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Inhibition of the MDM2/p53 Interaction

Design, Synthesis and Evaluation of MDM2 Inhibitors

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Abstract

Numerous essential cellular processes are regulated by protein-protein interactions (PPIs) and PPIs have therefore been recognised as potential new drug targets. The transcription factor p53 is often referred to as the guardian of the genome due to its involvement in DNA repair, induction of cell cycle arrest and cellular apoptosis. The amount of p53 in a cell is mainly controlled by the negative regulator MDM2, which upon complex formation with p53 leads to an overall reduction of the p53 level. Consequently, inhibition of the MDM2/p53 interaction has emerged as a promising new therapeutic strategy for the treatment of cancers retaining wild-type p53.

This thesis describes the design, synthesis and evaluation of β-hairpins, 8-(triazolyl)purines and 2,5-diketopiperazines as MDM2/p53 interaction inhibitors.

β-Hairpin derivatives were synthesised using automated solid phase peptide synthesis followed by a head to tail cyclisation in solution. Evaluation of the MDM2 inhibitory activity of the β-hairpin derivatives together with solution conformational analysis using NAMFIS calculations revealed that molecular flexibility is important to gain highly potent MDM2 inhibitors. Two series of 8-(triazolyl)purines and 2,5-diketopiperazines (2,5-DKPs) were evaluated as MDM2 inhibitors. The first series were designed to directly mimic an α-helical region of the p53 peptide, containing key residues in the *i*, *i*+4 and *i*+7 positions. Conformational analyses indicated that both 8-(triazolyl)purines and 2,5-DKP derivatives were able to place substituents in the same spatial orientation as an α-helical template. The second series were designed primarily based on structure-based docking studies. The most potent inhibitors identified were from the latter series and displayed micromolar IC_{50}-values in a biochemical fluorescence polarization assay. Binding to MDM2 was confirmed by WaterLOGSY experiments. Efficient synthetic protocols for the synthesis of both tetrasubstituted 8-(triazolyl)purines and tetrasubstituted 2,5-DKPs have been developed. Furthermore, an efficient bromination protocol for 8-bromination of electron rich purines utilising pyridiniumtribromide was developed. The fluorescent properties of the 8-(triazolyl)purines were determined and it was found that the regioisomerism of the triazole has an important impact on the quantum yield.

Keywords: Protein-protein interaction, MDM2/p53 interaction, MDM2 inhibitors, α-helix mimetics, β-hairpin, 2,5-diketopiperazine, spiro-2,5-diketopiperazine, purine, 8-(triazolyl)purine, solution conformational analysis, NAMFIS, fluorescence.