Current Trends in Biotechnology and Pharmacy
Vol. 19(1) 2204-2210, January 2025, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2025.1.9

Computational Analysis of HSP90 Isoforms Identifies Differential Sensitivity Towards Specific Inhibitors

Shravanthi Ravula[1#], Sai Charitha Mullaguri[1#], Sree Kanth Sivan[2], Sravani Akula[1], Vijjulatha Manga[2] and Rama Krishna Kancha[1*]

[1] Molecular Medicine and Therapeutics Laboratory, CPMB, Osmania University, Hyderabad-500007.
[2] Department of Chemistry, Osmania University, Hyderabad-500007.

Corresponding author: ickrishna@gmail.com

Abstract

The heat shock protein 90 (HSP90) protein family consists of cytosolic HSP90-alpha, HSP90-beta and mitochondrial TRAP1. As HSP90 facilitates the activation and stabilization of many oncogenic proteins in cancer cells, it emerged as a potential therapeutic target in cancer treatment. Although many HSP90 inhibitors are under various phases of clinical trials, the efficacy of these inhibitors on each isoform is largely unknown. This study aims to determine the sensitivity profiles of HSP90 isoforms towards various natural and synthetic HSP90 inhibitors. Molecular docking analysis was performed for HSP90 inhibitors along with ATP and ADP over three isoforms HSP90-alpha, HSP90-beta and TRAP1. Our results indicate variable isoform-specific sensitivity towards HSP90 inhibitors. The predicted sensitivity profiles are in line with the limited available experimental data indicating the utility of molecular docking approach. In addition, potential interactions between the inhibitors and residues within the HSP90 isoforms were identified. The data generated in the current study may provide valuable insights for the design of isoform-specific HSP90 inhibitors with improved efficacy and specificity towards the HSP90 isoforms. Further, our study revealed critical residues in each of these isoforms for specific targeting by novel inhibitors.

Keywords

HSP90 isoforms, HSP90 inhibitors, molecular docking, binding affinity, drug sensitivity, ligand interactions.

Introduction

HSP90 is an important molecular chaperone that assists in the folding, maturation and activation of a large number of client proteins, which are critical for cellular homeostasis and various biological processes such as signal transduction, cell cycle regulation, and stress responses (1,2). HSP90 stabilizes several oncogenic proteins that promote tumor development and survival, thus offering an important cellular target for cancer treatment (3,4,5). HSP90 has multiple isoforms such as HSP90-alpha, HSP90-beta and TRAP1, each located in different cellular compartments and performing specific functions. HSP90-alpha and HSP90-beta are mainly cytoplasmic with HSP90-alpha being expressed in response to stress while HSP90-beta is constitutively expressed (6,7). TRAP1 is localized in the mitochondria and is involved in protecting cells from apoptotic stress (8). Although these isoforms share functional similarities, they have structural differences that may affect their interactions with inhibitors. This structural diversity offers a possibility for designing selective inhibitors that target specific HSP90 isoforms, potentially reducing off-target effects and enhancing therapeutic efficacy (9,10). Until now, many inhibitors have been developed that target the ATP-binding domain at the N-terminus of HSP90 to interfere with its chaperone activity and induce the degradation of its clients. However, the effects of these inhibitors on different HSP90 isoforms are not fully

understood. Molecular docking methods offer a valuable approach to study the binding affinities and specificities of HSP90 inhibitors for different isoforms, which can help in the development of more selective drugs (11,12). The aim of the current study is to perform molecular docking analysis to determine the sensitivities of HSP90 isoforms towards various natural and synthetic HSP90 inhibitors.

