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Article

Synthesis and *in vitro* Evaluation of Novel Galactosyl-triazolo-benzenesulfonamides Against *Trypanosoma cruzi*

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Os únicos fármacos aprovados para o tratamento da doença de Chagas, nifurtimox e benznidazol, apresentam efeitos colaterais e eficácia limitada na fase crônica da doença, destacando a necessidade de novos fármacos. Entre os diferentes alvos moleculares de fármacos identificados no parasita, uma *trans*-sialidase de *Trypanosoma cruzi* (TcTS) tem sido considerada essencial para o reconhecimento e invasão nas células do hospedeiro. Desta forma, o trabalho descreve a síntese eficiente e a avaliação biológica (inibição de TcTS e atividade antitripanossoma) de alguns triazol-arilsulfonamidas contendo galactose pela reação de 1,3-dipolar de cicloadição azida-alcino Cu(I) (CuAAC) em micro-ondas, usando como precursores os derivados de azido benzenossulfonamidas e alcino derivado de galactose. A maioria dos compostos testados contra TcTS mostrou inibição moderada a fraca (40%-15%), com exceção de um dos compostos (81%). Quanto ao ensaio de atividade antitripanossoma, alguns compostos [(IC_{50} 70,9 μM) e (IC_{50} 44,0 μM)] apresentaram atividades mais significativas, embora não tenham sido tão ativos como benznidazol (IC_{50} 1,4 μM). Adicionalmente, a avaliação da citotoxicidade mostrou que todos os compostos não foram citotóxicos. Neste trabalho preliminar, alguns compostos foram considerados protótipos para posterior otimização.

The only drugs approved for the treatment of Chagas disease, nifurtimox and benznidazole, present toxic side effects and limited efficacy in the chronic stage of the disease, which highlight the need for new drugs. Amongst the different molecular drug targets discovered in the parasite, *Trypanosoma cruzi trans*-sialidase (TcTS) has been considered crucial in the recognition and invasion of host cells. Hence, we report the efficient synthesis and biological evaluation (TcTS inhibition and antitrypanosomal activities) of some galactose-containing triazol-arylsulfonamides via microwave-assisted Cu(I) 1,3-dipolar azide-alkyne cycloaddition (CuAAC) based on azide benzenesulfonamides and a galactose-derived alkyne as precursors. Most of the compounds tested against TcTS showed moderate to weak inhibition (40%-15%), except one of the compounds (81%). Regarding the antitrypanosomal assay, some compounds [(IC_{50} 70.9 μM) and (IC_{50} 44.0 μM)] exhibited the most significant activities, although not as active as benznidazole (IC_{50} 1.4 μM). Nevertheless, the cytotoxicity assessment showed that all compounds were not cytotoxic. In this preliminary work, we considered some compounds as lead scaffolds for further optimization.

Keywords: Chagas disease, *Trypanosoma cruzi, trans*-sialidase, benzenesulfonamides, click chemistry

Introduction

The last decade witnessed enormous advances in our understanding of *Trypanosoma cruzi* (*T. cruzi*) genome, the etiological agent of Chagas disease, which comprises 22,570 protein-coding genes, including the recent superfamily encoding mucins of the parasite surface, crucial to help the parasite to evade the immune system. This sequencing showed that over 50% of the genome is composed of repetitive sequences, including genes for

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surface superfamily of molecules, such as *trans*-sialidase, proteases and surface gp63 proteins associated with mucin (MASP's).[1] From the exploration of outstanding possibilities offered by genomic and proteomic resources, a series of molecular targets were identified to seek for high-affinity ligands, which may open up a new generation of selectivity drug candidates.[2-7] In this regard, *trans*-sialidase enzyme, found in flagellar trypomastigotes vesicles-containing glycoproteins, plays an essential role in parasite cellular invasion, growth, differentiation and survival processes.[8-11] Furthermore, *T. cruzi trans*-sialidase (TcTS) affects parasite entry in cardiac cells, leading to the development of host cell inflammatory response and infection process, besides being extremely important in modulating the immune response against infection in the chronic phase of Chagas disease.[12]

The molecular mechanism of TcTS relies on the transference of sialic acids from host cells to terminal β-galactose molecules present on parasite surface glycoproteins, introducing them into parasite surface mucins.[13-17] Only after the sialylation process, *T. cruzi* can invade and infect macrophages host cells. In general, it is established that parasite and host cell interactions are mediated by several molecules comprising calcium, glycoprotein Gp83, PKC, cruzipain, mucins and Gp85/*trans*-sialidase primarily by the action of Gp82 component which triggers Ca^{2+} mobilization from cell stores, and contribute for the invasion step.[12,18,19] Thus, besides the relative relevance of TcTS for the parasite entry, sialylated mucins greatly contribute for parasite to escape from host immunological defense mechanisms hence acquisition of sialic acids leads mucins to interact with the inhibitory sialic acid-binding protein Siglec-E (sialic acid binding Ig like lectin-E), which disables immune cells activation.[20,21] Furthermore, *trans*-sialidase also plays a role after parasite invasion and subsequent fusion of parasitophorus vacuole with lysosomes, since it promotes pore membrane formation by desialylation of lysosome membrane glycoproteins, from which the parasite escapes to the cytoplasm and differentiates into replicative amastigote forms.[22-24] Therefore, the role played by *trans*-sialidase in the pathogenesis of Chagas disease makes it a valuable and selective target to be explored in the search for new therapeutics. In fact, potential TcTS inhibitors acting by complementary interaction at both donor (β-galactose) and acceptor (sialic acid) sub-sites are of great concern.[25-27]

Currently, the treatment of the Chagas disease is based on two drugs, nifurtimox (**1**) and benznidazole (**2**), which are effective during the acute phase of the disease albeit several drawbacks related to side effects and resistance development are described.[6,28,29] Despite the significant reduction of parasite load and changes in the immune response during the course of benznidazole-combined therapy, for instance with allopurinol,[30] clomipramine,[31] amiodarone,[32] and posaconazole,[5] this promising discovery strategy based on the exploitation of new clinical activity observed for an old drug hitherto did not reach the market.[33] According to the World Health Organization (WHO), Program for Tropical Disease Research (TDR) and BIO Ventures for Global Health, the major issues concerning the research and drug development in neglected disease, in which Chagas disease is a case in point, encompass implementation of new public policies and alternative strategies to translate basic research into new drug candidate.[34]

In this scenario, the major aim in designing antitrypanosomal drugs is to find a drug which will be effective in the chronic stage of the disease and remain active despite resistant variants, since an estimated 10,000 deaths still occur annually and 7 to 8 million people are infected worldwide.[35] Among the numerous potent antitrypanosomal agents, sulfonamide derivatives have shown high *in vitro* activities against *T. cruzi* and *trans*-sialidase (Figure 1).

Thus, based on molecular hybridization strategy,[36] a series of *N*-quinolin-8-yl-arylsulfonamides,[37] arylsulfonyl-2-methyl-1,2,3,4-tetrahydroquinolines[38] and *N*-(biphenyl-4-yl-sulfonyl)-nicotinamides[39] were described as potential antitrypanosomal agents, as respectively illustrated by compounds **3** (IC_{50} 31.75 μM, Selectivity Index-SI 2.1), **4** (IC_{50} 11.44 μM, SI 21.7) and **5** (lytic concentration-LC_{50} 50.61 μM, SI not available). Regarding *trans*-sialidase, chalcone-derived sulfonamides, such as **6**, proved to be very active, with IC_{50} ranging from 0.6 to 7.3 μM, which

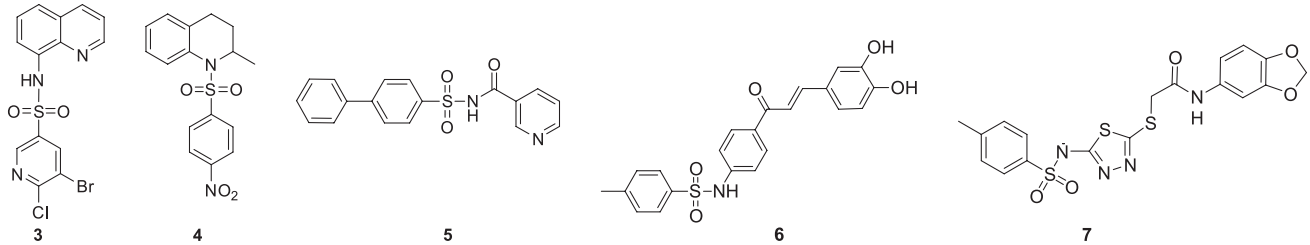


Figure 1. Potent antitrypanosomal sulfonamide derivatives described in the literature.

did not inhibit the human sialidase Neu2 at concentrations up to 200 μM.[40] Alternatively, from a library of 1819 molecules, a potent *N*-thiadiazol-arylsulfonamide **7** *trans*-sialidase inhibitor (IC_{50} 280 μM) was successfully identified by virtual screening and docking simulations, being the arylsulfonamide core highlighted as sialic acid mimics that is able to interact in the TcTS donor sub-site.[27]

In the course of our work on novel hybrid molecules against *T. cruzi*, comprising β-galactosyl unit to interact in the TcTS acceptor sub-site,[41,42] and the importance of the above mentioned arylsulfonamides, we envisaged that combining both active core linked by a triazole bridge in a single molecule to give galactosyl-triazolo-benzenesulfonamides might provide TcTS inhibitors and antitrypanosomal lead derivatives. Examples of syntheses of glycosylmimetics based on TcTS substrates, assembled by a triazole linker, have been previously described by our group.[43,44] In this sense, we have decided to assemble these two important motifs (galactose and sulfonamide) for TcTS inhibition and antitrypanosomal activities via 1,2,3-triazole ring. Additionally, variations on heterocycle-linked sulphonamide core by the bioisosteric replacement of isoxazole, pyrimidine and pyridine rings or an acyl group may represent a convenient strategy to construct chemical diversity and regulate acidity.

