Heat Shock Protein 90/70 (HSP90/70)

Targeted Library

June, 2012
YAI, ChemDiv, Inc.
The Core Strategy

Analyze the structures of reported HSP90/70 inhibitors (more than 1K molecules);

- Analyze the structures of HSP90 and HSP70 proteins and conformational shifts within the active binding site observed upon ligand binding;
- Binding modes and allosteric binding sites identification/analysis;
- Include selective HSP90 and HSP70 binders;
- 3D-model construction/validation;
- Select small-molecule compounds from ChemDiv store (more than 1.5 mil molecules) with potential HSP90/70 activity following several structure determinant, including similarity, privileged scaffolds and “med-chem” filters;
- Molecular Docking of selected compounds / “Pro-Drug” approach
Representative examples of reported HSP90 inhibitors

- **Novobiocin**
  - Hsp90 C-terminal inhibitor
  - NB disrupts Bcr-Abl/Hsp90 and Bcr-Abl/Hsp70 interactions
  - Launched (anticancer)
  - Pfizer

- **CNF-2024**
  - Phase II (anticancer)
  - ATP-site ligand, HSP90
  - Conforma Therapeutics

- **PF-04928473**
  - Phase I (anticancer)
  - ATP-site ligand, HSP90
  - Pfizer

- **Tanespimycin (17-AAG)**
  - ATP-site ligand, HSP90
  - Phase III (anticancer)
  - Pfizer

- **Ganetespib**
  - Phase II/III (anticancer)
  - ATP-site ligand, HSP90
  - Synta Pharmaceuticals

- **AT-13387AU**
  - Phase II (anticancer)
  - ATP-site ligand, HSP90
  - Astex Pharmaceuticals

- **KW-2478**
  - Phase I/II (anticancer)
  - Kyowa Hakko Kirin

- **NVP-AUY922**
  - Phase II (anticancer)
  - Novartis

- **NVP-HSP990**
  - Phase I (anticancer)
  - Novartis

- **XL-888**
  - Phase I (anticancer)
  - Selective HSP90 inhibitor
  - Exelixis

- **Biological Testing**
  - Selective HSP90 inhibitor
  - Sanofi

- **Pfizer**

- **Memorial Sloan-Kettering Cancer Center**

- **Sloan-Kettering Institute**

- **Biological Testing**

- **Exelixis**

- **Sanofi**

- **Pfizer**
Representative examples of reported HSP70 inhibitors

**KNK-437**
Preclinical
Inhibits the synthesis of HSP90
Kaneka

**VER-155008**
Preclinical
Vernalis
IC$_{50}$ = 0.5 mcM

**Mal-2-213**
Biological Testing
ATP-site ligand
Columbia University
IC$_{50}$ ~30 nM

**YK-5**
Biological Testing
Allosteric site inhibitor
Memorial Sloan-Kettering Cancer Center

**MKT-077**
Phase II (anticancer) allosteric inhibitor
Dana-Farber Cancer Institute

**Myricetin**
Allosteric site inhibitor
The final library content (more than 15K compounds)

compounds with ‘moderate-to-high’ structural similarity (Tanimoto coeff. >0.5) to the reported HSP90 and HSP70 inhibitors, *isosteric modifications, topological analogues*

**EXAMPLES:**

![Compound developed by Kyowa Hakko Kirin](image1)

ID: K808-1985

Compound developed by Kyowa Hakko Kirin
HSP90 inhibitor under early biological evaluation
Kitamura, Y. et al. US 7781485 Aug 24, 2010

![Compound developed by Emory University](image2)

ID: G856-2554

Compound developed by Emory University
HSP90 inhibitor under early biological evaluation
Min, J. et al. US 022070, 2012

IC\(_{50}\)=0.770 µM (Displacement of geldanamycin)
H417 human small-cell lung carcinoma cells

compounds successfully passed through the developed **3D-models**

**EXAMPLES:**

![Compound](image3)

ID: G857_1210

HIGH SCRORE

![Compound](image4)

ID: K219_0094

MEDIUM SCRORE
non-trivial \(\alpha\)-helix and \(\beta\)-sheet mimetics (HSP90/70-'client protein' interaction mimetics)

compounds with a high diversity in structure (minor subset), including nature-like compounds, e.g. spiro-compounds (Escape from flatland)

