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RESEARCH ARTICLE

Synthesis And Characterization of New Heterocyclic Compounds and Anti-Cancer, Docking Study

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ABSTRACT

Three new heterocyclic compounds, derivatives of hexahydropyrimidine (Py4M), thiazinam (Th4M), and oxazinan (OX4M), were synthesized through the reaction of substituted chalcone with guanidine hydrochloride, thiourea, and urea. Spectroscopic techniques such as FT-IR, mass spectrometry, and^{1}H NMR were used to characterize the synthesized compounds, confirming their proposed structures. The cytotoxicity of all synthesized compounds was evaluated in vitro using the MTT assay after 72 hours against the human breast cancer cell line MCF-7. The results demonstrated good activity of these compounds on MCF-7, particularly Th4M at a high concentration (1000 µg/ml), which showed an inhibition ratio of 76.9%. Three ligand-derived compounds (Py4M, OX4M, Th4M) were docked against MCF-7 breast cancer cells. Th4M exhibited the best binding affinity and potential anticancer activity.

Keywords: chalcone, thiazine, pyrimidine, oxazine. MCF-7, Docking

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1. Introduction

There are different kinds of heterocyclic compounds, such as 5-membered rings with one heteroatom or more than one. Or maybe 6-membered with one or more heteroatoms, or the change in the possession of the atoms makes the compounds have a personality and different applications. The most common heteroatoms, N, O, and S, are typically found in the ring of heterocyclic compounds. These compounds are cyclic structures containing at least two dissimilar heteroatoms in the ring. They are widely distributed in nature and play a crucial role in the lifecycle in various ways. Most sugars and their byproducts, including vitamin C, contain 5-membered (furan) and 6-membered (pyran) rings with one atom of oxygen. The Vit. B groups include heterocyclic compounds with nitrogen; the most well-known case is pyridoxine (vitamin B6), a imitative of pyridine that is primarily involved in the metabolism of amino acids. Changes in the atoms' composition give these compounds distinct properties and applications. In our study, we focused on three types of heterocyclic compounds in poisons: 1,3-pyrimidine, thiazine, and oxazine. These compounds are vital in medicine, serving as antidepressants, antibacterials, antimalarials, antifungals, and more. Additionally, they are promising anticancer agents. Cancer is a highly complex disease that poses significant therapeutic challenges. It may originate in any organ or tissue, with each cancer type being classified according to its primary site of development. Thus, breast cancer continues to be identified as breast cancer even after metastasizing to other parts of the body. [6, 7]. Another investigational system to study hormone-regulated genes is the cell line MCF-7 (human breast cancer), which covers estrogen receptors. Some proteins that are induced in response

to estrogen November is the month of breast cancer awareness and we have scientists all over the world working to find better ways to prevent, screen for, and treat this disease while also hopefully on improving life quality for those who are previously diagnosed.

2. Experimental

2.1. Materials and Methods

IR spectra were measured for the prepared compounds as KBr disks using the Shimadzu model FT-IR-8400S spectrometer at the laboratory of the Chemistry Department at Basrah University. The spectra of ^{1}H-NMR were recorded on a Bruker spectrometer at 500 MHz in DMSO-d6 as solvent. TMS was used as an internal reference, and the mass spectra analysis was performed at Tehran University, Iran.

2.2. Maintenance of cell cultures

MCF-7 cell line depend on the group of Al-Amood and Yaseen (2022) [8]

2.3. Combination Cytotoxicity Assays

The cytotoxic effect was evaluated using an MTT cell viability assay on 96-well plates. MCF-7 cell line was seeded at 1 × 10^4 cells/well. After 24 hours or once a confluent monolayer had formed, cells were exposed to the compound being tested at a concentration of 1000 µg/mL. After 72 hours, cells were treat by type of vehicle, The medium was replaced with 28 µL of a 2 mg/mL MTT solution diluted in the complete medium and ampling incubated at 37 °C for two hours. The remaining crystals in the wells were solubilized by adding 100 µL of DMSO, followed by incubation at 37 °C for fifty minutes with stirring [9]. Absorbance was measured at 260 nm with a microplate reader. Each assay was performed in triplicate. Cytotoxicity percentage: Propagation rate (PR) = B / A × 100, where A mean optical density of each batch of untreated wells; B optical density of treated wells IR = 100 - PR [10]

