Computational Insights into Breast Cancer Therapeutics: Auto-Docking Studies of Symmetrical Azines with Breast Cancer Protein and Toxicity Predictions

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Abstract

Breast cancer is a prevalent malignancy affecting both women and men globally, arising from abnormal cell growth in breast tissues. Various risk factors, including age, genetics, hormonal influences, and lifestyle choices, contribute to its development. Early detection through screening methods like mammograms is pivotal for successful treatment. Treatment modalities encompass surgery, radiation, chemotherapy, hormone therapy, and targeted therapies, with outcomes improving due to heightened awareness and medical advancements. Auto-docking studies, employing computational simulations, are instrumental in drug discovery by predicting ligand-protein interactions. Breast Cancer Protein 2 (BCP2), represented by PDB code 2IOK, is pivotal in breast cancer pathogenesis, offering insights for targeted therapies. Here, we conducted auto-docking studies with four symmetrical azines on 2IOK, revealing azine one's superior binding affinity and toxicity predictions through ProTox-II software, highlighting potential therapeutic avenues in breast cancer research.

Key Words: Azine, Molecular Docking, Breast Cancer, Toxicity.

Introduction

Breast cancer is a type of cancer that forms in the cells of the breast. It is one of the most common cancers affecting women worldwide, but it can also occur in men, although much less frequently. Breast cancer can develop in different parts of the breast, including the milk ducts, the lobules that produce milk, or in the fatty tissue [1-3].

The exact cause of breast cancer is often unclear, but several factors can increase the risk, including age, family history, genetic mutations, hormonal factors, lifestyle choices, and environmental factors [4, 5].

Symptoms of breast cancer may include a lump or thickening in the breast or underarm area, changes in breast size or shape, skin changes on the breast, such as dimpling or puckering, nipple discharge other than breast milk, and nipple inversion [6-10].

Early detection through screening methods such as mammograms can greatly improve the chances of successful treatment. Treatment options for breast cancer may include surgery, radiation therapy, chemotherapy, hormone therapy, targeted therapy, or a combination of these approaches, depending on the type and stage of the cancer [11-15].

Awareness, early detection, and advancements in treatment have significantly improved outcomes for individuals diagnosed with breast cancer, but ongoing research and education efforts remain crucial in the fight against this disease.

Auto-docking studies involve computational simulations aimed at predicting the binding modes and affinities of small molecules or ligands with target proteins. This methodology is widely employed in drug discovery and design, as it helps in understanding the molecular interactions between ligands and their target binding sites. Auto-docking studies utilize algorithms and molecular modeling techniques to explore the conformational

space of ligands and predict their most favorable binding poses within the protein's binding site. These studies play a crucial role in virtual screening campaigns and lead optimization efforts, contributing to the development of novel therapeutics and bioactive compounds [16-19].

Breast cancer protein 2 (BCP2), also known as 2IOK, is a significant protein associated with breast cancer. The structure of this protein, represented by its PDB code 2IOK, provides crucial insights into its function and potential therapeutic targets. Understanding the structure and function of BCP2 is vital in unraveling the mechanisms underlying breast cancer development and progression. Researchers analyze 2IOK and related proteins to elucidate their roles in cell signaling, proliferation, and metastasis, aiming to develop targeted therapies for breast cancer patients [20, 21].

In this study, we selected four symmetrical azines that we had previously reported, and all four were docked with the breast cancer protein 2IOK. Among these azines, azine one exhibited superior binding affinity compared to the others. Additionally, the toxicity of all the azines was predicted using ProTox-II software.

Materials and Methods

Molecular docking

The protein data bank (PDB: 2IOK) contains Compound 1D and the human estrogen receptor alpha ligand-binding domain. Researchers investigated molecular docking using AutoDock 4.0 and an empirical grading scheme based on binding free energy. The AutoDock provides a wide range of stochastic search techniques. Our initial choice was to use the Lamarckian Genetic Algorithm (LGA). It utilises just the Genetic Algorithm to integrate both

local and global search (Solis and Wets algorithm). The 3D picture acquired from Discovery studio and the 2D image produced using the LigPlot.

Toxicity Prediction

We can cut down on preclinical drug development expenses, time, and animal testing by utilising in silico prediction techniques. ProTox-II includes machine-learning models covering hepatotoxicity, cytotoxicity, mutagenicity, immunotoxicity, adverse outcomes pathways (Tox21), and toxicity targets in addition to molecular similarity, pharmacophores, fragment probabilities, and other topics [22-24].

