Asian Journal of Pharmaceutical and Health Sciences
www.ajphs.com

In-silico Design of a Core Scaffold Pyrazolone Fused Heterocyclic Analogues as Dual Inhibitors Targeting Breast Cancer

Sumathy Arunachalam[1]*, Suresh Ramalingam[2], Gowrishankar Narayanasamy Lachmanan[1]

1 Department of Pharmaceutical Chemistry, Prime College of Pharmacy, Palakkad, Kerala, India.
2 Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Chidambaram, Tamilnadu, India.

ARTICLE HISTORY

Received: 15.05.2021

Accepted: 05.06.2021

Available online: 30.06.2021

DOI:

10.5530/ajphs.2021.11.9

Keywords:

Pyrazolone, heterocyclic analogues, *in-silico* docking studies, GlideXP, HER2.

***Corresponding author:**

Phone : +91 - 9487874829
Email : sumathy512@gmail.com

ABSTRACT

In human breast cancers, the human epidermal development factor receptor (HER2) is a membrane tyrosine kinase that is overexpressed, and gene amplified. Pyrazolone fused heterocyclic moieties- carboxy methyl thiazolidinone, azetiditinone are significant DNA-intercalating agents here HER2 amplification and overexpression are linked to tumour cell proliferation and the pathway of instances in breast cancer. Few novel pyrazolone fused heterocyclic analogues are designed by the *in-silico* technique for their HER2 inhibitory action. Docking patterns of compounds 4B1-4C20 are carried out against HER2 (PDB id-3RCD) by using Schrodinger suit 2016-2. The affinity of binding was chosen based on the glide score. Many compounds were showing good hydrophobic and hydrogen bonding communication and association to hinder HER2. The derivatives 4B1-4C20 have significant glide scores in the scope of -4.745 to -7.571 compared to the standard Tamoxifen (-4.326). The *in-silico* screening properties are within the drug likeness. The results proved that this study gave a shred of evidence for the consideration of pyrazolone fused heterocyclic analogues are potential HER2 inhibitors. Among the compounds, 4B1-4C20 with significant glide scores may produce significant antibreast cancer activity and further *in-vitro* and *in-vivo* investigations may prove their therapeutic potential.

INTRODUCTION

Breast cancer affects one out of every eight women, and it is expected that over 246,660 women in the United States will be diagnosed with the disease in 2016. Breast cancer mortality has decreased over the last two decades as a result of earlier detection and better treatment [1-3]. A noteworthy job is assumed among many of the malignant tumours which can be treated by chemotherapy. Globally around one of every five ladies are affected with breast cancer prompts to find the novel molecules [4-6]. Various targets are accounted for bosom malignant growth like HER2, oestrogen receptor (ER), vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), tyrosine kinase inhibitor, cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitor [7-9]. HER2-positive breast cancer tends to grow and spread vigorously, hence, the step to HER2 treatments is one of the most encouraging for focused betterment. HER2 is a layer tyrosine kinase, is overexpressed and connected to significant tumour cell multiplication and pathways [3, 6-8]. Pyrazolone fused Azetidinone and carboxymethyl thiazolidinone analogues are most commonly used in cancer treatment, however, it also possesses different pharmacological activities like antimicrobials, antioxidant, antimalarial, and analgesics [10-12]. Thiazolidine was one of the primary DNA intercalating agents in pyrazolone fused heterocyclic agents which is significant, and the chromophore intercalates with the DNA base sets. Hence, a study was aimed to design core scaffold pyrazolone fused heterocyclic analogues as dual inhibitors for targeting breast cancer.

MATERIALS AND METHODS

This section will describe the materials and method used in the study to design and develop novel pyrazolone fused heterocyclic analogues targeting breast cancer. The scheme of the heterocyclic analogues fused with the core scaffold pyrazolone is presented in

Figure 1.

