“ An Efficient One Pot Multicomponent Synthesis of Dihydro Pyrazolyl Bithiazole Derivatives as Potential Anticancer Agents and Their Molecular Docking Studies”

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Abstract

A novel series of dihydropyrazolyl bithiazoles were efficiently synthesized through simple reaction conditions via one pot multicomponent reaction of 2-bromo-1-(4-methyl-2-phenyl thiazol-5-yl)ethan-1-one (1), thiosemicarbazide (2) and chalcones (3 a-k) in the presence of sodium hydroxide in ethanol under reflux condition. The reaction generates two potential five membered heterocyclic pharmacophores i.e. Hantzsch thiazole and dihydropyrazole in one step through operational simplicity, shorter reaction time with high yields. The newly synthesized compounds were well characterized by IR, ^1H , ^{13}C NMR and Mass spectral data. All the newly synthesized compounds were evaluated for their anticancer activity against human cancer cell lines and calculated their binding energy values with respect to 3ert protein. The compounds 4f, 4g, 4h and 4j exhibited excellent activity against MCF-7 cancer cell line with IC_{50} Values of 3.47 ± 0.28 , **1.36 ± 0.74** , 3.76 ± 0.35 and 4.14 ± 0.26 μM concentrations and studied molecular interaction of probable target protein human Estrogen Receptor Alpha protein (3ert.pdb) using docking simulation.

Introduction

The main aim of modern drug discovery is to synthesize therapeutically active compounds by using simple reaction pathways. Multicomponent reactions (MCRs) are one of the versatile chemical transformations and useful tool for the synthesis of structurally diversified more complex therapeutic active molecules from readily available starting materials in a single step process. Due to the selectivity, efficiency in terms of reduction of number of steps, no waste generation, atom economy, high yields, less reaction time and eco compatibility, Multicomponent reactions are considered as ideal green and powerful synthetic methods in organic synthesis [1- 4] and drug discovery [5, 6].

Heterocycles are the most important pharmacophores in medicinal chemistry and researchers are aiming to develop the efficient synthetic methods to obtain pharmaceutically active heterocycles. Heterocycles also contributed to the improvement of quality of life through their applications in the field of biomolecules [7 - 9], agrochemicals [10 - 12], materials [13], food and cosmetics [14, 15].

Thiazoles and dihydropyrazoles are versatile five membered heterocyclic compounds, which are widely used in the field of medicinal chemistry, natural products and functional materials. Most of the thiazoles have been reported to exhibit vast pharmacological applications including antimicrobial [16, 17], antiviral [18 - 20], antitubercular [21, 22], anticancer [23 - 26], anti-inflammatory [27 - 29], antitrypanosomal [30, 31] and anti fungal activity [32, 33]. Pyrazoles represent another important nitrogen containing heterocycles that have attracted much more attention among the medicinal chemists due to their extensive applications in the field of drug discovery [34 - 40], agrochemicals [41] and materials [42 - 45]. Moreover the thiazole and pyrazole heterocycles are used as starting materials to construct the diversified heterocyclic systems and also they constitute an interesting template for combinatorial chemistry [46-49]. Some of the therapeutic active dihydropyrazole and thiazole heterocycles were given **Figure 1**.

In view of the biological significance of thiazoles and dihydropyrazoles, in continuation of our interest in synthesis of biologically active heterocyclic compounds, we felt that it is worthwhile to explore the synthesis and scope of thiazoles and dihydropyrazoles. Here in, we wish to report the development of new thiazole and dihydropyrazole heterocyclic compounds in a single molecular frame work through simple and efficient one pot multicomponent chemical transformation of 2-bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethan-1-one (1), thiosemicarbazide (2) and various substituted chalcones (3 a-k) and evaluation of their anticancer activity properties. At present anticancer activity study and molecular docking studies play a crucial role in order to understand the molecular level interaction and active site residues property in relation to biological activity.

Experimental

The organic solvents and the reagents like thiosemicarbazide, sodium hydroxide were procured from commercial sources and used as such without any further purification. The reactions were monitored through thin layer chromatography using pre coated silica gel plates and the spots were visualized under UV light and Iodine vapours. Melting points were recorded in open capillary melting tube using Stuart melting point apparatus (SMP-30) and are uncorrected. Infra red spectra were recorded on Perkin-Elmer (Spectrum -100S) spectrophotometer. The ^1H and ^{13}C were analyzed by using Bruker spectrometer at 400 and 100 MHz using tetramethylsilane (TMS) as internal standard. The mass spectra were recorded on Water micromass API instrument.

