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ARTICLE

Design, Synthesis and Molecular Docking Studies of 4-{3-[2-(2-Morpholin-4-yl-ethoxy)phenyl]-5-phenyl-pyrazol-1-yl}-benzenesulfonamide as Anti-Breast Cancer Agent

Praveen Kumar[1], Jai Prakash Kumar[2,✉], Juhi Barnwal[3] and Ritu Singh[4]

Asian Journal of Organic & Medicinal Chemistry

Volume: 5 Year: 2020
Issue: 4 Month: October–December
pp: 301–306
DOI: https://doi.org/10.14233/ajomc.2020.AJOMC-P271

Received: 20 July 2020
Accepted: 20 October 2020
Published: 31 December 2020

ABSTRACT

Novel 4-{3-[2-(2-morpholin-4-yl-ethoxy)phenyl]-5-phenyl-pyrazol-1-yl}benzenesulfonamide (**7**) was synthesized and evaluated for its anti-breast cancer activity. It was prepared by cyclocondensation reaction of morpholine-substituted β-diketone, 1-[2-(2-morpholin-4-yl-ethoxy)-phenyl]-3-phenyl-propane-1,3-dione (**3**) with 4-hydrazinobenzene-sulfonamide hydrochloride (**6**). Chemical structure of titled compound (**7**) was confirmed by FTIR, ^{1}H & ^{13}C NMR and HRMS spectroscoic analyses. The anticancer activity of titled compound **7** was evaluated against MCF-7 breast cancer cell line by MTT assay. Molecular docking was performed to predict its plausible binding with the estrogen receptor α(ERα) using Molecular Operating Environment 2019.0101 software. The MTT assay results showed that titled compound **7** exhibited better anticancer activity against MCF7 cells (IC_{50}: 4.25 μM) than standard drug, 4-hydroxytamoxifen (IC_{50}: 8.22 μM). Results of molecular docking studies were found in good agreement with the results of anticancer evaluation, as the binding score of titled compound **7** (-16.9872 kcal/mol) was lower as compared to 4-hydroxytamoxifen (-15.1112 kcal/mol). The new cationic interaction of titled compound **7** with Trp383 and hydrogen bonding interaction with Phe404 in active site of ERα made its anticancer activity better than 4-hydroxytamoxifen. Thus, 4-{3-[2-(2-morpholin-4-yl-ethoxy)phenyl]-5-phenyl-pyrazol-1-yl}benzenesulfonamide (**7**) was emerged as a potent anti-breast cancer agent.

KEYWORDS

Celecoxib, Benzenesulfonamide derivative, Molecular docking, anti-breast cancer agent.

Author affiliations:

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Uttar Pradesh University of Medical Sciences, Saifai, Etawah-206130, India
[2]University Department of Chemistry, Magadh University, Bodh Gaya-824234, India
[3]Department of Chemistry, Ranchi University, Ranchi-834002, India
[4]Department of Chemistry, S.S.V. College, Hapur-245101, India

✉To whom correspondence to be addressed:

E-mail: jaiprakashkumar@gmx.com

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INTRODUCTION

Breast cancer is a disease in which cells in breast tissue change and divide in uncontrolled manner, particularly resulting in formation of lumps. According to the American Cancer Society, an estimated 268,600 new cases of invasive breast cancer were diagnosed among women and approximately 2,670 cases were diagnosed in men in 2019. Approximately, 41,760 women and 500 men died from breast cancer in 2019 [1].

Selective estrogen receptor modulators (SERMs) are anti-estrogen drugs that act on the estrogen receptor. Tamoxifen, the oldest and the most prescribed SERMs is used in the treatment of breast cancer. The active metabolite of tamoxifen, 4-

