In silico screening of new potential TCR/CollagenII-MHCII inhibitors against rheumatoid arthritis

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Project goal

The Major Histocompatibility Complex (MHC)-restricted recognition of antigen peptides by T cell receptors (TCRs) of T-helper cell is a central event in the cellular immunity against pathogens. Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation of the joints that progressively invalidates patients. Genetic studies have shown that RA is strongly associated with the MHC class II allele HLA-DR4 and residues 261-273 of CollagenII (CII) have been identified as an immunodominant T cell epitope restricted by the DR4 molecule[1]. The interaction between TCR Vβ and CII (261-273)/HLA-DR4 complex has therefore increasingly gained attention as a valuable target for a novel strategy against RA. The aim of this study is to develop a pharmacophore virtual screening followed by molecular docking and *in vitro* assays leading to the identification of new TCR/CII(261-273)/HLA-DR4 inhibitors.

Starting point

The protein-protein interactions between TCR Vβ and CII(261-273)/HLA-DR4 served as targets for the development of new inhibitors. For this purpose, the 3D model structure of the TCR Vβ/CII(261-273)/HLA-DR4 ternary complex we recently generated from the TCR Vβ domain of a patient affected by RA[2], was used.

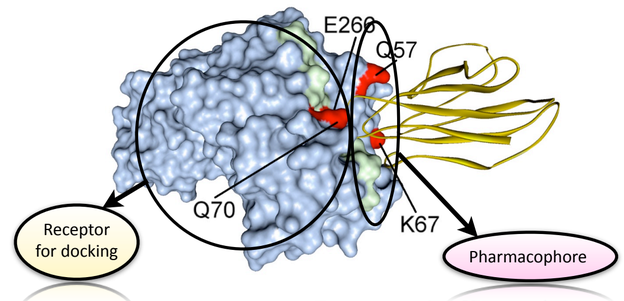

Figure 1
TCR-Vβ/CII(261-273)/HLA-DR4 interface. *TCR is represented as a yellow ribbon, HLA-DR4 (gray) and CII(261-273) (green) surfaces are shown. Residues predicted to be hotspots (ΔΔG>1.0 kcal/mol) are shown in red.*

Virtual Screening Strategy

ZINC DATABASE
Asinex + Maybridge
(587723 compounds)

Molecular weight from 160 to 500
LogP from -0.4 to 5.6
H-bond donors (OH and NH) ≤ 5
H-bond acceptors (O or N) ≤ 10

Lipinsky rule-based filtering improved by Ghose[3]

Subset I
(50553 compounds)

Multiconformer 3D database by Generate Conformations module in Discovery Studio v 2.5 (Accelrys Inc. San Diego, CA).

Pharmacophore database screening

Subset II
(37 compounds)

Hit compounds identified by LigandScout

Mol ID	FitValue	Mol ID	FitValue	Mol ID	FitValue
RAIA1	48.34	RAIA14	46.06	RAIA27	44.05
RAIA2	47.31	RAIA15	46.01	RAIA28	44.01
RAIA3	47.20	RAIA16	45.99	RAIA29	43.94
RAIA4	47.04	RAIA17	45.87	RAIA30	43.91
RAIA5	46.94	RAIA18	45.84	RAIA31	43.87
RAIA6	46.93	RAIA19	45.46	RAIA32	43.70
RAIA7	46.90	RAIA20	45.21	RAIA33	43.67
RAIA8	46.88	RAIA21	45.11	RAIA34	43.61
RAIA9	46.70	RAIA22	45.07	RAIA35	43.31
RAIA10	46.62	RAIA23	45.00	RAIA36	42.87
RAIA11	46.60	RAIA24	44.97	RAIA37	42.84
RAIA12	46.51	RAIA25	44.43		
RAIA13	46.40	RAIA26	44.23		

DOCKING

Hit compounds (432 conformations) were computationally docked to the binding site of CII(261-273)/HLA-DR4 complex using the docking program AUTODOCK v 4.2[5]. Molecular docking was carried out using the Lamarckian genetic algorithm (LGA) and the default docking parameters.

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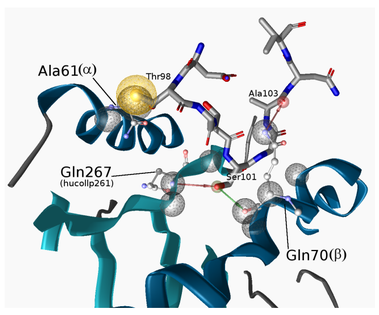
TOP-RANKING COMPOUNDS

Pharmacophore generation

The software LigandScout[4] was used for the automatic construction of 3D pharmacophore from structural data of macromolecule-ligand complex. The macromolecule was represented by HLA-DR4 in complex with the collagen peptide 261-273 whereas the ligand was comprised of residues 97-105 of TCR Vβ.



