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Synthesis and Evaluation of New 1,4-Dihydropyridine Derivatives as Potential Breast Cancer Therapeutics: HER2 Inhibition Efficacy Study Using MCF-7 and SK-BR-3 Cells and Molecular Docking

Abdulrhman Y. Khalifa*

Ministry of Education, The General Directorate of Education in Muthanna, Samawah, 66001, Iraq

Corresponding author: hayderhussein862@mu.edu.iq

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SK-BR-3 cells;
HER2 receptors.

ABSTRACT. The derivatives of 1,4-Dihydropyridine compound have gained significant interest for their potential-anticancer properties particularly in targeting breast-cancer. The study aimed to synthesize and evaluate the anticancer activity of four novel 1,4-dihydropyridine derivatives (5a–d) against breast cancer cell lines MCF-7 and SK-BR-3. The derivatives were synthesized via the reaction of N-(4-nitrophenyl)-3-oxobutanamide with substituted benzaldehyde and ammonium acetate. Cytotoxic activity was assessed using MCF-7 and SK-BR-3 cells, and IC_{50} values were determined. Molecular docking was performed to evaluate the binding affinity of the compounds to the HER2 receptor. Structural characterization was conducted using FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry. All four derivatives (5a–d) demonstrated higher inhibition rates in SK-BR-3 cells (72%, 83%, 89%, and 85%, respectively) compared to MCF-7 cells (51%, 54%, 58%, and 55%) at a concentration of 100 µg. Compound 5c exhibited the highest cytotoxicity, with the lowest IC_{50} value (18.5 µg/mL) against SK-BR-3 cells, followed by 5b and 5d, while 5a showed the weakest effect. Molecular docking results supported the potential of these compounds as HER2-targeted agents. The synthesized 1,4-dihydropyridine derivatives, particularly compound 5c, demonstrated promising anticancer activity, especially against HER2-positive SK-BR-3 cells.

INTRODUCTION

Breast cancer is considered one of the most common cancers that affect women all over the world, as studies have shown that it ranks second after lung cancer (Bilani *et al.*, 2020). Breast cancer is one of the cancers that is diagnosed after menopause in women, and this often causes a decrease in women's ability to function in their daily lives (Kamińska *et al.*, 2015). Lifestyle, psychological, environmental, and social factors are closely linked to breast cancer, and it has been proven that 5% to 10% of these cases are due to genetic mutations and family history, and about 20% to 30% of these cases are due to modifiable factors such as choosing medication, obesity, smoking, drinking alcohol, and others (Sun *et al.*, 2017).

The cells in the lining of the milk ducts, which are responsible for transporting milk from the glands to the nipples, can grow excessively until they appear cancerous, but they remain confined to the milk ducts. In this case, they are known as pre-invasive or non-invasive cancer, or intraductal cancer (Edward *et al.*, 2021; Obeagu *et al.*, 2023). It can occur in the lobules, that is, due to excessive growth of the cells of the lobules, which are a group of cells that produce milk. This is called in situ carcinoma of the lobulus or lobular tumor. These cancers may remain in the place where they first appear, or they may spread to nearby tissues and are called invasive cancer (Iatrakis and Zervoudis, 2021). Invasive breast cancer has been divided into four subtypes based on the expression of progesterone receptor (PR), estrogen receptor (ER), and human epidermal growth factor receptor 2 (HER2) (Joshi and Press, 2018). Luminal A breast cancer (PR+ and/or ER+, and HER2-) represents about 60% of breast cancer (Gao and Swain, 2018). Luminal B breast cancer (PR+ and/or ER+, and HER2+) represents about 30% of breast cancer (Ades *et al.*, 2014), and HER2 breast cancer (PR-, ER-, and HER2+) represents about 10% of breast cancer (Loibl and Gianni, 2017). Finally, triple-negative breast cancer (TNBC) (PR-, ER-, and HER2-) represents about

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15% to 20% of breast cancer and is considered more aggressive, It has a worse prognosis than other types of breast cancer and affects younger women (Bergin and Loi, 2019).

Heterocyclic nitrogen-containing compounds such as 1,4-dihydropyridines (DHP) are important structures that exhibit a wide range of biological activities. These activities include calcium channel blocking, antitumor, anti-inflammatory, antimicrobial, antihistamine, anticonvulsant, and analgesic effects (Mannhold *et al.*, 1992; Gaudio *et al.*, 1994; Mishra *et al.*, 2019). The DHP structure is characterized by its ability to be structurally modified by introducing different chemical substituents at multiple sites on the DHP ring, resulting in significant changes in pharmacological properties. These compounds can provide improved efficacy and biological activities, including anti-tuberculosis and anti-cancer roles (Desai *et al.*, 2001; Safak and Simsek, 2006; Radadiya *et al.*, 2014). Studies have shown that some 1,4-dihydropyridine derivative compounds show efficacy as anticancer activity by inhibiting "calcium channels" which inhibits the growth and spread of cancer cells (Liu *et al.*, 2024; Zonouz and Abedinpour, 2024). Studies have shown that these compounds can be effective in inhibiting multidrug resistance (MDR) in cancer cells, increasing the effectiveness of conventional chemotherapy (Oliveira *et al.*, 2024).

The structural design of 1,4-dihydropyridine derivatives could improve their efficacy as inhibitors against certain types of cancer, including breast cancer. The results show that modifications to the base ring of these compounds can enhance the anticancer efficacy of making these compounds an important target in the development of new cancer drugs in the future (Faizan *et al.*, 2024; Mehany *et al.*, 2024; Sruthi *et al.*, 2024). Although 1,4-dihydropyridine derivatives have demonstrated anticancer activity in previous studies, their application to the HER2 receptor remains limited. In this work, a selection of 1,4-DHP derivatives were designed and synthesized with carefully considered structural modifications to evaluate their potential as HER2 inhibitors. HER2 is a key therapeutic target in breast cancer that has not received sufficient attention in this field. The SK-BR-3 cell line was chosen because it is a highly HER2-expressing cell model, and because previous studies investigating the effects of 1,4-DHP derivatives on this specific cell line are lacking. Most published research has focused on other cell lines, such as MCF-7. This study thus contributes to filling an existing knowledge gap by linking molecular design, HER2 inhibition, and direct cellular action on a model closely related to the receptor pathway.

RESEARCH METHODS

All chemicals used were obtained from Sigma-Aldrich. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on DPX-300 MHz FT-NMR spectrophotometer (Bruker) using TMS as an internal reference standard in DMSO-D6 solvent. IR spectra were recorded on Shimadzu FTIR-8400 using KBr Disc and DRS techniques. The reaction progress was monitored using TLC on silica gel plates (Taher *et al.*, 2024; Abdullah *et al.*, 2025).

Synthesis

Preparation of N-(4-nitrophenyl)-3-oxobutanamide 3

4-nitroaniline 1 (0.4 mol) was added to 4 mL of KOH solution in water and stirred well until the solution became clear. Then, methyl acetoacetate 2 (0.36 mol) was added to the mixture and stirred for 5 minutes. After that, 100 mL of toluene was added, and the reaction was refluxed for 5 – 6 hours. The progress was monitored by thin-layer chromatography (TLC) until the reaction was complete. The reaction mixture was then cooled to room temperature, followed by the addition of toluene (100 mL) and 5% aqueous HCl solution (250 mL). Using a separating funnel, the toluene layer is collected and washed again with 100 mL of 5% HCl solution and twice with 100 mL of water and 50 ml. ml of Brine solution. The light layer is collected after washing and dried with anhydrous sodium sulfate. A rotavapor at 40 °C is used to distill the solvent. Add 50 mL of petroleum ether to it, keep it in the refrigerator, stir for 30 minutes at a temperature of 20 °C, add 20 mL of petroleum ether to it again, then separate the solid and dry it in the oven (Singh *et al.*, 2016).

Preparation of N3,N5-bis(4-nitrophenyl)-1,4-dihydro-2,6-dimethyl-4(Substituted phenyl) pyridine-3,5-dicarboxamide (5a-d)

To N-(4-nitrophenyl)-3-oxobutanamide 3 (1 mmole) and 1g of ammonium acetate in 10 mL methanol was added 0.5 mmole of different substituted benzaldehyde (4a-d). The mixture was refluxed for 24 hours until a precipitate was obtained. It was filtered, then washed with cold methanol, and then recrystallized using methanol-water to give the desired compound (Xie *et al.*, 2023).