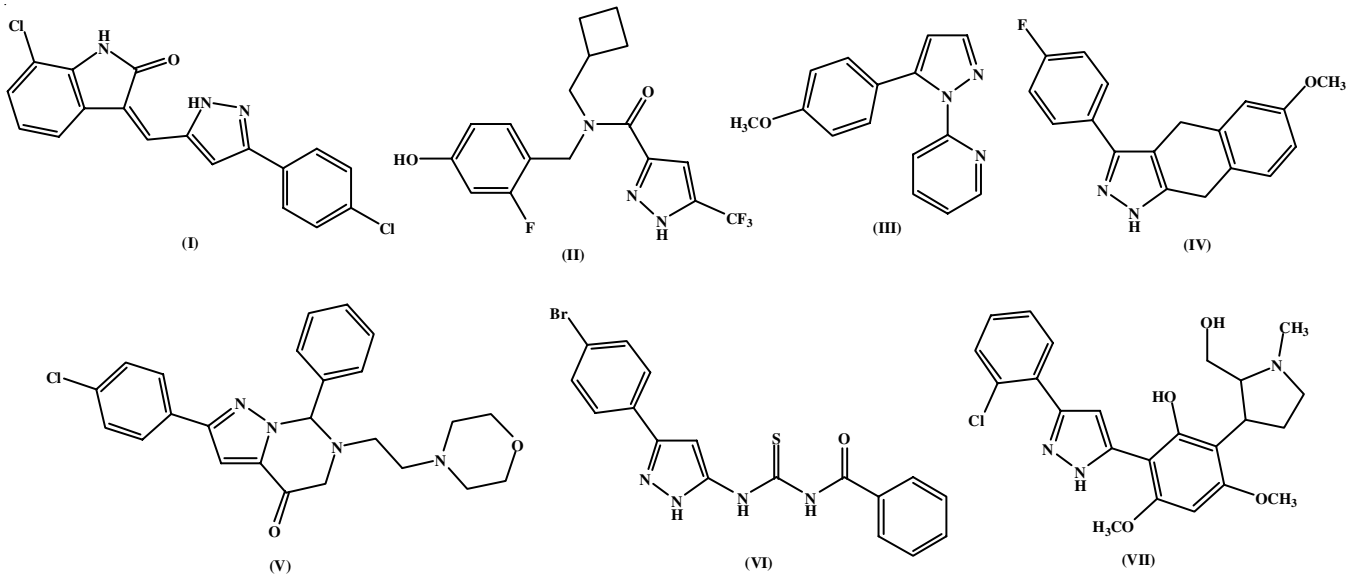


Fig. 1. Pyrazole derivatives with anticancer activities (**I-VII**)

hydroxytamoxifen (4-OHT) is generated *in vivo* by the enzyme CYP2D6. It was found effective in treatment of breast cancer in women. Despite the effectiveness of 4-OHT in breast cancer treatment, this drug also had adverse effects, such as blood clots, strokes and increased incidence of endometrial cancer in women [2]. Research shows that long-term administration of Tamoxifen also caused hepatic tumors in rats [3]. Thus, an alternative SERMs needs to be developed having lesser adverse effects.

Pyrazoles and their derivatives exhibited various biological activities such as antimicrobial [4], anti-tuberculosis [5], anti-inflammatory [6], antitumor [7] and analgesic [8] agents. Pyrazole can be synthesized by Knorr reaction or through the pyrazoline pathway, which involves the reaction of α,β-unsaturated ketone with hydrazine derivates [9], then oxidative aromatization to the corresponding pyrazole molecule [10]. Literature review revealed that various pyrazole derivatives exhibited anticancer activity [11] (Fig. 1), with few effective agents against breast cancer cell lines [12,13]. Furthermore, some pyrazole derivatives have been patented for their anticancer activity *e.g.*, substituted pyrazole derivatives for treatment of hepatic cancer *via* HePG-2 cell lines [14] and celecoxib for treatment of breast cancers [15].

Celecoxib repressed the proliferation of breast cancer cells *in vitro* and also prevent the incidences of breast cancer chemically induced by 7,12-dimethylbenzanthracene (DMBA) in rats [16]. According to Winfield & Payton-Stewart [17], sulfonamide part of celecoxib is an essential pharmacophoric requirement for anticancer activity (Fig. 2). Specifically, 1,3,5-triaryl pyrazole derivatives have been reported to have biological activity against various cancer cells such as MCF-7, MGC-803, HeLa dan Huh-718.

