Rational drug design in parasitology: *trans*-sialidase as a case study for Chagas disease

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***Trypanosoma cruzi trans*-sialidase is a potential target for Chagas disease chemotherapy. From the specific need of *T. cruzi* to obtain sialic acid through *trans*-sialidase-mediated transfers from host sources and the lack of alternative to this for the parasite, a good case can be made for *T. cruzi trans*-sialidase to serve as a potential drug target against Chagas disease. This review deals with both the particular aspects relevant to *T. cruzi trans*-sialidase as a target and generalises the situation for drug design in its broader aspects on the basis of some special problems in terms of rational drug design that *T. cruzi trans*-sialidase raises, particularly those of multiple gene copies and active site plasticity.**

Chagas disease status and potential drug targets

Chagas disease, caused by the protozoan *Trypanosoma cruzi* and transmitted to humans by infected blood-sucking reduviid bugs of the subfamily *Triatominae*, remains a substantial health problem in Latin America. In 2001, there were approximately 10 million people infected and 13,000 deaths associated with Chagas disease [1]. Initiatives launched across South America to control and prevent transmission by the insect vector, by use of residual insecticides inside houses, led to a remarkable decrease in the number of new disease cases reported in recent years. Increasing blood donor screening has also contributed to the decline of the number of new cases of Chagas disease [1,2].

In spite of the success of these various initiatives, improved diagnosis and treatment methods are still not available. The widespread distribution of sylvan populations of *Triatominae* in Central and South America, and recent reports on the resistance of these insects to the insecticides commonly used for their destruction, are also reasons for concern [3].

Nifurtimox and benznidazole, the only two drugs approved for treatment of Chagas disease, were launched by Bayer in 1967 and Roche in 1972, respectively. No alternatives have been found, even though Chagas disease chemotherapy has been the subject of research for many years. Drug design approaches being taken against various *T. cruzi* biochemical targets have been reviewed [4–6]. One potential target for Chagas disease chemotherapy is *T. cruzi trans*-sialidase (TcTS) [7–11]. However, no strong inhibitors of this enzyme are known; therefore, this drug target has not been fully validated yet. We now discuss TcTS as a possible drug target, along with recent results in this field.

Sialic acids, sialidases and *trans*-sialidases

The large sialic acid family, naturally occurring analogues of *N*-acetylneuraminic acid (sialic acid, **1**, Figure 1), is involved in many physiological phenomena. Generally found on glycoproteins and gangliosides in the extracellular medium or on the outer cell membranes, sialic acids often mask recognition sites for the immune system that could result in auto-immune responses [12].

Sialic acids are also important as recognition sites in the pathogenic processes caused by toxins and microorganisms [8,13]. Sialidases or neuraminidases catalysing de-sialylation of various glycoconjugates have been implicated as virulence factors in disease caused by various bacteria or viruses. In *Vibrio cholerae*, the sialidase-mediated removal of sialic acids from gangliosides creates GM_1, the binding site for the cholera toxin [8,10]. The sialidase from Influenza virus helps the elution of newly synthesised virions from infected cells and assists the movement of the virus through the mucus in the respiratory tract [14].

Sialidases are also expressed by some protozoan parasites, including the trypanosomes *T. brucei*, *T. cruzi* and *T. rangeli*.

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1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2007.12.004

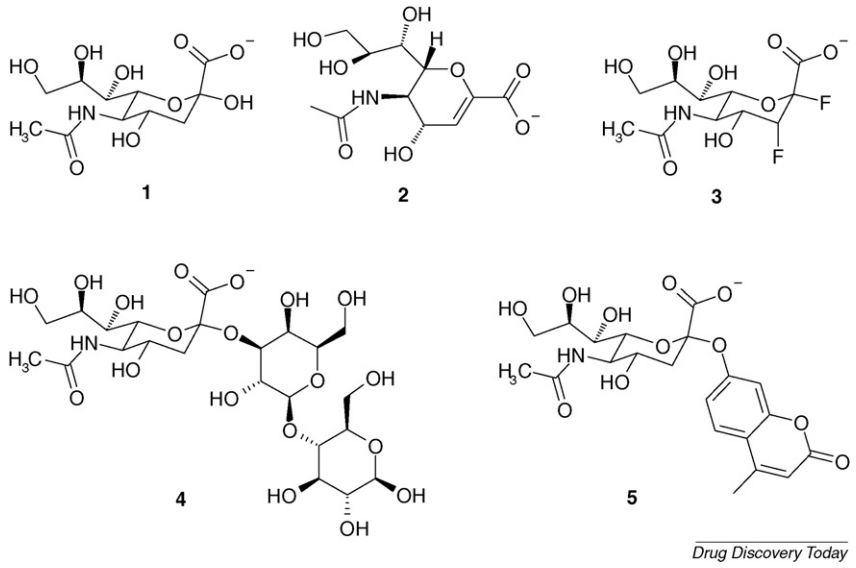


FIGURE 1

Structures of *N*-acetylneuraminic acid (sialic acid, **1**) and of ligands co-crystallised with TcTS (**2**–**5**).