Anticancer activity evaluation of 2-(5-(Aryl)-3-heteryl-4,5-dihydro-1H-pyrazol-1-yl)-4'-methyl-2'-phenyl-4,5'-bithiazole derivatives (4a-k) by using standard MTT assay:

The novel 2-(5-(Aryl)-3-heteryl-4,5-dihydro-1H-pyrazol-1-yl)-4'-methyl-2'-phenyl-4,5'-bithiazole derivatives (4a-k) were evaluated for their anticancer activity property against a panel of four human cancer cell lines like Non-small-cell lung cancer cell lines (A549), prostate cancer (DU-145), breast cancer (MCF-7) and Human neuroblastoma (SKNSH) cell lines by using standard MTT protocol in a 96 well plate. The four cancer cell lines were procured from American Type Culture Collection (ATCC) and cells were grown in Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS), 1% antibiotics and incubated at 37 °C in a humidified incubator with 5% CO₂. The newly synthesized compounds (4a-k) were dissolved in cell culture grade dimethyl sulphoxide solvent. The four cancerous cell lines were taken in a 96 well tissue culture plate (10⁴ cells/ each well) and the cells were treated with different concentrations of synthesized compounds and incubated for about 48 h. After 48 h of incubation, the medium was removed, washed with Dulbecco's phosphate buffer saline (DPBS) and treated with 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution in 200 µL of culture medium. The MTT solution treated cells were incubated for 1h and absorbance was measured at 562 nm using spectrophotometer. The absorbance of the treated cells was compared with the control. The MTT assay was performed in triplicate and the results were shown as percentage reduction in cell viability.

In silico Study:

Preparation of Target and Ligands:

Based on available literature and scientific fact, we have selected Estrogen Receptor Alpha (Er α) protein as a probable target against breast cancer (MCF-7). The 3-D structure of Human Estrogen Receptor Alpha protein (3ERT.PDB) [54] with co-crystallized bound ligand 4-Hydroxytamoxifen and 79 water molecules was downloaded from Protein Data Bank. The target protein was prepared in the form of .pdbqt format file using MGLTool by removing bound ligand and water molecules. The selected ligands (Dihydro Pyrazolyl Bithiazole Derivatives) were drawn using ChemSketch and saved in sdf format. These ligands were converted in pdb format using Open babel and was prepared in the form of .pdbqt format by using MGLTool by assigning maximum rotatable bonds. Hardware used for the current study having configuration HP Intel(R) Core(TM) i3, memory (RAM) 4.00 GB, with 64-bit Operating system and x64 based processor.

Grid parameter and Docking Simulation:

For the docking simulation study, active site was selected based on the bound ligand (OHT) and the grid parameter assigned with number of points in X=100; Y=100; Z=100 and center_x = 30.708; center_y = -1.057; center_z = 27.065 Å and default grid spacing (0.375 Å) on target molecule. With the help of molecular docking simulation approach using AutoDock Vina (ADT Vina), the evaluation of selected ligands was performed against the target (3ERT.PDB), to determine the structure activity relationship (SAR). The representation of docked confirmation with target was generated in the form of 2-D and 3-D using LigPlus [55] and Molegro Molecular Viewer [56] respectively.

General Procedure for the synthesis of 2-(5-(Aryl)-3-heteryl-4,5-dihydro-1H-pyrazol-1-yl)-4'-methyl-2'-phenyl-4,5'-bithiazole derivatives (4a-k):

A reaction mixture of 2-bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethan-1-one (**1**, 1 mmol), thiosemicarbazide (**2**, 1 mmol), chalcones (**3 a-k**, 1 mmol) and sodium hydroxide (1.5 mmol) in ethanol (5 mL) were heated to 70°C for about 4–5 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature and the separated solid was filtered. The solid obtained was recrystallized from ethanol to give the desired compound.