2.4. Synthesis of chalcone (K)[9]

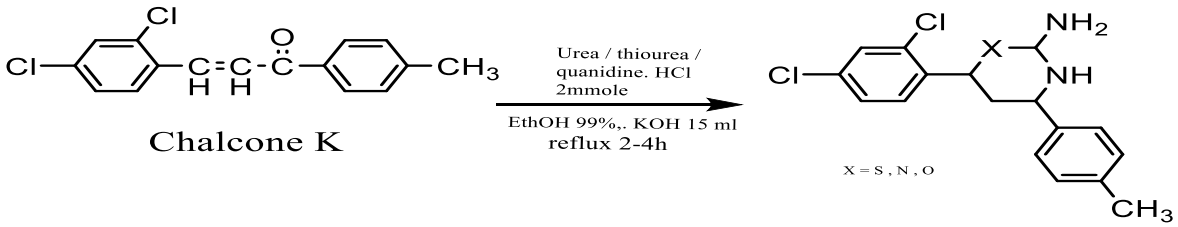
(1-(2,4-dichlorophenyl)-3-(p-tolyl)prop-2-en-1-one)

An aqueous NaOH 10% (10 ml) to a mixture of 2,4-dichlorobenzaldehyde (0.348 g, 2 mmole) and acetophenone (1-(p-tolyl) ethan-1-one) (2 mmole), in ethanol (96%, 15 ml), was added. For two hours, the mix. was stirred at 0 °C. Cold water (100 ml) was added to the mix. and acidified with 5 ml of AcOH. The product formed was recrystallized in abs. ethanol. Toluene: ethyl acetate: n-hexane (3:1:0.5) was used as the eluent solvent. The product spots were visualized by using a UV lamp at 250 nm.

2.5. Synthesis of compounds Thiazin / Oxazin / Pyrimidin.[10,11]

General method

To a mixture of chalcone K (2 mmole) and urea/thiourea/quinidine.HCl in absolute ethanol (15 mL), aqueous KOH 40% (1 mL) was added. Scheme 1. By refluxing for 4 hours, the mix. was heated. Then, it was poured into ice water after being acidified with 3 drops of H2O:HCl (1:1). The product was recrystallized in a mix. of abs. ethanol and water.



Scheme 1

Synthsis of 4-(2,4-dichlorophenyl)-6-(p-tolyl)-5,6-dihydro-2H-1,3-oxazin-2-amine (OX.4M):

A 40% aqueous KOH solution (1 mL) was added to a mix. of chalcone K (2 mmole) and urea (1 mmole) in 15 mL of absolute ethanol. The mixture was heated by refluxed to 4 hours, then it was transferred into ice water after being acidified with three drops of (1:1) H_2O:HCl. The product was recrystallized from a mix. of abs. ethanol and water. Mp: 168-170 °C, Rf: (0.33), % yield: (65), color: brown, FT-IR (NH 3317, Ar-H 3063, -CH2- 3000), 1H-NMR Ar-H (8.31-7.53 7H m), (3.58 2H d), (3.52 1H t), (4.63 1H t), (5.27 1H d) NH2 (1.26 2H s), HN (0.88 1H s), CH3 (2.32 3H s), Mass m/z: 105, 119,147, 322, 336.

Synthesis of 6-(4-methylphenyl)-4-(2,4-dichlorophenyl)-1,3-hexahydropyrimidine-2-amine (Py 4M):

A 40% aqueous KOH solution (1 ml) was added to a mix. of chalcone K (2 mmole) and quinidine hydrochloride (2 mmole) in abs. ethanol (15 ml). Under reflux to 3-4 hours, the mixture was heated, then transferred into ice-cold water after being acidified with 3 drops of (1:1) H2O:HCl. The eluent used was a (3:1) mixture of ethylacetate: normal hexane, and TLC showed one spot. Mp.108-114 ^oC, R_f : (0.21), %yield: (60), color: Brown, FT- IR(NH 3321, Ar-H 3032), -CH_2- 2924, CH_3 2862, ^{1}H-NMR Ar-H (8.31 -7.16 m 7H), (3.68 2H), (4.80, 4.55 d 1H), (5.35 d 1H), (5.55 d 1H), NH2 (1.26 s 2H), HN (0.88 s 1H), CH_3 (2.56 s 3H).Mass m/z: 105, 186,147, 314, 334.

Synthesis of 4-(2,4-dichlorophenyl)-6-(p-tolyl)-5,6-dihydro-2H-1,3-thiazin-2-amine (Th4M):

One milliliter of an aqueous solution of 40% KOH was added to a mixture of chalcone, K (2 mmole), and thiourea (1 mmole) in 15 mL of abs. ethanol. For 5 hours, the mixture was heated under reflux, and then transferred into ice water after being acidified with 3 drops of (1:1) H2O:HCl. A mixture of abs. ethanol and water were used to recrystallize the product. Mp. 78-82 °C, R_f :(0.6), % yield: (70), color: brow n, FT-IR (NH 3410, Ar-H 3080, -CH2- 2939, CH3 2820). ^{1}H-NMR Ar-H (7.89 -7.20 m 7H), (3.46 d 2H), (4.57- 4.31 m 1H), (5.32-5.34 m 1H), (5.45-5.43 m 1H), NH2 (1.26 s 2H), HN (0.86 s 1H), CH_3 (2.36 s 3H). Mass m/z: 119, 105,149, 347, 352.

