IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Design of Imidazole-Based Derivatives as Potential Candidates Used to treat Breast Cancer

Solo Lorin[1], M. Arockia doss[1*], K. Raja[2]

[1]Department of Chemistry, St. Joseph University, Nagaland-797115, India.
[2]Department of Biological and Chemical Sciences, School of Liberal Arts and Sciences, Mohan Babu University (Erstwhile Sree Vidyanikethan Engineering College), Tirupati-517102, Andhra Pradesh

Abstract

Tetra-substituted imidazole drugs were designed as potential inhibitors against Estrogen receptor α (ERα) which is a key target for breast cancer therapy. The ADME properties of the designed drugs were analysed using Lipinski's rule of five. Molecular operating environment (MOE), an integrated Computer-Aided Molecular Design Platform, was used to perform the docking study and the analysis of the protein-ligand interaction. The designed drugs displayed a binding energy ranging from -7.15 to -8.53 kcal/mol. Tamoxifen was used as a reference inhibitor against Estrogen receptor α. Among the 13 designed drugs, the 6-(6-(1H-pyrazol-1-yl)pyridin-3-yl)-N-(3,4-dimethoxyphenyl)imidazo[1,2-a]pyridine-2- carboxamide (**10**) fulfills the Lipinski's rule of five and showed good binding interaction with the target receptor and can become a potential alternative to the existing ERα inhibitors.

Keywords: Imidazole; MOE ; Docking; ERα inhibitor; Anticancer activity

1. Introduction

Around the world, there will be 19.3 million new cancer cases and roughly 10.0 million cancer deaths occurred by 2020. It is excluding of nonmelanoma skin cancer[1]. Female breast cancer was surpassed lung cancer as the most commonly diagnosed cancer, with an expected 2.3 million new cases (11.7%) and 6.9% deaths annually. The worldwide cancer burden is expected to be 28.4 million patients in 2040, an expansion of 47% from 2020[1,2], Estrogen signaling is fundamental in the onset and development of human breast cancer. Throughout the course of recent years, extensive efforts were made to comprehend the basic mechanisms of this important signal pathway in human breast cancer, which was simplified the development of anti-estrogen therapy, the first-line treatment for human cancer[3–5]. Estrogen receptor (ERα) is a member of the super family of nuclear receptors of transcription factors that regulate cell proliferation, differentiation, and homeostasis in various tissues. Tamoxifen is a matching anti-estrogen treatment for breast cancer. However, it is associated with an increased side effect, including ovarian cancer, stroke, and pulmonary embolism [6–8].The Imidazoles and benzimidazoles are advantaged heterocyclic bioactive compounds utilized effectively in the clinical act of numerous infections[9–12]. The biological activity of a compound is interaction results between the small-molecule ligand towards the macromolecular receptor. It can be discovered using *in silico* methods and compared to the experimental methods. The molecular docking scoring operations can be used to

predict bioactive poses of compounds and the calculated binding interactions are ranking with experimental assessment of prioritize compounds. These structure-based drug design is one of the useful tools in the place of drug discovery research[13,14]. The docking reports can be used to exploration for bioactive molecules and support clinical chemistry results during the drug discovery process[15]. This pharmacophore modeling study involves the evaluation of the key pharmacophore interactions features of imidazole derivatives and Estrogen Receptor α (ERα).

