DOI: 10.55522/jmpas.V11I6.4475 ISSN NO. 2320–7418

International peer reviewed open access journal

Journal of Medical Pharmaceutical and Allied Sciences

Journal homepage: www.jmpas.com CODEN: JMPACO

Research article

Design, synthesis and evaluation of novel 3-(substitutedphenyl)-2-(4-(substitutedphenyl) thiazol-2-yl)-2h-pyrazolo [3, 4-d] thiazol-5(6h)-one derivatives as anti-breast cancer agents

Pravin Nandakumar Muli[1], Sonali Shankar Kamble[2], Sanjay Sudhakar Pekamwar[3], Balaji WamanraoMatore[4], Jagadish Singh[4], ParthaPratim Roy[4],Megha Mukund Muley[5], Bhaskar Sadashiv Dawane*1

[1] School of Chemical Sciences, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India

[2] Department of Biochemistry, Gramin Science (Vocational) College, Vishnupuri, Nanded, Maharashtra, India

[3] School of Pharmacy, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India.

[4] Department of Pharmacy, Guru Ghasidas Vishwavidyalaya (A Central University) Bilaspur, Chhattisgarh, India

[5] Nanded Pharmacy College, Nanded, Maharashtra, India

ABSTRACT

Mainstream cancer research has continued to place a significant emphasis on the development of new and effective therapeutic candidates to tackle rising treatment resistance and off-target toxicities. Here, a series of novel 3-(substitutedphenyl)-2-(4-(substitutedphenyl)thiazol-2-yl)-2H-pyrazolo[3,4-d]thiazol-5(6H)-one derivatives were synthesized and characterized. The ability of the synthesized compounds to reduce the survival of the human breast cancer cell line MDA-MB 231 was evaluated. When compared to the reference chemical, 5-fluorouracil, 3-(4-chlorophenyl)-2-(4-(4-chlorophenyl)thiazol-2-yl)-2H-pyrazolo[3,4d]thiazol-5(6H)-oneand 3-(4-nitrophenyl)-2-(4-(4-nitrophenyl)thiazol-2-yl)-2H-pyrazolo[3,4d]thiazol-5(6H)-oneshowed the highest inhibitory activity (IC_{50}:550 0.78 M and 504 0.89, respectively) on the viability of MDA-MB 231 cells. A molecular docking study has been carried out to discover more potent drugs.

Keywords: Thiazolidione-2, 4-dione, Thiosemicarbazide, pyrazolo [3,4-d] thiazol-5(6H)-one, Anticancer activity, MDA-MB-231.

Received - 19-10-2022, Accepted- 25-11-2022

Correspondence: Bhaskar Sadashiv Dawane ✉ dawanebhaskar@gmail.com, **Orcid Id:** 0000-0003-2359-9012

School of Chemical Sciences, Swami Ramanand Teerth, Marathwada University, Nanded, Maharashtra, India.

INTRODUCTION

Breast cancer is not only the most common cancer in women worldwide, but it also ranks second in terms of the primary causes of mortality for women across the globe [1]. It is the most serious health problem of all gynaecological cancers, affecting a significant portion of the global population [2]. Every year, invasive breast cancer affects an estimated one million women globally. More specifically, in 2017, it was anticipated that there were roughly 40,610 breast cancer deaths among US women, although there were an increasing number of new patients with breast cancer every year in India [3,4]. The existing chemotherapy techniques utilized in the treatment of breast cancers have limitations due to significant target and off-target adverse effects in the face of developing drug resistance to the disease. Early diagnosis, and improved, and safer treatment options are among the interventions and strategies used to lower the prevalence and fatality rates. The problems of existing anticancer medications have spurred research into new, effective breast cancer treatments. One of the most active components of heterocyclic compounds, pyrazole, and its derivatives exhibit a wide range of pharmacological actions [5-7]. Pyrrole exhibits considerable antifungal [8], antimicrobials [9,10],anticancer [11,12], especially anti-breast cancer activity [13-16], anticonvulsant [17,18], antitubercular [19,20], analgesics [21], cardiovascular [22] and anti-inflammatory [23,24] activity. The thiazole moiety is one of the most significant heterocyclic compounds and is utilized frequently in pharmaceutical chemistry. It also demonstrates a variety of biological functions, including anti-breast cancer activity, antitubercular activity, analgesic, anti-inflammatory, antihypertensive, CNS activity, antioxidant, antiviral, antidiabetic [25], antimicrobial [26], immunosuppressive activities [27].It also demonstrates biological processes such as COX-2 inhibitors [28], anti-hypnotics [29], anti-allergic [30], and HIV-1 inhibitors [31].The significant inhibitory activity of triazolone scaffold against anticancer has been thoroughly investigated by numerous research organizations [32-34]. A few of the pyrazolothiazolone compounds have been created [35-36], and they have activity against the dengue virus [37] as well as being anti-proliferative [38], anticonvulsant [39], antimicrobial and anti-inflammatory [40], antiviral [41], anti-HIV-1 NNRT inhibitors [42],

anticancer [43-44].In an effort to aid in the drive against breast cancer, we screened a number of synthetic compounds incorporating fused heterocycles for possible biological activity.

MATERIALS AND METHODS

A general method for preparation of compounds 5(a-n)

An equimolar quantity of Thiazolidione-2,4-dione (1 mmol) and substituted aromatic aldehydes (1 mmol) were dissolved in a minimum quantity of PEG-400 along with a catalytic amount of bleaching earth clay (12.5) stirred at 70-80^0 C for one hour[45]. Add excess thiosemicarbazide (1.5 mmol), stir this mixture for another hour, then add 2-bromo-1-(4-substitutedphenyl) ethenone (1 mmol), and stir this reaction mixture for two hours[46].After the reaction is over, it is monitored on Thein Layer Chromatography After completion of the reaction, the mixture was poured into crushed ice, stirred for five minutes, and neutralized with diluted HCl if necessary. The separated solid was suction-filtered (water aspirator), washed with ice-cold water, and recrystallized from ethanol to yield an analytically pure product, the identity of which was determined by comparing its MP and spectroscopic data (IR, 1HNMR, 13CNMR and MS).

2-(4-(4-chlorophenyl) thiazol-2-yl)-3-(4-nitrophenyl)-2H-pyrazolo [3,4d]thiazol-5(6H)-one 5a.

$C_{19}H_{10}ClN_5O_3S_2$This compound was obtained in 90% yield, mp 210-220°C; 1HNMR (400 MHz, DMSO-*d6*): δ 12.6(s,1H, NH),7.4-7.4(d, 2H), 7.8-7.9(m, 2H), 8.1(s, 1 H,), 8.2-8.2(d, 2H),; ^{13}C NMR (400 MHz, DMSO-*d6*) ppm: δ 168.44,148.52, 140.40, 133.67, 133,14, 128.83, 127.83, 126.26, 124.01, 108.64,; IR (KBr) cm^{-1}:3294(NH stretching), 1670(C=O stretching), 1581(aromatic C=C stretching),; ms (EI): m/z (M+) 455.5. Anal.calcd. for: C, 50.00; H, 2.37; Cl, 7.70; N, 15.45; O, 10.32; S, 14.16. Found: C, 50.06; H, 2.21; Cl, 7.78; N, 15.36; O, 10.52; S, 14.07.