Material and Methods

HSP90-alpha (PDB: 1YES) (13), HSP90-beta (5UC4) (14) and TRAP1 (4Z1F) (15) isoforms were analysed for their sensitivities towards HSP90 inhibitors. Using Schrodinger Glide XP (extra precision) docking method (16), these structures were docked with adenosine triphosphate (ATP), adenosine diphosphate (ADP) and twenty HSP90 inhibitors collected from the PubChem (17): alvespimycin (17-DMAG), BIIB021, derrubone, gambogic acid, ganetespib, gedunin, geldanamycin, herbimycin, hypericin, IPI504 (retaspimycin hydrochloride), luminespib, macbecin, monocillin, onalespib, pochonin A, pochonin D, PU-H71 (Zelavespib), radicicol, sansalvamide A and tanespimycin (17-AAG) (18). The binding affinity of each receptor-ligand was measured as an XP score and the sensitivity profile of each HSP90 isoform towards the inhibitors was estimated. Additionally, multiple sequence alignment among HSP90-alpha, HSP90-beta and TRAP1 was carried out using Clustal Omega (19), and the interactions between the ligands and the HSP90 isoforms were collected and analysed. Interactions of each isoform with inhibitors that showed higher binding affinity were compared with the available experimentally determined co-crystal structures: HSP90-alpha with ganetespib (PDBs: 3TUH, 6LSZ and 8W8K) (20), TRAP1 with BIIB021 (PDB: 4Z1G) (15), and TRAP1 with PU-H71 (PDB: 4Z1F) (15).

Results and Discussion

A total of 66 molecular dockings were carried out using 20 HSP90 inhibitors, ATP and ADP against HSP90-alpha, HSP90-beta and TRAP1. Both ADP and ATP showed the highest binding affinities towards TRAP1 when compared to HSP90-alpha and HSP90-beta (Figure 1). Importantly, seven inhibitors (alvespimycin, derrubone, geldanamycin, hypericin, IPI-504, luminespib and monocillin) showed higher binding affinity towards HSP90-beta than HSP90-alpha and TRAP1 (Figure 1). Interestingly, BIIB021, gedunin and PU-H71 showed significantly higher binding affinity towards TRAP1 than HSP90-alpha and HSP90-beta; gambogic acid, macbecin and pochonin D showed slightly higher binding affinity towards TRAP1 when compared to HSP90-alpha and HSP90-beta (Figure 1). The greater sensitivity of TRAP1 towards PU-H71 further corroborates with the available experimental data when PU-H71 availability in mitochondria was increased (15). Ganetespib showed slightly higher binding affinity towards HSP90-alpha when compared to HSP90-beta and TRAP1 (Figure 1). Herbimycin showed similar binding affinities towards all three isoforms while onalespib, pochonin A, radicicol, sansalvamide A and tanespimycin showed higher binding affinities towards both HSP90-beta and TRAP1 when compared to HSP90-alpha (Figure 1).

All the interactions between HSP90 isoforms and the inhibitors were summarized in Figure 2 and the residues that interact with the ligands were highlighted in Figure 3. Analysis of the ligand-isoform complexes revealed that amino acid stretches ELI (HSP90-alpha: 47-49; HSP90-beta: 42-44; TRAP1: 115-117), GIGMT (HSP90-alpha: 95-99; HSP90-beta: 90-94; TRAP1: 160-164), GVGFY (HSP90-alpha: 135-139; HSP90-beta: 130-134; TRAP1: 202-206) and GTK (HSP90-alpha: 183-185; HSP90-beta: 178-180; TRAP1: 250-252) were interacting with at least one ligand in each isoform and are conserved among the isoforms (Figure 3). Interestingly, residues N51, M98, N106 and F138 (HSP90-alpha numbering) were shown to interact with all ligands in all three HSP90 isoforms (Figure 3). For individual isoforms, all the ligands showed interactions with (i) N51, A55, K58, I96, G97, M98, N106, L107 and F138 of HSP90-alpha; (ii) N46, A50, M93, N101, L102, F133 and

