Way2Drug

Understanding Chemical-Biological Interactions

"Design and synthesis of peptide inhibitors targeting HER2 as a therapeutic strategy in breast cancer"

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Breast cancer and Human epidermal growth factor receptor 2 (HER2)

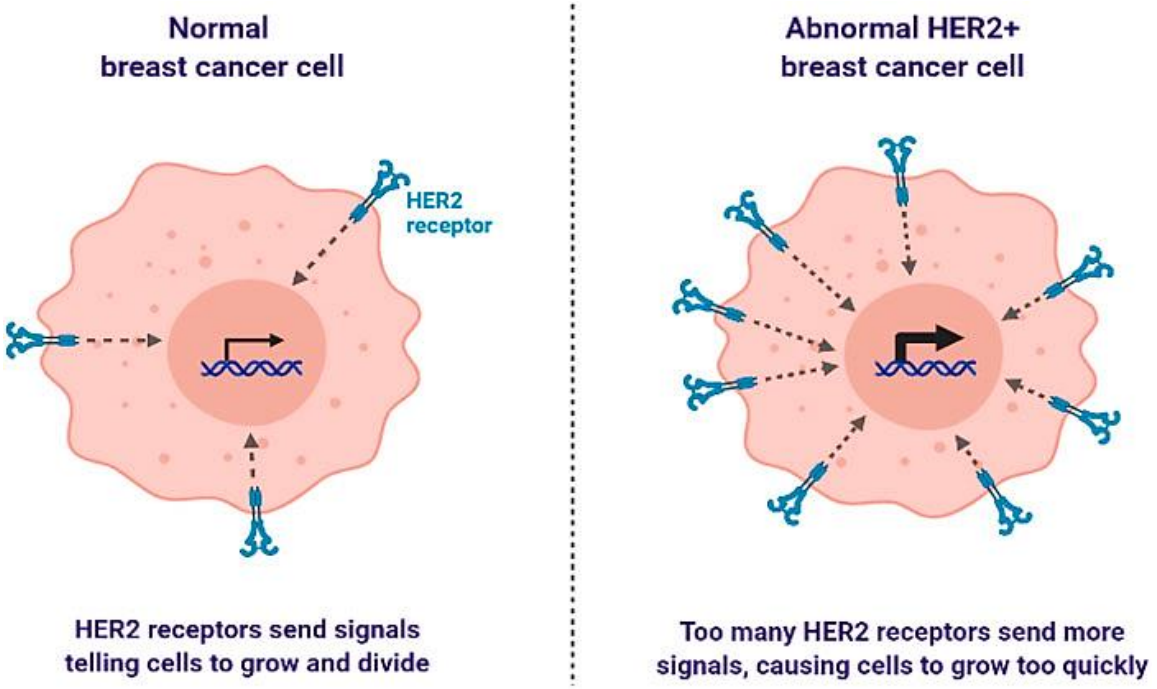


Figure 1. Normal breast cancer cell and abnormal breast cancer cell (HER2 overexpressing).