1. 4'-methyl-2'-phenyl-2-(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4,5'-bithiazole (**4a**):

Yellow solid, yield: (406 mg, 84%); mp. 208 - 210 °C; IR (KBr, $\bar{\nu}_{\text{max}}$ cm⁻¹): 1602 (C=N); ¹H NMR (DMSO-*d*₆; δ ppm): 2.48 (s, 3H), 3.32 – 3.40 (dd, *J* = 23.2 Hz, 9.2 Hz, 1H), 3.93 – 4.03 (dd, *J* = 23.2 Hz, 16 Hz, 1H), 5.59 – 5.65 (dd, *J* = 16 Hz, 9.2 Hz, 1H), 6.69 (s, 1H, Ar-H), 7.09 (t, *J* = 6.8 Hz, 1H), 7.25 – 7.36 (m, 2H, Ar-H), 7.41 – 7.47 (m, 7H, Ar-H), 7.60 (s, 1H, Ar-H), 7.86 – 7.89 (m, 2H, Ar-H); ¹³C NMR (DMSO-*d*₆ + CDCl₃; δ ppm):

17.34, 44.31, 64.78, 105.65, 125.55, 126.12, 126.84, 127.91, 128.80, 129.13, 130.02, 133.49, 134.44, 141.42, 143.37, 149.27, 163.58, 164.23; **MS (ESI m/z):** 485.09 [M+H]⁺.

2. 2-(5-(4-fluorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4'-methyl -2'-phenyl-4,5'-bithiazole
(4b)

Orange solid, yield: (446 mg, 89%); mp. 185- 187°C; **IR (KBr, λ_{max} cm⁻¹):** 1612 (C=N); **¹H NMR (DMSO-*d*₆; δ ppm):** 2.51 (s, 3H), 3.29 – 3.34 (dd, *J* = 12.8 Hz, 6 Hz, 1H), 3.97- 4.04 (dd, *J* = 16.8 Hz, 4.8 Hz, 1H), 5.61 – 5.66 (dd, *J* = 11.2 Hz, 5.6 Hz, 1H), 6.76 (d, *J* = 4.8 Hz, 1H, Ar-H), 7.07 – 7.11 (m, 3H, Ar-H), 7.29 (s, 1H), 7.44 – 7.52 (m, 5H, Ar-H), 7.74 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.90 (s, 2H, Ar-H); **¹³C NMR (DMSO-*d*₆ + CDCl₃; δ ppm):** 17.06, 44.24, 64.16, 105.87, 115.47, 115.64, 126.27, 127.92, 129.14, 130.28, 132.88, 134.36, 137.24, 143.08, 148.45, 148.61, 161.13, 163.86, 164.25; **MS (ESI m/z):** 503.08 [M+H]⁺.

3. 2-(5-(4-chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4'-methyl -2'-phenyl-4,5'-bithiazole
(4c)

Yellow solid, yield: (440 mg, 85%); mp. 256 -258 °C; **IR (KBr, λ_{max} cm⁻¹):** 1610 (C=N), 569 (C-Cl); **¹H NMR (DMSO-*d*₆; δ ppm):** 2.46 (s, 3H, -CH₃), 3.38 - 3.42 (dd, *J* = 14 Hz, 5.2 Hz, 1H, Pyrazole-CH₂), 4.04 – 4.10 (dd, *J* = 14.4 Hz, 9.6 Hz, 1H, Pyrazole-CH₂), 5.64 – 5.68 (dd, *J* = 9.6 Hz, 5.2 Hz, 1H, Pyrazole-CH), 7.09 (s, 1H, Ar-H), 7.17 – 7.19 (m, 1H, Ar-H), 7.42 – 7.46 (m, 4H, Ar-H), 7.48 – 7.50 (m, 4H, Ar-H), 7.76 (d, *J* = 4.8 Hz, 1H, Ar-H), 7.89 (d, *J* = 6.8 Hz, 2H, Ar-H); **¹³C NMR (DMSO-*d*₆ + CDCl₃; δ ppm):** 22.83, 48.34, 61.10, 69.98, 110.98, 115.29, 116.37, 123.60, 130.73, 132.41, 133.11, 133.69, 134.49, 138.69, 139.46, 148.39, 153.69, 169.25; **MS (ESI m/z):** 519.05 [M+H]⁺.

