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Synthesis, Anticancer Evaluation and Molecular Docking Studies of 1-(2-Fluorobenzyl)piperazine Triazoles: A Novel Breast Cancer Cell Inhibitors

LAKSHMANA BELIYAIAH[1,], RANGASWAMY JAVARAPPA[2,], ABHIRAMI DILKALAL[3,], VINAYA[1,], V. DWARAKANATH[4,] and YERIYUR B. BASAVARAJU[1,*,]

[1]Department of Studies in Chemistry, Manasagangothri, University of Mysore, Mysore-570006, India
[2]Department of Chemistry, Poornaprajna College, Shri Admar Mutt Education Council, Mangalore University, Udupi-576101, India
[3]Department of Botany, Bangalore University, Jnana Bharathi, Bangalore-560056, India
[4]Department of Studies in Biotechnology, Tumkur University, Tumkur-572103, India

*Corresponding author: E-mail: ybb2706@gmail.com

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New series of 1-(2-fluorobenzyl)piperazine triazoles **7(a-k)** have been synthesized and evaluated for anticancer activity. The molecular structures of all the compounds were established by employing ^{1}H NMR, ^{13}C NMR and mass spectral analysis. *In vitro* anticancer activity was evaluated using MTT assay against MCF7 breast cancer cell line. Compounds **7i** and **7j** bearing 4-fluorophenyl and 2-fluorophenyl pendant from triazole substituent phenyl ring exhibited the highest anticancer efficacy with IC_{50} values of 12.09 µg/mL and 15.12 µg/mL, respectively. A molecular docking study conducted on human HER2 complexed with hercepatin fab was acquired from Protein Data Bank (PDB ID: 1N8Z). Molecular docking studies demonstrated Leu443, Gly442 and Leu27 as key residues interacting with active compounds.

Keywords: 1-(2-Fluorobenzyl)piperazine, Triazole, Click chemistry, Anticancer activity, Molecular docking.

INTRODUCTION

Cancer stands as a formidable global health challenge, claiming countless lives and affecting individuals and their families on a profound scale. Despite significant advancements in medical science, discovering new therapeutic agents for cancer treatment remains a critical and complex task. The quest for a cure demands the integration of diverse disciplines, cutting-edge research methodologies and a relentless commitment to overcoming the intricacies of this multifaceted disease [1]. Breast cancer remains the most diagnosed form of cancer in women, contributing significantly to both morbidity and mortality rates [2]. Age stands out as the primary risk factor for breast cancer, with additional significant risk factors including low parity and infrequent breastfeeding. These factors contribute significantly to the characterization of breast cancer as a classical cancer in high-resource nations and underscore its continuing rise across nearly all countries [3].

The outstanding exactitude, less toxicity and useful biological properties, the chemistry and importance of nitrogen-containing heterocyclic analogs have been a hot topic in the bioorganic and medicinal chemistry [4]. Derivatives containing piperazine rings are one of the important class heterocyclic compounds and have numerous advantages like less toxicity, easy forming of multiple ionic bonds or hydrogen bonds, acid-base equilibrium constant and functional effect of moderating drug lipid water partition coefficient [5]. It has been revealed that *N*-substituted piperazines have a extensive range of biological applications, such as antimicrobial [6], anti-cancer [7], insecticidal and herbicidal activities [8], particularly they were often used as antibacterial and antifungal agents. Therefore, it is often make acquainted with into some parent moieties to enhance the medicinal and biological applications during the design of drug and synthesis of heterocyclic derivatives. Furthermore, triazole is one of the five-membered heterocycles containing nitogen, introduced into compounds that will contribute to a wide range of biological properties including anti-inflammatory [9], anticancer [10,11], antifungal [12], anti-anxiety [13] insecticidal [14] and plant growth regulating activities [15]. Some medicinal plants such as cyproconazole,

triadimefon, metconazole, tebuconazole, propiconazole and epoxiconazole containing triazole moieties (Fig. 1) were demonstrated to be antifungal agents [16]. However, 1,2,4-triazole derivatives of Mannich bases containing substituted piperazine moiety have been demonstrated to possess antifungal activity [17,18] and 4,5-disubstituted-1,2,4-triazole Mannich bases with piperazine were found to have tuberculostatic activity [19]. Inspired by the appealing biological profiles of the above mentioned compounds, in the present study aimed to synthesize 1-(2-fluorobenzyl)piperazine triazoles scaffolds and anticancer activity was evaluated against human breast cancer cell line.

EXPERIMENTAL

From Sigma-Aldrich, USA, all the chemicals and reagents for synthesis were procured. Open capillary columns were used to determine the melting point of the synthesized compounds and are uncorrected. The mass and purity of the synthesized compounds were analyzed by LCMS. Bruker DRTX-400 NMR spectrometer was used to record ^{1}H NMR in 400 MHz instruments and ^{13}C in 100 MHz instruments in $CDCl_3$ solvent with tetramethyl silane as an internal standard. Silica gel from Merck (60 to 120 meshes) was used for the analytical TLC and column chromatography.

