DESIGN OF NOVEL PEPTIDE DRUG CONJUGATE WARHEADS AS NOVEL POTENTIAL ANTICANCER AGENTS

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THE NEXT DRUG STARTS HERE

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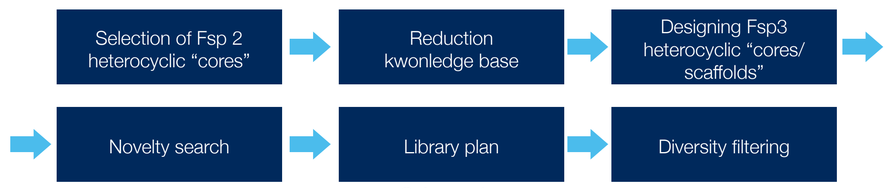
INTRODUCTION

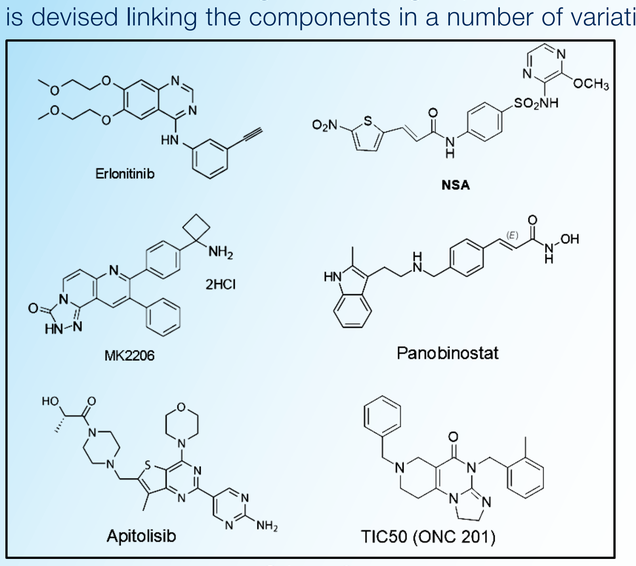
Over the 10 years ComInnex specialized on developing high quality drug-like libraries (typically 100 – 300 members per library) in order to cover chemical space of potential biological targets effectively. The applied strategy focuses on non-flat 3-dimensional templates and libraries that result in screening compounds with more favourable physicochemical properties, higher sp3/sp2 atom ratio (Fsp3/Fsp2), and novel 3-dimensional shapes with various functionalities. (Scheme 1.). In the present poster we report the preliminary results of the new combined concept and the 3D structure-based hit virtual screening model.



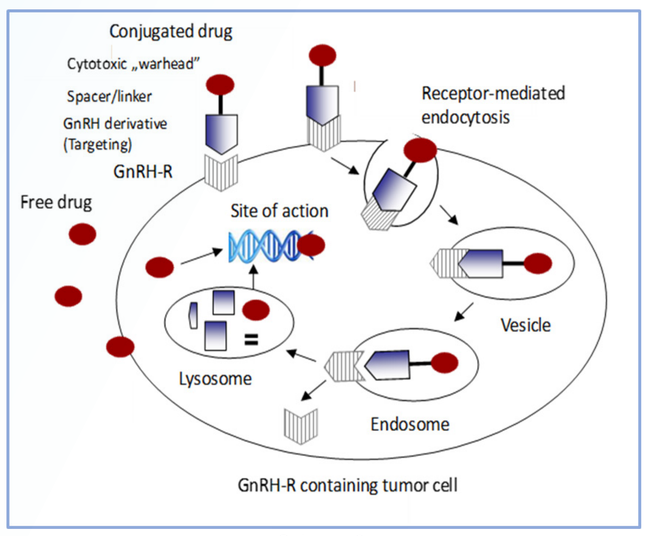
Scheme 1.

