*Supplementary Materials*

# Discovery of Novel Hsp90 C-Terminal Inhibitors Using 3D-Pharmacophores Derived from Molecular Dynamics Simulations

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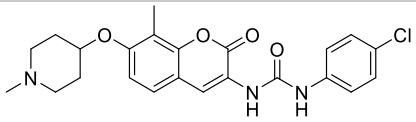
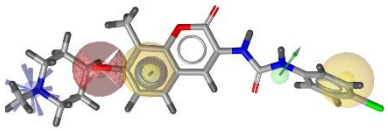
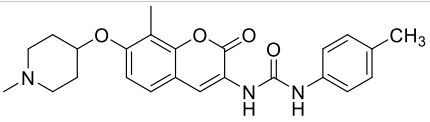
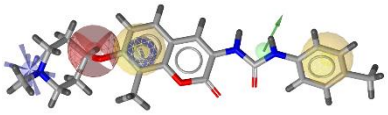
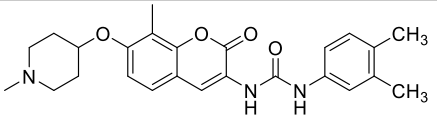
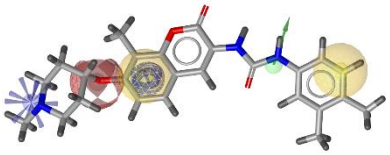
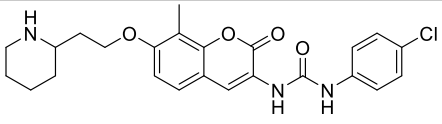
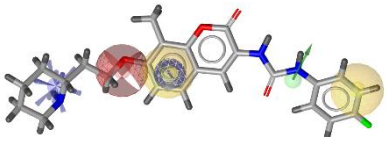
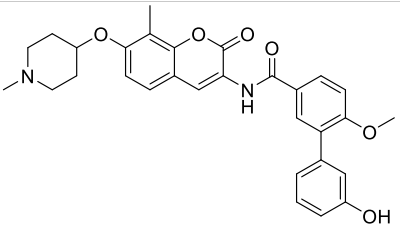
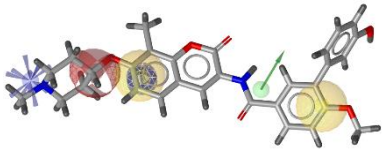
**Table S1.** Structures and antiproliferative activities in SKBr3 cell line of Hsp90 CTD inhibitors **2** and **S1–S12** used as a training set for ligand-based (LB) pharmacophore model creation. Each ligand is shown aligned to the resulting LB model (exclusion volumes are not displayed).

| Cpd | Structure | SKBr3 $IC_{50}$ (μM) | Alignment with the 3D Ligand-Based Pharmacophore Model |
|---|---|---|---|
| **2** |  <br>CC1=C2C(C=C(NC(NC3=CC=C(Cl)C=C3)=O)C(O2)=O)=CC=C1OC4CCN(C)CC4 | 0.17 [1] |  |
| **S1** |  <br>CC1=C2C(C=C(NC(NC3=CC=C(C)C=C3)=O)C(O2)=O)=CC=C1OC4CCN(C)CC4 | 0.27 [1] |  |
| **S2** |  <br>CC1=C2C(C=C(NC(NC3=CC(C)=C(C)C=C3)=O)C(O2)=O)=CC=C1OC4CCN(C)CC4 | 0.31 [1] |  |
| **S3** |  <br>CC1=C2C(C=C(NC(NC3=CC=C(Cl)C=C3)=O)C(O2)=O)=CC=C1OCCC4NCCCC4 | 0.21 [1] |  |
| **S4** |  <br>CC1=C2C(C=C(NC(C3=CC(C4=CC=CC(O)=C4)=C(OC)C=C3)=O)C(O2)=O)=CC=C1OC5CCN(C)CC5 | 0.31 [1] |  |