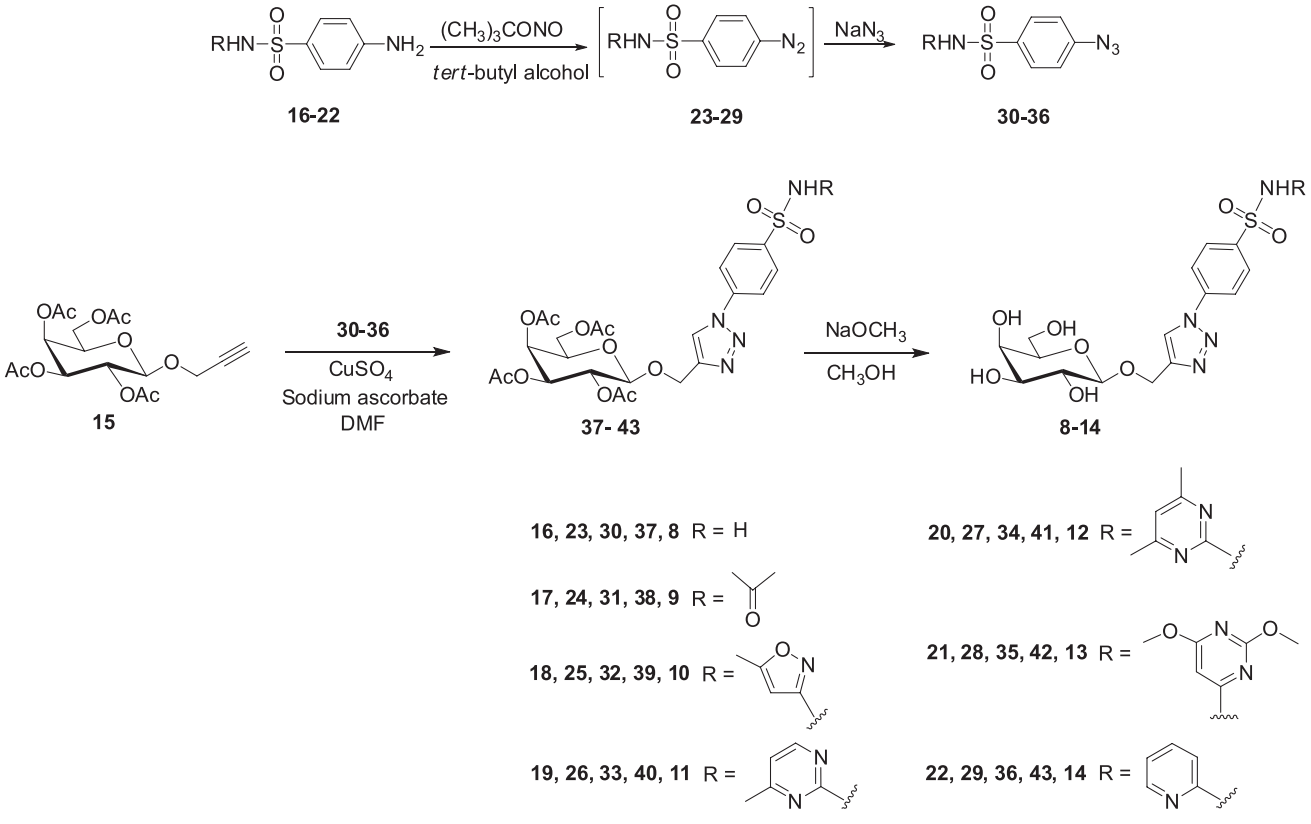
Hence, we describe the synthesis of a series of water-soluble galactose-containing triazol-arylsulfonamides via microwave-assisted Cu(I) 1,3-dipolar azide-alkyne cycloaddition (CuAAC) as efficient, practical and selective click chemistry strategy to provide 1,4-disubstituted 1,2,3-triazole hybrid molecules using azide benzenesulfonamides and galactose-derived alkyne as precursors. To pursue our goal, these compounds were evaluated as TcTS inhibitors and also as antitrypanosomal agents against *T. cruzi* (trypomastigote form). In addition, cytotoxicity was tested against a mammalian cell line.

Results and Discussion

Synthesis

The synthesis of target products **8**-**14** (Scheme 1) started from 2'-propynyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**15**), which was obtained in 90% from the TMSOTf-catalysed glycosylation reaction using propargyl alcohol and 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl trichloroacetimidate.[45]

Taking advantage of the commercial available arylsulfonamides **16**-**22**, named sulfanilamide (**16**), sulfacetamide (**17**), sulfamethoxazole (**18**), sulfamerazine



Scheme 1. Synthesis of galactosyl-triazolo-benzenesulfonamides (**8**-**14**) via microwave-assisted Cu(I) 1,3-dipolar azide-alkyne cycloaddition (CuAAC) as a click chemistry strategy. Intermediates **23**-**29** were not isolated from the reaction mixture and were used in the next step without purification.

(**19**), sulfamethazine (**20**), sulfadimethoxine (**21**) and sulfapyridine (**22**), we explored their use as starting material to introduce azide group[46] in the place of their corresponding free 4-aminoaryl group via diazo intermediates.[47] Thus, treatment of precursors **16**-**22** with *tert*-butyl nitrite in *tert*-butyl alcohol for addition of nitroso group to the amino functionalities, followed by dehydration of protonated diazohydroxyl intermediates, afforded the diazo derivatives (**23**-**29**), which were used without work up and further purification. Thus, the azide benzenesulfonamide **30** were prepared by a modified procedure using microwave-assisted reaction[48] to improve the condensation between azide group and diazo functionality of **23**, generating aryl-pentazenes and -pentazol intermediates, with resultant release of nitrogen to give azide **30** in 86% yield, significantly higher than those reported under conventional heating (61%).[49] While these conditions were very convenient to prepare the intermediate 4-azidobenzenesulfonamide (**30**), the reduced solubility of precursors **24**-**29** in *tert*-butyl alcohol limited their further application in the synthesis of the remaining azido intermediates **31**-**36**. To circumvent this issue, several experiments were carried out varying the solvents (DMF, acetonitrile, acetone, dioxane and THF), but only recovery of starting materials or products formation in very low yields (approximately 10%) was observed.[50] Given the high proportion of *tert*-butyl nitrite (6 to 12 equivalents), which might impair precursor solubilization during the microwave-assisted reaction, its reduction (4 equivalents) proved to be convenient to obtain the azide intermediates **31**-**36** in high yields (71 to 85%) using acetonitrile, dioxane or THF (depending on the starting material), with exception of the pyridinyl-benzenesulfonamide **36** (51% yield).[47,50] The characterization of azide intermediates **30**-**36** by ^{1}H NMR showed the downfield shifted (0.5 ppm on average) of the aromatic hydrogens once compared to the corresponding amine precursors (**16**-**22**), besides the intense azide band (2097 cm^{-1}) at IR spectrum.

With the azide benzenesulfonamides intermediates **30**-**36** in hands, the coupling of their azide functions with the *O*-propynyl galactoside **15** was carried out using the highly efficient CuAAC coupling in a sealed tube under microwave-assisted conditions, which shortened the required reaction time for up 1 h, instead of 16 h under conventional heating.[51] In general, the reactions were conducted in DMF at 70-80 °C (150 W) for 10-60 min in the presence of copper sulfate and sodium ascorbate for *in situ* generation of Cu(I) catalyst. Under these conditions, a small chemical diversity of protected galactosyl-triazolo-benzenesulfonamides **37**-**43** were obtained with complete regioselectivity since the 1,4-disubstituted triazoles were the unique isomer observed by ^{1}H NMR, with a single triazole hydrogen approximately at 8.0 ppm. Despite the previously reported synthesis of non-substituted derivative **37** in higher yield,[52] our novel series containing *N*-acetamide and -heterocycles groups was conveniently prepared in 50-63% yield.

Finally, removal of the sugar acetate protecting groups with catalytic NaOMe in MeOH, followed by treatment with DOWEX® 50WX8 resin, afforded the target products **8**-**14** in nearly quantitative yield.

Biological evaluations

Inhibition of *T. cruzi trans*-sialidase (TcTS)

The *in vitro* inhibitory properties of compounds **8**-**14** against a recombinant *T. cruzi trans*-sialidase were assessed by a sensitive and practical continuous fluorimetric method that measures the residual hydrolase activity of TcTS on the donor substrate 2'-(4-methylumbelliferyl)-α-D-*N*-acetyl-neuraminic acid (MuNANA) by cleaving the glycosidic bond that releases the fluorophore, methylumbelliferone (MU).[53] Thus, an initial screening was performed using 1.0 mM concentration of products **8**-**14** in the presence of MuNANA (0.1 mM). For comparison purposes, the activities of 2-deoxy-2,3-dehydro-*N*-acetyl-neuraminic acid (DANA) and pyridoxal phosphate were concomitantly measured in the same concentrations of the target compounds due to their respective weak and moderate activities on TcTS.[54]

According to Figure 2, compounds **8**-**14** showed a variable influence on TcTS enzyme inhibition, being compound **14** the most active of the series with an inhibition percentage of 81%, higher than pyridoxal phosphate (73%). On the other hand, the inhibition profile of compounds **9**-**13** was just moderate (40%), and compound **8** was weak (15%). Based on the greatest efficacy of **14** against TcTS, bearing a pyridine heterocycle at the benzenesulfonamide core, we pursued the enzymatic activity at lower concentrations (1.0, 0.75, 0.5 and 0.25 mM), however, the inhibition dropped significantly, giving an IC_{50} of 0.61 mM. In fact, the inhibition of this enzyme presents a great challenge, since there are few examples of strong TcTS inhibitors that act at nM range.[27]

Despite the lack of evidences that correlate TcTS and *in vitro* trypomastigote survival, we also investigated the potential antitrypanosomal properties of compounds **8**-**14** as TcTS-independent experiments.

Antitrypanosomal and cytotoxicity evaluations

In vitro growth-inhibitory properties of all compounds (**8**-**14**) against *T. cruzi* trypomastigotes bloodstream-form were achieved by a colorimetric reaction using chlorophenol

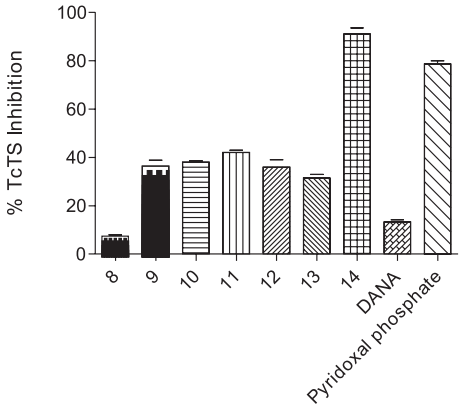


Figure 2. TcTS inhibition promoted by galactosyl-triazolo-benzenesulfonamides (**8**-**14**) using a continuous fluorimetric method. The results are based on three independent experiments, performed in triplicate.

Table 1. Antitrypanosomal activities showed by compounds **8**-**14** and benznidazole (Bz) expressed as IC_{50}, using *T. cruzi* trypomastigotes (Tulahuen strains)

Compound	R	IC_{50} / μM
8	H	155.3 ± 32.00
9	acetamide	119.5 ± 2.82
10	5-methylisoxazole	70.9 ± 8.90
11	6-methylpyrimidine	189.0 ± 5.44
12	4,6-dimethylpyrimidine	204.9 ± 6.50
13	2,4-dimethoxypyrimidine	44.0 ± 1.03
14	pyridine	179.7 ± 2.75
Benznidazole (Bz)	–	1.4 ± 0.56

red-β-D-galactoside (CPRG) as substrate for modified β-galactosidase Tulahuen strain of *T. cruzi*.[2,55] From these experiments, the IC_{50} for all derivatives were calculated considering the concentrations of compounds able to promote 50% parasite lysis, so-called antitrypanosomal activity, using benznidazole (Bz) as reference. From the IC_{50} data (Table 1 and Figure 3), it was evident that benzenesulfonamide substituents affect the activity in a reasonable extension, although they were not as active as benznidazole (IC_{50} 1.4 μM).

