Electronic Supplementary Material (ESI) for RSC Advances.


**Supplementary data**

# Disruption of Protein-Protein Interactions: Hot spot detection, structure-based virtual screening and in vitro testing for anti-cancer drug target – Survivin

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## Contents

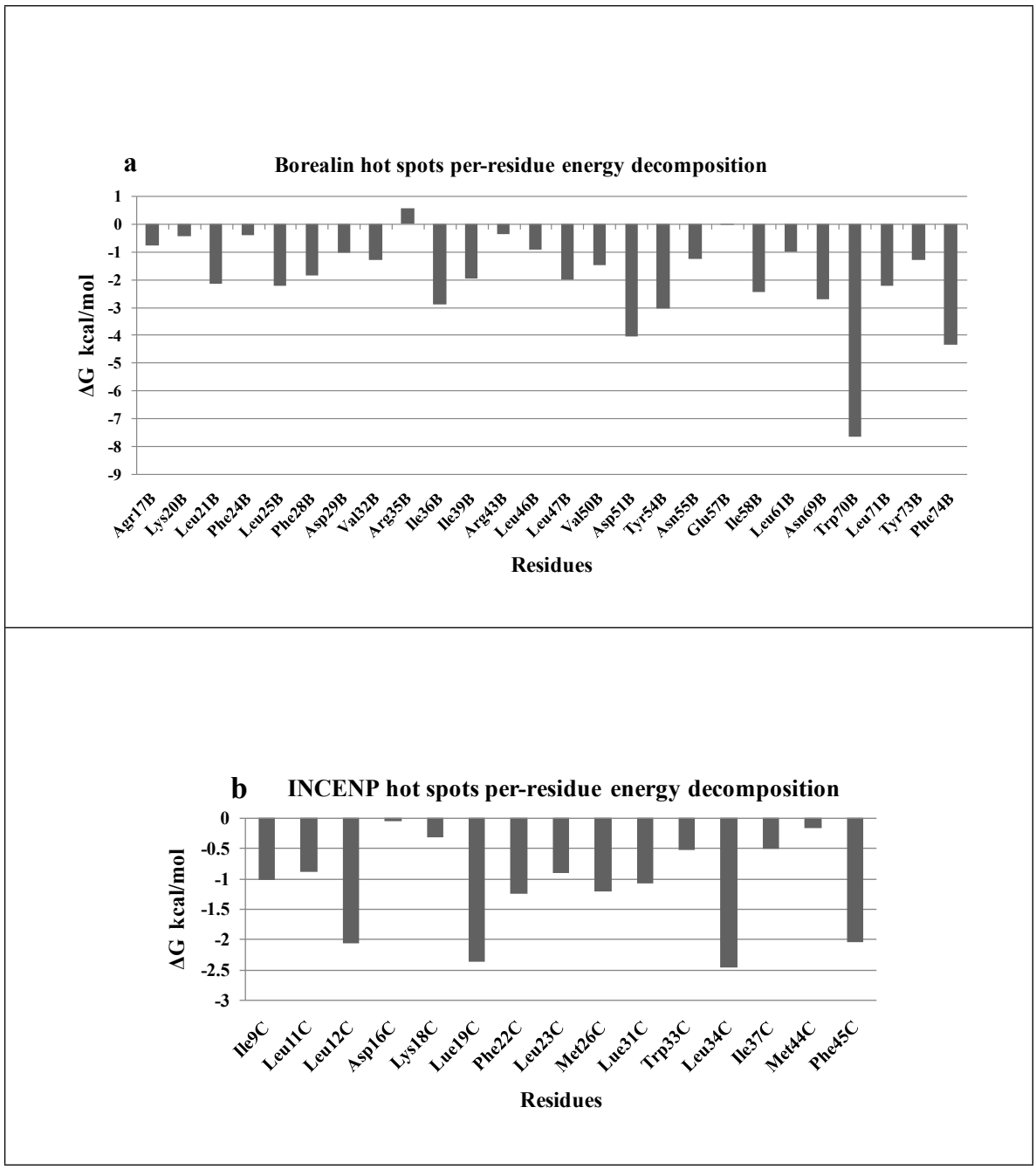



**Figure S1.** Hot spot residue-wise energy contribution for the complex (a) Borealin hot spot residues energy contribution and (b) INCENP protein hot spot residues energy contribution.