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N3,N5-bis(4-nitrophenyl)-1,4-dihydro-2,6-dimethyl-4-phenyl)-pyridine-3,5-dicarboxamide (5a)

Yield: (74%). mp: 233-234 oC. IR (KBr Disc): 3298-3408 cm-1(-NH), 2850-2976 cm-1 (C-H), 1672 cm-1(-CO-NH), 1599 cm-1 (-NH Bend). ^{1}H-NMR (300 MHz, DMSO-d6): δ 2.10 (s, 6H, -CH3), 5.12 (s, 1H, CH-ring), 7.08 (m, 1H, Ar-H), 7.22 (t, 4H, Ar-H, J=3.048 Hz), 7.31 (d, 2H, Ar-H, J=7.386 Hz) 7.47 (m, 2H, Ar-H, J=7.85 Hz), 7.78 (d, 2H, Ar-H, J=7.965 Hz), 8.06 (s, 2H, Ar-H), 8.21 (s, 1H, NH-ring), 9.65 (s, 2H, -CO-NH-).13C-NMR: δ 18.9 (2CH3), 53.65 (CH), 98.8 (2ArC), 108.2 (2ArC), 112.6-112.7 (4ArC), 121.4 (2ArC), 123.5 (ArC), 124.8 (ArC), 125.1 (2ArC), 127.3 (2ArC), 131.5 (ArC), 136.5 (2ArC), 140.3 (ArC), 158.8 (2C=O). Mass (m/z): [Peak Found (M−1): 513.5 (calculated (M−1): 513.39)].

N3,N5-bis(4-nitrophenyl)-1,4-dihydro-2,6-dimethyl-4-(4-methoxy phenyl)- pyridine-3,5-dicarboxamide (5b)

Yield: (74%). mp: 233-234 oC. IR (KBr Disc): 3199-3430 cm-1(-NH), 2860-2985 cm-1 (C-H), 1680 cm-1(-CO-NH), 1605 cm-1 (-NH Bend), 1218 cm-1 (C-O-C). 1H-NMR (300 MHz, DMSO-d6): δ 2.09 (s, 6H, -CH3), 3.64 (s, 3H, -OCH3), 5.07 (s, 1H, CH-ring), 6.76 (d, 2H, Ar-H, J=8.58 Hz), 7.14 (d, 2H, Ar-H, J=8.56 Hz), 7.30 (d, 2H, Ar-H, J=7.485 Hz), 7.47 (t, 2H, Ar-H, J=7.914 Hz), 7.78 (d, 2H, Ar-H, J=8.076 Hz), 8.07 (s, 2H, Ar-H), 8.17 (s, 1H, NH-ring), 9.60 (s, 2H, 2H, -CO-NH-).13C-NMR: δ 18.11 (2CH3), 53.74 (CH), 56.4 (OCH3), 98.71 (2ArC), 108.21 (2ArC), 112.75-112.84 (4ArC), 121.48 (2ArC), 123.62 (ArC), 124.91 (ArC), 125.17 (2ArC), 127.4 (2ArC), 131.57 (ArC), 136.64 (2ArC), 149.39 (ArC), 158.87 (2C=O). Mass (m/z): [Peak Found (M−1): 543.52 (calculated (M−1): 543.43)].

N3,N5-bis(4-nitrophenyl)-1,4-dihydro-2,6-dimethyl-4-(2-choloro phenyl)- pyridine-3,5-dicarboxamide (5c)

Yield: (81%). mp: 224-225 oC. IR (KBr Disc): 3294-3421 cm-1(-NH-), 2850-2976 cm-1 (-C-H), 1660 cm-1(-CO-NH-), 1602 cm-1 (-NH- Bend), 750 cm-1 (-C-Cl). 1H-NMR (300 MHz, DMSO-d6): δ 2.09 (s, 6H, -CH3), 5.44 (s, 1H, CH-ring), 7.15 (m, 2H, Ar-H), 7.30 (m, 3H, Ar-H, J=8.074 Hz), 7.45 (m, 3H, Ar-H, J=7.85 Hz) 7.74 (d, 2H, Ar-H, J=8.007 Hz), 8.06 (s, 2H, Ar-H), 8.31 (s, 1H, NH-ring), 9.76 (s, 2H, -CO-NH-). 13C-NMR: δ 18.13 (2CH3), 50.1(CH), 99.42 (ArC), 109.22 (ArC), 112.61-112.71 (4ArC), 121.32 (ArC), 123.11 (ArC), 128.1 (2ArC), 129.31 (ArC), 130.8 (ArC), 130.9 (ArC), 131.3 (2ArC), 131.12 (ArC), 136.82 (2ArC), 149.11 (2ArC), 159.1 (2C=O). Mass (m/z): [Peak Found (M−1): 547.94 (calculated (M−1): 547.81)].

N3,N5-bis(4-nitrophenyl)-1,4-dihydro-2,6-dimethyl-4-(2-methoxy phenyl)- pyridine-3,5-dicarboxamide (5d)

Yield: (68%). mp: 244-245 oC. IR (KBr Disc): 3308-3399 cm-1(-NH-), 2829-2952 cm-1 (-C-H), 1678.13 cm-1(-CO-NH-), 1597.11 cm-1 (-NH- Bend), 1238 cm-1 (-C-O-C-). 1H-NMR (300 MHz, DMSO-d6): δ 2.16 (s, 6H, -CH3), 3.59 (s, 3H, -OCH3), 5.37 (s, 1H, CH-ring), 6.85 (m, 2H, Ar-H), 7.07(t, 1H, Ar-H, J=4.365 Hz), 7.20 (d, 1H, Ar-H, J=6.06 Hz), 7.32 (d, 2H, Ar-H, J=7.41 Hz), 7.50 (t, 2H, Ar-H, J=7.89 Hz), 7.78 (d, 2H, Ar-H, J=7.95 Hz), 8.10 (s, 2H, Ar-H), 8.34 (s, 1H, NH-ring), 9.54 (s, 2H, -CO-NH-). 13C-NMR: δ 18.08 (2CH3), 53.41 (CH), 57.12 (OCH3), 99.71 (2ArC), 108.24 (ArC), 109.2 (2ArC), 112.45-112.55 (4ArC), 121.21 (ArC), 122.52 (ArC), 122.91 (ArC), 123.8 (ArC), 126.31 (2ArC), 131.23 (2ArC), 140.8 (2ArC), 142.21 (2ArC), 149.62 (1ArC), 158.65 (2C=O). Mass (m/z): [Peak Found (M−1): 543.52 (calculated (M−1): 543.43)].

Anticancer Assays

In vitro, anticancer profiles of the synthesized compounds (5a-d) were determined against human breast cancer cell lines (MCF-7 and SK-BR-3) by a cell viability assay (MTT assay) (Tasneem *et al.*, 2022). DMEM (low glucose), 10% fetal bovine serum, and antibiotic/antimycotic formed the main constituents of the culture medium. MCF-7 and SK-BR-3 cells were seeded in a culture plate at a density of 1×104 cells/well. These were incubated at 37 °C under a humidified atmosphere containing 5% CO_2 for 24 hrs before the assay. After that, the cells were further incubated in media containing various concentrations of the test compounds. After 24 hr, the medium was removed and washed with PBS. About 20 µL of MTT solution was added to each well followed by 4 h of incubation at 37 °C. Subsequently, the medium was removed, followed by the addition of 200 µL DMSO. After slowly shaking (twice) for 5 s, the absorbance of each well was determined at 570 nm. The cell viability (%) was calculated according to Equation 1, as the ratio of the absorbance of simple free cells.

$$\text{Survival (\%)} = \frac{\text{absorbance of simple treated cells}}{\text{absorbance of simple free cells}} \times 100\% \quad (1)$$

Docking Method

The crystal structure of human epidermal growth factor receptor 2 was retrieved from the Protein Data Bank (PDB: 3PP0). After determining the protein structure, the protein preparation protocol was applied using Discovery Studio. All water molecules were removed except those that directly interact with the protein. The active site of

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the protein was identified and adopted as the binding site, and the crystallized ligand was removed. The prepared protein was then saved in .pdb format. We used ChemDraw to draw the 2D chemical structure and subsequently convert it to 3D. All molecules were prepared for molecular interaction experiments using the small molecule protocol in Discovery Studio. Molecular energy minimization and calculation of partial atomic charges were performed. Docking simulations were performed using AutoDock Chimera and AutoDock Vina, and DSV was used to visualize the interaction. The main binding sites of the compounds were analyzed based on the docking results with human epidermal growth factor 2 using Chimera, with a focus on identifying the key amino acids bound to the compounds and studying their molecular interactions.