In *T. brucei*, the causative agent of human African trypanosomiasis, and *T. cruzi*, these enzymes have mainly *trans*-sialidase activity, that is they transfer sialic acids from one glycoconjugate to another [8,15,16]. *T. brucei* expresses *trans*-sialidases only in the insect stages of its life cycle, contrary to the case in *T. cruzi*, where *trans*-sialidase is mainly expressed in the mammalian stages [8,15]. *T. rangeli*, an American trypanosome related to *T. cruzi*, expresses a sialidase with no *trans*-sialidase activity but with high amino acid sequence identity to the enzyme of *T. cruzi* [17].

T. cruzi trans-sialidase

The first report of sialidase activity in *T. cruzi* [18] found it present in bloodstream trypomastigotes but absent from the intracellular amastigote forms. Later, *trans*-sialidase activity mediated by parasite membrane-anchored enzymes was described [19,20], and the sialidase and *trans*-sialidase activities were shown to be mediated by the same enzyme [21], thenceforth known as *T. cruzi trans*-sialidase (TcTS).

TcTS is coded by genes that are part of a large gene family, with 1430 gene members, generally called the *trans*-sialidase superfamily [22], which encodes hundreds of proteins ranging in size from 60 to more than 200 kDa [16,23,24]. Of these, 12 encode enzymatically active TcTS, 725 code for inactive TcTS-like proteins, and 693 are *trans*-sialidase pseudogenes [22]. The key difference between active and inactive members is the Tyr342His mutation [25]. Most active TcTS proteins contain tandem repeats of a 12 amino acid sequence, SAPA (shed acute phase antigen) repeats, in the C-terminal region [23]. Additional variation within the TcTS families is also conferred by different degrees of glycosylation [26]. These variants present rational drug design with a problem that has not yet been reviewed to our knowledge. However, the situation may not be entirely bleak if one considers that, in spite of the huge size of the kinome with 518 members, selective and therapeutically usable intervention has been achieved for cancer treatment with Iressa, for example [27].

TcTS plays key roles in the pathogenesis of Chagas disease

TcTS family members play different roles in the pathogenesis of Chagas disease owing to differences in their structure (e.g. presence/absence of the SAPA domain, enzymatically active or inactive) and their expression levels at the different stages of *T. cruzi* life cycle [16].

The surface of invasive *T. cruzi* trypomastigotes is covered by numerous *trans*-sialidases and also diverse mucin-like proteins, all attached to the membrane by GPI (glycosylphosphatidylinositol) anchors [16,28]. *T. cruzi* cannot synthesise sialic acid *de novo*, and the main role of active TcTS forms is to acquire sialic acid units from mammalian host glycoconjugates and transfer them to terminal β-Gal residues on the mucins that cover the parasite membrane.

When sialic acids are transferred by TcTS to the trypomastigote surface they provide (i) direct protection of terminal β-Gal residues in trypomastigote mucins from recognition by the host immune system, and indirect protection of terminal α-Gal residues by their negative charges [29] and (ii) resistance to complement and immediate survival of trypomastigotes released to the bloodstream [30]. TcTS also aids in recognition and attachment to host cells through active site-mediated binding to sialic acids and/or β-galactosyl residues on the surface of host cells [31–35] or through domains distinct from the active site [36,37]. TcTS sheds into the bloodstream, following cleavage of the GPI anchor, and removes sialic acid from the platelet surface, causing thrombocytopenia in the acute phase of Chagas disease [38,39]. TcTS also induces apoptosis in the spleen, thymus and peripheral ganglia [40,41], but activation and prevention of apoptosis of T lymphocytes have been reported additionally for shed forms of TcTS [42].

TcTS structure and catalysis mechanism

High-resolution crystal structures of a fully active TcTS mutant, lacking the C-terminal SAPA domain, showed a globular N-terminal domain of 372 residues. This forms a six-bladed β-propeller that contains the active site, connected through a long α-helix (23

amino acids) to a C-terminal lectin-like domain of 238 residues, which does not participate in the *trans*-glycosylation activity of the enzyme [9]. Currently available crystal structures include free TcTS, and TcTS in complex with various molecules (Figure 1), including the general inhibitor of most sialidases/neuraminidases DANA (**2**), thought to represent a transition-state analogue neuraminidase inhibitor, and lactose (sialic acid acceptor substrate) [9]. Structures with the TcTS substrates sialyllactose (**4**) and 4-methylumbelliferyl sialoside MuNANA (**5**) were obtained with an inactive TcTS mutant (Asp59Ala). The structure of TcTS in complex with 2,3-difluorosialic acid (di-F-NANA, **3**) shows the ligand covalently attached to Tyr342 [43].