Accordingly, compounds **13** (IC_{50} 44.0 μM) and **10** (IC_{50} 70.9 μM), having the 2,4-dimethoxypyrimidine and 5-methylisoxazole groups, respectively, exhibited the most significant antitrypanosomal activity, while derivatives **11** (6-methylpyrimidine), **12** (4,6-dimethylpyrimidine) and **14** (pyridine), besides the non-substituted benzenesulfonamide **8**, showed the lowest potencies of the series with IC_{50} ranging from 155.3 to 204.9 μM. Comparing to the previous set of compounds, intermediate activities were attained for compound **9** (acetamide) with IC_{50} 119.5 μM.

It is noteworthy that pyridine derivative **14** that positively inhibit TcTS, failed to inhibit parasite growth as anticipated, suggesting that the antitrypanosomal activities observed for products **10** and **13** may occur by a different mechanism of action. On the other hand, the moderate TcTS activity showed by **14** could be insufficient to cause parasite death.

In addition, compounds **8**-**14** were examined for their ability to affect mammalian cells, such as cultured mouse spleen cells (Figure 4).[56] As a general observation, all galactosyl-triazolo-benzenesulfonamides were not cytotoxic to cells in concentrations similar to those used in the evaluation of the antitrypanosomal activities (500 to 3.9 μM). Given the lack of cytotoxicity, the corresponding LD_{50} values and, consequently, the selectivity index could not be calculated at the tested conditions.

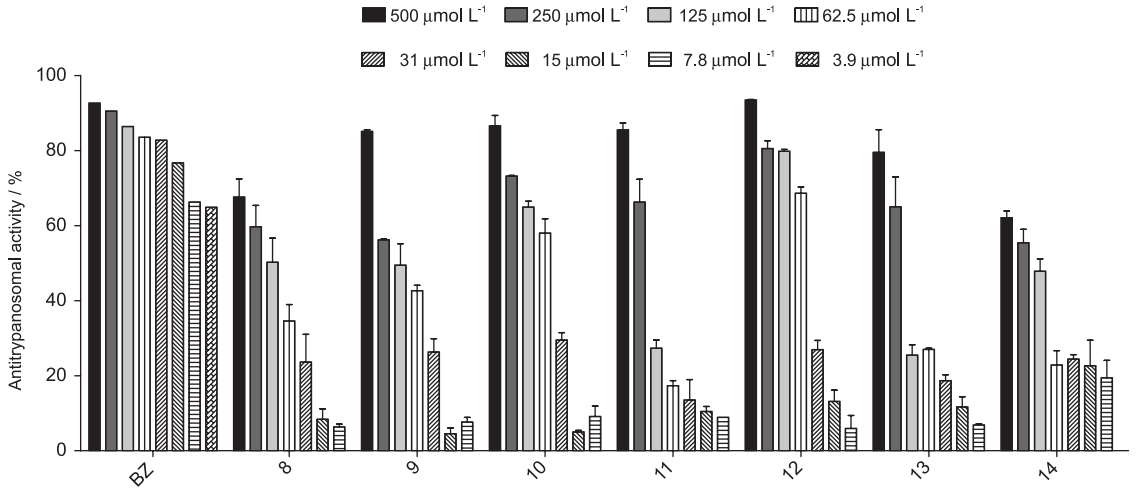


Figure 3. Percentage of antitrypanosomal activities of compounds **8**-**14** and reference compound benznidazole (Bz) against Tulahuen-modified strain of *T. cruzi*, cultured with LLC-MK2 cells. The results are based on three independent experiments, performed in duplicate.

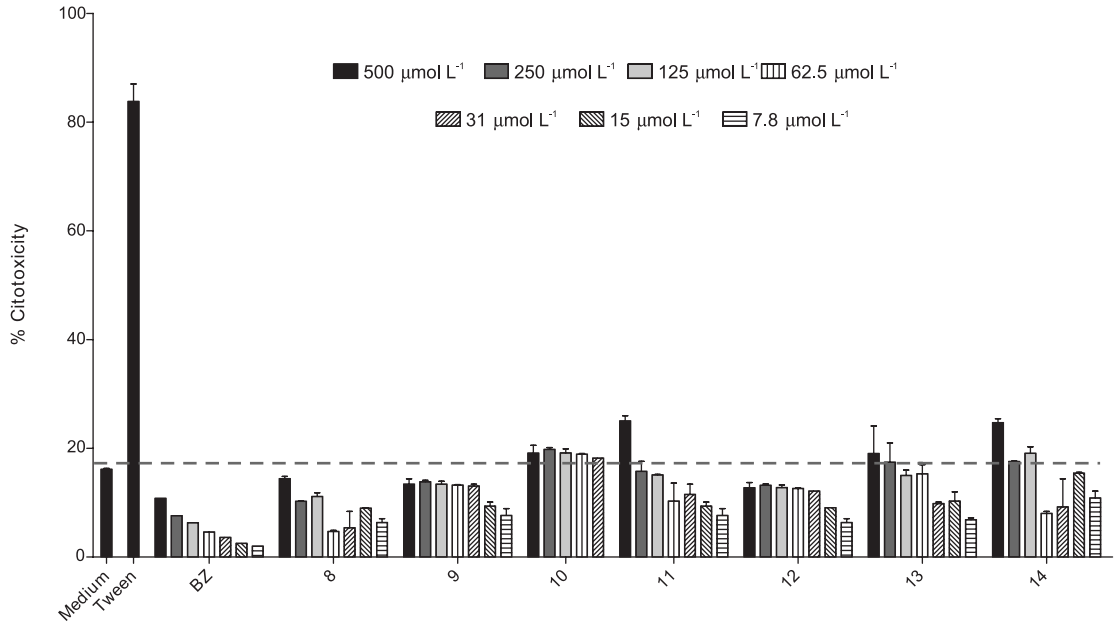


Figure 4. Percentage of cell death caused by compounds **8**-**14** and benznidazole (Bz) evaluated against cultured mouse spleen cells for 24 h. Medium and Tween were used as negative and positive controls, respectively. The data are representative of three independent experiments, performed in duplicate.

Conclusions

In summary, our studies demonstrated that commercial available benzenesulfonamides can be converted to the corresponding azide derivatives using different solvents under microwave-assisted reaction. The coupling of the azide benzenesulfonamides, comprising the free sulfonamide function or substituted by acetamide or different heterocycles, with galactose-derived alkyne was successfully achieved via CuAAC reaction for the formation of a small set of regioselective 1,4-disubstituted galactosyl-triazolo-benzenesulfonamides in moderate to good yields.

Bioassays with products **8**-**14** revealed TcTS inhibition only for the galactosyl-triazolo-benzenesulfonamide **14**, containing a sulfonamide-linked pyridine ring. Despite the weak activity in terms of IC_{50}, its inhibition was superior to pyridoxal phosphate used as reference. On the other hand, the evaluation of products **8**-**14** against *T. cruzi* trypomastigote showed products **10** and **13**, bearing respectively the 2,4-dimethoxypyrimidine and 5-methylisoxazole groups, as the most actives of the series, while the remaining products were two to four fold less active than **13**. Bearing in mind the role played by TcTS in parasite cell surface glycosylation and the difficulties to associate TcTS inhibition with parasite viability *in vitro* assays, we found, indeed, a lack of correlation between the results obtained in enzyme and *T. cruzi* trypomastigote *in vitro* assays, suggesting that antitrypanosomal activities found for products **10** and **13** is not related to TcTS inhibition and may involve alternative mechanisms. Thus, based on the low cytotoxicity displayed by the series, compounds **10** and **13** can be considered as lead scaffolds for further optimization.

Experimental

General

All chemicals were purchased as reagent grade and used without further purification. Solvents were dried according to standard methods.[57] MuNANA (2'-(4-methylumbelliferyl)-α-D-*N*-acetyl-neuraminic acid sodium salt), used as a donor substrate for silylation reactions, was acquired from Toronto Research Chemicals Inc.. The *trans*-sialidase used in this study was a His-tagged 70 kDa recombinant material truncated to remove C-terminal repeats but retaining the catalytic *N*-terminal domain of the enzyme.[58] Reactions were monitored by thin layer chromatography (TLC) on 0.25 nm precoated silica gel plates (Whatman, AL SIL G/UV, aluminium backing) with the indicated eluents. Compounds were visualized under UV light (254 nm) and/or dipping in ethanol-sulfuric acid (95:5, v/v), followed by heating the plate for a few minutes. Column chromatography was performed on Silica Gel 60 (Fluorochem, 35-70 mesh) or on a high performance flash chromatography (Biotage Horizon) system using 12 or 25 mm flash cartridges with the indicated eluents. The microwave-assisted reactions were performed in sealed tubes on a CEM Discover® Microwave System. HPLC

purifications were performed on a Shimadzu HPLC system using a Shim-PaK CLC-ODS (M) semi-preparative reverse phase column (250_10.0 mm). Nuclear magnetic resonance spectra were recorded on Bruker Advance DRX 300 (300 MHz), DPX 400 (400 MHz) or DPX 500 (500 MHz) spectrometers. Chemical shifts (δ) are given in parts *per* million downfield from tetramethylsilane. Assignments were made with the aid of HMQC and COSY experiments. Accurate mass electrospray ionization mass spectra (ESI-HRMS) were obtained using positive ionization mode on a Bruker Daltonics UltrOTOF-Q-ESI-TOF mass spectrometer.

Synthesis of azide benzenesulfonamides **30**-**36**

General procedure

Sodium azide (78 mg, 1.20 mmol, 3 equiv.) was introduced in a microwave flask equipped with a stirring bar and solubilized with water (150 µL). To this solution, the remaining reagents were added in the following order: solvent reaction (1 mL), 4-aminobenzenesulfonamide (**16**-**22**) (0.4 mmol, 1 equiv.) and *tert*-butyl nitrite (*t*-BuONO). The mixture was stirred and heated under microwave radiation at 150 W. The reaction was followed by TLC and after completion, the reaction mixture was partitioned between hexane and EtOAc. The aqueous phase was extracted with EtOAc (3 times), and the organic phase was dried over Na_2SO_4, filtered and concentrated. The product was obtained after flash chromatography on a Biotage Horizon, using 12 mm flash cartridge, flow 8 mL min^{-1}; hexane/EtOAc; gradient 0-40% and 40%-40% (v/v).

4-azidobenzenesulfonamide (**30**)

Following the general procedure, using 4-aminobenzenesulfonamide (**16**) (68.8 mg), *tert*-butyl alcohol as solvent, *t*-BuONO (285 µL, 2.4 mmol, 6 equiv.), reaction time 35 min at 60 °C. Yield: 86% (0.344 mmol). IR (KBr) ν_{max}/cm^{-1} 3247, 3338, 2100 (azide peak); ^{1}H NMR (300 MHz, CD_3OD) δ 7.89 (d, 2H, J_{ortho} 8.9 Hz, Ar-H and C*H*CSO_2NHR), 7.21 (d, 2H, J_{ortho} 8.9 Hz, Ar-H and $CHCN_3$).