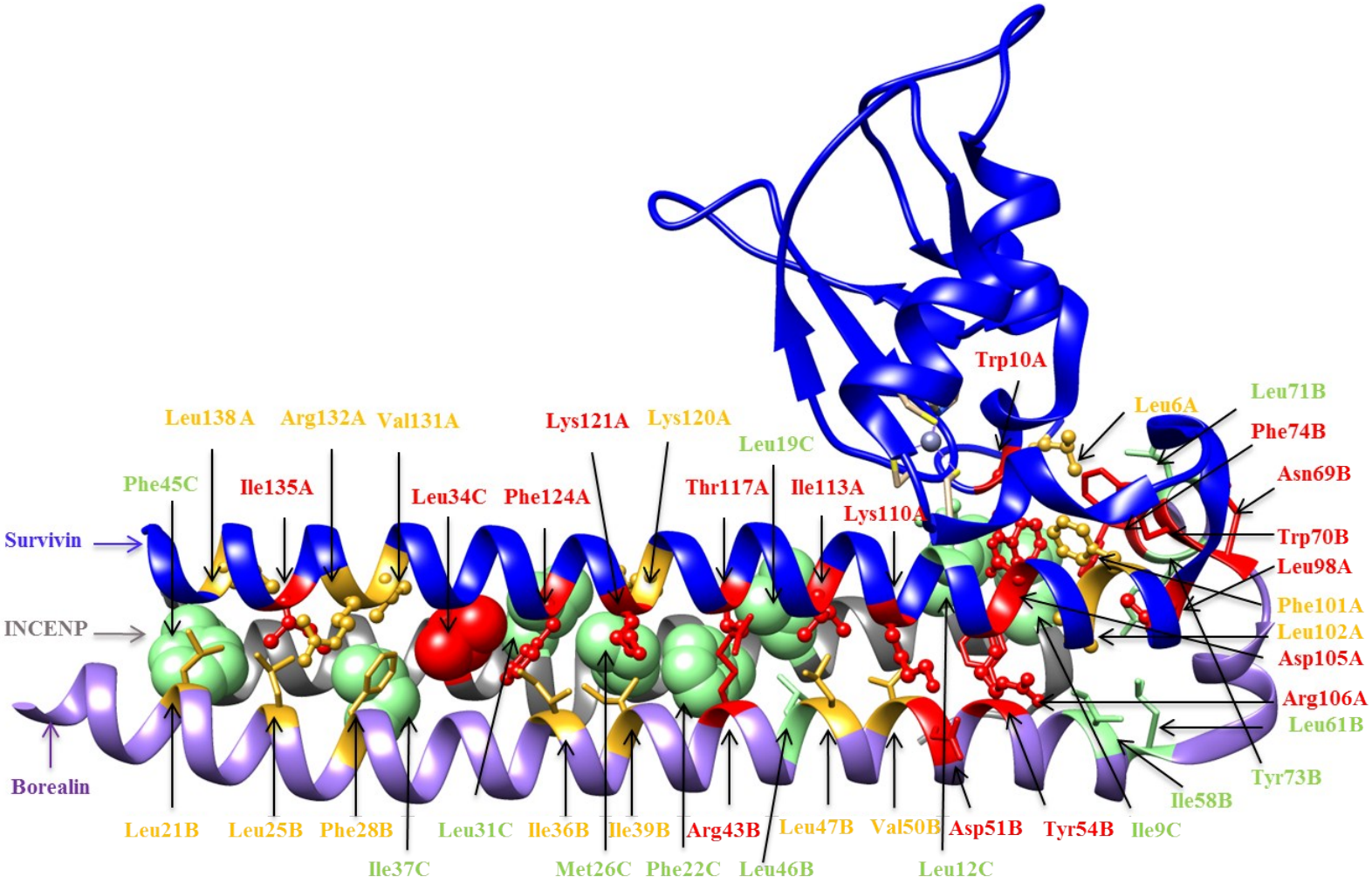



**Figure S2.** Mapping of the energetically important residues in CPC interface region. Hot spots, warm hot spots and null spots are represented in red, gold and light green color, respectively. The CPC complex partners Survivin, Borealin and INCENP are represented in blue, purple and gray color, respectively.

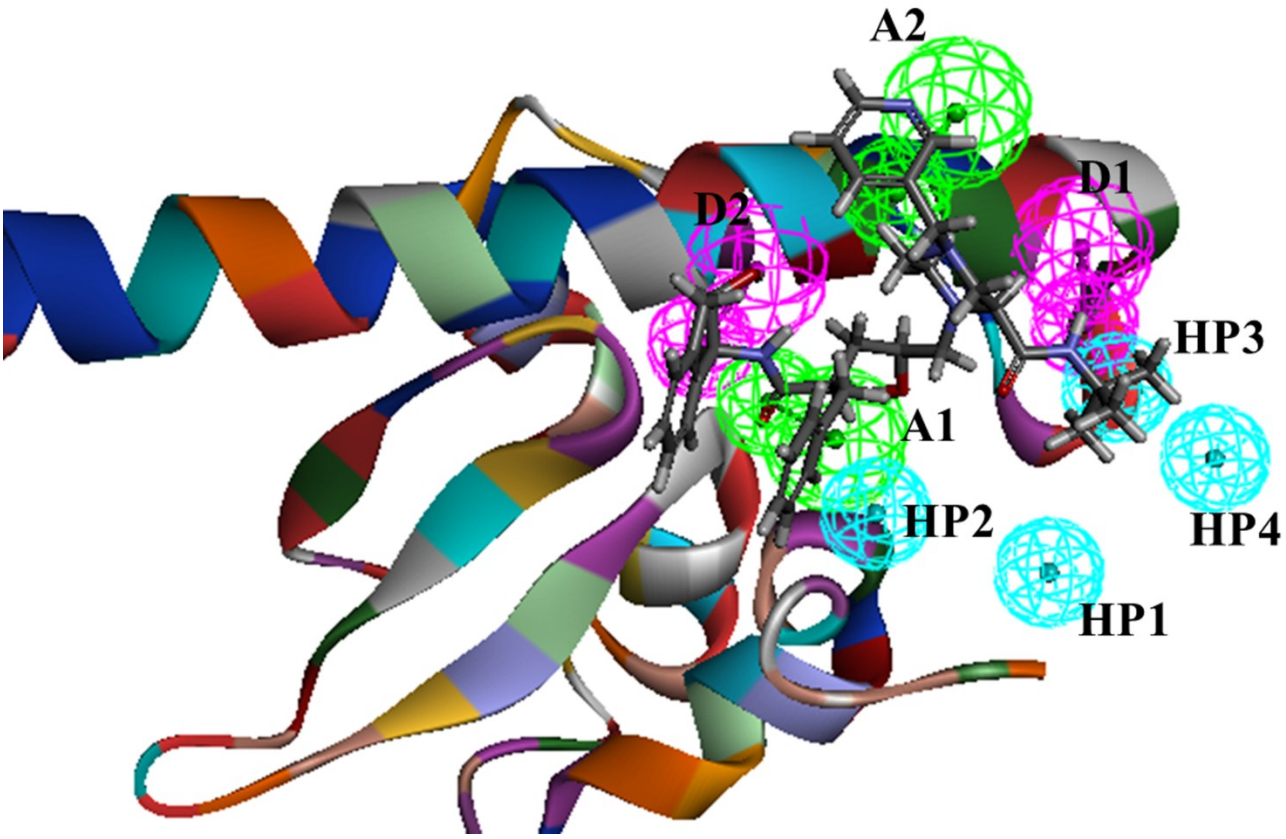



**Figure S3.** Binding mode of Indinavir (stick model) in Survivin, mapped over the pharmacophore model used for virtual screening.