3-(4-chlorophenyl)-2-(4-(4-chlorophenyl) thiazol-2-yl)-2H-pyrazolo [3,4d] thiazol-5(6H)-one 5b.

$C_{19}H_{10}Cl_2N_4OS_2$This compound was obtained in 92% yield, mp 210-220°C; 1HNMR (400 MHz, DMSO-*d6*): δ 12.6(s,1H, NH),7.4-7.4(d, 2H), 7.8-7.9(m, 2H), 8.1(s, 1 H,), 8.2-8.2(d, 2H),; ^{13}C NMR (400 MHz, DMSO-*d6*) ppm: δ 168.12,148.52, 146.18, 140.54, 140.40, 133.54, 133.24, 131.87, 129.43, 128.81, 128.52, 128.22, 127.76, 127.14 104.56,; IR (KBr) cm^{-1}:3294(NH stretching), 1670(C=O stretching), 1581(aromatic C=C stretching),; ms (EI): m/z (M+) 445 Anal. calcd. for: C, 51.20; H, 2.27; Cl, 15.92; N, 12.58; O, 03.61; S, 14.41. Found: C, 51.24; H, 2.26; Cl, 15.92; N, 12.58; O, 03.59; S, 14.40.

3-(4-bromophenyl)-2-(4-(4- chlorophenyl) thiazol-2-yl)-2H-pyrazolo[3,4d] thiazol-5(6H)-one 5c.

$C_{19}H_{10}$ Br Cl N_4OS_2 This compound was obtained in 88% yield, mp 210-220°C; 1HNMR (400 MHz, DMSO-*d6*): δ 12.6(s,1H, NH),7.4-7.5(d, 2H), 7.9-7.9(m, 2H), 8.0(s, 1 H,), 8.0-8.1(d, 2H),; ^{13}C NMR (400 MHz, DMSO-*d6*) ppm: δ 167.68,150.36,147.75, 140.61, 138.33, 132.32, 131.23, 128.04, 127.01, 126.47, 124.42, 123.11, 106.13,; IR (KBr) cm^{-1}: 3243(NH stretching), 1668(C=O stretching), 1581(aromatic C=C stretching),;ms (EI): m/z (M+) 500.36 Anal. calcd. for: C, 47.01; H, 2.17; Br, 16.11; Cl, 7.23; N, 11.13; O, 3.25; S, 13.11. Found: C, 46.59; H, 2.06; Br, 16.31; Cl, 7.24; N, 11.44; O, 03.27; S, 13.09.

2-(4-(4- Chlorophenyl) thiazol-2-yl)-3-(4-fluorophenyl)-2H-pyrazolo [3,4d]thiazol-5(6H)-one 5d.

$C_{19}H_{10}ClFN_4OS_2$ This compound was obtained in 82% yield, mp 210-220°C; 1HNMR (400 MHz, DMSO-*d6*): δ 12.7(s,1H, NH),7.5-7.5(d, 2H), 7.8-7.8(m, 2H), 8.0(s, 1 H,), 8.1-8.1(d, 2H),; ^{13}C NMR (400 MHz, DMSO-*d6*) ppm: δ 169.33,149.40,143.67, 140.63, 137.11, 133.00, 131.56, 128.12, 127.09, 126.49, 124.51, 123.23, 105.43,; IR (KBr) cm^{-1}: 3352(NH stretching), 1670(C=O stretching), 1581(aromatic C=C stretching),; ms (EI): m/z (M+) 439.44 Anal. calcd. for: C, 53.18; H, 2.16; Cl, 8.32; F, 4.52; N, 13.16; O, 3.69; S, 14.97. Found: C, 53.21; H, 2.35; Cl, 8.27; F, 4.43; N, 13.06; O, 3.73; S, 14.95.

2-(4-(4- Chlorophenyl) thiazol-2-yl) -3- (4-hydroxyphenyl) -2 H-pyrazolo [3,4d]thiazol-5(6H)-one5e.

$C_{19}H_{11}$ Cl $N_4O_2S_2$ This compound was obtained in 78% yield, mp 210-220°C; 1HNMR (400 MHz, DMSO-*d6*): δ 12.5(s,1H, NH),10.4(s, 1H, OH), 7.3-7.4(d, 2H), 7.8-7.8(m, 2H), 7.9(s, 1 H,), 7.9-8.0(d, 2H),; ^{13}C NMR (400 MHz, DMSO-*d6*) ppm: δ 167.62,150.43,142.10, 138.09, 137.43, 132.21, 131,69, 128.57, 127.09, 125.20, 124.32, 123.12, 106.09,; IR (KBr) cm^{-1}: 3303(NH stretching), 3212(OH stretching), 1668(C=O stretching), 1587(aromatic C=C stretching),; ms (EI): m/z (M+) 437.03 Anal. calcd. for: C, 53.40; H, 2.58; Cl, 8.32; N, 13.14; O, 7.52; S, 15.04. Found: C, 53.46; H, 2.60; Cl, 8.30; N, 13.12; O, 7.50; S, 15.02.

3-(4-chlorophenyl)-2-(4-(4-nitrophenyl) thiazol-2-yl)-2H-pyrazolo [3,4d] thiazol-5(6H)-one 5h.

$C_{19}H_{10}ClN_5O_3S_2$This compound was obtained in 90% yield, mp 210-220°C; 1HNMR (400 MHz, DMSO-*d6*): δ 12.3(s,1H, NH),7.4-7.4(d, 2H), 7.8-7.9(m, 2H), 8.1(s, 1 H,), 8.2-8.2(d, 2H),; ^{13}C NMR (400 MHz, DMSO-*d6*) ppm: δ 167.78,149.40,147.05, 140.67, 138.68, 133.27, 131,98, 128.54, 127.15, 126.87, 124.01, 123.72, 105.16, ; IR (KBr) cm^{-1}:3355(NH stretching), 1670(C=O stretching), 1589(aromatic C=C stretching),; ms (EI): m/z (M+) 455.5 Anal. calcd. for: C, 50.08; H, 2.34; Cl, 7.76; N, 15.34; O, 10.41; S, 14.07. Found: C, 50.06; H, 2.21; Cl, 7.78; N, 15.36; O, 10.53; S, 14.07.

3-(4-nitrophenyl)-2-(4-(4-nitrophenyl) thiazol-2-yl)-2H-pyrazolo [3,4d]thiazol-5(6H)-one 5i.