Synthesis of 1-(2-fluorobenzyl)-4-(prop-2-yn-1-yl)piperazine: To a stirred solution of 1-(2-fluorobenzyl)piperazine (**1**) (1.0 g, 5.154 mmol) in DMF (15 mL) and powdered K_2CO_3 pellets (0.8 g, 5.79 mmol) at room temperature, propargyl bromide (0.46 mL, 6.15 mmol) was added dropwise. At room temperature, the reaction mixture was stirred for 6-7 h and as the reaction progressed TLC in 2:1 hexane and ethyl acetate was used for its monitoring. Methylene chloride (3 × 15 mL) was used to extract the product. The organic layer was passed through anhydrous Na_2SO_4 and evaporated to get dry product under reduced pressure. The crude product was chromatographed using silica gel (ethyl acetate:hexane,1:8).

Synthesis of azidobenzenes 6(a-k): Added dropwise a cold solution of sodium nitrite (-5 ºC) to the aniline derivatives **4(a-h)** (1 equiv.) dissolved in conc. HCl with continuous stirring. After 15 min slowly added freshly prepared sodium azide (1 equiv.) solution for about 20 min with continuous stirring. The newly formed azidobenzene was immediately extracted by ethyl acetate (3 × 10 mL) and washed the organic layer with water (20 mL). Dried over anhydrous Na_2SO_4 to get crude azidobenzene derivatives **6(a-h)** and the products were stored at 2-5 ºC.

Synthesis of 1-(2-fluorobenzyl)piperazine triazoles 7(a-k): To a stirred solution of 1-(2-fluorobenzyl)-4-(prop-2-yn-1-yl)piperazine (**3**) (1 equiv.) H_2O and DCM in 1:2 ratio along with $CuSO_4{\cdot}5H_2O$ (0.3 equiv.) were added. This was followed by sodium ascorbate (0.6 equiv.) at ambient temperature and after 5 min, azidobenzene derivatives (1.2 equiv.) **6(a-h)** were added with continuous stirring for another 2 h at room temperature. The reaction progress was identified by TLC using 1:2 hexane and ethyl acetate. The product was extracted by using ethyl acetate (3 × 15 mL) and the organic layer was washed with distilled water (15 mL) followed by brine (15 mL). Anhydrous Na_2SO_4 was used to dry the organic layer and concentrated under reduced pressure to get crude triazoles of 1-(2-fluorobenzyl)piperazine **7(a-h)**, further, the crude product was chromatographed using silica gel (ethyl acetate:hexane,1:8) (**Scheme-I**).

1-(2-Fluorobenzyl)-4-((1-phenyl-1*H*-1,2,3-triazol-5-yl)methyl)piperazine (7a): Pale yellow solid, yield: 91%, m.p.: 152-154 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.94 (s, 1H, Triz-H), 7.71 (m, 3H, Ar-H), 7.50 (m, 3H, Ar-H), 7.10-6.98 (m, 3H, Ar-H), 3.77 (s, 2H, N-CH_2), 3.61 (s, 2H, N-CH_2), 2.61-2.47 (t, 8H, N-CH_2).^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 131.8, 129.8, 129.0, 128.8, 124.0, 120.6, 55.2, 52.9, 52.5. LCMS (*m/z*): 352.184 (M+), 353.1879 (M+2); Anal. calcd. (found) % for $C_{20}H_{22}N_5F$: C, 68.36 (67.81); H, 6.31 (6.92); F, 5.41 (5.73); N, 19.93 (19.54).

