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Binding of triazole-linked galactosyl arylsulfonamides to galectin-3 affects *Trypanosoma cruzi* cell invasion

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ABSTRACT

The synthesis of the O-3 triazole-linked galactosyl arylsulfonamides **1**–**7** as potential inhibitors of *Trypanosoma cruzi* cell invasion is described. These target compounds were synthesized by Cu(I)-catalysed azide-alkyne cycloaddition reaction ('click chemistry') between different azide arylsulfonamides and the alkyne-based sugar 3-O-propynyl-βGalOMe. Inhibition assays of *T. cruzi* cell invasion with compounds **1**–**7** showed reduced values of infection index (~20) for compounds **3** and **5**, bearing the corresponding 5-methylisoxazole and 2,4-dimethoxypyrimidine groups, which also presented higher binding affinities to galectin-3 (EC_{50} 17–18 μM) in Corning Epic label-free assays. In agreement with experimental results, the assessment of the theoretical binding of compounds **1**–**7** to galectin-3 by MM/PBSA method displayed higher affinities for compounds **3** (−9.7 kcal/mol) and **5** (−11.1 kcal/mol). Overall, these achievements highlight compounds **3** and **5** as potential *T. cruzi* cell invasion blockers by means of a galectin-3 binding-related mechanism, revealing galectin-3 as an important host target for design of novel anti-trypanosomal agents.

1. Introduction

Chagas disease is among the most prevalent neglected tropical diseases, affecting an estimated 6 to 7 million people mostly with low socioeconomic status. Although this disease is endemic in Latin America, it is spreading as a worldwide threat due to globalization and population mobility to North America, Europe and others areas where it is non-endemic.[1,2] Chagas disease is mainly transmitted by triatomine bugs that carry the protozoan parasite *Trypanosoma cruzi*, and make its eradication exceptionally difficult; furthermore, due to parasite's efficient ways of evading the host immune system, vaccination remains as a great challenge.[2,3] Thus, the use of anti-trypanosomatid drugs is the unique choice for treatment and still relies only on benznidazole and nifurtimox, drugs with poor efficacy in chronic infection phase and recognized toxicity.[3] In this regard, development of multi-target drug candidates able to block parasite invasion in the early stages of infection may represent an effective strategy against this neglected disease.

T. cruzi adhesion and invasion into host cells are regulated by complex interactions among parasite cell surface components, such as *trans*-sialidase (TcTS),[4] mucins[5] and cruzipain,[6] as well as galectins-3 and -1,[7,8] and Toll-like receptors present in host cells.[9,3] *T. cruzi trans*-sialidase is anchored to the parasite membrane by glycosylphosphatidylinositol (GPI) and has a fundamental role on *T. cruzi* invasion since it catalyses the transfer of sialic acid from host glycoconjugates to β-galactopyranosyl acceptor groups on *T. cruzi* mucins.[4,10–12] This transglycosylation reaction enables the parasite to be recognized by host cells and protects it against complement-independent lyses induced by human anti-α-galactosyl antibodies due to the generated negatively charged sialylated barrier.[13,14] In addition, TcTS may induce the early escape of *T. cruzi* from lysosome-enriched parasitophorous vacuole (PV) formed in the host cell by removing sialic acid from lysosome-associated membrane glycoproteins (LAMP), followed by disruption of PV membranes with the aid of a parasite trypsin sensitive enzyme (TcTox).[15] In turn, *T. cruzi* mucins (TcMUC) are also GPI anchored plasma membrane glycoproteins (about 60% carbohydrate by weight) highly expressed on *T. cruzi* surface, with about 2×10^6 copies per parasite. TcMUC play a significant role in the parasite

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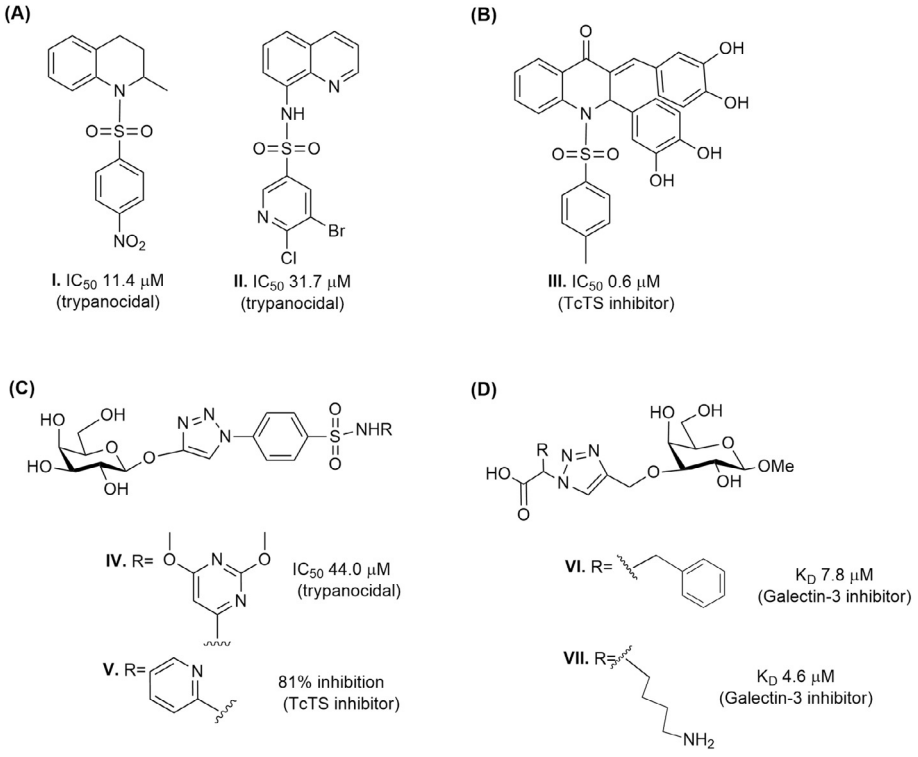