$C_{19}H_{10}N_6O_5S_2$ This compound was obtained in 88% yield, mp 210-220°C; 1HNMR (400 MHz, DMSO-*d6*): δ 12.7(s,1H, NH), 8.1-8.2(m, 2H), 7.8-7.9(d, 2H), 7.8(s, 1 H,),; ^{13}C NMR (400 MHz, DMSO-*d6*) ppm: δ 167.68,155.59,147.15, 140.75, 138.80, 133.34, 132,06, 128.66, 127.24, 126.99, 124.15, 105.30,; IR (KBr) cm^{-1}: 3309(NH stretching), 1677(C=O stretching), 1573(aromatic C=C stretching),; ms (EI): m/z (M+) 466. Anal. calcd. for: C, 49.95; H, 2.11; N, 18.00; O, 17.17; S, 12.77. Found: C, 48.92; H, 2.16; N, 18.02; O, 17.15; S, 13.75.

3-(4-bromophenyl)-2-(4-(4-nitrophenyl) thiazol-2-yl)-2H-pyrazolo [3,4d]thiazol-5(6H)-one 5j.

$C_{19}H_{10}BrN_5O_3S_2$ This compound was obtained in 84% yield, mp 210-220°C; 1HNMR (400 MHz, DMSO-*d6*): δ 12.3(s,1H, NH),7.4-7.5(d, 2H), 7.9-8.0(m, 2H), 8.1(s, 1 H,), 8.2-8.2(d, 2H), ; ^{13}C NMR (400 MHz, DMSO-*d6*) ppm: δ 167.88,149.36,145.95, 140.77, 138.43, 132.23, 131,33, 128.44, 127.11, 126.43, 124.15, 123.72, 105.09,; IR (KBr) cm^{-1}: 3345(NH stretching), 1668(C=O stretching), 1579(aromatic C=C stretching),; ms (EI): m/z (M+) 500.36 Anal. calcd. for: C, 47.31; H, 2.04; Br, 14.97; N, 13.99; O, 08.89; S, 12.80. Found: C, 45.61; H, 2.01; Br, 15.97; N, 14.00; O, 09.59; S, 12.82.

3-(4-fluorophenyl)-2-(4-(4-nitrophenyl) thiazol-2-yl)-2H-pyrazolo [3,4d]thiazol-5(6H)-one 5k.

$C_{19}H_{10}FN_5O_3S_2$This compound was obtained in 80% yield, mp 210-220°C; 1HNMR (400 MHz, DMSO-*d6*): δ 12.3(s,1H, NH),7.5-7.6(d, 2H), 7.8-7.9(m, 2H), 8.1(s, 1 H,), 8.2-8.2(d, 2H),; ^{13}C NMR (400 MHz, DMSO-*d6*) ppm: δ 168.33,152.40,144.32, 140.73, 138.12, 133.02, 131,00, 128.02, 127.11, 126.88, 124.01, 123.72, 105.11,; IR (KBr) cm^{-1}: 3352(NH stretching), 1670(C=O stretching), 1581(aromatic C=C stretching),; ms (EI): m/z (M+) 439.44 Anal. calcd. for: C, 51.91; H,2.25; F, 4.36; N, 15.97; O, 10.96; S, 14.55. Found: C, 51.94; H, 2.29; F, 4.32; N, 15.94; O, 10.92; S, 14.59.

3-(4-hydroxyphenyl)-2-(4-(4-nitrophenyl)thiazol-2-yl)-2H-pyrazolo [3,4d]thiazol-5(6H)-one 5l.

$C_{19}H_{11}N_5O_4S_2$ This compound was obtained in 79% yield, mp 210-220°C; 1HNMR (400 MHz, DMSO-*d6*): δ 12.3(s,1H, NH),10.2(s, 1H, OH), 7.3-7.4(d, 2H), 7.7-7.8(m, 2H), 7.9(s, 1 H,), 7.9-8.0(d, 2H),; ^{13}C NMR (400 MHz, DMSO-*d6*) ppm: δ 167.11,151.40,144.05, 139.99, 137.48, 132.21, 131,99, 128.00, 127.01, 126.37, 124.00, 123.21, 104.76,;

IR (KBr) cm^{-1}: 3309(NH stretching), 3200(OH stretching), 1673(C=O stretching), 1597(aromatic C=C stretching),; ms (EI): m/z (M+) 437.03 Anal. calcd. for: C, 52.09; H, 2.65; N, 15.97; O, 14.73; S, 14.56. Found: C, 52.17; H, 2.53; N, 16.01; O, 14.63; S, 14.66.

Cell lines and chemicals

The National Centre for Cell Science (NCCS: A National Cell Line Facility), Pune (MS), India, provided the human breast cancer cell line (MDA-MB 231). Sigma-Aldrich Co. provided the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (St. Louis MO, USA). The remaining reagents and solvents used were of AR grade and bought from commercial sources.

Anticancer Activities

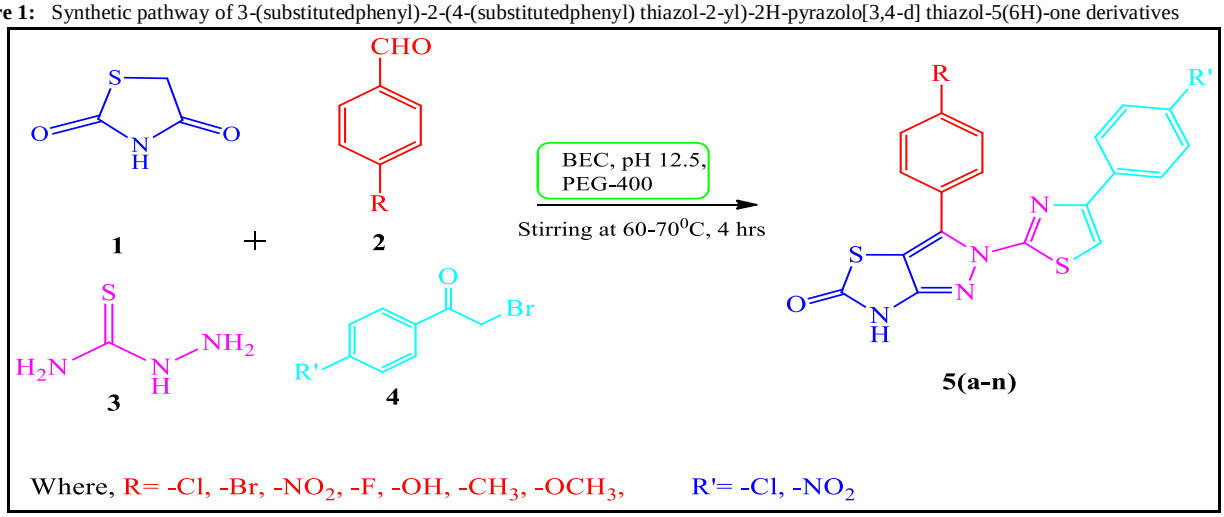
The3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide(MTT) assay was used to determine the cytotoxic capability of the chosen drugs against specific cancer cell lines [47]. In 96 well culture plates, the cells were inoculated at a density of 1×10^5cells/mL.The cells were exposed to various doses of substances dissolved in 0.1% DMSO for 24 hours. Following the initial incubation time, 20 µl of MTT (2 mg/ml) were added to each well, and the cells were then incubated at 37 °C for an additional 4 hours. Further, formazan crystals were dissolved in isopropyl alcohol and therefore the quantity of formazan created was calculable at 570 nm. It has been determined that IC_{50} represents the concentration

RESULTS & DISCUSSION

The reaction pathways used to create compounds 5(a–n)are shown in Figure 1 .In this one-pot multicomponent scheme,an equimolar quantity of Thiazolidione-2,4-dione and substituted aromatic aldehydes was dissolved in a minimum quantity of PEG-400containing a catalytic amount of bleaching earth clay (pH12.5). This mixture was stirred for another hour after adding excess thiosemicarbazide, and when 2-Bromo-1-(4-substituted phenyl) ethanone was added, good to outstanding yields of the desired compounds 5(a-n) were obtained (Table 1).

