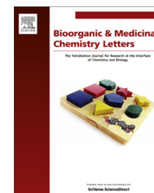
Bioorganic & Medicinal Chemistry Letters 23 (2013) 6799–6804

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl



Sulfur incorporation generally improves Ricin inhibition in pterin-appended glycine-phenylalanine dipeptide mimics

Paul A. Wiget, Lawrence A. Manzano, Jeff M. Pruet, Grace Gao, Ryota Saito, Arthur F. Monzingo, Karl R. Jasheway, Jon D. Robertus, Eric V. Anslyn *

Department of Chemistry and Biochemistry, University of Texas at Austin, 1 University Station A1590, Austin, TX 78712, United States

ARTICLE INFO

Article history:
Received 26 August 2013
Revised 3 October 2013
Accepted 7 October 2013
Available online 12 October 2013

Keywords:
Pterin
Thioamide
Dipeptide mimic
Ricin inhibitors
RTA
SAR

ABSTRACT

Several 7-aminoamido-pterins were synthesized to evaluate the electronic and biochemical subtleties observed in the 'linker space' when *N*-{*N*-(pterin-7-yl)carbonylglycyl}-L-phenylalanine **1** was bound to the active site of RTA. The gylcine–phenylalanine dipeptide analogs included both amides and thioamides. Decarboxy gly-phe analog **2** showed a 6.4-fold decrease in potency (IC_{50} = 128 μM), yet the analogous thioamide **7** recovered the lost activity and performed similarly to the parent inhibitor (IC_{50} = 29 μM). Thiourea **12** exhibited an IC_{50} nearly six times lower than the oxo analog **13**. All inhibitors showed the pterin head-group firmly bound in their X-ray structures yet the pendants were not fully resolved suggesting that all pendants are not firmly bound in the RTA linker space. Calculated log *P* values do not correlate to the increase in bioactivity suggesting other factors dominate.

© 2013 Published by Elsevier Ltd.

As recently as April 2013 the biotoxin ricin has been employed as a potential weapon against high-ranking government officials including US Senator Roger Wickers and President Barack Obama.[1] Ricin toxicity can occur from aerosol exposure in addition to a wealth of other methods at doses below 1 μg/kg body weight. Ricin has already seen use as a weapon, and due to the ease of castor oil procurement and toxin extraction, the threat persists.[2]

The cytotoxin ricin is a prototypical type 2 ribosome inactivating protein found in castor beans.[3] Ricin Toxin A (RTA), the A chain of the cytotoxin Ricin, catalyzes adenosine depurination, and is an optimal candidate for inhibitor Structure–Activity Relationship (SAR) studies due to its ease of handling and known and reproducible crystal structure.[4] Additionally, we have previously reported numerous pterin-based RTA inhibitors as potential anti-Ricin agents via structure-based design and virtual screening.[5] The pterin heterocycle is not only consistent in showing activity with a multitude of pendants attached, but also with rare exception delivers firmly resolved X-ray crystallographic data.

Structure–Activity Relationships (SAR) correlate the biological response of a specific process to the varying of functional groups of a biomolecule (i.e., protein mutation or modification), or to participating substrates such as drug candidates, co-factors, etc. As with proteins, subtle changes in drug candidate structure can drastically change drug efficacy, uptake, binding affinity, toxicity, etc. due to the modification of the three dimensional space the molecule occupies. These changes can be due to (amongst others) the addition or reduction of steric bulk, conformational restrictions, and/or hydrogen bonding preferences.[6]

Highly specific interactions can be mapped and optimized within classes of molecules by carefully controlling the modifications in a drug candidate by keeping the overall sterics and degrees of freedom the same or highly similar. One way this has been achieved is by amide bond translocation within drug candidates. Coppola et al. found that reversing the order of the amide in one such substrate could produce the dramatic effect of having no activity to 33% inhibition of their target protein at 10 μM. Translocation has also shown varying effects on the stability of candidates in microsomes, increasing $t_{1/2}$, by a factor of 3.[7]

Conversion of amides to thioamides has been used frequently to alter the native conformation of peptides by modifying the hydrogen bond strength and rotational barriers of the molecule or drug candidate. Thioamides have been found to be compatibly stable in such tertiary structures as β-sheets and α-helicies, even at elevated temperatures. Their incorporation into organocatalysts has seen improvements of selectivity factors (*s*) by an order of magnitude.[8] The geometry, determined by both spectroscopic[9] and computational[10] methods show shorter N–C(S) bond lengths, longer C–S bond lengths, and higher amide rotational barriers, suggesting a greater degree of N–C double bond character. In drug candidates thioamide incorporation has produced dramatic changes in K_D, log *p*, and hydrolysis rate, thus changing biological

* Corresponding author.
E-mail address: anslyn@austin.utexas.edu (E.V. Anslyn).

0960-894X/$ - see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.bmcl.2013.10.017

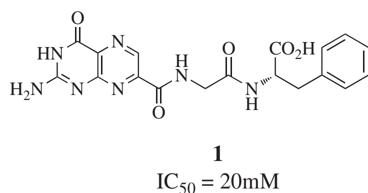


Figure 1. *N*-{*N*-(Pterin-7-yl)carbonylglycyl}-L-phenylalanine.

activity.[11] Urea or thiourea substitution has been shown to resolve the ambiguity in cytokine response of the parent amide molecule and produce CD1d agonists.[12] Hence, incorporating ureas or thioureas is yet another way of easily modifying the conformational degrees of freedom and hydrogen bonding ability within potential drug candidates.

