6''), 3.96 (d, *J* = 8.0 Hz, 1 H, H6'), 4.36 (s, 1 H, H2'), 4.44 (s, 1 H, H3'), 4.48 (d, *J* = 12.0 Hz, 1 H, H5''), 4.61 (d, *J* = 12.0 Hz, 1 H, H5'), 5.91 (s, 1 H, H1'), 5.97 (s, 1 H, D<sub>2</sub>O exchangeable, OH), 7.34 (s, 2 H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>), 8.14 (s, 1 H, H2), 8.24 (s, 1 H, H8). <sup>13</sup>C NMR (100.6 MHz, DMSO-*d*<sub>6</sub>): δ 14.0 (CH<sub>3</sub>), 22.1 (CH<sub>2</sub>), 24.6 (CH<sub>2</sub>), 28.6 (CH<sub>2</sub>), 28.7 (CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 29.0 (CH<sub>2</sub>), 31.3 (CH<sub>2</sub>), 36.6 (CH<sub>2</sub>), 66.8 (C5'), 70.7 (C2'), 71.2 (C6'), 79.3 (C3'), 85.4 (C1'), 118.9 (Ar-C), 138.0 (C8), 148.5 (Ar-C), 152.7 (C2), 156.3 (C6), 173.8 (C=O).  $m/z$  calculated for  $[M]^+$  C<sub>23</sub>H<sub>36</sub>N<sub>6</sub>O<sub>7</sub>S, 540.63; found: 540.90.

**((1*R*,3*R*,4*R*,7*S*)-3-(6-Amino-9*H*-purin-9-yl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptan-1-yl)methyl tetradecanoylsulfamate (20).** Procedure B, white solid (0.120 g, 25%), mp 158–161 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 0.85 (app t, *J* = 7 Hz, 3 H, CH<sub>3</sub>), 0.97–1.34 (m, 20 H, CH<sub>2</sub>), 1.38–1.51 (m, 2 H, CH<sub>2</sub>), 2.22 (t, *J* = 7.3 Hz, 2 H), 3.82 (d, *J* = 8.0 Hz, 1 H, H6''), 3.97 (d, *J* = 8.0 Hz, 1 H, H6'), 4.36 (s, 1 H, H2'), 4.44 (s, 1 H, H3'), 4.49 (d, *J* = 12.0 Hz, 1 H, H5''), 4.62 (d, *J* = 12.0 Hz, 1 H, H5'), 5.90 (s, 1 H, H1'), 5.96 (br-s, 1 H, D<sub>2</sub>O exchangeable, OH), 7.34 (s, 2 H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>), 8.14 (s, 1 H, H2), 8.24 (s, 1 H, H8). <sup>13</sup>C NMR (100.6 MHz, DMSO-*d*<sub>6</sub>): δ 14.0 (CH<sub>3</sub>), 22.1 (CH<sub>2</sub>), 25.4 (CH<sub>2</sub>), 28.7 (CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 28.94 (CH<sub>2</sub>), 28.99 (CH<sub>2</sub>), 29.03 (CH<sub>2</sub>), 31.3 (CH<sub>2</sub>), 64.2 (C5'), 70.7 (C2'), 71.3 (C6'), 79.1 (C3'), 85.3 (C1'), 85.8 (C4'), 118.9 (Ar-C), 138.0 (C8), 148.5 (Ar-C), 152.7 (C2), 156.0 (C6), 176.7 (C=O).  $m/z$  calculated for  $[M]^+$  C<sub>25</sub>H<sub>40</sub>N<sub>6</sub>O<sub>7</sub>S, 568.69; found, 569.10.