The electronic environment affects the yield of the reaction. The yield of 3-(substitutedphenyl)-2-(4-(substitutedphenyl)thiazol-2-yl)-2H-pyrazolo[3,4-d]thiazol-5(6H)-one nucleus with electron releasing substituents found to be at the higher side as compared with electron-withdrawing substituents such as $–NO_2$.Electron-withdrawing substituents have a deleterious effect on the yield of the product (Table 1 Entry 1 and 8 to 14).

Figure 1: Synthetic pathway of 3-(substitutedphenyl)-2-(4-(substitutedphenyl) thiazol-2-yl)-2H-pyrazolo[3,4-d] thiazol-5(6H)-one derivatives



Anticancer Activities

The most potent compounds from molecular docking studies (5a, 5b, and 5h, 5i) were evaluated for their anti-breast cancer potential. The growth inhibitory effect was assessed using the human breast cancer cell line MDA MB-231. The results are summarized in Table 2: and expressed as the IC_{50} values. 5-fluorouracil was used as a positive control. Compounds 5b and 5i showed significant anti-breast cancer activity (IC_{50} - 550 ± 0.78, 504 ± 0.89 µM respectively) while the remaining 5a and 5h showed moderate activity against breast cancer cell lines. From IC_{50} values, we can assume that the synthesized derivatives exhibited good to moderate cytotoxic activity against the examined breast cancer cell line.

Table 1: Physiochemical properties of synthesized compounds (a-n)

Product	R	R'	Yield in %	mp^{0}C
5a	-NO_2	-Cl	78	214-216
5b	-Cl	-Cl	90	243-245
5c	-Br	-Cl	88	236-238
5d	-F	-Cl	86	240-242
5e	-OH	-Cl	88	263-265
5f	-CH_3	-Cl	84	231-233
5g	-OCH_3	-Cl	78	261-263
5h	-Cl	-NO_2	82	242-244
5i	- NO_2	-NO_2	73	257-259
5j	-Br	-NO_2	82	248-250
5k	-F	-NO_2	80	260-262
5l	-OH	-NO_2	78	268-270
5m	- CH_3	-NO_2	72	234-235
5n	-OCH_3	-NO_2	72	250-252

Table 2: Anticancer activities of compounds

Compound code	IC_{50} value (µM)
5a	900 ± 0.75
5b	550 ± 0.78
5h	576 ± 0.23
5i	504 ± 0.89
5-fluorouracil	23.5 ± 0.21

In Vitro cytotoxic activity of compounds against breast cancer cell line MDA MB-231. 5-fluorouracil = Positive control. Results are expressed as the mean values from three independent experiments ± Standard deviation (SD).

Docking process validation and Docking Study

Docking process validation was performed by pose selection method by re-docking of respective co-crystal ligand onto each protein separately and comparing the RMSD values of (under 2 Å Standard) between bound co-crystal ligand and re-docked co-crystal ligand [48].

Table 3: Docking scores of synthesized compounds 5(a-n) with five different proteins

Compound ID	Lib Dock Score				
	PDB ID: 2OH4	PDB ID: 4ASD	PDB ID: 1XKK	PDB ID: 6S89	PDB ID: 5CLS
5a	99.5351	102.119	99.3303	101.038	67.2896
5b	**116.745**	**118.145**	**114.885**	**103.224**	**94.1374**
5c	102.482	101.863	91.1277	80.4187	88.6514
5d	101.291	91.112	94.5801	79.0267	91.6546
5e	97.9457	88.0031	95.5167	76.9944	88.8697
5f	94.5001	102.915	91.135	90.9667	64.4444
5g	106.873	108.3547	104.635	100.399	82.0071
5h	**111.072**	**109.323**	**111.369**	**105.191**	**110.269**
5i	**118.195**	**110.561**	**114.096**	**110.254**	**107.904**
5j	112.773	87.4209	106.574	120.07	104.907
5k	110.178	115.481	109.318	103.973	111.17
5l	104.694	104.865	110.99	121.983	106.329
5m	108.455	77.4661	110.501	106.904	100.907
5n	**115.982**	**119.385**	**113.082**	**108.69**	**98.8302**
Sunitinib	118.775	136.456	119.466	104.892	105.393
Doxorubicin	145.643	ND	104.499	93.133	126.412

Initial results of docking validation showed that all the co-crystal ligands have an RMSD value within a 2 Å. Lib dock results suggested that all designed compounds have a good binding affinity towards all five protein structures. Lib dock scores of all compounds and two known standard drugs were reported in **Table 3**: Compound 5i showed the highest lib dock score i.e., **118.195** approximate to the standard sunitinib i.e., 118.775 on 2OH4, which is a protein. Compounds 5b, 5h, 5i, and 5nhave shown a good docking score against all the docked proteins.

When we compared the results of three different targets, we found that our compounds showed more lib dock scores towards VEGFR proteins (PDB ID: 2OH4 & 4ASD) as compared to EGFR and METAP2 proteins. The binding free energy calculations again showed that compound **5i** has the lowest binding free energy (BE = -143.428 kcal/mol)amongst the designed compounds and standard sunitinib (BE = **-128.769** kcal/mol). The remaining compounds have shown binding free energy between -74.482 kcal/mol to -110.665 kcal/mol. To analyze the binding patterns and amino acid interactions within the active site most scoring pose of each compound was selected.