N-[(4-azidophenyl)sulfonyl]acetamide (**31**)

Following the general procedure, using *N*-[(4-aminophenyl)sulfonyl]acetamide (**17**) (85.6 mg) acetonitrile as solvent, *t*-BuONO (190 µL, 1.60 mmol, 4 equiv.), reaction time 35 min at 40 °C. Yield: 83% (0.332 mmol). IR (KBr) ν_{max}/cm^{-1} 2100 (azide peak); ^{1}H NMR (300 MHz, CD_3OD) δ 8.01 (d, 2H, J_{ortho} 8.8 Hz, Ar-H and C*H*CSO_2NHR), 7.24 (d, 2H, J_{ortho} 8.8 Hz, Ar-H and $CHCN_3$), 1.97 (s, 3H, CH_3).

4-azido-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide (**32**)

Following the general procedure, using 4-amino-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide (**18**) (101.3 mg), dioxane as solvent, *t*-BuONO (190 µL, 1.60 mmol, 4 equiv.), reaction time 20 min at 50 °C. Yield: 82% (0.328 mmol). IR (KBr) ν_{max}/cm^{-1} 3250, 3000, 2750, 2110 (azide peak); ^{1}H NMR (300 MHz, CD_3OD) δ 7.91 (d, 2H, J_{ortho} 8.9 Hz, Ar-H and C*H*CSO_2NHR), 7.23 (d, 2H, J_{ortho} 8.9 Hz, Ar-H and $CHCN_3$), 6.14 (s, 1H, isoxazol), 2.33 (s, 3H, CH_3).

4-azido-*N*-(4-methylpyrimidin-2-yl)benzenesulfonamide (**33**)

Following the general procedure using 4-amino-*N*-(4-methylpyrimidin-2-yl)benzenesulfonamide (**19**) (105.6 mg), dioxane as solvent, *t*-BuONO (190 µL, 1.60 mmol, 4 equiv.), reaction time 20 min at 55 °C. Yield: 74% (0.296 mmol). IR (KBr) ν_{max}/cm^{-1} 3500, 3250, 3000, 2750, 2110 (azide peak); ^{1}H NMR (300 MHz, CD_3OD) δ 8.21 (d, 1H, *J* 5.2 Hz, H-pyrimidine), 8.06 (d, 2H, J_{ortho} 8.8 Hz, Ar-H and C*H*CSO_2NHR), 7.19 (d, 2H, J_{ortho} 8.8 Hz, Ar-H and $CHCN_3$), 6.82 (d, 1H, *J* 5.2 Hz, H-pyrimidine), 2.34 (s, 3H, CH_3).

4-azido-*N*-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide (**34**)

Following the general procedure, using 4-amino-*N*-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide (**20**) (111.3 mg), dioxane as solvent, *t*-BuONO (190 µL, 1.60 mmol, 4 equiv.), reaction time 20 min at 55 °C. Yield: 71% (0.284 mmol). IR (KBr) ν_{max}/cm^{-1} 2110 (azide peak); ^{1}H NMR (400 MHz, CD_3OD) δ 8.04 (d, 2H, J_{ortho} 8.6 Hz, Ar-H and C*H*CSO_2NHR), 7.16 (d, 2H, J_{ortho} 8.6 Hz, Ar-H and $CHCN_3$), 6.67 (s, 1H, H-pyrimidine), 2.26 (s, 6H, 2× CH_3).

4-azido-*N*-(2,6-dimethoxypyrimidin-4-yl) benzenesulfonamide (**35**)

Following the general procedure, using 4-amino-*N*-(2,6-dimethoxypyrimidin-4-yl)benzenesulfonamide (**21**) (124 mg) THF as solvent, *t*-BuONO (190 µL, 1.60 mmol, 4 equiv.), reaction time 40 min at 30 °C. Yield: 85% (0.340 mmol). IR (KBr) ν_{max}/cm^{-1} 1570, 2110 (azide peak); ^{1}H NMR (400 MHz, CD_3OD) δ 7.92 (d, 2H, J_{ortho} 8.8 Hz, Ar-H and C*H*CSO_2NHR), 7.16 (d, 2H, J_{ortho} 8.8 Hz, Ar-H and $CHCN_3$), 5.95 (s, 1H, H-pyrimidine), 3.79 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3).

4-azido-*N*-pyridin-2-yl-benzenesulfonamide (**36**)

Following the general procedure, using 4-amino-*N*-pyridin-2-yl-benzenesulfonamide (**22**) (99.6 mg), THF as solvent, t-BuONO (190 µL, 1.60 mmol, 4 equiv.), reaction time 30 min at 40 °C. Yield: 51% (0.204 mmol).

IR (KBr) ν_{max}/cm^{-1} 1570, 1200, 2110 (azide peak); ^{1}H NMR (400 MHz, CD_3OD) δ 7.92 (s, 1H, H-pyridine), 7.89 (d, 2H, J_{ortho} 8.8 Hz, Ar-H and C*H*CSO$_2$NHR), 7.66 (ddd, 1H, *J* 9.0, 7.2, 1.9 Hz, H-pyridine), 7.18 (dd, 1H, *J* 9.0, 1.0 Hz, H-pyridine), 7.13 (d, 2H, J_{ortho} 9.0 Hz, Ar-H and $CHCN_3$), 6.84 (ddd, 1H, *J* 7.2, 5.9, 1.0 Hz, H-pyridine).

2-propynyl 2,3,4,6-tretra-*O*-acetyl-β-D-galactopyranoside (**15**)

2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (2.15 g, 4.37 mmol) was diluted with anhydrous dichlorometane (DCM) (40 mL), under argon atmosphere, and treated with propargyl alcohol (2.55 mL, 43 mmol). The mixture was cooled to –40 °C and stirred for 30 min, then treated dropwise with trimethylsilyl trifluoromethanesulfonate (0.16 mL, 0.94 mmol) solution in DCM (1 mL). The mixture was kept stirring for a period of 1 h and the reaction was followed by TLC (hexane/EtOAc 1:1). After completion, the reaction was neutralized with triethylamine (0.1 mL), warmed to room temperature and filtered off. The mixture was concentrated under reduced pressure and purified by gradient flash chromatography (hexane/EtOAc; gradient 0-50%). The product **15** was obtained in 90% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.40 (dd, 1H, $J_{3,4}$ 3.4 Hz, $J_{4,5}$ 0.78 Hz, H-4), 5.22 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 10.5 Hz, H-2), 5.06 (dd, 1H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3.4 Hz, H-3), 4.74 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.38 (d, 2H, *J* 2.4 Hz, OC*H*$_2$CCH), 4.19 (dd, 1H, $J_{5,6}$ 6.6 Hz, $J_{6',6}$ 11.2 Hz, H-6), 4.13 (dd, 1H, $J_{5,6}$ 6.6 Hz, $J_{6',6}$ 11.2 Hz, H-6'), 3.94 (dt, 1H, $J_{4,5}$ 1.1 Hz, $J_{5,6}$ 6.6 Hz, H-5), 2.46 (t, 1H, *J* 2.4 Hz, CCH), 2.15-1.99 (s, 12H, 4× CH_3CO).

Synthesis of protected galactosyl-triazolo-benzenesulfonamides (**37-43**) and final products (**8-14**)

General procedure

4-azidobenzenesulfonamides (**30**-**36**) (0.2 mmol, 1 equiv.) were diluted in DMF (0.3 mL) in a microwave sealed flask, equipped with a stirring bar, and treated with the propynyl sugar **15** (85.0 mg, 0.22 mmol, 1.1 equiv.), sodium ascorbate (3.96 mg, 0.02 mmol, 0.1 equiv.) and copper sulfate solution 0.1 mol.L^{-1} (60 μL). The mixture was stirred and heated under microwave radiation at 150 W varying time and temperature. The reaction was monitored by TLC [hexane/EtOAc 1:9 (v:v)], which showed the formation of only one product. The mixture was concentrated and coevaporated with toluene under reduced pressure to eliminate the solvent (DMF). Then, the product was extracted using EtOAc (3 portions of 5 mL) and washed with water (1 portion of 3 mL) to eliminate the remaining salts. After filtration, the organic phase was dried over Na_2SO_4, filtered and concentrated. The product was obtained after flash chromatography on a Biotage Horizon [12 mm flash cartridge, flow 8 mL min^{-1}; hexane/EtOAc; gradient 0-50%, 50-100% and 100-100% (v/v)]. The *O*-protected galactosyl-triazolo-benzesulfonamides **37**-**43** (0.1 mmol) were dissolved in methanol (2 mL) and treated dropwise with NaOMe (1 mol L^{-1}) at 0 °C until pH 9. The mixture was stirred until the completion of the reaction, followed by TLC (hexane/EtOAc 1:3). Then, the mixture was neutralized with DOWEX resin (50WX8 H$^+$), filtered and concentrated under reduced pressure. The products **8**-**14** were obtained in quantitative yield.

4-{4-[(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl)-oxymethyl]-1-*H*-1,2,3 triazol-*1*-yl}benzenesulfonamide (**37**) and 4-{4-[(β-D-galactopyranosyl)-oxymethyl]-1-*H*-1,2,3 triazol-*1*-yl}benzenesulfonamide (**8**)

Protected product (**37**): Following the general procedure using 4-azidobenzenesulfonamide (**30**) (39.6 mg), reaction time 40 min at 70 °C. Yield: 60% (0.12 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (d, 2H, J_{ortho} 8.7 Hz, Ar-H and C*H*CSO$_2$NHR), 8.09 (s, 1H, CH-triazole), 7.94 (d, 2H, J_{ortho} 8.7 Hz, Ar-H and CHC-triazole), 5.44 (dd, 1H, $J_{4,5}$ 1.0 Hz, $J_{4,3}$ 3.3 Hz, H-4), 5.28 (dd, 1H, $J_{2,1}$ 7.8 Hz, $J_{2,3}$ 10.5 Hz, H-2), 5.10 (d, 1H, J_{gem} 12.5 Hz, OCH_2-triazole), 5.07 (dd, 1H, $J_{3,4}$ 3.4 Hz, $J_{3,2}$ 10.3 Hz, H-3), 4.92 (d, 1H, J_{gem} 12.5 Hz, OCH_2-triazole), 4.72 (d, 1H, *J* 7.8 Hz, H-1), 4.23 (dd, 1H, $J_{5,6}$ 6.8 Hz, $J_{6,6'}$ 11.2 Hz, H-6), 4.16 (dd, 1H, $J_{5,6'}$ 6.6 Hz, $J_{6',6}$ 11.2 Hz, H-6'), 4.00 (ddd, 1H, $J_{5,4}$ 1.0 Hz, $J_{5,6}$ 6.8 Hz, $J_{6,6'}$ 6.6 Hz, H-5), 2.20-1.98 (s, 12H, 4× CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5-169.7 ($CH_3\underline{C}O$), 145.7 (C-triazole), 142.2 (Ar, C-SONH2), 139.6 (Ar, *p*-*C*SONH$_2$), 127.9 (Ar, *m*-*CSO*NH2), 120.8 (CH-triazole), 120.1 (Ar, *o*-NH2), 100.1 (C-1), 70.4 (C-5), 70.1 (C-2), 68.2 (C-3), 66.4 (C-4), 62.3 (C-6), 60.8 (OCH_2), 20.2-19.9 ($\underline{C}H_3CO$).