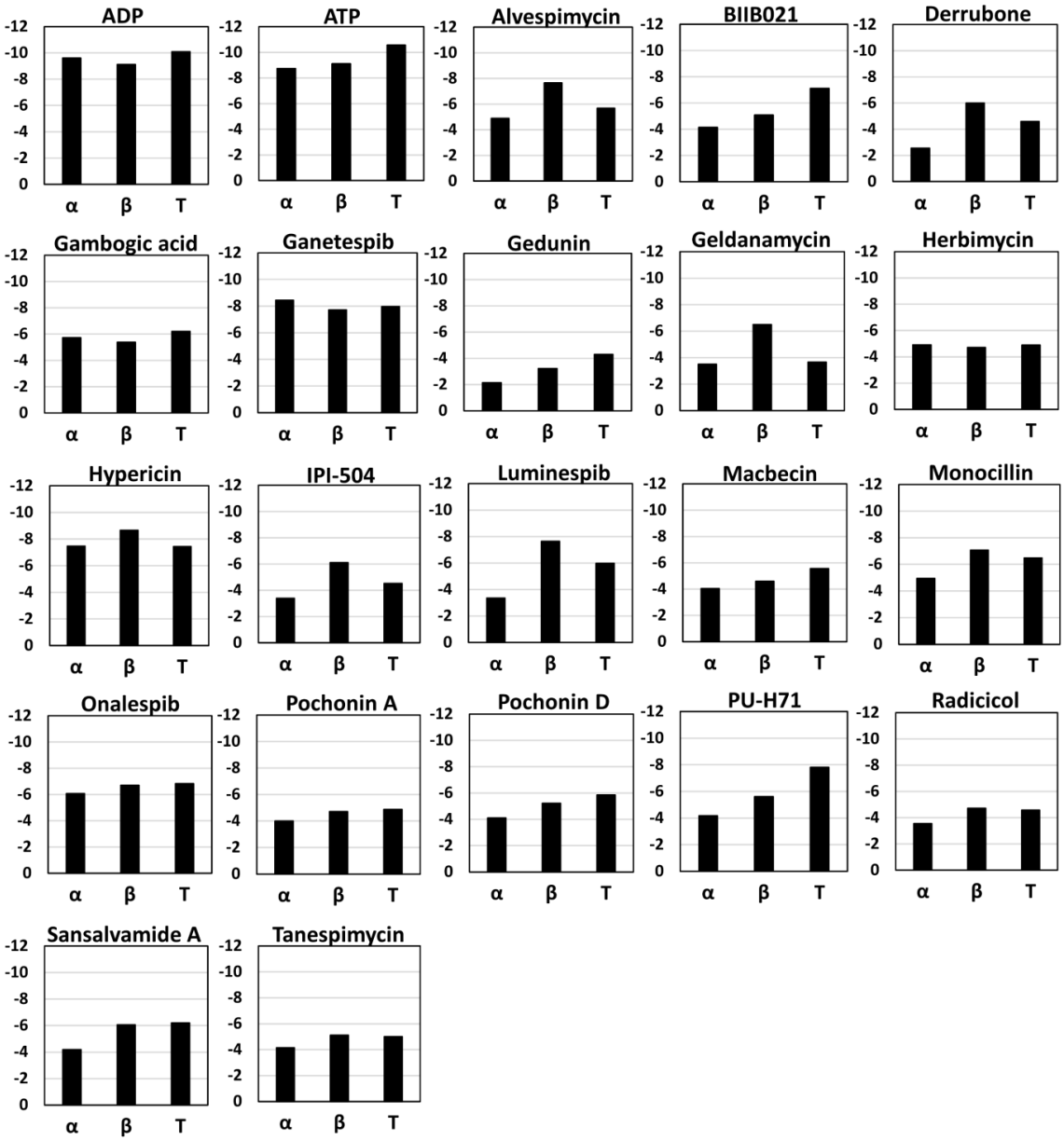

Figure 1. Predicted drug sensitivity profiles of HSP90 isoforms towards HSP90 inhibitors. Binding affinities of ADP, ATP and twenty inhibitors with each HSP90 isoform were measured in terms of XP scores and graphs were plotted. α: alpha; β: beta; T: TRAP1.