4. 2-(5-(4-bromophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4'-methyl -2'-phenyl-4,5'-bithiazole
(4d)

Orange solid, yield: (495 mg, 88%); mp. 296 - 298 °C; **IR (KBr, λ_{max} cm⁻¹):** 1615 (C=N), 598 (C-Br); **¹H NMR (DMSO-*d*₆; δ ppm):** 2.51 (s, 3H, -CH₃), 3.33 - 3.41 (dd, *J* = 23.6 Hz, 10 Hz, 1H, Pyrazole-CH₂), 3.91 – 4.01 (dd, *J* = 23.2 Hz, 15.6 Hz, 1H, Pyrazole-CH₂), 5.50 – 5.57 (dd, *J* = 16 Hz, 10 Hz, 1H, Pyrazole-CH), 6.68 (s, 1H, Ar-H), 7.09 (t, *J* = 6.8 Hz, 1H, Ar-H), 7.25 (t, *J* = 10 Hz, 2H, Ar-H), 7.36 (d, *J* = 10.4 Hz, 1H, Ar-H), 7.41 – 7.47 (m, 5H, Ar-H), 7.63 (s, 1H, Ar-H) 7.92 (d, *J* = 10.4 Hz, 2H, Ar-H); **¹³C NMR (DMSO-*d*₆ + CDCl₃; δ ppm):** 22.01, 49.02, 60.71, 69.50, 110.08, 116.27, 123.69, 130.88, 132.51, 133.21, 133.69, 134.59, 138.26, 139.37, 148.27, 153.02, 169.19; **MS (ESI m/z):** 565.00 [M+2H]⁺.

5. 2-(5-(4-methoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4'-methyl -2'-phenyl-4,5'-bithiazole (4e)

Orange solid, yield: (406 mg, 79%); mp. 224-226 °C; **IR (KBr, λ_{max} cm⁻¹):** 1612 (C=N), 1265 (-OCH₃); **¹H NMR (DMSO-*d*₆; δ ppm):** 2.52 (s, 3H, -CH₃), 3.32 – 3.38 (dd, *J* = 17.6 Hz, 6.8 Hz, 1H, Pyrazole-H), 3.77 (s, 3H, -OCH₃), 3.90 – 3.98 (dd, *J* = 17.2 Hz, 11.6 Hz, 1H, Pyrazole-H), 5.56 – 5.61 (dd, *J* = 11.6 Hz, 6.4 Hz, 1H,

Pyrazole-H), 6.67 (s, 1H, Ar-H), 6.88 (d, J = 8.4 Hz, 2H, Ar-H), 7.10 (d, J = 4 Hz, 1H, Ar-H), 7.34 (d, J = 8.4 Hz, 2H, Ar-H), 7.42 – 7.47 (m, 4H, Ar-H), 7.58 (s, 1H, Ar-H), 7.89 (d, J = 7.6 Hz, 2H, Ar-H); **^{13}C NMR (DMSO- d_6 + CDCl_3 ; δ ppm)**: 22.12, 60.03, 69.03, 110.04, 118.81, 130.87, 132.59, 132.88, 133.31, 134.61, 138.05, 139.37, 148.25, 153.16, 153.94, 163.93, 168.98; **MS (ESI m/z)**: 515.10 $[\text{M}+\text{H}]^+$.

6. 2-(5-(3,4-dimethoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4'-methyl-2'-phenyl-4,5'-bithiazole (**4f**)

Yellow solid, yield: (408 mg, 75%); mp. 232 – 234°C; **IR (KBr, λ_{max} cm^{-1})**: 1625 (C=N), 1256 ($-\text{OCH}_3$); **^1H NMR (DMSO- d_6 ; δ ppm)**: 2.53 (s, 3H, $-\text{CH}_3$), 3.33 – 3.41 (dd, J = 23.6 Hz, 9.2 Hz, 1H, Pyrazole-H), 3.85 (s, 3H, $-\text{OCH}_3$), 3.89 (s, 3H, $-\text{OCH}_3$), 3.95 – 3.99 (dd, J = 23.6 Hz, 16.8 Hz, 1H, Pyrazole-H), 5.53 – 5.59 (dd, J = 16 Hz, 9.6 Hz, 1H, Pyrazole-H), 6.67 (s, 1H, Ar-H), 6.86 (d, J = 12 Hz, 1H, Ar-H), 6.96 (s, 2H, Ar-H), 7.10 (d, J = 6.4 Hz, 1H, Ar-H), 7.25 (s, 1H, Ar-H), 7.41 – 7.45 (m, 4H, Ar-H), 7.88 (d, J = 9.2 Hz, 2H, Ar-H); **^{13}C NMR (DMSO- d_6 + CDCl_3 ; δ ppm)**: 22.02, 60.71, 69.50, 110.08, 116.27, 123.69, 130.88, 132.51, 133.21, 133.69, 134.59, 153.02, 169.36; **MS (ESI m/z)**: 544.90 $[\text{M}+\text{H}]^+$.