In the present research work, novel 1,3,5-triaryl pyrazole derivative containing morpholine and sulfonamide moiety was synthesized. Compound 4-{3-[2-(2-morpholin-4-yl-ethoxy)-phenyl]-5-phenyl-pyrazol-1-yl}benzenesulfonamide (**7**) was synthesized *via* cyclocondensation reaction of morpholine-substituted β-diketone, 1-[2-(2-morpholin-4-yl-ethoxy)-

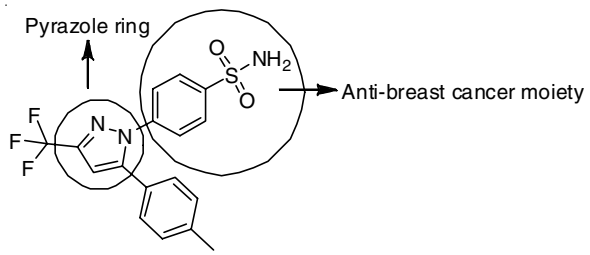


Fig. 2. Celecoxib as anticancer agent

phenyl]-3-phenyl-propane-1,3-dione (**3**) with 4-hydrazino-benzenesulfonamide hydrochloride (**6**). The titled compound **7** was evaluated for its anticancer activity *in vitro* against MCF-7 cell lines taking 4-hydroxytamoxifen (4-OHT) as standard drug. Molecular docking study with human estrogen alpha receptor (ERα) was performed to ascertain the molecular mechanism of action of the compound **7** as anti-breast cancer agent using 4-hydroxytamoxifen as reference standard.

EXPERIMENTAL

Chemicals and solvents of analytical grade were procured from Sigma-Aldrich, Merck India (Mumbai, India) and used without further purification. Titled compound **7** was synthesized using sealed-vessel reactor-Anton Paar Monowave 50. Reaction progress was monitored by TLC under UV Lamp 254/366 nm (Cole-Parmer). Melting point was determined on Fisher-Johns apparatus and reported uncorrected. UV spectrum was recorded on Genesys™ 10S UV-Visible spectrophotometer. FTIR spectra was recorded in KBr pellet on Shimadzu® FT-IR Prestige-21 spectrophotometer. Mass spectral data was recorded on mass spectrometer-Water LCT premier XE positive mode. ^{1}H NMR and ^{13}C NMR spectral data were recorded on Agilent® at 500 MHz and 125 MHz, respectively.

Synthesis: The synthetic route of titled compound **7** is outlined in **Scheme-I**.