Results and Discussions

New compounds that could be used as inhibitors for a range of diseases are continuously being developed and synthesised by our research team [25-27]. As part of our ongoing study, we are currently utilising clinical techniques against humans in an effort to uncover a unique, potent chemical that has a favourable effect on multiple enzymes. The four chemicals were chosen, and similar compounds were even created in a wet lab and previously documented by our team of researchers. The structure of the four azine compounds is shown in Figure 1.

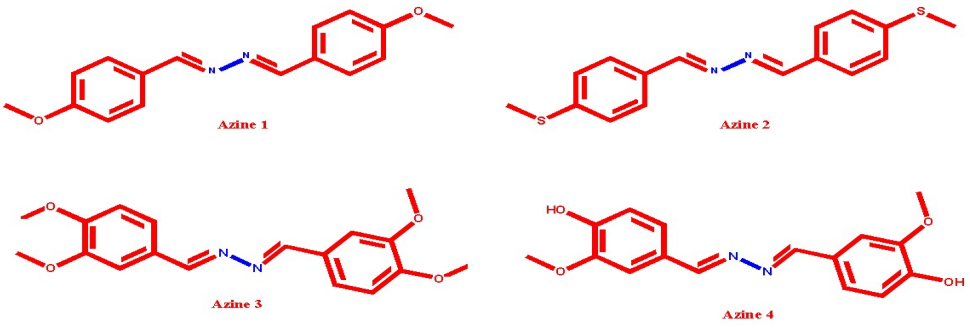

Figure 1. Structure of the azine 1-4

Docking Studies

Docking tests were performed to examine the active sites of the medicinal drugs against the breast cancer protein (2IOK) (Table-1).

Table 1. Binding affinity of the azine 1-4.

2IOK				
	Azine 1	**Azine 2**	**Azine 3**	**Azine 4**
Binding energy	-6.27	-6.06	-5.56	-5.58
Ligand efficiency	-.0.31	-0.3	-0.23	-0.25
Inhib_Constant	25.43	36.4	84.54	80.98
Intermol_energy	-7.76	-7.55	-7.64	-7.67
Vdw_hb_disolve_energy	-7.75	-7.54	-7.56	-7.66
Electrostatic energy	-0.01	-0.01	-0.09	-0.01
Total_internal	-0.41	-0.39	-0.81	-1.91
Torsional energy	1.49	1.49	2.09	2.09
Unbound energy	-0.41	-0.39	-0.81	-1.91
refRMS	53.02	50.32	54.5	51.18

Azine 1 demonstrated the lowest binding affinity of -6.27 kcal/mol and exhibited several interactions with the breast cancer protein 2IOK. These interactions included one π-cation interaction with the amino acid ARG 394, with a bond length of 4.13 Å, one π-anion interaction with GLU 353, with a bond length of 3.58 Å, two π-σ interactions with ILE 326, with bond lengths of 3.93 Å and 4.00 Å respectively, one π-anion interaction with GLU 323, with a bond length of 3.96 Å, and one π-alkyl interaction with the amino acid LEU 320, with a bond length of 4.85 Å and displays twelve hydrophobic interactions with PHE 404(A),

ARG 394(A), ILE 326(A), TRP 393 (A), GLU 323(A), LEU 320(A), GLY 442(A), VAL 446(A), PHE 445(A), PRO 325(A), LEU 327(A) and GLU 353(A) (Figure 2).

