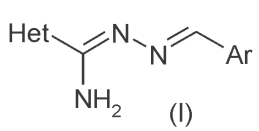
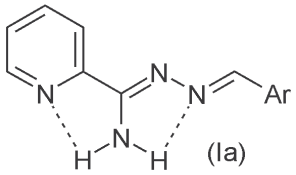
s1.m6.p16 **Structural systematics for the antimycobacterial carboxamidrazones.** C. H. Schwalbe, K. A. McMillan and D. L. Rathbone, *Aston Pharmacy School, Aston University, Birmingham B4 7ET, UK. E-mail: c.h.schwalbe@aston.ac.uk*

Keywords: Carboxamidrazones; Antimycobacterial; Hydrogen bonding

Certain heteroarylcarboxamidrazones display activity approaching that of the clinically important anti-tuberculosis drug isoniazid. We have by now determined crystal structures [1,2] of 15 variants of structure (I) differing in the number, location and oxidation state of N atoms in the heterocycle, the nature and substitution of the aryl group, and modifications to the central chain.



(I)



(Ia)

Planarity facilitates the achievement of a conjugated system. Where, as in (Ia), the amino group suffers no steric clashes, angles between the Het and Ar planes range from 2.8 to 20.5°. In the 3- and 4-pyridyl isomers with their C-H..H-N steric clash these angles increase to 13.4-78.5°. Where Het remains 2-pyridyl but the second N atom in the chain bears a hydrogen atom causing N-H..H-N clash, the angles are 31.6-59.2°. The largest twists in the chain occur about the single bonds. Where the heterocycle is 2-pyridyl and the second N atom in the chain is available (Ia), sharply angled but reasonably short intramolecular N-H..N bonds form. Although they remain sterically accessible, these amino H atoms engage in additional intermolecular H bonds only in some instances, and such hydrogen bonds are often fairly weak. In both cases where the central chain is modified to contain a urea moiety, the amino and adjacent NH groups donate hydrogen bonds to the same carbonyl O atom of a nearby molecule, which may help to reconcile two partially positive H atoms in close proximity. Even though the first N atom in the chain always remains unprotonated and could accept a resonance-assisted hydrogen bond, it is frequently left unused. When in the 2-position, the pyridyl N atom is always engaged in intramolecular hydrogen bonding, and sometimes it accepts an intermolecular hydrogen bond as well. With one exception it is an intermolecular hydrogen bond acceptor when in the 3- or 4-positions. Thus it may be considered to be an important part of the pharmacophore. Both 2-pyridyl and 4-pyridyl isomers are capable of antimycobacterial activity, but there is evidence for a maximum tolerable length along the spine of the molecule from the Het N atom to the opposite extremity of the substituted Ar moiety. Thus both the 2-pyridyl and the 4-pyridyl isomers are moderately active when Ar is 4-t-butylphenyl; however, when Ar is elongated to 3-methoxy-4-benzyloxyphenyl the 2-pyridyl isomer is highly active (MIC 4-8μg ml^{-1} against *Mycobacterium fortuitum*) but the 4-pyridyl analogue is inactive.

[1] N D Cox, C H Schwalbe, D L Rathbone, N Khan and K J Parker (2003) *J.Pharm. Pharmac.* **55** (Suppl.): S-19.
[2] C H Schwalbe, D L Rathbone, N D Cox, P R Lowe, K J Parker and N Khan (2003) *J.Pharm. Pharmac.* **55** (Suppl.): S-19-20.

s1.m6.p17 **Crystal Structure Analysis and ITC Measurements to Correlate Structural and Thermodynamic Properties in a Congeneric Series of Trypsin and Thrombin Inhibitors.** Jasmine Simunec, Andreas Heine, Gerhard Klebe, *Inst. of Pharmaceutical Chemistry. Univ. Marburg, Marbacher Weg 6, D35032 Marburg, Germany. E-mail: simunec@staff.uni-marburg.de*

Keywords: Thermodynamic studies; Molecular recognition; Serine proteinases

Rational approaches to ligand design suffer from the fact that our knowledge of the factors determine affinity and specificity of biomolecular interactions is still very rudimentary despite enormous methodological advances in structure determination techniques. Thermodynamic studies show that the affinity is not simply governed by structural features alone, but result as a complex interplay of structure and dynamics. Therefore, lead discovery requires both structural and thermodynamic studies in parallel. In addition to crystal structure determination isothermal titration calorimetry (ITC) provides reliable experimental data on the overall thermodynamic parameters that govern the biomolecular recognition process. In a congeneric series of low molecular-weight ligands, the binding properties with respect to trypsin and thrombin has been studied by crystallography and ITC. Based on the crystal structures, it is possible to explain selectivity variations between trypsin and thrombin that result from differences in addressing the S2 pocket of these serine proteinases and in the formation of a hydrogen bonds to the 60's loop of thrombin. The thermodynamic results support structural findings. The racemic pair of two ligands, obtained from synthesis, can be studied in terms of individual signals in one ITC measurements resulting in a modulated titration curve. For example the separated enantiomers show Ki values deviating by a factor of about 1000.[1] Advantage of the ITC experiment is the simultaneous determination of both K_i values in one experiment without separating the enantiomers.

[1] U. Obst, P. Betschmann, C. Lerner, P. Seiler, F. Diederich, *Helv. Chim. Acta* **2000**, *83*, 855 - 909.