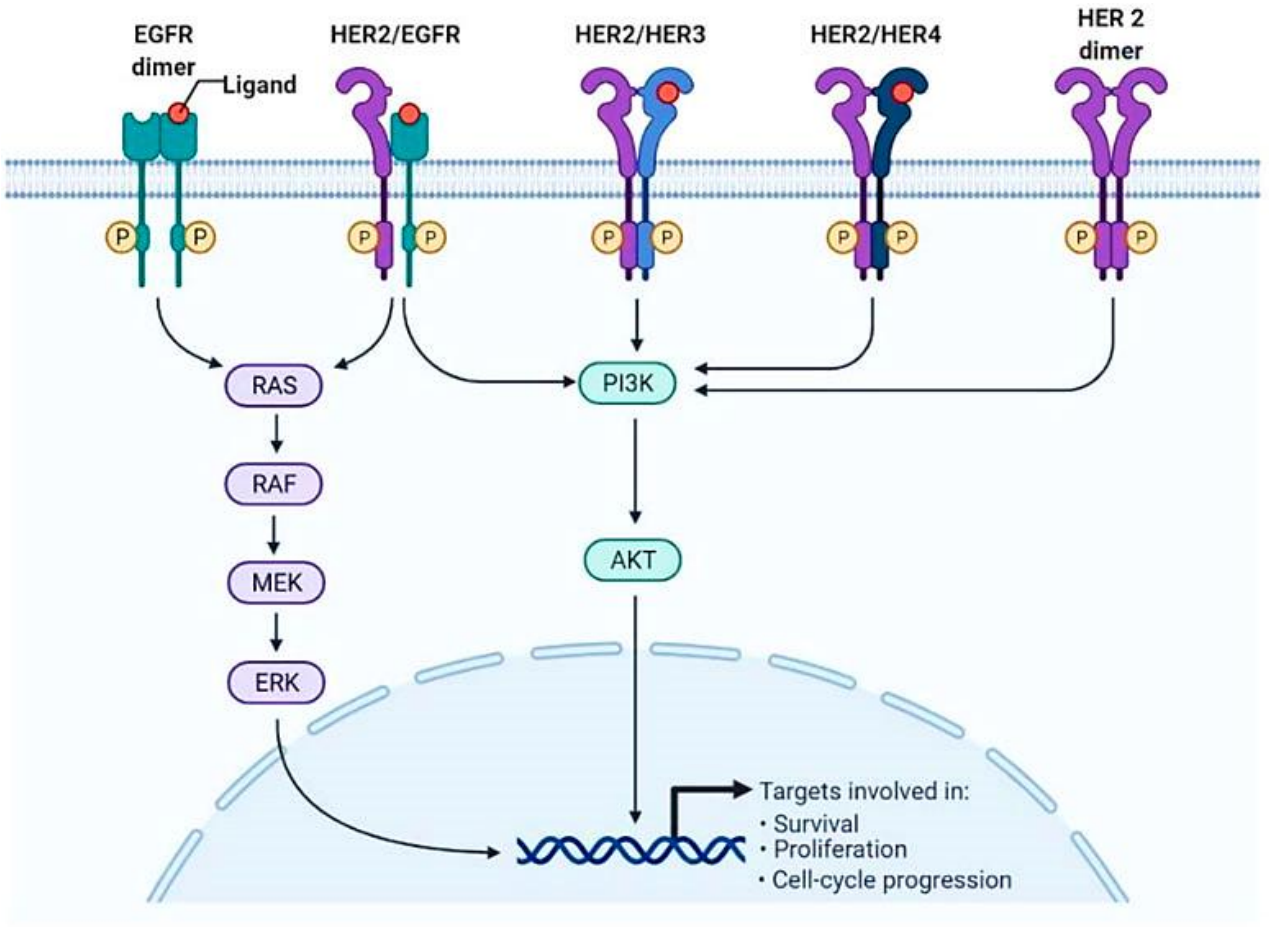


Figure 2. Signaling pathways activated by HER family receptors.

Singh DD, Lee H-J, Yadav DK. Clinical updates on tyrosine kinase inhibitors in HER2-positive breast cancer. *Front Pharmacol*. **2022**; *13*: 1089066.