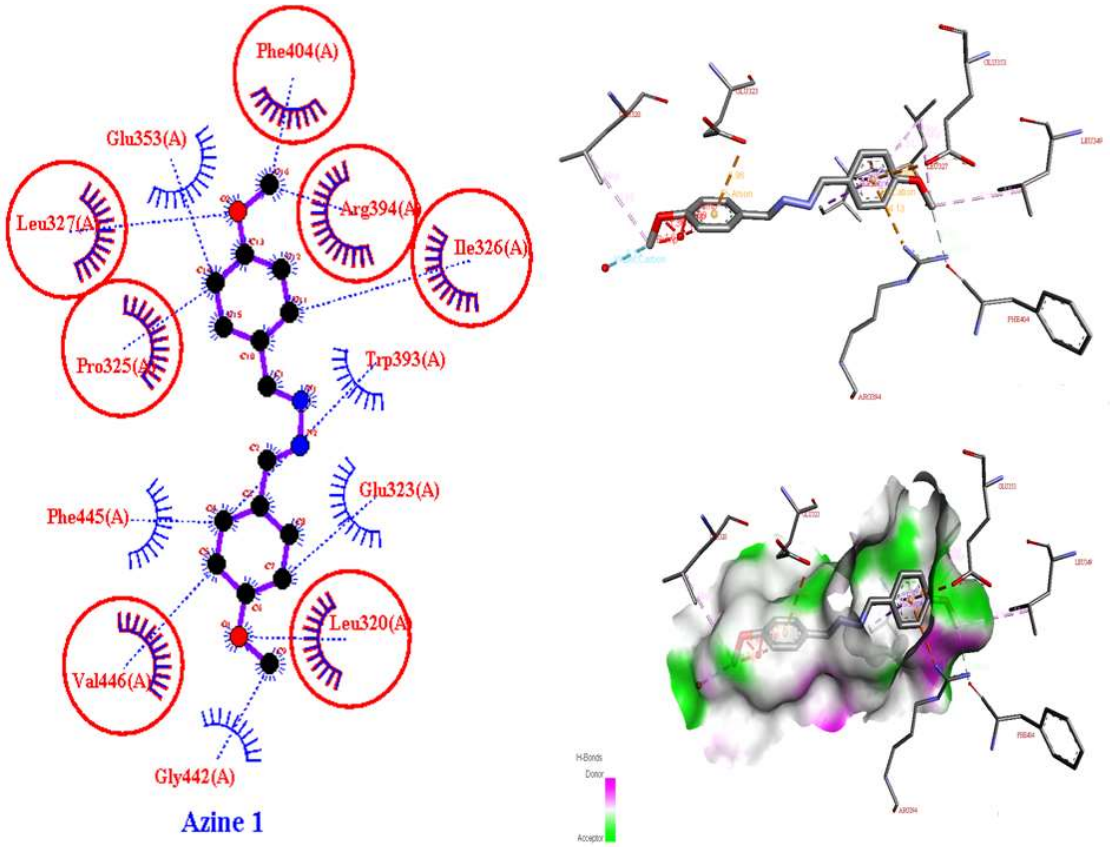

Figure 2. Docking image of azine 1 against 2IOK

Azine 2, with a binding affinity of -6.06 kcal/mol, exhibited several interactions with amino acids. Specifically, it formed one π-cation interaction with ARG 394, a π-alkyl interaction with PRO 324, a π-sulfur interaction with ARG 394, and two π-π T-shaped interactions with ARG 394, with bond lengths of 4.74Å, 4.68Å, 5.35Å, 4.73Å, and 4.91Å respectively. Additionally, the hydrogen atom of Azine 2 formed a hydrogen bond with the nitrogen atom of the amino acid TRP 393(A). Furthermore, Azine 2 exhibited ten hydrophobic interactions with GLU 443(A), GLY 442(A), GLU 323(A), PHE 445(A), ILE 386(A), GLY 390(A), LEU 387(A), LYS 449(A), MET 357(A), and GLU 353(A) (Figure-3).