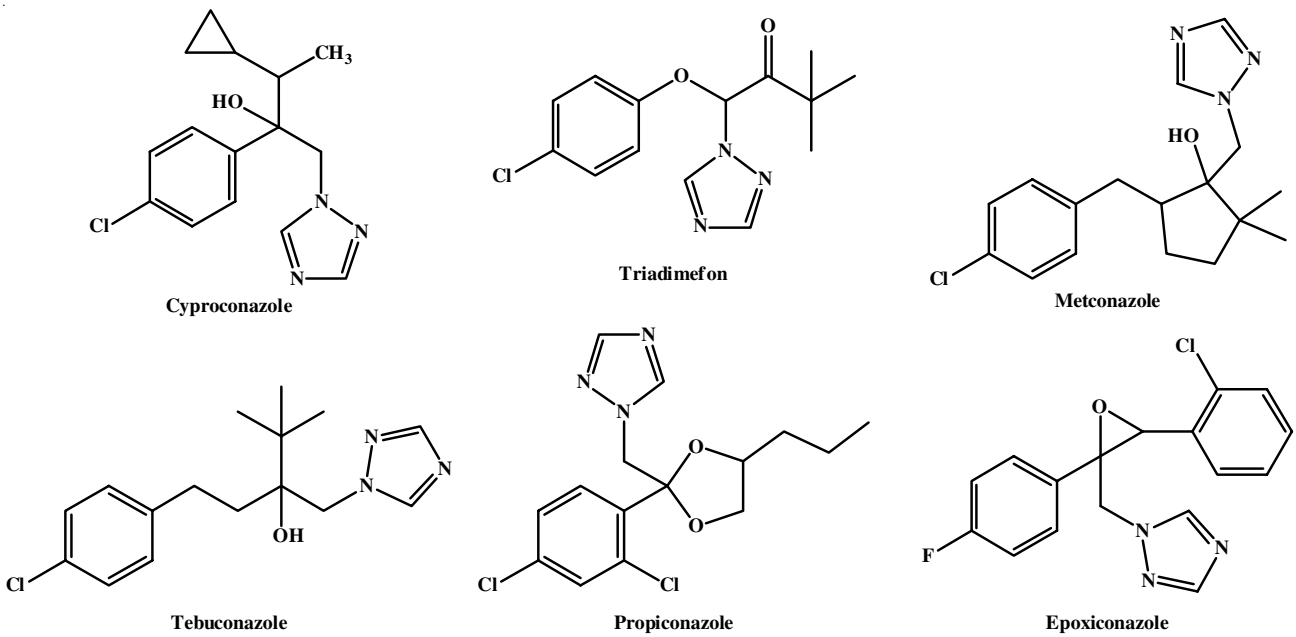
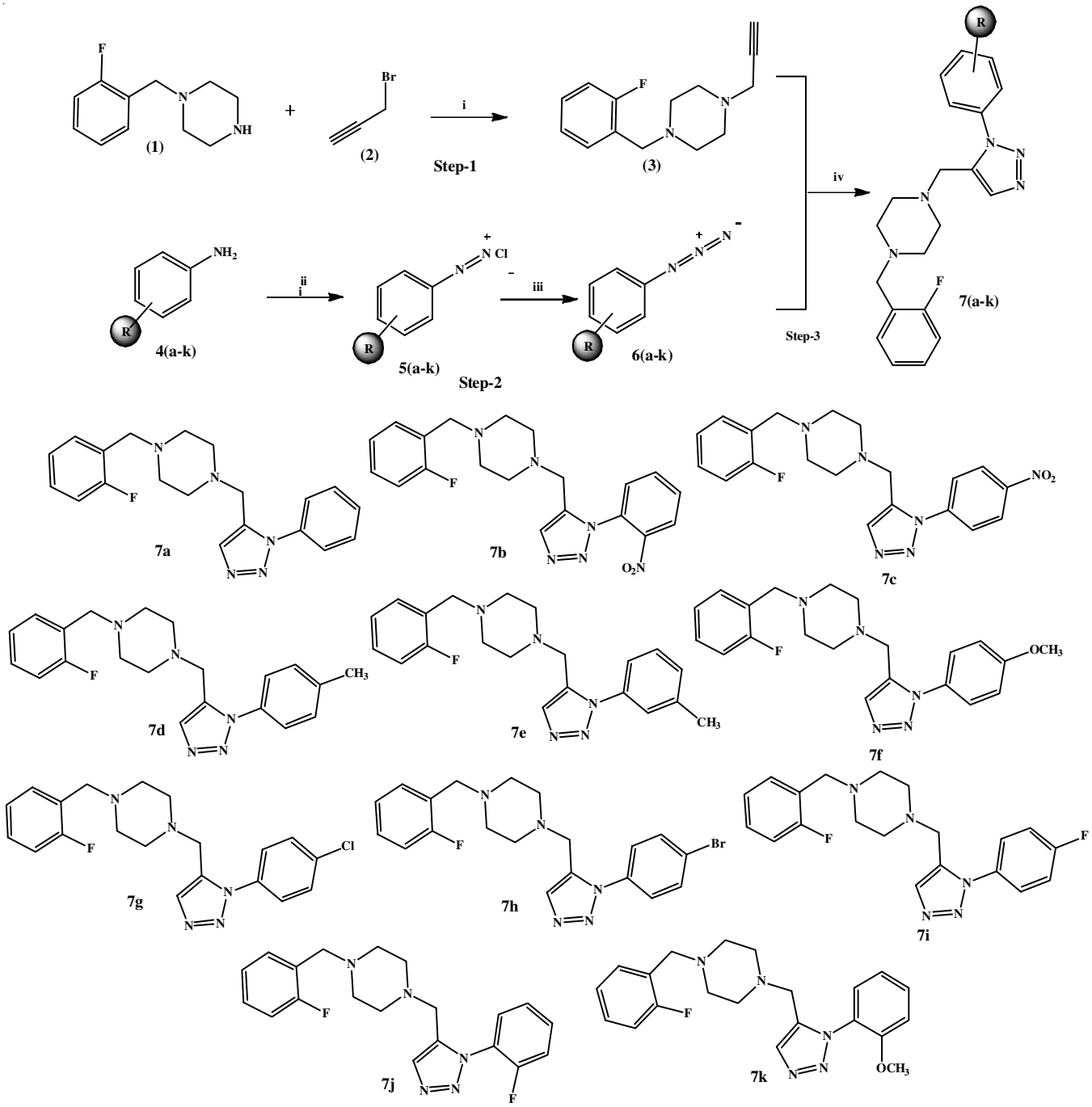