T179 of HSP90-beta; (iii) N119, M163, N171, G202, V203, F205, T251 and I253 of TRAP1 (Figures 2 and 3). Among these, A55, I96, M98 and L107 of HSP90-alpha, A50, M93 and L102 of HSP90-beta, and M163, V203 and I253 of TRAP1 formed Vander Waal interactions with all the ligands (Supplementary Figures 1-11).

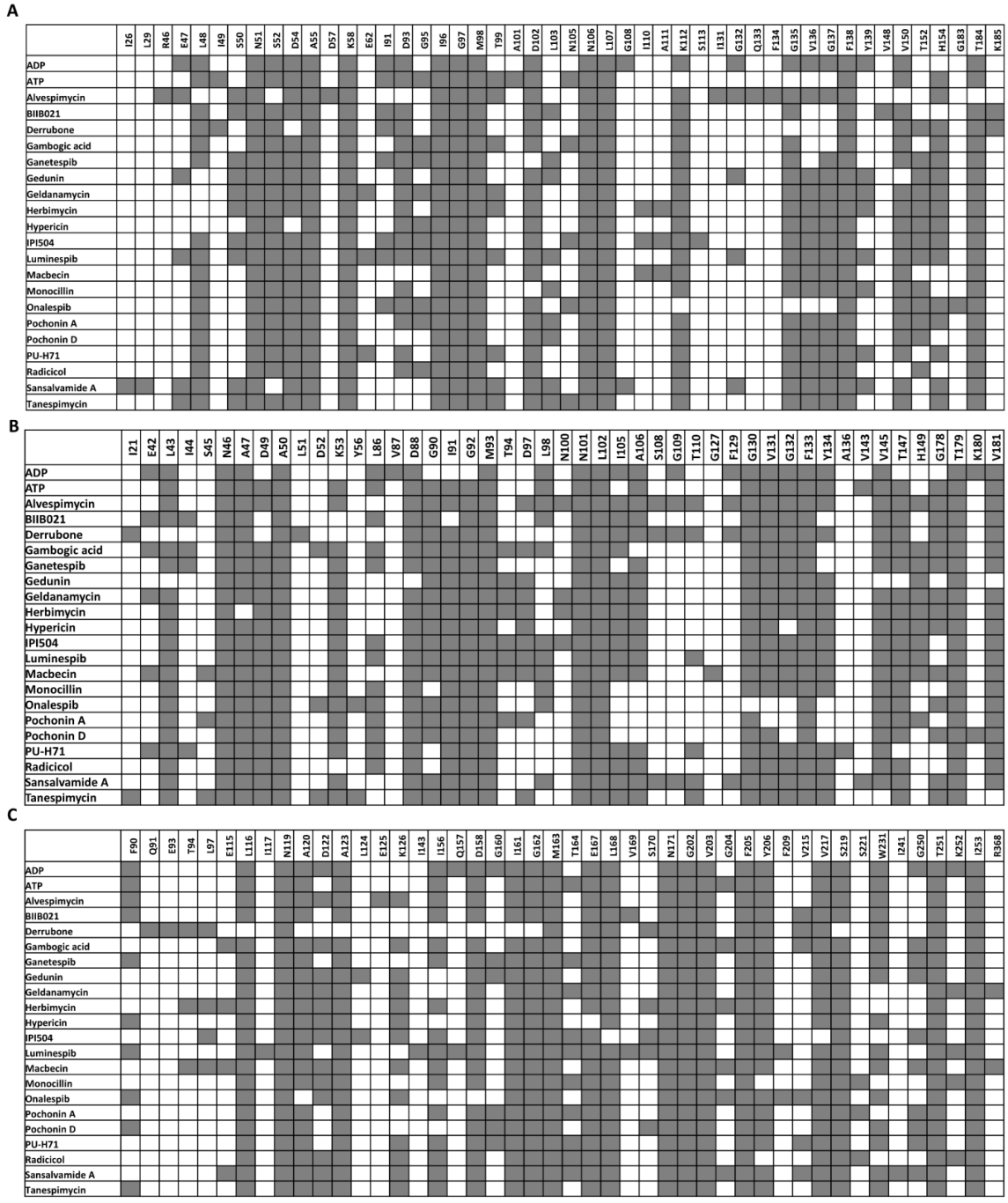

Figure 2. Receptor-ligand interactions between HSP90 isoforms, and ADP, ATP and twenty HSP90 inhibitors. Amino acids in HSP90-alpha (A), HSP90-beta (B) and TRAP1 (C) that are interacting with each ligand were shaded.

Computational analysis of HSP90 isoforms identifies differential sensitivity towards specific inhibitors

```
HSP90-alpha   1   MPEETQTQDQ PMEEEEVETF AFQAEIAQLM SLIINTFYSN KEIFLRELIS NSSDALDKIR  60
HSP90-beta    1   MPEE-----V HHGEEEVETF AFQAEIAQLM SLIINTFYSN KEIFLRELIS NASDALDKIR  55
HSP90-trap1  69   EPLHSIISST ESVQGSTSKH EFQAETKKLL DIVARSLYSE KEVFIRELIS NASDALEKLR  128

HSP90-alpha   61  YESLTDPSKL DSGKELHINL IPNKQDRTLT IVDTGIGMTK ADLINNLGTI AKSGTKAFME  120
HSP90-beta    56  YESLTDPSKL DSGKELKIDI IPNPQERTLT LVDTGIGMTK ADLINNLGTI AKSGTKAFME  115
HSP90-trap1  129  HKLVSDGQAL ---PEMEIHL QTNAEKGTIT IQDTGIGMTQ EELVSNLGTI ARSGSKAFLD  185

HSP90-alpha   121 ALQAGA--DI SMIGQFGVGF YSAYLVAEKV TVITKHN--D DEQYAWESSA GGSFTVRTDT  176
