Apr 20th, 1:00 PM - 3:00 PM

Computational Design of Novel Insulin Degrading Enzyme Inhibitors

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Computational Design of Novel Insulin Degrading Enzyme Inhibitors

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Abstract 1

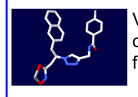
Human insulin degrading enzyme (IDE) plays a role in the proteolytic cleavage of insulin, glucagon, and other short, hydrophobic peptides with roles in glucose and cellular metabolism. Because of IDE's role in insulin clearance, IDE inhibitors may hold promise as therapies for potentiating insulin signaling in patients suffering from type 2 diabetes mellitus. IDE is a large (~100 kDa) chambered protease of the conserved M16A subfamily of zinc metalloproteases. The enzyme adopts a structure that is analogous to a clamshell formed by the joining of the N terminal and C terminal domains. The characteristic zinc binding and catalytic motif (HXXEH) is positioned within the enzyme's N terminus, while C terminal residues also play important roles in substrate binding and catalysis. Here, we describe the use of a computational work-flow for identifying novel IDE inhibitors. The work flow integrates mutation-based active site structural analysis, virtual screening, docking and fragment-based design. Initial computational results appear promising and should lead to assay testing in the near future.

Introduction 2

- Insulin-degrading enzyme (IDE) a 110 kDa zinc metalloprotease is involved in the clearance of many bioactive peptide substrates, including insulin (K_m = 85 nM) and amyloid-beta, peptides vital to the development of diabetes and Alzheimer's disease, respectively
- Located in the cytosol its proteolytic activity subject to complicated signaling regulation inside of cells
- The role of IDE in the clearance of insulin suggests that IDE inhibitors could be used moderate levels of insulin in type 2 patients
- The IDE active site consists of two clamshell like structure connected by a liker which is flexible allowing "open and closed state" which allows access to the catalytic zinc
- Large active site called crypt ~ 15,700 Å
- IDE can exist in solution as monomer and then aggregates to its most active form the dimer
- When the enzyme opens the enzyme encapsulates the substrate that primarily bind to an exosite 30 Å away

J. Mol. Biol. 395, 430-443 (2010), J Biol Chem. 2015 Aug 14;290(33):20044-59, *Trends in Endocrinology & Metabolism* 2016 27, 24-34DOI: (10.1016/j.tem.2015.11.003, Eur J Med Chem. 2015 January 27; 90: 547–567

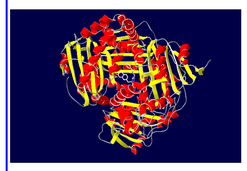
Molecular Docking 3



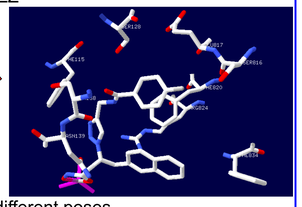
Virtual Libraries based on physical properties from Zinc Database

Predicted ligand poses and binding affinities rescoring using four other criteria

Docking Software Vega-ZZ

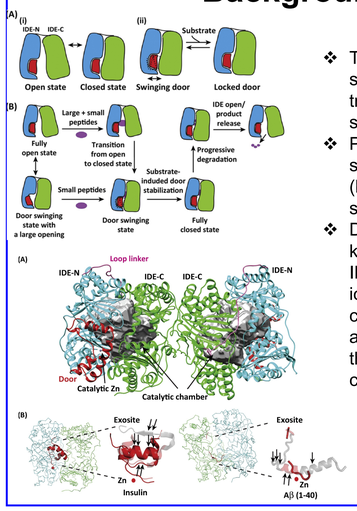


Protein-Receptor



- Generates poses and ΔG's
- Each virtual compound has ten different poses
- Sort out duplicates between the five different binding criteria
- Compare pose to crystal ligand pose
- Analyze distance of docked ligand and metal in enzyme
- Look for hydrogen bonding interactions
- Analyze fit in binding pockets look for cavities in enzyme
- Look for acceptable pose buy compound test on *in vitro* assay
- Generate pharmacological hit

