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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3809–3812

# Design, synthesis and evaluation of novel uracil amino acid conjugates for the inhibition of *Trypanosoma cruzi* dUTPase

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Received 21 March 2006; revised 11 April 2006; accepted 11 April 2006
Available online 3 May 2006

**Abstract**—Potential inhibitors of the *Trypanosoma cruzi* dUTP nucleotidohydrolase were docked into the enzyme using the program FlexX. Compounds that docked selectively were then selected and synthesized using solid phase methodology, giving rise to a novel library of amino acid uracil acetamide compounds which were evaluated for enzyme inhibition and anti-parasitic activity.


The protozoan parasite *Trypanosoma cruzi* is the causative agent of Chagas' disease, also called American trypanosomiasis. The parasite is transmitted by blood sucking triatomine insects and is one of three species of the genus Trypanosoma that are pathogenic to humans. Chagas' disease occurs mainly in South and Central America. It is estimated that 16–18 million people are infected with the disease, of which approximately 50,000 will die each year.[1] Although the number of incidences of the disease has declined over the past 20 years due to vector control initiatives,[2] there is still no satisfactory cure for American trypanosomiasis and current drug treatment is only effective against the early stages of the disease. The need for new drugs to treat this disease is therefore urgent.

Recently, deoxyuridine 5′-triphosphate nucleotidohydrolase (dUTPase) has been identified as a novel and valid target against trypanosomatidae.[3] The enzyme is essential in both eukaryotes and prokaryotes, where investigated.[4,5] dUTPase catalyses the hydrolysis of dUTP to dUMP in the presence of $Mg^{2+}$ and plays a critical role in maintaining the level of dUTP in the cell $10^{-5}$ times lower than that of dTTP. In doing so, incorporation of uracil into DNA is minimized, excessive incorporation of which would normally lead to DNA fragmentation and cell death. dUTPases are known to exist in several oligomeric forms. Monomeric enzymes are encoded by herpes virus and Epstein–Barr virus, while the homotrimeric forms are present in mammals, various bacteria and viruses and also in the *Plasmodium falciparum* parasite, the causative agent for malaria. The homodimeric enzymes have been found in *Leishmania major*, *T. cruzi* and *Campylobacter jejuni*. The dimeric enzymes show no similarity in sequence or structure to the monomeric or trimeric forms and are thought to have reached their catalytic potential through a different evolutionary route. Crystal structures of seven trimeric (*Escherichia coli*,[6] human,[7] equine infectious anemia virus,[8] feline immunodeficiency virus,[9] *Methanococcus jannaschii*,[10] *Mycobacterium tuberculosis*,[11] and *P. falciparum*[12]) and two dimeric (*T. cruzi*[13] and *C. jejuni*[14]) dUTPases have been published to date.

It has been shown that relative to other eukaryotic dUTPase enzymes, α,β-imido-dUTP is a strong inhibitor of the dimeric enzymes. It has been proposed therefore that the triphosphate moiety is necessary for inhibition of the dimeric dUTPases.[15] To emphasise the difference, tritylated nucleoside derivatives prepared by our group were good inhibitors of the trimeric enzymes[12] but are inactive against the dimeric parasitic

*Keywords*: dUTPase; Structure-based drug design.
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doi:10.1016/j.bmcl.2006.04.027

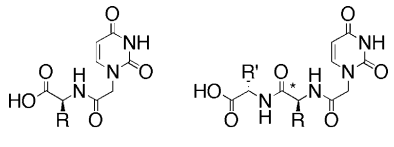
dUTPases. In contrast to the trimeric dUTPases where a glycine-rich site for phosphate binding is common, the phosphates in the dimeric enzymes are held in place with hydrogen bonds to charged side chains.[13] Taking advantage of the structural differences between these enzymes, it was thought that selective inhibitors for the dimeric *T. cruzi* dUTPase (TcdUTPase) could be designed and synthesized as lead compounds for the treatment of Chagas' disease.

Considering the hydrophilic nature of the TcdUTPase active site, compounds in which chains with good hydrogen bond donating or hydrogen bond accepting capability attached to N1 of the uracil ring were considered as potential inhibitors. Ease of synthesis of uracil acetamide derivatives, to which were attached one or two amino acids (Fig. 1), by the Fmoc solid phase strategy allowed for a wide range of structural diversity to be implemented and were therefore chosen for further study. Compounds such as those shown in Figure 1 were drawn, minimized and docked into the active site of TcdUTPase using the program FlexX. The ligand-free (native) TcdUTPase exists in an open form (pdb 1OGL). Binding of the substrate induces substantial structural changes so that the enzyme closes over the ligand (pdb 1OGK).[13] Docking studies were carried out on the closed (complexed) form of the enzyme. The compounds which docked with the best superimposition over the endogenous dUDP ligand were synthesized using solid phase chemistry and tested for biological activity.