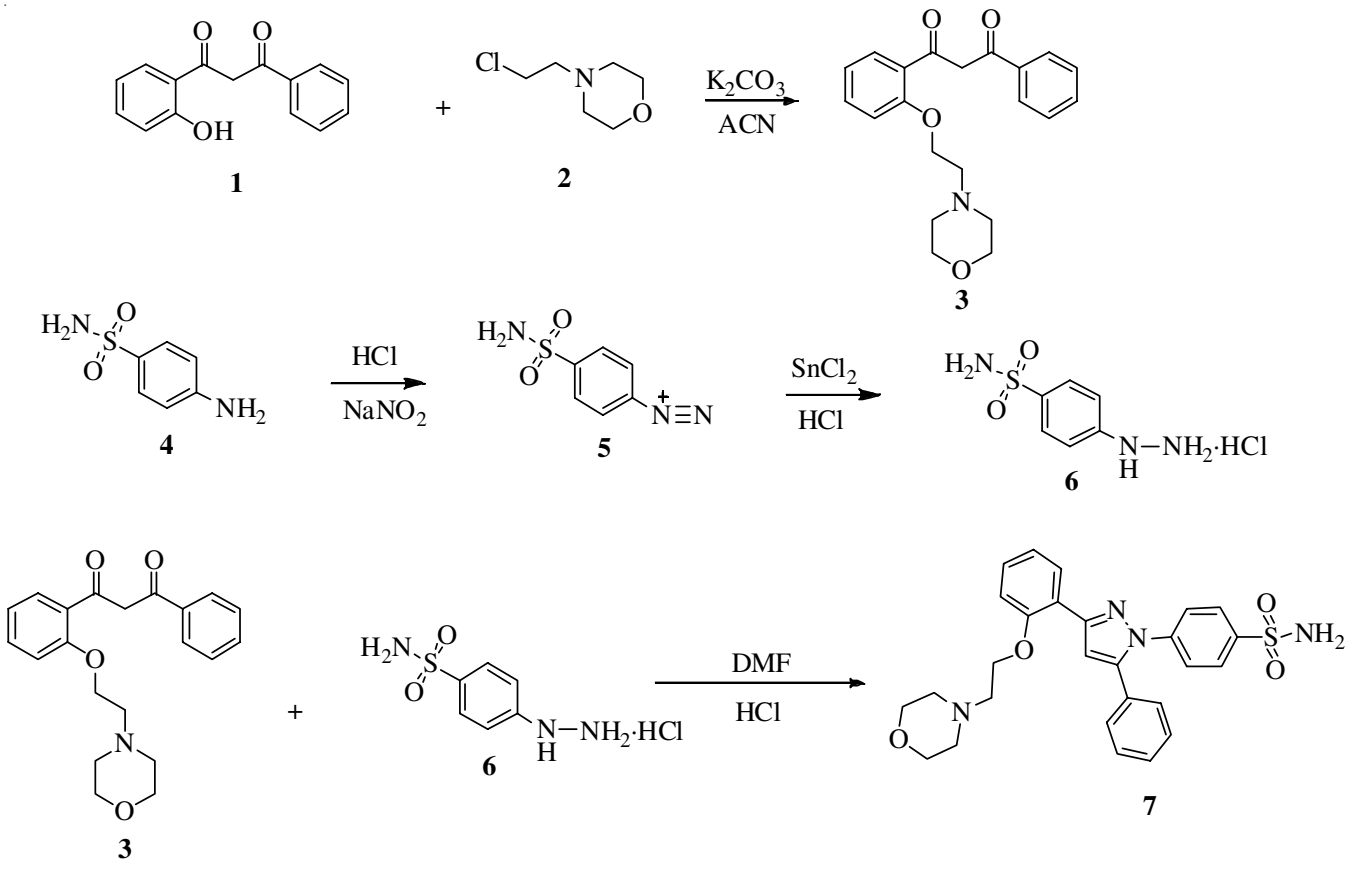
Synthesis of 1-[2-(2-morpholin-4-yl-ethoxy)phenyl]-3-phenyl-propane-1,3-dione (3): 1-(2-Hydroxy-phenyl)-3-phenyl-propane-1,3-dione (**1**) (3 mmol) and 4-(2-chloro-ethyl)-

morpholine hydrochloride (**2**) (4.5 mmol) were dissolved in acetonitrile (5 mL). The reaction was catalyzed using potassium carbonate (6 mmol). The reaction mixture was reacted using sealed-vessel reactor at 80 °C in pressure tube with a stir bar. The reaction progress was monitored every 30 min using TLC. After completion of the reaction, the reaction mixture was cooled and concentrated using a rotary evaporator. Crude product was extracted using ethyl acetate:water (3:15 mL). The organic layer was taken, dried using anhydrous sodium sulfate and evaporated using rotary evaporator to obtain 1-[2-(2-morpholin-4-yl-ethoxy)phenyl]-3-phenyl-propane-1,3-dione (**3**).

Synthesis of 4-hydrazino-benzenesulfonamide hydrochloride (6): The hydrazine compound **6** was synthesized by patented method [19]. A mixture of 4-amino-benzenesulfonamide (**4**) (20 mmol), conc. HCl (10 mL) and crushed ice (20 g) in 100 mL sized Erlenmeyer flask was placed in the ice bath, followed by drop-wise addition of $NaNO_2$ (20 mmol) in 2 mL water while stirring with a magnetic stirrer. The reaction mixture was allowed to undergo diazotization reaction until solution became clear. After completion of the reaction, the reaction mixture was poured into the Erlenmeyer flask filled with cold solution of $SnCl_2 \cdot H_2O$ (10 g) in conc. HCl (10 mL). The mixture was stirred rapidly until solid product was formed. Then the mixture was allowed to stand overnight. Solid product thus obtained was filtered using vacuum and dried to obtain 4-hydrazino-benzenesulfonamide hydrochloride (**6**).