Molecular docking studies

Protein preparation:

The HER2 enzyme with co-crystallized ligand (PDB ID: 3RCD, 2.3.A^{O}) was identified from the protein data bank. The protein preparation wizard module of Schrodinger suite 2016-2 was utilized to set up the protein. Water atoms past 5A^{0} and without hydrogen bonds are evacuated. A controlled minimization of energy of the protein structure was done by utilizing OPLS3 to correct the orientation side chain, hydroxyl groups and alleviate potential steric dashes.

HER2 receptors are framed by extracellular areas (ECD) a transmembrane section and an intracellular district. There are four sections in ECD. Section 1&3 give a job in ligand official. Areas 2&4 are cysteine build-ups significant for disulfide security. Totally 19-25 amino acid residues are involved in the formation of trans membrane portions. The C-terminal tail

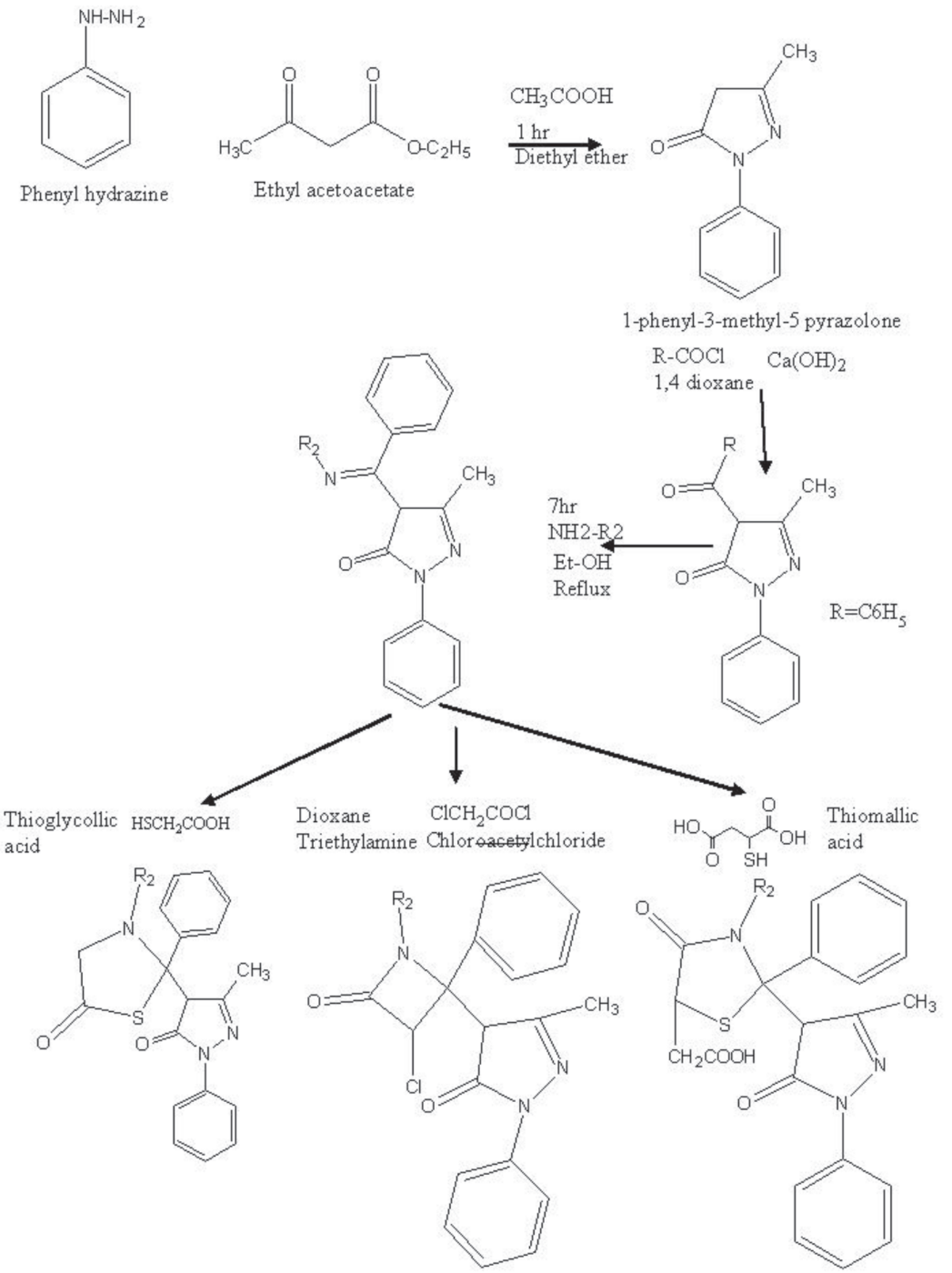

Fig 1 : Scheme of the heterocyclic analogues fused with the core scaffold pyrazolone

containing phosphorylation enacted the intracellular protein kinase movement. HER2 receptor initiations are affected by the structure of the dimer and the character of the ligand.

Receptor Grid Generation

In docking, a glide box was produced to characterize the centroid of the dynamic site, a co-crystallized ligand was held in the gem structure of the protein planning wizard, and it was utilized for the receptor lattice development. Glide grid generation wizard is utilized to produce Grid Box. The measurements of the glide gride box of the protein were set to 14 A^0 x 14 A^0 x 14 A^0.

Ligand Preparation

The ligand structure was produced by a 2D sketcher and exposed to the ligand preparation module of Schrodinger suite 2016. The chiralities were corrected and by including stereo concoction, ionization the ligands were changed over from 2D to 3D. The ionization and tautomeric state P^H 6.7-7.1 utilizing Epik module. In the last phase of ligand preparation, mixes were limited utilizing optimized potentials for liquid simulations (OPLS-3) until a root mean square deviation of 1.8A^0 was accomplished. A solitary low vitality ring affirmation per ligand was produced and ligands were streamlined.