2. Materials and methods

2.1. Preparation of ligand and protein

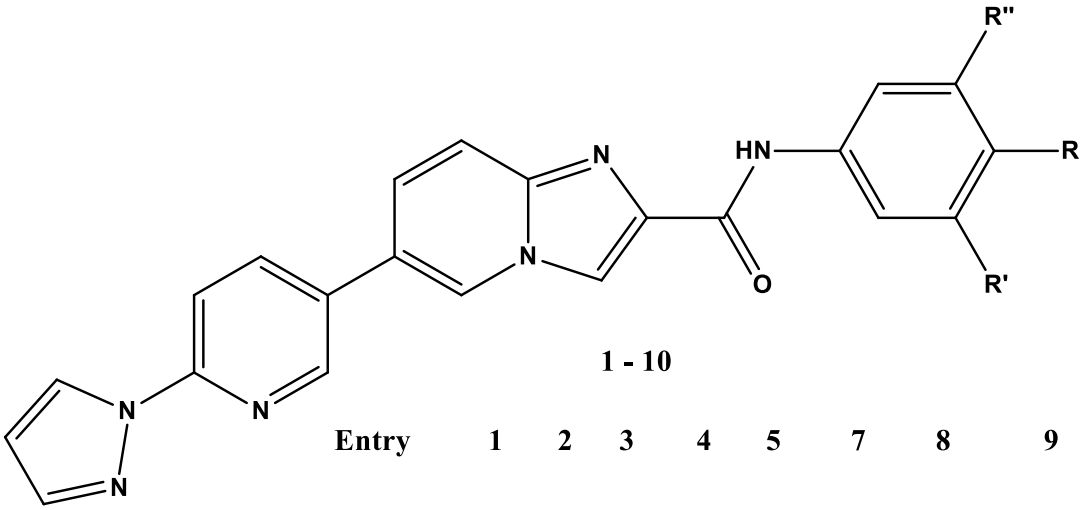
In the present study, 13 derivatives of imidazole (**Fig.1**), which were chosen from the literature[16]. The selected structures were drawn using chem draw software. Then its mol. file and smiles were extracted. Its physiochemical properties were noted. The 3D-structures were drawn using chem draw software. Each ligand in SDF format was entered into the database after protonation and energy minimization with Amber12: EHT Force field. The crystal structure of the ERα (PDB code: 3ERT) with the 4-hydroxytamoxifen was retrieved from the Protein Data Bank (http://www.rcsb.org/pdb/). The crystal structure of the ERα is displayed in **Fig. 2**. The protein structure was prepared using MOE 09 docking tools[17]. The removal of water molecules, structure correction, and 3D protonation was done. The energy minimization was performed using Amber12:EHT Forcefield.

2.2.Molecular Docking

The docking analysis of ERα Receptor with imidazole derivatives was carried out by MOE 09 docking tool. Tamoxifen has been used as a standard drug. The 5 finest docked positions were created by applying a scoring job London dG and using induce-fit model. The Discovery studio visualizer was used to analysis the report.

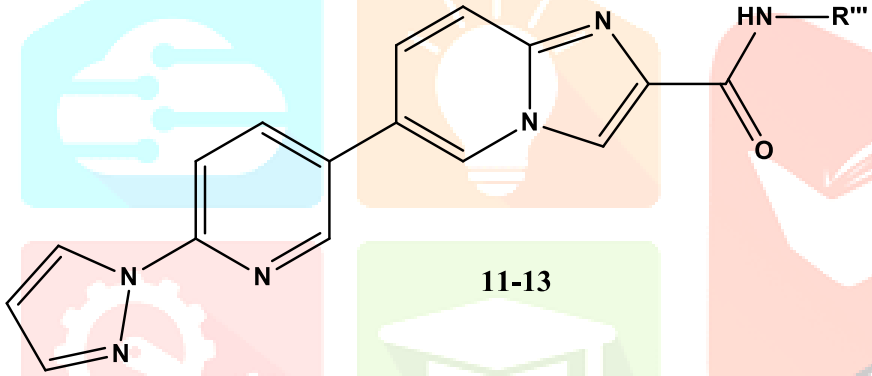
2.3. Lipinski rule of five of the ligands

Using the SWISS-ADME server (http://www.swissadme.ch/index.php) molecular properties and drug likeness of the compounds was examined on the basis of "Lipinski's Rule of Five"[18]. The SDF structures of the compounds were fed into the SWISS-ADME server and were converted into SMILES format which were then screened. The screened vales are collected in **Table 1**.