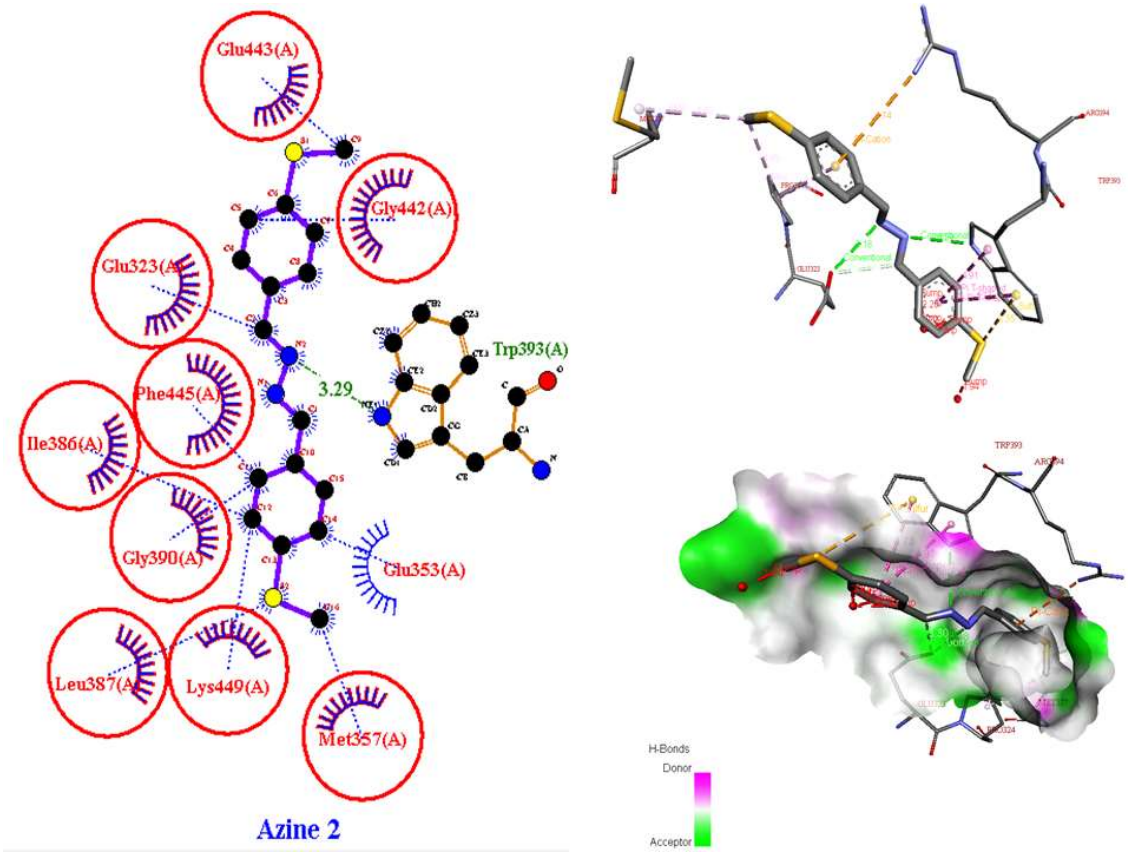

Figure 3. Docking image of azine 2 against 2IOK

The binding affinity of azine 3 with 2IOK is -5.56 kcal/mol, it having one hydrogen bond with LEU 1327(B), two π-π stacked, one π-σ, one π-cation and one π-alkyl interactions with amino acids TRP 1393, ILE 1326, ARG 1394 and PRO 1324 respectively (Figure 4).