RESULTS AND DISCUSSION

Synthesis

In this study, 1,4-dihydropyridine derivatives (5a-d) were prepared in two steps due to their pharmacological importance (Figure 1). In the first step, N-(4-nitrophenyl)-3-oxobutanamide 3 was prepared from 4-nitroaniline 1 in reaction with methyl acetoacetate 2 with KOH as a catalyst and toluene as a solvent. In the second step, 1,4-dihydropyridine derivatives (5a-d) were prepared using a modified Hantzsch reaction method, where N-(4-nitrophenyl)-oxobutanamide 3 was reacted with ammonium acetate and different substituted benzaldehyde (4a-d), in the presence of methanol as a solvent. The required compounds were obtained with yields.

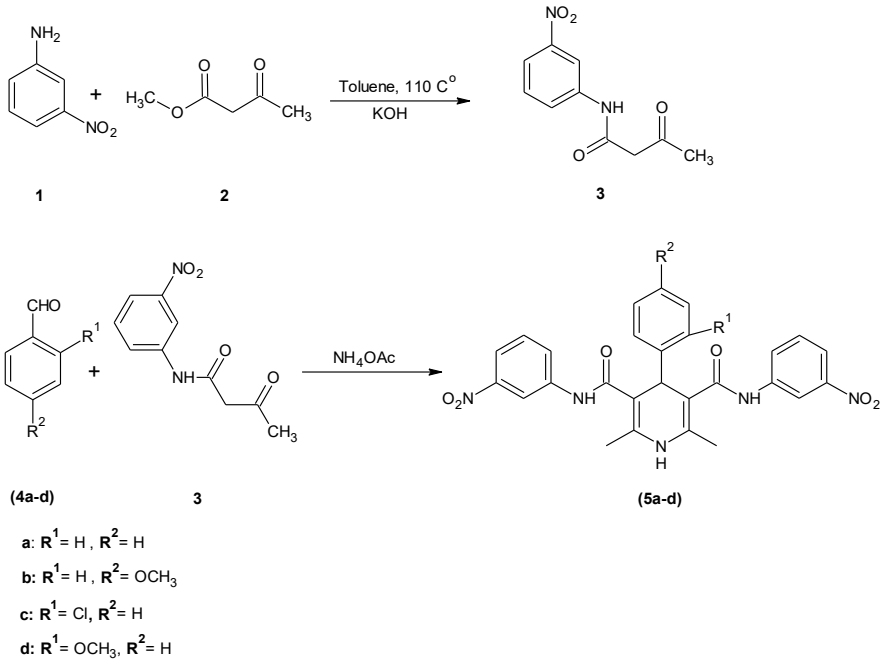


Figure 1. Synthesis of 1,4-dihydropyridine derivatives (5a-d).

Anticancer

The effectiveness of the 1,4-dihydropyridine derivatives (5a-d) that were synthesized against breast cancer SK-BR-3 and MCF-7 cell lines was studied to evaluate their effectiveness in suppressing the growth of breast cancer cells. The greatest inhibition of these compounds was observed for SK-BR-3 cells compared to MCF-7 cells at a concentration of 100 µg/ml. The high inhibition of SK-BR-3 cells is because these cells have high expression of HER2 receptors on the cell wall (Moradipoodeh *et al.*, 2020). HER2 is a protein that belongs to "the epidermal growth factor receptor" (EGFR) proteins, and this receptor plays an important role in regulating growth and cellular reproduction. We note the high inhibition values for compounds 5a, 5b, 5c, and 5d at the highest concentration of 100 µg/mL (Figure 2). They showed a significant inhibition of 72%, 83%, 89%, and 85%, respectively. This high inhibition can be explained by the fact that these compounds bind effectively. On the cell wall through the HER2 receptor, and because of this effective connection between the HER2 receptor and compounds 5a, 5b, 5c, and 5d, these compounds work to hinder the activity of these receptors and hinder the pathways and signals carried out by HER2, such as the Mitogen-activated protein kinase (MAPK) pathway and

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the Phosphoinositide 3-kinase/Protein kinase B (PI3K/AKT) pathway, thus increasing the rates of programmed cell death.

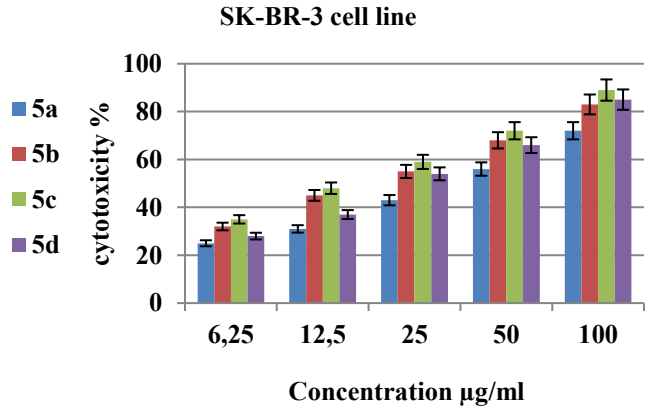


Figure 2. Cytotoxic effect of 1,4-dihydropyridine derivatives (5a-d) against SK-BR-3 cell line.

As for inhibition of MCF-7 cells, we note that the inhibition values for compounds 5a, 5b, 5c, and 5d for MCF-7 cells at the highest concentration of 100 µg/ml showed inhibition of 51%, 54%, 58%, and 55%, respectively (Figure 3). The low inhibition of MCF-7 cells is because these cells have a low expression of HER2 receptors compared to SK-BR-3 cells (Arienti *et al.*, 2019), as the lack of these receptors will reduce the number of sites to which compounds 5a, 5b, 5c, and 5d bind on the surface of the cells. MCF-7 cells, which have low expression of HER2 receptors, do not rely heavily on HER2 signals; therefore the effect of compounds 5a, 5b, 5c, and 5d is less effective on MCF-7 cells, because these cells contain a small number of HER2 receptors to bind to. These compounds contain and disrupt cellular pathways and signals (Taher *et al.*, 2024).

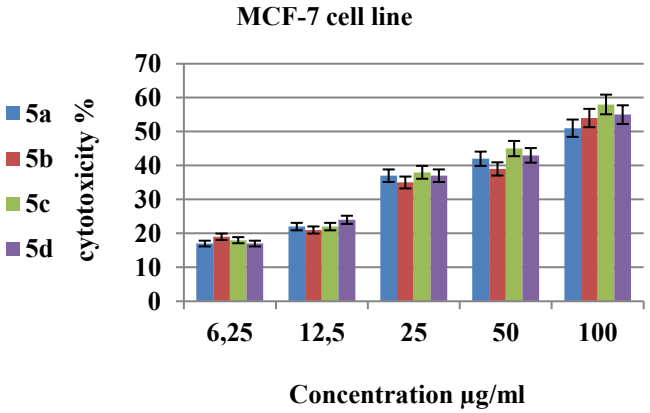


Figure 3. Cytotoxic effect of 1,4-dihydropyridine derivatives (5a-d) against MCF-7 cell line.

Accordingly, we notice a difference in the response to synthetic compounds depending on the expression of HER2 receptors, and that SK-BR-3 breast cancer cells, which have a high and excessive expression of HER2 receptors, we notice that they rely heavily on HER2 signals in order to survive, grow, and reproduce. Any disruption or inhibition of HER2 receptors will have a significant impact on cell growth. In contrast, MCF-7 cells, which have low expression of HER2 receptors, we note that they do not depend on HER2 signals, and therefore inhibition of HER2 receptors for these cells is not as effective.