HSP90-beta    116 ALQAGA--DI SMIGQFGVGF YSAYLVAEKV VVITKHN--D DEQYAWESSA GGSFTVRADH  171
HSP90-trap1   186 ALQNQAEASS KIIGQFGVGF YSAFMVADRV EVYSRSAAPG SLGYQWLSDG SGVFEIAEAS  245

HSP90-alpha   177 GEPMGRGTKV ILHLKEDQTE YLEERRIKEI VKKHSQFIGY PITLFVEKER DKEVSDDEAE  236
HSP90-beta    172 GEPIGRGTKV ILHLKEDQTE YLEERRVKEV VKKHSQFIGY PITLYLEKER EKEISDDEAE  231
HSP90-trap1   246 G--VRTGTKI IIHLKSDCKE FSSEARVRDV VTKYSNFVSF PLYLN-----  ----------  288

HSP90-alpha   237 EKEDKEEEKE KEEKESEDKP EIEDVGSDEE EEKKDGDKKK KKKIKEKYID QEELNKTKPI  296
HSP90-beta    232 EEKG---EKE EEDKDDEEKP KIEDVGSDEE DDSGKDKKKK TKKIKEKYID QEELNKTKPI  288
HSP90-trap1   288 ----------  ----------  ----------  ----------  ----------  GRRMNTLQAI  298

HSP90-alpha   297 WTRNPDDITN EEYGEFYKSL TNDWEDHLAV KHFSVEGQLE FRALLFVPRR APFDLFENRK  356
HSP90-beta    289 WTRNPDDITQ EEYGEFYKSL TNDWEDHLAV KHFSVEGQLE FRALLFIPRR APFDLFENKK  348
HSP90-trap1   299 WMMDPKDVRE WQHEEFYRYV AQAHDKPRYT LHYKTDAPLN IRSIFYVPDM KPSMFDVSRE  358

HSP90-alpha   357 KKNNIKLYVR RVFIMDNCEE LIPEYLNFIR GVVDSEDLPL NISREMLQQS KILKVIRKNL  416
HSP90-beta    349 KKNNIKLYVR RVFIMDSCDE LIPEYLNFIR GVVDSEDLPL NISREMLQQS KILKVIRKNI  408
HSP90-trap1   359 LGSSVALYSR KVLIQTKATD ILPKWLRFIR GVVDSEDIPL NLSRELLQES ALIRKLRDVL  418
```