Table 4: Binding free energy, binding affinity, and hydrogen bond interactions of synthesized compounds 5(a-n) with 2OH4 protein

Compound ID	PDB ID: 2OH4		
	Binding free energy (kcal/mol)	Affinity (kcal/mol)	Hydrogen bond Interaction
5a	-85.624	-9.2	LYS918
5b	-102.318	-9.6	ARG1049,SER923
5c	-88.5319	-9.3	-
5d	-90.0492	-9.0	ARG1049
5e	-90.9076	-9.0	ARG1049, LYS918
5f	-74.482	-9.4	LYS918
5g	-88.7404	-9.0	ARG1049, LYS918
5h	-105.019	-9.2	LYS918
5i	**-143.428**	**-9.6**	**ASP1040**, GLU883, SER882
5j	-96.053	-9.2	-
5k	-101.599	-9.1	ARG1049
5l	-110.665	-9.2	ARG1049, LYS918
5m	-93.0873	-9.4	LYS918
5n	-109.527	-9.1	ARG1049, LYS918
Sunitinib	**-128.769**	**-8.5**	**ASP1040, LEU838, CYS917**
Doxorubicin	**-246.79**	**-9.7**	**ARG1030, THR924, ASN921, GLY920, CYS917**

The hydrogen bond interactions shown by each ligand were reported in **Table 4**: Ligand **5i**showed the highest three hydrogen bond interactions withASP1040, GLU883, and SER882, followed by seven hydrophobic interactions with VAL914, VAL897, LEU887, CYS1022, LEU1017, HIS2024, ILE886 amino acid residues. Hydrogen bond interaction with **ASP1040** and hydrophobic interactions with **VAL914, VAL897, LEU887, and HIS2024** amino acid residue was common in ligand 5i and standard sunitinib,which suggests that compound 5ihas a similarity in binding pattern with sunitinib.While other compounds showed one or two hydrogen bond interactions.

The 2D and 3D docking interactions for the most potent ligand 5i and standard sunitinib with 2OH4 protein were shown in

Figure 2. The orientation of the best scoring pose of ligand 5i and sunitinib within the active site of 2OH4 protein is shown in **Figure2**. The interaction of ligands5a, 5b, and 5h are also shown in **Figure 3, Figure 4, and Figure 5** respectively.

Because of their lower binding affinity of -9.6 kcal/mol on 2OH4 protein, the docking data from Auto Dock also suggested and confirmed that compounds 5i and 5b are the most potent.