**Table S1.** Identification of survivin dimer hot spot residues using Robetta server, KFC server and HotRegion database

| Residue Name | Robetta server | HotRegion database | KFC server |
|---|---|---|---|
| Survivin chain A residues | | | |
| Leu6A | NH | H | NH |
| Trp10A | H | H | NH |
| Phe93A | NH | H | NH |
| Leu98A | H | H | H |
| Phe10A | NH | H | H |
| Leu102A | NH | NH | H |
| Asp105A | NH | NH | H |
| Survivin chain B residues | | | |
| Leu6B | NH | H | NH |
| Trp10B | H | H | H |
| Phe93B | NH | H | NH |
| Leu98B | H | H | H |
| Phe101B | NH | H | H |
| Leu102B | NH | NH | H |
| Asp105B | H | NH | NH |

[a]NH denotes non hot spot and H denotes hot spot

**Table S2**.Identification of CPC hot spot residues using Robetta server, KFC server and Hotregion database

| Residue Name | Robetta Server | HotRegion Database | KFC Server |
|---|---|---|---|
| **Survivin residues** | | | |
| Leu6A | H | H | H |
| Trp10A | H | H | H |
| Phe13A | NH | H | H |
| Phe93A | NH | H | NH |
| Leu96A | NH | H | H |
| Leu98A | H | H | H |
| Phe101A | H | H | H |
| Leu102A | H | H | H |
| Asp105A | H | H | H |
| Arg106A | H | NH | H |
| Agr108A | H | NH | NH |

| | | | |
|---|---|---|---|
| Lys110A | H | NH | H |
| Ile113A | H | NH | H |
| Thr117A | H | NH | H |
| Lys120A | H | NH | H |
| Lys121A | H | NH | H |
| Phe124A | H | NH | H |
| Val131A | H | NH | H |
| Arg132A | H | NH | NH |
| Ile135 A | H | H | H |
| Leu138A | H | NH | H |
| **Borealin residues** | | | |
| Agr17B | H | NH | NH |
| Lys20B | NH | NH | NH |
| Leu21B | H | NH | H |
| Phe24B | NH | H | NH |
| Leu25B | H | H | H |
| Phe28B | H | NH | H |
| Asp29B | H | NH | NH |
| Val32B | NH | H | NH |
| Arg35B | NH | NH | NH |
| Ile36B | NH | H | NH |
| Ile39B | H | NH | H |
| Arg43B | H | NH | H |
| Leu46B | NH | H | NH |
| Leu47B | H | H | H |
| Val50B | NH | H | H |
| Asp51B | NH | NH | H |
| Tyr54B | H | NH | H |
| Asn55B | NH | NH | N |
| Glu57B | NH | NH | N |
| Ile58B | H | H | H |
| Leu61B | NH | H | H |
| Asn69B | H | NH | H |
| Trp70B | H | H | H |
| Leu71B | H | NH | N |
| Tyr73B | NH | NH | H |
| Phe74B | H | NH | H |
| **INCENP residues** | | | |
| Ile9C | NH | NH | N |
| Leu11C | NH | H | H |
| Leu12C | H | H | H |
| Asp16C | NH | NH | NH |
| Lys18C | NH | NH | NH |
| Lue19C | H | H | H |

| | | | |
|---|---|---|---|
| Phe22C | H | NH | NH |
| Leu23C | NH | H | NH |
| Met26C | H | NH | H |
| Lue31C | H | H | H |
| Trp33C | NH | H | NH |
| Leu34C | H | H | H |
| Ile37C | NH | NH | NH |
| Met44C | NH | NH | NH |
| Phe45C | H | H | NH |

[a]NH denotes non hot spot and H denotes hot spot

**Table S3.** Survivin dimer hot spot binding free energy decoposition using MM/PBSA *in silico* alanine scanning mutagenesis.

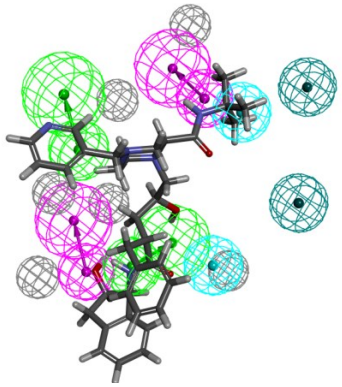
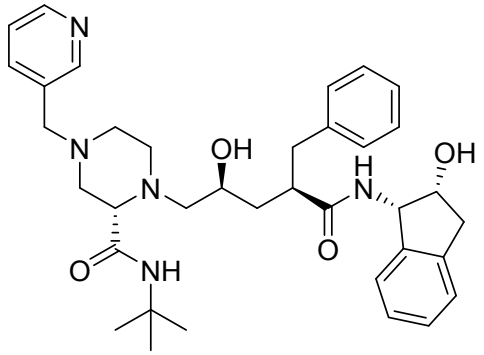
| **Residue Name** | **ASM ΔΔG kcal/mol** | **Residue status** |
|---|---|---|
| **Survivin chain A residues** | | |
| Leu6A | 2.87 | Warm spot |
| Trp10A | 4.83 | Hot spot |
| Phe93A | 2.62 | Warm spot |
| Leu98A | 8.89 | Hot spot |
| Phe101A | 1.66 | Null spot |
| Leu102A | 2.87 | Warm spot |
| Asp105A | 0.91 | Null spot |
| **Survivin chain B residues** | | |
| Leu6B | 0.58 | Null spot |
| Trp10B | 7.89 | Hot spot |
| Phe93B | 3.14 | Warm spot |
| Leu98B | 5.07 | Hot spot |
| Phe101B | 1.61 | Null spot |
| Leu102B | 2.47 | Warm spot |
| Asp105B | 7.11 | Hot spot |