The active site of TcTS contains several conserved microbial sialidase features, for example, an arginine triad (Arg35, Arg245 and Arg314), which interacts with the carboxylate group of sialic acid, two important residues for stabilisation of the transition-state (Tyr342 and Glu230), an aspartate (Asp59) essential for catalysis, and a hydrophobic pocket, accommodating sialic acid's *N*-acetyl group [9].

In addition to these conserved features, a second site, the sialic acid acceptor site, which accommodates the galactose and penultimate sugar moieties of the sialic acid donor and acceptor substrates, plays a key role in the chemical mechanism [9,43,44]. The two main residues of the sialic acid acceptor site (Tyr119 and Trp312) are crucial for the *trans*-sialidase activity of TcTS, and for its selectivity towards α-2,3 sialosides. Mutations in these residues led to loss of *trans*-sialidase activity, and/or loss of substrate specificity [44]. Tyr119 acquires various conformations in the presence of ligands or in unbound TcTS [9].

The mode of action of TcTS implies two catalytic functions for its active site: hydrolysis of sialic acid from the donor substrate plus transfer of the same sialic acid to an acceptor substrate [9,43,45].

The currently accepted catalytic mechanism for TcTS is shown in Figure 2A. On binding a sialic acid donor substrate in the active site, the hydroxyl group of Tyr342 reacts as a nucleophile via an S_N2-type reaction, assisted by the nearby Glu230 acting as a general base catalyst [43]. This results in a covalently bound TcTS-sialoside intermediate [43,46]. Asp59 protonates the leaving