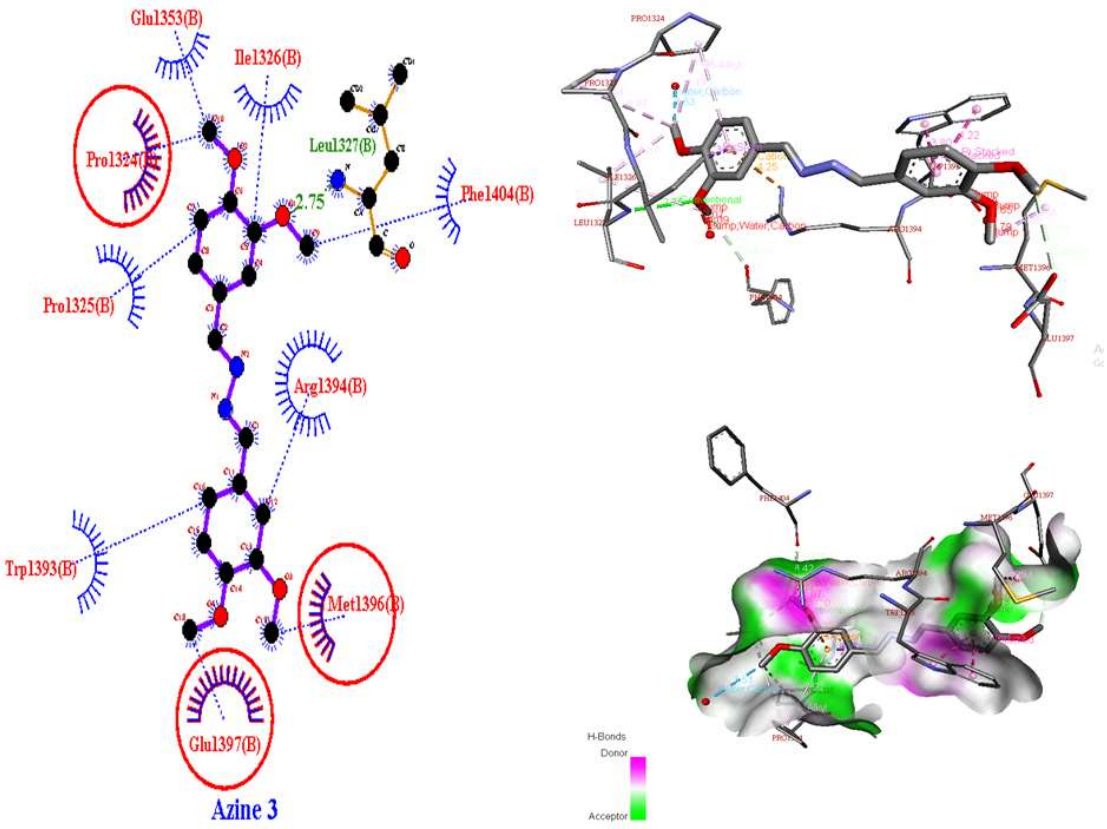

Figure 4. Docking image of azine 3 against 2IOK

Azine 4 demonstrated the binding affinity of -5.58 kcal/mol and exhibited several interactions with the breast cancer protein 2IOK. These interactions included one π-cation interaction with the amino acid ARG 1394, with a bond length of 3.52 Å, one π-anion interaction with GLU 1323, with a bond length of 3.69 Å, two π-alkyl interactions with PRO 1406, with bond lengths of 5.04 Å and 5.10 Å respectively, and displays twelve hydrophobic interactions and two hydrogen bond with PHE 404(A), ARG 394(A), ILE 326(A), TRP 393 (A), GLU 323(A), LEU 320(A), GLY 442(A), VAL 446(A), PHE 445(A), PRO 325(A), LEU 327(A), GLU 353(A), LEUL 1327(B) respectively (Figure 5).

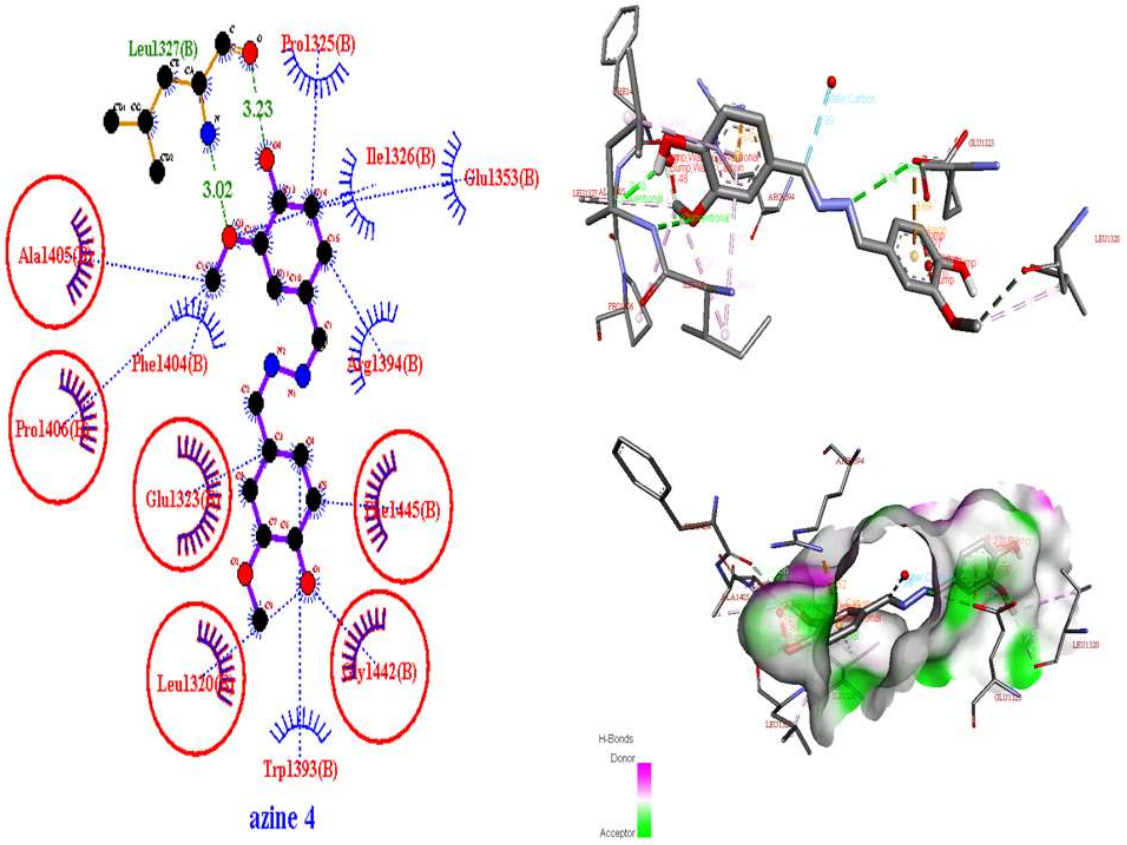

Figure 5. Docking image of azine 4 against 2IOK

***In silico* toxicological assessment**

ProTox-II was used to calculate the compound's toxicity profile. Table 2 shows the predicted toxicity profile and Figure 6 display the Graphical representation of predicted dose value distribution for compound.