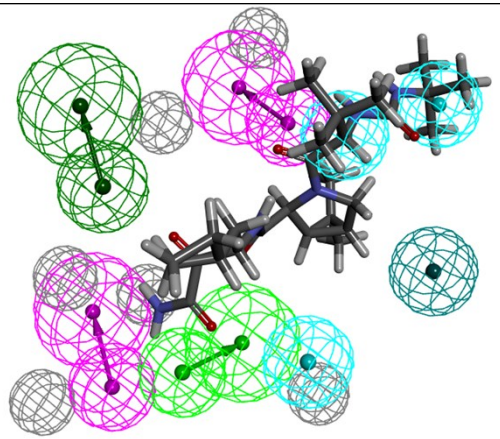
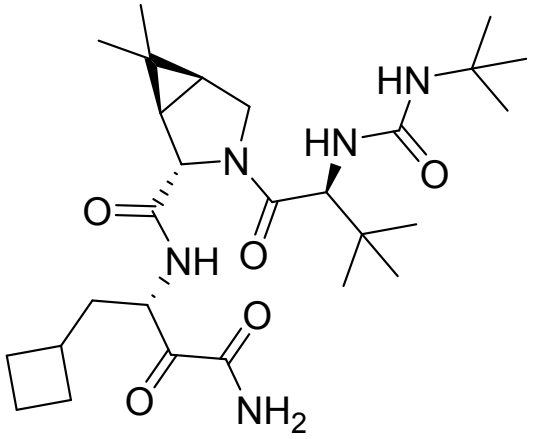
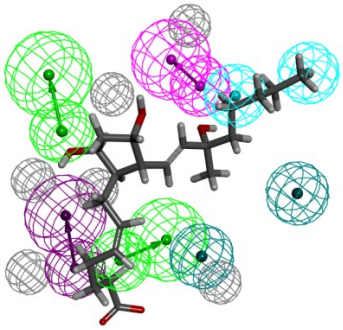
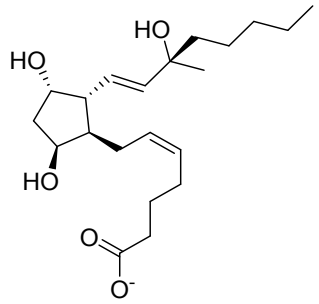
**Table S4.** CPC hot spots binding free energy decoposition using MM-PBSA *in silico* alanine scanning mutagenesis.

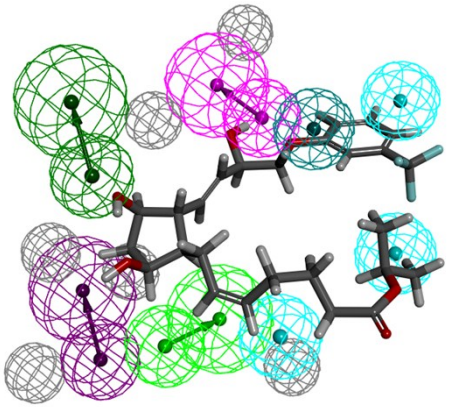
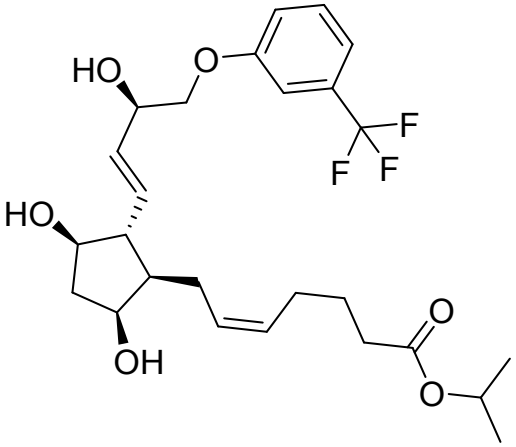
| Residue Name | ASM ΔΔG kcal/mol | Residue status |
|---|---|---|
| **Survivin residues** | | |
| Leu6A | 3.74 | Warm spot |
| Trp10A | 4.39 | Hot spot |
| Phe13A | -0.54 | Null spot |
| Phe93 | 0.84 | Null spot |
| Leu96A | 1.19 | Null spot |
| Leu98 A | 5.14 | Hot spot |
| Phe101 A | 2.08 | Warm spot |
| Leu102 A | 3.57 | Warm spot |
| Asp105 A | 7.96 | Hot spot |
| Arg106 A | 16.15 | Hot spot |
| Agr108 A | 1.87 | Null spot |
| Lys110 A | 11.37 | Hot spot |
| Ile113 A | 4.60 | Hot spot |
| Thr117 A | 6.40 | Hot spot |
| Lys120 A | 2.60 | Warm spot |
| Lys121 A | 5.43 | Hot spot |
| Phe124 A | 5.97 | Hot spot |
| Val131 A | 3.39 | Warm spot |
| Arg132A | 2.01 | Warm spot |
| Ile135 A | 5.86 | Hot spot |
| Leu138 A | 3.45 | Warm spot |
| **Borealin residues** | | |
| Agr17B | 1.09 | Null spot |
| Lys20B | 0.46 | Null spot |
| Leu21B | 2.91 | Warm spot |
| Phe24B | 1.02 | Null spot |
| Leu25B | 2.05 | Warm spot |
| Phe28B | 2.94 | Warm spot |
| Asp29B | 1.52 | Null spot |
| Val32B | 1.04 | Null spot |
| Arg35B | 0.15 | Null spot |
| Ile36B | 2.52 | Warm spot |
| Ile39B | 2.40 | Warm spot |
| Arg43B | 4.23 | Hot spot |
| Leu46B | 1.42 | Null spot |
| Leu47B | 3.87 | Warm spot |