Deprotected product (**8**): ^{1}H NMR (300 MHz, DMSO) δ 8.89 (s, 1H, H-triazole), 8.05 (d, 2H, *J* 8.6 Hz, Ar-H), 7.93 (d, 2H, *J* 8.6 Hz, Ar-H), 7.45 (s, 2H, NH_2), 4.85 (d, 1H, J_{gem} 12.3 Hz, CH_2-triazole), 4.65 (d, 1H, J_{gem} 12.3 Hz, CH_2-triazole), 4.20 (d, 1H, $J_{1,2}$ 7.0 Hz, H-1), 3.55-3.05 (m, 6H, H-2, H-3, H-4, H-5, H-6, H-6'); ^{13}C NMR (75 MHz, DMSO) δ 145.6 (C-triazole), 143.5 (Ar, CSO_2), 139.4 (Ar, *p*-CSO_2), 127.4 (Ar, *m*-CSO_2), 122.9 (CH-triazole), 119.9 (Ar, *o*-CSO_2), 102.8 (C-1), 75.1 (C-5), 70.6 (C-3), 67.8 (C-2), 61.3 (C-6), 60.4 (OCH_2); HRMS (ESI-MS) *m*/*z*, calcd. for $C_{20}H_{24}N_6O_8S$ [M + Na]$^+$: 439.1001, found: 439.0892.

4-{4-[(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl)-oxymethyl]-1-*H*-1,2,3 triazol-*1*-yl}-*N*-[(phenyl)sulfonyl] acetamide (**38**) and 4-{4-[(β-D-galactopyranosyl)-oxymethyl]-1-*H*-1,2,3-triazol-*1*-yl}-*N*-[(phenyl)sulfonyl] acetamide (**9**)

Protected product (**38**): Following the general procedure, using *N*-[(4-azidophenyl) sulfonyl]acetamide (**31**) (48 mg), reaction time 40 min at 80 °C. Yield: 60% (0.12 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (d, 2H, J_{ortho} 8.43 Hz, Ar-H and C*H*CSO_2NHR), 8.07 (s, 1H, H-triazole), 7.94 (d, 2H, J_{ortho} 8.43 Hz, Ar-H and CHC-triazole), 5.43 (dd, 1H, $J_{3,4}$ 3.7 Hz, $J_{4,5}$ 0.6 Hz, H-4), 5.27 (dd, 1H, $J_{1,2}$ 8.1 Hz, $J_{2,3}$ 10.3 Hz, H-2), 5.08 (d, 1H, J_{gem} 12.5 Hz, OCH_2-triazole), 5.06 (dd, 1H, $J_{3,4}$ 3.4 Hz, $J_{2,3}$ 10.3 Hz, H-3), 4.91 (d, 1H, J_{gem} 12.5 Hz, OCH_2-triazole), 4.71 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1), 4.20 (dd, 1H, $J_{5,6}$ 6.6 Hz, $J_{6,6'}$ 11.0 Hz, H-6), 4.14 (dd, 1H, $J_{5,6'}$ 6.6 Hz, $J_{6',6}$ 11.0 Hz, H-6'), 3.98 (ddd, 1H, $J_{4,5}$ 0.6 Hz, $J_{5,6}$ 6.6 Hz, $J_{5,6'}$ 6.6 Hz, H-5), 2.10 (s, 3H, CH_3-sulfonamide), 2.05-1.99 (s, 9H, 3x CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7-169.9 ($CH_3\underline{C}O$), 168.0 (CO-sulfonamide), 145.7 (C-triazole), 140.6 (Ar, C-SO_2), 138.5 (Ar, *p*-CSO_2), 130.6 (Ar, *m*-CSO_2), 120.4 (CH-triazole), 120.3 (Ar, *o*-CSO_2), 100.8 (C-1), 71.0 (C-5), 70.8 (C-3), 68.8 (C-2), 67.4 (C-4), 63.3 (C-6), 61.8 (O*C*H_2), 24.95 (CH_3-sulfonamide), 20.9-20.7 ($\underline{C}H_3CO$).

Deprotected product (**9**): ^{1}H NMR (400 MHz, DMSO) δ 8.98 (s, 1H, H-triazole), 8.17 (d, 2H, J_{ortho} 8.7 Hz, Ar-H and C*H*CSO_2NHR), 8.11 (d, 2H, J_{ortho} 8.7 Hz, Ar-H and CHC-triazole), 4.92 (d, 1H, J_{gem} 12.5 Hz, CH_2-triazole), 4.75 (d, 1H, 12.5 Hz, CH_2-triazole), 4.27 (d, 1H, $J_{1,2}$ 7.3 Hz, H-1), 3.64 (m, 1H, H-4), 3.56 (m, 2H, H-6 and H-6'), 3.42-3.28 (m, 3H, H-2, H-3, H-5), 1.94 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ 169.3 (CO), 145.6 (C-triazole), 139.8 (Ar, CSO_2), 138.9 (Ar, *p*-CSO_2), 129.6 (Ar, *m*-CSO_2), 123.0 (CH-triazole), 120.3 (Ar, *o*-CSO_2), 102.9 (C-1), 75.4 (C-5), 73.4 (C-3), 70.5 (C-2), 68.2 (C-4), 61.3 (OCH_2-triazole), 60.6 (C-6), 23.4 (CH_3); HRMS (ESI-MS) *m/z*, calcd. for $C_{17}H_{22}N_4O_9S$ $[M - H]^-$: 457.11075, found: 457.1028.

4-{4-[(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl)-oxymethyl]-1-*H*-1,2,3 triazol-*1*-yl}-*N*-(5-methylisoxazol-3-yl) benzenesulfonamide (**39**) and 4-{4-[(β-D-galactopyranosyl)-oxymethyl]-1-*H*-1,2,3-triazol-*1*-yl}-*N*-(5-methylisoxazol-3-yl) benzenesulfonamide (**10**)

Protected product (**39**): Following the general procedure, using 4-azido-*N*-(5-methylisoxazol-3-yl) benzenesulfonamide (**32**) (55.8 mg), reaction time 60 min at 80 °C. Yield: 50% (0.1 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, 2H, J_{ortho} 8.9 Hz, Ar-H and C*H*CSO_2NHR), 7.81 (s, 1H, H-triazole), 7.89 (d, 2H, J_{ortho} 8.9 Hz, Ar-H and CHC-triazole), 6.26 (s, 1H, H-isoxazole), 5.44 (dd, 1H, $J_{4,5}$ 0.6 Hz, $J_{3,4}$ 3.3 Hz, H-4), 5.27 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 10.3 Hz, H-2), 5.09 (d, 1H, J_{gem} 12.8 Hz, OCH_2), 5.08 (dd, 1H, $J_{3,4}$ 3.3 Hz $J_{2,3}$ 10.3 Hz), 4.7 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.01 (dd, 1H, $J_{5,6}$ 6.7 Hz, $J_{6,6'}$ 11.2 Hz, H-6), 4.07 (dd, 1H, $J_{5,6'}$ 6.4 Hz, $J_{6',6}$ 11.2 Hz, H-6'), 3.92 (ddd, 1H, $J_{4,5}$ 0.6 Hz, $J_{5,6}$ 6.7 Hz, $J_{5,6'}$ 6.4 Hz, H-5), 2.32 (s, 3H, CH_3-isoxazole), 2.10-1.90 (s, 12H, 4× CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6 (C-O-isoxazole), 170.4-169.6 ($CH_3\underline{C}O$), 145.8 (C-N-isoxazole), 145.6 (C-triazole), 140.3 (Ar, C-SO_2), 139.9 (Ar, *p*-CSO_2), 129.2 (Ar, *m*-CSO_2), 120.8 (CH-triazole), 120.6 (Ar, *o*-CSO_2), 100.8 (C-1), 94.7 (CH-isoxazole), 71.0 (C-5), 70.8 (C-3), 68.8 (C-2), 67.1 (C-4), 62.9 (OCH_2), 61.3 (C-6), 20.8-20.5 ($\underline{C}H_3CO$), 12.71 (CH_3-isoxazole).

Deprotected product (**10**): ^{1}H NMR (500 MHz, CD_3OD) δ 8.67 (s, 1H, H-triazole), 8.07 (d, 2H, J_{ortho} 9.0 Hz Ar-H and C*H*CSO_2NHR), 8.04 (d, 2H, J_{ortho} 9.0 Hz Ar-H and CHC-triazole), 6.14 (s, 1H, H-isoxazole), 5.01 (d, 1H, J_{gem} 12.9 Hz, CH_2-triazole), 4.88-4.86 (m, 1H, CH_2-triazole), 4.36 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 3.80 (dd, 1H, $J_{3,4}$ 3.4 Hz, $J_{4,5}$ 0.7 Hz, H-4), 3.77 (dd, 1H, $J_{5,6}$ 6.5 Hz, $J_{6,6'}$ 11.2 Hz, H-6), 3.70 (dd, 1H, $J_{5,6'}$ 6.5 Hz, $J_{6',6'}$ 11.2 Hz, H-6'), 3.56-3.52 (m, 2H, H-2 and H-5), 3.45 (dd, 1H, $J_{2,3}$ 9.6 Hz, $J_{3,4}$ 3.4 Hz, H-3), 2.29 (s, 3H, CH_3); ^{13}C NMR (125 MHz, CD_3OD) δ 172.3 (C-O-isoxazole), 159.1 (C=N-isoxazole), 147.1 (C-triazole), 140.6 (Ar, CSO_2), 140.1 (Ar, *p*-CSO_2), 130.3 (Ar, *m*-CSO_2), 123.8 (CH-triazole), 121.8 (Ar, *o*-CSO_2), 104.5 (C-1), 96.5 (CH-isoxasole), 76.9 (C-5), 74.9 (C-3), 72.5 (C-2), 70.4 (C-4), 63.1 (OCH_2-triazole), 62.7 (C-6), 12.3 (CH_3-isoxazole); HRMS (ESI-MS) *m/z*, calcd. for $C_{19}H_{23}N_5O_9S$ $[M - H]^-$: 520.1014, found: 520.1113.