Peptides in breast cancer therapy

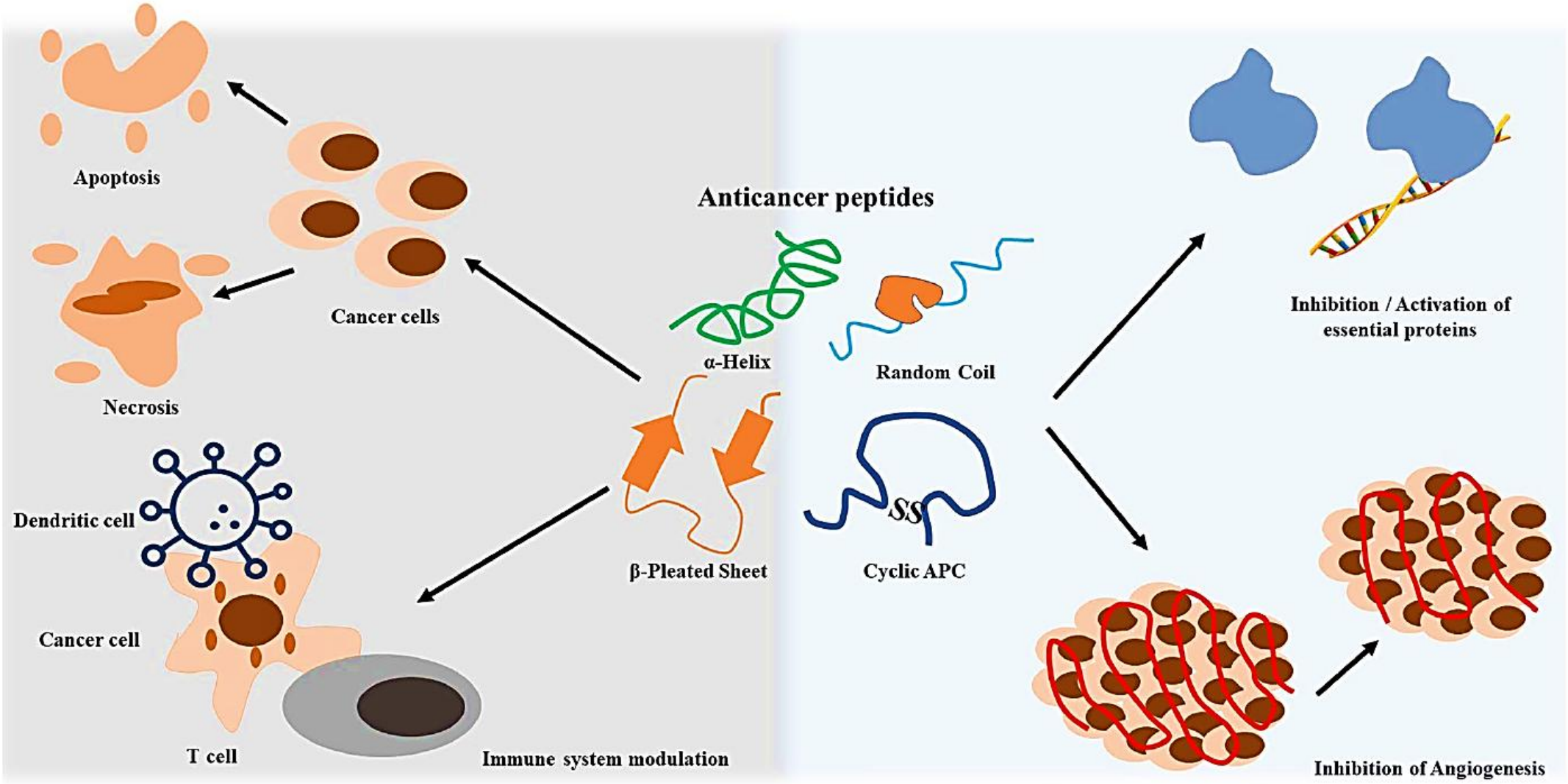


Figure 3. Anticancer peptides and their role as targeted therapy in breast cancer.

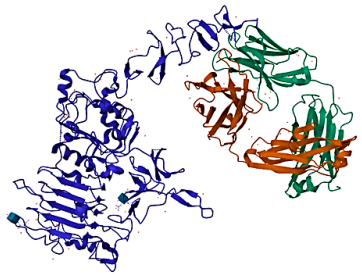
Das A, Adhikari S, Deka D, Bisgin A, Paul S, Balidya N, et al. An updated review on recent advances in the usage of novel therapeutic peptides for breast cancer treatment. *Int J Pept Res Ther*. **2023**; *29*(2): 1-17.

Main objective

To Implement structure-based pharmacophore modeling to design a series of peptides targeting HER2, evaluate their affinity for the receptor by *in silico* methods and obtain the most promising candidates by solid-phase chemical synthesis.