The IC_{50} values of compounds 5a, 5b, 5c, and 5d were determined as follows (Figure 4): 48 µg/ml, 25 µg/ml, 18.5 µg/ml, and 31.7 µg/ml, respectively against SK-BR-3 cell line. By determining the IC_{50} values and analyzing the data using cytotoxicity curves. The results showed a significant variation in efficacy among the compounds, with compound 5c recording the lowest IC_{50} value of 18.5 µg/ml, indicating the highest cytostatic efficacy. This was followed by compound 5b with an IC_{50} value of 25 µg/ml, then 5d with a value of 31.7 µg/ml, and finally compound 5a which recorded the highest IC_{50} value of 48 µg/ml, indicating the least efficacy among these compounds. These values reflect significant differences in the response of cells to the concentrations of the compounds. In addition, the strength of the relationship between concentration and cytotoxicity was analyzed

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using the coefficient of determination R^2. Compounds 5a, 5b, 5c, and 5d showed high R^2 values of approximately 0.9458, 0.9039, 0.9144, and 0.9189, respectively, indicating a good agreement between the actual data and the model used in the analysis. These high R^2 values reflect the reliability of the results and confirm that the variation in concentration has a significant and direct effect on the cytotoxicity of the studied compounds.

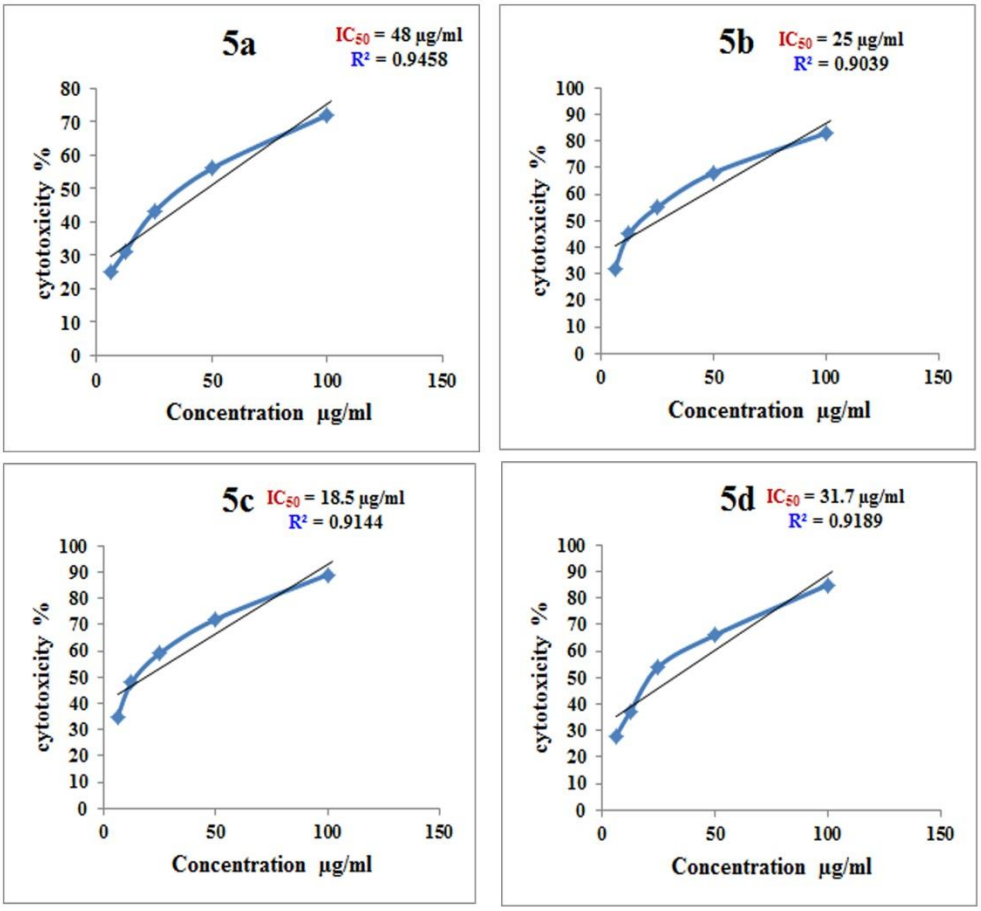


Figure 4. The percentage cytotoxicity at different concentrations of synthesized 1,4-dihydropyridine derivatives (5a-d) against SK-BR-3 cell line with its IC_{50}.

Docking Studies

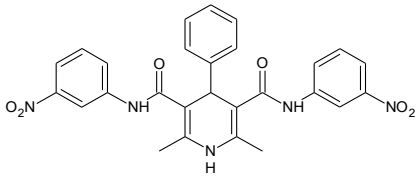
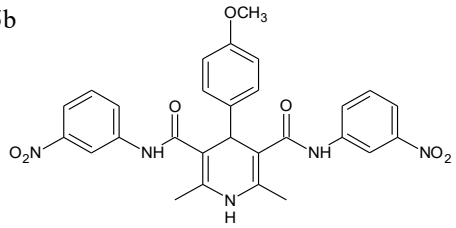
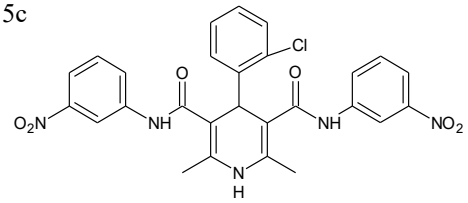
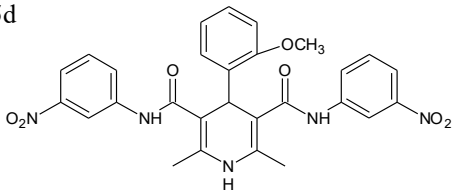
In the quest to develop novel HER2 inhibitors, we investigated the binding of new 1,4-dihydropyridine derivatives (5a-d) compounds to HER2 (PDB: 3PP0) using molecular docking techniques, with the aim of assessing the compounds' ability to inhibit HER2 activity associated with breast cancer growth and progression. HER2 is a receptor tyrosine kinase family member that plays a pivotal role in regulating cell growth and proliferation. Therefore, targeting its active and inactive states is an important challenge in the design of pharmacological inhibitors (Rubin *et al.*, 2024).

The HER2 protein, like other members of the receptor tyrosine kinase family, consists of a typical structure with two main domains: the front domain (N-lobe) and the back domain (C-lobe), which are connected by a flexible region known as the hinge region. The front-domain contains frequently β-strands and single α-helix while the back-domain consists mostly of multiple α-helices. The two domains are separated by a deep cleft representing the ATP binding site which is the main site targeted by inhibitory-compounds. The activity of the HER2 protein is determined by the position of the DFG (D=Aspartate - F=Phenylalanine - G=Glycine) sequence, which is an important part of the catalytic site of the enzyme (Aertgeerts *et al.*, 2011). There are two positions of the protein, DFG in and DFG out. In the DFG in, phenylalanine (F) is oriented inward and forms part of the catalytic site, enhancing the activity of the protein. In contrast, when phenylalanine is directed outward, as in the DFG out position, the catalytic site is disrupted, impairing enzyme activity (Vijayan *et al.*, 2015). By analyzing the docking images of 1,4-dihydropyridine derivatives (5a-d) bound to HER2, the amino acids bound to the compounds and

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their interactions were determined, as well as the type of bonds and the energy value associated with each interaction (Table 1).

Table 1. Molecular Docking Scores of 1,4-dihydropyridine derivatives (5a-d) at the binding region of HER2 (PDB: 3PP0).

No	Compound No.	Score (ΔG)	C-lobe	DFG motif	Hinge region	Distance (Å)	Type
5a		-8.5			MET801	2.241	Hydrogen
			ARG849			2.527	Hydrogen
			ASP845			4.137	Pi-Anion
				VAL734		3.768	Pi-Sigma
				PRO885		5.166	Pi-Alkyl
				VAL734		4.838	Pi-Alkyl
					ALA751	4.625	Pi-Alkyl
			LEU852			4.725	Pi-Alkyl
5b		-8.6	LEU726			2.826	Hydrogen
			CYS805			2.368	Hydrogen
			ARG811			2.210	Hydrogen
			ASP863			2.939	Hydrogen
					GLY732	2.651	Hydrogen
				VAL734		2.762	Pi-Sigma
			ARG849	VAL734		4.834	Alkyl
						4.700	Pi-Alkyl
			LEU852		ALA751	4.542	Pi-Alkyl
						4.693	Pi-Alkyl
5c		-8.7	VAL839			2.355	Hydrogen
				ASN708		2.147	Hydrogen
			TYR835	TYR772		2.363	Hydrogen
			ARG978			2.164	Hydrogen
			ARG985			2.308	Hydrogen
					GLY776	3.601	Hydrogen
			ARG978			4.370	Pi-Cation
					ALA706	5.077	Pi-Alkyl
			ILE872			4.607	Pi-Alkyl
5d		-8.5	VAL839			2.338	Hydrogen
				ASN708		2.116	Hydrogen
			TYR835	TYR772		2.477	Hydrogen
			ARG978			2.125	Hydrogen
			ARG985		GLY776	3.627	Hydrogen
					GLY776	3.685	Hydrogen
			ARG978			3.768	Hydrogen
			ARG978			4.332	Pi-Cation
					ALA706	5.078	Pi-Alkyl
			ILE872			4.660	Pi-Alkyl

