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Tetrafluorophenoxymethyl ketone cruzain inhibitors with improved pharmacokinetic properties as therapeutic leads for Chagas' disease

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ABSTRACT

Inhibition of the cysteine protease cruzain from *Trypanosoma cruzi* has been studied pre-clinically as a new chemotherapeutic approach to treat Chagas' disease. Efficacious effects of vinylsulfone-based cruzain inhibitors in animal models support this therapeutic hypothesis. More recently, substrate–activity screening was used to identify nonpeptidic tetrafluorophenoxymethyl ketone inhibitors of cruzain that showed promising efficacy in animal models. Herein we report efforts to further optimize the in vitro potency and in vivo pharmacokinetic properties of this new class of cruzain inhibitors. Through modifications of the P1, P2 and/or P3 positions, new analogs have been identified with reduced lipophilicity, enhanced potency, and improved oral exposure and bioavailability.

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Chagas' disease is a parasitic infection caused by the parasite *Trypanosoma cruzi* and is transmitted to the human host via the triatominae subfamily of reduviidae insects.[1–3] While the acute phase of the disease is treatable, the chronic phase of infection is more intractable, requiring extended therapy with high doses of poorly tolerated drugs like nifurtimox and benznidazole.[4,5] Moreover, cardiac damage arising from chronic, asymptomatic infection has made Chagas' disease the leading cause of heart disease in Latin America. Resistance to nifurtimox and benznidazole is also on the rise, highlighting the need for safe and effective new agents that act by distinct molecular mechanisms.

The parasite protease cruzain is the major cysteine protease activity in *T. cruzi*, with important roles throughout the parasite life cycle.[6] The vinylsulfone K777 (**1**) is an irreversible inhibitor of several mammalian and parasite cysteine proteases, including cruzain (Fig. 1). The compound is moderately orally bioavailable (~5–20%) and has proven efficacious in several parasitic disease models. For example, in a dog model of Chagas' disease, oral treatment with **1** at 50 mg/kg BID for 14 days reduced parasitemia below levels detectable by hemocytometry and partially protected animals from cardiac tissue damage.[7]

Although **1** has proved invaluable in validating cysteine protease inhibition as a viable therapeutic approach in parasitic diseases, the compound exhibits several suboptimal features from a drug development perspective. In particular, **1** exhibits nonlinear dose–exposure pharmacokinetics, irreversibly inhibits CYP3A4,[8] and is a substrate for P-glycoprotein. On the basis of a chemoproteomic analysis employing the *N*-propargyl analog **2**, Renslo and co-workers reported[9] that the major target of **1**, at least in cell culture, was mammalian cathepsin B of the host cell. This finding may reflect the protonatable, lysosomotropic nature of **1**, a property that has previously derailed clinical development of cathepsin K inhibitors for osteoporosis.[10,11]

In parallel with the preclinical development of first-generation protease inhibitor **1,** various groups have sought to identify next-generation cruzain inhibitors that are more selective and less peptidic in nature. Thus, in a survey of non-peptidic P2/P3 moieties, Renslo and co-workers identified the vinyl sulfone **3** and solved a high-resolution structure of **3** bound to cruzain.[12] Contemporaneously, Ellman and co-workers reported the co-crystal structure of the non-peptidic inhibitor **4**,[13] which was discovered using substrate–activity screening.[14] Comparing these X-ray structures with earlier structures of **1** revealed that both peptidic and non-peptidic inhibitors make contact with the S1′–S3 subsites of cruzain and share important hydrogen bonding interactions, such as with the backbone carbonyl of Asp161. Interestingly, in inhibitor **4** it is the C–H bond at C-5 of the triazole ring that donates this hydrogen bond. The higher affinity of **4** compared to **3** for cruzain may arise in part from an additional hydrogen bond between the P3 quinoline nitrogen atom and Ser61, and from a water-mediated interaction between the basic amine and

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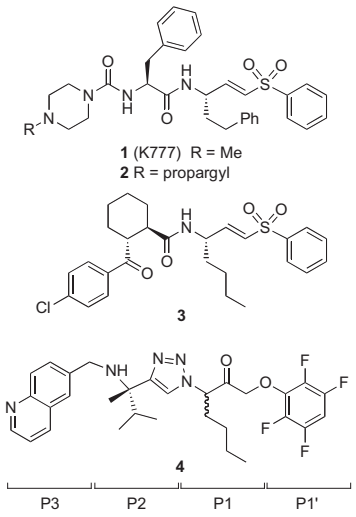

Figure 1. Structure of the prototypical peptidic cruzain inhibitor **1** (K777), the related activity-based probe **2**, and non-peptidic cruzain inhibitors **3** and **4**. The P1′–P3 side chains are indicated.

Glu208. Compound **4** was effective against *T. cruzi* parasites in two different cell-based assays and when administered to *T cruzi* infected mice at 20 mg/kg BID (ip) for 27 days, compound **4** afforded a haematological cure in 2/4 treated animals.[13]

Herein we describe efforts to improve the potency and pharmacokinetic (PK) properties of tetrafluorophenylmethyl ketone inhibitors derived from **4**. In vivo PK profiling of **4** in mice revealed moderate half-life and clearance values and reasonable bioavailability. These in vivo data were correlated with in vitro ADME surrogates to guide an optimization strategy focused on reducing lipophilicity while maintaining or improving upon the in vitro biochemical and antiparasitic activity. Structure-aided design was employed to select P1–P3 side chains that would retain key hydrophobic and hydrogen bonding interactions while reducing overall lipophilicity (calculated as *A*log*P* in Vortex, Dotmatics). Guided by this improved analogs such as **21** were developed that exhibit improved anti-trypanosomal activity in vitro, combined with superior oral exposure, half-life, and bioavailability as compared to **4**.