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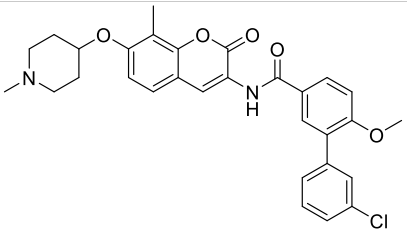
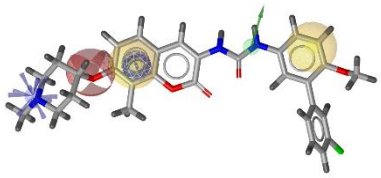
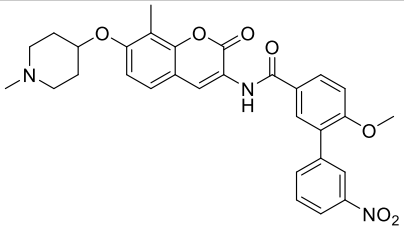
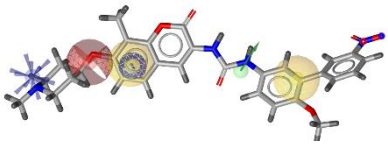
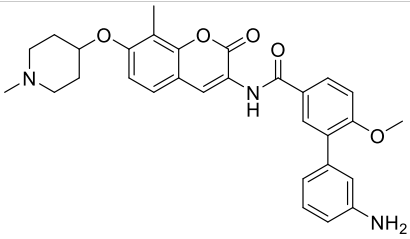
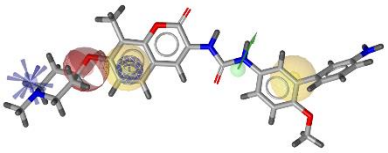
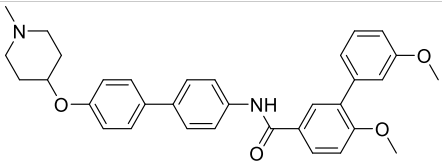
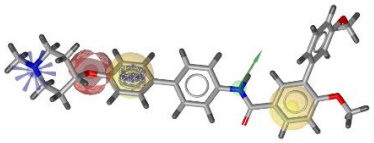
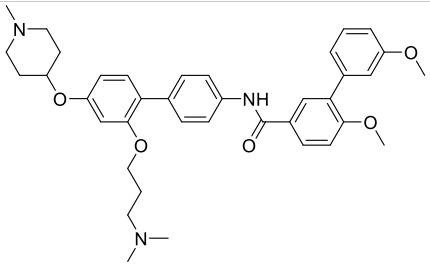
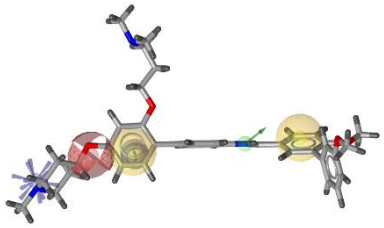
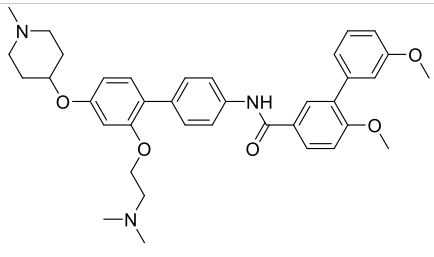
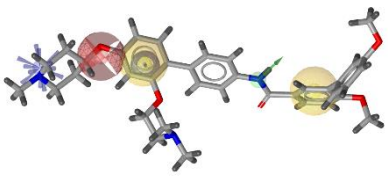
| **S5** |  <br>CC1=C2C(C=C(NC(C3=CC(C4=CC=CC(Cl)=C4)=C(OC)C=C3)=O)C(O2)=O)=CC=C1OC5CCN(C)CC5 | 0.13 [1] |  |
| **S6** |  <br>CC1=C2C(C=C(NC(C3=CC(C4=CC=CC([N+]([O-])=O)=C4)=C(OC)C=C3)=O)C(O2)=O)=CC=C1OC5CCN(C)CC5 | 0.16 [1] |  |
| **S7** |  <br>CC1=C2C(C=C(NC(C3=CC(C4=CC=CC(N)=C4)=C(OC)C=C3)=O)C(O2)=O)=CC=C1OC5CCN(C)CC5 | 0.36 [1] |  |
| **S8** |  <br>CN(CC1)CCC1OC(C=C2)=CC=C2C3=CC=C(NC(C4=CC(C5=CC=CC(OC)=C5)=C(OC)C=C4)=O)C=C3 | 0.47 [2] |  |
| **S9** |  <br>CN(CC1)CCC1OC(C=C2)=CC(OCCCN(C)C)=C2C3=CC=C(NC(C4=CC(C5=CC=CC(OC)=C5)=C(OC)C=C4)=O)C=C3 | 0.39 [2] |  |
| **S10** |  <br>CN(CC1)CCC1OC(C=C2)=CC(OCCN(C)C)=C2C3=CC=C(NC(C4=CC(C5=CC=CC(OC)=C5)=C(OC)C=C4)=O)C=C3 | 0.34 [2] |  |