4-{4-[(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl)-oxymethyl]-1-*H*-1,2,3 triazol-*1*-yl}-*N*-(4-methylpyrimidin-2-yl) benzenesulfonamide (**40**) and 4-{4-[(β-D-galactopyranosyl)-oxymethyl]-1-*H*-1,2,3-triazol-*1*-yl}-*N*-(4-methylpyrimidin-2-yl) benzenesulfonamide (**11**)

Protected product (**40**): Following the general procedure using 4-azido-*N*-(4-methylpyrimidin-2-yl) benzenesulfonamide (**33**) (58 mg), reaction time 40 min at 70 °C. Yield: 60% (0.12 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, 1H, *J* 5.3 Hz, H-pyrimidine), 8.32 (d, 2H, J_{ortho} 8.7 Hz, Ar-H and C*H*CSO_2NHR), 8.06 (s, 1H, H-triazole), 7.89 (d, 2H, *J* 8.7 Hz, Ar-H and CHC-triazole), 6.84 (d, 1H, *J* 5.3 Hz, H-pyrimidine), 5.41 (dd, 1H, $J_{3,4}$ 3.3 Hz, $J_{4,5}$ 0.7 Hz, H-4), 5.25 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 10.4 Hz, H-2), 5.06 (d, 1H, J_{gem} 12.8 Hz, CH_2-triazole), 5.04 (dd, 1H, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.3 Hz), 4.88 (d, 1H, $J_{gem,}$ 12.8 Hz, CH_2-triazole), 4.69 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1),

4.20 (dd, 1H, $J_{5,6}$ 6.7 Hz, $J_{6,6'}$ 11.1 Hz, H-6), 4.14 (dd, 1H, $J_{5,6'}$ 6.7 Hz, $J_{6',6}$ 11.1 Hz, H-6'), 3.97 (dt, 1H, $J_{4,5}$ 0.7 Hz, $J_{5,6}$ 6.7 Hz, H-5), 2.44, (s, 3H, CH_3-pyrimidine), 2.15-1.98 (s, 12H, 4× CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5-170.0 ($CH_3\underline{C}O$), 168.3 (C-2'), 156.3 (C-6'), 156.1 (C-4'), 145.7 (C-triazole), 140.0 (Ar, CSO_2), 139.9 (Ar, *p*-CSO_2), 130.8 (Ar, *m*-CSO_2), 120.8 (CH-triazole), 119.8 (Ar, *o*-CSO_2), 115.1 (C-5'), 100.8 (C-1), 70.9 (C-5), 70.8 (C-3), 68.8 (C-2), 67.0 (C-4), 62.9 (C-6), 62.3 (OCH_2), 23.6 (CH_3-pyrimidine), 20.2-19.9 ($\underline{C}H_3CO$).

Deprotected product (**11**): ^{1}H NMR (300 MHz, D_2O) δ 8.51 (s, 1H, H-triazole), 8.18 (d, 1H, *J* 5.9 Hz, H-pyrimidine), 8.16 (d, 2H, J_{ortho} 8.7 Hz, Ar-H and C*H*CSO_2NHR), 7.86 (d, 2H, *J* 8.7 Hz, Ar-H and CHC-triazole), 6.83 (d, 1H, *J* 5.6 Hz, H-pyrimidine), 5.11 (d, 1H, J_{gem} 12.8 Hz, CH_2-triazole), 4.99 (d, 1H, J_{gem} 12.8 Hz, CH_2-triazole), 4.57 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1), 3.96 (d, 1H, $J_{3,4}$ 3.3 Hz, H-4), 3.75-3.65 (m, 3H, H-5, H-6, H-6'), 3.57 (dd, 1H, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.3 Hz, H-3), 3.58 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 10.0 Hz, H-2), 2.34 (s, 3H, CH_3-pyrimidine); ^{13}C NMR (75 MHz, CD_3OD) δ 166.0 (C-2'), 156.3 (C-6'), 156.0 (C-4'), 144.6 (C-triazole), 141.2 (Ar, CSO_2), 138.8 (Ar, *p*-CSO_2), 128.8 (Ar, *m*-CSO_2), 123.8 (CH-triazole), 121.0 (Ar, *o*-CSO_2), 111.7 (C-5'), 102.1 (C-1), 75.3 (C-5), 72.2 (C-3), 70.7 (C-2), 68.6 (C-4), 61.8 (C-6), 60.9 (OCH_2-triazole); HRMS (ESI-MS) *m/z*, calcd. for $C_{20}H_{24}N_6O_8S$ [M + Na]$^+$: 531.1273, found: 531.1270.

4-{4-[(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl)-oxymethyl]-1-*H*-1,2,3 triazol-*1*-yl}-*N*-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide (**41**) and 4-{4-[(β-D-galactopyranosyl)-oxymethyl]-1-*H*-1,2,3-triazol-*1*-yl}-*N*-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide (**12**)

Protected product (**41**): Following the general procedure using 4-azido-*N*-(4,6-dimethylpyrimidin-2-yl) benzenesulfonamide (**34**) (60.8 mg), reaction time 10 min at 70 °C. Yield: 63% (0.13 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (d, 2H, J_{ortho} 8.8 Hz, Ar-H and C*H*CSO_2NHR), 8.07 (s, 1H, H-triazole), 7.90 (d, 2H, J_{ortho} 8.8 Hz, Ar-H and CHC-triazole), 6.67 (s, 1H, H-pyrimidine), 5.42 (dd, 1H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 0.7 Hz, H-4), 5.26 (dd, 1H, $J_{1,2}$ 8.1 Hz, $J_{2,3}$ 10.4 Hz, H-2), 5.07 (d, 1H, J_{gem} 12.6, CH_2-triazole), 5.05 (dd, 1H, $J_{3,4}$ 3.5 Hz, $J_{2,3}$ 10.4 Hz, H-3), 4.90 (d, 1H, J_{gem} 12.6, CH_2-triazole), 4.69 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1), 4.22 (dd, 1H, $J_{5,6}$ 6.6 Hz, $J_{6,6'}$ 11.1 Hz, H-6), 4.15 (dd, 1H, $J_{5,6'}$ 6.6 Hz, $J_{6',6}$ 11.1 Hz, H-6'), 3.98 (ddd, 1H, $J_{4,5}$ 0.7 Hz, $J_{5,6}$ 6.6 Hz, $J_{5,6'}$ 6.6 Hz, H-5), 2.38 (s, 6H, 2x CH_3-pyrimidine), 2.16-1.99 (s, 12H, 4× CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5-169.6 ($CH_3\underline{C}O$), 168.4 (C-2'), 155.9 (C-4' and C-6'), 145.7 (C-triazole), 139.9 (Ar, CSO_2), 139.9 (Ar, *p*-CSO_2), 130.9 (Ar, *m*-CSO_2), 120.8 (CH-triazole), 119.7 (Ar, *o*-CSO_2), 115.0 (C-5'), 100.7 (C-1), 70.9 (C-5), 70.8 (C-3), 68.8 (C-2), 67.0 (C-4), 62.9 (C-6), 61.3 (OCH_2), 23.5 (CH_3-pyrimidine), 20.8-20.6 ($\underline{C}H_3CO$)

Deprotected product (**12**): ^{1}H NMR (300 MHz, D_2O) δ 8.55 (s, 1H, H-triazole), 8.20 (d, 2H, J_{ortho} 5.6 Hz, H-pyrimidine), 8.09 (d, 2H, J_{ortho} 8.7 Hz, Ar-H and C*H*CSO_2NHR), 7.88 (d, 2H, J_{ortho} 8.7 Hz, Ar-H and CHC-triazole), 6.85 (d, 1H, J_{ortho} 5.6 Hz, H-pyrimidine), 5.05 (d, 1H, J_{gem} 12.8 Hz, CH_2-triazole), 4.89 (d, 1H, J_{gem} 12.8 Hz, CH_2-triazole), 4.46 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1), 3.85 (dd, 1H, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ 0.9 Hz, H-4), 3.75-3.65 (m, 3H, H-5, H-6, H-6'), 3.57 (dd, 1H, $J_{3,4}$ 3.2 Hz, $J_{2,3}$ 9.9 Hz H-3), 3.48 (dd, 1H, $J_{2,3}$ 9.9 Hz, $J_{1,2}$ 7.8 Hz, H-2), 2.35 (s, 3H, CH_3); ^{13}C NMR (75 MHz, D_2O) δ 166.7 (C-2'), 156.7 (C-6'), 155.6 (C-4'), 144.6 (C-triazole), 141.2 (Ar, CSO_2), 139.9 (Ar, *p*-CSO_2), 128.8 (Ar, *m*-CSO_2), 123.8 (CH-triazole), 119.7 (Ar, *o*-CSO_2), 111.7 (C-5'), 102.1 (C-1), 74.6 (C-5), 72.7 (C-3), 70.7 (C-2), 67.9 (C-4), 61.1 (C-6), 60.3 (OCH_2), 21.7 (CH_3); HRMS (ESI-MS) *m/z*, calcd. for $C_{21}H_{26}N_6O_8S$ [M + Na]$^+$: 545.1331, found: 545.1431.

4-{4-[(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl)-oxymethyl]-1-*H*-1,2,3-triazol-*1*-yl}-*N*-(2,6-dimethoxypyrimidin-4-yl)benzenesulfonamide (**42**) and 4-{4-[(β-D-galactopyranosyl)-oxymethyl]-1-*H*-1,2,3-triazol-*1*-yl}-*N*-(2,6-dimethoxypyrimidin-4-yl)benzenesulfonamide (**13**)

Protected product (**42**): Following the general procedure using 4-azido-*N*-(2,6-dimethoxypyrimidin-4-yl)benzenesulfonamide (**35**) (67.2 mg), reaction time 60 min at 80 °C. Yield: 61% (0.12 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, 2H, J_{ortho} 8.85 Hz, Ar-H and C*H*CSO_2NHR), 8.07 (s, 1H, H-triazole), 7.92 (d, 2H, J_{ortho} 8.85 Hz, Ar-H CHC-triazole), 6.25 (s, 1H, H-pyrimidine), 5.41 (dd, 1H, $J_{3,4}$ 3.4 Hz, $J_{4,5}$ 1.0 Hz, H-4), 5.25 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 10.3 Hz, H-2), 5.07 (d, 1H, J_{gem} 12.8, CH_2-triazole), 5.05 (dd, 1H, $J_{3,4}$ 3.4 Hz, $J_{2,3}$ 10.3 Hz, H-3), 4.90 (d, 1H, J_{gem} 12.8 Hz, CH_2-triazole), 4.69 (d, 1H, $J_{1,2}$ 7.8 Hz), 4.21 (dd, 1H, $J_{5,6}$ 6.5 Hz, $J_{6,6'}$ 11.2 Hz, H-6), 4.13 (dd, 1H, $J_{5,6'}$ 6.5 Hz, $J_{6',6}$ 11.2 Hz, H-6'), 3.98 (ddd, 1H, $J_{4,5}$ 1.0 Hz, $J_{5,6}$ 6.5 Hz, $J_{5,6'}$ 6.5 Hz, H-5), 3.92 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 2.16-1.99 (s, 12H, 4× CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8 (C-6'), 170.7-169.7 ($CH_3\underline{C}O$), 164.4 (C-2'), 158.4 (C-4'), 145,8 (C-triazole), 140.2 (Ar, CSO_2), 139.7 (Ar, *p*-CSO_2), 129.4 (Ar, *m*-CSO_2), 120.7 (CH-triazole), 120.6 (Ar, *o*-CSO_2), 100.7 (C-1), 85.9 (C-5'), 70.9 (C-5), 70.7 (C-3), 68.8 (C-2), 67.0 (C-4), 62.9 (C-6), 61.3 (OCH_2), 55.6 (OCH_3), 53.9 (OCH_3), 20.8-20.6 ($\underline{C}H_3CO$).

