Development of novel drug candidates for selective inhibition of Bcl-2 protein–protein interactions

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Introduction

Protein–protein interactions as a therapeutic target

- The Bcl-2 family of proteins is central to the regulation of apoptosis, and their overexpression has been linked to certain types of lymphoma and carcinoma,[1] as well as resistance to conventional antitumor treatments. In particular, the interactions between pro- and anti-apoptotic Bcl-2 family proteins control the integrity of the outer mitochondrial membrane.
- The aim of this project was to develop a set of novel anti-cancer drug candidates that function by selectively inhibiting such protein–protein interactions (PPI).[2]
- The present work focuses on the Hit Finding and Hit-to-lead phases for the development of novel inhibitors of PPI within the Bcl-2 protein family.

Target Identification → **Hit Finding** → **Hit to Lead** → Lead Optimization → Pre-Clinical → Clinical

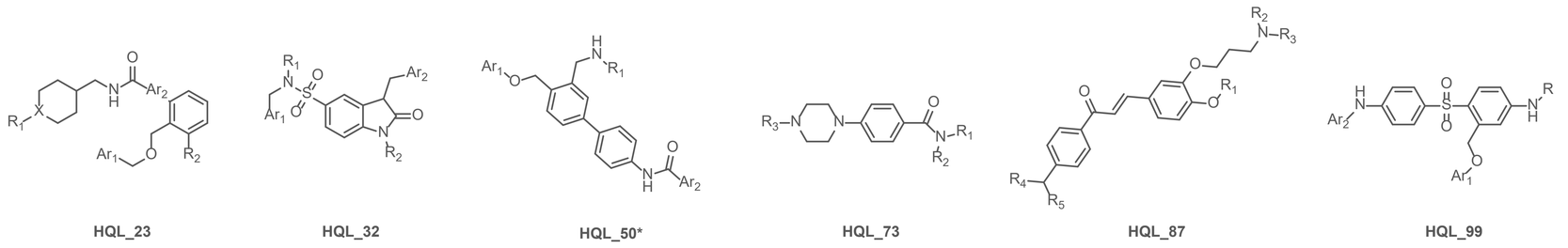
Generation of Virtual Hits

HQL Pharmaceuticals

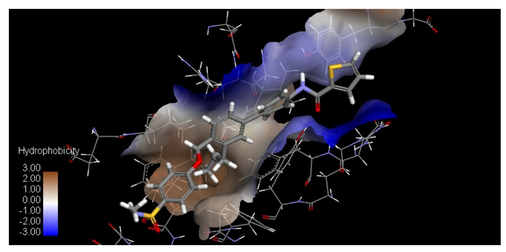
- ChemSpaceScanner (CSS): Proprietary computational drug discovery platform for virtual screening of huge chemical libraries (>10^{30} molecular entities) at high resolution.
- A virtual library of potential candidates was designed and refined following ADMET and stability criteria. The structures were then clustered by scaffold similarity and further refined by synthetic feasibility and patentability.
- The obtained structures presented high chemical diversity, with very different scaffolds (bicyclic systems or highly functionalized aromatic rings) and varied substitution patterns including chiral centers in some cases.
- Activity optimization was performed through the typical design–synthesis–assay cycles with subsequent refinement of the computational models.

Screening and Optimization

Synthetic routes were designed for each of the families, representative compounds were prepared, and their inhibitory activity evaluated by Surface Plasmon Resonance (SPR). Peptides corresponding to the BH3 domain helixes of pro-apoptotic Bcl-2 family members were attached to carboxyl-coated SPR chips via their *N*-terminus, and the binding of the protein to the chip-bound peptide was measured. Compounds were incubated at multiple concentrations with the protein prior to injection. *Series HQL_50 was added after the initial scaffold screening. (IC50 data shown only for the best compound within each series and iteration.)



HQL_23 HQL_32 HQL_50* HQL_73 HQL_87 HQL_99



Scaffold screening » » » » » » » » » » **Round 2** » » » » » » » » » » **Round 3** » » » » » » » » » » **Lead**

16 compounds

Series	IC50 (μM)
HQL_23	not active
HQL_32	32 – 200
HQL_73	180 – 300
HQL_87	not active
HQL_99	not active

33 compounds

Series	IC50 (μM)
HQL_32	2,6
HQL_73	4 – 50
HQL_50	0,5 – 2

62 compounds

Series	IC50 (μM)
HQL_32	1,3
HQL_73	1,5 – 12
HQL_50	0,25 – 0,5

New scaffold HQL_50 very active.
» » » » Stability issues in HQL_32 solved by elongating linker in Ar_2.
» » » » » » » » Refinement of computational model to adjust for different binding modes.

HQL_50(123)	
Bcl-xL	250 nM
CYP1A2	not active
CYP2C9	282 nM
CYP2C19	2,13 μM
Permeability (Papp)	4,79 ×10^{-6} cm/s
Cell survival MV4-11	
10 μM, 24 h	Apoptosis

Conclusions

The aim of this project was to develop a set of novel anti-cancer drug candidates that function by selectively inhibiting Bcl-2 protein–protein interactions (PPI).

In collaboration with HQL Pharmaceuticals, a virtual library of potential candidates was designed. The virtual hits were clustered into families, and selected compounds were chosen for synthesis. The activity of the compounds was evaluated by SPR measurements. After the initial hit-finding stage, the structures were further optimized (hit-to-lead) to improve the activity from micromolar to the nanomolar range.

Keywords: protein–protein interactions, Bcl-2, hit-to-lead, drug discovery

References

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[2] Mullard, A. *Nat. Rev. Drug Discov.*, 2012, 11, 173.

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