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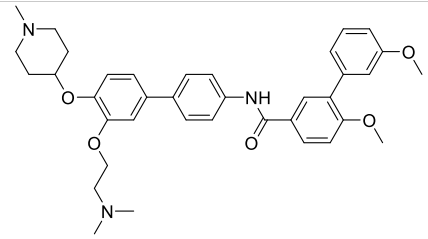
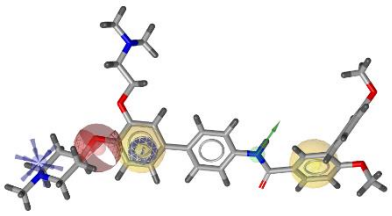
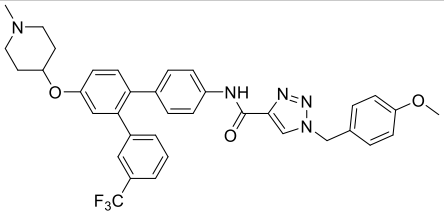
|---|---|---|---|
| **S11** |  <br>CN(CC1)CCC1OC(C=C2)=C(OCCN(C)C)C=C2C3=CC=C(NC(C4=CC(C5=CC=CC(OC)=C5)=C(OC)C=C4)=O)C=C3 | 0.40 [2] |  |
| **S12** |  <br>CN(CC1)CCC1OC(C=C2)=CC(C3=CC=CC(C(F)(F)F)=C3)=C2C4=CC=C(NC(C5=CN(CC6=CC=C(OC)C=C6)N=N5)=O)C=C4 | 0.50 [3] | / |

**Table S2.** Cell viability of Hep G2 and MCF-7 cells in MTS assay after treatment with compounds **5**–**12**. Data are means ± SD of three independent experiments performed in triplicates.

| Compound | Hep G2<br>% Viability | MCF-7<br>% Viability |
|---|---|---|
| **5** | 86.8 ± 7.6 | 93.5 ± 4.3 |
| **6** | 67.0 ± 3.7 | 71.0 ± 9.5 |
| **7** | 100.2 ± 6.0 | 99.5 ± 5.8 |
| **8** | 67.2 ± 3.0 | 65.2 ± 20.8 |
| **9** | 86.2 ± 12.8 | -0.53 ± 1.5 |
| **10** | 97.7 ± 7.5 | 116.5 ± 41.4 |
| **11** | 1.38 ± 0.1 | 31.4 ± 11.9 |
| **12** | 92.6 ± 4.3 | 93.8 ± 1.4 |