The in vivo pharmacokinetic properties of **4** were evaluated to establish a baseline for further optimization work. In vitro ADME parameters were also determined in the hope that in vitro surrogates could be correlated with key in vivo parameters. The in vivo PK profile of **4** in mice turned out to be quite reasonable as a starting point for further optimization. Hence, the compound exhibits a reasonably long half-life in mice ($T_{1/2}$ = 3.3 h), moderate clearance (CL = 36.2 mL/min/kg), and oral bioavailability of ~20%. A steady-state volume of distribution (Vss) of 4.2 L/kg suggested good tissue penetration, as is desirable for a Chagas' therapeutic.[15] Like **1**, compound **4** inhibits CYP3A4 in vitro in the low μM regime (CYP 3A4 IC_{50} = 3.8 μM). Despite being highly lipophilic (*A*log*P* = 7.0) compound **4** was found to exhibit reasonable stability to cultured liver microsomes, consistent with the long half-life observed in mice ($T_{1/2}$ ~3.3 h). Permeability in an MDCK cell monolayer assay was modest-to-low and solubility was qualitatively estimated to be low as well, both factors likely contributing to the modest bioavailability observed. Thus, an initial target of the optimization campaign was to reduce lipophilicity, with the expectation that improvements in solubility and permeability would contribute to greater bioavailability and overall exposure on oral dosing.

To improve both potency and in vivo exposure, we sought to identify new P1–P3 moieties that would retain the hydrogen bond to Ser61 while reducing overall lipophilicity (Fig. 1). We were aided in this effort by inspection of the previously disclosed X-ray crystal structure of **4** bound to cruzain (pdb 3IUT).[13] This structure reveals that the hydrogen bond to Ser61 in S3 is largely solvent exposed, implying that more hydrophilic heterocycles at P3 might retain this interaction while affording the desired reduction in overall lipophilicity. The S2 sub-site of cruzain is the most lipophilic and solvent inaccessible and was therefore expected to contribute more than any other sub-site to a favorable binding free energy. Accordingly, we considered that a larger, more lipophilic group at this position might be beneficial in terms of potency. Among several larger P2 groups explored previously,[13] we selected cyclopentyl and isopropyl for the current study. Finally, the S1 subsite in cruzain is rather more solvent exposed and forms relatively few hydrophobic interactions with the *n*-Bu side chain of **4** (e.g., a P1 ethyl analog is nearly as potent as **4**). We briefly explored small *gem*-dialkyl substitution at P1 (e.g., cyclopropyl) but found such analogs were devoid of either biochemical or antitrypanosomal activities yet were more potent inhibitors of key CYPs, possibly a consequence of a more exposed triazole ring in such analogs. Our efforts at P1 were thus directed at side chains that retain the larger cone angle of *n*-butyl (as in **4**) but with the introduction of heteroatoms to modulate overall lipophilicity (Fig. 2).

New analogs were synthesized using the general synthetic approaches described previously for **4** and similar analogs.[13,14] Briefly, amino acid starting materials bearing the desired P1 substituent were converted to α-azido acids and then in four steps to the tetrafluorophenylmethyl azido ketone intermediates **A** (Scheme 1). Propargyl amines bearing the desired P2 substituent were prepared in non-racemic form using Ellman's chiral sulfinamide auxiliary.[16] The amines were next subjected to reductive amination with heterocyclic aldehydes or alternatively were coupled to heteroaryl carboxylic acids to afford intermediates **B** bearing the desired P2 and P3 substituents. Finally, copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) reaction between intermediates **A** and **B** afforded the final analogs **4**-**23**.

The new analogs were tested for cruzain inhibition using a biochemical assay, as described previously.[17] To facilitate the rapid evaluation analogs, we determined IC_{50} values rather than full kinetic parameters. While k_{inact}/K_i values are generally preferred when evaluating irreversible inhibitors, IC_{50} values can provide useful rank-order SAR, provided that pre-incubation times are consistent. The assay was performed with a final cruzain concentration of 0.1 nM in a pH 5.5 assay buffer comprising 100 mM sodium acetate, 5 mM DTT, 0.01% Triton X-100 and 10 mM EDTA.

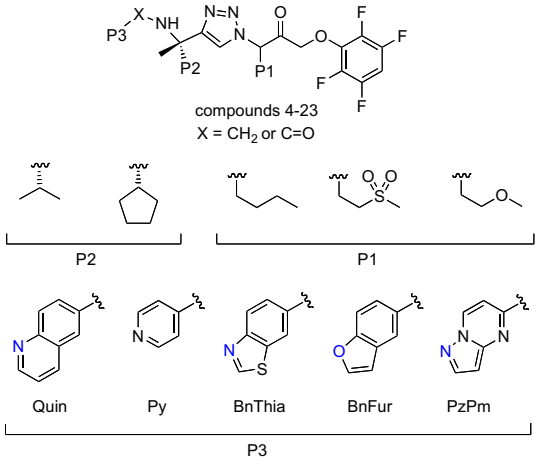

Figure 2. Summary of the structural chemotypes explored in this work.

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