2.6. Molecular docking approach

By examining the relationships between alternative docking positions and ligand binding scores, while ensuring optimal docking parameters, the crystal structure analysis of MCF-7 cells reveals their significant role in immune regulation. These cells contribute to immune suppression and facilitate tumor progression, positioning them as promising therapeutic targets for enhancing immune surveillance and advancing cancer treatment strategies. 6FOF codes linked to the crystal construction of a crystallized modified form of h-Gal3: Gal-3[NTS/VII-IX], the protein active site 1 [12]. From the Protein Data Bank homepage (https://www.rcsb.org/structure/6FOF), the structure of the crystal was obtained at a resolution of 2.20 Å, which is considered good quality for docking studies [13]. Molecular docking performance was evaluated using two widely accepted parameters: a root mean square deviation (RMSD) value close to 2.0 Å and a binding energy of ≤ -7.0 kcal/mol, both of which indicate reliable ligand–receptor interactions. The subsequent energy refinement and supported model construction (using Amber 10) utilized the force of Extended Hückel Theory (EHT) [14].

3. Results and Discussion

3.1. Results of anti-cancer activity

The prepared compounds showed cytotoxic effects on the MCF-7 cell line, at variable inhibition percentages, as indicated in Table 1.

Table 1. The inhibition ratio of the MCF-7 cell line.

Sample	Inhibition % of MCF-7
Th.4M	76.9
OX.4M	74
PY.4M	69.9

The results indicate the effects of three different compounds on MCF-7 cells (breast cancer cells).

The compound containing (Sulfur) thiazinan (Th.4M) exhibits an inhibition percentage of 76.9%. This compound appears to have a good effect in inhibiting cancer cell growth. When a compound containing (Oxygen) oxazinan (OX.4M), the inhibition percentage is 75%. This compound also has a good effect, but slightly less than the sulfur-containing compound. Compound containing Nitrogen (Py.4M). The inhibition percentage is 69.9%. This compound has an effect, but it's less potent than the other two compounds.

Overall, the sulfur-containing compound seems to be the greatest effective in inhibiting MCF-7 cell growth, followed by the oxygen-containing compound, and then the nitrogen-containing compound.

3.2. Molecular docking MCF 7 Cell line

The molecular docking results, as presented in Table 2 and Figures 1-3, between three compounds (Py.4M, OX.4M, and Th.4M) and the breast cancer cell line receptor MCF-7 (PDB ID: 6FOF), revealed significant differences in their binding affinities and interaction patterns within the active site.[16,17] The compound Th.4M exhibited the strongest binding attraction, with a docking score of -7.8436 kcal/mol, tailed by OX.4M at -7.5283 kcal/mol, and Py.4M with the lowest affinity at -6.8374 kcal/mol. These values suggest that Th.4M forms the most stable complex with the active site of the receptor, potentially enhancing its cytotoxic effect against MCF-7 cells. OX.4M, on the other hand, demonstrated a broader interaction profile with seven distinct contacts (hydrogen donors, acceptors, and π–H), indicating a high binding versatility but possibly lower selectivity, which could have implications for off-target effects. Py4M showed fewer interactions, primarily involving HIS158 and ARG144, which correlates with its weaker overall binding score. Th4M appears to be the most promising candidate among the tested ligands for targeting immunomodulatory elements of the MCF-7 cell receptor. It holds potential as a lead compound in the development of therapeutics aimed at reactivating suppressed immune responses in breast cancer [18,19].

Table 2: Molecular Docking Results of Py4M, OX4M, and Th4M Ligands with MCF-7 Cell Line 6FOF Receptor.

			Bonds between (ID: 6FOF) with Active Site Residues						
Compd.	**Score (kcal/mol)**	**RMSD (Å)**	**Compd. Atoms**	**Receptor Atoms**	**Receptor Residues**	**Interaction**	**d (Å)**	**E (kcal/mol)**	**Total E (kcal/mol)**
Py4M–6FOF	-6.8374	2.177	N 37 N 37 C 20	NE2 NE2 6-ring	HIS 158 (B) ARG 144 (B) TRP 181 (B)	H-donor H-acceptor H-π	3.09 3.37 3.80	-0.5 -2.2 -0.5	-30.5962
OX4M–6FOF	-7.5283	2.374	N 22 C 24 N 36 N 22 N 36 N 36 6-ring	OD1 NE2 OD1 NH2 NE NH2 CZ2	ASN 179 (A) HIS 158 (B) ASN 160 (B) ARG 144 (B) ARG 144 (B) ARG 144 (B) TRP 181 (B)	H-donor H-donor H-donor H-acceptor H-acceptor H-acceptor pi-H	3.02 3.19 2.90 3.27 3.26 3.05 3.70	-0.6 -0.6 -0.8 -1.8 -1.2 -4.3 -0.5	-37.119