Table 2. Complete Toxicity profile of azine 1-4

		Azine 1		Azine 2		Azine 3		Azine 4	
Classification	**Target**	**Prediction**	**Probability**	**Prediction**	**Probability**	**Prediction**	**Probability**	**Prediction**	**Probability**
Organ toxicity	Hepatotoxicity	Inactive	0.72	Inactive	0.55	Active	0.54	Active	0.51
Organ toxicity	Neurotoxicity	Active	0.72	Active	0.58	Inactive	0.60	Inactive	0.69
Organ toxicity	Nephrotoxicity	Inactive	0.65	Inactive	0.81	Inactive	0.59	Inactive	0.51
Organ toxicity	Respiratory toxicity	Inactive	0.81	Inactive	0.52	Inactive	0.61	Inactive	0.57
Organ toxicity	Cardiotoxicity	Inactive	0.73	Inactive	0.72	Inactive	0.55	Active	0.50
Toxicity end points	Carcinogenicity	Inactive	0.55	Active	0.59	Active	0.52	Active	0.57
Toxicity end points	Immunotoxicity	Inactive	0.58	Inactive	0.98	Inactive	0.58	Active	0.62
Toxicity end points	Mutagenicity	Inactive	0.84	Inactive	0.55	Inactive	0.53	Inactive	0.56
Toxicity end points	Cytotoxicity	Inactive	0.91	Inactive	0.84	Inactive	0.63	Inactive	0.79
Toxicity end points	BBB-barrier	Active	0.70	Active	0.95	Active	0.83	Active	0.64
Toxicity end points	Ecotoxicity	Active	0.74	Active	0.80	Active	0.66	Inactive	0.54
Toxicity end points	Clinical toxicity	Inactive	0.70	Inactive	0.70	Inactive	0.68	Inactive	0.60
Toxicity end points	Nutritional toxicity	Inactive	0.93	Inactive	0.68	Inactive	0.79	Inactive	0.77
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive	0.86	Inactive	0.70	Active	0.56	Active	0.65
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	Inactive	0.98	Inactive	0.93	Inactive	0.93	Inactive	0.93
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	Inactive	0.99	Inactive	0.98	Inactive	0.99	Inactive	0.99

Tox21-Nuclear receptor signalling pathways	Aromatase	Inactive	0.89	Inactive	0.92	Inactive	0.83	Inactive	0.89
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	Active	0.61	Inactive	0.71	Inactive	0.70	Inactive	0.62
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	Inactive	0.89	Inactive	0.93	Inactive	0.88	Inactive	0.61
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	Inactive	0.97	Inactive	0.95	Inactive	0.97	Inactive	0.92
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	Inactive	0.85	Inactive	0.63	Inactive	0.85	Inactive	0.74
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive	0.85	Inactive	0.63	Inactive	0.85	Inactive	0.74
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	Inactive	0.59	Inactive	0.78	Inactive	0.50	Active	0.64
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	Inactive	0.89	Inactive	0.94	Inactive	0.90	Inactive	0.79
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	Inactive	0.84	Inactive	0.84	Inactive	0.85	Inactive	0.82
Molecular Initiating Events	Thyroid hormone receptor alpha	Inactive	0.90	Inactive	0.90	Inactive	0.90	Inactive	0.90

	(THRα)								
Molecular Initiating Events	Thyroid hormone receptor beta (THRβ)	Inactive	0.78	Inactive	0.78	Inactive	0.78	Inactive	0.78
Molecular Initiating Events	Transtyretrin (TTR)	Inactive	0.97	Inactive	0.97	Inactive	0.97	Inactive	0.97
Molecular Initiating Events	Ryanodine receptor (RYR)	Inactive	0.98	Inactive	0.98	Inactive	0.98	Inactive	0.98
Molecular Initiating Events	GABA receptor (GABAR)	Inactive	0.96	Inactive	0.96	Inactive	0.96	Inactive	0.96
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	Inactive	0.92	Inactive	0.92	Inactive	0.92	Inactive	0.92
Molecular Initiating Events	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPAR)	Inactive	0.97	Inactive	0.97	Inactive	0.97	Inactive	0.97
Molecular Initiating Events	Kainate receptor (KAR)	Inactive	0.99	Inactive	0.99	Inactive	0.99	Inactive	0.99
Molecular Initiating Events	Achetylcholinestera se (AChE)	Inactive	0.72	Inactive	0.55	Active	0.54	Active	0.51
Molecular Initiating Events	Constitutive androstane receptor (CAR)	Inactive	0.98	Inactive	0.98	Inactive	0.98	Inactive	0.98
Molecular Initiating Events	Pregnane X receptor (PXR)	Inactive	0.92	Inactive	0.92	Inactive	0.92	Inactive	0.92
Molecular Initiating Events	NADH-quinone oxidoreductase (NADHOX)	Inactive	0.97	Inactive	0.97	Inactive	0.97	Inactive	0.97
Molecular Initiating Events	Voltage gated sodium channel (VGSC)	Inactive	0.95	Inactive	0.95	Inactive	0.95	Inactive	0.95
Molecular Initiating Events	Na+/I- symporter (NIS)	Inactive	0.98	Inactive	0.98	Inactive	0.98	Inactive	0.98
Metabolism	Cytochrome CYP1A2	Active	0.68	Inactive	0.51	Active	0.66	Inactive	0.55

