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Pyrazole derivatives affinity to Estrogen receptor Alpha for breast cancer treatment evaluation using molecular docking

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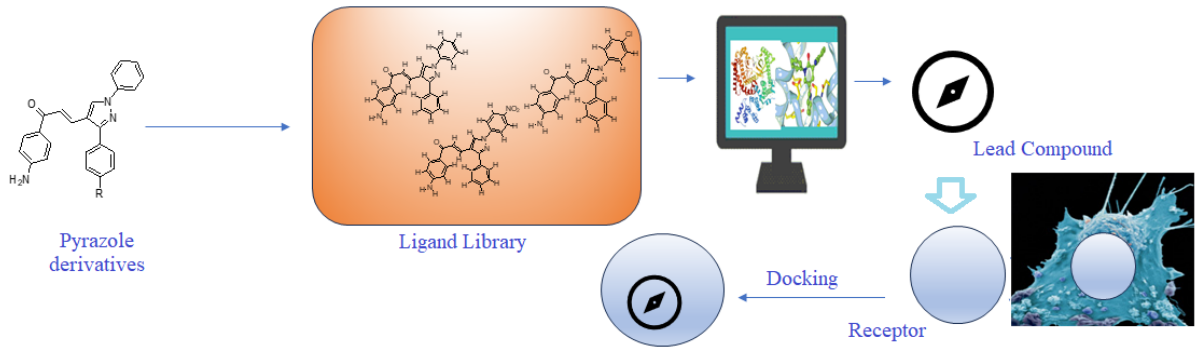
Article

ABSTRACT



After lung cancer, which is the most common cancer overall, breast cancer is the second most common cancer in women. Breast cancer is primarily brought on by 17-estradiol, a key estrogen involved in cell initiation and proliferation and whose effects are controlled by estrogen receptors. The first line of treatment for patients with ER+ breast cancer is endocrine therapy, which comprises the classes of selective estrogen receptor modulators (SERMs), selective estrogen receptor down-regulators (SERDs), aromatase inhibitors (AI), and sulfatase inhibitors. Pyrazole, a useful pharmacophore, exhibits a wide range of biological functions, including anti-cancer activity. In order to boost the number of hits from a high throughput screening, docking research was done on pyrazole derivatives as an estrogen receptor alpha inhibitor. The findings of molecular docking showed that selected Pyrazole derivatives (compound with substituent 4-methyl (2), 4-hydroxy (3), 4-chloro (4), 4-bromo (5), 4-fluoro (6), 4-amino (8), 3-methyl (9), 3-hydroxy (10), 3-chloro (11), 3-bromo (12), and 3-fluoro (13) were found to be potent when compared to standard 4-hydroxy Tamoxifen, well known marketed drug against breast cancer. The lead optimization towards estrogen receptor alpha in this study potentiates the further development of pyrazole derivatives for breast cancer treatment.

Keywords: Breast cancer, Estrogen Receptor Alpha, Docking, Pyrazole, Molegro Virtual Docker

INTRODUCTION

Cancer is considered as the most troublesome disease worldwide. It causes over ten million deaths annually.[1] Breast cancer, which is a malignant tumor developed in breast tissues including lobules, ducts, and the cells around them is one of the most prevalent cancers.[2] According to the WHO Report 2020, 68500 deaths occur from this disease.[3] A higher risk of breast cancer is associated with hormonal anomalies, notably those affecting the hormone estrogen.[4,5] Breast cancer is primarily brought on by 17-estradiol, a key estrogen involved in cell initiation and proliferation and whose effects are controlled by estrogen receptors. Clinical and experimental research has shown that the ER subtype is primarily responsible for the majority of cases of breast cancer.[6–8] In addition to assisting with the development of reproductive organs, nuclear receptors known as estrogen receptors (ER) help in growth, reduce cholesterol levels, maintain bone homeostasis, and protect the cardiovascular system. The main locations where ER is expressed include the uterus, ovaries, testis, breast, and epididymis.

Since ER is present in lymphocytes, the prostate, the lung, the skin, and the bone, it is widely distributed throughout the whole body.[9] The first line of treatment for individuals with ER+ breast cancer is endocrine therapy (ET), which comprises the classes of selective estrogen receptor modulators (SERMs), selective estrogen receptor down-regulators (SERDs), aromatase inhibitors (AI), and sulfatase inhibitors (SI).[10] There are several