Synthesis of 4-{3-[2-(2-morpholin-4-yl-ethoxy)phenyl]-5-phenyl-pyrazol-1-yl}benzenesulfonamide (7): 1-[2-(2-Morpholin-4-yl-ethoxy)phenyl]-3-phenyl-propane-1,3-dione (**3**) (1 mmol) and 4-hydrazino-benzenesulfonamide hydrochloride (**6**) (1 mmol) were dissolved in DMF (5 mL). 10 N HCl was added to the reaction mixture. The reaction mixture was refluxed at 80 °C in a three-neck round bottom flask. The reaction was monitored for completion after every 1 h using TLC. After completion, the reaction mixture was poured into 30 g crushed ice. The mixture was allowed to stand until maximum precipitate was formed. The solid product obtained was filtered using a vacuum, washed with water and *n*-hexane and air dried at room temperature. The solid product obtained was purified using column chromatography using isocratic system of *n*-hexane-ethyl acetate (9:1) to obtain 4-{3-[2-(2-Morpholin-4-yl-ethoxy)phenyl]-5-phenyl-pyrazol-1-yl}benzenesulfonamide (**7**).

Molecular formula: $C_{27}H_{28}N_4O_4S$; Yellow solid; Yield: 61%; m.p.: 168-169 °C; HPLC t_R: 17.652 min; FTIR (KBr, ν, cm^{-1}): 3325 (-NH), 1597 (-C=N), 1464 (-C=C aromatic), 1354 (S=O sulfonamide); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.01 (dd, *J* = 7.65, 1,75 Hz, 1H, Ar-H), 7.83 (d, *J* = 8.3, 2H, Ar-H), 7.51 (d, *J* = 8.3, 2H, Ar-H), 7.46-7.42 (m, 5H, Ar-H), 7.35 (t, *J* = 8.0, 1H, Ar-H), 7.33 (br s, NH_2), 7.32 (s, 1H, pyrazole ring-H), 7.16 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.03 (t, *J* = 7.5 Hz, 1H, Ar-H), 4.20 (t, *J* = 5.4 Hz 2H, O-CH_2), 3.46 (t, *J* = 4.6 Hz, 4H, O-$(CH_2)_2$ morpholine), 2.77 (t, *J* = 5.4 Hz, 2H, N-CH_2), 2.45 (br s, 4H, N-$(CH_2)_2$ morpholine); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 156.49, 149.36, 143.72, 142.98, 142.51, 130.57, 130.39, 129.98, 129.27, 129.17, 129.09, 128.23, 127.08, 125.30, 121.36, 121.06, 113.25, 111.21, 66.61, 65.35, 57.58, 53.87; *m/z* $[M+H]^+$ (%): 505.1936 (100).



Scheme-I: Synthetic route of 4-{3-[2-(2-morpholin-4-yl-ethoxy)-phenyl]-5-phenyl-pyrazol-1-yl}-benzenesulfonamide (**7**)

Anticancer evaluation: Anticancer evaluation of compound **7** was performed against MCF-7 cell line using 3-(4,5-dimethyltiazol-2-il)-2,5-diphenitetrazolium bromide (MTT) method. Cells were placed in 96-well culture plates with a density of 1×10^4 cells/well and allowed to stick. After 24 h, the compound **7** in different concentrations ranging from 50 µg/mL to 3.125 µg/mL was added and the culture plates incubated at 37 ºC in an atmosphere of 5% CO_2 for 24, 48 and 72 h 20 µL MTT was added to each well and further incubated under the same conditions for 2 h. After incubation, the solution in each well was removed and 100 µL DMSO was added to the well to dissolve the resulting formazan. The last cell line well was used as a negative control, in which only culture media was added. Cisplatin was used as a positive control. Each concentration of the compound **7** was tested in three repetitive evaluations. Absorbance was measured at a wavelength of 550 nm. The results were expressed as a percentage of cell viability (%). Concentration of compound with 50% cell growth inhibition was expressed as the median value of inhibitory concentration (IC_{50}).

Molecular docking studies: Docking studies of compound **7** were carried out using Molecular Operating Environment 2019.0101 software (MOE of Chemical Computing Group Inc). Crystal structure of human estrogen α receptor (ERα) (PDB ID: 3ERT) was taken from Protein Data Bank. Receptor protein was prepared using structure preparation wizard. All polar hydrogen atoms were added and residue issues were corrected. The energy minimization of protein was done using MMFF94x force field. Ligand compound was sketched using MOE molecule builder. Active sites were identified using site finder features of MOE. Poses for compound **7** was scored by initial rescoring methodology (London dG) and the final rescoring methodology (London dG) by placement using Triangle Matcher protocol and post-placement refinement was rigid receptor. Proposed docking protocol was validated by re-docking of the co-crystallized native ligand into the active site of 3ERT. Lowest binding energy conformations were picked and visualized using BIOVIA Discovery Visualizer 2019.