**((1*R*,3*R*,4*R*,7*S*)-3-(6-Amino-9*H*-purin-9-yl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptan-1-yl)methyl pentadecanoylsulfamate (21).** Procedure B, white solid (0.150 g, 34%), mp decomposes at 155 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 0.84 (app t, *J* = 6.8 Hz, 3 H, CH<sub>3</sub>), 1.12–1.30 (m, 22 H, CH<sub>2</sub>), 1.38–1.52 (m, 2 H, CH<sub>2</sub>), 2.19 (app t, *J* = 7.3 Hz, 2 H, CH<sub>2</sub>), 3.82 (d, *J* = 8.0 Hz, 1 H, H6''), 3.98 (d, *J* = 8.0 Hz, 1 H, H6'), 4.37 (s, 1 H, H2'), 4.46 (s, 1 H, H3'), 4.54 (d, *J* = 12.0 Hz, 1 H, H5''), 4.69 (d, *J* = 12.0 Hz, 1 H, H5'), 5.92 (s, 1 H, H1'), 7.36 (s, 2 H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>), 8.14 (s, 1 H, H2), 8.24 (s, 1 H, H8). <sup>13</sup>C NMR (100.6 MHz, DMSO-*d*<sub>6</sub>): δ 14.0 (CH<sub>3</sub>), 22.1 (CH<sub>2</sub>), 24.3 (CH<sub>2</sub>), 28.5 (CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 28.96 (CH<sub>2</sub>), 29.1 (CH<sub>2</sub>), 31.4 (CH<sub>2</sub>), 35.9 (CH<sub>2</sub>), 67.9 (C5'), 70.8 (C2'), 71.2 (C6'), 79.3 (C3'), 85.3 (C1'), 85.5 (C4'), 119.0 (Ar-C), 138.0 (C8), 148.5 (Ar-C), 152.8 (C2), 156.1 (Ar-C), 172.60 (C=O).  $m/z$  calculated for  $[M]^+$  C<sub>26</sub>H<sub>42</sub>N<sub>6</sub>O<sub>7</sub>S, 582.71; found, 583.00.

**((1*R*,3*R*,4*R*,7*S*)-3-(6-Amino-9*H*-purin-9-yl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptan-1-yl)methyl stearoylsulfamate (22).** Procedure B, white solid (0.075 g, 15%), mp 218–221 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 0.85 (app t, *J* = 6.8 Hz, 3 H, CH<sub>3</sub>), 0.97–1.35 (m, 28 H, CH<sub>2</sub>), 1.38–1.52 (m, 2 H, CH<sub>2</sub>), 2.22 (t, *J* = 7.3 Hz, 2 H, CH<sub>2</sub>), 3.82 (d, *J* = 8.0 Hz, 1 H, H6''), 3.97 (d,



$J = 8.0$  Hz, 1 H, H6'), 4.38 (s, 1 H, H2'), 4.46 (s, 1 H, H3'), 4.57 (d,  $J = 12.0$  Hz, 1 H, H5''), 4.74 (d,  $J = 12.0$  Hz, 1 H, H5'), 5.92 (s, 1 H, H1'), 6.01 (s, 1 H, D<sub>2</sub>O exchangeable, OH), 7.35 (s, 2 H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>), 8.13 (s, 1 H, H2), 8.24 (s, 1 H, H8). <sup>13</sup>C NMR (100.6 MHz, DMSO-d<sub>6</sub>):  $\delta$  14.02 (CH<sub>3</sub>), 22.2 (CH<sub>2</sub>), 24.0 (CH<sub>2</sub>), 28.4 (CH<sub>2</sub>), 28.7 (CH<sub>2</sub>), 29.1 (CH<sub>2</sub>), 31.3 (CH<sub>2</sub>), 35.4 (CH<sub>2</sub>), 68.5 (C5'), 70.8 (C2'), 71.1 (C6'), 79.4 (C3'), 85.14 (C1'), 85.5 (C4'), 118.97 (Ar-C), 138.0 (C8), 148.5 (Ar-C), 152.8 (C2), 156.0 (C6), 171.7 (C=O).  $m/z$  calculated for [M]<sup>+</sup> C<sub>29</sub>H<sub>48</sub>N<sub>6</sub>O<sub>7</sub>S, 624.79; found, 624.90.

## 6.2. Biological activity-methods

**6.2.1. Amastigote culture and drug sensitivity assay.** The laboratory strain of *Leishmania infantum* was used for *in vitro* screening of synthesized compounds. The *in vitro* sensitivity of compounds (16–22) to amastigotes<sup>44</sup> was determined in primary mouse peritoneal macrophages (MP $\emptyset$ ).<sup>56</sup> For the drug sensitivity assay, the 16  $\mu$ M compound stock solutions were four-fold serially diluted in DMSO, followed by an additional dilution in culture medium (RPMI 1640 supplemented with 20 mM L-glutamine, 16.5 mM NaHCO<sub>3</sub>, 5% heat-inactivated fetal calf serum [FCSi], and 2% P/S solution). The highest in-test drug concentration was 64  $\mu$ g mL<sup>-1</sup>. Assays were performed in quadruplet in 96-well microtiter tissue culture plates (Nunc), with each well containing the test compound dilutions together with  $3 \times 10^4$  MP $\emptyset$  and  $3 \times 10^5$  amastigotes per well. After 5 days of incubation at 37 °C in 5% CO<sub>2</sub>-air, intracellular amastigote burdens (and cytotoxicity) were microscopically assessed after Giemsa staining. The results are expressed as percent reduction of parasite burden compared to the level in untreated control wells, and the 50% inhibitory concentration (IC<sub>50</sub>) and 50% cytotoxic concentration (CC<sub>50</sub>) on MP $\emptyset$  was determined.