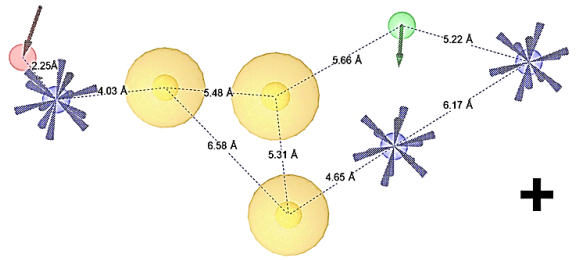
Methodology

RCSB PDB PROTEIN DATA BANK



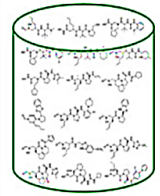
HER2 / Trastuzumab

LigandScout



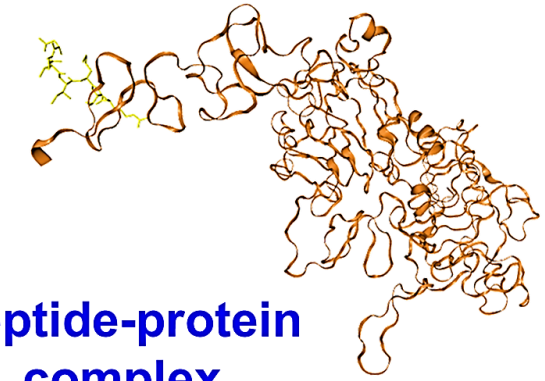
Pharmacophore modeling

+



De novo peptide library

LigandScout

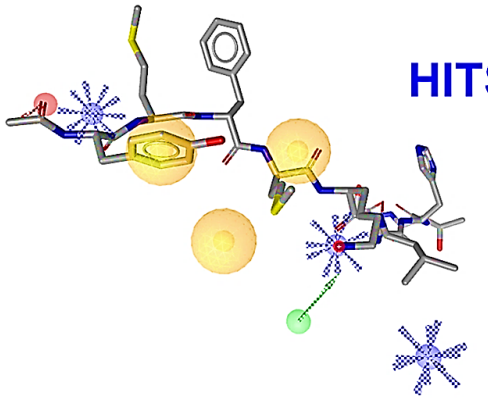


Peptide-protein complex

HPEPDOCK 2.0

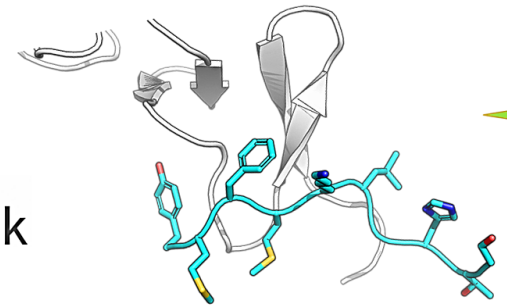
HER2

+



HITS

flexpepdock



Complex refinement

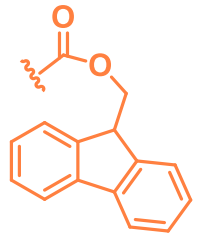
Solid-phase peptide synthesis

Solid-phase peptide synthesis

= polymeric support

= protective group

Fmoc =



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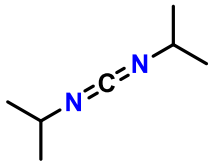

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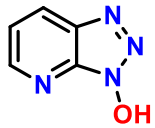

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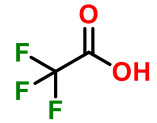
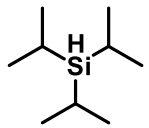
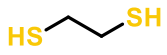


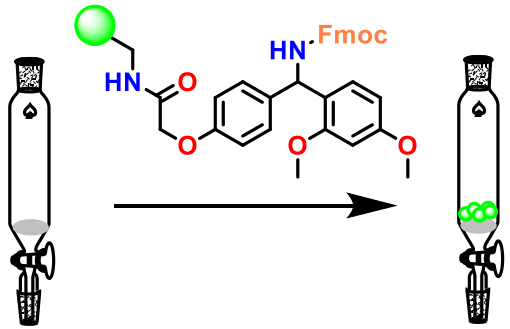
TIS =



EDT =

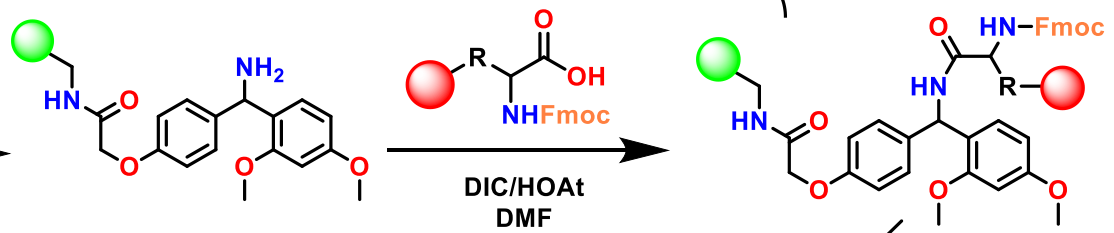


Rink Amide AM Resin



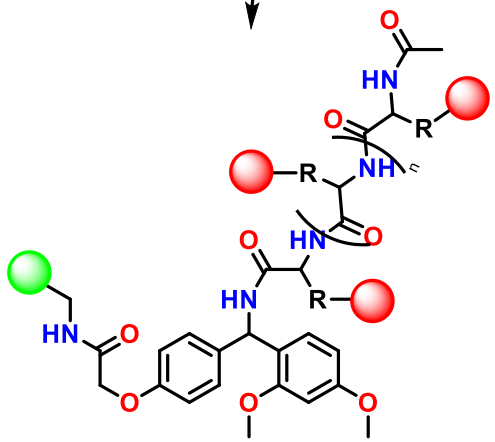
30 min

Multiple washes with DMF / IPA / DCM



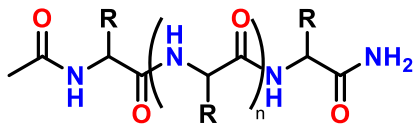
Chain elongation (remove Fmoc group again and couple the next amino acid)