RESULTS AND DISCUSSION

Novel 1,3,5-triaryl pyrazole compound containing morpholine and sulfonamide moiety was synthesized through three step reaction. First step involved the synthesis of 1-[2-(2-morpholin-4-yl-ethoxy)phenyl]-3-phenyl-propane-1,3-dione (**3**). In this reaction the oxygen in hydroxyl group of 1-(2-hydroxy-phenyl)-3-phenyl-propane-1,3-dione (**1**) acted as a nucleophile for the attack of 4-(2-chloro-ethyl)-morpholine hydrochloride (**2**) resulting in alkylation reaction. In the second step, 4-hydrazino-benzenesulfonamide hydrochloride (**6**) was prepared from 4-Amino-benzenesulfonamide (**4**). 4-amino-benzenesulfonamide ***4*** was first converted to diazo compound 4-sulfamoyl-benzenediazonium (**5**) *via* diazotization reaction. It was further reduced with $SnCl_2$ to yield 4-hydrazino-benzenesulfonamide hydrochloride (**6**). The last step involved the cyclocondensation of 1-[2-(2-morpholin-4-yl-ethoxy)phenyl]-3-phenyl-propane-1,3-dione (**3**) with 4-hydrazineylbenzenesulfonamide (**6**) to obtain 4-{3-[2-(2-morpholin-4-yl-ethoxy)phenyl]-5-phenyl-pyrazol-1-yl}benzenesulfonamide (**7**) in good yield.

Compound **7** was characterized by FTIR, ^1H & ^{13}C NMR spectroscopy and the chemical formula was confirmed by high resolution mass spectrometry. In FTIR spectra, the vibration of NH stretching of sulfonamide appeared in region 3325 cm^{-1} and the vibration of -S=O stretching was found at 1354 cm^{-1}. In ^1H NMR spectra, the formation of pyrazole was confirmed by singlet signal appeared at downfield region around δ 7.32 which indicated C_4 proton of pyrazole ring. A singlet signal at δ 7.33 ppm showed the protons of NH_2 group in sulfonamide moiety while the protons of morpholine moiety was found at δ 2.45-4.21. Furthermore, the most deshielded peak of ^{13}C NMR at 156.49, 149.36 was indicated as peak of C_3 (C=N) and C_5 (C-N) in pyrazole ring. The HRMS analysis of compound **7** demonstrates $[M+H]^+$ molecular ion peak at *m/z*: 505.1936 with 100% abundance. This molecular weight corresponds to the calculated mass, *m/z*: 505.1910. Therefore, it was concluded that the titled compound **7** was synthesized as per synthetic route depicted in **Scheme-I**.

Anticancer activity: The anti-breast cancer activity of compound **7** was studied against MCF-7 breast cancer cells by MTT assay. The MTT assay is a colourimetric assay for measuring the cellular growth that reduces the yellow coloured MTT to purple formazan by mitochondrial reductase of living cells. At a particular wavelength, the absorbance of this coloured solution can be measured and the IC_{50} values were calculated. The drug, 4-Hydroxytamoxifen was used as positive control. Compound **7** displayed better activity with IC_{50} value: 4.25 µM compared to 4-Hydroxytamoxifen (4-OHT), IC_{50}: 8.218 µM (Table-1). The anticancer activity results indicated compound **7** as a promising anti-breast cancer agent.

Molecular docking studies: Molecular docking simulation of 4-{3-[2-(2-morpholin-4-yl-ethoxy)phenyl]-5-phenyl-pyrazol-1-yl}benzenesulfonamide (**7**) was performed to identify its potential binding modes and investigate its similarity to the standard ligand binding modes. Before performing docking of compound **7**, the method for molecular docking simulation was validated by re-docking of reference ligand, 4-OHT with ERα receptor to verify that docking protocol can be used in this simulation. The best conformation of re-docked 4-OHT has root mean square deviation (RMSD) of 0.7104 Å which is lower than 2 Å which confirmed the similarity in binding patterns of compound **7** with 4-OHT in ERα receptor [20,21].