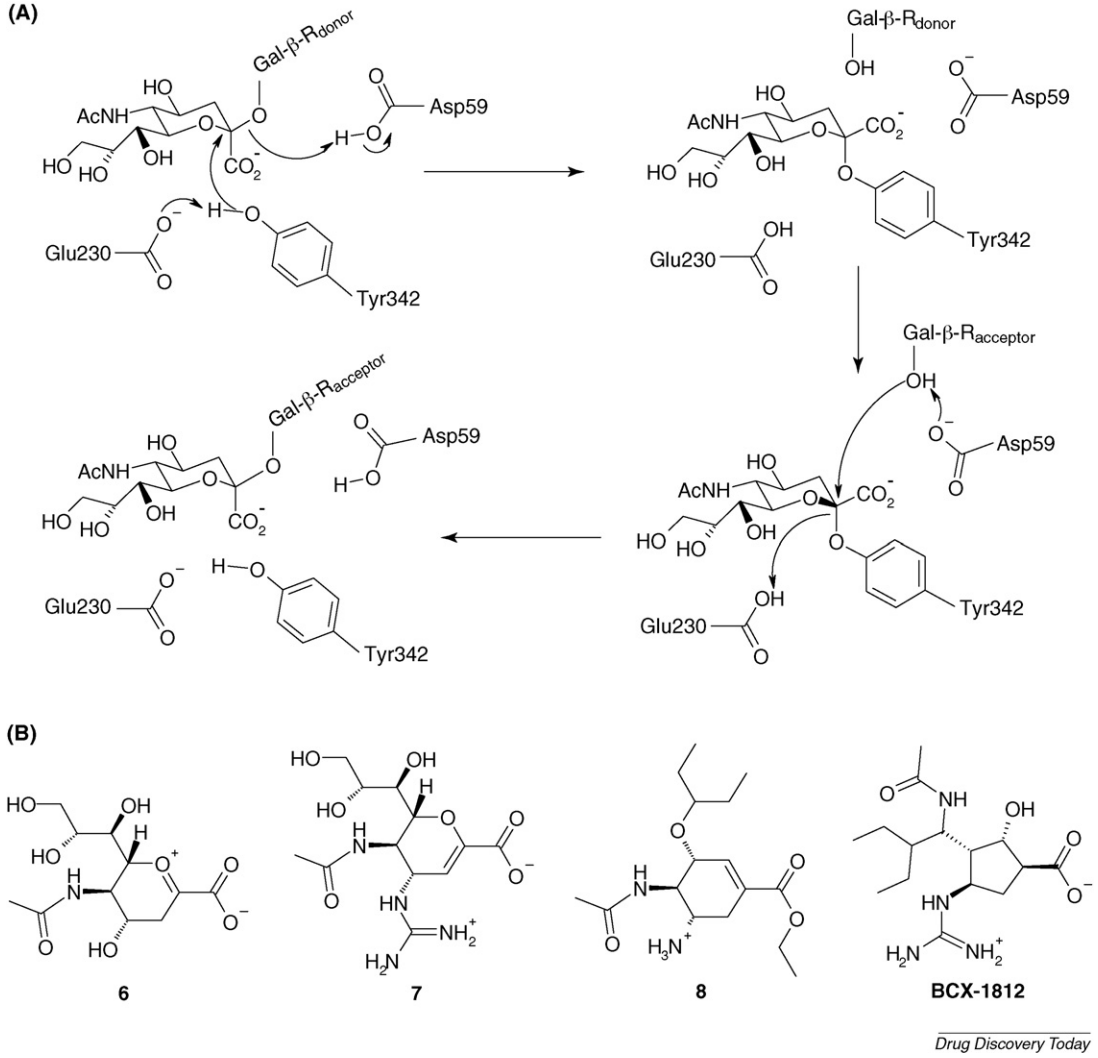


FIGURE 2

(A) Catalytic mechanism for TcTS-mediated transfer of sialic acid between terminal β-galactoside glycoconjugates; **(B)** structure of the sialosyl oxocarbenium cation (**6**) and transition-state analogue inhibitors of Influenza neuraminidase (**7**, **8** and BCX-1812).

group in the first S_N2 reaction, and is a base catalyst for the second step, again S_N2-type, by accepting the proton from the 3-hydroxyl group of the acceptor galactoside [43]. This results in overall retention of configuration of the sialic acid moiety [17].

Surface plasmon resonance experiments showed that binding of lactose to the enzyme occurs only after occupancy of the sialic acid binding site [9]. Thus, TcTS acts via a bisubstrate double displacement (ping-pong) mechanism [43,46].

Is TcTS a valid drug target?

Considering the specific need of *T. cruzi* to obtain sialic acid through *trans*-sialidase-mediated transfers from host sources, the lack of alternatives to this pathway in the parasite, and other diverse roles of this enzyme in the pathogenesis of Chagas disease, a good initial case can be made for TcTS serving as potential drug target. However, in terms of developing the required novel medicinal chemistry to generate potential drug leads, this particular enzyme raises a number of questions in drug design (Box 1), important for drug design against *T. cruzi* in particular, but also for medicinal chemistry in general. The core of the problem is how to define if this protein has been validated as a drug design target—or indeed if it can be, for there are a number of special features of this enzyme that set it aside from the usual examples.

Criteria a, b and c: biochemical rationale

In terms of criteria **a** and **b**, a reasonable case can be made for TcTS as a drug target, as sialic acids in *T. cruzi* are essential for the parasite's immune–evasion mechanism, as already discussed. TcTS is mainly expressed in the mammalian stages, so it may be usable as a target against Chagas disease if other criteria are met. Other studies verify that expression of TcTS is a major virulence factor in *T. cruzi*, as invasive phenotypes are restricted to TcTS-expressing populations, and this enzyme was expressed in significantly higher levels in the more lethal strains than in less pathogenic strains [47,48]. Criterion **c** appears straightforward in this case.

Criterion d: target knockout presents a problem

Criterion **d** is simple to understand; if the target can be knocked out or ablated in some way, can the parasite survive? However, this simplicity is deceptive and raises a general problem that will increasingly be thrown up by genomics for medicinal chemistry and biochemistry, one that TcTS neatly exemplifies. The complexity of TcTS at the chromosomal level [22] means that straightforward gene deletion experiments are unlikely to be successful, given the number of (potential) sources of gene products. It may be possible to design a set of siRNAs, but this mechanism of silencing is not yet available in *T. cruzi* as it is in *T. brucei*, and therefore evaluating them *in vivo* would be complicated, to say the least [49].

Another possible means to ablate the target is to sequester it with an antibody. The importance of TcTS for the survival of *T. cruzi* trypomastigotes after infection, and for the subsequent invasion of host cells, has been verified along these lines. Use of specific antibodies that targeted sialylated epitopes or sialic acid acceptors on the parasite membrane, or the catalytic domain of TcTS, led to a significant reduction of host cell infection [31,32,35,36,50].

BOX 1

Criteria levelled as the basis of target validation in medicinal chemistry

(a) Does the aspect of parasite biochemistry offer a strong scientific rationale for chemical intervention?
(b) Are there alternatives to the biochemistry that the parasite can enlist to overcome any attack?
(c) Is the process specific to the parasite rather than the host?
(d) If the target is knocked out (e.g. gene knockout, siRNA, antibodies), is the pathology affected?
(e) Do any known drugs operate against analogous targets in diseases likely to be broadly similar in nature?
(f) Do known inhibitors of the target affect the pathology appropriately?