Metabolism	Cytochrome CYP2C19	Active	0.67	Inactive	0.65	Inactive	0.54	Active	0.59
Metabolism	Cytochrome CYP2C9	Active	0.81	Inactive	0.54	Active	0.52	Inactive	0.50
Metabolism	Cytochrome CYP2D6	Inactive	0.61	Inactive	0.55	Inactive	0.64	Inactive	0.69
Metabolism	Cytochrome CYP3A4	Inactive	0.77	Inactive	0.68	Active	0.51	Inactive	0.51
Metabolism	Cytochrome CYP2E1	Inactive	0.99	Inactive	0.79	Inactive	0.99	Inactive	0.99

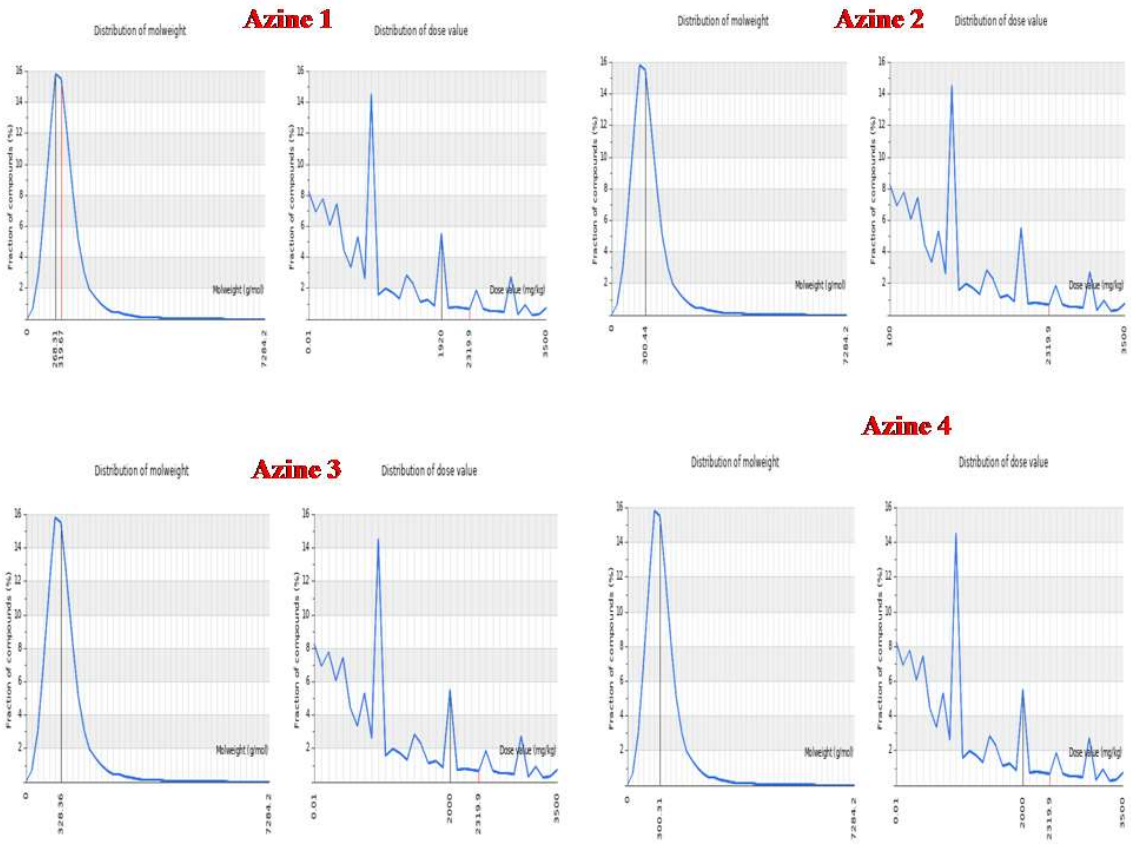

Figure 6. Graphical representation of predicted dose value distribution for azine 1-4

Conclusion

In conclusion, our study focused on the docking of four symmetrical azines, previously reported by our team, with the breast cancer protein 2IOK. Among these azines, azine one displayed notably higher binding affinity than the others, indicating its potential as a lead compound for further development. Furthermore, the toxicity of all four azines was assessed using ProTox-II software, providing valuable insights into their safety profiles. These findings contribute to the understanding of the molecular interactions of these azines with the target protein and their potential therapeutic relevance in breast cancer treatment.