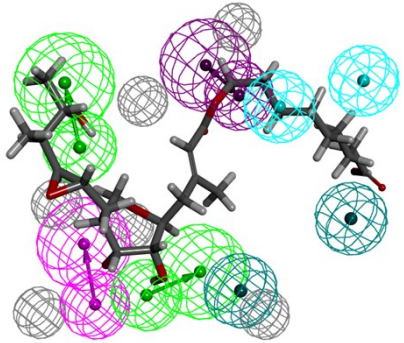
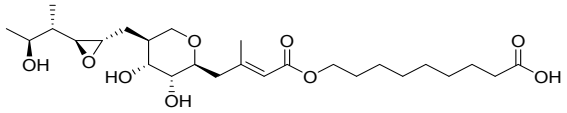
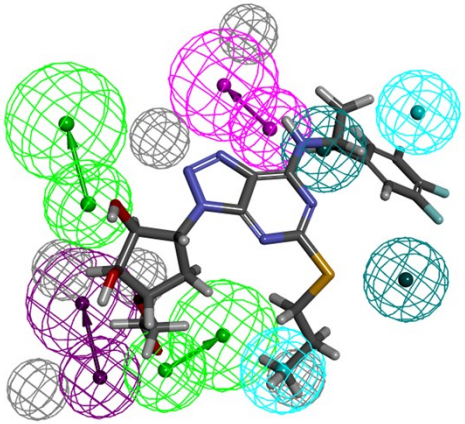
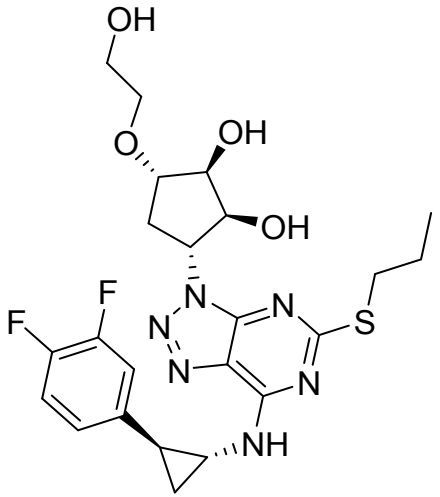
| | | |
|---|---|---|
| Val50B | 2.21 | Warm spot |
| Asp51B | 20.12 | Hot spot |
| Tyr54B | 6.71 | Hot spot |
| Asn55B | 0.72 | Null spot |
| Glu57B | 0.47 | Null spot |
| Ile58B | 1.92 | Null spot |
| Leu61B | 1.72 | Null spot |
| Asn69B | 5.23 | Hot spot |
| Trp70B | 11.99 | Hot spot |
| Leu71B | 1.87 | Null spot |
| Tyr73B | 1.59 | Null spot |
| Phe74B | 4.67 | Hot spot |
| **INCENP residues** | | |
| Ile9C | 1.19 | Null spot |
| Leu11C | 0.53 | Null spot |
| Leu12C | 1.79 | Null spot |
| Asp16C | -0.29 | Null spot |
| Lys18C | 0.28 | Null spot |
| Lue19C | 2.57 | Warm spot |
| Phe22C | 1.25 | Null spot |
| Leu23C | 0.53 | Null spot |
| Met26C | 1.72 | Null spot |
| Lue31C | 0.74 | Null spot |
| Trp33C | 0.26 | Null spot |
| Leu34C | 4.03 | Hot spot |
| Ile37C | 1.56 | Null spot |
| Met44C | 0.09 | Null spot |
| Phe45C | 1.84 | Null spot |

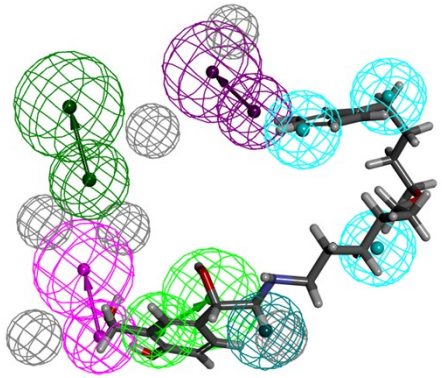
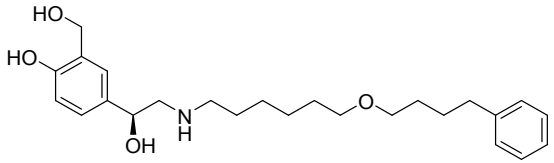
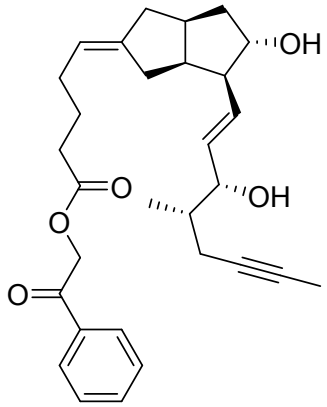
**Table S5**. 2D structure of top scored 10 hits from DrugBank, their mapping to the pharmacophore model and their binding free energy values to the target survivin

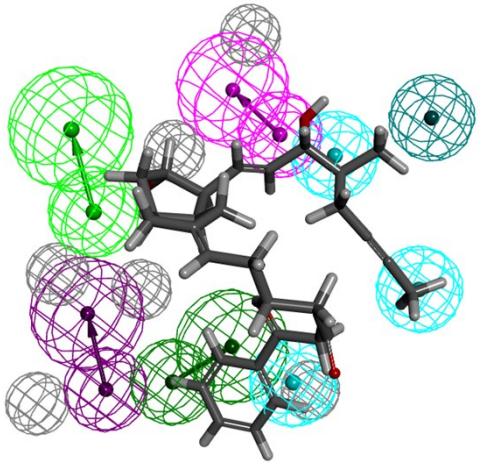
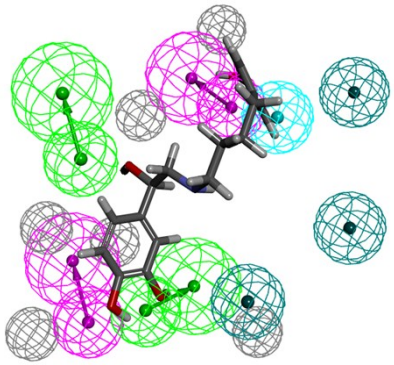
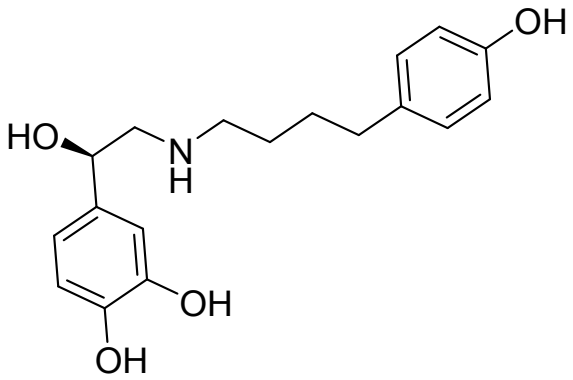
| Sl.No | Binding orientation of the compounds with their pharmacophore features | Compound 2D structure / Name / Drug bank ID | Binding free energy (ΔG) kcal/mol |
|---|---|---|---|
| 1 |  |  Indinavir/ DB00224 (It is protease inhibitor used as a component of highly active antiretroviral therapy to treat HIV/AIDS. | -217.119 |