Fig. 1. Some medicinal plants containing triazole moieties



Scheme-I: Synthetic pathway of triazoles of 1-(2-fluorobenzyl)piperazine derivatives [Reagents and condition: (i) NaOH, DMF reflux, 2 h, (ii) conc. HCl, $NaNO_2$, 273 K, (iii) NaN_3, (iv) $CuSO_4 \cdot 5H_2O$ sodium ascorbate, DCM:H_2O 2:1 RT, 2 h]

1-(2-Fluorobenzyl)-4-((1-(2-nitrophenyl)-1*H*-1,2,3-triazol-5-yl)methyl)piperazine (7b): Pale yellow solid, yield: 93%, m.p.: 168-170 ºC ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.06 (m, 2H), 7.93 (s, 1H, Triz-H), 7.70-7.61 (m, 2H, Ar-H), 7.33 (m, 2H, Ar-H), 7.10-6.98 (m, 2H, Ar-H), 3.80 (s, 2H, N-CH_2), 3.62 (s, 2H, N-CH_2), 2.72-2.58 (t, 8H, N-CH_2).^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 134.1, 131.8, 130.8, 129.0, 128.1, 126.2, 125.0, 124.0, 120.8, 115.5, 55.1, 52.8, 52.4.. LCMS (*m/z*): 397.1685 (M+), 398.168 (M+2); Anal. calcd. (found) % for $C_{20}H_{21}N_6O_2F$: C, 60.60 (59.15); H, 5.34 (5.84); F, 4.79 (5.43); N, 21.20 (20.78).

1-(2-Fluorobenzyl)-4-((1-(4-nitrophenyl)-1*H*-1,2,3-triazol-5-yl)methyl)piperazine (7c): Pale yellow solid, yield: 97%, m.p.: 161-163 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.39 (m, 2H), 8.22 (m, 1H, Ar-H), 8.06 (s, 1H, Triz-H), 7.95 (m, 2H, Ar-H), 7.32 (m, 1H, Ar-H), 7.10 (m, 1H, Ar-H), 7.00 (m, 1H, Ar-H), 3.77 (s, 2H, N-CH_2), 3.60 (s, 2H, N-CH_2), 2.58 (t, J = 19.6 Hz, 8H, N-CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.7, 147.2, 141.3, 131.8, 129.1, 129.0, 125.6, 124.1, 124.0, 115.3, 55.2, 53.1, 52.5. LCMS (*m/z*): 397.160 (M+), 398.1644 (M+2); Anal. calcd. (found) % for $C_{20}H_{21}FN_6O_2$: C, 60.60 (61.33); H, 5.34 (4.94); F, 4.79 (4.59); N, 21.20 (21.86)

1-(2-Fluorobenzyl)-4-((1-(*p*-tolyl)-1*H*-1,2,3-triazol-5-yl)-methyl)piperazine (7d): Pale yellow solid, yield: 83%, m.p.: 155-157 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.57 (s, 1H, Triz-H), 7.36 (m, 2H, Ar-H), 7.09 (m, 3H, Ar-H), 7.01 (m, 3H, Ar-H), 3.62 (s, 4H, N-CH_2), 3.27 (s, 3H, Ar-CH_3), 2.60 (t, 8H, N-CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 140.0, 137.0, 131.9, 129.5, 129.1, 123.9, 121.2, 117.6, 115.2, 112.6, 55.1, 53.1, 52.7, 52.3, 21.4. LCMS (*m/z*): 366.1946 (M+), 367.1979 (M+2); Anal. calcd. (found) % for $C_{21}H_{24}FN_5$: C, 69.02 (68.12); H, 6.62 (6.32); F, 5.20 (5.73); N, 19.16 (19.81).

1-(2-Fluorobenzyl)-4-((1-(*m*-tolyl)-1*H*-1,2,3-triazol-5-yl)methyl)piperazine (7e): Pale yellow solid, yield: 86%, m.p.: 165-167 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.50 (s, 1H, Triz-H), 7.34 (m, 2H, Ar-H), 7.07-6.97 (m, 2H, Ar-H), 6.68-6.57 (m, 2H, Ar-H), 3.77 (s, 2H, N-CH_2), 3.60 (s, 2H, N-CH_2), 2.60 (t, *J* = 23.8 Hz, 8H), 2.41 (s, 3H, Ar-CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 140.0, 137.0, 131.9, 129.5, 129.1, 124.0, 121.2, 117.6, 116.4, 115.3, 112.6, 55.1, 52.8, 52.4, 21.5. LCMS (*m/z*): 366.2024 (M+), 367.205 (M+2); Anal. calcd. (found) % for $C_{21}H_{24}N_5F$: C, 69.02 (67.83); H, 6.62 (7.26); F, 5.20 (5.07); N, 19.16 (19.83).

1-(2-Fluorobenzyl)-4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-5-yl)methyl)piperazine (7f): Pale yellow solid, yield: 91%, m.p.: 171-173 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.87 (s, 1H, Triz-H), 7.60 (m, 2H, Ar-H), 7.34 (m, 1H, Ar-H), 7.10-6.98 (m, 5H, Ar-H), 3.85 (s, 3H, Ar-CH_3), 3.78 (s, 2H, N-CH_2), 3.61 (s, 2H, N-CH_2), 2.63-2.45 (t, 8H, N-CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 134.1, 131.8, 130.8, 129.1, 128.1, 125.7, 124.0, 120.8, 115.5, 55.1, 53.0, 52.8, 52.4. LCMS (*m/z*): 382.2004 (M^+), 383.205 (M+2); Anal. calcd. (found) % for $C_{21}H_{24}N_5OF$: C, 66.12 (65.76); H, 6.34 (6.94); F, 4.98 (5.24); N, 18.36 (17.76).