Acetylation
3 h



Cleavage

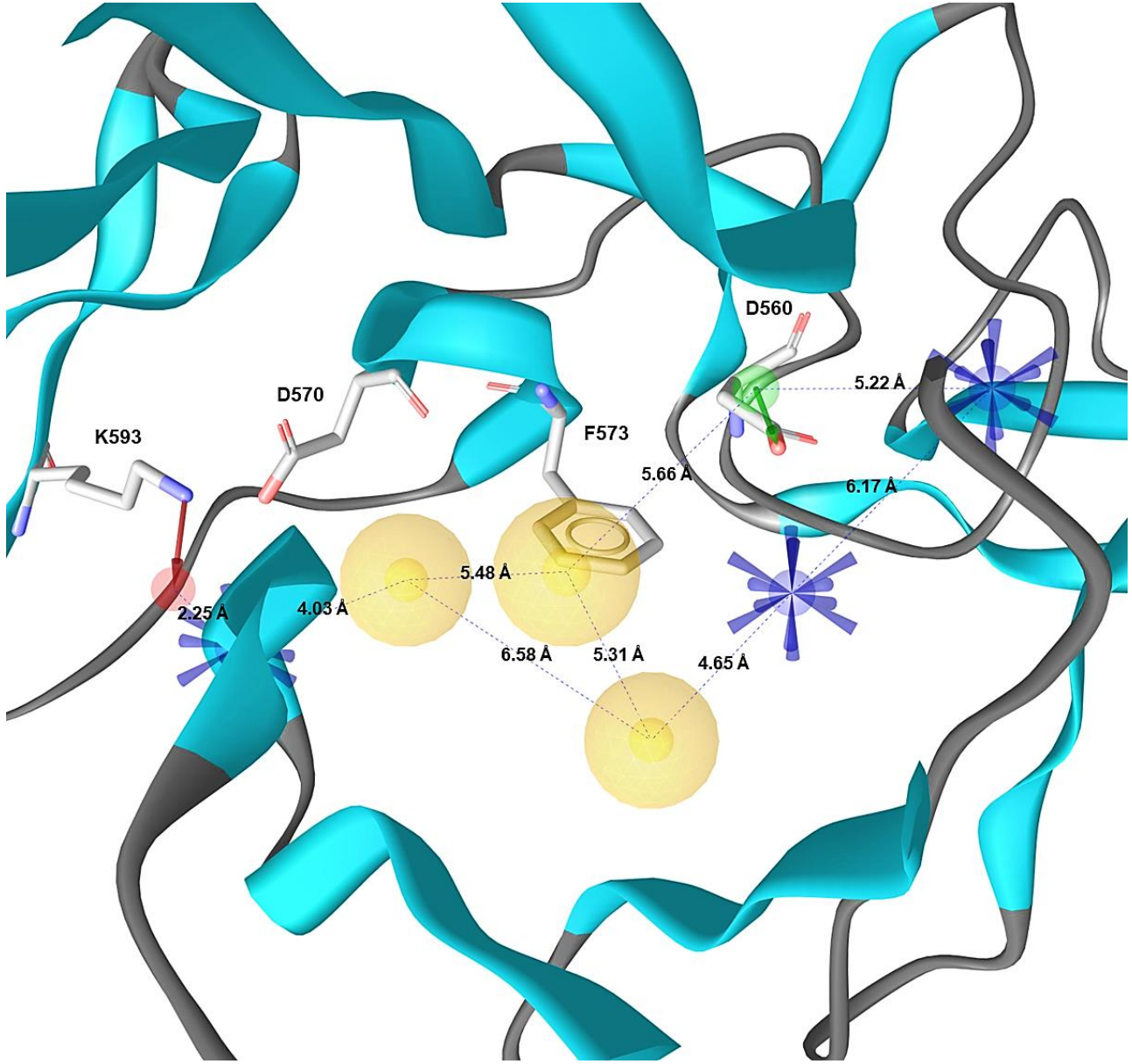
TFA/H_2O/TIS/EDT
92.5:2.5:2.5:2.5
2 h



Final peptide sequence

Results

Pharmacophoric map targeting HER2



	H	Hydrophobic (3)
	PI	Positive ionization site (3)
	HBD	Hydrogen bond donor
	HBA	Hydrogen bond acceptor