**6.2.2. *In vitro* activity against *Trypanosoma brucei* subsp. *rhodesiense* and *Trypanosoma brucei* subsp. *Brucei*.** *T. brucei* subsp. *rhodesiense* STIB900 BSF trypomastigotes were maintained in HMI-18 medium with 15% heat-inactivated fetal calf serum (Harlan-SeraLab, United Kingdom) at 37 °C in a 5% CO<sub>2</sub>-95% air mixture. Trypomastigotes were washed and resuspended in fresh medium at a concentration of  $2 \times 10^5$  mL<sup>-1</sup>. The top concentration for the test compounds was 64  $\mu$ M. Five different concentrations of drug were tested in triplicate. Plates were incubated for 72 h at 37 °C in a 5% CO<sub>2</sub>-95% air mixture. At 72 h, the plates were assessed microscopically before alamarBlue was added. Plates were read after 5 to 6 h on a Gemini Fluorescent plate reader (Softmax Pro. 3.1.1, Molecular Devices, United Kingdom) at an excitation/emission of 530/585 nm, with a filter cutoff at 550 nm. 50% inhibition concentration (IC<sub>50</sub>) values were calculated with Mx $\backslash$ fit (IDBS, United Kingdom).

For studies with *T. brucei* subsp. *brucei* bloodstream forms, trypomastigotes were maintained in HMI-9 medium with 10% heat-inactivated fetal calf serum (Gibco) at 37 °C in a 5% CO<sub>2</sub>-95% air mixture. The HMI-9 medium was supplemented with 1  $\mu$ g mL<sup>-1</sup> of ergosterol, which was dissolved in DMSO. Procyclic forms were grown in SDM-79 with 10% heat-inactivated fetal calf serum at 27 °C.

**6.2.3. *In vitro* activity against *Trypanosoma cruzi*.**<sup>57</sup> *T. cruzi* epimastigotes (Tulahuen 2 strain) were grown at 28 °C in an

axenic medium (BHI-tryptose) complemented with 5% fetal calf serum. Cells from 5-day-old culture (stationary phase) were inoculated to 50 mL of fresh culture medium to give an initial concentration of  $1 \times 10^6$  cells per mL. Cell growth was followed by measuring the absorbance of the culture at 600 nm every day. Before inoculation, the medium was supplemented with the indicated amount of the studied compound from a stock solution in DMSO. The final concentration of DMSO in the culture medium never exceeded 0.4%, and the control was run in the presence of 0.4% DMSO and in the absence of compound. No effect on epimastigotes' growth was observed by the presence of up to 1% DMSO in the culture medium. To determine IC<sub>50</sub> values, 50% inhibitory concentrations, parasite growth was followed in the absence (control) and presence of increasing concentrations of the corresponding compound. At day 5, the absorbance of the culture was measured and related to the control. The IC<sub>50</sub> value was taken as the concentration of compound needed to reduce the absorbance ratio to 50%.

**6.2.4. *In vitro* cytotoxicity against mammalian cells.** Diploid human lung fibroblasts (MRC-5; BioWittaker) were cultured in minimum essential medium (MEM; Gibco), supplemented with 20 mM L-glutamine, 16.5 mM NaHCO<sub>3</sub>, 5% FCSi, and 2% P/S solution. The assay was performed in 96-well tissue culture plates, with each well containing the test compound dilutions together with  $5 \times 10^3$  MRC-5 cells per well. After 7 days of incubation at 37 °C in 5% CO<sub>2</sub>-air, cell viability was assessed after addition of Alamar Blue<sup>58</sup> (2  $\mu$ L of a 1/10 solution per well), and fluorescence was measured (Fluostar Optima [550-nm excitation, 590-nm emission]) after 4 h of incubation at 37 °C. The results are expressed as percent reduction in cell viability compared to untreated control wells, and a CC<sub>50</sub> was determined.

## Abbreviations

DCM	dichloromethane
DIC	diisopropylcarbodiimide
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethyl sulfoxide
Et <sub>3</sub> N	triethylamine
EtOH	ethanol
MeOH	methanol
MIC	minimum inhibitory concentration
mp	melting point
NMR	nuclear magnetic resonance
SAR	structure-activity relationship
THF	tetrahydrofuran
TLC	thin layer chromatography

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