1 - 10

Entry	1	2	3	4	5	7	8	9	10
R	H	F	Cl	Br	CH_3	CN	OH	OCH_3	OCH_3
R'	H	H	H	H	H	H	H	H	OCH_3
R''	H	H	H	H	H	H	H	H	H



11-13

Entry	R'''
11	Pyridine
12	Pyrrole
13	Thiophene

Fig.1 Imidazole derivatives **1-13**

Fig. 2 Crystal structure of the Human Estrogen Receptor

3. Results and Discussion

The structure of the designed drugs was based on synthesized compounds that displayed anti-cancer properties against breast, lung and prostate cancer cell lines. Ligands **1-10** were designed by forming various derivatives of the template, which was achieved by replacing various substituents at the aromatic rings of N-phenylacetamide moiety. Ligands **11-13** were designed by replacing the aromatic rings of the N-phenylacetamide moiety with Pyridine, Pyrrole and Thiophene respectively. All the substituents used in the designing, i.e. F, Cl, Br, CH_3, CN, OH, OCH_3, pyridine, pyrrole and thiophene, were used to enhance the binding ability of the ligands to the target protein. All the ligands performed very well in the ADME screening for drug-likeness, with no violation of Lipinks's rule of five. All the ligands were then selected for docking with the target protein.

Table 1 Docking scores of deigned imidazole derivatives

Ligand id	Binding Energy	Ligand id	Binding Energy
	kcal/mol		kcal/mol
1	-7.54	8	-7.41
2	-7.15	9	-7.99
3	-7.21	10	-8.53
4	-7.56	11	-7.35
5	-7.52	12	-7.47
6	-7.26	13	-7.46

7	-7.73		

The target protein is a Human Estrogen receptor alpha ligand-binding domain in complex with 4-Hydroxytamoxifen, with the PDB ID: 3ERT. The receptor hosts a hydrophobic cavity which is the Ligand Binding Pocket (LBP), and is formed by Helices 3,6,7,8,11 and 12 [19]. The LBD region comprising of amino acid residues from 302 to 552 is a conserved region and not subjected to mutations[20]. A crucial interaction of helix-12 (amino acid residues 536-544) with the guest ligand determines the activity of a ligand as an agonist/antagonist. In the interaction of tamoxifen with the ligand binding domain (**Fig. 3**), the absence of hydrogen bond interaction with HIS 524 closes helix-12 and disable its binding to co-activator[21].

Table 2 Docking result of Imidazole derivatives and standard drug

Ligand no	Binding energy	interactions	H bond
4	-7.56	LEU 346; MET 421; LEU 391; PRO 324; ILE326; ARG 394; LEU 387; LEU 349; ALA 350	LEU 391; GLU 353
5	-7.52	MET 421; ILE 326; PRO 324; ARG 394; LEU 387; LEU 349; ALA 350	
7	-7.73	GLY 521; MET 421; MET 388; LEU 391; LEU 387; GLY 390; ILE 326; PRO 324; ARG 394; ALA 350; LEU 349; ILE 424	GLU 353
9	-7.99	LEU 346; MET 388; LEU 387; LEU 391; GLY 390; ILE 326; PRO 324; GLU 323; ARG 394; ALA 350; LEU 349; MET 421; ILE 424; HIS 524; GLU 419	LEU 391; LYS 449; GLU 358
10	-8.53	GLU 323; ILE 326; LEU 349; LEU 346; MET 343; MET 421; ALA 350; LEU 391; LEU 387; ARG 394; GLY 390; GLU 358; ILE 386; PRO 324; PHE 443	LYS 449; GLU 358
Tamoxifen	-9.09	LEU 525; MET 421; PHE 404; LEU 391; LEU 387; ALA 350; MET 343; LEU 346; THR 347; ASP 351; LEU 525	

Tamoxifen, a selective estrogen receptor modulator used to prevent/treat breast cancer in women, is used as a standard for comparing the docking results. The standard drug tamoxifen displays a docking score of -9.09 kcal/mol and is stabilized by carbon hydrogen bond with amino acid residues ASP A:351 and THR A:347 at the LBD region. There are two pi-sulphur interactions of the aromatic rings with amino acid residues MET A:421 and MET A:343 and a couple of alkyl and pi-alkyl interactions with stabilized the overall interactions.