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treatments for breast cancer, including Ruxolitinib and Crizotinib, however, they all have negative effects (**Figure1**).[11,12] Therefore, there is a need to create innovative treatments for breast cancer. Pyrazole has a number of biological properties that are widely recognised, including antibacterial,[12] anti-inflammatory,[13,14] anti-cancer,[14] antiviral,[15] antidepressant,[16] and anti-HIV properties.[17] In the present article, we describe various newly designed pyrazole derivatives and their potential to link with Erα-positive sites which was obtained from the Protein Data Bank by a technique known as Molecular Docking. It is one of the most frequently used methods in structure-based drug design, due to its ability to predict the preferred orientation of small molecule ligands to the appropriate target binding site. Delineation of the binding behaviour plays an important role in the rational design of drugs as well as in elucidating fundamental biochemical processes.[18] This can be used to develop more potent, selective, and efficient drug candidates.[19,20] To find potent drug candidates, docking in combination with a scoring function can be used to *evaluate large databases.*[21]

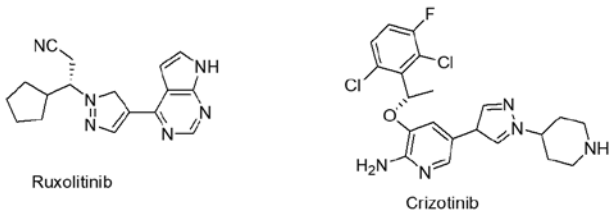
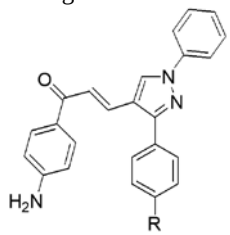


Figure 1. Chemical structures of pyrazole containing anticancer drugs

MATERIALS AND METHODOLOGY

Molecular docking is the process that involves placing molecules in appropriate configurations to interact with receptors. Docking studies have been performed with a group of pyrazole derivatives using Molegro virtual docker 6.0[22] on estrogen receptor alpha (PDB ID 3ERT) accessed from a protein data bank.[23] Molegro Virtual docker provides higher accuracy and enables the user to easily set up and perform docking runs. The basic structure of analogues is shown in **Table 1**.



Compd no.	R	Compd no.	R
1	H	**12**	3-Br
2	4-CH_3	**13**	3-F
3	4-OH	**14**	3-NO_2
4	4-Cl	**15**	3-NH_2
5	4-Br	**16**	2-CH_3
6	4-F	**17**	2-OH
7	4-NO_2	**18**	2-Cl
8	4-NH_2	**19**	2-Br
9.	3-CH_3	**20**	2-F
10.	3-OH	**21**	2-NO_2
11	3-Cl	**22**	2-NH_2

Ligand preparation

CS Chem office 8.0 was used for the sketching of molecules with the assistance of drawing tools of Chem Draw. The sketched 2D structures were remodeled into 3D structures using Marvin sketch. The 3D structures were then subjected to energy minimization mistreatment molecular mechanics.

Protein Preparation

Protein (PDB code: 3ERT) was downloaded from the protein data Bank. All necessary bonds, bond orders, hybridizations, gas atoms and charges were allotted. Water molecules, unnecessary bonds were deleted. The receptor location was then covered with an electrostatic surface. To find the potential binding locations, a grid-based cavity prediction method was built.

Docking All of the ligands optimized conformers were added to the docking wizard's workspace to begin docking. The search algorithms were previously running ten times. In this context, the term "runs" refers to the number of docking simulations that were done for each docked ligand, with each simulation producing a single result, such as a pose. The choice of the ligands from the docking wizard was done on the basis of the Mol Dock score and hydrogen bond interaction.[24–32]

The Moldock scoring function (Mol Dock Score), Escore is defined by the following energy terms:

$$Escore = Einter + Eintra \quad \text{----------1}$$

$$Einter = \sum i=ligand \sum j=protein [EPLP (rij) + 332.0 \frac{qiqj}{4r^2ij} \text{-------2}$$

$$Eintra = \sum i=ligand \sum j=protein [EPLP (rij)] + \sum \text{flexible bond A } [1 - \cos (m\Theta - \Theta\circ)] + Eclash \quad \text{----3}$$

Where E intra is the inter energy of the ligand; E inter is the ligand–protein interaction energy.

Equation 1 represents the total binding affinity (*E*score), the term *E*inter refers to the ligand and receptor energy interaction, and *E*intra shows the ligand internal energy. **Eqs. 2** and **3** are also used to compute the Einter and Eintra

RESULT

The most important feature of docking is the logical interaction of the ligand with the putative-binding site of the enzyme. We studied the docking of the unknown 22 compounds using PDB(3ERT) with estrogen receptor alpha inhibitors. Among these 22 compounds, 8 compounds (2,3,4, 5, 6, 8,9, 10, 11, 12, 13) (**Table 2**) were found to be potent having a good moldock score as compared to the standard 4-hydroxy tamoxifen (**Figure 2**) having a moldock score is -139.588 which is comparatively lower than the unknown compounds. The most potent compound is 5 (Moldock score -146.007) (**Figure 3**). All poses of novel derivatives and 4-hydroxy tamoxifen lie in same cavity 2 (**Figure 4, 5**). So, these compounds can be used for further designing and also potent of them envisaged for their *in vitro* and *in vivo* activity.