Criterion e: another disease has already benefited from use of similar targets

The importance of Influenza sialidase led to the rational design of strong inhibitors, based on the structure of 2-deoxy-2,3-didehydro-D-*N*-acetylneuraminic acid **2**, DANA (Figure 1), a transition-state analogue based on **6**, the sialosyl oxocarbenium cation (Figure 2B). Zanamivir (**7**), oseltamivir (**8**, an ester pro-drug) and BCX-1812 (BioCryst), are successful examples of this strategy (Figure 2B), and the first two are now marketed as anti-flu drugs (Relenza®, GSK, and Tamiflu®, Roche, respectively) [10,14]. So, on the face of it, intervention against this enzyme class can achieve therapeutic benefit in other diseases, fulfilling criterion **e**. Such ability to target Influenza enzymes in patients indicates that, at least in principle, the requisite selectivity relative to the human host is possible to achieve, fulfilling criterion **b**.

Criterion f: known inhibitors as tools

There are no strong inhibitors of TcTS yet reported. DANA binds extremely weakly to TcTS, with no cross-recognition of the Influenza sialidase inhibitors by the trypanosomal enzyme [51]. This is in spite of the active sites of TcTS and Influenza neuraminidase showing several conserved features. Thus, the development of strong specific inhibitors is the most obvious next means to provide biological tools for probing the role of TcTS and maybe even a route to potential drug leads.

Chemical inhibition of TcTS

Taking the above features together, the catalytic activity of TcTS is a potential target for the treatment of Chagas disease, especially to prevent invasion of host cells. In view of the difficulties with gene knockout and similar direct tests of the crucial nature of the target, design of powerful, specific inhibitors of TcTS activity is presently necessary to confirm this hypothesis and fulfil criterion **f**. Inhibitors of TcTS activity would also be likely to bind to inactive TcTS, given the very limited amino acid differences between the active sites of the two proteins. Compounds tested as potential TcTS inhibitors can be grouped into two categories, depending on the active site regions they target, namely sialic acid mimetics and sialic acid donor/acceptor substrate mimetics.

Sialic acid mimetics

Mimicking the sialic acid structure, a very successful strategy in inhibiting Influenza neuraminidase [14], has been explored in

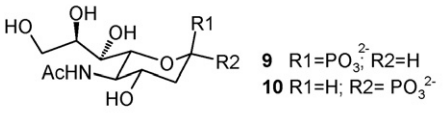
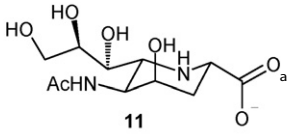
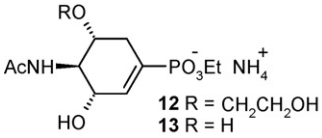
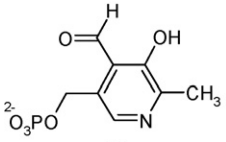
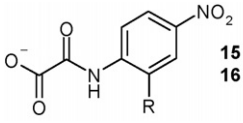
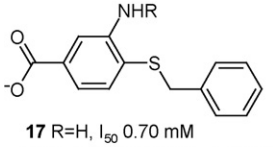
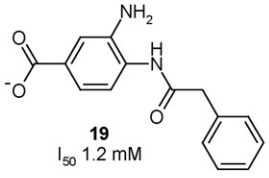
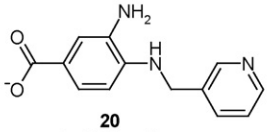
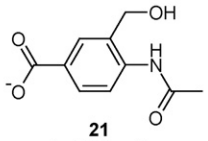
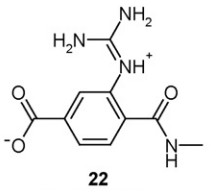
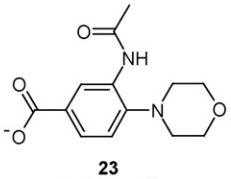
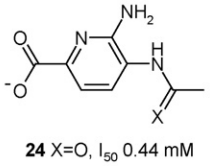
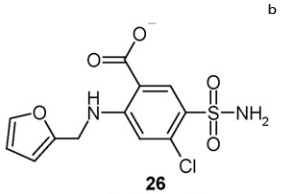
search of potential TcTS inhibitors (see Table 1). Despite its very weak inhibition of TcTS, DANA (**2**, with a reported K_i of 12.3 mM) was the first compound co-crystallised with TcTS and showed a good fit in the active site, and therefore its weak inhibition was surprising [9,52]. Interestingly, *T. rangeli* sialidase (70% sequence identity to TcTS in the catalytic domain) is inhibited by DANA with a K_i of 0.00015 mM, but in mutants of this enzyme displaying *trans*-sialidase activity, the K_i increased 10,000 fold to 1.54 mM [52]. This clearly shows that key amino acid differences (including Tyr119), responsible for the different activity of TcTS, are also responsible for the lower inhibition observed for DANA. Using a continuous fluorescence assay for TcTS [53], we found that a series of 5,6-dihydro-*4H*-pyran-2-carboxylic acid derivatives (based on the DANA ring scaffold), including zanamivir (**7**), failed to inhibit TcTS significantly at 1 mM concentrations, indicating that this is not a good scaffold from which to obtain TcTS inhibition without refining the strategy for its use. BCX-1812, with a cyclopentane scaffold, also failed to inhibit TcTS [51].