Among the drugs that were designed, ligand **4, 5, 7, 9** and **10** produced the highest binding score of -7.56 kcal/mol, -7.52 kcal/mol, -7.73 kcal/mol, -7.99 kcal/mol and -8.53 kcal/mol respectively. The binding scores were quite reasonable as compared to the standard drug. Ligand **10** produce the highest binding score among all the designed drugs, and its binding scores are very close to that of the standard drug. The interaction of ligand **10** at the ligand binding pocket is largely stabilized by two conventional hydrogen bond interactions with amino acid residues LYS A:449 and GLU A: 358, and a considerable number of van der Waals interactions. The imidazo-pyridine ring is actively involved in pi-sigma interaction with LEU A:387, pi-sulphur interaction with ARG A:394 and pi-alkyl interactions with LEU A:391, ALA A:350 and LEU A:349. The pyridine ring connected to the pyrazole ring is actively involved in pi-sulphur interaction with MET A:343, and pi-alkyl interactions with LEU A:346 and MET A:421. The two methoxy substitutions at the aromatic

rings of N-phenylacetamide moity has enhanced the binding ability to the target with a conventional hydrogen bond and four carbon hydrogen bond. Ligand **9** is stabilized by three conventional hydrogen bonds with the residual amino acids LEU A:391, GLU A:358 and LYS A:449. The imidazo-pyridine is actively involved with carbon hydrogen bond with LEU A:387, pi-cation interaction with ARG A:394, pi-sigma interaction with LEU A:387, and pi-alkyl interactions with LEU A:349, ALA A:350, LEU A:391 and LEU A:387. Ligand **7** is stabilized by a conventional hydrogen bond with GLU A:353 and Carbon hydrogen bond with GLY A:521, LUE A:387 and GLY A:390. The imidazo-pyridine ring is involved carbon hydrogen bond interaction with LEU A:387, pi-cation interaction with ARG A:394, pi-sigma interaction with LEU A:387, and multiple pi-alkyl interactions with LEU A:391, LEU A:349, ALA A:350 and LEU A:387. Substitution of CN group at para-position of the aromatic rings of N-phenylacetamide moity doesn't contribute much to the binding energy. Ligand **4** is stabilized with two conventional hydrogen bond with LEU A:391 and GLU A:353 and a significant amount on van der Waals interactions. The imidazo-pyridine ring is actively involved in pi-cation interaction with ARG A:394, pi-sigma interaction with LEU A:387, and a number of pi-alkyl interactions with ALA A:350, LEU A:349 and LEU A:391. The substitution of Br group at the para-position of the aromatic ring of N-phenylacetamide moity contributes to two alkyl interactions with PRO A:324 and ILE A:326. In ligand **5** the imidazo-pyridine ring is actively involved in pi-cation interactions with ARG A:394, pi-sigma interactions with LEU A:387, and multiple pi-alkyl interactions with ALA A:350 and LEU A:349. As seen from the docking results, ligands with methoxy substituents at the aromatic ring of N-phenylacetamide moity (Ligand **10 & 9**) yielded the best results. The imidazo-pyridine ring in all the top scoring ligands has significant contribution to the binding energy due to pi-cation interaction with ARG A:394, pi-sigma interaction with LEU A:387 and a number of pi-alkyl interactions at the active site. One of the crucial part of the ligand is the amide group, where there is conventional hydrogen bond interaction between N-H with GLU A:353 (ligand **10, 9, 7** & **4**) and the carbonyl oxygen with LEU A:391 (ligand **4** & **9**).