Analysis of the targeted 1,4-dihydropyridine derivatives (5a-d) using docking techniques to understand the dynamics of their binding to the HER2 active site revealed important details about the free energy of binding and how the compounds (5a-d) bind and interact with the amino acids in the active site, which contributes to determining the DFG in or DFG out the position of each compound. All compounds (5a-d) were shown to exploit the glycine-rich binding region and important amino acids surrounding the ATP binding site, indicating their ability to inhibit HER2 activity by interfering with the catalytic activity in the C-lobe. The free energy analysis of the binding shows that compounds 5a, 5b, 5c, and 5d have a binding energy of (-8.5, -8.6, -8.7 and -8.5 kcal/mol), respectively, indicating excellent energy compatibility between the compounds and the catalytic site of the protein, and the appearance of the total energy of the compounds' binding to the protein with a negative value indicates stable binding, which enhances the effectiveness of the compounds as a potential inhibitor.

In Figure 5, compound 5a is shown to bind to several important amino acids in the active site of the HER2 receptor. The bonds formed with the main amino acids involved in the binding include two hydrogen bonds with

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ARG849 and MET801 that help stabilize the compound in the binding site and these bonds contribute to the anchoring of the aromatic part of the compound in the hydrophobic pocket in the C-lobe of the HER2 domain. Pi-Sigma with VAL734 , Pi-Anion with ASP845 , Pi-Alkyl with VAL734 , PRO885 , ALA751 and LEU852. These bonds indicate that compound 5a interacts in an integrated manner with the active site of the protein.

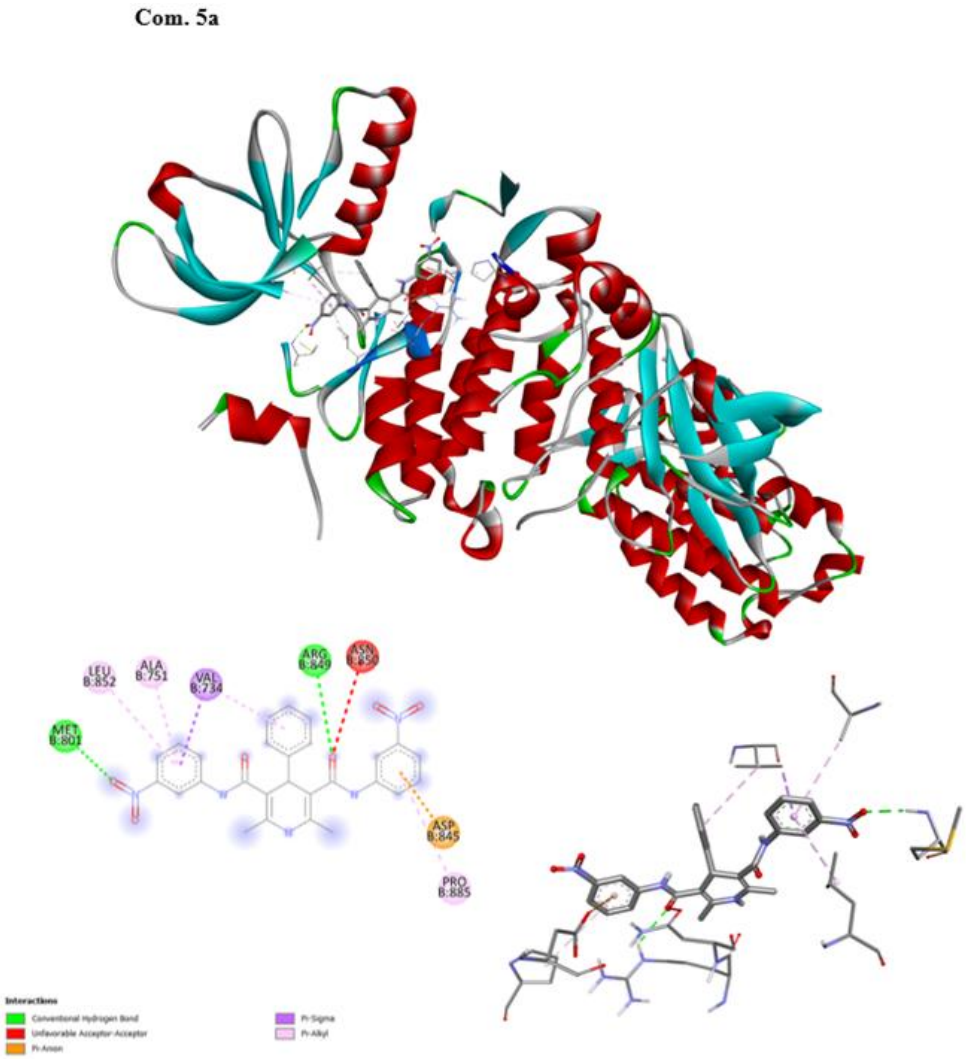


Figure 5. Molecular docking interactions of 2D and 3D interaction of compound 5a at the binding region of HER2 (PDB: 3PP0).

In Figure 6, compound 5b is shown to be bound to several amino acids and form several bonds, and it was found to form four hydrogen bonds with the amino acids LEU726, ASP863, CYS805, and ARG811. The conventional hydrogen bonds with these amino acids form important bonds that contribute to the stability of the compound within the binding site. The formation of a carbon-hydrogen bond with GLY732 helps guide the compound precisely within the binding site and determine its relative direction. Also, the formation of the pi-sigma interaction with VAL734 and pi-alkyl bonds with VAL734, LEU852, ARG849, and ALA751 provides an interaction between the aromatic ring of the compound and the amino acids, which contributes to the stability of the compound within the binding pocket.

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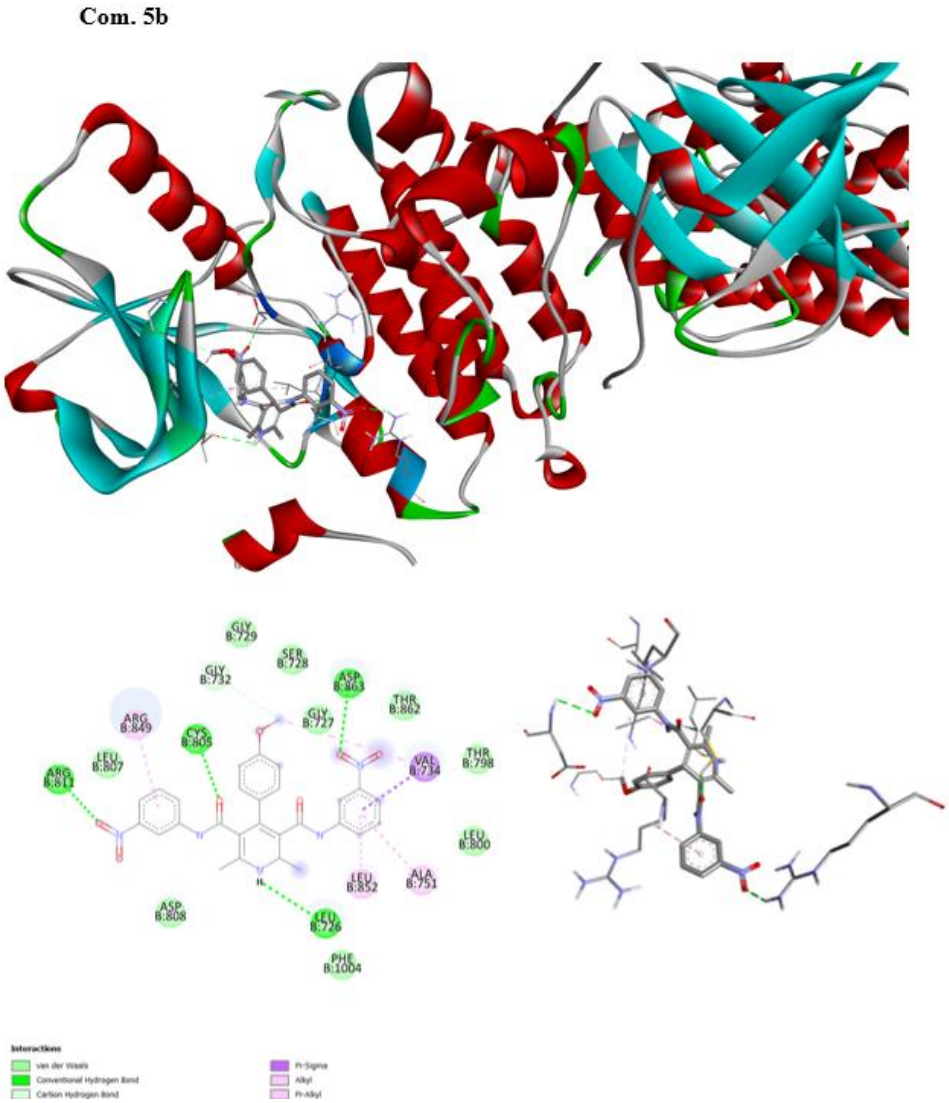