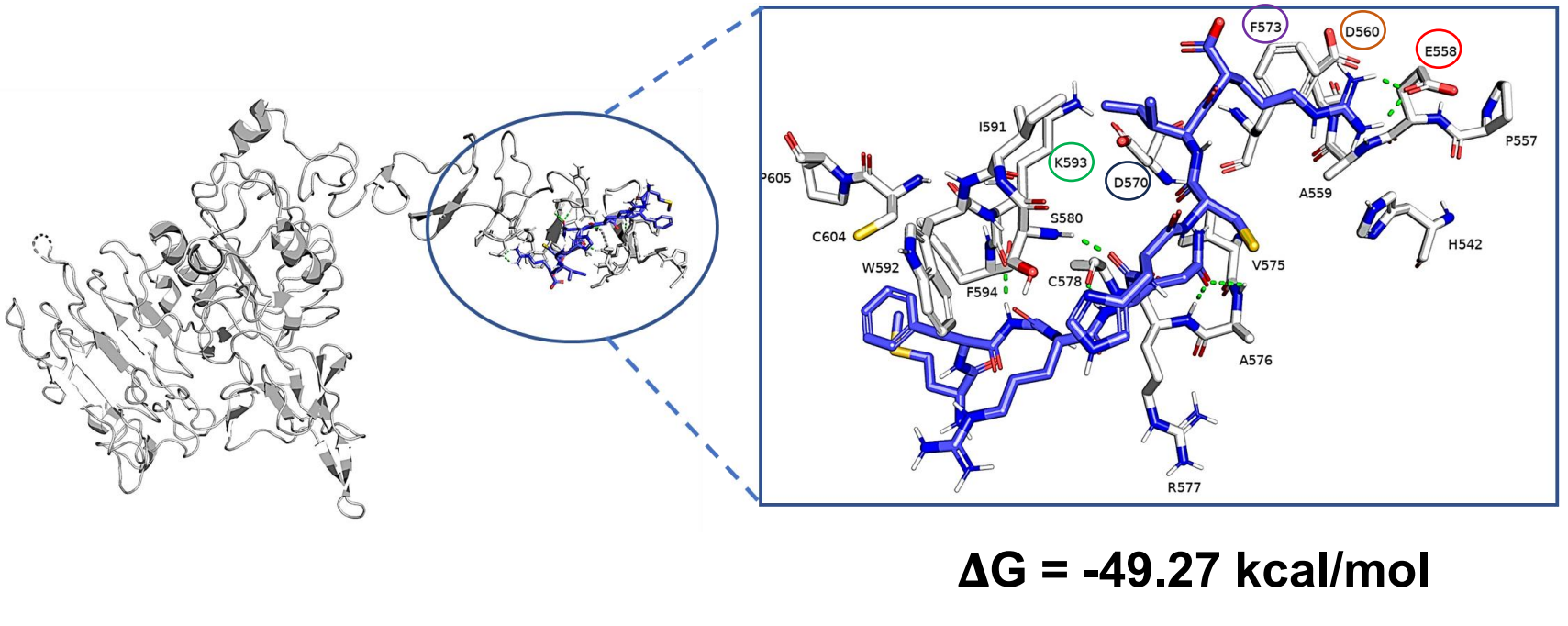
Figure 4. Structure-based pharmacophore map targeting HER2 (HER2 residues are shown in white).

Molecular docking and prediction of allergenic and toxic properties

Table 1. Results of *in silico* evaluations for the top-ranked HER2-targeted peptides in virtual screening.

		Ligand Scout 4.5	HPEPDOCK 2.0	FlexPepDock	HawkDock (MM/GBSA)	AllerTOP v. 2.0	ToxinPred
ID	Peptide sequence	% pharmacophore fit	Score	Score	ΔG (kcal/mol)	Allergenicity prediction	Toxicity prediction
PHER37	MFGRQHCIR	45.52	-158.503	-967.090	-49.27	Non-allergen	Non-toxic
PHER19	AWVCNRIDG	45.16	-152.316	-964.597	-38.26	Non-allergen	Non-toxic
PHER14	CIDMKLAYLV	43.59	-146.642	-966.084	-30.69	Allergen	Non-toxic
PHER27	YMFMKLGHTS	38.7	-171.679	-957.576	-19.5	Allergen	Non-toxic
PHER77	ASQFNDVNTAVAW	38.4	-167.473	-967.391	-34.58	Allergen	Non-toxic
PHER47	RFMNHIVTVN	38.37	-166.825	-967.594	-33.89	Non-allergen	Non-toxic
PHER4	LKGFTRT	38.27	-149.875	-962.026	-25.28	Non-allergen	Non-toxic
PHER8	KYNCRITVH	38.25	-153.423	-965.826	-28.95	Allergen	Non-toxic
PHER5	RHGFTYLVK	38.19	-193.695	-965.380	-33.81	Non-allergen	Non-toxic
PHER78	ASGFNIKDTYIHW	36.94	-177.587	-960.829	-52.26	Non-allergen	Non-toxic

PHER37 binding mode on HER2



ΔG = -49.27 kcal/mol

Figure 5. Docking of PHER37 peptide (blue) and its binding mode with HER2 residues (white).