2,3-Difluorosialic acid (**3**, Figure 1) inhibits TcTS time-dependently by forming a covalent bond with the hydroxyl group of Tyr342. However, complete inactivation requires very high concentrations (20 mM), and the enzyme spontaneously recovers activity after removal of excess inactivator [46].

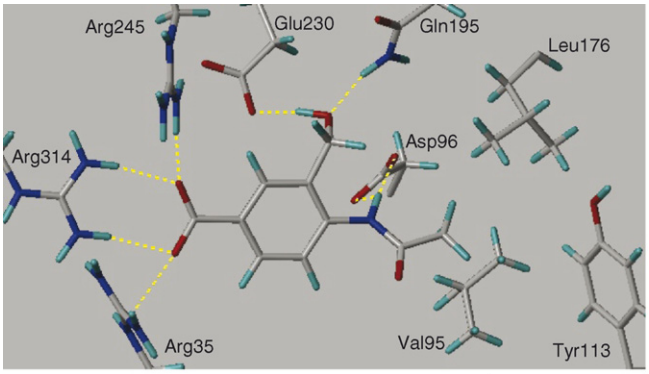
Sialic acid mimetics with a phosphonate instead of a carboxylate group, **9** and **10** (Table 1), did not inhibit TcTS, nor did **11**, another sialyl-mimetic. By contrast, these compounds were competitive inhibitors of various sialidases [54], and cyclohexenephosphonate monoalkyl esters **12** and **13** were very weak inhibitors of TcTS [55].

TABLE 1

Sialic acid mimetics tested against TcTS

Structure and TcTS inhibition	Reference
2: K_i 12.3 mM[a]	[52]
7: (zanamivir), **BCX-1812**: n.i (1 mM)[b]	[51]; Our unpublished results
5: covalent inhibitor; complete inhibition at 20 mM	[46]
 **9** R1=PO_3^{2-}, R2=H; **10** R1=H; R2=PO_3^{2-}	[54]
 **11**	
n.i. (1 mM)	
 **12** R = CH_2CH_2OH; **13** R = H	[55]
I_{50} 5 mM[a]	
 **14**	[56]
K_i 7.3 mM (non-competitive)[a]	
 **15** R = H; **16** R = F	[54]
n.i. (1 mM)[a]	
 **17** R=H, I_{50} 0.70 mM; **18** R=$COCH_2NH_3^+$, I_{50} 1.0 mM	[51]
 **19** I_{50} 1.2 mM	
 **20** I_{50} 0.74 mM	
 **21** I_{50} 0.54 mM	
 **22** I_{50} 0.76 mM	
 **23** I_{50} 0.58 mM	
 **24** X=O, I_{50} 0.44 mM; **25** X=S, I_{50} 0.54 mM	
 **26** I_{50} 0.67 mM	[b]

[a] Results obtained with sialic acid transfer assays.
[b] Results obtained with a sialic acid hydrolysis assay [53]; n.i., no inhibition.



Drug Discovery Today

FIGURE 3

Predicted binding mode of compound **21** in the active site of TcTS.

From the viewpoint of either drug lead design or possible use as *in vitro* or *in vivo* biological probes, the inhibitors above are not promising as they are complex, chiral, large and very polar. Away from complex frameworks, the only reports of TcTS inhibition are for pyridoxal phosphate (**14**) and several benzoic acid and pyridine-2-carboxylic acid derivatives (**17–26**, Table 1) [51,56]. Pyridoxal phosphate is a weak, non-competitive inhibitor (K_i 7.3 mM), but its analogues pyridoxine, pyridoxal and pyridoxamine phosphate, failed to improve inhibition [56]. *N*-(4-Nitrophenyl)-oxamic acids, **15** and **16** (Table 1), non-competitive inhibitors of *Vibrio cholerae* sialidase, do not inhibit TcTS [54].