Figure 2: 2D and 3D interactions of ligand 5i and Sunitinib with 2OH4 rotein

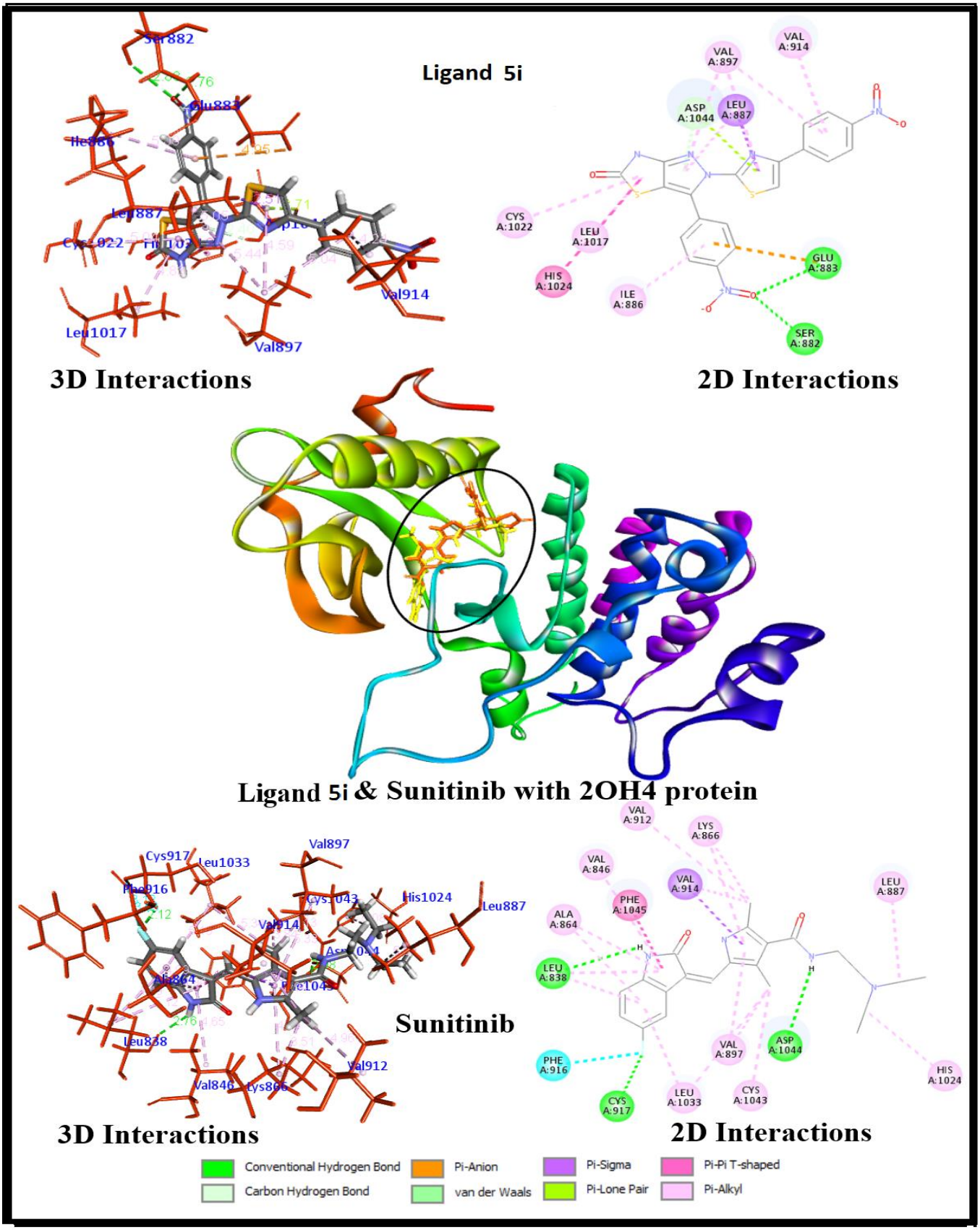


Figure 3: 2D and 3D interactions of ligand 5a

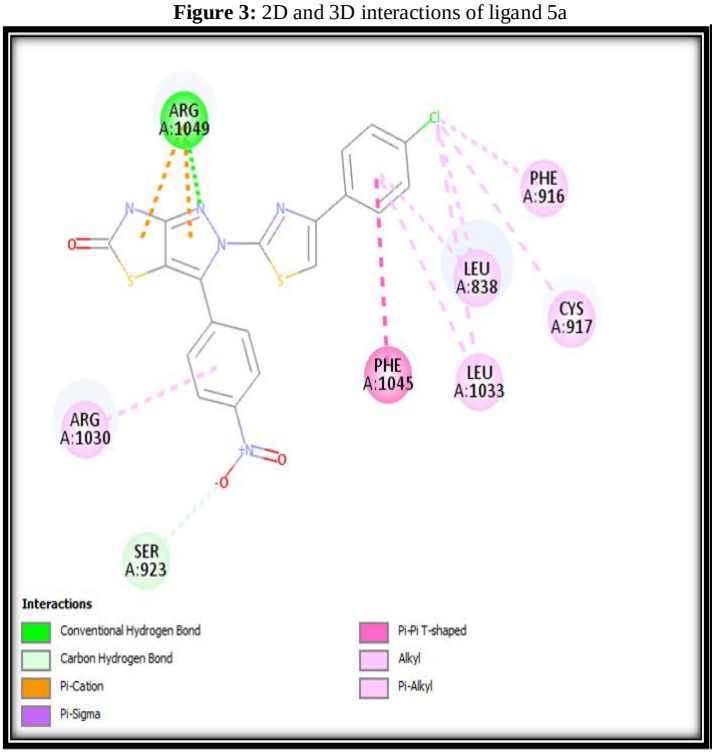


Figure 4: 2D and 3D interaction of ligand 5b

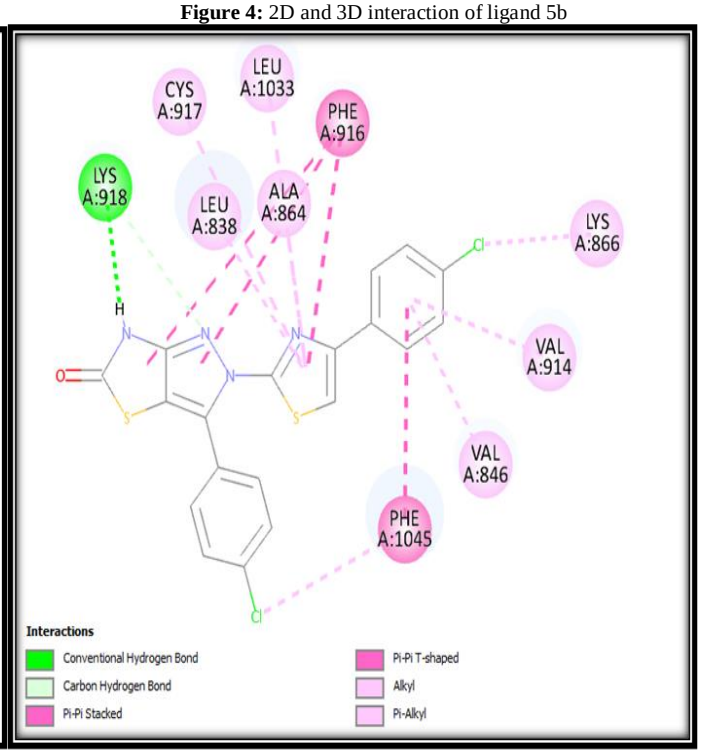
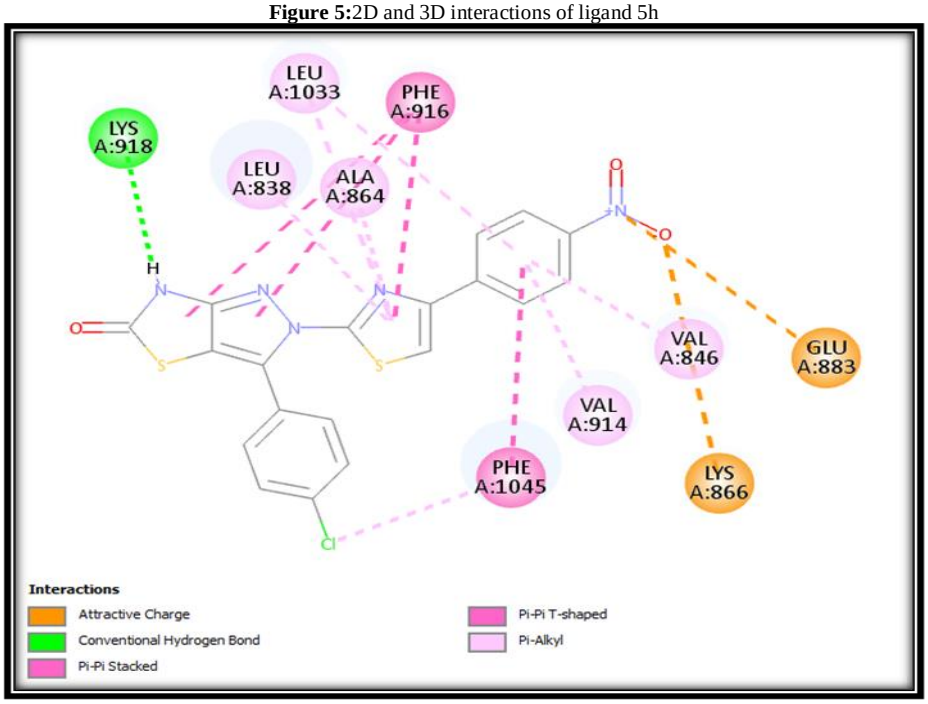


Figure 5:2D and 3D interactions of ligand 5h



CONCLUSION

Compounds **5b** and **5i** are the most potent according to the docking study since they had a lower binding affinity of -9.6 kcal/mol on the 2OH4 protein, and the breast cancer cell line MDM-MB-231 has validated this. The biological test results the following conclusions can be reached about their structure-activity relationships, incorporation of a substituted electron withdrawing group into 3-(substitutedphenyl) and 4-(substitutedphenyl) of the 3-(substitutedphenyl)-2-(4-(substitutedphenyl)thiazol-2-yl)-2H pyrazolo[3,4-d]thiazol-5(6H)-one ring appears to increase anticancer activity and further investigations are in process.

Conflict of interest

Pravin Nandkumar Muliwrote the main manuscript. All authors reviewed, edited the manuscript, and approved this final version. All authors declared that there are no conflicts of interest.

REFERENCES

1. PalSM, Lüchtenborg EA, Davies RH Jack, 2014. The treatment and survival of patients with triple negative breast cancer in a London population. Springer Plus, 3, Pages-553. Doi: 10. 1186/2193-1801-3-553.

2. Zeeneldin AA, Ramadan M, Gaber AA, et al, 2013. Clinico-pathological features of breast carcinoma in elderly Egyptian

patients: a comparison with the non-elderly using population-based data. Journal of the Egyptian National Cancer Institute. 25(1), Pages-5-11. Doi: 10.1016/j.jnci.2012.10. 003.

3. Syed FQ, Elkady AI, Mohammed FA, et al, 2018. Chloroform fraction of Foeniculum vulgare induced ROS mediated, mitochondria-caspase-dependent apoptotic pathway in MCF-7, human breast cancer cell line. Journal of ethno pharmacology. 218(1), Pages-16-26. Doi: 10. 1016/j.jep.2018.02.029.

4. Devi PS, Kumar MS, Das SM, 2011. Evaluation of antiproliferative activity of red sorghum bran anthocyanin on a human breast cancer cell line (mcf-7). International journal of breast cancer. 211(1), Pages-1-6. Doi: 10.4061 /2011 /891481.

5. Khan MF, Alam MM, Verma G, et al, 2016. The therapeutic voyage of pyrazole and its analogs: A review. European Journal of Medicinal Chemistry. 120(1), Pages-170-201. Doi: /10.1016/j.ejmech.2016.04.077.