Glide Ligand Docking

All the ligands 4B1-4C20 are docked into the synergist pocket of HER2 protein ((PDB ID: 3RCD). The best-docked ligands are selected based on the glide score. The ideal gathering among the ligands and the receptor were scored utilizing the glide ligand docking module. Extra precision XP visualizer of glide module was utilized to analyze the results. The upgrading parameters and the diminishing movement are noted simultaneously to obtain betterment in the result data.

RESULTS

The framed pyrazolone fused heterocyclic analogues are undertaken for sub-atomic investigations by a docking program. The structured derivatives and their analogues are docked towards HER2 (3RCD) to identify their HER2 restraint action against malignant growth. The compounds 4B1-4C20 demonstrated great liking to the receptor compared to the standard Tamoxifen. The glide scores obtained from the studies are shown in Table 1. Compounds 4B1-4C20 except 4B14 have significant glide scores in the range of -4.74 to -7.571 compared to the standard Tamoxifen (-3.78). The docking results prove that the presence of the heterocyclic rings fused with the pyrazolone moiety is a factor and in the greater part of the mixes assumes a worthy score. The docked poses of the compounds 4B and 4C series with HER2 (3RCD) are presented in Figures 2 and 3. The docking score of certain derivatives is diminished due to the XP penalties.

DISCUSSION

This study will focusing the pyrazole derivatives as antibreast cancer agents. There were only a few published studies on pyrazolone targeting breast cancer as a novel therapeutic agent. Based on previous research that demonstrated the importance of pyrazole scaffolds in cancer management and VEGFR-2 inhibition, a new set of core scaffold pyrazolone fused heterocyclic analogues were synthesised and tested for anticancer efficacy against human breast cancer. In comparison to the