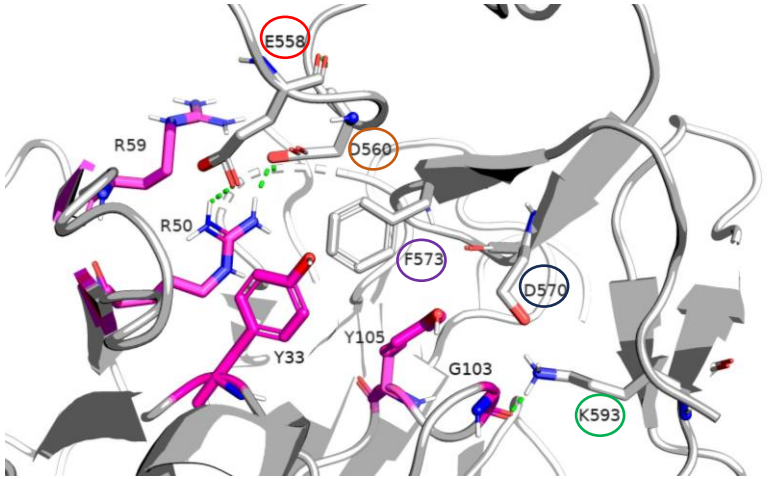
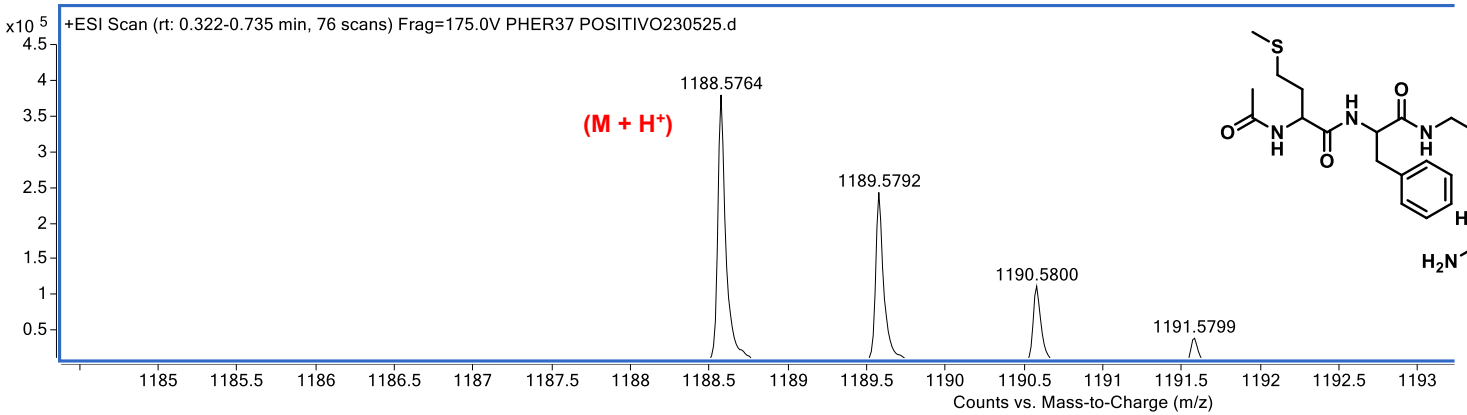
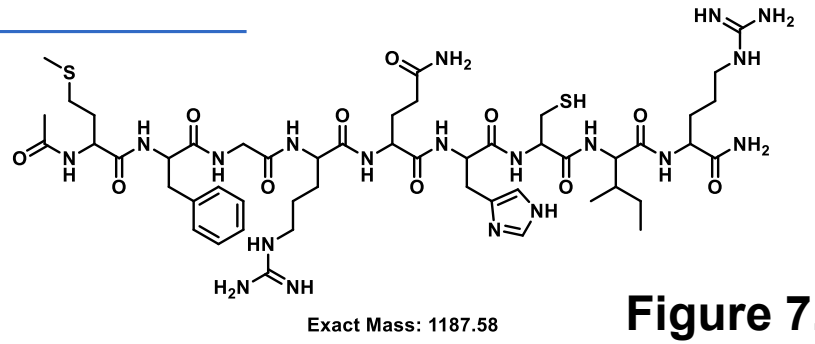


Figure 6. Binding mode of Trastuzumab residues (magenta) on HER2.