Figure 3. Schematic representation of HSP90 residues interacting with inhibitors. Multiple sequence alignment was performed for the HSP90 isoforms. All the residues that were interacting with at least one ligand in each isoform were highlighted in yellow. Residues in each isoform that were interacting with all the 22 ligands were coloured in red.

Comparison of co-crystal structures (3TUH, 6LSZ and 8W8K) of HSP90-alpha and ganetespib complexes with the molecular docking analysis revealed many common interactions: hydrogen bonds with K58 and G97, and non-bonded interactions with L48, S52, A55, D93, I96, M98, L107, F138 and V186 (Supplementary Figure 4). All the interactions observed in the experimental co-crystals except G108 and T109 were present in the HSP90-alpha and ganetespib complex (Supplementary Figure 4). Further, the common interactions between co-crystal structure (4Z1G) and TRAP1-BIIB021 complex (Supplementary Figure 2) include hydrogen bonds with D158 and T251, and non-bonded interactions with N119, A123, I161, G162, M163, L168, G202 and F205. Similarly, common interactions between co-crystal structure (4Z1F) and TRAP1-PU-H71 complex (Supplementary Figure 10) include hydrogen bonding with D158, non-bonded interactions with N119, A123, I161, G162, M163, E167, N171, G202, F205, W231 and T251. All the interactions present in the co-crystals of TRAP1 with BIIB021 (4Z1G) and PU-H71 (4Z1F) were observed in the complexes obtained through molecular docking indicating the usefulness of computational approach in predicting the drug sensitivities of HSP90 isoforms.

Conclusion

The current study established the sensitivity profiles of HSP90 isoforms towards various natural and synthetic inhibitors. Among

the HSP90 isoforms, TRAP1 was observed to be more sensitive towards PU-H71, which is in line with the previously reported experimental data. Additionally, the interactions observed in the available experimental co-crystal structures were largely present in the complexes obtained from our molecular docking analyses indicating the effectiveness of this approach in predicting drug sensitivities of HSP90 isoforms.

Availability of data

All the data are included in the supplementary files.

Acknowledgements

RKK acknowledges funding from ICMR-Ad-hoc grant (F.No. 58/31/2020/PHA/BMS). SR acknowledges fellowship from the Council of Scientific & Industrial Research (File No. 09/132(0884)/2019-EMR-I). SCM acknowledges salary from ICMR-Ad-hoc grant (F.No. 58/31/2020/PHA/BMS).

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Schopf, F.H., Biebl, M.M. and Buchner, J., 2017. The HSP90 chaperone machinery. Nature reviews Molecular cell biology, 18(6), pp.345-360.

2. Neckers, L. and Workman, P., 2012. Hsp90 molecular chaperone inhibitors: are we there yet?. Clinical cancer research, 18(1), pp.64-76.

3. Kancha, R.K., Bartosch, N. and Duyster, J., 2013. Analysis of conformational determinants underlying HSP90-kinase interaction. PLoS One, 8(7), p.e68394.

4. Sreedhar, A.S. and Csermely, P., 2004. Heat shock proteins in the regulation of apoptosis: new strategies in tumor therapy: a comprehensive review. Pharmacology & therapeutics, 101(3), pp.227-257.

5. Jhaveri, K. and Modi, S., 2012. HSP90 inhibitors for cancer therapy and overcoming drug resistance. Advances in pharmacology, 65, pp.471-517.