Table 1 : Docking studies for compounds 4B1-4C20 with 3RCD

Code	Glide score	Glide evdw	Glide ecoul	Glide energy	Glide einternal	Glide emodel	XP HBond
4B-1	-3.816	-28.153	-0.163	-28.315	13.886	-38.5	-0.447
4B-6	-4.257	-35.238	-1.28	-36.518	16.078	-42.939	0
4B-7	-3.938	-36.962	-2.86	-39.822	13.733	-44.465	-0.187
4B-8	-3.927	-41.842	-1.26	-43.102	2.325	-49.441	0
4B-9	-3.878	-42.015	-0.984	-43	10.674	-55.244	0
4B-14	-2.576	-37.462	-2.689	-40.151	9.44	-48.944	0
4B-15	-4.368	-42.287	2.56	-39.728	9.586	-47.88	0
4B-17	-4.745	-37.341	-8.819	-46.16	11.252	-51.425	-0.48
4C-3	-5.304	-33.11	-10.637	-43.747	11.637	-61.67	-1.103
4C-17	-7.571	-40.816	-20.355	-61.171	5.797	-76.89	-2.153
4C-18	-6.429	-46.778	-10.419	-57.197	0	-66.186	-1.18
Tamoxifen	-4.326	-37.263	-0.373	-37.636	2.435	-52.367	0