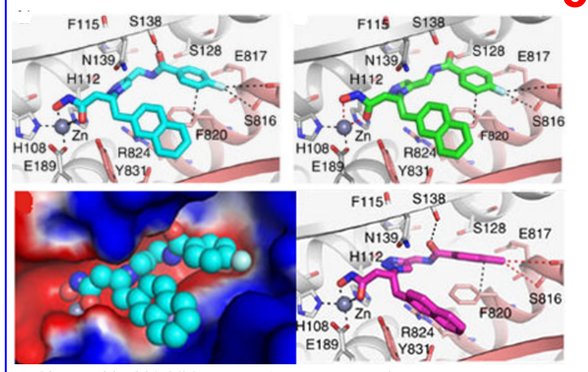
Background 4



- Two key conformational switches open and closed transition in absence of substrate
- Partly open allow smaller substrates peptides (bradykinin) fully open larger substrates such as insulin
- Dimeric IDE structure. The key features of IDE including IDE-N, IDE-C, catalytic zinc ion, and door subdomain, are colored in cyan, green, gray, and red, respectively, while the surface of catalytic chamber of IDE is in gray

Trends in Endocrinology & Metabolism 2016 27, 24-34DOI: (10.1016/j.tem.2015.11.003,

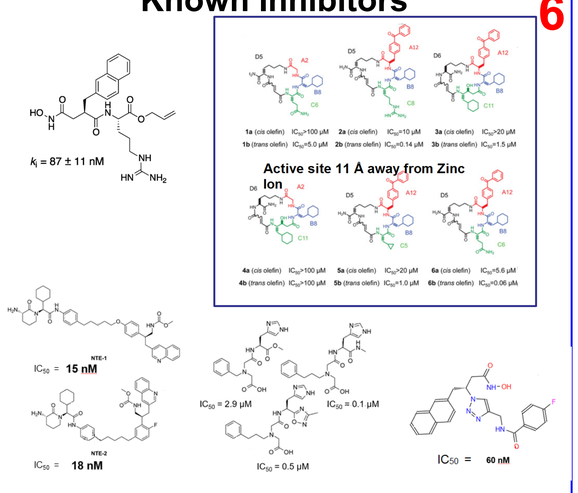
Active Site 5



- Known 60 nM inhibitor crystal structure used
- Forms close contacts at catalytic site with IDE-N, IDE-C
- Chelates to zinc ion with hydroxamate
- Fluorine group form π stacking interaction with Phe820
- Triazole interacts with Arg824
- Naphthyl group hydrophobic interactions F820, F834

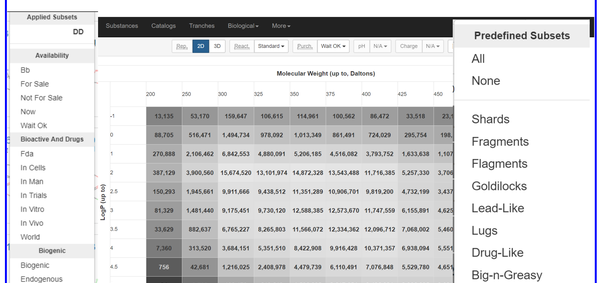
Nature Communications volume 6, Article number: 8250 (2015) doi:10.1038/ncomms9250

Known Inhibitors 6



Leissring,J. Med. Chem., 2013, 56 (6), pp 2246–2255, Nat Commun. 2015 Sep 23;6:8250, J Biol Chem. 2015 Aug 14;290(33):20044-59, Eur J Med Chem. 2015 January 27; 90: 547–567, Nature volume 511, pages 94–98 (03 July 2014)

Zinc Database 7



- Rationally design virtual library based on Lipinski's Rules molecular weight, log P, and other criteria
- Test only compounds that can be purchased with the selected compounds to be tested on just the target/enzyme
- When pharmaceutical hit is found it can be drawn to find congeners for further optimization

J. Chem. Inf. Model., 2015, 55 (11), pp 2324–2337

Results 8

Sum of Appearance	Column Labels					
Row Labels	Contacts	Electron DD	Plants	PLP	PLP NF	Grand Total
Grand Total	255	255	255	255	255	1275
00651	1	8	8	4	1	22
00652	4	10	4	2	1	21
02309		9	10			19
02847	1		2	4	8	15
03005		10	5			15
03009		10	5			15
03010		10	5			15
03018		8	6	1		15
04845	1		4	6	3	14
01952	1		3	4	5	13
02846			2	3	8	13
03006		9	4			13
00094			2	8	2	12
01737	1		1	3	7	12
03695		10	1	1		12
04221	2		4	3	3	12
00926			6	5		11