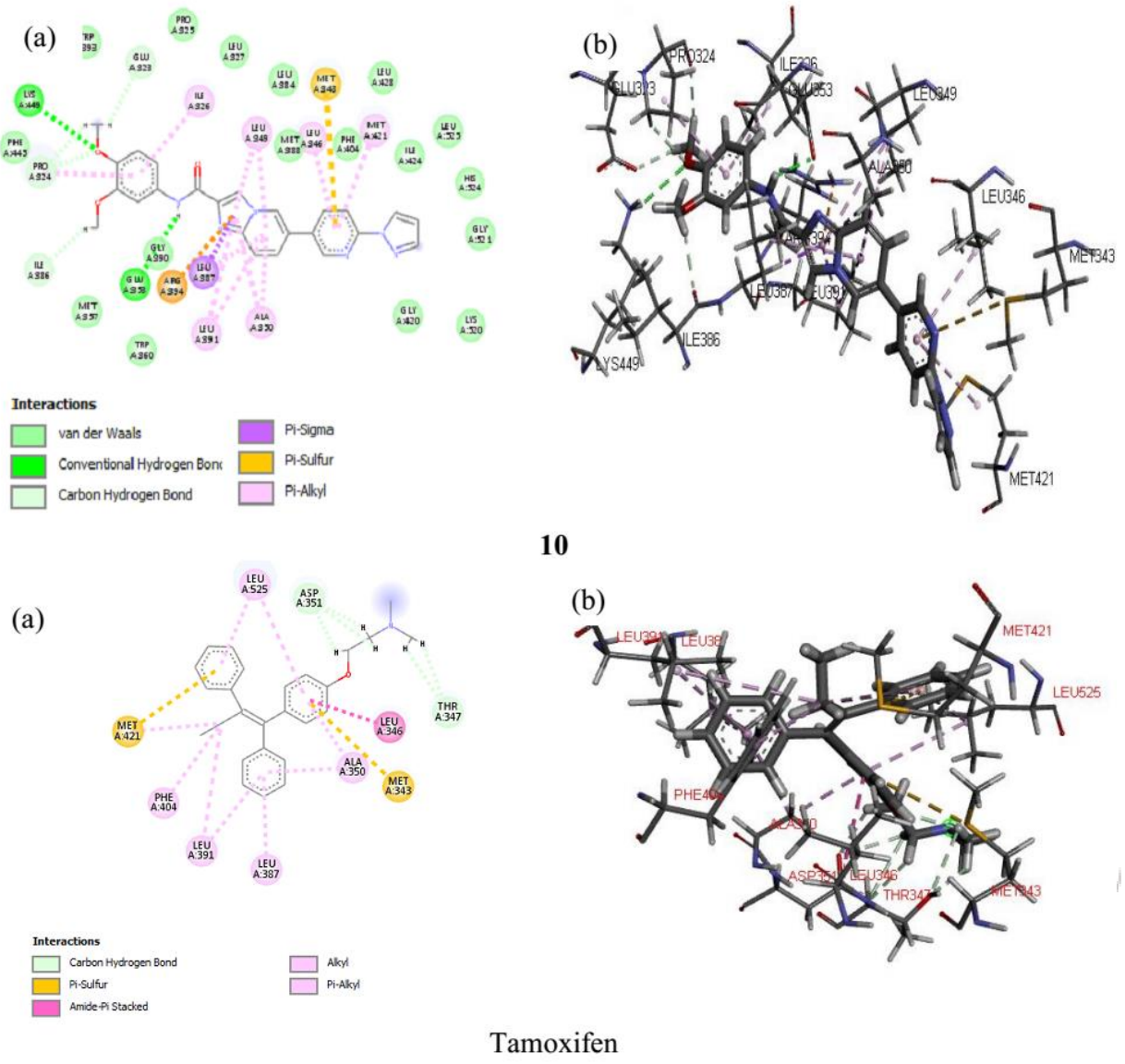


Fig. 3 (a) 2D and (b) 3D images of ligand **10** and Tamoxifen drug

The ADMET screening of the top scoring Imidazole derivatives **4,5,7,9** and **10** predicts good drug likeness properties (**Table 3**). Lipinski's rule of five suggesting that the imidazole derivatives would not have problems with oral bioavailability