Several benzoic acid or pyridine-2-carboxylic acid derivatives showed I_{50} values in the 0.1–1 mM range (**17–26**, Table 1) [51]. These compounds are generally weaker inhibitors of Influenza neuraminidase than their 5,6-dihydro-*4H*-pyran-2-carboxylic acid counterparts, probably owing to the inability of their flat rings to mimic the stereochemistry of the sialic acid ring system. However, some performed better against TcTS, namely **21**, **22**, **24** and **25** [51,57,58]. In the predicted docked conformation of **21** (Figure 3), its carboxylate moiety provides the expected strong interaction with the arginine triad (residues 35, 245 and 314). The hydroxyl group could form hydrogen bonds with both Glu230 and Gln195, and the amide NH group is hydrogen-bonded to Asp96, with the acetyl group located in the hydrophobic pocket. However, the apparently good fit predicted for **21** and other compounds did not translate into strong TcTS inhibition [51].

Sialic acid donor/acceptor substrate analogues

This group of compounds, aiming to mimic natural substrates for *trans*-sialylation, should occupy both the sialic acid and sialic acid donor/substrate binding sites. Alternatively, they may target essentially the sialic acid acceptor pocket, where the lactose moiety of sialic acid donors and acceptors binds. Inhibition constants for such compounds have been determined using sialic acid transfer assays.

The GM_3 ganglioside **27** (Figure 4) is a good substrate for TcTS, but when the sialic acid residue is modified, at C4 (deoxy) or C8 (deoxy or epimeric), it becomes an inhibitor with I_{50} values in the 10–100 μM range [59]. Sialic acid thiogalactosides could inhibit TcTS, owing to the greater distance between the NANA and Gal moieties, as the C–S bond is longer than C–O. However, **28**, an α2,3-sialyl-β-thiogalactoside, did not inhibit TcTS [60]. Marino *et al.* [61] described the synthesis of several 2-thioether derivatives of sialic acid as potential inhibitors of TcTS, but no inhibition data were given.

Lactitol (**29**, Figure 4), a lactose derivative, prevents sialylation of lactose, *N*-acetyl-lactosamine and 4-methylumbelliferyl-β-D-galactopyranoside, with an I_{50} of 0.57 mM determined against the latter [62,63]. Lactitol also prevented sialylation of parasite mucins by TcTS at high concentrations (low mM range), and reduced infection of mammalian cells by 20–27% [62]. However, this compound does not inhibit the enzymatic activity of TcTS, behaving as a preferential sialic acid acceptor in comparison with conventional substrates.

Recently, Agusti *et al.* [64] reported the synthesis of several oligosaccharides from *T. cruzi* mucins containing galactofuranose and galactopyranose and ranging from three to five sugar units. These molecules are able to accept sialic acid from sialyllactose in the TcTS-catalysed reaction, though in most cases with lower efficiency when compared with that of *N*-acetyllactosamine. Similarly to lactitol [62], these oligosaccharides prevent sialic acid transfer to 4-methylumbelliferyl-β-D-galactopyranoside, with I_{50} values for competitive inhibition of between 0.6 and 4.4 mM. The pentasaccharide alditol **32** (Gal*p*(β1 → 2)[Gal*p*(β1 → 3)]Gal*p*(β1 → 6)[Gal*f*(β1 → 4)]GlcNAc-4)]GlcNAcol) was the most active inhibitor in this series (I_{50} 0.61 mM). Related compounds **30** and **31** showed similar activity (I_{50} 0.85 mM and 0.70 mM, respectively). However, none of the oligosaccharides showed an improvement to the TcTS inhibitory activity of lactitol. The complexity and high molecular weight of these compounds also make them unattractive as potential drug leads.

Following the results obtained for sialyl-mimetic cyclohexene phosphonates **12** and **13** (Table 1), galactose–phosphonate derivatives that target the sialic acid acceptor site of TcTS have been recently reported [63]. Compounds **33**, **35**, **37** and **38** targeted the acceptor site (lactose moiety) and the arginine triad of the sialic acid site (phosphonate group), whereas **34**, **36**, and **39–41** targeted both the sialic acid and sialic acid acceptor sites. Inhibition was weak, the strongest being **35** and **36** (I_{50} 3 and 1.5 mM, respectively). No inhibition was observed for **34**, and all the remaining compounds (**33**, **37-41**) showed I_{50} values above 6 mM.

From this survey, it is evident that there are currently no good lead compounds as starting points for TcTS inhibitor design, and no strong, drug-like inhibitors described for this enzyme. Various approaches can be taken to address this. Firstly, despite the differences between the active sites of Influenza neuraminidase and TcTS, it is important to evaluate available compounds designed against the former against TcTS, as in principle considerable time could be saved in inhibitor design and synthesis. Secondly, computer-based methods such as virtual screening and *de novo* design algorithms can be used to inform ligand design. Of course, knowledge-guided screening can also be carried out. An additional factor, alluded to earlier and, which may bear upon the discrepancy between predicted and observed inhibitory potency, relates to the plasticity of the TcTS binding site. In particular, Tyr 119, which forms part of the acceptor sugar site, occupies a number of distinct conformations in the solved crystal structures of TcTS. The ability to account for protein flexibility in inhibitor design remains