Th4M –6FOF	-7.8436	2.345	S 26 S 26 N 36 6-ring	OD1 CB NH2 5-ring	ASN 160 (A) ASN 179 (B) ARG 144 (A) TRP 181 (A)	H-donor H-acceptor H-acceptor pi-pi	3.91 3.67 3.15 3.58	-0.7 -0.8 -0.7 -1.9	-33.206

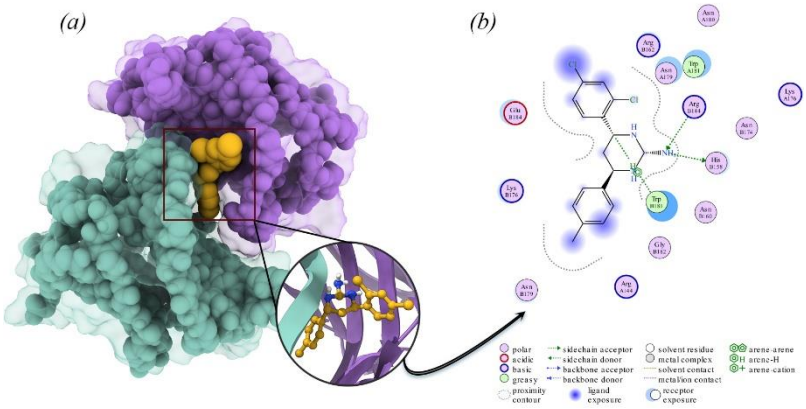


Figure 1. MD(a) the binding of the Py4M and the MCF-7 Cell line 6FOF Receptor. (b) 3D illustration.

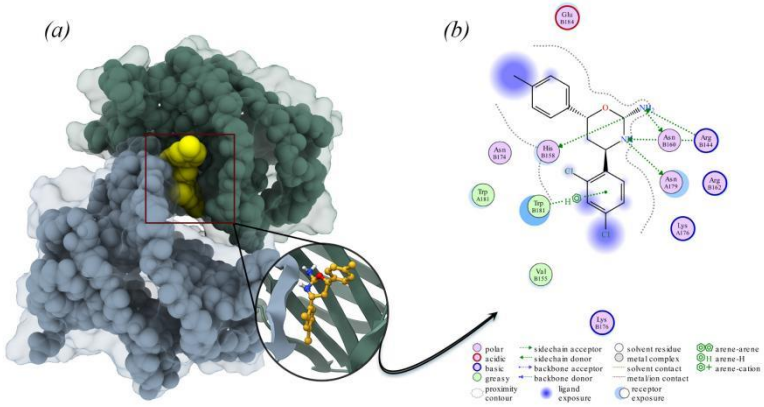


Figure 2. MD (a) Binding of the OX4M and the MCF-7 Cell line 6FOF Receptor. (b) 3D illustration.

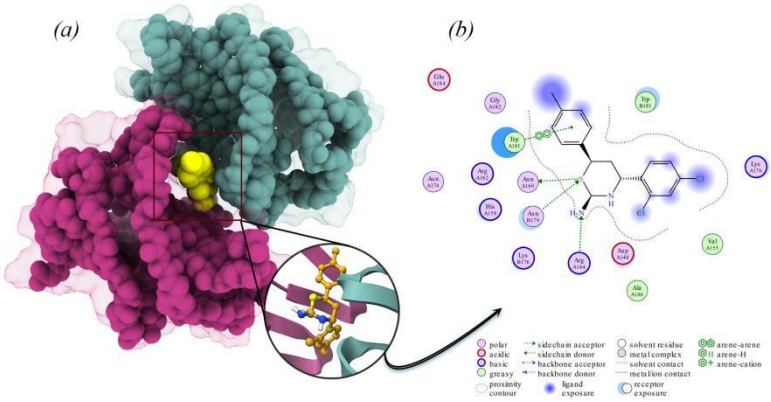


Figure 3. MD (a) Binding of the Th4M and the MCF-7 Cell line 6FOF Receptor. (b) 3D representation.

4. Conclusion

Th4M demonstrated the strongest and most specific binding to MCF-7 receptors, suggesting its promise as a candidate for breast cancer immunotherapy.

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