TABLE-1
ANTICANCER ACTIVITY AND MOLECULAR DOCKING STUDY OF
4-{3-[2-(2-MORPHOLIN-4-YL-ETHOXY)-PHENYL]-5-PHENYL-PYRAZOL-1-YL}-BENZENESULFONAMIDE (**7**)

Compound	IC_{50} (µM)	S (kcal/mol)	Interactions	
			Hydrophilic	Hydrophobic
7	4.25	-16.9872	Asp351, Glu353, Trp383, Phe404	Met343, Leu346, Ala350, Asp351, Leu387, Phe404, Leu525
4-OHT	8.218	-15.1112	Asp351, Glu353, Arg394.	Met343, Leu346, Thr347, Ala350, Leu387, Phe404, Met421, Ile424, Leu428, Leu525.

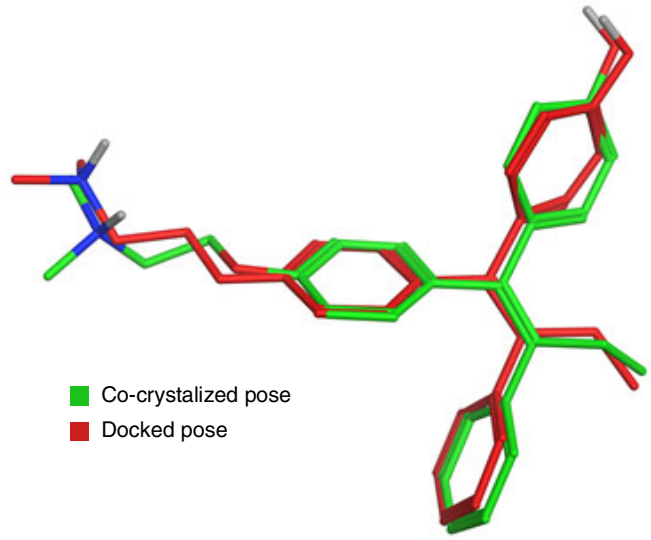


Fig. 3. Binding pose of re-docked 4-hydroxytamoxifen (4-OHT)

Docking results shown that docking score of compound **7** was -16.9872 as compared to -15.1112 of 4-OHT (Table-1). Based on the calculated docking score, compound **7** was found with better anti-breast cancer activity than reference drug. The results of docking studies of compound **7** was in good agreement of results of MTT assay. Binding mode of both compounds involved hydrophobic interactions. Although, 4-OHT showed hydrophilic interactions through cationic attraction with Asp351 and hydrogen bonding with Glu353 and Arg394, while compound **7** had more hydrophilic interactions through cationic attraction with Asp351 and Trp383 and hydrogen bonding with Glu353 and Phe404. Amino acids Glu353 and Arg394 residue are essential for binding with ERα [22]. Thus, it was concluded that the new cationic interaction with Trp383 and hydrogen bonding interaction with Phe404 in active site of ERα were responsible for better anti-breast cancer activity of compound **7** than reference drug, 4-OHT.