Fig. 1. Representative compounds with significant trypanocidal effect (**I**, **II** and **IV**), and inhibitory activities against TcTS (**III** and **V**) and galectin-3 (**VI** and **VII**).

recognition process and invasion of host cells based on its acceptor substrate preferences for sialic acid, transferred by TcTS.[16,17]

On the other hand, galectin-3 is a carbohydrate-binding lectin found in different host cell compartments that is also importantly involved in *T. cruzi* infection. It contains a conserved carbohydrate recognition domain (CRD) with affinity for β-galactosides containing glycoconjugates and acts as one of the receptors that mediates the adhesion of the parasite to the host cells, besides being involved in cell invasion and intracellular trafficking of the parasite.[3,7,18] Galectin-3 is able to interact effectively with both *T. cruzi* surface mucins and laminin present in the extracellular matrix, establishing thus a bridge between these two components, which increases the recruitment of trypomastigotes to the extracellular matrix and their adhesion to cells.[18] Galectin-3 can also act as marker of the cell biology events in *T. cruzi* infection, since it is able to interact with lysosome-associated membrane glycoproteins (LAMP) of macrophage cell phagosomes, accumulating around *T. cruzi* parasites that lysed the parasitophorous vacuole during the host cell infection.[19]

Novel compounds from distinct chemical classes have been recently reported as antitrypanosomal agents.[20–28] For instance, potent trypanocidal activities have been described for tetrahydroquinoline-sulfonamide derivatives against epimastigote forms (Y strain) (**I**,[25] IC_{50} 11.4 μM and **II**,[26] IC_{50} 31.7 μM) (Fig. 1A), as well as strong inhibitory activity towards TcTS by chalcone-derived sulfonamides (**III**,[27] IC_{50} 0.6 μM) (Fig. 1B). In addition, a series of seven galactosyl-triazolo-benzenesulfonamides comprising β-galactosyl unit anomeric-linked to distinct arylsulfonamides via triazole ring was reported by Carvalho et al.,[28] being verified significant trypanocidal activity against trypomastigote forms (Tulahuen strain) for the 2,4-dimethoxypyrimidine-derivative **IV** (IC_{50} 44.0 μM), and strong inhibition against TcTS for the pyridine-derivative **V** (81% at 1 mM) (Fig. 1C). Differently from this series containing anomeric triazole-substituted galactosyl sugar, we had described the synthesis of 1,2,3-triazole amino acids-derived-3-O-galactosides as galectin-3 inhibitors, based on the known potential of O-3 triazole-galactose analogs to interact with galectin-3 CRD. High binding affinities for galectin-3 were verified for Phe (**VI**)/ Lys (**VII**)-derived-3-O-galactosides (Fig. 1D), with corresponding K_D values of 7.8 μM and 4.6 μM.[29]

All these findings led us to postulate that novel hybrid glycoconjugates represented by 1,2,3-triazole arylsulfonamides-derived-3-O-galactosides, such as **1**–**7** (Fig. 2), may hamper *T. cruzi* cell invasion by possible dual inhibition of TcTS and galectin-3. Therefore, here we describe the synthesis of O-3 triazole-linked galactosyl arylsulfonamides **1**–**7**, via microwave-assisted Cu(I) 1,3-dipolar azide-alkyne cycloaddition (CuAAC), along with their evaluation on *T. cruzi* cell invasion process and inhibition of both TcTS and galectin-3.

2. Results and discussion

2.1. Synthesis

The syntheses of the O-3 triazole-linked galactosyl arylsulfonamides **1**–**7** by CuAAC required the previous preparation of the

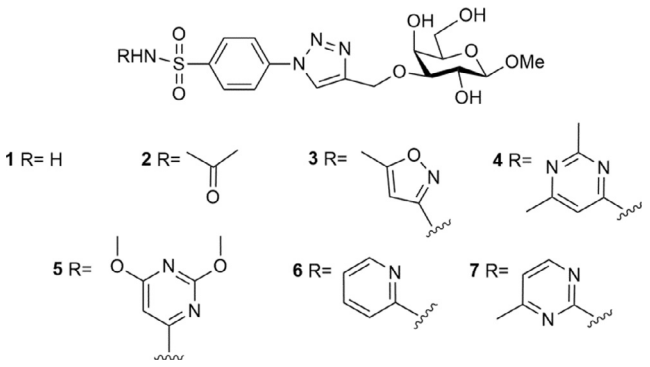

Fig. 2. Chemical structures of the target triazole-linked galactosyl arylsulfonamides **1**–**7**.