7. 4'-methyl-2'-phenyl-2-(3-(thiophen-2-yl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4,5'-bithiazole (**4g**)

Yellow solid, yield: (510 mg, 89%); mp. 250 - 252°C; **IR (KBr, λ_{max} cm^{-1})**: 1612 (C=N), 1260 ($-\text{OCH}_3$); **^1H NMR (DMSO- d_6 ; δ ppm)**: 2.55 (s, 3H, $-\text{CH}_3$), 3.36 – 3.44 (dd, J = 23.6 Hz, 10 Hz, 1H, Pyrazole- CH_2), 3.78 (s, 3H, $-\text{OCH}_3$), 3.86 (s, 3H, $-\text{OCH}_3$), 3.92 – 4.02 (dd, J = 23.2 Hz, 15.6 Hz, 1H, Pyrazole- CH_2), 5.47 – 5.54 (dd, J = 15.6 Hz, 10.4 Hz, 1H, Pyrazole-CH), 6.65 (s, 2H, Ar-H), 6.77 (s, 1H, Ar-H), 7.11 (t, J = 6.8 Hz, 1H, Ar-H), 7.28 (d, J = 3.6 Hz, 1H, Ar-H), 7.44 – 7.49 (m, 3H, Ar-H), 7.60 (s, 1H, Ar-H), 7.91 (m, 1H, Ar-H); **^{13}C NMR (DMSO- d_6 + CDCl_3 ; δ ppm)**: 21.74, 60.94, 65.28, 70.26, 108.11, 110.79, 130.91, 132.67, 133.88, 135.05, 137.63, 139.17, 141.82, 142.15, 147.76, 153.59, 158.11, 168.43, 169.36%; **MS (ESI m/z)**: 575.12 $[\text{M}+\text{H}]^+$.

8. 2-(3-(furan-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4'-methyl-2'-phenyl-4,5'-bithiazole (**4h**)

Yellow solid, yield: (428 mg, 86%); mp. 218 - 220°C; **IR (KBr, λ_{max} cm^{-1})**: 1610 (C=N), 1258 ($-\text{OCH}_3$); **^1H NMR (DMSO- d_6 ; δ ppm)**: 2.63 (s, 3H, $-\text{CH}_3$), 3.58 – 3.66 (dd, J = 23.2 Hz, 9.2 Hz, 1H, Pyrazole- CH_2), 3.71 – 3.81 (dd, J = 22.8 Hz, 15.6 Hz, 1H, Pyrazole CH_2), 3.86 (s, 3H, $-\text{OCH}_3$), 5.67 – 5.73 (dd, J = 15.6 Hz, 9.2 Hz, 1H, Pyrazole-CH), 6.36 – 6.38 (m, 1H, Ar-H), 6.55 (d, J = 4 Hz, 1H, Ar-H), 6.70 (s, 1H, Ar-H), 6.96 (d, J = 11.6 Hz, 2H, Ar-H), 7.37 – 7.52 (m, 4H, Ar-H), 7.72 (d, J = 11.6 Hz, 2H, Ar-H), 7.92 (d, J = 9.6 Hz, 2H, Ar-H); **^{13}C NMR (DMSO- d_6 + CDCl_3 ; δ ppm)**: 22.17, 47.75, 60.19, 62.48, 110.00, 113.86, 115.40, 118.99, 128.44, 130.90, 132.86, 133.74, 134.60, 138.35, 147.00, 148.21, 153.88, 156.37, 157.41, 165.89, 168.59, 169.51; **MS (ESI m/z)**: 499.15 $[\text{M}+\text{H}]^+$.