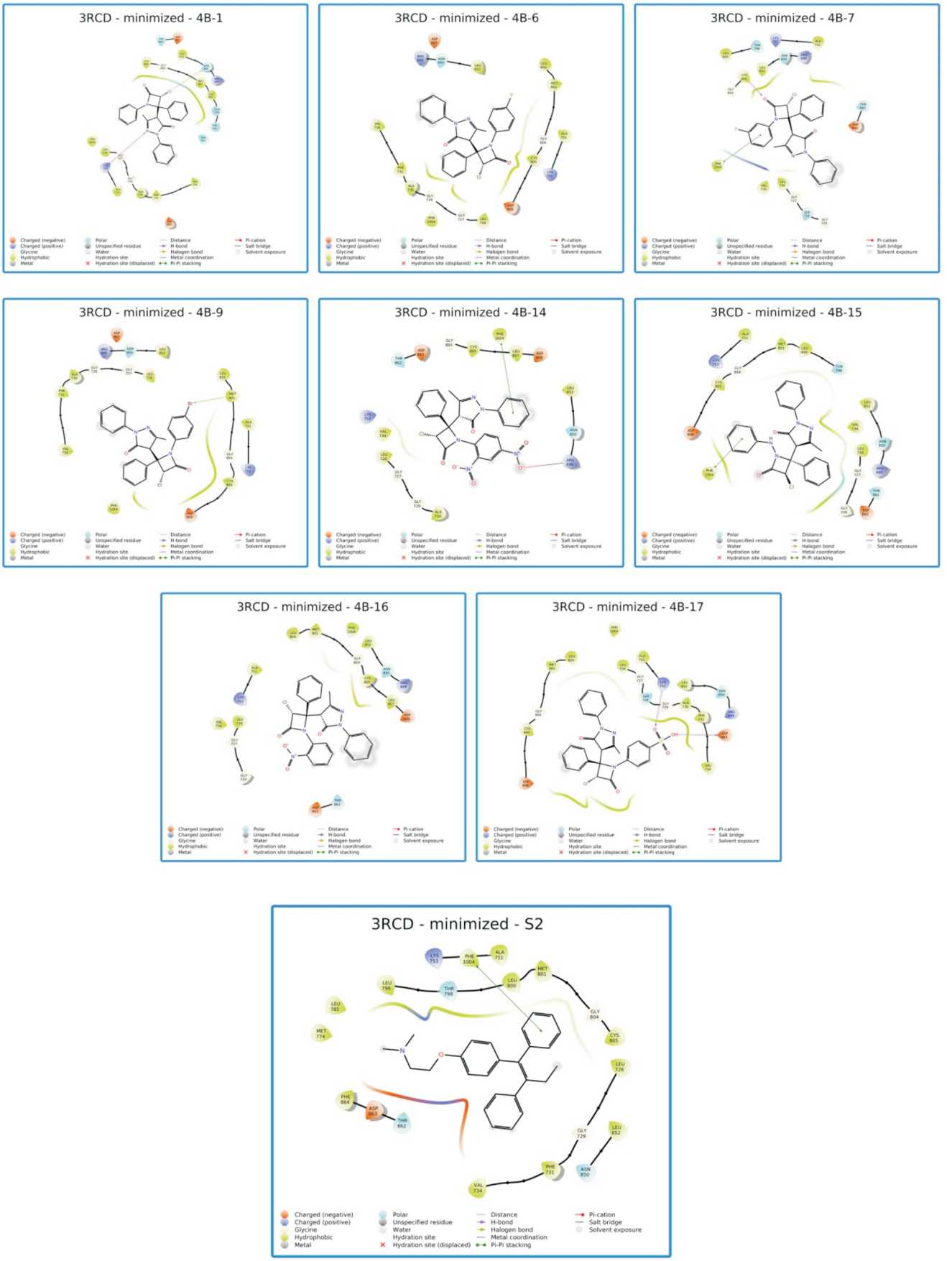

Fig. 1 : Docked poses of the compounds 4B series with HER2 (3RCD)