1-((1-(4-Chlorophenyl)-1*H*-1,2,3-triazol-5-yl)methyl)-4-(2-fluorobenzyl)piperazine (7g): Pale yellow solid, yield: 85%, m.p.: 152-154 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.94 (s, 1H, Triz-H), 7.67 (m, 2H, Ar-H), 7.48 (m, 2H, Ar-H), 7.34 (m, 2H, Ar-H), 7.10-6.98 (m, 2H, Ar-H), 3.78 (s, 2H, N-CH_2), 3.61 (s, 2H, N-CH_2), 2.58-2.48 (t, 8H, N-CH_2).^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 134.5, 131.8, 130.0, 129.1, 124.0, 121.7, 121.1, 115.5, 115.3, 55.1, 53.1, 52.8, 52.6, 52.4. LCMS (*m/z*): 386.135 (M+), 388.132 (M+2); Anal. calcd. (found) % for $C_{20}H_{21}N_5ClF$: C, 62.25 (61.85); H, 5.49 (5.89); Cl, 9.19 (9.29); F, 4.92 (5.03); N, 18.15 (17.91).

1-((1-(4-Bromophenyl)-1*H*-1,2,3-triazol-5-yl)methyl)-4-(2-fluorobenzyl)piperazine (7h): Pale yellow solid, yield: 79%, m.p.: 158-160 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.96 (s, 1H, Triz-H), 7.62 (m, 4H, Ar-H), 7.33 (m, 1H, Ar-H), 7.10-6.98 (m, 3H, Ar-H), 3.80 (s, 2H, N-CH_2), 3.62 (s, 2H, N-CH_2), 2.62 (t, *J* = 24.5 Hz, 8H, N-CH_2).^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 133.0, 131.8, 129.2, 129.1, 124.0, 121.9, 115.5, 115.3, 55.1, 53.1, 52.8, 52.3. LCMS (*m/z*): 430.1083 (M+), 432.1056 (M+2); Anal. calcd. (found) % for $C_{20}H_{21}N_5BrF$: C, 55.82 (56.23); H, 4.92 (5.07); Br, 18.57 (17.87); F, 4.41 (4.31); N, 16.27 (16.52).

1-(2-Fluorobenzyl)-4-((1-(4-fluorophenyl)-1*H*-1,2,3-triazol-5-yl)methyl)piperazine (7i): Pale yellow solid, yield: 87%, m.p.: 163-165 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.92 (s, 1H, Triz-H), 7.70-7.62 (m, 2H, Ar-H), 7.35 (m, 1H, Ar-H), 7.20 (m, 1H, Ar-H), 7.10-6.98 (m, 4H, Ar-H), 3.78 (s, 2H, N-CH_2), 3.61 (s, 2H, N-CH_2), 2.59-2.43 (t, 8H, N-CH_2).^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 131.8, 129.0, 123.9, 122.6, 122.5, 116.9, 116.7, 115.5, 115.3, 55.1, 52.6, 52.4, 51.9. LCMS (*m/z*): 370.192 (M+), 371.1933 (M+2); Anal. calcd. (found) % for $C_{20}H_{21}N_5F_2$: C, 65.03 (64.76); H, 5.73 (6.03); F, 10.29 (9.95); N, 18.96 (19.26).

1-(2-Fluorobenzyl)-4-((1-(2-fluorophenyl)-1*H*-1,2,3-triazol-5-yl)methyl)piperazine (7j): Pale yellow solid, yield: 94%, m.p.: 166-168 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.94 (s, 1H, Triz-H), 7.59-7.45 (m, 4H, Ar-H), 7.29-7.37 (m, 1H, Ar-H), 7.12-6.99 (m, 3H, Ar-H), 3.77 (s, 2H, N-CH_2), 3.59 (s, 2H, N-CH_2), 2.58 (t, *J* = 17.1 Hz, 8H, N-CH_2).^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 145.4, 131.8, 131.3, 129.0, 123.9, 120.9, 115.8, 115.5, 115.2, 108.4, 108.1, 55.2, 53.2, 52.9, 52.5. LCMS (*m/z*): 370.184 (M+), 371.189 (M+2); Anal. calcd. (found) % for $C_{20}H_{21}N_5F_2$: C, 65.03 (64.76); H, 5.73 (6.03); F, 10.29 (9.95); N, 18.96 (19.26).

