Summary of the doctoral thesis: "Etablierung von Testverfahren zur Evaluation niedermolekularer Modulatoren des Wnt Signalwegs und anderer biologischer Prozesse"

A major aim of chemical biology is the identification and validation of new biologically active compounds. Ideally, these molecules are not only applied in basic but also in applied research. The goal of this thesis work was therefore to develop – in collaboration with chemists from the department – new active compounds for the elucidation of biological processes.

In a collaboration with Dr. L.-G. Milroy (Max Planck Institute for Molecular Physiology) a detailed structure activity relationship of Chondramide and Jaspamide analogs was developed. These marine natural products are strong actin binders and thereby inhibit the cell division. They could serve as starting points for anti-cancer drug development projects. Two cell lines where used to measure dose response curves of 25 compounds for the establishment of a structure activity relationship which serves now as a starting point for further synthesis efforts.

Together with S. Schoof and S. Baumann under the supervision of Dr. H.-D. Arndt (Max Planck Institute for Molecular Physiology) semisynthetic analogs of the thiopeptide antibiotic thiostreptone were analysed in a second project. The activity of the molecules was analysed using an antibiotic assay and tissue culture. Thiostreptone targets the bacterial ribosome and therefore a staining of the structural similar mitochondrial ribosome was developed using a fluorescent thiostreptone probe.

In a collaboration with the group of Prof. Dr. Niemeyer (Technical University Dortmund) a methodology to cultivate cells on microstructured glass slides was developed. The slides were based on the DDI technology and different DNA-peptide conjugates were successfully applied. The cells attached via integrin receptors to the tested petides harboring integrin binding motives but also pure DNA microstructures could be used for cell culture.

A protein probe to test the cellular palmitoylation machinery was designed in a collaboration with Prof. Dr. Waldmann and Prof. Dr. Bastiaens (Max Planck Institute for Molecular Physiology). This protein probe had to be free of protein binding motifs to be

a purely artificial sensor. Therefore GFP was cloned to express an additional c-terminal cysteine. This clone was later used for MIC-ligation with a substrate of the palmitoylation machinery and was afterwards microinjected in cells to probe the dependency of the palmitoylation machinery to the protein core by the collaboration partners.

As part of the thesis a methodology to generate a representative subset of molecules from the in house compound collection was developed. This work was performed in cooperation with S. Wetzel (Max Planck Institute for Molecular Physiology). The obtained compound collection was optimized to be used in cellular screenings by the use of four simple selection rules. First a compound should be available in sufficient quantities to be tested in cellular settings with an additional amount for secondary screenings. 300 µl were set as the lower limit. The other three rules dealt with the solubility and the structure of the compound. Therefore an AlogP of less than 6 to ensure solubility and either one stereocenter or three heteroatoms or one cycle in combination with the intention than the compound has to be screened had to be fulfilled to pass the criteria. Compounds which passed all four filters were clustered by chemical similarity in groups of ten molecules in average. The OptiSim algorithm was used for this process. 479 groups were generated by this procedure. A representative member of the group was subjected to the cellular screenings and in case of activity the whole group was tested. This approach reduced the amount of compounds for the testing strongly by simultaneously retaining the chance of identifying active molecules.

The above mentioned set was used in two different cellular screenings. One of these tests dealt with the identification of new antimicrobial substances. This test was very promising because many natural products have antibacterial properties and natural products are often the starting points for the synthesis projects in the department. By implementation of the DIN 58940 in combination with System Duetz Covers a robust, sensitive and reliable screening procedure was established. Four clinically relevant bacterial strains were used to test the generated compound set. By the identification of the substituted ß-carboline hydantiones a group of low micromolar inhibitors could be identified. By showing that the analysed tetramic acids uncouple the proton gradient it was possible to elucidate the mode of action of another active compound class.

An essential part of this thesis was the screening and identification of modulators of the canonical wnt signaling pathway. This pathway is one of the key regulators of developmental and differentiational processes. Misregulation is therefore a hallmark of

cancer development and inhibitors of wnt driven processes might act as anti-cancer molecules. Activators of the canonical wnt signaling pathway can be applied in stem cell therapy and they are already used to fasten wound healing after surgery. Due to the equal importance of activators and inhibitors an unbiased screen of in total 800 compounds was done using a modified reportergene cell line.

One of the most potent inhibitors was a decaline which was analysed in more detail in subsequent experiments. First the ED_{50} was shown to be in the low micromolar range in the reportergene cell line as well as in untransformed HEK293 cells. Second if the compound was applied to HEK293 cells activated at the level of the destruction complex the activity could still be observed. This could be shown either by specific activators or by cancer cell lines. These results imply that the substance inhibits the wnt signaling pathway at the transcriptional level. To test this hypothesis different fractions of cellular proteins were incubated with the decaline inhibitor. After the incubation the wnt signaling pathway modulating activity of each fraction was analysed by application to HEK293 cells and subsequent werstern blotting of these cells. It could be shown that the fraction containing nuclear proteins inhibited the activity of the decaline. This can be explained by the interaction of a nuclear protein with the compound thereby sequestering the compound away from the cells and no effect could be observed in these cells afterwards. A synthesis of the compound was not possible due to an erroneous structural assignment in the database. Aiding the structural elucidation of the active small molecule a structure activity relationship was developed. Therefore 320 decalines were tested and structural distinct active molecules could be discovered during this process.

Similarily to the above mentioned inhibitors the most potent activators were also analysed in more detail. These small molecules belong to the class of oxepanes, seven membered ring systems containing an oxygen atom, and activate the wnt signaling pathway only if applied in combination with wnt3a protein. If the activators were applied to HEK293 cells activated at the level of the destruction complex the activity was lost, pointing to an modulating effect at the level of the receptor activity. An affinity probe was synthesized by Dr. C. Derave to analyse this effect in more detail. For the synthesis of the affinity probe a structure activity relationship was developed based on a 105 membered oxepane library. Three of the proteins identified during the affinity purification were analysed in detail. The proteins CDC2 and PESC1 where shown to be non specific interaction partners whereas the Van-Gogh protein 1 was a reversible and

thereby a specific interaction partner of the oxepane. The Van-Gogh proteins act as receptors in the PCP branch of the wnt signaling pathway and might thereby lead to an better understanding of the crosstalk between different wnt signaling pathways.