Table 3 Drug likeness properties of the Imidazole derivatives

Ligand no.	MF	MW	Log p	Log s	HBA	HBD	TPSA	refractivity	NRB
4	$C_{22}H_{15}BrN_6O$	459.30	3.59	-5.89	4	1	77.11	117.46	5
5	$C_{23}H_{18}N_6O$	394.43	3.29	-5.21	4	1	77.11	114.73	5
7	$C_{23}H_{15}N_7O$	405.41	2.74	-4.86	5	1	100.90	114.47	5
9	$C_{23}H_{18}N_6O_2$	410.43	2.91	-4.98	5	1	86.34	116.25	6
10	$C_{24}H_{20}N_6O_3$	440.45	2.91	-5.04	6	1	95.57	122.74	7

4. Conclusion

This work purposes to design a robust to predict the anticancer activity based on imidazole (1-**13**) derivatives of selective ERα inhibitor. Essential interactions of imidazole derivatives with ERα have been studied by molecular docking analysis. The designed drugs displayed a binding energy ranging from -7.15 to -8.53 kcal/mol. In the designed candidates, ligand **10** showed good interaction energy compared to standard tamoxifen ERα inhibitor. The binding mode analysis of ligand **10** revealed that two amino residues namely LYS 449; GLU 358 play important roles in stabilizing the ERα-imidazole interaction, Lipinski's rule of five suggesting that the 6-(6-(1H-pyrazol-1-yl)pyridin-3-yl)-N-(3,4-dimethoxyphenyl)imidazo[1,2-a]pyridine-2-carboxamide (**10**) would not have problems with oral bioavailability. This work highlights the specific considerations for the selection of docking methods, specifically for the study of imidazole and its derivatives with ERα.

References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians 2021;71:209–49. https://doi.org/10.3322/CAAC.21660.

[2] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA: A Cancer Journal for Clinicians 2011;61:69–90. https://doi.org/10.3322/CAAC.20107.

[3] Wang ZY, Yin L. Estrogen receptor alpha-36 (ER-α36): A new player in human breast cancer. Molecular and Cellular Endocrinology 2015;418:193–206. https://doi.org/10.1016/J.MCE.2015.04.017.

[4] Sanyakamdhorn S, Agudelo D, Bekale L, Tajmir-Riahi HA. Targeted conjugation of breast anticancer drug tamoxifen and its metabolites with synthetic polymers. Colloids and Surfaces B: Biointerfaces 2016;145:55–63. https://doi.org/10.1016/J.COLSURFB.2016.04.035.

[5] Chang M. Tamoxifen Resistance in Breast Cancer. Biomolecules & Therapeutics 2012;20:256. https://doi.org/10.4062/BIOMOLTHER.2012.20.3.256.

[6] Targeted conjugation of breast anticancer drug tamoxifen and its metabolites with synthetic polymers - PubAg n.d. https://pubag.nal.usda.gov/catalog/5531744 (accessed July 8, 2022).

[7] Dorssers LCJ, van der Flier S, Brinkman A, van Agthoven T, Veldscholte J, Berns EMJJ, et al. Tamoxifen Resistance in Breast Cancer. Drugs 2001 61:12 2012;61:1721–33. https://doi.org/10.2165/00003495-200161120-00004.