OBJECTIVES

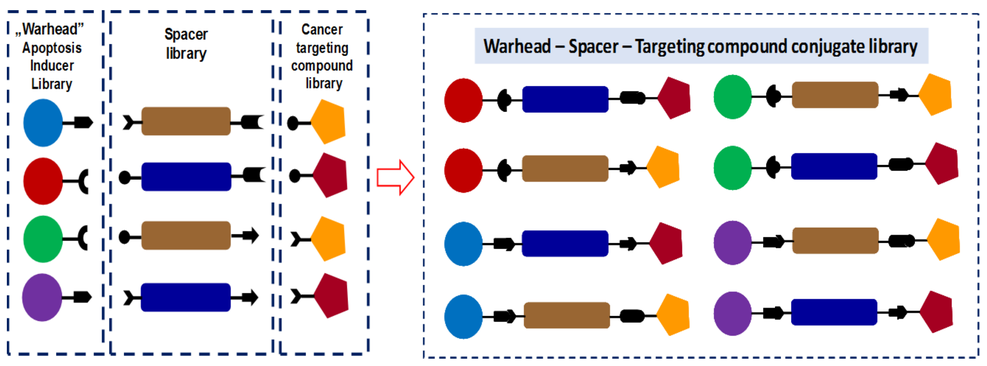
Our overall objectives was to generate Small Molecule-Drug Conjugates (SMDCs) that can be used for targeted tumour therapy. In order to realize this goal potential anti-cancer small molecules (e.g. apoptosis inducers, preferably that trigger apoptosis via covalent interaction, Scheme 2.) was linked to appropriate targeting peptides (such as gonadotropin-releasing hormone, GnRH) ensuring the selectivity towards the tumour cells. (It is well-documented that GnRH receptors are overexpressed in various tumour cells.) Based on the structure of known apoptosis inducer molecules a diverse small molecule library was designed and generated. Peptide homing devices (e.g. Gonadotropin-releasing hormone (GnRH)) were conjugated with potential cytotoxic „warheads” through cleavable linkers (Scheme 3-4.)[1]. The activity could rely on two facts a.) intrinsic cytotoxicity b.) inducing apoptosis by interfering with the programmed cell-death pathway. In the initial library generation process we focused on meeting the following requirements: lead-like properties, synthetic feasibility, novelty and diversity. In order to provide a reasonable choice for biological screening a 3-component library is devised linking the components in a number of variations.



Scheme 2.



Scheme 3.

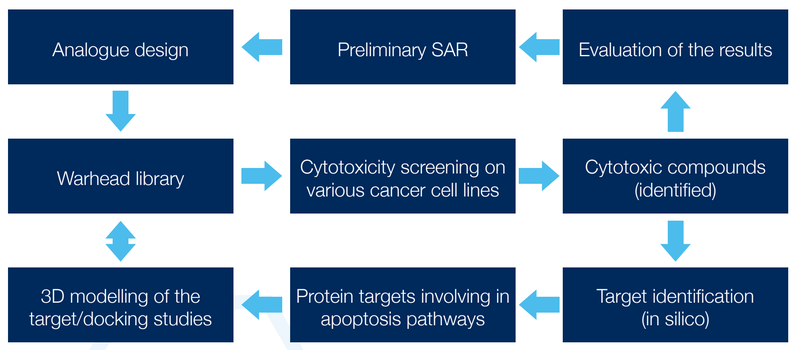


Scheme 4.

MATERIALS AND METHODS

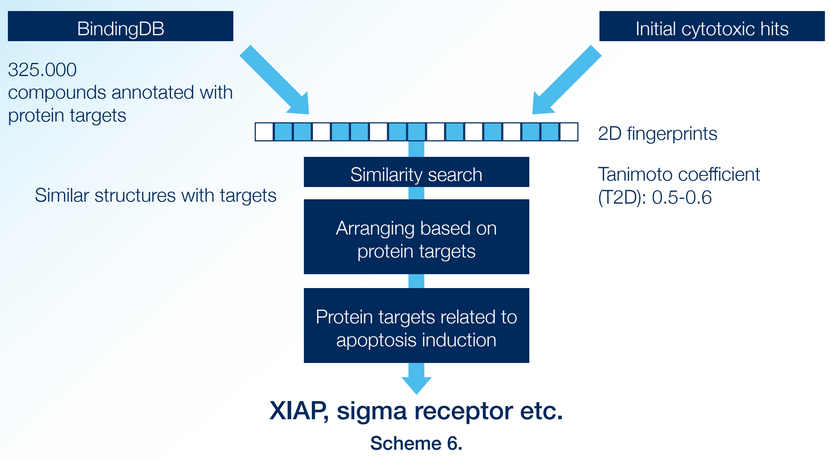
The first step of this process is to design, select and synthesize a potential “warhead” small library and test the compounds in cytotoxicity screening. Based on the results similar compounds (analogues) could be designed and tested in a second round. In parallel, *in silico* target identification could be carried out followed by 3D modelling of the targets and docking the similar compounds. Compounds with the highest docking score would be selected from the warhead library for cytotoxicity screening. (Scheme 5.)

In an initial compound set several hundred diverse compounds were synthesized. After stability assessment and strict quality control (purity: > 95 %) and LogD/LogS assessment 139 compounds containing linker connection functional groups were finally selected and submitted for cytotoxicity tests on PANC1 (pancreas tumour) cell line. For cytotoxicity assay real-time, impedance-based cell analysis was applied, and 15 hit compounds (< 50 % viability@ 10^{-4} M) were identified. The compounds could be arranged into 6 chemotypes.



Scheme 5.