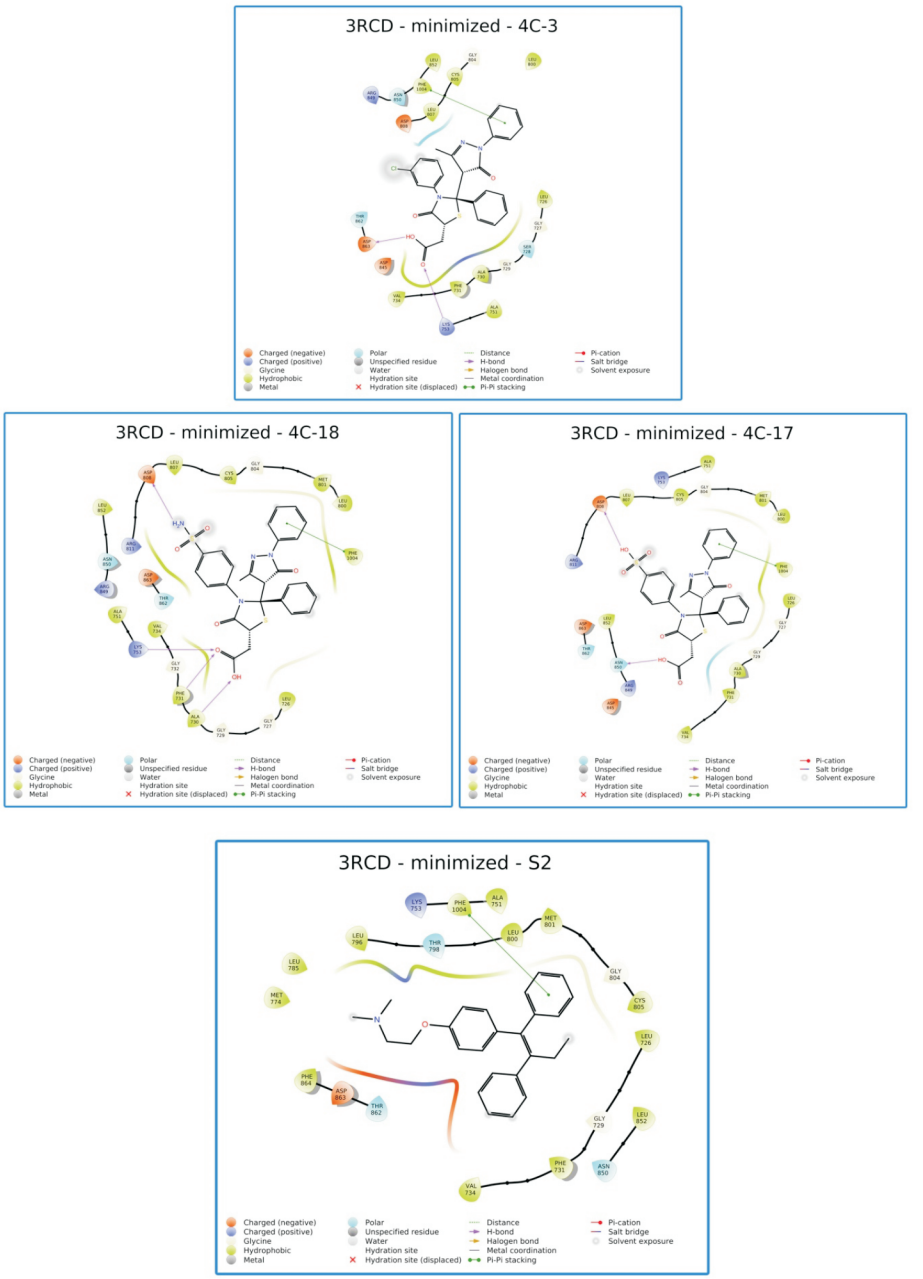

Fig 3 : Docked poses of the compounds 4C series with HER2 (3RCD)

standard drug Tamoxifen, the synthesised compounds demonstrated remarkable antibreast cancer activity. Similar findings were observed in a study by Dawood et al, where anticancer screening revealed significant sensitivity of breast carcinoma (MCF-7) to compounds 4b, 5c, 6c, 7b, 7c, and 12c with IC_{50} values ranging from 16.50 to 26.73 M in comparison to tamoxifen (IC_{50} = 23.31 µM) [10]. Whereas the pyrazole fused compounds on docking studies indicated the best binding mode compared to the standard ligand sorafenib [13]. A study by Althagafi et al. shown the high efficiency was recorded towards MCF-7 (breast carcinoma) cell line, all of the tested pyrazolone compounds interacted significantly with breast cancer protein (strong correlation with the practical result), followed by DNA polymerase protein. [14].

Markovic et al, synthesised twenty-five 4-aminomethylidene derivatives from 3-phenyl-2-pyrazolin-5-one and 1,3-diphenyl-2-pyrazolin-5-one and tested them for their antiproliferative activity against human breast cancer cell lines (MDA-MB-361 and MDA-MB-453). In the treatment of both cell lines, the compounds derived from 1,3-diphenyl-2-pyrazolin-5-one demonstrated the most remarkable activity [15]. Gediz Erturk and Omerustaoglu, synthesised a series of new substituted-5-pyrazolones compounds and then formulated by the Vilsmeier-Haack reaction to obtain substituted-4-carbaldehyde-5-pyrazolones. In the final step of their study, they added urea and allowed it to react with formulated pyrazolones and observed that instead of the C=N bond in azomethine form, the compounds tautomerized to form a series of novel pyrazole-4-

ylidenemethylurea structures. At all-time points, all 5-pyrazolone-urea compounds were more toxic (p 0.05) in cancerous A431 cells than noncancerous cells. All the compounds demonstrated a biphasic hormetic effect. Cell migration was inhibited by four of the nine compounds tested [16].

Tamoxifen was used as the gold standard in this study because it is the most widely used anti-estrogen for the treatment of hormone-dependent breast cancer. The specific drug is used as hormonal therapy for patients with oestrogen receptor positive breast cancer. The pharmacological activity of Tamoxifen is dependent on its conversion to its active metabolite, endoxifen, by CYP2D6. Tamoxifen, when used as adjuvant therapy, lowers the risk of recurrence and death from breast cancer and provides effective palliation for patients with metastatic breast cancer [17-20].

Among all the compounds synthesised in this study, compounds 4B1-4C20 except 4B14 have significant glide scores in the range of -4.74 to -7.571 compared to the standard Tamoxifen (-3.78). This proven that the synthesised compounds with the presence of heterocyclic ring fused with pyrazolone moiety can have a significant role in antibreast cancer management. Further studies are warranted to analyse the compounds for better anticancer properties.