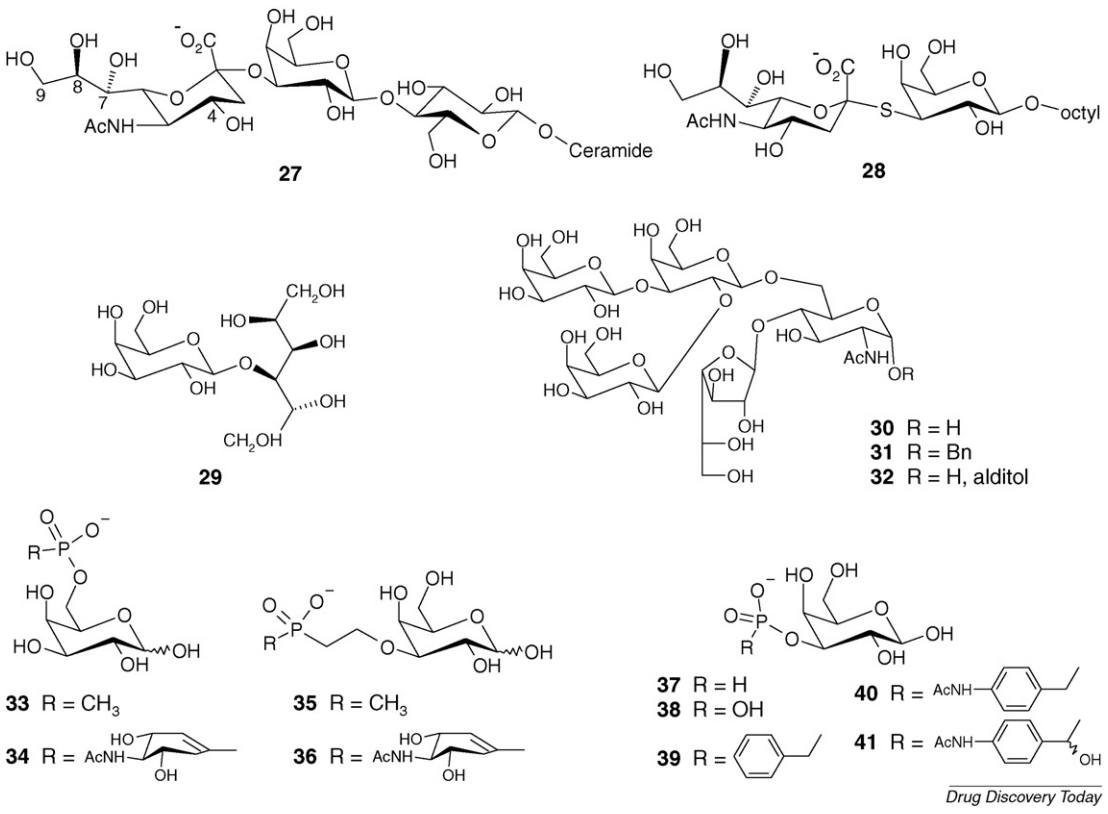


FIGURE 4

Sialic acid donor substrate mimetics (**27**, **28**, **34**, **36**, **39**–**41**) and sialic acid acceptor substrate mimetic (**29**–**33**, **35**, **37**, **38**) reported or tested as TcTS inhibitors.

a major challenge for current computational methodologies and should be addressed in future structure-based TcTS inhibitor design studies [65].

Streicher [10] has pointed out that, although strong inhibitors have been discovered against Influenza virus enzyme based on DANA, inhibitor design against the sialidases or *trans*-sialidases of bacteria or trypanosomes has been much less straightforward, and also much less explored. It is also important to note that most of the weak TcTS inhibitors described in the literature were not specifically designed against TcTS, but this does not fully explain the weak inhibition observed for some of them. The most striking example is DANA, as available structural data showed an apparently good fit and favourable interactions of this compound in the active site of TcTS [9]. The causes of this anomaly must also be found.

Outlook

The TcTS case history provides an example of a potential biological target that may be useful for drug design, but that is a special case of a complex genetic profusion of copies and types of closely related gene product, making straightforward genetic disruption methods more difficult than usual. Nevertheless, there are good biochemical reasons to probe it further. Chemical evidence of the enzyme's essential role in the parasite is still lacking, as there are no known strong specific chemical inhibitors of TcTS, or drugs known to act against it. However, the protein also presents structural challenges for the designer of ligands *in silico*.

Acknowledgements

We are grateful for gifts of neuraminidase inhibitor samples to GSK (Stevenage, U.K.) and BioCryst (Birmingham, AL, U.S.A.).

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