Table 2. Ligand–receptor interaction data of pyrazole using molegro virtual docker software:

Comp. No,	Mol Dock Score	No. of H-bond interactions	Ligand Atom	Protein residue	Distance Annotation(Å)
4-hydroxy tamoxifen	-139.588	2	O of OH O of OH	N of Arg 394 O of Glu 353	2.79 2.64
1	-133.194	1	=O of C=O	O of Thr 347	3.00
2	-145.014	1	=O of C=O	O of Thr 347	3.14
3	-145.773	1	O of OH =O of C=O	N of His 524 O of Thr 347	3.11 3.11
4	-144.727	1	=O of C=O	O of Thr 347	3.11
5	-146.007	1	=O of C=O	O of Thr 347	3.17
6	-145.349	1	=O of C=O	O of Thr 347	3.10
7	-103.102	2	N of NO_2 O of NO_2	N of Arg 394 N of Arg 394	3.02 2.59
8	-143.167	2	=O of C=O N of NH_2	O of Thr 347 N of His 524	3.16 3.09
9	-142.835	2	=O of C=O	O of Thr 347	3.12
10	-140.05	2	O of OH =O of C=O	N of His 524 O of Thr 347	3.10 3.11
11	-141.70	2	=O of C=O	O of Thr 347	3.06
12	-140.429	1	N of NH_2	O of Thr 347	3.09
13	-141.95	1	=O of C=O	O of Thr 347	3.10
14	-124.395		N-1 of Pyrazole N-2 of Pyrazole	N of Arg 394 N of Arg 394	3.02 3.16
15	-133.788	3	O of C=O N of NH_2 N of NH_2	O of Thr 347 N of His 524 O of Asp 351	2.80 3.51 3.20
16.	-136.209	1	=O of C=O	O of Thr 347	3.10
17.	-104.177	0	-	-	-
18	-135.107	1	=O of C=O	O of Thr 347	3.10
19	-136.838	1	=O of C=O	O of Thr 347	3.10
20	-136.103	1	=O of C=O	O of Thr 347	3.10
21	-95.081	0	-	-	-
22	-119.52	1	N of NH_2 N of NH_2	O of Glu 353 O of Leu 387	2.94 2.52

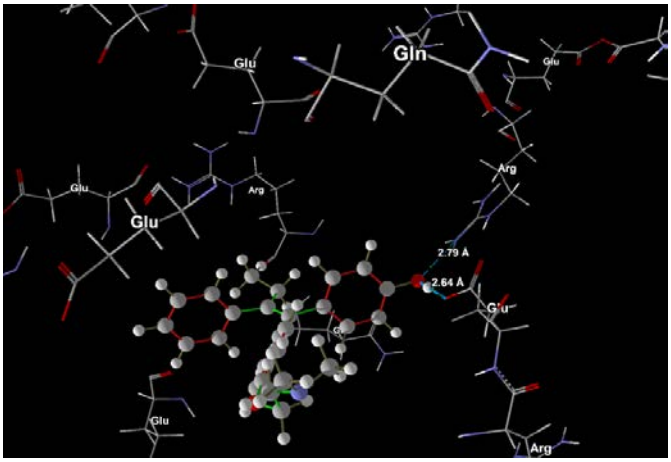


Figure 2. Docking of reference 4-hydroxy tamoxifen into 3ERT

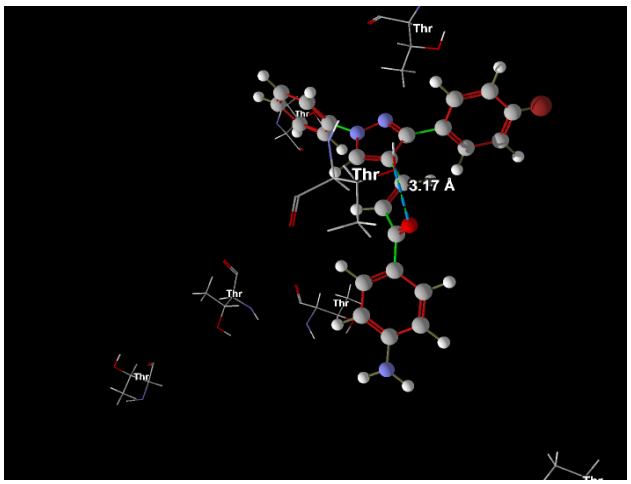


Figure 3. Binding of compound 5 (Max. Mol Dock Score= -146.007) into Estrogen receptor alpha (PDB: 3ERT)