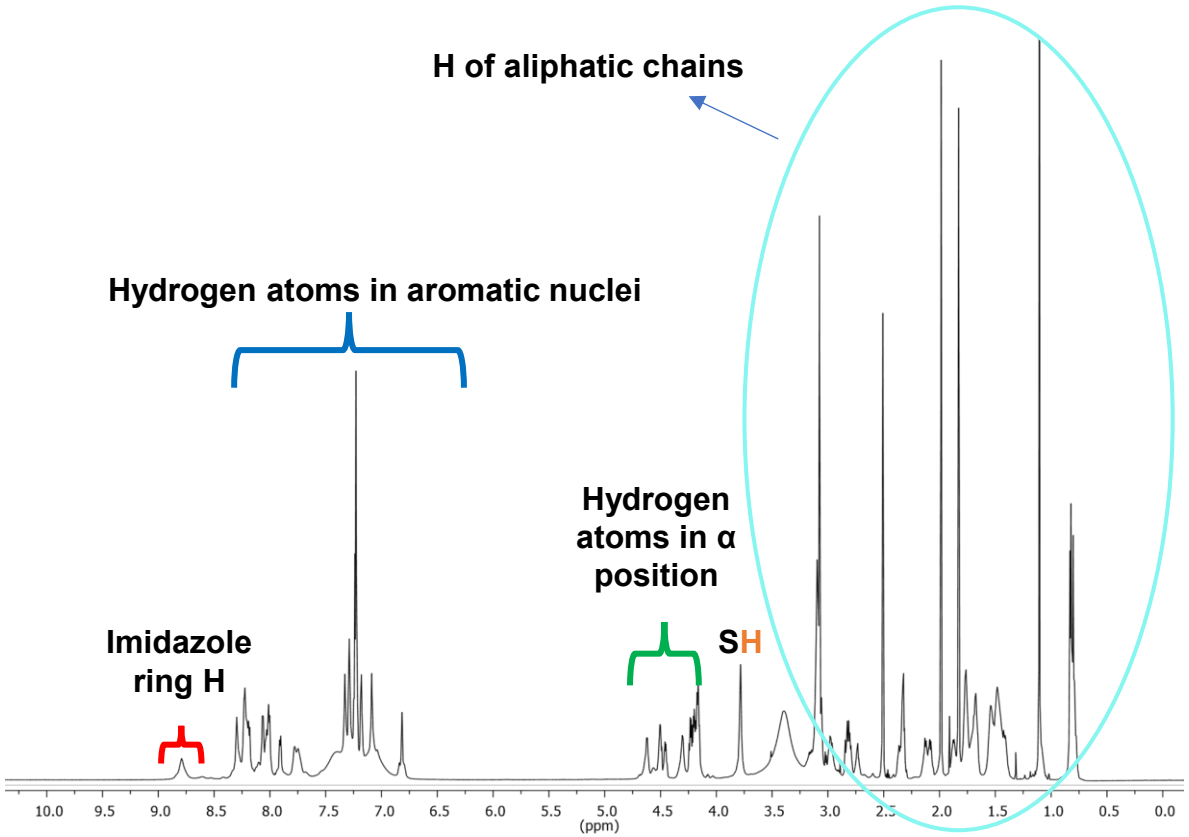
Structural characterization





Chemical yield = 85%

Figure 7. Mass spectrum (**ESI+**) of the PHER37 peptide.



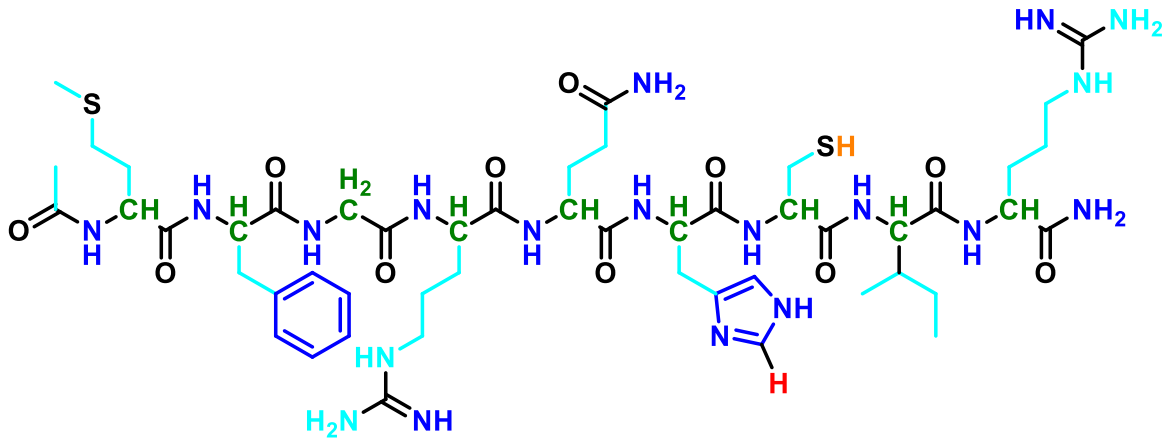


Figure 8. ^{1}H-RMN spectrum of the PHER37 peptide (DMSO-d_6, 700 MHz).

Conclusion

Pharmacophore modeling based on the structure of the HER2/Trastuzumab complex and molecular docking enabled the design and identification of new peptide sequences targeting HER2, from which the most promising (PHER37) was obtained by solid-phase chemical synthesis. The peptide obtained is a potential candidate for its evaluation on breast cancer cell lines and for possible use in therapies targeting this disease.