Synthesis of Novel Inhibitors of IdeS, a Bacterial Cysteine Protease

Including Studies of Stereoselective Reductive Aminations

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Abstract

The cysteine protease IdeS is an IgG degrading enzyme secreted by the bacterium *Streptococcus pyogenes* to evade the human immune system. In this thesis several inhibitors of IdeS have been synthesized and evaluated. Such inhibitors should be highly useful when elucidating the detailed mechanism of IdeS action. They might also have a potential as treatment of acute and severe infections caused by the bacteria. Further, IdeS has a therapeutic application of its own due to the proteolytic ability and an IdeS inhibitor might contribute during the development.

Only irreversible, unselective inhibitors of IdeS were known five years ago. In this thesis, three strategies with the aim to synthesize and identify more inhibitors have been undertaken. Focus was first set on compounds with a substructure resembling the known inhibitors but with reversible warheads, i.e. nitrile, azide and aldehyde functions. The aldehyde derivatives were found to provide the first reversible inhibitors of IdeS.

Then, to avoid covalent interactions and obtain more selective inhibitors, a substrate based strategy was undertaken. A 3-aminopiperidine fragment was used as replacement of either of the two residues adjacent to the scissile bond in IgG. Such fragments can be synthesized from *N*-protected 3-aminopiperidone and amino acid esters in reductive aminations in which a stereogenic center is formed. A series of di-, tri- and tetrapeptide analogues, together with eight peptides covering the cleavage site of IgG, were screened for their capacity to inhibit the cysteine proteases IdeS, SpeB and papain. Several analogues showed inhibition capacity, two compounds showed also high selectivity for IdeS. In contrast, none of the tested peptides showed any inhibition. Computational docking studies indicate that the identified IdeS peptide analogues and the non-active peptides do not share the same binding site in IdeS. Probably, the piperidine moiety hinders the inhibitor to enter the catalytic site.

A more detailed study of the stereoselectivity in the reductive aminations affording the 3-aminopiperidine fragment showed that a large protecting group (trityl) together with a large reducing agent (NaBH(O-2-ethylhexanoyl)$_3$) gave the highest diastereomeric ratio. The highest ratio obtained was 21:79 when L-proline methyl ester was used. The newly formed stereogenic center had the *R*-configuration, determined by chemical correlation. Computer based conformational analysis combined with Boltzmann distribution calculations implies an axial attack by the reducing reagent on the intermediary imine.

To improve the potency of the two identified di- and tripeptide analogues synthetic routes to conformationally restricted *N*-containing bicyclic derivatives was undertaken in a third strategy. Five compounds with different bicyclic scaffolds were screened for their inhibition capacity towards IdeS and papain. One of the compounds was able to inhibit the first step of proteolytic cleavage of IgG by IdeS, a process usually completed in seconds.

Keywords:

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