FlexX is a docking program that takes into account the flexibility of the ligand but not that of the receptor. The docking method it uses is based on an incremental construction algorithm which consists of three phases: (1) base selection, (2) base placement, and (3) complex construction.[16] The interaction types and scoring functions in FlexX are based on work by Böhm and Klebe.[17]

Docking studies showed that: (1) the R side chain should be H or $CH_3$. Structures where the side chains, R, were bigger than those of Ala or Gly were too bulky to fit into the active site. (2) The optimum distance between the carbonyl marked with an * (Fig. 1) and the terminal atom was 2 or 3 but no more than 5 atoms. (3) The best scoring compounds were those where R′ was a charged residue. A large degree of variation of the R′ side chain without effecting the superimposition was, however, allowed. Compounds **1**–**11** (Table 1) were among those which docked best into the TcdUTPase active site (Fig. 2).

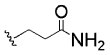
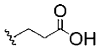
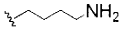
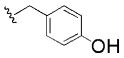
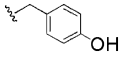
Compounds **1**–**11** were synthesized by solid phase Fmoc chemistry as shown in Scheme 1. Amino acids **12**–**19**



**8** **1-7, 9-11**

**Figure 1.** Structure of potential TcdUTPase inhibitors.

**Table 1.** Compounds docked into the TcdUTPase active site using FlexX and subsequently synthesized

| Compound | R | R′ | Colour |
|---|---|---|---|
| **1** | $CH_3$ |  | Orange |
| **2** | $CH_3$ |  | Red |
| **3** | $CH_3$ |  | Green |
| **4** | $CH_3$ | H | Blue |
| **5** | $CH_3$ |  | Purple |
| **6** | $CH_3$ |  | Magenta |
| **7** | $CH_3$ |  | Violet |
| **8** | H | — | Greenblue |
| **9** | H |  | Redorange |
| **10** | H |  | By atom type |
| **11** | H |  | White |
| dUDP | | | Yellow |

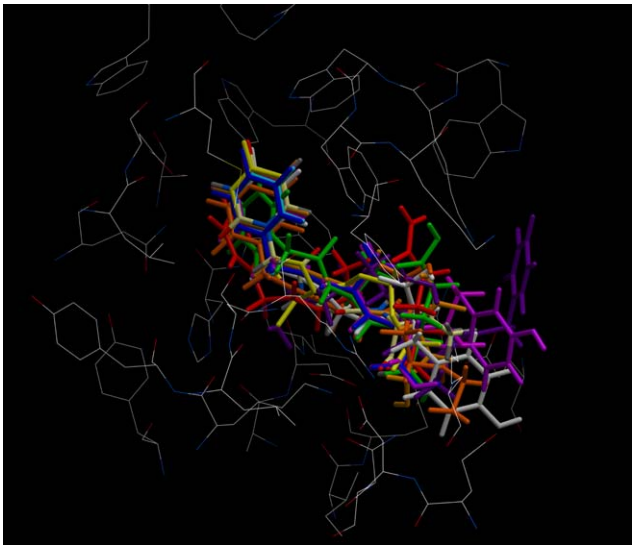
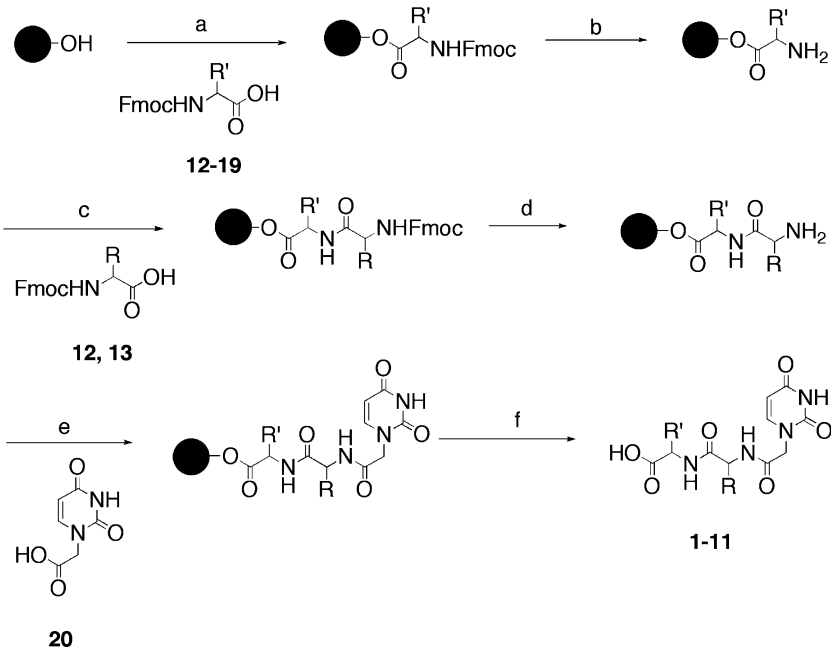