1-(2-Fluorobenzyl)-4-((1-(2-methoxyphenyl)-1*H*-1,2,3-triazol-5-yl)methyl)piperazine (7k): Pale yellow solid, yield: 89%, m.p.: 174-176 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.09 (s, 1H, Triz-H), 7.76 (m, 1H, Ar-H), 7.31-7.43 (m, 3H, Ar-H), 7.10-7.01 (m, 4H, Ar-H), 3.87 (s, 4H, N-CH_2), 3.62 (s, 3H, Ar-CH_3), 2.60-2.42 (t, 8H, N-CH_2).^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 130.1, 125.5, 124.0, 121.3, 115.3, 112.3, 56.0, 55.1, 53.1, 52.6, 52.3. LCMS (*m/z*): 382.192 (M+), 383.193 (M+2); Anal. calcd. (found) % for $C_{21}H_{24}N_5OF$: C, 66.12 (65.82); H, 6.34 (6.14); F, 4.98 (4.59); N, 18.36 (19.26).

Anticancer evaluation: MCF 7 breast cancer cell line obtained from the National Centre for Cell Science (NCCS), Pune, India; was selected to evaluate the anticancer activity of synthesized compounds of 1-(2-fluorobenzyl)piperazine triazoles (**7a-k**). The cytotoxicity was evaluated by standard MTT ((3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyl tetrazolium bromide) assay as described by Mosmann [20]. The cells were maintained in DEME high sugar media supplemented with 10% FBS along with 1% antibiotic, antimycotic solution in presence of 5% CO_2, 18-20% O_2 at 37 ºC in a CO_2 incubator. Once it reached an appropriate density the cells were detached using EDTA and stained with trypan blue, counted on hemocytometer. Then, briefly, 200 µL cells were seeded in 96-well plates at a density of 20,000 cells/well. Different concentrations of compounds from (0, 15, 30 and 60 µg mL^{-1}) were added and the plates were incubated for 24 h at 37 ºC in a 5% CO_2 atmosphere. After 24 h, MTT solution was added and the plates were incubated for another 3 h in dark conditions. The MTT solution was aspirated and the resulting formazan product was dissolved in DMSO. Absorbance was recorded at 570 nm with a plate reader and cell viability was assessed by comparing it to untreated cells. Doxorubicin (Sigma, USA) was served as the positive control.

The percent (%) viability of MCF-7 cells was calculated as:

$$\text{Cell viability (\%)} = \frac{\text{OD sample}}{\text{OD control}} \times 100$$

Molecular docking: The molecular docking study of synthesized compounds with human HER2 complexed with Herceptin Fab protein (PDB ID: 1N8Z) was performed by using an Autodock tool to know the different interactions between the ligand and proteins [21]. The 3D structure of the epidermal growth factor receptor protein was downloaded from the RCSB Protein Data Base in PDB format, the bound ligands were removed by using Biovia Discovery Studio 2019. Using an Autodock tool, polar hydrogen atoms were added and energy was minimized to the protein [22]. The grid box was generated to define a binding site using grid size off or X = 123.62, Y = 103.46 and Z = -47.13, respectively. All the synthesized molecules were drawn in the Marvin JS tool, converted into a 3D structure and saved in PDB format. All these compounds were optimized and converted from PDB to PDBQT format by using Autodock tools 4.2.6. and molecules have been docked to an active site of the receptor protein. The binding energy of the compounds was observed as a negative score with a unit of kJ mol^{-1}. Finally, the protein-ligand interactions were analyzed by using the Biovia Discovery Studio visualizer.

RESULTS AND DISCUSSION

The synthetic pathway for target compounds **7(a-k)** is shown in **Scheme-I**. 1-(2-Fluorobenzyl)-4-(prop-2-yn-1-yl)piperazine (**3**) was obtained by the reaction of 1-(2-fluorobenzyl)piperazine (**1**) with 3-bromoprop-1-yne (**2**). In next step, azidobenzene derivatives **6(a-k)** were synthesized by diazotization of electron releasing and withdrawing substituted anilines **4(a-k)** and converting resultant aryl diazonium salt **5(a-k)** into azidobenzenes in the presence of sodium azide (step-2). In the final step, the reaction of azidobenzenes **6(a-h)** with 1-(2-fluorobenzyl)-4-(prop-2-yn-1-yl)piperazine (**3**) in the presence of $CuSO_4$ and sodium ascorbate catalyst, underwent 1,3 dipolar cycloadditions of azide-alkyne click chemistry [23] thereby resulting 1-(2-fluorobenzyl)piperazine triazoles **7(a-k)** in good yields.

Anticancer evaluation: The cytotoxic activity of the synthesized compounds **7(a-k)** was evaluated against human breast cancer cell line MCF-7 at different concentrations and the IC_{50} (50% growth inhibition) value and percent cell viability, was estimated using MTT assay. Since viable cells contain NAD(P)H-dependent oxidoreductase enzymes which reduce tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide to formazan a violet coloured complex. The resultant coloured complex was measured at 570 nm; the darker the solution more the metabolically active cells [24]. The percentage of cell viability of HCF-7 cell line after the test compounds treated at concentrations of 15, 30 and 60 µg/mL along with standard doxorubicin is represented in Fig. 2 and their IC_{50} values are depicted in Table-1. The synthesized 1-(2-fluorobenzyl)piperazine triazole derivatives **7(a-k)** exhibited a potential anticancer activity on the selected cell line in a dose-dependent manner. Compounds **7c**, **7g**, **7i** and **7j** have comparatively good anticancer properties with a decrease in cell viability percentage. This could be due to the presence of electron withdrawing groups like nitro, chloro, bromo and fluoro-substituents on triazole *N*-substituted phenyl ring.