- Top 5% of virtual compounds are summarized in excel sheet
- Each virtual compound 10 different conformations
- Excel computes duplicates from the five different criteria that evaluates virtual docking studies

Results 9

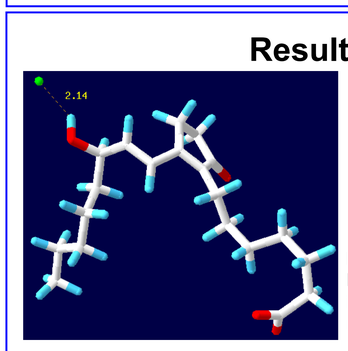


Figure 1 Pose of docked ligand showing the distance with the metal zinc (green) look for distances < 2.5 Å

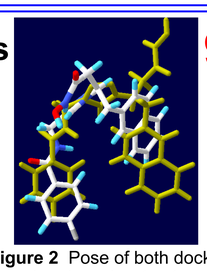


Figure 2 Pose of both docked ligand and crystal structure inhibitor (gold) from protein model

Type of Scan	log p	Sub Type	MW	Library Size
Virtual	1	Drug Like	375	765
Virtual	2.5	Drug Like	375	1043
Virtual	2	Drug Like	375	1478
Virtual	2	Drug Like	350	1462
Virtual	2	Drug Like	425	921
Fragment	5	fragment	350	536

Table 1 Different parameters applied for the docking studies

Results 10

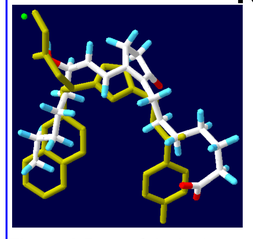


Figure 3 Pose of docked potential inhibitor with crystal structure ligand(gold), metal zinc (green)

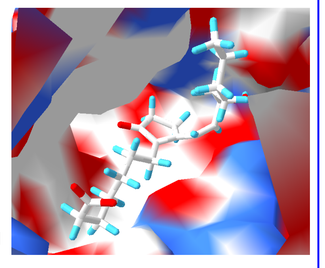
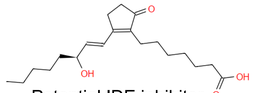


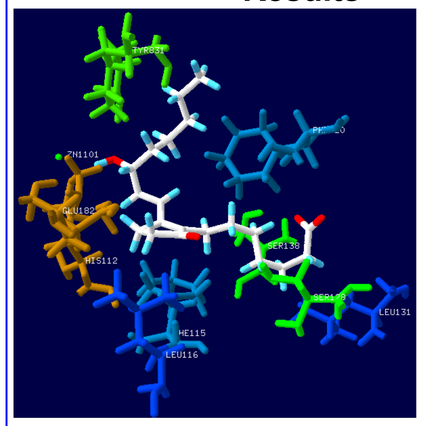
Figure 4 IDE inhibitor at active site gray hydrophobic area



Potential IDE inhibitor

Figure 5 Pose of docked potential inhibitor at the binding pocket showing cavities

Results 11



- Active site of IDE very large 15,700 Å cavities to build into
- Generate virtual compound library of congeners of known IDE inhibitors
- Generate virtual compound library of higher molecular weights

Figure 6 IDE inhibitor (white) at active site showing potential hydrogen bonding with SER 138, amino acids: shades of blue non-polar, green polar, brown charged.

Conclusion Future Work 12

- Using physical properties for the ligands, multiple parameters for docking studies allows smaller more manageable libraries/ data
- Biological assay/testing of the interesting compounds from docking studies to be evaluated *in vitro* on the target enzyme
- Initial identification of the pharmaceutical hit optimize via virtual compounds
- Identification of the pharmacophore
- Site directed mutagenesis studies
- Looking for biological clues that will drive the synthetic chemistry
- Are there cavities in the enzyme that can be build into
- Find new hydrogen bonding interactions
- Fragment based drug design
- Generate synthetic schemes to generate congeners of the pharmaceutical hit
- Continue molecular modeling to support synthetic chemistry
- Evaluate pharmacokinetics ADME