**Figure 2.** Docking of compounds **1**–**11** in TcdUTPase active site. Superimposition with dUDP.




**Scheme 1.** General synthesis of compounds **1**–**11**: (a) DIC, **12**–**19**, DMAP, DMF (**12**, Ala; **13**, Gly; **14**, Asp; **15**, Gln; **16**, Glu; **17**, Lys; **18**, Thr; **19**, Tyr); (b) 20% piperidine, DMF; (c) TBTU, HOBt, **12**–**13**, DIPEA, DMF; (d) 20% piperidine, DMF; (e) TBTU, HOBt, **20**, DIPEA, DMF (f); 50% TFA, DCM, TES.

were first converted to the appropriate anhydrides using DIC. The anhydrides and DMAP were then added to Wang resin[18] and the percentage loading was calculated by UV absorption.[19] Subsequent deprotection of the Fmoc group with piperidine was then followed by coupling to the second amino acid using TBTU and HOBt.[20,21] A final deprotection and coupling to 1-carboxymethyl uracil, **20**,[22] followed by cleavage from the resin beads using 50% TFA released the desired compounds **1**–**11**. All final compounds were analysed and characterized by $^{1}$H NMR and MS.

Compounds **1**–**11** were tested for both TcdUTPase enzyme inhibition[23] and in vitro effects on parasite growth.[24] As can be seen from Table 2, none of the compounds inhibited the TcdUTPase enzyme at a concentration of 1 mM. Neither did the compounds inhibit parasite growth at the maximum concentrations shown.

**Table 2.** Biological activity of compounds **1**–**11**

| Compound | Enzyme inhibition $K_i$ | In vitro $IC_{50}$ (μM) | Cytotoxicity $IC_{50}$[a] (μM) |
|---|---|---|---|
| **1** | >1 mM | >84 | >252 |
| **2** | >1 mM | >81 | >243 |
| **3** | >1 mM | >81 | 210 |
| **4** | >1 mM | >100 | >301 |
| **5** | >1 mM | >81 | >243 |
| **6** | >1 mM | >87 | >262 |
| **7** | >1 mM | >74 | 214 |
| **8** | >1 mM | >132 | >396 |
| **9** | >1 mM | >84 | >253 |
| **10** | >1 mM | >91 | >274 |
| **11** | >1 mM | >76 | >230 |
| Standard | 18.40[b] μM | 1.04[c] | 0.15[d] |

[a] Cytotoxicity tests were carried out on rat L6 cells.
[b] dUMP.
[c] Benznidazole.
[d] Podophyllotoxin.

Thus, the search so far for lead inhibitors of the dimeric dUTPase enzymes remains unsuccessful. There are a number of reasons why this design paradigm was not successful in this case. (i) Recently, the crystal structure of the dimeric *C. jejuni* dUTPase revealed the coordination of $Mg^{2+}$ ions within the active site of the enzyme.[14] These ions are absent in the TcdUTPase crystal structure. It is unclear if the $Mg^{2+}$ ions are bound to the un-liganded enzyme, but it is possible that the presence of these ions must be taken into account when designing inhibitors in silico.

(ii) As previously mentioned, FlexX does not take into account the flexibility of the protein in question. Although this approximation reduces run time significantly, in this case where binding of the ligand induces a large conformational change in protein structure it is possible that a docking program in which protein flexibility is taken into account should be used.

(iii) There must be a mechanism by which the dUTPase undergoes the large conformational change on binding dUTP. Two mechanisms of binding could be envisaged. First, the uracil and deoxyribose moieties, which bind to motifs from the rigid domain of protein, would be recognized. The α, β, and subsequently γ phosphates, which are recognized by mobile domain motifs, would then bond to the $Mg^{2+}$ ions and respective amino acid side chains sequentially and induce 'active site closure'. Alternatively, the phosphate moieties would be recognized by the mobile domain first and the protein would then undergo such a conformational change so as to

encapsulate the uracil and sugar moieties. In the latter case, the phosphate moieties would be more essential for ligand recognition and therefore replacement of them would be detrimental to ligand recognition. This would explain the lack of activity of compounds **1**–**11**. In either case, the experimental biological data indicate that compounds **1**–**11** do not possess the required structure to bind to the open form of the enzyme sufficiently enough to induce the conformational change required for inhibition.

Although compounds **1**–**11** docked with good superimposition and favourable binding energies into the TcdUTPase active site with FlexX, it is apparent that other factors such as protein flexibility and the presence of $Mg^{2+}$ ions should be taken into account when designing competitive inhibitors for these enzymes in silico.

## Acknowledgments

The authors thank the European Union (QLRT-2001-00305), Cardiff University and the FIS Network RICET/C03 for funding of this project. The National EPSRC Mass Spectrometry service centre (Swansea) is acknowledged for accurate mass spectrometry.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.04.027.

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