EXAMPLES:
Statistic

 Num. of unique heterocycles: 176;
 Num. of unique screens: 3,663;
 Diversity: 0.801 (cosine coefficient);
 Num. of comb.templates: 340
Hsp90 exists as two isoforms: Hsp90α and Hsp90β

- Hsp90 consists of a highly conserved N-terminal domain, a charged linker, and a highly conserved C-terminal region, in general, the charged linker is not important for Hsp90 function, and the inhibitors of Hsp90 mainly bind to the N-and C-terminal regions. The N-terminal domain binds the natural products geldanamycin (GDA), radicicol and their derivatives, which modulate at least two different conformational states. Novobiocin binds to the C-terminal nucleotide binding site and inhibits Hsp90 function.

- The first Hsp90 inhibitor drug is **geldanamycin**, a natural product isolated from *Streptomyces hygroscopicus*. The geldanamycin analogue **17AAG** (17-allylamino-17-demethoxy-geldanamycin) possesses all the Hsp90-related characteristics of geldanamycin but with lower toxicity. Another natural Hsp90 inhibitor is **radicicol**.

![Geldanamycin](image1.png)  
![17AAG](image2.png)  
![Radicicol](image3.png)
Besides the natural compounds, some synthesized inhibitors have also shown promising effects on Hsp90, for example, purine-based inhibitors the Pyrazole-isoxazole analogues, Novobiocin and coumarin scaffold analogues, such as 4TCNA

Benzamide tetrahydro-4H-carbazol-4-one analogs (BT), AT13387 derivatives (AT) and Dihydroxylphenyl amides (DA) have been developed as further potent Hsp90 inhibitors

3D-QSAR, CoMFA, CoMSIA, ANN and 3D-pharmacophore as well as 3D-docking methods were employed to investigate several HSP90 purine-based inhibitors, which provided useful models for designing the Hsp90 targeted compounds

the common pharmacophoric features, i.e., one H-bond donor, one H-bond acceptor, one hydrophobic (aromatic), and two hydrophobic (aliphatic) features, which might be of import to develop inhibitors as anti-cancer agents
to date, there are no 3D-QSAR studies of many ligands reported except very few classes. Moreover, no comprehensive feature for the ligand-receptor interactions, such as the hydrophobic contact between the key amino acid residues has been demonstrated.

Purine-based HSP90 pharmacophore developed by Chen includes hydrophobic (blue), hydrogen bond acceptor (green) and hydrogen bond donor (purple). The distances of features were labeled by blue lines.

Chen CY. Insights into designing the dual-targeted HER2/HSP90 inhibitors. J. Mol. Graph. Model. 2010, 29(1), 21-31
More than 50 crystallographic complexes of small-molecule compounds including several BBs with HSP90 have been described to date. A majority of these complexes were investigated during the study.

MolSoft Software™ was used to analyzed these complexes with regard to their structural homology and the ligand binding modes.

The ATP-binding site was thoroughly reconstructed based on the 3D-alignment of many protein-ligand complexes.

Reported HSP90 ligands were flexibly redocked to the binding site of Hsp90 protein to internally evaluate the model. The optimum docking conformations as the most probable binding conformation corresponding with a low energy score were analyzed.

Hsp90α N-terminal domain bound to ATP (3T0Z) Homo sapiens
The 3D-alignment of two crystallographic data obtained for ATP and **geldanamycin**

- **D93, T184** and **G97** are the most crucial conservative (rigid) points in the first common binding mode

- **AAs which coordinate the cofactor** (Mg$^{2+}$) **are the second target**

- **there are several other AAs which can also provide significant contacts with ligands within the ATP-binding site**, including N106 (adjusted), K112, K58 (adjusted), F138 and G137, N106 (adjusted), L107 (adjusted), N51, G97
**VHD** is one compound among a wide fragment-based library screened recently against HSP90. For many fragments the related crystallographic data was obtained. We also used these data to develop our 3D-docking model.

ATP bound to the binding site of HSP90 (orange), direct ATP analogues in the site (yellow). Crystallographic data, superposition.