References

1. Sharma GN, Dave R, Sanadya J, Sharma P, Sharma K. *Journal of advanced pharmaceutical technology & research*, **2010**, 1, 1, 109-26.
2. Sutherland RL, Musgrove EA. *Journal of mammary gland biology and neoplasia*, **2004**, 9, 95-104.
3. Sun YS, Zhao Z, Yang ZN, Xu F, Lu HJ, Zhu ZY, Shi W, Jiang J, Yao PP, Zhu HP. *International journal of biological sciences*, **2017**, 13, 1387.
4. Obeagu EI, Obeagu GU. *Elite Journal of Nursing and Health Science*, **2024**, 2, 1-7.
5. Obeagu EI, Obeagu GU. *Annals of Medicine and Surgery*, **2024**, 1, 86, 2805-11.
6. Safaei S, Fadaee M, Farzam OR, Yari A, Poursaei E, Aslan C, Samemaleki S, Shanehbandi D, Baradaran B, Kazemi T. *Breast Cancer Research*, **2024,** 29, 26, 57.
7. Obeagu EI, Obeagu GU. *Elite Journal of Health Science*. **2024**, 2, 20-35.
8. Hoque R, Das S, Hoque M, Haque E. *World Journal of Advanced Research and Reviews*, **2024**, 21, 1985-94.
9. Sledge GW, Mamounas EP, Hortobagyi GN, Burstein HJ, Goodwin PJ, Wolff AC. *Journal of clinical oncology,* **2014**, 7, 32, 1979.

10. Armstrong K, Eisen A, Weber B. *New England Journal of Medicine*, **2000**, 24, 342, 564-71.
11. Weigelt B, Geyer FC, Reis-Filho JS. *Molecular oncology*, **2010**, 1, 4, 192-208.
12. Trayes KP, Cokenakes SE. *American family physician*, **2021**, 104, 171-8.
13. Burguin A, Diorio C, Durocher F, *Journal of personalized medicine*, **2021**, 19, 11, 808.
14. Britt KL, Cuzick J, Phillips KA, *Nature Reviews Cancer*, **2020**, 20, 417-36.
15. Kashyap D, Pal D, Sharma R, Garg VK, Goel N, Koundal D, Zaguia A, Koundal S, Belay A. *BioMed research international*, **2022,** 18, 2022.
16. Ramkumar S, Ramarajan R. *Results in Chemistry*, **2023**, 1, 5, 100861.
17. Ramkumar S, Ramarajan R. *ChemistrySelect*, **2023**, 6, 8, e202204818.
18. Volden O, Stahl A, Fossen TI. *International Journal of Intelligent Robotics and Applications*, **2022**, 6, 86-103.
19. Vakser IA. *Current Opinion in Structural Biology*, **2020**, 1, 64, 160-5.
20. Saravanan D, Deivam S, Padmavathy J, Balaji P, Pillai KK. *International Journal of Research in Pharmaceutical Sciences and Technology*, **2023**, 29, 4, 01-7.
21. Priya M, Zochedh A, Chandran K, Arumugam K, Banu S, Chakaravarthy C, Sultan AB. *Letters in Applied NanoBioScience*. **2022**, 12.
22. Banerjee P, Eckert AO, Schrey AK, Preissner R. *Nucleic acids research*, **2018**, 2, 46, W257-63.
23. Drwal MN, Banerjee P, Dunkel M, Wettig MR, Preissner R. ProTox: a web server for the in silico prediction of rodent oral toxicity. *Nucleic acids research.* **2014,** 1, 42, W53-8.
24. Merdekawati F. *Journal of Applied Pharmaceutical Science*, **2018**, 30, 8, 119-29.
25. Subramaniyan R, Ramarajan R, Ramalingam A, Sambandam S, Petersamy A, Guerroudj AR, Boukabcha N, Chouaih A. *Journal of Molecular Structure*. **2023**, 15, 1278, 134946.
26. Ramkumar S, Ramarajan R. *ChemistrySelect*, **2023**, 10, 8, e202204494.

27. Ramkumar S, Rajalakshmi R, *Materials Today: Proceedings*, **2023**, 21.