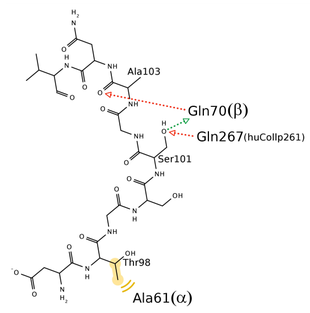
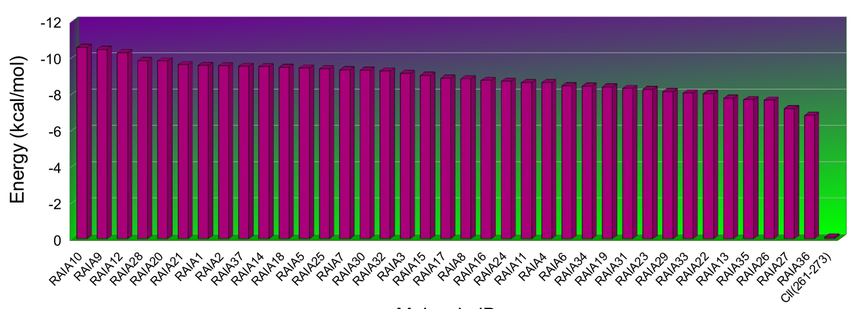


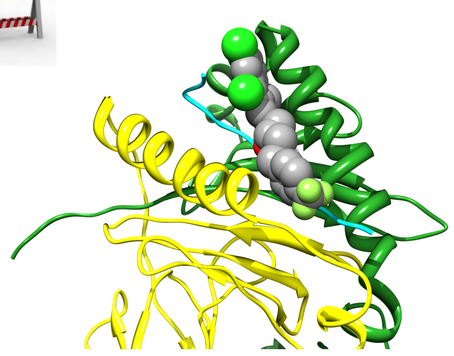
Figure 2
Structure-based pharmacophore model. *The pharmacophore hypothesis contained 14 features: one hydrophobic group (yellow sphere), two H-bond acceptors (red arrows), one H-bond donor (green arrow) and ten excluded volumes (grey spheres).*

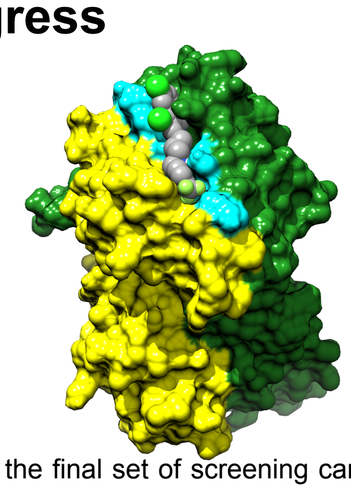
Docking results

Mol ID	Energy (kcal/mol)	Mol ID	Energy (kcal/mol)	Mol ID	Energy (kcal/mol)
RAIA10	-10.56	RAIA7	-9.32	RAIA19	-8.38
RAIA9	-10.45	RAIA30	-9.30	RAIA31	-8.30
RAIA12	-10.27	RAIA32	-9.27	RAIA23	-8.24
RAIA28	-9.83	RAIA3	-9.13	RAIA29	-8.13
RAIA20	-9.82	RAIA15	-9.03	RAIA33	-8.05
RAIA21	-9.61	RAIA17	-8.87	RAIA22	-8.01
RAIA1	-9.56	RAIA8	-8.83	RAIA13	-7.76
RAIA2	-9.54	RAIA16	-8.74	RAIA35	-7.68
RAIA37	-9.52	RAIA24	-8.69	RAIA26	-7.66
RAIA14	-9.50	RAIA11	-8.62	RAIA27	-7.18
RAIA18	-9.46	RAIA4	-8.62	RAIA36	-6.82
RAIA5	-9.43	RAIA6	-8.45	CII(261-273)	-0.10
RAIA25	-9.38	RAIA34	-8.42		



Work in progress





1) We are currently testing the best molecules from the final set of screening candidates for *in vitro* toxicity by measuring the induction of necrosis and apoptosis of PBMC (peripheral blood mononuclear cells) obtained from RA patients and cultured in the presence of inhibitor molecules.

2) Expansion of collagen specific T cells, in the presence of CII(261-273) peptide and inhibitor molecules, will be measured by immunoscope as we recently described[6].

References

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3) Ghose, A. et al. J Combin Chem (1999) 1:55-68
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5) Morris, G. M. et al. J Comput Chem (1998) 19:1639-62
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