From the fragment-based library.
Crystal Structure of HSP90 with N-Aryl-benzimidazolone

Key contacts: **G97, T184, D93**

**K58-sulfonyl oxygen (weak)**
Another binding mode was revealed for several tricyclic HSP90 inhibitors.

**H-bonding:** Y139, L103

**Hydrophobic and stacking contacts:**
Phe138, Y139, Leu103, Ala111, Leu107, Phe170, Gly106, Phe22, W162
Combined features form the third binding mode for HSP90 ligands

N(conf): 24
minE: -99.97
maxE: -71.49
Energy (calc): -82.78
RMSD: 6.401e-007

N(conf): 15
minE: -85.68
maxE: -54.12
Energy (calc): -85.68
RMSD: 6.915e-007
N(conf): 17  
minE: -77.63  
maxE: -41.11  
Energy (calc): -77.63  
RMSD: 6.718e-007

N(conf): 21  
minE: -111.7  
maxE: -67.89  
Energy (calc): -111.7  
RMSD: 6.193e-007
N(conf):11
minE:-86.47
maxE:-32.4
Energy (calc):-69.8
RMSD: 6.119e-007
0.1 sens

N(conf):11
minE:-100.1
maxE:-22.38
Energy (calc):-100.1
RMSD: 6.924e-007
0.1 sens
Hsp70 chaperones are key to cellular protein homeostasis
Hsp70 proteins are allosteric machines and offer, besides classical active-site targets, also opportunities to target the mechanism of allostery
Potent anticancer compound MKT-077 has recently been described as an "allosteric drug"
Using NMR spectroscopy the compound's binding site on human Hsc70 (hHsc70 has 85% identity with human Hsp70) was identified. Fractional shifts upon addition of MKT-077 in the 15N,2H-labeled hHsc70 include T222, A223, D225, H227, L228. For each resonance, the shift at a molar ratio of 0.5 was defined as 50% completed
We have reconstructed the allosteric binding site using this data and docked MKT-077

ATP-binding site for competitive inhibitors
**Selective Binding to HSP90**

► XL888 is a potent and selective ATP-competitive inhibitor of HSP90 developed by Exelixis
► XL888 binds to the target in a manner that is structurally distinct from other HSP90 inhibitors currently in the clinic
► XL888 selectively inhibits members of the HSP90 family and it is inactive against HSP70, the structurally related Bergerat-fold ATPase Topoisomerase II, and a diverse panel of serine/threonine and tyrosine kinases
► Crystallographic analysis of XL888 bound to HSP90 has showed that XL888 extends across the width of the ATP-binding domain. A flexible region of ~30 amino acids referred to as the “lid” (magenta ribbon) adopts a different conformation when bound to XL888 compared with ADP or Geldanamycin

Insight into the selectivity, XL888 binding mode

yellow – ATP; orange – compound 1

Terminal region adjustment and alternative binding cavity formation for penetration

yellow – docking results
orange – crystallographic data

Selective HSP90 inhibitors docked into the reconstructed binding site
SNX-2112 (Pfizer)


NVP-AUY922 (Novartis)

Representative examples of compounds from HSP90/70 library

Possible O-dealkylation by CYP3A4

HIGH SCORE

\[ E_p = -60 \text{ kcal/mol} \]
presumably > Pgp-target > poor BBB permeability

MEDIUM SCRORE

\[ E_p = -64 \text{ kcal/mol} \]
Pro-Drug Strategy

\[ D398_0454 \xrightarrow{\text{O-dealkylation}} D398_0454_1 \]

Medium SCRORE

\[ E_p = -47 \text{ kcal/mol} \]
Pro-Drug Strategy

\[
\begin{align*}
&\text{G801_0201} & & \text{G801_0201_1} \\
&\text{N-dealkylation} & & \text{N-dealkylation} \\
&\text{CYP3A4} & & \text{CYP3A4} \\
&\text{N51/S52} & & \text{N51/S52} \\
&\text{H2O} & & \text{H2O} \\
&\text{MEDIUM SCORRE} & & \text{MEDIUM SCORRE} \\
&E_p = -74 \text{ kcal/mol} & & E_p = -74 \text{ kcal/mol}
\end{align*}
\]
Pro-Drug Strategy

N\nN
NH
N
O
O
CH3
OH
OH
N
N
NH
N
O
O
CH3
metabolic sites
hydroxylation
CYP3A4
possible
deacetylase
site S062-1044 S062-1044-1
D93
N51
T184
G97
K58
H2O
Pro-Drug Strategy

HIGH SCRORE
E_p = -74 kcal/mol