| | | | |
|---|---|---|---|
| 2 |  |  <br>Boceprevir/ DB08873<br>(It is a protease inhibitor used as a treatment hepatitis caused by hepatitis C virus) | -205.98 |
| 3 |  |  <br>Carboprost Tromethamine/ DB00429 | -186.557 |

| | | | |
|---|---|---|---|
| | | (It is a synthetic prostaglandin analogue of PGF2α with oxytocic properties. Carboprost induces contractions and can trigger abortion in early pregnancy) | |
| 4 |  |  <br>Travoprost/ DB00287<br>(Travoprost used for controlling the progression of glaucoma or ocular hypertension, by reducing intraocular pressure) | -181.652 |
| | | | |

| | | | |
|---|---|---|---|
| 5 |  |  <br>Mupirocin/ DB00410<br><br>(It is an antibiotic of the monoxycarbolic acid class. Effective against gram positive bacteria) | -176.435 |
| 6 |  |  <br>Ticagrelor/ DB08816<br><br>(It is a platelet aggregation inhibitor) | -176.136 |

| | | | |
|---|---|---|---|
| 7 |  |  <br>Salmeterol/ DB00938<br>(Salmeterol is a long-acting beta2-adrenergic receptor agonist used in the maintenance and prevention of asthma symptoms and maintenance of chronic obstructive pulmonary disease (COPD) symptoms) | -171.649 |
| 8 | |  | -148.109 |

| | | | |
|---|---|---|---|
| |  | Iloprost/ DB01088<br><br>(Iloprost is a drug used to treat pulmonary arterial hypertension (PAH), scleroderma, Raynaud's phenomenon) | |
| 9 |  |  <br><br><br>Arbutamine/ DB01102<br><br>(Arbutamine is a cardiac stimulant. It stimulates adrenergic receptors.) | -141.82 |

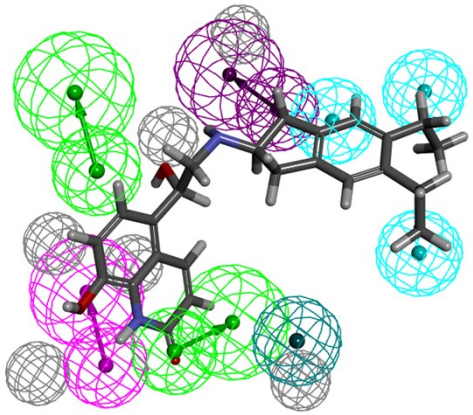
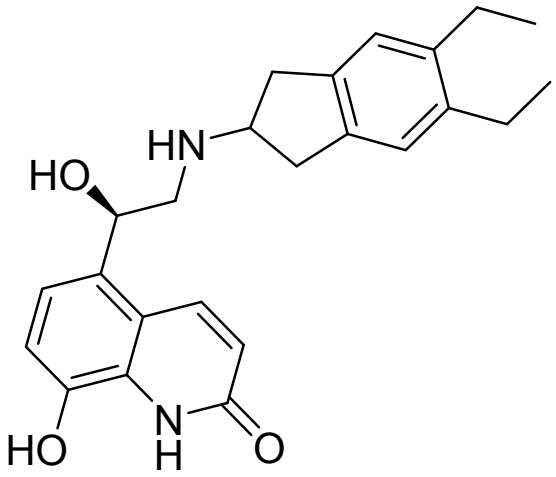
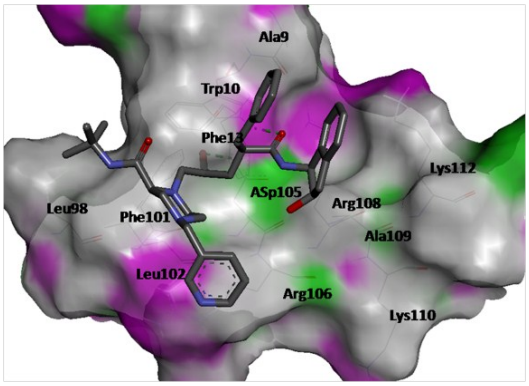
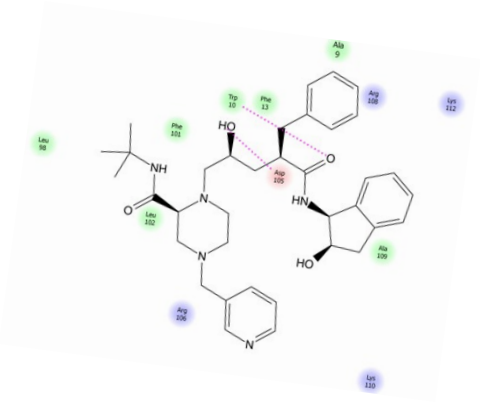
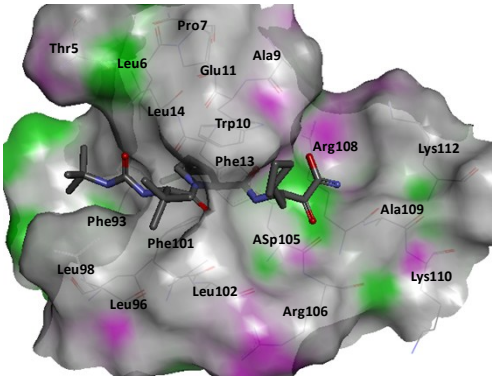
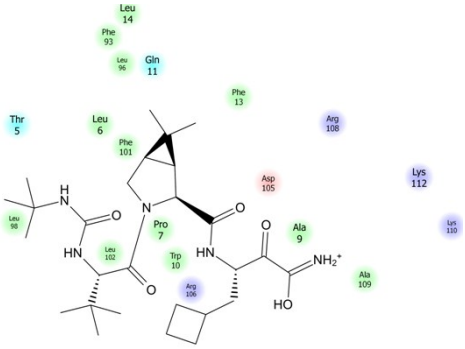
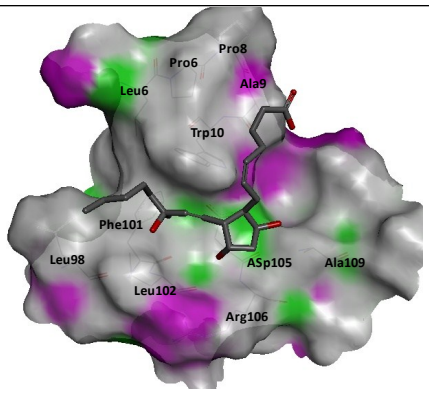
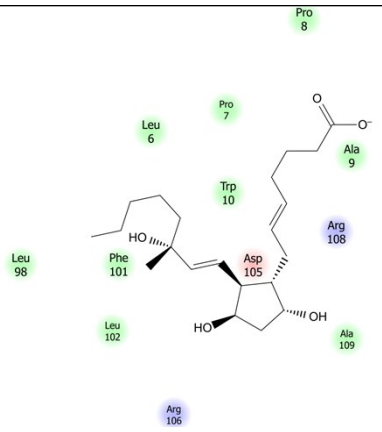
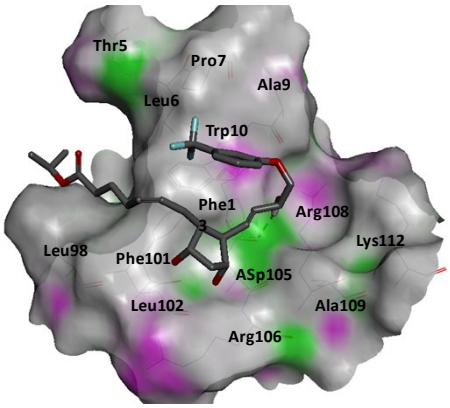
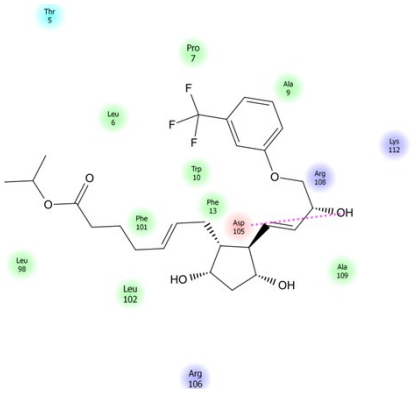
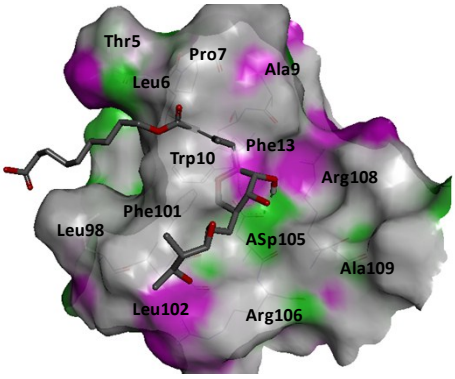
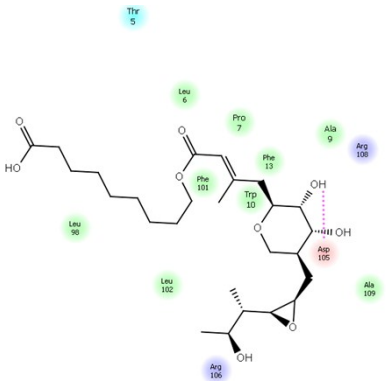
| | | | |
|---|---|---|---|
| 10 |  |  <br>Indacaterol/ DB05039<br>(Indacaterol (INN) is an ultra-long-acting beta-adrenoceptor agonist) | -136.344 |