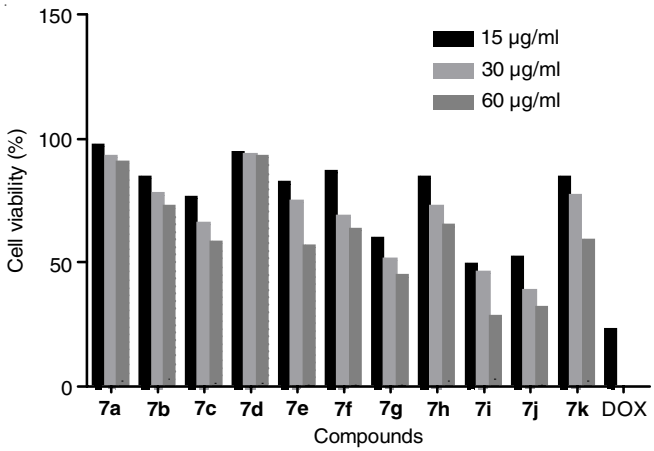


Fig. 2. Percentage of cell viability assay showing the viability of MCF-7 after exposure to different concentrations of synthesized 1-(2-fluorobenzyl)piperazine triazoles with standard drug doxorubicin (DOX)

TABLE-1
IC_{50} VALUES OF SYNTHESIZED OF 1-(2-FLUOROBENZYL)PIPERAZINE TRIAZOLES DERIVATIVES AGAINST MCF-7 CELL LINE

Compounds	IC_{50} (µg/mL)	Compounds	IC_{50} (µg/mL)
7a	133.56	**7g**	25.23
7b	41.12	**7h**	36.67
7c	33.56	**7i**	12.09
7d	102.03	**7j**	15.12
7e	96.01	**7k**	70.55
7f	85.66		

Compounds **7i** and **7j** substituted with fluoro group on phenyl ring of triazole exhibited 27.79% and 31.78% cell viability at a concentration of 60 µg/mL. Similarly, these two compounds showed the lowest IC_{50} concentration which is the concentration causing 50% inhibition of cellular proliferation, of 12.09 and 15.12 µg/mL, respectively. Whereas compounds **7d**, **7e** and **7f** having electron donating groups like methyl and methoxy on the phenyl ring revealed less anticancer activity compared to compounds **7i** and **7j**.

Molecular docking studies: All the synthesized 1-(2-fluorobenzyl)piperazine triazole scaffolds **7(a-k)** were bound in the active site of target protein human HER2 complexed with Herceptin Fab (PDB ID: 1N8Z) with different conformational poses. Based on *in vitro* anticancer activity studies, compounds **7c**, **7g**, **7i** and **7j** were the most active having good IC_{50} values ranging from 12.09 µg/mL to 33.56 µg/mL. The decent activity of **7c**, **7g**, **7i** and **7j** was also justified by the docking results (-7.5 kJ mol^{-1} to -7.9 kJ mol^{-1}) whereby both bind inside the active region of the target protein. The docking results revealed the good correlations with the experimental results of anticancer activity. Compound **7c** displayed a binding energy of -7.9 kJ mol^{-1} and formed hydrogen between different amino acid residues Phe 98, Trp47 Ala40 and Gly 44, pi–pi stacking interactions with Ala 61 and Lys 43. Compound **7g** showed binding energy of -7.9 kJ mol^{-1} and exhibits the conventional hydrogen bond interaction with Ile 591, Tyr 55 amino acid residues and pi-pi stacking interactions with Phe 104. Compounds **7i** and **7j** exhibited binding energy of -7.6 and -7.5 kJ