Deprotected product (**13**): ^{1}H NMR (300 MHz, CD_3OD) δ 8.69 (s, 1H, H-triazole), 8.20 (d, 2H, J_{ortho} 9.0 Hz, Ar-H and C*H*CSO_2NHR), 8.08 (d, 2H, J_{ortho} 9.0 Hz, Ar-H and CHC-triazole), 6.14 (s, 1H, H-pyrimidine), 5.03 (d, 1H, J_{gem} 12.9 Hz, CH_2-triazole), 4.90-4.86 (m, 1H, CH_2-triazole), 4.38 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 3.84 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.82 (dd, 1H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1.0 Hz, H-4), 3.79 (dd, 1H, $J_{5,6}$ 4.2 Hz, $J_{6,6'}$ 11.4 Hz, H-6), 3.74 (dd, 1H, $J_{5,6'}$ 4.2 Hz, $J_{6',6}$ 11.4 Hz, H-6'), 3.61-3.55 (m, 2H, H-2 and H-5), 3.47 (dd, 1H, $J_{2,3}$ 9.7 Hz, $J_{3,4}$ 3.5 Hz, H-3); ^{13}C NMR (75 MHz, CD_3OD) δ 171.7 (C-6'), 159.9 (C-2'), 159.9 (C-4'), 145.5 (C-triazole), 139.8 (Ar, CSO_2), 139.5 (Ar, *p*-CSO_2), 129.1 (Ar, *m*-CSO_2), 122.9 (CH-triazole), 120.3 (Ar, *o*-CSO_2), 102.8 (C-1), 84.8 (C-5'), 75.3 (C-2), 73.3 (C-3), 70.5 (C-5), 68.2 (C-4), 61.2 (C-6), 60.8 (OCH_2), 54.6 (OCH_3), 53.9 (OCH_3); HRMS (ESI-MS) *m/z*, calcd. for $C_{21}H_{26}N_6O_{10}S$ [M + Na]$^+$: 577.1328, found: 557.1338.

4-{4-[(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl)-oxymethyl]-1-*H*-1,2,3 triazol-*1*-yl}benzenesulfa-*ortho*-pyridine (**43**) and 4-{4-[(β-D-galactopyranosyl)-oxymethyl]-1-*H*-1,2,3 triazol-*1*-yl}benzenesulfa-*ortho*-pyridine (**14**)

Protected product (**43**): Following the general procedure using 4-azido-*N*-pyridin-2-yl-benzenesulfonamide (**36**) (55 mg), reaction time 30 min at 80 °C. Yield: 50% (0.1 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (dd, 1H, *J* 6.0 Hz, *J* 1.3 Hz, H-pyridine), 8.10 (d, 2H, J_{ortho} 8.8 Hz, Ar-H and C*H*CSO_2NHR), 8.04 (s, 1H, H-triazole), 7.80 (d, 2H, J_{ortho} 8.8 Hz, Ar-H and CHC-triazole), 7.69 (ddd, 1H, *J* 9.0 Hz, *J* 6.2 Hz, *J* 1.9 Hz, H-pyridine), 7.44 (d, 1H, *J* 9.0 Hz, H-pyridine), 6.84 (dt, 1H, *J* 6.2 Hz, *J* 1.0 Hz, H-pyridine), 5.40 (dd, 1H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 0.9 Hz, H-4), 5.23 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 10.3 Hz, H-2), 5.05 (d, 1H, J_{gem} 12.8 Hz, CH-triazole), 5.03 (dd, 1H, $J_{2,3}$ 10.3 Hz, $J_{3,4}$ 3.5 Hz, H-3), 4.87 (d, 1H, J_{gem} 12.8 Hz, CH-triazole), 4.69 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1), 4.19 (dd, 1H, $J_{5,6}$ 6.5 Hz, $J_{6,6'}$ 11.3 Hz, H-6), 4.14 (dd, 1H, $J_{5,6'}$ 6.5 Hz, $J_{6',6}$ 11,3 Hz, H-6'), 3.97 (dt, 1H, $J_{4,5}$ 0.9 Hz, $J_{5,6}$ 6.5 Hz, H-5), 2.14-1.97 (s, 12H, 4× CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5-169.6 ($CH_3\underline{C}O$), 155.6 (C-2'), 145.6 (C-triazole), 143.3 (C-6'), 142.3 (Ar, CSO_2), 139.0 (C-3'), 139.2 (Ar, *p*-CSO_2), 128.7 (Ar, *m*-CSO_2), 120.8 (CH-triazole), 120.5 (Ar, *o*-CSO_2), 115.8 (C-4'), 113.9 (C-5'), 100.7 (C-1), 70.9 (C-5), 70.7 (C-3), 68.8 (C-2), 67.0 (C-4), 62.9 (C-6), 62.3 (OCH_2), 20.8-20.6 ($\underline{C}H_3CO$).

Deprotected product (**14**): ^{1}H NMR (300 MHz, CD_3OD) δ 8.68 (s, 1H, H-triazole), 8.04 (d, 2H, J_{ortho} 9.0 Hz, Ar-H and C*H*CSO_2NHR), 7.92 (d, 2H, J_{ortho} 9.0 Hz, Ar-H and CHC-triazole), 7.86 (dd, 1H, J_{meta} 5.0 Hz, J_{ortho} 7.2 Hz, H-pyridine), 7.66 (ddd, 1H, J_{meta} 1.87 Hz, J_{ortho} 7.2 Hz, J_{ortho} 8.7 Hz, H-pyridine), 7.21 (d, 1H, J_{ortho} 8.7 Hz, H-pyridine), 6.79 (dd, 1H, J_{meta} 5.0 Hz, 7.0 Hz, H-pyridine), 5.01 (d, 1H, J_{gem} 12.8 Hz, CH_2-triazole), 4.95 (d, 1H, J_{gem} 12.8 Hz, CH_2-triazole), 4.37 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1), 3.81-3.60 (m, 3H, H-4, H-6, H-6), 3.50-3.35 (m, 3H, H-5, H-3, H-2); ^{13}C NMR (75 MHz, CD_3OD) δ 145.9 (C-triazole), 142.4 (Ar, CSO_2), 141.2-141.0 (C-5' and C-2'), 139.4 (Ar, *p*-CSO_2), 128.4 (Ar, *m*-CSO_2), 122.1 (CH-triazole), 120.1 (Ar, *o*-CSO_2), 115.1-114.9 (C3'and C-4'), 102.9 (C-1), 74.9 (C-5), 72.9 (C-3), 70.4 (C-2), 68.3 (C-4), 61.1 (C-6), 60.6 (OCH_2); HRMS (ESI-MS) *m/z*, calcd. for $C_{20}H_{23}N_5O_8S$ [M + Na]$^+$: 516.1267, found: 516.1163.

Biological assays

Trans-sialidase inhibition assay

Trans-sialidase used in this study was a His-tagged 70 kDa recombinant material truncated to remove C-terminal repeats but retaining the catalytic *N*-terminal domain of the enzyme.[58] Inhibition was assessed using the continuous fluorimetric assay described by Douglas and co-workers.[53] Briefly, the assay was performed in triplicate in 96-well plates containing phosphate buffer solution at pH 7.4 (25 µL), recombinant enzyme solution (25 µL) and inhibitor solution (25 µL of a 4.0 mM solution). This mixture was incubated for 10 min at 26 °C followed by addition of MuNANA (Km = 0.68 mM;[53] 25 µL of a 0.4 mM solution giving an assay concentration of 0.1 mM). The fluorescence of the released product (Mu) was measured after 10 min, with excitation and emission wavelengths of 360 and 460 nm, respectively, and the data were analyzed with GraphPad Prism software version 4.0 (San Diego, CA, USA). Inhibition percentages were calculated by the equation: $\% I = 100 \times [1 - (V_i/V_0)]$, where V_i is the velocity in the presence of inhibitor and V_0 is the velocity in absence of inhibitor.

In vitro antitrypanosomal assay

To evaluate the antitrypanosomal activity in both trypomastigote form of *T. cruzi*, LLC-MK2 cell strain (ATCC) were resuspended in RPMI medium without phenol red at 2×10^3 cells well^{-1} and were cultured in 96-well plates for 24 h.[2,55] The cells were infected with 1×10^4 trypomastigotes forms of *T. cruzi* Tulahuen strain stably expressing the β-galactosidase gene from *Escherichia coli* (Tulahuen-lacZ), and after 24 h, the synthesized compounds (**8-14**) and benznidazole were added at concentrations of 500.0, 250.0, 125.0, 62.5, 31.25, 15.6, 7.8 and 3.9 µM. After 4 days of culture, 50 µL of PBS containing 0.5% of Triton X-100 and

100 μM chlorophenol red-β-D-galactoside (CPRG-Sigma) were added. Plates were incubated at 37 °C for 4 h and absorbance was read at 570 nm.[59] Results of parasite viability were measured based on the catalysis of CPRG by β-galactosidase and performed in triplicates.

In vitro cytotoxicity assay

The cytotoxicity of compounds **8-14** was evaluated on spleen cells isolated from C57BL/6 mice, spleens were macerated in RPMI 1640 medium (Gibco-BRL Life Technologies, Grand Island, NY) and incubated for 5 min with red blood cell lysis buffer (one part of 0.17 M Tris-HCl and nine parts of 0.16 M ammonium chloride). The isolated cells were centrifuged at 1500 rpm for 10 min and resuspended in RPMI medium containing 5% fetal bovine serum (Life Technologies Inc., Bethesda, MD) and antibiotics (Sigma Chemical Co., St. Louis). The spleen cells were seeded flat-bottom 96-well plates at 6.5×10^6 mL^{-1} cells well^{-1} with different concentrations of the synthesized compounds at 37 °C for 24 h.[56] Tween 20 at 0.5% was used as cell death positive control and benznidazole (Roche) was used as a reference drug. After 24 h the cells were incubated with 10 μg mL^{-1} propidium iodide (Sigma) and acquired using a FACSCantoII (Becton-Dickinson Immunocytometry System Inc., San Jose, CA, USA). Data analysis was performed using FlowJo software (Ashland, Oregon, USA). The experiments were also performed in triplicates.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