6. Ebenezer O, Shapi M, Tuszynski JA, 2022. A Review of the Recent Development in the Synthesis and Biological Evaluations of Pyrazole Derivatives. Biomedicines. 10(5), Pages-1124. Doi: ./10.3390/biomedicines10051124.

7. Kumari MA, Venkata rao C A, 2020. Review on Recent Trends in the Bioactive Studies of Pyrazole Derivatives. Asian Journal of Research in Chemistry. 13(5), Pages-383-94. Doi: /10.5958/0974-4150.2020.00072.3.

8. Schito AM, Caviglia D, Brullo C, et.al, 2022. Enhanced Antibacterial Activity of a Cationic Macromolecule by its Complexation with a Weakly Active Pyrazole Derivative. Biomedicines. 10(7), Pages-1607. Doi: 10.3390/bio medicines10071607.

9. B Bhatt H, Sharma S, 2017. Synthesis and antimicrobial activity of pyrazole nucleus containing 2-thioxothiazolidin-4-one derivatives. Arabian Journal of Chemistry. 10(2), Pages-1590-1596. Doi: 10.1016/j.arabjc.2013.05.029.

10. Koca İ, Özgür A, Coşkun KA, et al, 2013. Synthesis and anticancer activity of acyl thioureas bearing pyrazole moiety. Bioorganic & medicinal chemistry. 21(23), Pages3859-3865. Doi: 10.1016/j.bmc.2013.04.021.

11. Dawood KM, Eldebss TM, El-Zahabi HS, et al, 2013. Synthesis of some new pyrazole based 1,3-thiazoles and 1,3,4-thiadiazoles as anticancer agents. European Journal of Medicinal Chemistry. 70(1), Pages-740-749. Doi: 10.1016/j.ejmech.2013.10. 042.

12. AnwarMM, Abd El-KarimSS, MahmoudAH, et al, 2019. A Comparative Study of the Anticancer Activity and PARP-1 Inhibiting Effect of Benzofuran–Pyrazole Scaffold and Its Nano-Sized Particles in Human Breast Cancer Cells. Molecules. 24(13), Pages-2413. Doi: 10.3390/molecules24132413.

13. Jeong M, Jung E, Lee YH, et al, 2020. A Novel Synthetic Compound (E)-5-((4-oxo-4Hchromen-3-yl) methyleneamino)-1-phenyl-1Hpyrazole-4-carbonitrile Inhibits TNF-Induced MMP9 Expression via EGR-1 Downregulation in MDA-MB-231 Human Breast Cancer CellsInt. International journal of molecular sciences. 21(14), Pages-5080. Doi: 10. 3390/ijms21145080.

14. Ashourpour M, Mostafavi-Hosseini F, Amini M, et al, 2021. Pyrazole Derivatives Induce Apoptosis via ROS Generation in the Triple Negative Breast Cancer Cells, MDA-MB-468. Asian Pacific Journal of Cancer Prevention: APJCP. 22(1), Pages-2079. Doi: 10.31557/APJCP.2021.22.7.2079.

15. Dawood DH, Nossier ES, Ali MM, et al, 2020. Synthesis and molecular docking study of new pyrazole derivatives as potent anti-breast cancer agents targeting VEGFR-2 kinase. Bioorganic Chemistry. 101(1), Pages-103916. Doi: .10.1016/j. bioorg.2020.103916.

16. Abdel-Aziz M, Abuo-Rahma GE, Hassan AA, 2009. Synthesis of novel pyrrazole derivatives and evaluation of their antidepressant and anticonvulsant activities. European Journal of Medicinal Chemistry. 44(9), Pages-3480-3487. Doi: /10.1016/j.ejmech.2009.01.032.

17. Kaushik D, Khan SA, Chawla G, et al, 2010. N'- [(5-chloro-3-methyl-1- phenyl-1H-pyrazol-4-yl) methylene]2/4-substituted hydrazides: synthesis and anticonvulsant activity. European Journal of Medicinal Chemistry. 45(9), Pages-3943-3949. Doi: ./10.1016/j.ejmech.2010.05.049.

18. Khunt RC, Khedkar VM, Chawda RS, et al, 2012. Synthesis, antitubercular evaluation and 3D-QSAR study of Nphenyl-3-(4-fluorophenyl)-4-substituted pyrazole derivatives. Bioorganic& medicinal chemistry letters. 22(6), Pages-666-678. Doi: ./10.1016/j.bmcl.2011.10.059.

19. Raffa D, Maggio B, Raimondi MV, et al, 2015. Recent advances in bioactive systems containing pyrazole fused with five membered heterocycles. European Journal of Medicinal Chemistry. 97(1), Pages-732-746. Doi: ./10.1016/j. ejmech.2014.12.023.

20. Vijesh AM, Isloor AM, Shetty P, et al, 2013. New Pyrrazole derivatives containing 1,2,4-triazoles and benzoxazoles as potent antimicrobial and analgesic agents. European Journal of Medicinal Chemistry. 62(1), Pages-410-415. Doi: .10.1016/j.ejmech.2012.12.057.

21. Pathak RB, Chovatia PT, Parekh HH, 2012. Synthesis, antitubercular and antimicrobial evaluation of 3-(4-chlrophenyl)-4-substituted pyrazole derivatives. Bioorganic & medicinal chemistry letters. 22(10), Pages-5129-5133. Doi: /10.1016/j.bmcl.2012.05.063.

22. Daidone G, Maggio B, Raffa D, et al, 1994. Synthesis and pharmacological study of ethyl-1-methyl-5-[2-substituted-4-oxo-3(4H)-quinazolinyl]-1H-pyrazole-4-acetates. European Journal of Medicinal Chemistry. 29(9), Pages-707-711. Doi: /10.1016/0223-5234(94)90033-7.

23. Gökhan-Kelekçi N, Yabanoğlu S, Küpeli E, et al,2007. A new therapeutic approach in Alzheimer disease: some novel pyrazole derivatives as dual MAO-B inhibitors and anti-inflammatory analgesics. Bioorganic & medicinal chemistry. 15(17), Pages-5775-5786.Doi: ./10.1016/j.bmc.2007.06.004.

24. Sadek B, Al-Tabakha MM, Fahelelbom KM, 2011. Antimicrobial Prospect of Newly Synthesized 1,3-Thiazole Derivatives. Molecules. 16(11), Pages-9386-9396. Doi: ./10.3390/molecules16119386

25. Paget CJ, Kisner K, Stone RL, et al, 1969. Heterocyclic substituted ureas. II. Immunosuppressive and antiviral activity of benzothiazolyl- and benzoxazolylureas. Journal of medicinal

chemistry. 12(6), Pages-1016–1018. Doi: ./10.1021/jm00 306a011.

26. Carter JS, Kramer S, Talley JJ, et al, 1999. Synthesis and activity of sulphonamide substituted 4,5-diarylthiazoles as selective cyclooxygenase- 2 inhibitors. Bioorganic & medicinal chemistry letters. 9(8), Pages-1171-1174. Doi: 10. 1016/S0960-894X(99)00157-2.