CONCLUSION

Pyrazolone fused heterocyclic analogues of carboxymethyl thiazolidinone showed numerous biological activities. This study exhibited a dynamic site by connecting with numerous amino corrosive build-ups and proved the recognizing lead atom in this *in-silico* study. The compounds 4B1-4C20 have significant antibreast cancer activity with remedial possibilities and this study will be helpful in the future *in-vitro* and *in-vivo* assessments.

ACKNOWLEDGMENT

The authors thank Ms Jainey P James, Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences, Mangalore, Karnataka for the support rendered towards the analytical studies of this research; Management of Prime College of Pharmacy, Palakkad, and Annamalai University, Tamilnadu, for the support to execute the various aspects of this research progress.

CONFLICT OF INTEREST

Nil

FINANCIAL SUPPORT

Nil

REFERENCES

1. Hsu JL, Hung MC. The role of HER2, EGFR, and other receptor tyrosine kinases in breast cancer. Cancer Metastasis Rev. 2016 Dec;35(4):575-588. doi: 10.1007/s10555-016-9649-6. PMID: 27913999; PMCID: PMC5215954.
2. Larionov AA. Current Therapies for Human Epidermal Growth Factor Receptor 2-Positive Metastatic Breast Cancer Patients. Front Oncol. 2018 Apr 3;8:89. doi: 10.3389/fonc.2018.00089.
3. Dormann C. Metastatic Human Epidermal Growth Factor Receptor 2-Positive Breast Cancer: Current Treatment Standards and Future Perspectives. Breast Care (Basel). 2020 Dec;15(6):570-578. doi: 10.1159/000512328.
4. Sivanandy P, Shanmugam S, Lau RY, Chin JYS, Lee XX, Lau YY, et al. Current Trends in the Treatment of Human Epidermal Growth Factor Receptor-2 Negative Breast Cancer. Journal of Pharmacy and Nutrition Sciences. 2020:10:46-59.
5. Tolaney SM, Guo H, Pernas S, Barry WT, Dillon DA, Ritterhouse L, et al. Seven-Year Follow-Up Analysis of Adjuvant Paclitaxel and Trastuzumab Trial for Node-Negative, Human Epidermal Growth Factor Receptor 2-Positive Breast Cancer. J Clin Oncol. 2019 Aug 1;37(22):1868-1875. doi: 10.1200/JCO.19.00066.
6. Arciero CA, Guo Y, Jiang R, Behera M, O'Regan R, Peng L, Li X. $ER^+/HER2^+$ Breast Cancer Has Different Metastatic Patterns and Better Survival Than $ER^-/HER2^+$ Breast Cancer. Clin Breast Cancer. 2019 Aug;19(4):236-245. doi: 10.1016/j.clbc.2019.02.001.
7. Shah M, Nunes MR, Stearns V. CDK4/6 Inhibitors: Game Changers in the Management of Hormone ReceptorPositive Advanced Breast Cancer? Oncology (Williston Park). 2018 May 15;32(5):216-22. PMID: 29847850; PMCID: PMC6424488.
8. Nagini S. Breast Cancer: Current Molecular Therapeutic Targets and New Players. Anticancer Agents Med Chem. 2017;17(2):152-163. doi: 10.2174/1871520616666160502122724.
9. Chai D, Li K, Du H, Yang S, Yang R, Xu Y, Lian X. β2-microglobulin has a different regulatory molecular mechanism between ER^+ and ER^- breast cancer with HER2. BMC Cancer. 2019 Mar 12;19(1):223. doi: 10.1186/s12885-019-5410-1.
10. Dawood DH, Nossier ES, Ali MM, Mahmoud AE. Synthesis and molecular docking study of new pyrazole derivatives as potent anti-breast cancer agents targeting VEGFR-2 kinase. Bioorg Chem. 2020 Aug;101:103916. doi: 10.1016/j.bioorg.2020.103916.
11. Aboul Wafa OM, Daabees HMG, Badawi WA. 2-Anilinopyrimidine derivatives: Design, synthesis, *in vitro* anti-proliferative activity, EGFR and ARO inhibitory activity, cell cycle analysis and molecular docking study. Bioorg Chem. 2020 Jun;99:103798. doi: 10.1016/j.bioorg.2020.103798.
12. Tahmasvand R, Bayat P, Vahdaniparast SM, Dehghani S, Kooshafar Z, Khaleghi S, Almasirad A, Salimi M. Design and synthesis of novel 4-thiazolidinone derivatives with promising anti-breast cancer activity: Synthesis, characterization, *in vitro* and *in vivo* results. Bioorg Chem. 2020 Nov;104:104276. doi: 10.1016/j.bioorg.2020.
13. Salama SK, Mohamed MF, Darweesh AF, Elwahy AH, Abdelhamid IA. Molecular docking simulation and anticancer assessment on human breast carcinoma cell line using novel bis (1,4-dihydropyrano [2,3-c] pyrazole-5-carbonitrile) and bis (1,4-dihydropyrazolo [4',3':5,6] pyrano [2,3-b] pyridine-6-carbonitrile) derivatives. Bioorg Chem. 2017 Apr;71:19-29. doi: 10.1016/j.bioorg.2017.01.009.
14. Althagafi I, El-Metwaly NM, Elghalban MG, Farghaly TA,