Although vaccines and cellular trafficking inhibitors have proven to be viable Ricin countermeasures, our previously reported competitive active-site inhibitors have shown increased success.[13–15] Our work has shown that both synthetic and peptide side chains appended on the pterin ring system have accessed new binding modes not predicted by models, shown a preference for hydrophobic chains, and improved our understanding of the two primary binding pockets.[14,15] Of the most highly active compounds was a Gly-Phe appended pterin **1**[15] (Fig. 1). In the space of the RTA protein that joins the pterin binding site and a secondary binding site referred to as the 'linker space', tyrosine 80 is available for hydrogen bond donation and π-stacking. Previously reported substrates exhibited hydrogen bonding with Tyr 80 but the mode, nature, and optimum location of the hydrogen bond acceptor remained unclear. Additionally, Trp221 provides an edge-to-face[16] interaction with the benzene ring of **1**. In order to explore the subtleties of the linker-space between the specificity pocket and the secondary binding pocket through substrate structure–activity relationships, herein we report a series highly similar ($n + m = 3$) of '*Inside*' and '*Outside*' amide- and thioamide-appended pterin analogs of **1** (see Fig. 2). Additionally, as proteolytic stability is of concern in peptide-based drug candidates, we chose to decarboxylate all inhibitors to circumvent this familiar drug-candidate weakness.[17] Therefore, the inhibitors are designed to probe the linker space via amide transposition, inversion, and through conversion to the thioamide in comparison to the parent compound **1**.

The syntheses of '*Inside*' aminoamides began with carbonyldiimidizole (CDI) facilitated amide coupling to the appropriate Boc-protected aminoacid[18] (such that $n + m = 3$ to maintain gly-phe homology). Deprotection and ether/DCM precipitation of the pure aminoamide salt afforded the desired gly-phe analogs. Similarly, mono-Boc protected diamines[19] were reacted with either

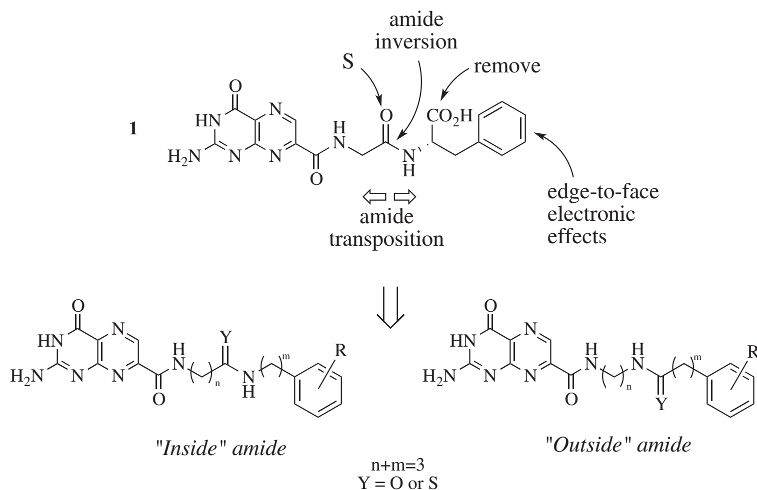
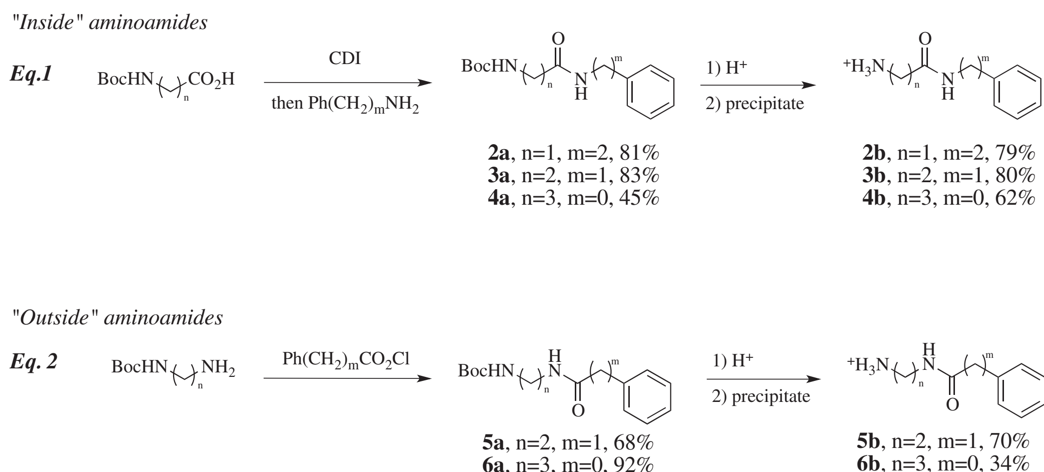


Figure 2. Proposed variations of **1**.



Scheme 1. Synthesis of *Inside* and *Outside* Gly-Phe aminoamide analogs.

Download English Version:

https://daneshyari.com/en/article/10593344

Download Persian Version:

https://daneshyari.com/article/10593344

Daneshyari.com