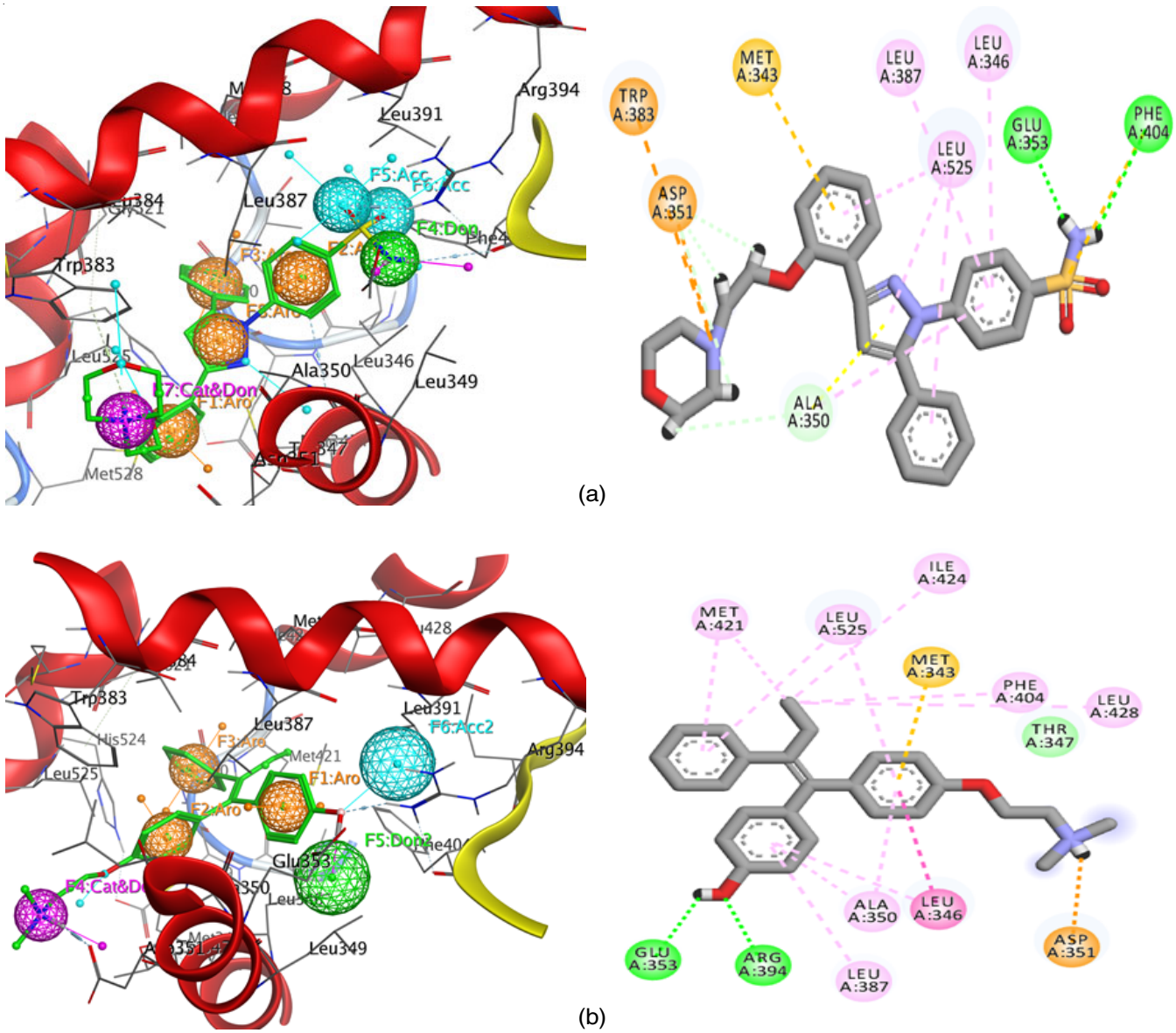


Fig. 4. (a) 3D & 2D structure-based pharmacophore modeling of compound **7**; (b) 3D & 2D structure-based pharmacophore modeling of 4-OHT; Hydrophobic, cationic atom, hydrogen bond donor and acceptor interactions were represented as yellow, purple, green and blue spheres, respectively

Muctharidi *et al.* [23] reported that interaction of 4-OHT with Asp351 and avoiding interaction with His521 will decrease its agonistic effect on uterus and resulting in decrease of its adverse reactions. As molecular docking results of compound **7** were in good agreement of 4-OHT, it is expected that compound **7** will also have lesser adverse reactions.

Conclusion

The results obtained in this work revealed that 4-{3-[2-(2-morpholin-4-yl-ethoxy)phenyl]-5-phenyl-pyrazol-1-yl}benzenesulfonamide (**7**) having 1,3,5-triaryl pyrazole containing sulfonamide and morpholine moiety exhibited significant anti-breast cancer activity, thus can be further explored as a lead in the development of newer anticancer agents. Based on molecular docking studies, compound **7** was expected to have lesser adverse reactions. Further, pharmacokinetic studies of titled compound **7** are required for predicting its absorption, distribution, metabolism and excretion characteristics in the human subjects.

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