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References

1. El-Sayed, N. M.; *Science* **2005**, *309*, 409.
2. Dias, L. C.; Dessoy, M. A.; Silva, J. J. N.; Thiemann, O. H.; Oliva, G.; Andricopulo, A. D.; *Quim. Nova* **2009**, *32*, 2444.
3. Rivera, G.; Bocanegra-Garcia, V.; Ordaz-Pichardo, C.; Nogueda-Torres, B.; Monge, A.; *Curr. Med. Chem.* **2009**, *16*, 3286.
4. Bernardes, L. S. C.; Zani, C. L.; Carvalho, I.; *Curr. Med. Chem.* **2013**, *20*, 2673.
5. Urbina, J. A.; *Mem. Inst. Oswaldo Cruz* **2009**, *104*, 311.
6. Coura, J. R.; *Mem. Inst. Oswaldo Cruz* **2009**, *104*, 549.
7. Andriani, G.; Amata, E.; Beatty, J.; Clements, Z.; Coffey, B.; Courtemanche, G.; Devine, W.; Erath, J.; Juda, C.; Wawrzak, Z.; Wood, J.; Lepesheva, G.; Rodriguez, A.; Pollastri, M.; *J. Med. Chem.* **2013**, *56*, 2556.
8. Previato, J. O.; Jones, C.; Xavier, M. T.; Wait, R.; Parodi, A. J.; Previato, L. M.; *J. Biol. Chem.* **1995**, *270*, 7241.
9. Corey, W. T.; Lima, M. F.; Villalta, F.; *Biochem. Bioph. Res. Co.* **2002**, *290*, 29.
10. Pereira-Chioccola, V. L.; Acosta-Serrano, A.; De Almeida, I. C.; Ferguson, M. A. J.; Souto-Padron, T.; Rodrigues, M. M.; Travassos, L. R.; Schenkman, S.; *J. Cell Sci.* **2000**, *113*, 1299.
11. Agrellos, O. A.; Jones, C.; Todeschini, A. R.; Previato, J. O.; *Mol. Biochem. Parasitol.* **2003**, *126*, 93.
12. Torrecilhas, A. C.; Schumacher, R. I.; Alves, M. J. M.; Colli, W.; *Microbes Infect.* **2012**, *14*, 1465.
13. Schenkman, S.; Eichinger, D.; Pereira, M. E. A.; Nussenzweig, V.; *Annu. Rev. Microbiol.* **1994**, *48*, 499.
14. Buschiato, A.; Amaya, M. F.; Cremona, M. L.; Frasch, A. C.; Alzari, P. M.; *Mol. Cell* **2002**, *10*, 757.
15. Amaya, M. F.; Buschiazzo, A.; Nguyen, T.; Alzari, P. M.; *J. Mol. Biol.* **2003**, *325*, 773.
16. Neres, J.; Bryce, R. A.; Douglas, K. T.; *Drug Discov. Today* **2008**, *13*, 110.
17. Mitchell, L.; Neres, J.; Ramraj, A.; Raju, R. K.; Hilier, I. H.; Vincent, M. A.; Bryce, R.; *Biochemistry* **2013**, *52*, 3740.
18. Alves, M. J. M.; Colli, W.; *Open Parasitol. J.* **2010**, *4*, 77.
19. Tonelli, R. R.; Torrecilhas, A. C.; Jacysyn, J. F.; Juliano, M. A.; Colli, W.; Alves, M. J. M.; *Parasitology* **2011**, *138*, 481.
20. Buscaglia, C. A.; Campo, V. A.; Frasch, A. C. C.; Noia, J. M.; *Nature Rev. Microb.* **2006**, *4*, 229.
21. de Lederkremer, R. M.; Agusti, R.; *Chem. Biochem.* **2009**, *62*, 311.
22. Andrews, N. W.; *J. Cell Biol.* **2002**, *158*, 389.
23. Andrade, L. O.; Andrews, N. W.; *J. Exp. Med.* **2004**, *200*, 1135.
24. Rubin-de-Celis, S. S.; Uemura, H.; Yoshida, N.; Schenkman, S.; *Cell Microbiol.* **2006**, *8*, 1888.
25. Vandekerckhove, F.; Schenkman, S.; Pontes de Carvalho, L.; Tomlinson, S.; Kiso, M.; Yoshida, M.; Hasegawa, A.; Nussenzweig, V.; *Glicobiology* **1992**, *2*, 541.
26. Busse, H.; Hakoda, M.; Stanley, M.; Streicher, H.; *J. Carbohydr. Chem.* **2007**, *26*, 159.
27. Neres, J.; Brewer, M. L.; Ratier, L.; Botti, H.; Buschiazzo, A.; Edwards, P. N.; Mortenson, P. N.; Charlton, M. H.; Alzari, P. M.; Frasch, A. C.; Bryce, R. A.; Douglas, K. T.; *Bioorg. Med. Chem. Lett.* **2009**, *19*, 589.
28. Castro, J. A.; Meccan, M. M.; Bartel, L. C.; *Hum. Exp. Toxicol.* **2006**, *25*, 471.

29. Wilkinson, S. R.; Taylor, M. C.; Horn, D.; Kelly, J. M.; Cheeseman, I.; *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 5022.
30. Perez-Mazliah, D.; Alvarez, M.; Cooley, G.; Lococo, B.; Bertocchi, G.; Petti, M.; Albareda, M.; Armenti, A.; Tarleton, R.; Laucella, S.; Viotti, R.; *J. Antimicrob. Chemother.* **2013**, *68*, 424.
31. Strauss, M.; Lo Presti, M.; Bazán, P.; Baez, A.; Fauro, R.; Esteves, B.; Negrete, O.; Cremonezzi, D.; Paglini-Oliva, P.; Rivarola, H.; *Parasitol. Int.* **2013**, *62*, 293.
32. Veiga-Santos, P.; Barrias, E.; Santos, J.; Moreira, T.; Carvalho, T.; Urbina, J.; Souza, W.; *Int. J. Antimicrob. Agents* **2012**, *40*, 61.
33. Willyard, C.; *Nat. Med.* **2013**, *19*, 2.
34. Nwaka, S.; Ramirez, B.; Brun, R.; Maes, L.; Douglas, F.; Ridley, R. P.; *PLOS Negl. Trop. Dis.* **2009**, *3*, 1.
35. Clayton, J.; *Nature* **2010**, *465*, S4-S5; World Health Organization (WHO); *Chagas Disease (American trypanosomiasis)*, Fact sheet No. 340, 2013. Available at http://www.who.int/mediacentre/factsheets/fs340/en/ accessed in April, 2014.
36. Viegas-Junior, C.; Danuello, A.; da Silva Bolzani, V.; Barreiro, E. J.; Fraga, C. A.; *Curr. Med. Chem.* **2007**, *14*, 1829.
37. da Silva, L. E.; Joussef, A. C.; Pacheco, L. K.; da Silva, D. G.; Steindel, M.; Rebelo, R. A.; *Bioorg. Med. Chem.* **2007**, *15*, 7553.
38. Pagliero, R. J.; Lusvarghi, S.; Pierini, A. B.; Brun, R.; Mazzieri, M. R.; *Bioorg. Med. Chem.* **2010**, *18*, 142.
39. Bocanegra-Garcia, V.; Villalobos-Rocha, J.; Nogueda-Torres, B.; Lemus- Hernandez, M.; Camargo-Ordoñez, A.; Rosas-Garcia, N.; Rivera, G.; *Med. Chem.* **2012**, *8*, 1039.
40. Kim, J. H.; Ryu, H. W.; Shim, J. H.; Park, K. H.; Withers, S. G.; *ChemBioChem* **2009**, *10*, 2475.
41. Campo, V. L.; Carvalho, I.; da Silva, C. H. T. P.; Schenkman, S.; Hill, L.; Nepogodiev, S. A.; Field, R. A.; *Chem. Sci*. **2010**, *1*, 507.
42. Carvalho, I.; Andrade, P.; Campo, V. L.; Guedes, P. M. M.; Sesti-Costa, R.; Silva, J. S.; Schenkman, S.; Dedola, S.; Hill, L.; Rejzek, M.; Nepogodiev, S. A.; Field, R. A.; *Bioorg. Med. Chem.* **2010**, *18*, 2412.
43. Campo, V. L.; Sesti-Costa, R.; Carneiro, Z. A.; Silva, J. S.; Schenkman, S.; Carvalho, I.; *Bioorg. Med. Chem.* **2012**, *20*, 145.
44. Martins-Teixeira, M. B.; Campo, V. L.; Biondo, M.; Sesti-Costa, R.; Carneiro, Z. A.; Silva, J. S.; Carvalho, I.; *Bioorg. Med. Chem.* **2013**, *21*, 1978.
45. Muthana, S.; Yu, H.; Huang, S.; Chen, X.; *J. Am. Chem. Soc*. **2007**, *129*, 11918.
46. Bräse, S.; Gil, C.; Knepper, K.; Zimmermann, V.; *Angew. Chem*., *Int. Ed.* **2005**, *44*, 5188.
47. Barral, K.; Moorhouse, A. D.; Moses, J. E.; *Org. Lett.* **2007**, *9*, 1809.
48. Kappe, C. O.; *Angew. Chem., Int. Ed.* **2004**, *43*, 6250.
49. Das, J.; Patil, S. N.; Awasthi, R.; Narasimhulu, C. P.; Trehan, S.; *Synthesis* **2005**, *11*, 1801.
50. Zhang, F.; Moses, J. E.; *Org. Lett.* **2009**, *11*, 1587.
51. Huisgen, R.; *Angew. Chem., Int. Ed. Engl*. **1963**, *2*, 633; Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B.; *Angew. Chem., Int. Ed*. **2002**, *41*, 2596; Tornøe, C. W.; Christensen, C.; Meldal, M. J.; *J. Org. Chem.* **2002**, *67*, 3057; Bodine, K. D.; Gin, D. Y.; Gin, M. S.; *J. Am. Chem. Soc*. **2004**, *126*, 1638; Aragão-Leonetti, V.; Campo, V. L.; Gomes, A. S.; Field, R. A.; Carvalho, I.; *Tetrahedron* **2010**, *66*, 9475.
52. Wilkinson, B. L.; Bornaghi, L. F.; Houston, T. A.; Innocenti, A.; Vullo, D.; Supuran, C. T.; Poulsen, S. A.; *J. Med. Chem.* **2007**, *50,* 1651.
53. Neres, J.; Buschiazzo, A.; Alzari, P. M.; Walsh, L.; Douglas, K. T.; *Anal. Biochem.* **2006**, *357*, 302.
54. Neres, J.; Bonnet, P.; Edwards, P. N.; Kotian, P. L.; Buschiazzo, A.; Alzari, P. M.; Bryce, R. A.; Douglas, K. T.; *Bioog. Med. Chem.* **2007**, *15*, 2106.
55. Guedes, P. M. M.; Oliveira, F. S.; Gutierrez, F. R.; da Silva, G. K.; Rodrigues, G. J.; Bendhack, L. M.; Franco, D. W.; do Valle Matta, M. A.; Zambonim, D. S.; da Silva, R. S.; Silva, J. S.; *Br. J. Pharmacol.* **2010**, *160*, 270.
56. Silva, J. J. N.; Pavanelli, W. R.; Gutierrez, F. R. S.; Lima, F. C. A.; Silva, A. B. F.; Silva, J. S.; Franco, D. W.; *J. Med. Chem.* **2008**, *51*, 4104.
57. Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R.; *Purification of Laboratory Chemicals*, 2nd ed., Pergamon: New York, 1980.
58. Schenkman, S.; Chaves, L. B.; de Carvalho, L. C. P.; Eichinger, D. J.; *Biol. Chem*. **1994**, *269*, 7970.
59. Buckner, F. S.; Verlinde, C.; LaFlamme, A. C.; VanVoorhis, W. C.; *Antimicrob. Agents Chemother*. **1996**, *40*, 2592.

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