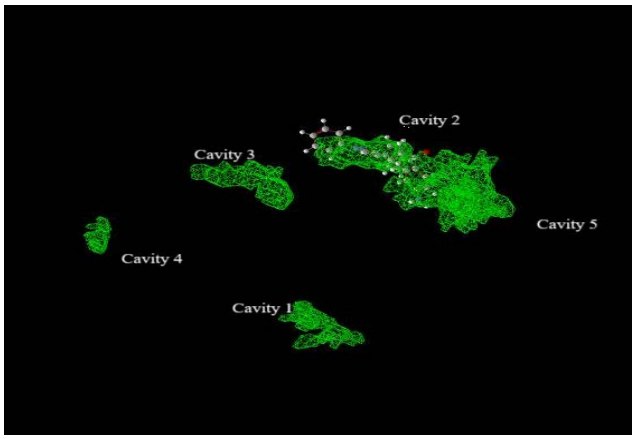


Figure 4. Binding of reference 4-hydroxy tamoxifen into Cavity 2

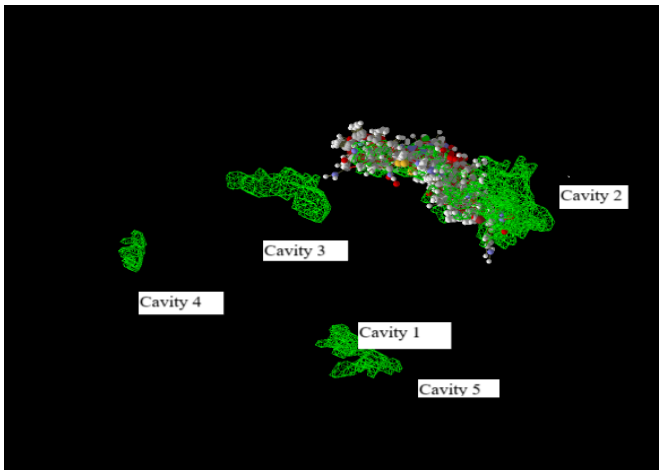


Figure 5. Binding of all poses of novel designed compounds into Cavity 2

Discussion

Peyrot *et al.* (1992) synthesized (E)-3-(4-(dimethylamino) phenyl)-1-(2,5-dimethoxyphenyl)-2-methylprop-2-en-1-one [33] as anti-mitotic agents with rapid and reversible binding to the colchicine-binding site for inhibiting its assembly to

microtubules. Kamal *et al.* (2013) [34] designed and synthesized a novel scaffold (Z)-3-((3-phenyl-1H-pyrazol-5-yl) methylene) indolin-2-one (E), which has significant polymerization inhibitory activity. Stauffer *et al.* investigated the effect of substituent patterns on ER binding affinity and potency as an ERα-selective agonist, by preparing a number of tetrasubstituted pyrazole analogues with defined variations at certain substituent positions. Analysis of their binding affinity pattern shows that a C (4)-propyl substituent is optimal and that a *p*-hydroxyl group on the N(1)-phenyl group also enhances affinity and selectivity for Erα.[35] In the present study, we designed 30 novel pyrazole-derivatives and found some of them are effectively active against estrogen receptor alpha(2,3,4, 5, 6, 8,9, 10, 11, 12,13). Hence, it was concluded that 3-(4-substituted phenyl pyrazole) derivatives are more effective as compared to 3-(3- 3-substituted phenyl pyrazole) and 3-(2-substituted phenyl pyrazole).

CONCLUSION

Docking studies have helped us to know about the binding modes of the pyrazole to elicit their estrogen receptor alpha inhibitory activity. From our study, it was concluded that compounds 2,3,4, 5, 6, 8,9, 10, 11, 12, 13) were found to be potent having a good moldock score as compared to the standard 4-hydroxy tamoxifen having a moldock score was -139.588 which is comparatively lower than the unknown compounds. Compound 5 was found to be the most potent with a Moldock score -146.007. Moreover, it was concluded that 3-(4-substituted phenyl pyrazole) derivatives are more effective as compared to 3-(3- 3-substituted phenyl pyrazole) and 3-(2-substituted phenyl pyrazole). These investigations were found to be very helpful during the synthesis of some selected compounds, which were more potent and selective estrogen receptor alpha inhibitors. These investigations are very helpful in understanding the relationship between drugs and receptors. Moreover, it was also proved from the above discussion that the geometry of the receptor plays a very important role in defining drug action.

FUTURE PERSPECTIVES

The current study can be used further for the development of highly active and potent pyrazole derivatives against breast cancer. Our *in- silico* approach has paved the path for shifting our current research to the synthesis of these compounds and further *in vitro* assays.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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