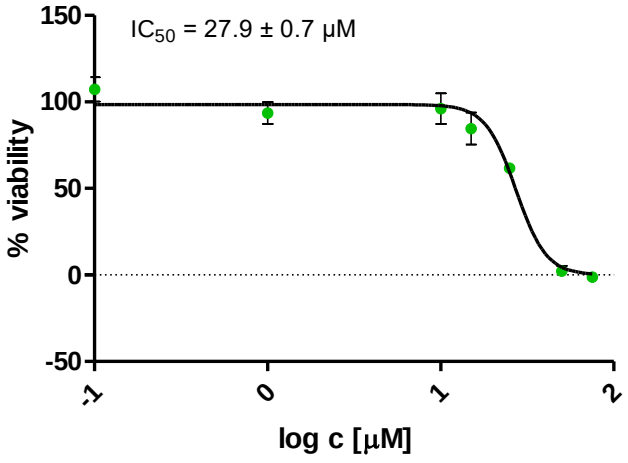



**Figure S1.** Dose-response curve for compound **11** in MTS assay in Hep G2 cell line, shown for an independent measurement in triplicate. The $IC_{50}$ value (mean ± SD) is a result of three independent measurements.

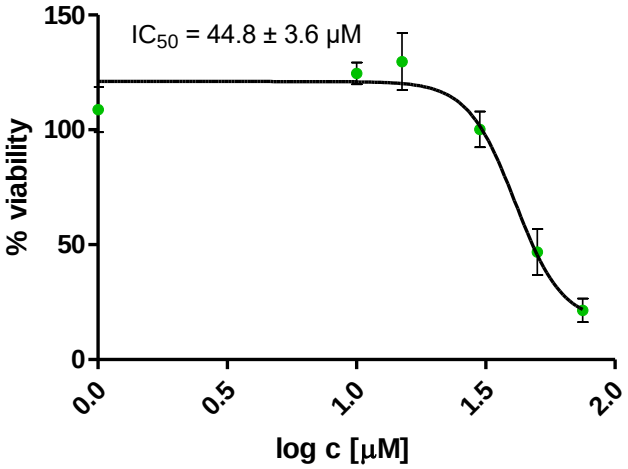



**Figure S2.** Dose-response curve for compound **11** in MTS assay in MCF-7 cell line, shown for an independent measurement in triplicate. The $IC_{50}$ value (mean ± SD) is a result of three independent measurements.

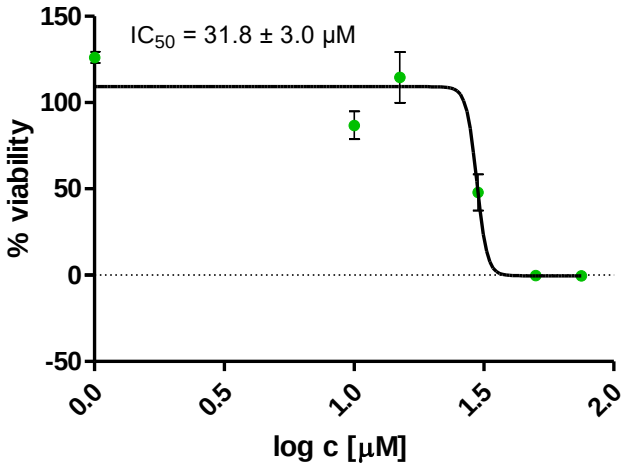



**Figure S3.** Dose-response curve for compound **9** in MTS assay in MCF-7 cell line, shown for an independent measurement in triplicate. The $IC_{50}$ value (mean ± SD) is a result of three independent measurements.

