MS05-P06

Stability of API. Lactamization of γ-amino acids in crystal state

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γ-Aminobutyric acid (GABA) plays numerous physiological functions and its derivatives are potential active pharmaceutical ingredients (API). At the moment two of them (Baclofen and Gabapentin – see below) are widely used. **Baclofen** (Kemstro, Lioresal) is an agonist of GABA receptors. It shows significant muscle relaxant activity and is used as antispastic drug while **Gabapentin** (Prebalin, Lyrica) shows anticonvulsant activity and is used mainly as antileptic drug, in neurophatic pain and in generalized anxiety disorder.

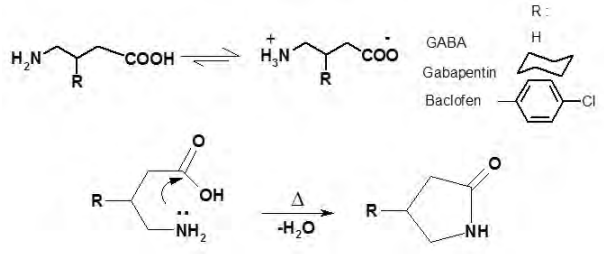
GABA and its neutral derivatives exist in a dipolar (zwitterionic) form both in solid states and in solutions (see below), which theoretically should prevent possible nucleophilic attack of the electron lone pair at N atom (engaged in N-H bond in protonated forms) on the carboxylic carbon (only in neutral carboxylic group) with formation either cyclic ɤ-lactam or linear peptide. The reaction is observed quite often in solutions, where a tiny concentration of the neutral form of the amino acids being in equilibrium with the ionic forms allows such reaction to go. The reaction (shown below) was also observed in the solid state by Borka [1] in the case of Baclofen at elevated temperatures. Surprisingly, one of several solid Baclofen forms under study in our laboratory revealed the lactamization reaction in moderate temperatures.

The analysis of crystal structures of γ-amino acids found in Cambridge Structural Database and in our archives (unpublished ones) allowed us to point out several factors, which have to be taken into account when considering perspective stability of such API.

The most important features are:

- conformation of the main amino acid chain,
- shortest intra- and intermolecular distances between amino and carboxyl groups,
- geometry and strength hydrogen bonds formed by the two reacting groups,
- relative spatial orientation of the two groups,
- possibility of rotation of the carboxyl group.

The common values of the analyzed factors and their influence on lactamization of ɤ-amino acid will be presented.



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[1] L. Borka. (1979). Acta Pharm Suec, 16, 345-348.

Keywords: GABA derivatives, Lactamization in crystals, API stability

MS05-P07

Fragment screening on protein Kinase A and PIM1-Kinase

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Fragment screening is a method routinely applied in pharmaceutical drug development by now. Here, small molecules (<300 Da) that are identified during the screening process are developed into lead compounds with high affinity. While high-throughput screening usually requires large compound libraries, fragment libraries can be much smaller in size, containing only several hundred to thousand molecules. We developed a small fragment library composed of 361 compounds and validated it on the aspartyl protease endothiapepsin for which we obtained a high hit rate during crystallographic screening.[1,2]

We used this library to screen protein kinase A (PKA) and PIM1-kinase. For pre-screening we applied a thermal shift assay (TSA) to identify suitable fragments for crystallographic screening. In the TSA 31 fragments were identified for PKA and 52 for PIM1. These fragments were then subjected to crystallographic screening where we obtained 15 complex structures for PKA and 13 for PIM1, indicating also here a high crystallographic hit rate. In comparison we tested a random collection of fragments for crystallographic screening with PKA resulting in a much lower hit rate (21%). Deviating hit lists in the TSA assay and only one common fragment observed in both kinase structures suggest that fragments might be selective binders. TSA screening results, observed binding motifs and structural differences in the ATP-binding pockets of both kinases will be discussed in detail.

References:

[1] Köster, H. et al. (2011) J. Med. Chem. 54, 7784-7796.
[2] Schiebel, J. et al. (2016) ACS Chem. Biol. 11, 1693-1701.

Keywords: fragment library validation, structure-based drug design, crystallographic fragment screening