Figure 6. Molecular docking interactions of 2D and 3D interaction of compound 5b at the binding region of HER2 (PDB: 3PP0).

In Figure 7, compound 5c is shown to be bound to several amino acids in the active site of the protein and to form several bonds, which are five conventional hydrogen bonds with the amino acids ASN708, TYR835, VAL839, ARG985, and ARG978. These bonds enhance the stability of the compound in the active site and contribute to determining the correct orientation of the compound. The formation of a carbon-hydrogen bond with the amino acid GLY776. Although carbon-carbon hydrogen bonds are weaker than conventional hydrogen bonds, they contribute to the stability of the interaction between the compound and the protein. Pi-alkyl interactions with ILE872 and ALA706, which contribute to the stability of the compound through non-polar interactions. And the Pi-Cation interaction with ARG978. And unfavorable interactions with TYR772, which may indicate a slight repulsion, but does not necessarily harm the overall stability of the compound in the site.

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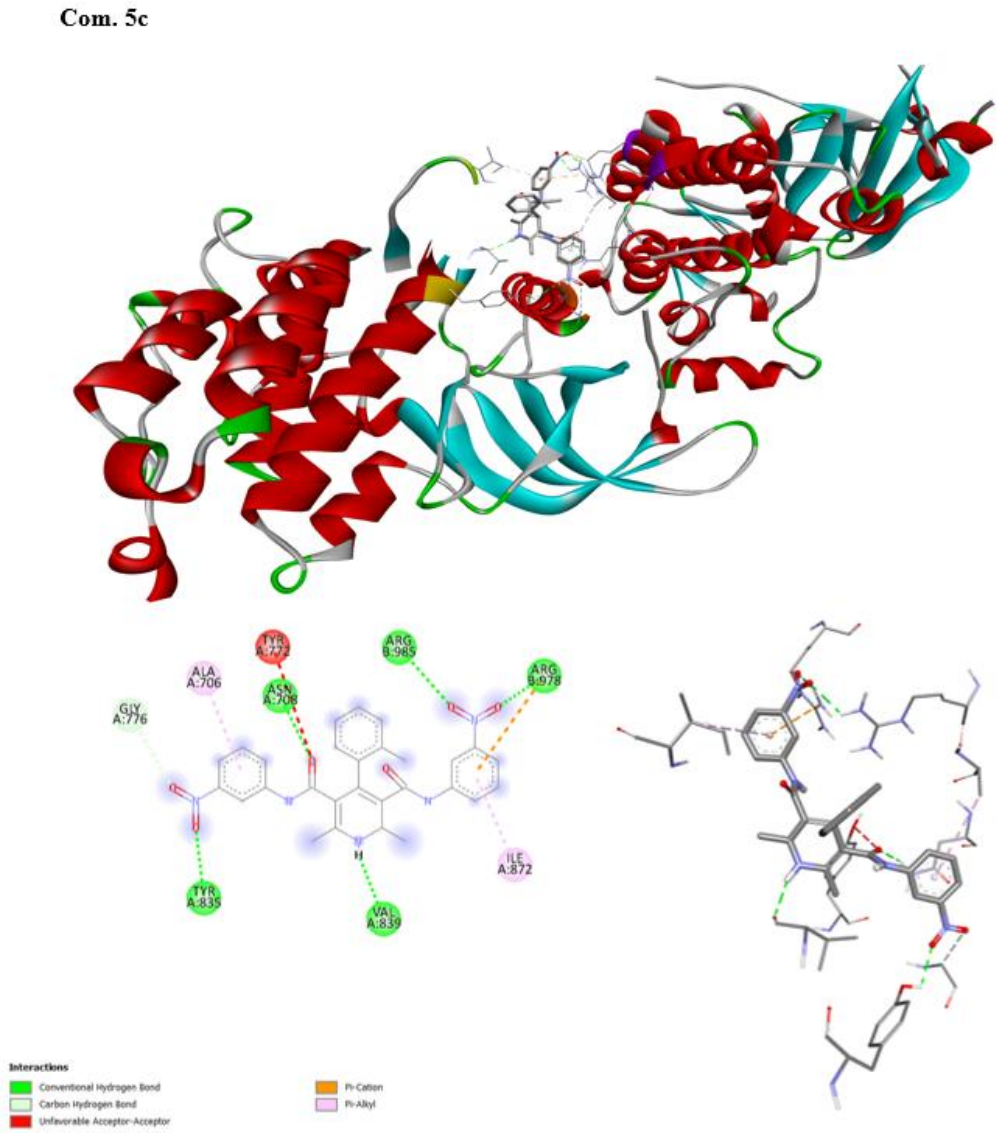


Figure 7. Molecular docking interactions of 2D and 3D interaction of compound 5c at the binding region of HER2 (PDB: 3PP0).

In Figure 8, we notice that the 5d compound is linked to several amino acids in the active site of the protein and forms several bonds, which are four traditional hydrogen bonds with the amino acids ASN708, ARG978, VAL839, and TYR835. These bonds contribute to stabilizing the compound in the active site of the protein and enhance its inhibitory effect. There are also two carbon-hydrogen bonds with GLY776, which enhance the stability of the compound in the C-lobe. There are also Pi-Alkyl bonds with ALA706 and ILE872, and Pi-Cation bonds with ARG978. These bonds add a layer of stability by interacting with the aromatic groups in the compound.

The key turning point in this study is the interpretation of the position of the compounds (5a-d) in the context of DFG. The DFG chain represents a critical location to determine whether the protein is in the DFG-in (active state) or DFG-out (inactive state). The location indicated in the images clearly shows that all compounds (5a-d) are in the DFG-out position. This can be explained by the position of the amino acid phenylalanine (Phe) relative to the active and back pockets, where the phenylalanine (Phe) in the DFG chain appears to be located outside the active pocket, away from the ATP-binding region. This distribution indicates that the protein is in the DFG-out state, which means that the kinase is in an inactive state. The amino acid aspartate is also shifted away from this location, indicating that Asp is out of its traditional position in the active state, preventing catalytic activity, and confirming that the protein is in an inactive state. This discovery is important in developing more effective inhibitors that target the inactive states of proteins such as HER2, as these inhibitors are better able to control the activity of cancer cells, especially in breast cancer. These results could contribute to developing more specific

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inhibitors that target cancer cells in their inactive states, which may reduce the likelihood of developing resistance to treatment.