VIRTUAL TARGET IDENTIFICATION



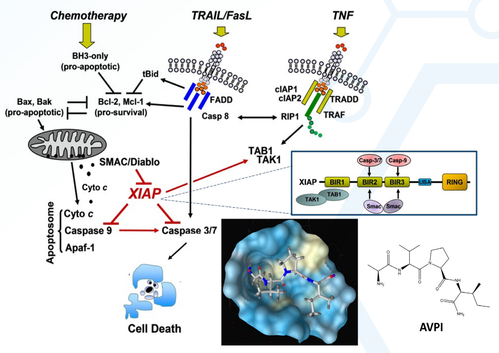
Scheme 6.

Using BindingDB, an annotated database containing 325000 biologically active compounds together with their validated protein targets, was used as a potential source for in silico target identification. In order to find close analogues to our initial cytotoxic hits similarity search was carried out using ChemAxon InstantJChem software. (Scheme 6.) Compounds were considered similar if the Tanimoto coefficient was between 0.5-0.6. The protein targets of the similar compounds were considered as their putative protein targets. Among such targets sigma receptor, and more interestingly XIAP (X-linked inhibitor of apoptosis) were identified.

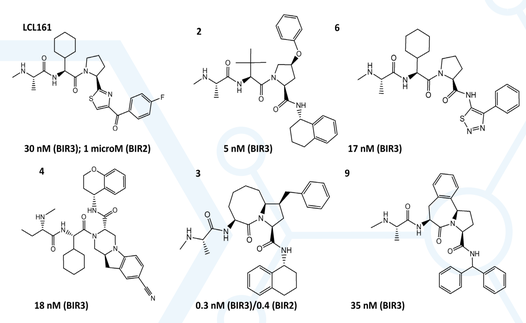
XIAP AS POTENTIAL TARGET FOR INDUCING APOPTOSIS

As a result, one of the novel, distinct and active compound clusters were predicted to act as XIAP blockers, thus, they may activate the apoptosis machinery as well. XIAP directly neutralizes caspase-9 via its BIR3 domain and the effector caspases-3 and -7 via its BIR2 domain. A natural inhibitor of XIAP is SMAC (second mitochondria-derived activator of caspases)/Diablo (direct IAP binding protein with low pI) increases the amount of free, activated caspase-3,-7,and-9 and promotes the final steps of cell death execution (Scheme 7.)[2].

The N-terminal alanine–valine–proline amino acids of SMAC extended with an isoleucine forming AVPI (alanine–valine–proline-isoleucine) serves as a starting point for SMAC-(peptido) mimetics drug design. This tetrapeptide interacts with XIAP BIR2 as well as XIAP BIR3 with cca. 500 nM activity. Most of the XIAP antagonist drug candidates are chemical derivatives of the N-terminal part of SMAC/Diablo. These “SMAC-mimetics” either specifically induce apoptosis in cancer cells or act as drug-sensitizers. 6 examples are shown in Scheme 8. LCL-161 shows similar chemical motifs to our active chemotype.



Scheme 7.



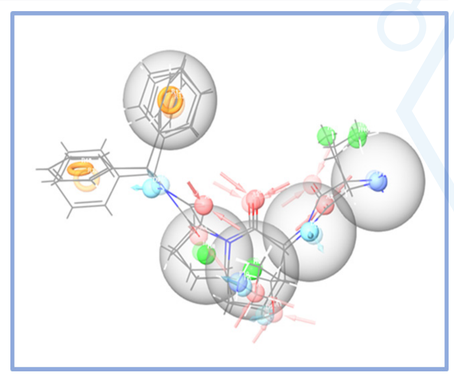
Scheme 8.

DOCKING STUDIES

Based on these findings our attention turned to designing a focused XIAP antagonist targeted library. The available crystal structure of XIAP allowed to generate 3D models in order to identify the major interactions of the hit compounds and allowing to design more effective XIAP antagonists. For 3D modelling the Schrödinger Small-molecule drug discovery suite software package was used complemented with molecular mechanical calculations. For allowing flexibility of the binding the Induced Fit Docking modul was used followed by MM/GBSA free energy calculation. As a crystal structure of XIAP BIR3 domain 4HY0 (PDB) was selected. Based on the available XIAP-BIR3 ligand complexes a consensus pharmacophore model was developed (Scheme 9.).
The generated 3D model was validated with the 6 known hit compounds. (Scheme 8., Table 1.)

Mol	Docking score	ΔG_{bind}
LCL161	-8.386	-88.853
I2	-9.706	104.908
I3	-9.755	-99.114
I4	-10.532	-109.560
I6	-8.592	-88.639
I9	-9.673	-95.976

Table 1.



Scheme 9.

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1. Mező G, Manea M., Expert Opin. Drug Deliv., 2010, 7, 79-96.
2. Obexer, P., & Ausserlechner, M. J. Frontiers in oncology, 2014, 4, 197

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AZ NEXT ALAPJÁL MEGVALÓSULÓ PROJEKT
AZ INNOVÁCIÓ LENDÜLETE