**Table S6**. Representation of 3D and 2D interaction mode of top scored 10 hits with Survivin hot and warm spot residues (below the figure the interacting hot and warm spots are shown in red and gold color, respectively).

<table>
<tr><th>S.No</th><th>3D structure</th><th>2D structure</th></tr>
<tr><td rowspan="2">1</td><td> 

Indinavir/ DB00224</td><td>  </td></tr>
<tr><td colspan="2">Interactions: HB:Trp10 and Asp105; HP: Ala9, Trp10, Phe13, Leu98, Phe101, Leu102 and Ala109; Charged (+Ve): Arg106, Agr108, Lys110 and Lys112; Charged (-Ve): Asp105</td></tr>
<tr><td rowspan="2">2</td><td> 

Boceprevir/ DB08873</td><td>  </td></tr>
<tr><td colspan="2">Interactions: HP: Leu6, Pro7, Ala9,Trp10, Phe13, Leu14, Phe93, Leu96, Leu98, Phe101, Leu102 and Ala109; Charged (+Ve): Arg106, Agr108, Lys110 and Lys112; Charged (-Ve): Asp105; Polar: Thr5 and Gln11</td></tr>
<tr><td>3</td><td></td><td></td></tr>
</table>

| | | |
|---|---|---|
| |  <br>Carboprost Tromethamine/ DB00429 |  |
| | **Interactions: HP:** Leu6, Pro7, Pro8, Ala9, Trp10, Leu98, Phe101, Leu102 and Ala109; **Charged(+Ve):** Arg106 and Agr108; **Charged(-Ve):** Asp105 | |
| 4 |  <br>Travoprost/ DB00287 |  |
| | **Interactions: HP:** Leu6, Pro7, Ala9, Trp10, Phe13, Leu98, Phe101, Leu102 and Ala109; **Charged (+Ve):** Arg106, Agr108, and Lys112; **Charged (-Ve):** Asp105; **Polar:** Thr5 | |
| 5 |  <br>Mupirocin/ DB00410 |  |
| | **Interactions: HB**: Asp105; **HP:** Leu6, Pro7, Ala9, Trp10, Phe13, Leu98, Phe101, Leu102 and Ala109; **Charged (+Ve):** Arg106 and Arg108; **Charged (-Ve):** Asp105; **Polar:** Thr5 | |