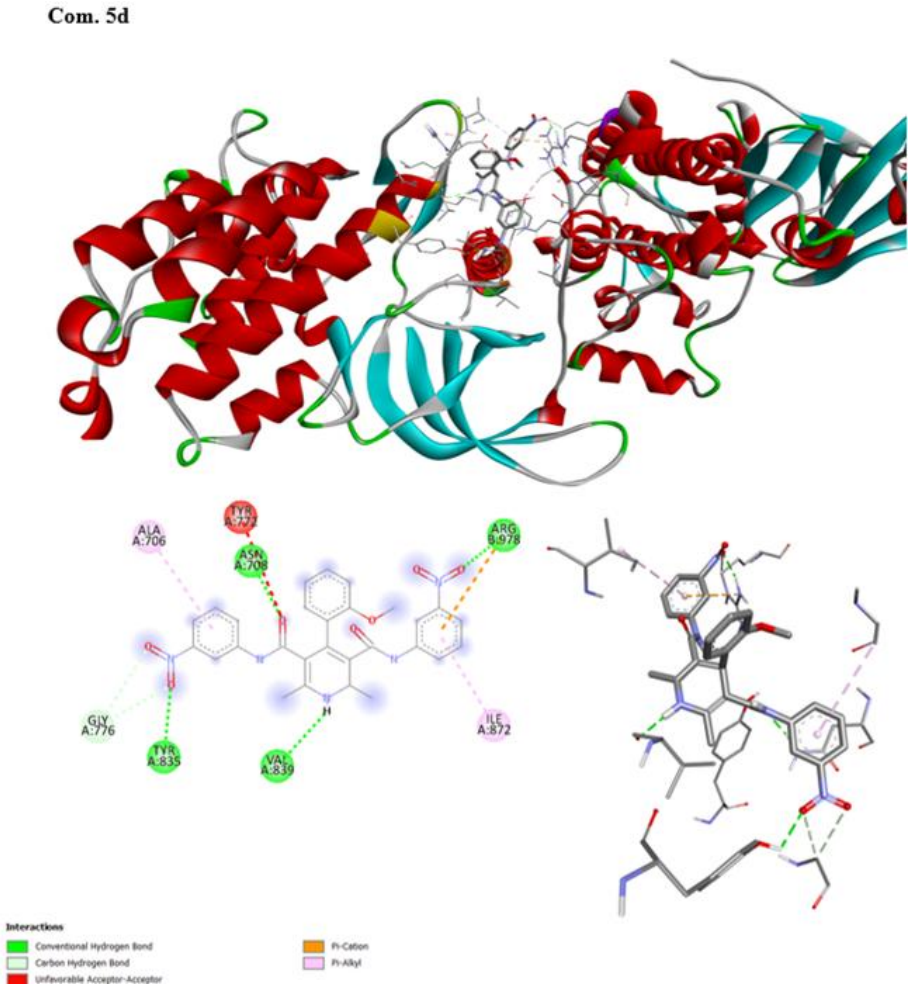


Figure 8. Molecular docking interactions of 2D and 3D interaction of compound 5d at the binding region of HER2 (PDB: 3PP0).

From the above and since the difference in binding energy between the four compounds is very small (only 0.2 kcal/mol), we conclude that all compounds may be similarly effective, but compound 5c may be the best among them due to its free energy sensitivity that indicates a more stable binding to the active site. All four compounds bind stably to the HER2 active site, which enhances their potential as effective HER2 inhibitors. In conclusion, the free energy of binding supports the stability of the compounds to the HER2 active site, with a slight advantage for compound 5c due to its more negative free energy, which may indicate its higher efficiency as an inhibitor. Further testing is recommended to determine the efficacy of 1,4-dihydropyridine derivatives (5a-d) against other cancer cell lines, in addition to MCF-7 and SK-BR-3 cells, to validate the efficacy of these compounds across a wider range of cancer types. Further studies of the molecular mechanisms of action of these derivatives, focusing on analyzing their interaction with HER2 and other proteins associated with cancer cell growth, using advanced techniques such as molecular analysis and molecular dynamics. It is also recommended to evaluate the toxicity of these derivatives (5a-d) on normal cells and ensure their safety before moving to clinical studies. Preclinical studies are recommended to determine the optimal dose and evaluate any potential side effects. Based on the results of this study, it is recommended to design new 1,4-dihydropyridine derivatives with modified chemical structures to improve the bioactivity against cancer cells and increase selectivity for HER2 receptors. Studies are recommended to evaluate the thermal and chemical stability of these derivatives under different conditions to ensure their stability and efficacy during manufacturing, storage, and clinical use. These recommendations can help improve the therapeutic efficacy of derivatives and design new molecules with improved therapeutic properties.

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CONCLUSION

The four synthesized 1,4-dihydropyridine derivatives (5a-d) showed remarkable efficacy against breast cancer cells, with SK-BR-3 cells showing a greater response compared to MCF-7 cells. This suggests that these derivatives may be promising in the development of targeted therapies for HER2-associated breast cancer. SK-BR-3 cells, which express HER2 at high density, were found to be more responsive to 1,4-dihydropyridine derivatives (5a-d) compared to MCF-7 cells, confirming the importance of HER2 as a therapeutic target in breast cancer. Compound 5c emerged as the most potent among the four derivatives, making it a potential candidate for further development as a targeted drug for the treatment of breast cancer, especially in HER2-associated types. Molecular analysis studies provided valuable insights into how these derivatives interact with HER2, enhancing the understanding of their therapeutic potential and promoting the development of novel targeted therapies. Based on these results, it is necessary to continue research to explore the modification of the chemical structure to improve the biological activity, thermal and chemical stability of these derivatives, and to expand the evaluation on other cell lines to ensure the efficacy and safety of these compounds.

CONFLICT OF INTEREST

The author declares that they have no conflicts of interest.

AUTHOR CONTRIBUTION

AYK: Conceptualization, Methodology, Investigation, Formal Analysis, Data Curation, Validation, Visualization, Writing – Original Draft, Writing – Review and Editing. The author confirms that he has read and approved the final version of the manuscript.

DECLARATION OF GENERATIVE AI

No declaration of generative AI use was provided.

REFERENCES

Abdullah, H. T., Al-Bayati, A. M., Saleh, A. N. A., Alsalihi, K. A., and Abed, H. H., 2025. Effect of *Ocimum basilicum* Herbs Extract on Pro-Inflammatory Cytokines in Ethanol-Induced Liver Damage in Rats. *Wiadomości Lekarskie*, 1522–1529. https://doi.org/10.36740/WLek/208443.

Ades, F., Zardavas, D., Bozovic-Spasojevic, I., Pugliano, L., Fumagalli, D., De Azambuja, E., Viale, G., Sotiriou, C., and Piccart, M., 2014. Luminal B Breast Cancer: Molecular Characterization, Clinical Management, and Future Perspectives. *Journal of Clinical Oncology*, 32, 2794–2803. https://doi.org/10.1200/JCO.2013.54.1870.

Aertgeerts, K., Skene, R., Yano, J., Sang, B.-C., Zou, H., Snell, G., Jennings, A., Iwamoto, K., Habuka, N., Hirokawa, A., Ishikawa, T., Tanaka, T., Miki, H., Ohta, Y., and Sogabe, S., 2011. Structural Analysis of the Mechanism of Inhibition and Allosteric Activation of the Kinase Domain of HER2 Protein. *Journal of Biological Chemistry*, 286, 18756–18765. https://doi.org/10.1074/jbc.M110.206193.

Arienti, C., Pignatta, S., and Tesei, A., 2019. Epidermal Growth Factor Receptor Family and Its Role in Gastric Cancer. *Frontiers in Oncology*, 9, 1308. https://doi.org/10.3389/fonc.2019.01308.

Bergin, A. R. T. and Loi, S., 2019. Triple-Negative Breast Cancer: Recent Treatment Advances. *F1000Research*, 8, 1342. https://doi.org/10.12688/f1000research.18888.1.

Bilani, N., Zabor, E. C., Elson, L., Elimimian, E. B., and Nahleh, Z., 2020. Breast Cancer in the United States: A Cross-Sectional Overview. *Journal of Cancer Epidemiology*, 2020, 1–8. https://doi.org/10.1155/2020/6387378.

Desai, B., Sureja, D., Naliapara, Y., Shah, A., and Saxena, A. K., 2001. Synthesis and QSAR Studies of 4-Substituted Phenyl-2,6-Dimethyl-3, 5-Bis-N-(Substituted Phenyl)Carbamoyl-1,4-Dihydropyridines as Potential Antitubercular Agents. *Bioorganic & Medicinal Chemistry*, 9, 1993–1998. https://doi.org/10.1016/S0968-0896(01)00141-9.

Faizan, S., Hani, U., Haider, N., Vadivel, V., and Prashantha Kumar, B. R., 2024. N-Substituted Dihydropyridines as Promising EGFR Tyrosine Kinase Inhibitors against Breast Cancer: Design, Synthesis, Biological Evaluation, Docking, and Molecular Dynamics Simulations. *Results in Chemistry*, 7, 101502. https://doi.org/10.1016/j.rechem.2024.101502.