6. Marzec, M., Eletto, D. and Argon, Y., 2012. GRP94: An HSP90-like protein specialized for protein folding and quality control in the endoplasmic reticulum. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1823(3), pp.774-787.

7. Barabutis, N., 2020. Heat shock protein 90 inhibition in the inflamed lungs. Cell Stress and Chaperones, 25(2), pp.195-197.

8. Gesualdi, N.M., Chirico, G., Pirozzi, G., Costantino, E., Landriscina, M. and Esposito, F., 2007. Tumor necrosis factor-associated protein 1 (TRAP-1) protects cells from oxidative stress and apoptosis. Stress, 10(4), pp.342-350.

9. Prodromou, C., 2016. Mechanisms of Hsp90 regulation. Biochemical Journal, 473(16), pp.2439-2452.

10. Chen, B., Piel, W.H., Gui, L., Bruford, E. and Monteiro, A., 2005. The HSP90 family of genes in the human genome: insights into their divergence and evolution. Genomics, 86(6), pp.627-637.

11. Miyata, Y., Nakamoto, H. and Neckers, L., 2013. The therapeutic target Hsp90 and cancer hallmarks. Current pharmaceutical design, 19(3), pp.347-365.

12. Taipale, M., Jarosz, D.F. and Lindquist, S., 2010. HSP90 at the hub of protein homeostasis: emerging mechanistic insights. Nature reviews Molecular cell biology, 11(7),

pp.515-528.

13. Stebbins, C.E., Russo, A.A., Schneider, C., Rosen, N., Hartl, F.U. and Pavletich, N.P., 1997. Crystal structure of an Hsp90–geldanamycin complex: targeting of a protein chaperone by an antitumor agent. Cell, 89(2), pp.239-250.

14. Khandelwal, A., Kent, C.N., Balch, M., Peng, S., Mishra, S.J., Deng, J., Day, V.W., Liu, W., Subramanian, C., Cohen, M. and Holzbeierlein, J.M., 2018. Structure-guided design of an Hsp90β N-terminal isoform-selective inhibitor. Nature communications, 9(1), p.425.

15. Lee, C., Park, H.K., Jeong, H., Lim, J., Lee, A.J., Cheon, K.Y., Kim, C.S., Thomas, A.P., Bae, B., Kim, N.D. and Kim, S.H., 2015. Development of a mitochondria-targeted Hsp90 inhibitor based on the crystal structures of human TRAP1. Journal of the American Chemical Society, 137(13), pp.4358-4367.

16. Friesner, R.A., Murphy, R.B., Repasky, M.P., Frye, L.L., Greenwood, J.R., Halgren, T.A., Sanschagrin, P.C. and Mainz, D.T., 2006. Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein− ligand complexes. Journal of medicinal chemistry, 49(21), pp.6177-6196.

17. Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B.A., Thiessen, P.A., Yu, B. and Zaslavsky, L., 2023. PubChem 2023 update. Nucleic acids research, 51(D1), pp.D1373-D1380.

18. Ravula, S., Akula, S. and Kancha, R.K., 2024. Natural and Synthetic Inhibitors of Heat Shock Protein 90 Chaperone in Cancer Treatment. Indian journal of pharmaceutical education and research, 58(2), pp.401-410

19. Madeira, F., Madhusoodanan, N., Lee, J., Eusebi, A., Niewielska, A., Tivey, A.R., Lopez, R. and Butcher, S., 2024. The EMBL-EBI Job Dispatcher sequence analysis tools framework in 2024. Nucleic Acids Research, p.gkae241.

20. Xu, C., Zhang, X., Zhao, L., Verkhivker, G.M. and Bai, F., 2024. Accurate Characterization of Binding Kinetics and Allosteric Mechanisms for the HSP90 Chaperone Inhibitors Using AI-Augmented Integrative Biophysical Studies. JACS Au, 4(4), pp.1632-1645.