27. Ergenç N, Çapan G, Günay NS, et al, 1999. Synthesis and Hypnotic Activity of New 4-Thiazolidinone and 2-Thioxo-4, 5-imidazolidinedione Derivatives. Archiv der Pharmazie: An International Journal Pharmaceutical and Medicinal Chemistry. 332(10), Pages-343-347. Doi: doi: 10.1002/(sici)1521-4184(199910)332:10<343::aid-ardp343>3.0.co;2-0.

28. Hargrave KD, Hess FK, Oliver JT, 1983. N-(4-Substituted-thiazolyl) oxamic acid derivatives, new series of potent, orally active antiallergy agents. Journal of medicinal chemistry. 26(8), 1158-1163.Doi: ./10.1021/jm00362a014.

29. Bell FW, Cantrell AS, Hoegberg M, et al, 1995. Phenethylthiazolethiourea (PETT) compounds, a new class of HIV-1 reverse transcriptase inhibitors. 1. Synthesis and basic structure-activity relationship studies of PETT analogs. Journal of medicinal chemistry. 38(25), Pages-4929-4936. Doi: ./10.1021/jm00025a010.

30. Havrylyuk D, Zimenkovsky B, Vasylenko O, et al, 2009. Synthesis of novel thiazolone-based compounds containing pyrazoline moiety and evaluation of their anticancer activity. European Journal of Medicinal Chemistry. 44(4), Pages-1396–1404. Doi: ./10.1016/j.ejmech.2008.09.032.

31. Liu X, Deng L, Song H, et al, 2011. Asymmetric Aza-Mannich Addition: Synthesis of Modified Chiral 2-(Ethylthio)-thiazolone Derivatives with Anticancer Potency. Organic Letters. 13(6), Pages-1494-1497.Doi:./10.1021/ol200185h.

32. Khalil NA, Ahmed EM, El-Nassan HB, 2013. Synthesis, characterization, and biological evaluation of certain 1,3-thiazolone derivatives bearing pyrazoline moiety as potential anti-breast cancer agents. Medicinal Chemistry Research. 22(1), Pages-1021–1027. Doi: 10.1007/s00044-012-0098-7.

33. Beedkar SD, Khobragade CN, Chobe SS, et al, 2012. Novel thiazolo-pyrazolyl derivatives as xanthine oxidase inhibitors and free radical scavengers. International journal of biological macromolecules. 50, Pages-947-56. Doi: 10.1016/j.ijbio mac.2012.02.009.

34. Kamble RD, Meshram RJ, Hese SV, et al, 2016. Synthesis and in silico investigation of thiazoles bearing pyrazoles derivatives as anti-inflammatory agents. Computational biology and chemistry. 61(1), Pages-86 96. Doi: 10.1016/j.compbio lchem.2016.01.007.

35. Vishvakarma VK, Singh P, Kumar V, et al,2019.Pyrrolothiazolones as Potential Inhibitors for the nsP2BnsP3 Protease of Dengue Virus and Their Mechanism of Synthesis. Chemistry Select. 4(32), Pages-9410–9419. Doi: ./10.1002/slct.201901119

36. Aboelnaga A, Mansour E, Fahim AM, et al, 2021. Synthesis, anti-proliferative activity, gene expression, docking and DFT investigation of novel pyrazol-1-yl-thiazol-4(5H)-one derivatives. Journal of molecular structure. 1251, Pages-131945. Doi: ./10.1016/j.molstruc.2021.131945.

37. Trapani G, Franco M, Latrofa A, et al, 1994. Synthesis and anticonvulsant activity of some 1,2,3,3a-tetrahydropyrrolo [2,1-b] benzothiazol-ones and pyrrolo[2, M]thiazole analogues. European Journal of Medicinal Chemistry. 29(3), Pages-197-204. Doi: ./10.1016/j.molstruc.2021.131945.

38. Abdelhamid AO, Abdelall EK, Abdel-Riheem NA, et al, 2010. Synthesis and antimicrobial activity of some new 5-arylazothiazole, pyrazolo [1, 5-a] pyrimidine,[1, 2, 4] triazolo [4, 3-a] pyrimidine, and pyrimido [1, 2-a] benzimidazole derivatives containing the thiazole moiety. Phosphorus, Sulfur, and Silicon and Related elements. 185(4), Pages-709-18.Doi: 10. 1134/S1068162021020102.

39. El-Sabbagh OI, Baraka MM, Ibrahim SM, et al, 2009. Synthesis and antiviral activity of new pyrazole and thiazole derivatives. European journal of medicinal chemistry. 44(9), Pages-3746-3753. Doi: ./10.1016/j.ejmech.2009.03.038.

40. Kasralikar HM, Jadhavar SC, Goswami SV, et al, 2019. Design, synthesis and molecular docking of pyrazolo [3, 4d] thiazole hybrids as potential anti-HIV-1 NNRT inhibitors. Bioorganic Chemistry. 86(1), Pages-437-444. Doi; ./10.1016/j.bioorg. 2019.02.006.

41. Alsayari A, Muhsinah AB, Asiri YI, et al, 2021. Synthesis, Characterization, and Biological Evaluation of Some Novel Pyrazolo [5, 1-b] thiazole Derivatives as Potential Antimicrobial and Anticancer Agents. Molecules. 26(17), Pages-5383 Doi: ./10.3390/molecules26175383.

42. El-Sabbagh OI, Baraka MM, Ibrahim SM, et al, 2009. Synthesis and antiviral activity of new pyrazole and thiazole derivatives. European journal of medicinal chemistry. 44(9), Pages-3746-3753. Doi:10.1016/j.ejmech.2009.03.038.

43. Lee HS, Seo EY, Kang NE, et al, 2008. [6]-Gingerol inhibits metastasis of MDA-MB-231 human breast cancer cells. The Journal of nutritional biochemistry. 19(5), Pages-313-319. Doi:10.1016/j.jnutbio.2007.05.008.

44. Matore BW, Banjare P, Singh J, et al, 2022. In silico selectivity modeling of pyridine and pyrimidine based CYP11B1 and CYP11B2 inhibitors: A case study. Journal of Molecular Graphics and Modelling. 116, Pages-108238. Doi: ./10.1016/j.jmgm.2022.108238.

How to cite this article

Bhaskar Sadashiv Dawane, Pravin Nandakumar Muli, Sonali Shankar Kamble, Sanjay Sudhakar Pekamwar, Balaji Wamanrao Matore, Jagadish Singh, ParthaPratim Roy, Megha Mukund Muley, 2022. Design, synthesis and evaluation of novel 3-(substitutedphenyl)-2-(4-(substitutedphenyl) thiazol-2-yl)-2h-pyrazolo [3, 4-d] thiazol-5(6h)-one derivatives as anti-breast cancer agents. Journal of medical pharmaceutical and allied sciences, V 11 - I 6, Pages - 5453 – 5460. Doi: 10.55522/jmpas.V11I6.4475.