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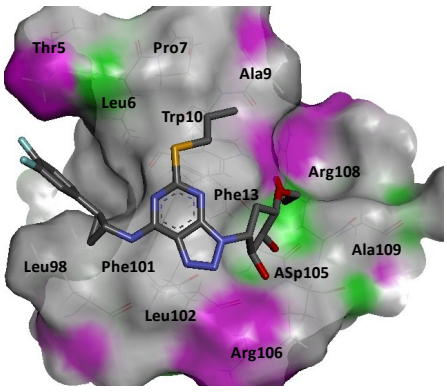
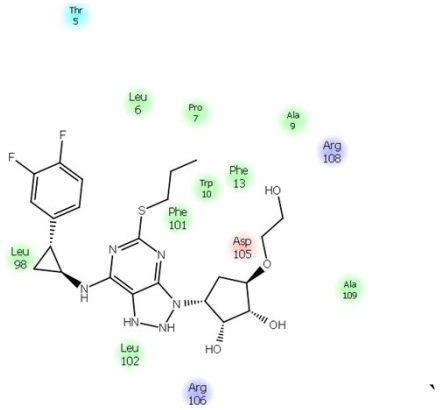
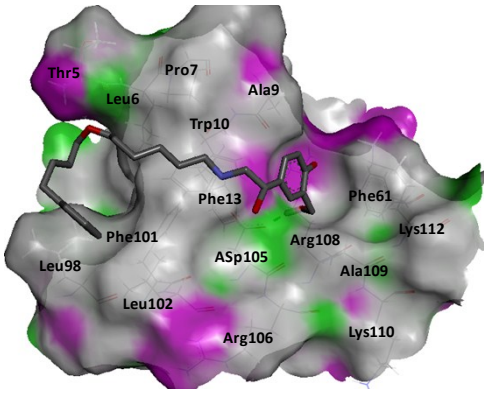
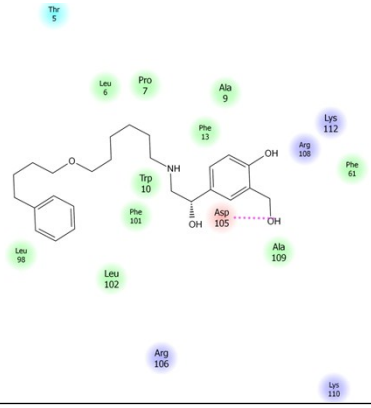
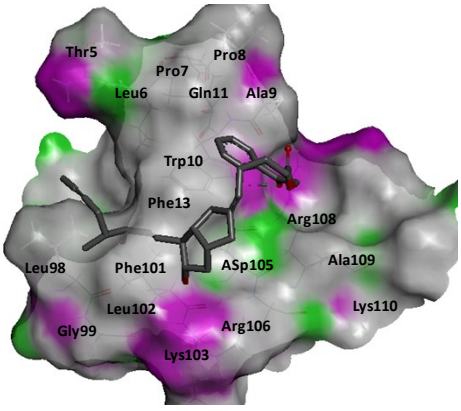
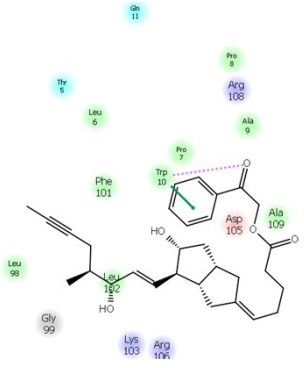
| 6 |  Ticagrelor/ DB08816 |  |
| | **Interactions: HP:** Leu6, Pro7, Ala9, Trp10, Phe13, Leu98, Phe101, Leu102 and Ala109; **Charged (+Ve):** Arg106 and Agr108; **Charged (-Ve):** Asp105; **Polar:** Thr5 | |
| 7 |  Salmeterol/ DB00938 |  |
| | **Interactions: HB:** Asp105; **HP:** Leu6, Pro7, Ala9, Trp10, Phe13, Phe61, Leu98, Phe101, Leu102 and Ala109; **Charged (+Ve):** Arg106, Agr108, Lys110 and Lys112; **Charged (-Ve):** Asp105; **Polar:** Thr5 | |
| 8 |  Iloprost/ DB01088 |  |
| | **Interactions: HB:** Trp10; **HP:** Leu6, Pro7, Pro8, Ala9, Trp10, Leu98, Phe101, Leu102 and Ala109; **Charged (+Ve):** Lys103, Arg106 and Agr108; **Charged (-Ve):** Asp105; **Polar:** Thr5 and Gln11; **π-π stacking**: Trp10 | |

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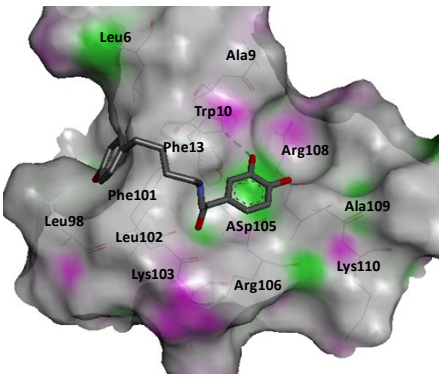
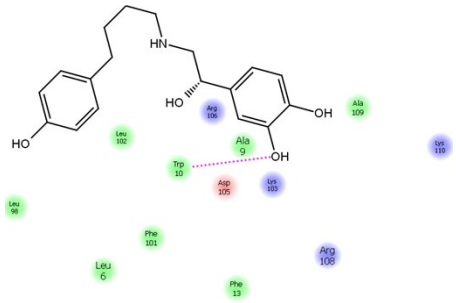
<tr><td rowspan="2">9</td><td> 

Arbutamine/ DB01102</td><td> 
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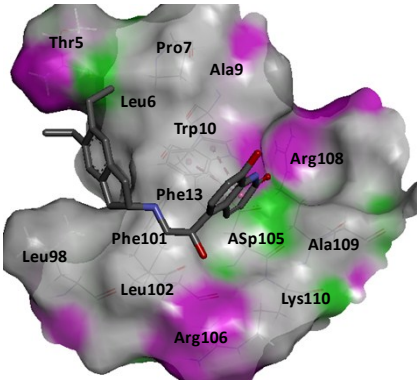
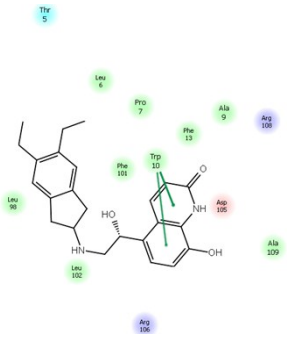
<tr><td rowspan="2">10</td><td> 

Indacaterol/ DB05039</td><td> 

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