Gao, J. J. and Swain, S. M., 2018. Luminal a Breast Cancer and Molecular Assays: A Review. *The Oncologist*, 23, 556–565. https://doi.org/10.1634/theoncologist.2017-0535.

Copyright © 2026, Universitas Sebelas Maret, ISSN 1412-4092, e ISSN 2443-4183

Gaudio, A. C., Korolkovas, A., and Takahata, Y., 1994. Quantitative Structure-Activity Relationships for 1,4-Dihydropyridine Calcium Channel Antagonists (*Nifedipine analogues*): A Quantum Chemicalklassical Approach. *Journal of Pharmaceutical Sciences*, 83, 1110–1115. https://doi.org/10.1002/jps.2600830809.

Iatrakis, G. and Zervoudis, S., 2021. Epidemiology of Ductal Carcinoma in Situ. *Chirurgia*, 116, S15. https://doi.org/10.21614/chirurgia.116.5.suppl.S15.

Joshi, H. and Press, M.F., 2018. Molecular Oncology of Breast Cancer. *The Breast*, Elsevier, e285, 282–307.

Kamińska, M., Ciszewski, T., Łopacka-Szatan, K., Miotła, P., and Starosławska, E., 2015. Breast Cancer Risk Factors. *Menopausal Review*, 3, 196–202. https://doi.org/10.5114/pm.2015.54346.

Liu, H., Zhu, S., Xia, G., Huang, Z., Han, W., Li, Z., and Liu, C., 2024. Effects of 1,4-Dihydropyridine Derivatives on Cell Injury and mTOR of HepG2 and 3d-QSAR Study. *Computational Biology and Chemistry*, 109, 108010. https://doi.org/10.1016/j.compbiolchem.2023.108010.

Loibl, S. and Gianni, L., 2017. HER2-Positive Breast Cancer. *The Lancet*, 389, 2415–2429. https://doi.org/10.1016/S0140-6736(16)32417-5.

Mannhold, R., Jablonka, B., Voigt, W., Schönafinger, K., and Schraven, E., 1992. Calcium- and Calmodulin-Antagonism of Elnadipine Derivatives: Comparative Sar. *European Journal of Medicinal Chemistry*, 27, 229–235. https://doi.org/10.1016/0223-5234(92)90006-M.

Mehany, M. M., Hammam, O. A., Selim, A. A., Sayed, G. H., and Anwer, K. E., 2024. Novel Pyridine Bearing Pentose Moiety-Based Anticancer Agents: Design, Synthesis, Radioiodination and Bioassessments. *Scientific Reports*, 14, 2738. https://doi.org/10.1038/s41598-024-53228-4.

Mishra, A. P., Bajpai, A., and Rai, A. K., 2019. 1,4-Dihydropyridine: A Dependable Heterocyclic Ring with the Promising and the Most Anticipable Therapeutic Effects. *Mini-Reviews in Medicinal Chemistry*, 19, 1219–1254. https://doi.org/10.2174/1389557519666190425184749.

Moradipoodeh, B., Jamalan, M., Zeinali, M., Fereidoonnezhad, M., and Mohammadzadeh, G., 2020. Specific Targeting of HER2-Positive Human Breast Carcinoma SK-BR-3 Cells by Amygdaline-ZHER2 Affibody Conjugate. *Molecular Biology Reports*, 47, 7139-7151. https://doi.org/10.1007/s11033-020-05782-z.

Obeagu, E., Abdi, Y., Obeagu, G., Bunu, U. O., P.C, U., and Alum, E., 2023. Biomakers of Breast Cancer: Overview. *International Journal of Current Research in Biological Medicine*, 8, 8–16. https://doi.org/10.22192/ijcrbm.2023.08.01.002.

Oliveira, T. a. S., Silva, J. B. A., Esperandim, T. R., Acésio, N. O., Tavares, D. C., and Crotti, A. E. M., 2024. Anticancer Activity of 4-Aryl-1,4-Dihydropyridines. *Future Pharmacology*, 4, 564–573. https://doi.org/10.3390/futurepharmacol4030031.

Radadiya, A., Khedkar, V., Bavishi, A., Vala, H., Thakrar, S., Bhavsar, D., Shah, A., and Coutinho, E., 2014. Synthesis and 3d-Qsar Study of 1,4-Dihydropyridine Derivatives as MDR Cancer Reverters. *European Journal of Medicinal Chemistry*, 74, 375–387. https://doi.org/10.1016/j.ejmech.2014.01.011.

Rubin, E., Shan, K., Dalal, S., Vu, D., Milillo-Naraine, A., Guaqueta, D., and Ergle, A., 2024. Molecular Targeting of the Human Epidermal Growth Factor Receptor-2 (HER2) Genes across Various Cancers. *International Journal of Molecular Sciences*, 25, 1064. https://doi.org/10.3390/ijms25021064.

Safak, C. and Simsek, R., 2006. Fused 1,4-Dihydropyridines as Potential Calcium Modulatory Compounds. *Mini-Reviews in Medicinal Chemistry*, 6, 747–755. https://doi.org/10.2174/138955706777698606.

Singh, R. P., Kaur, R., and Singh, K., 2016. A Review on Synthesis of Aminoacetanilides. *ScienceIn Publishing*, 4, 111–120.

Sruthi, A. S. V. L., Faizan, S., Vikram, H., Veena, N. G., Susil, A., Harindranath, H., Vasanth Kumar, S., Kumar Shivaraju, V., and Prashantha Kumar, B. R., 2024. A Multifaceted Approach for the Development of Novel Hantzsch 1,4-Dihydropyridines as Anticancer Agents: Rational Design, Parallel Synthesis, Analysis, Cytotoxicity and EGFR/HER2 Inhibition Studies. *Results in Chemistry*, 7, 101413. https://doi.org/10.1016/j.rechem.2024.101413.

Sun, Y.-S., Zhao, Z., Yang, Z.-N., Xu, F., Lu, H.-J., Zhu, Z.-Y., Shi, W., Jiang, J., Yao, P.-P., and Zhu, H.-P., 2017. Risk Factors and Preventions of Breast Cancer. *International Journal of Biological Sciences*, 13, 1387–1397. https://doi.org/10.7150/ijbs.21635.

Taher, I., Hamzah, B., H. Abed, H., and Husseini, M., 2024. Synthesizing Novel Vanillin-Based Di-Schiff Compounds and Evaluating Their Antibacterial Properties Running Title: Novel Vanillin-Based Di-Schiff Compounds and Their Antibacterial Properties. 2, 54–63.

Tasneem, S., Sheikh, K. A., Naematullah, M., Mumtaz Alam, M., Khan, F., Garg, M., Amir, M., Akhter, M., Amin, S., Haque, A., and Shaquiquzzaman, M., 2022. Synthesis, Biological Evaluation and Docking Studies of Methylene Bearing Cyanopyrimidine Derivatives Possessing a Hydrazone Moiety as Potent Lysine

Copyright © 2026, Universitas Sebelas Maret, ISSN 1412-4092, e ISSN 2443-4183

Specific Demethylase-1 (Lsd1) Inhibitors: A Promising Anticancer Agents. *Bioorganic Chemistry*, 126, 105885. https://doi.org/10.1016/j.bioorg.2022.105885.

Vijayan, R. S. K., He, P., Modi, V., Duong-Ly, K. C., Ma, H., Peterson, J. R., Dunbrack, R. L., and Levy, R. M., 2015. Conformational Analysis Ofthe Dfg-out Kinase Motifand Biochemical Profiling of Structurally Validated Type Ii Inhibitors. *Journal of Medicinal Chemistry*, 58, 466–479. https://doi.org/10.1021/jm501603h.

Xie, H., Hu, Q.-Q., Zhang, Y.-L., Qin, X.-T., and Li, L., 2023. Simple and Efficient Synthesis of Diamino Derivatives of Bis-1,2,4-Oxadiazolevia Tandem Staudinger/Aza-Wittig Reaction. *Current Organic Synthesis*, 20, 589–594. https://doi.org/10.2174/1570179420666221006113032.

Zonouz, M. A. and Abedinpour, S., 2024. A Review on the Methods of Preparing 1,4-Dihydropyridine Derivatives. *Communications in Catalysis*, 2. https://doi.org/10.22049/cic.2024.29549.1038.

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