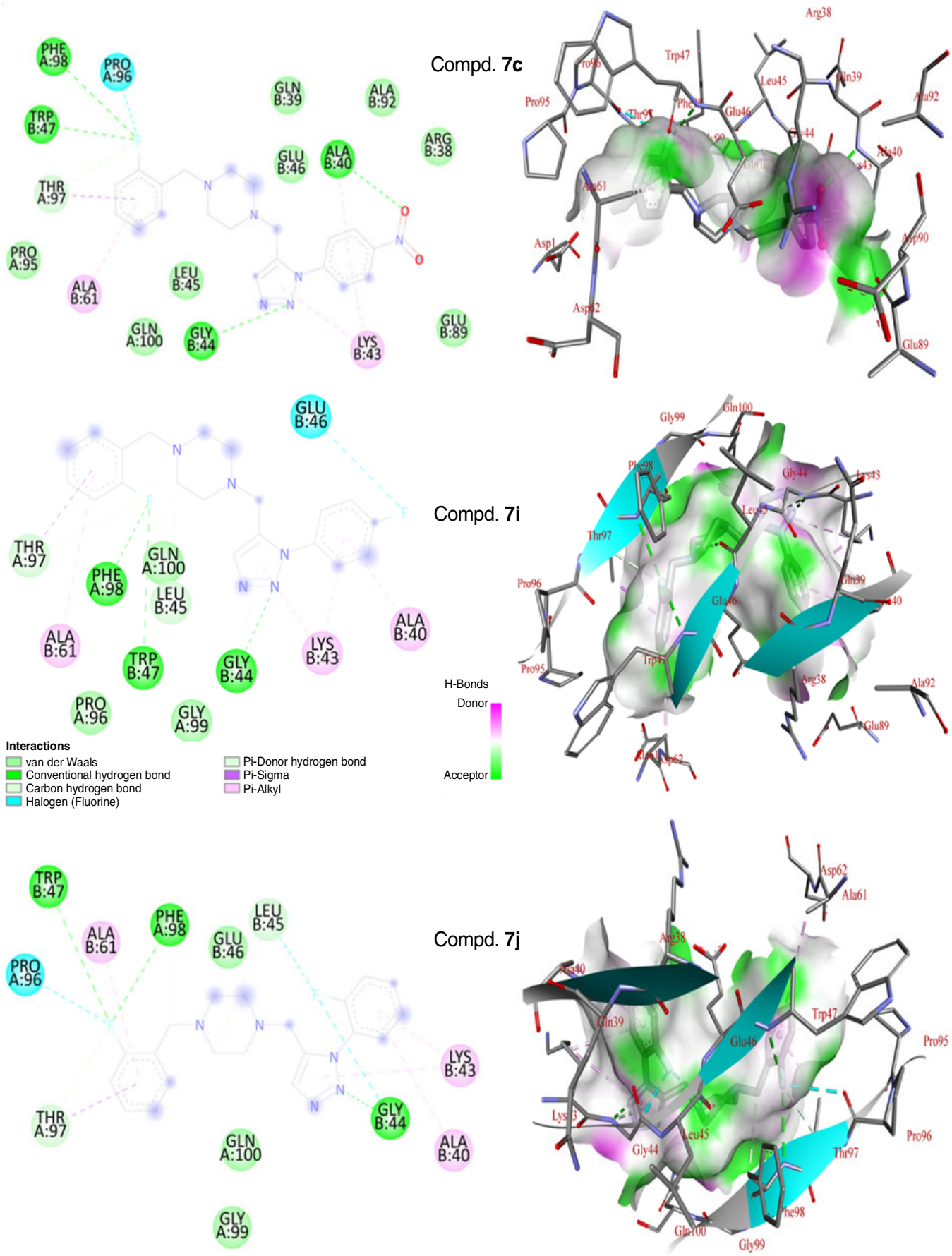


Fig. 3. Docking of compound **7c, 7i** and **7j** with active side of protein

mol^{-1}, respectively by forming three H-bonds with the amino acid residues Phe 98, Trp 47, Gly 44 and pi-alkyl interactions with Ala 61, Lys 43, Ala 40 amino acid residues. Binding energy values and potent interactions with different amino acid residues for all the compounds are presented in Table-2 and Fig. 3.

TABLE-2
BINDING ENERGIES AND HYDROGEN BOND INTERACTION WITH AMINO ACID RESIDUES OF ACTIVE SITE PROTEIN

Compound	Binding energy (kJ mol^{-1})	Hydrogen bond interaction with amino acid residues
7a	-1.5	Try 47
7b	-5.3	Phe 98, Gly 44
7c	-7.9	Phe 98, Trp47 Ala40 and Gly 44
7d	-2.6	Phe 98
7e	-3.3	Trp 47, Ala 40
7f	-3.5	Trp 47, Ala 40, Gly 44
7g	-7.9	Ile 591 Tyr 55, Asp 108
7h	-5.8	Ile 591, Tyr 55, Trp 47
7i	-7.6	Phe 98, Trp 47, Gly 44
7j	-7.5	Phe 98, Trp 47, Gly 44
7k	-4.2	Trp 47, Ala 40, Gly 44
Doxorubicin	-8.5	–

Conclusion

A series of novel 1-(2-fluorobenzyl)piperazine triazole scaffolds **7(a-h)** were synthesized by the Cu(I)-catalyzed 1,3-dipolar cycloaddition of azide-alkyne click chemistry. The role of substituents on the triazole substituted phenyl ring was investigated for the cytotoxic potential of 1-(2-fluorobenzyl)-piperazine triazole scaffolds against selected cancer cell lines. The results demonstrated that among synthesized compounds, **7i** and **7j** having electron withdrawing groups like *p*-fluoro and *o*-fluoro were found to be the most potent analogs having IC_{50} values of 12.09 µg/mL and 15.12 µg/mL, respectively, while they had no toxic effect against non-cancer cells. In addition, among synthesized analogs, compounds **7c** and **7g** showed moderate to good activity with IC_{50} values of 33.56 µg/mL and 25.23 µg/mL respectively against the mentioned cell line. The molecular docking studies demonstrated that all the functionality in the synthesized derivatives recognized one or the other mode of interaction and is compatible with the experimental results. Thus, some of the 1-(2-fluorobenzyl)piperazine triazole derivatives appear to be promising targeted compounds encouraging further investigation as anticancer agents.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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