[8] Muchtaridi M, Syahidah HN, Subarnas A, Yusuf M, Bryant SD, Langer T. Molecular Docking and 3D-Pharmacophore Modeling to Study the Interactions of Chalcone Derivatives with Estrogen Receptor Alpha. Pharmaceuticals 2017, Vol 10, Page 81 2017;10:81. https://doi.org/10.3390/PH10040081.

[9] Imidazoles and Benzimidazoles as Tubulin-Modulators for Anti-Canc...: Ingenta Connect n.d. https://www.ingentaconnect.com/content/ben/cmc/2015/00000022/00000011/art00002 (accessed July 8, 2022).

[10] Congiu C, Cocco MT, Onnis V. Design, synthesis, and in vitro antitumor activity of new 1,4-diarylimidazole-2-ones and their 2-thione analogues. Bioorganic & Medicinal Chemistry Letters 2008;18:989–93. https://doi.org/10.1016/J.BMCL.2007.12.023.

[11] Venkatesan AM, Agarwal A, Abe T, Ushirogochi H, Ado M, Tsuyoshi T, et al. 5,5,6-Fused tricycles bearing imidazole and pyrazole 6-methylidene penems as broad-spectrum inhibitors of β-lactamases. Bioorganic & Medicinal Chemistry 2008;16:1890–902. https://doi.org/10.1016/J.BMC.2007.11.006.

[12] Han MS, Kim DH. Effect of zinc ion on the inhibition of carboxypeptidase A by imidazole-bearing substrate analogues. Bioorganic & Medicinal Chemistry Letters 2001;11:1425–7. https://doi.org/10.1016/S0960-894X(01)00226-8.

[13] Meng X-Y, Zhang H-X, Mezei M, Cui M. Molecular Docking: A Powerful Approach for Structure-Based Drug Discovery. Current Computer Aided-Drug Design 2012;7:146–57. https://doi.org/10.2174/157340911795677602.

[14] Güner OF, Bowen JP. Setting the record straight: The origin of the pharmacophore concept. Journal of Chemical Information and Modeling 2014;54:1269–83. https://doi.org/10.1021/CI5000533/ASSET/IMAGES/MEDIUM/CI-2014-000533_0002.GIF.

[15] Kalyaanamoorthy S, Chen YPP. Structure-based drug design to augment hit discovery. Drug Discovery Today 2011;16:831–9. https://doi.org/10.1016/J.DRUDIS.2011.07.006.

[16] Rani CS, Reddy AG, Susithra E, Mak KK, Pichika MR, Reddymasu S, et al. Synthesis and anticancer evaluation of amide derivatives of imidazo-pyridines. Medicinal Chemistry Research 2020 30:1 2020;30:74–83. https://doi.org/10.1007/S00044-020-02638-W.

[17] Chemical Computing Group ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2021 n.d.

[18] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews 1997;23:3–25. https://doi.org/10.1016/S0169-409X(96)00423-1.

[19] Muchtaridi M, Dermawan D, Yusuf M. Molecular Docking, 3D Structure-Based Pharmacophore Modeling, and ADME Prediction of Alpha Mangostin and Its Derivatives against Estrogen Receptor Alpha. Journal of Young Pharmacists 2018;10:252–9. https://doi.org/10.5530/jyp.2018.10.58.

[20] Seielstad DA, Carlson KE, Kushner PJ, Greene GL, Katzenellenbogen JA. Analysis of the Structural Core of the Human Estrogen Receptor Ligand Binding Domain by Selective Proteolysis/Mass Spectrometric Analysis. Biochemistry 1995;34:12605–15. https://doi.org/10.1021/BI00039A016/ASSET/BI00039A016.FP.PNG_V03.

[21] Kumar R, Zakharov MN, Khan SH, Miki R, Jang H, Toraldo G, et al. The Dynamic Structure of the Estrogen Receptor. Journal of Amino Acids 2011;2011:1–7. https://doi.org/10.4061/2011/812540.