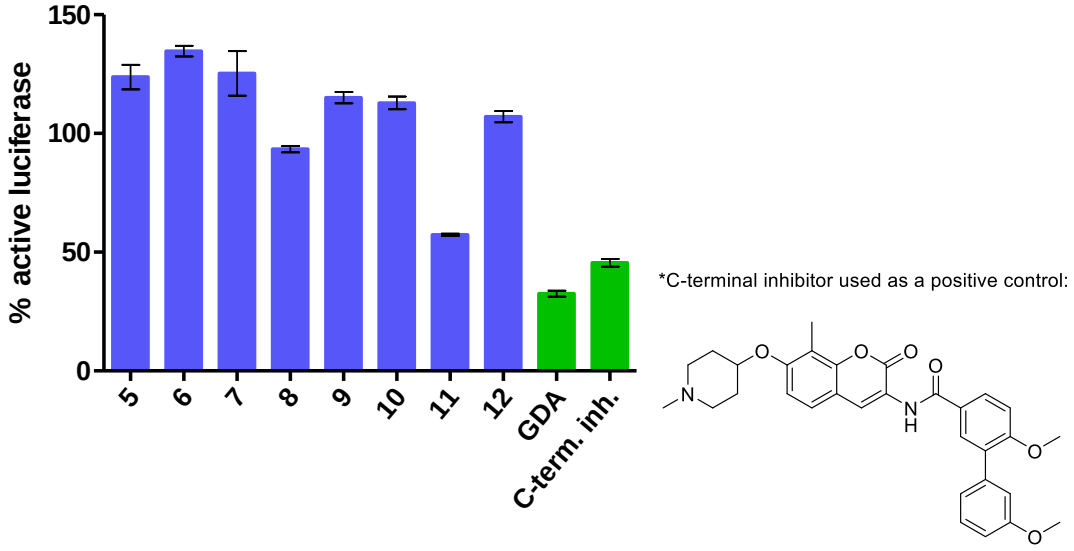



**Figure S4.** Luciferase refolding activity of Hsp90 in PC3 MM2 cells after treatment with compounds **5–12**, C-terminal inhibitor* and geldanamycin (GDA) at 50 μM concentration. Data are means ± SD of three independent experiments performed in triplicates.

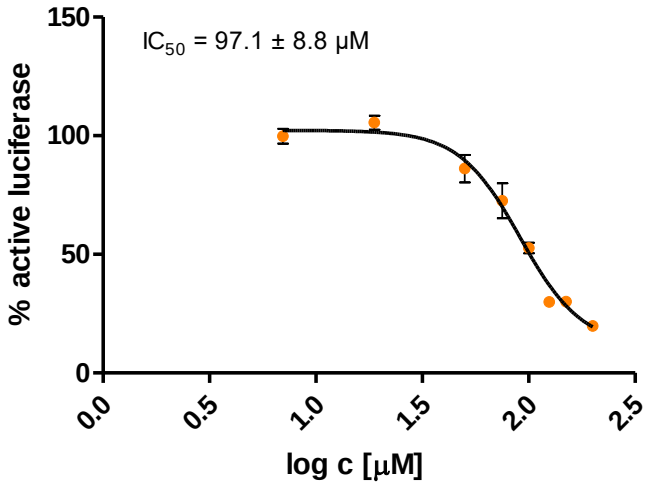



**Figure S5.** Dose-response curve for compound **11** in luciferase refolding assay on PC3 MM2 cell line, shown for an independent measurement in triplicate. The $IC_{50}$ value (mean ± SD) is a result of three independent measurements.

## References


1. Moroni, E.; Zhao, H.; Blagg, B.S.J.; Colombo, G. Exploiting Conformational Dynamics in Drug Discovery: Design of C-Terminal Inhibitors of Hsp90 with Improved Activities. *J. Chem. Inf. Mode.* **2014**, *54*, 195–208, doi:10.1021/ci4005767.
2. Garg, G.; Zhao, H.; Blagg, B.S.J. Design, Synthesis and Biological Evaluation of Alkylamino Biphenylamides as Hsp90 C-Terminal Inhibitors. *Bioorg. Med. Chem.* **2017**, *25*, 451–457, doi:10.1016/j.bmc.2016.11.030.
3. Davis, R.E.; Zhang, Z.; Blagg, B.S.J. A Scaffold Merging Approach to Hsp90 C-Terminal Inhibition: Synthesis and Evaluation of a Chimeric Library. *Med. Chem. Commun.* **2017**, *8*, 593–598, doi:10.1039/C6MD00377J.