Khedr AM. Synthesis of Pyrazolone Derivatives and Their Nanometer Ag(I) Complexes and Physicochemical, DNA Binding, Antitumor, and Theoretical Implementations. Bioinorg Chem Appl. 2018 May 14;2018:2727619. doi: 10.1155/2018/2727619.

15. Marković V, Erić S, Stanojković T, Gligorijević N, Aranđelović S, Todorović N, Trifunović S, Manojlović N, Jelić R, Joksović MD. Antiproliferative activity and QSAR studies of a series of new 4-aminomethylidene derivatives of some pyrazol-5-ones. Bioorg Med Chem Lett. 2011 Aug 1;21(15):4416-21. doi: 10.1016/j.bmcl.2011.06.025.

16. Gediz Erturk A, Omerustaoglu H. Synthesis and Cytotoxic Evaluation of Some Substituted 5-Pyrazolones and Their Urea Derivatives. Molecules. 2020 Feb 18;25(4):900. doi: 10.3390/molecules25040900.

17. Yang G, Nowsheen S, Aziz K, Georgakilas AG. Toxicity and adverse effects of Tamoxifen and other anti-estrogen drugs. Pharmacol Ther. 2013 Sep;139(3):392-404. doi: 10.1016/j.pharmthera.2013.05.005.

18. Neven P, Jongen L, Lintermans A, Van Asten K, Blomme C, Lambrechts D, et al. Tamoxifen Metabolism and Efficacy in Breast Cancer: A Prospective Multicenter Trial. Clin Cancer Res. 2018 May 15;24(10):2312-2318. doi: 10.1158/1078-0432.CCR-17-3028.

19. Busonero C, Leone S, Bartoloni S, Acconcia F. Strategies to degrade estrogen receptor α in primary and ESR1 mutant-expressing metastatic breast cancer. Mol Cell Endocrinol. 2019 Jan 15;480:107-121. doi: 10.1016/j.mce.2018.10.020.

20. McAndrew NP, Finn RS. Management of ER positive metastatic breast cancer. Semin Oncol. 2020 Oct;47(5):270-277. doi: 10.1053/j.seminoncol.2020.07.005.

Cite this article : Sumathy Arunachalam, Suresh Ramalingam, Gowrishankar Narayanasamy Lachmanan. *In-silico* Design of a Core Scaffold Pyrazolone Fused Heterocyclic Analogues as Dual Inhibitors Targeting Breast Cancer.
Asian J. Pharm. Hea. Sci.. 2021;11(2):2468-2474. DOI : 10.5530/ajphs.2021.11.9