9. 2-(5-(3,4-dimethoxyphenyl)-3-(pyridin-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4'-methyl-2'-phenyl-4,5'-bithiazole (**4i**)

Yellow solid, yield: (485 mg, 90%); mp. 262 - 264°C; IR (KBr, λ_{max} cm^{-1}): 1622 (C=N), 1248 (-OCH₃); ¹H NMR (DMSO-*d*₆; δ ppm): 2.52 (s, 3H, -CH₃), 3.49 – 3.55 (dd, *J* = 18.4 Hz, 6.8 Hz, 1H, -Pyrazole-CH₂), 3.82 (s, 3H, -OCH₃), 3.87 (s, 3H, -OCH₃), 4.00 – 4.04 (dd, *J* = 12 Hz, 4 Hz, 1H, Pyrazole –CH₂), 5.56 – 5.61 (dd, *J* = 12.4 Hz, 7.2 Hz, 1H, Pyrazole-CH), 6.71 (s, 1H, Ar-H), 6.84 (d, *J* = 8 Hz, 1H, Ar-H), 6.96 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.29 – 7.32 (m, 1H, Ar-H), 7.33 – 7.40 (m, 3H, Ar-H), 7.59 (s, 1H, Ar-H), 7.91 (t, *J* = 8 Hz, 1H, Ar-H), 7.87 (d, *J* = 8.6 Hz, 2H, Ar-H), 8.13 (d, *J* = 8 Hz, 1H, Ar-H), 8.59 (d, *J* = 5.6 Hz, 1H, Ar-H) ¹³C NMR (DMSO-*d*₆ + CDCl₃; δ ppm): 22.11, 48.00, 60.71, 69.63, 110.36, 115.56, 116.44, 123.79, 126.01, 128.88, 130.82, 133.74, 134.62, 138.83, 141.19, 148.32, 154.17, 158.56, 168.47, 169.04; MS (ESI *m/z*): 540.15 [M+H]⁺.

10. 2-(3-(benzofuran-2-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4'-methyl-2'-phenyl-4,5'-bithiazole (**4j**)

Yellow solid, yield: (508 mg, 92%); mp. 236 - 238°C; IR (KBr, λ_{max} cm^{-1}): 1612 (C=N), 725 (C-Cl); ¹H NMR (DMSO-*d*₆; δ ppm): 2.51 (s, 3H, -CH₃), 3.33 – 3.41 (dd, *J* = 23.6 Hz, 9.2 Hz, 1H, Pyrazole-CH₂), 3.95 – 4.05 (dd, *J* = 23.2 Hz, 16 Hz, 1H, Pyrazole-CH), 5.64 – 5.70 (dd, *J* = 16.4 Hz, 9.2 Hz, 1H, Pyrazole-CH₂), 6.72 (s, 1H, -Ar-H), 7.09 (s, 1H, Ar-H), 7.35 – 7.45 (m, 9H, Ar-H), 7.56 – 7.64 (m, 2H, Ar-H), 7.90 (d, *J* = 10.4 Hz, 2H, Ar-H); ¹³C NMR (DMSO-*d*₆; δ ppm): 17.42, 43.11, 63.78, 107.10, 110.50, 111.82, 122.34, 124.36, 126.42, 128.08, 129.12, 132.89, 140.31, 143.05, 144.96, 148.11, 155.54, 163.96; MS (ESI *m/z*): 554.10 [M+H]⁺.

11. 2-(3-(benzofuran-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4'-methyl-2'-phenyl-4,5'-bithiazole (**4k**)

Yellow solid, yield: (499 mg, 91%); mp. 287 - 289°C; IR (KBr, λ_{max} cm^{-1}): 1630 (C=N), 1245 (C-OCH₃); ¹H NMR (DMSO-*d*₆; δ ppm): 2.58 (s, 3H, -CH₃), 3.36 – 3.44 (dd, 23.6 Hz, *J* = 8.8 Hz, 1H, Pyrazole-CH₂), 3.78 (s, 3H, -OCH₃), 3.93 – 4.02 (dd, *J* = 23.2 Hz, 16 Hz, 1H, Pyrazole-CH), 5.62 – 5.68 (dd, *J* = 16 Hz, 8.8 Hz, 1H, Pyrazole-CH), 6.73 (s, 1H, Ar-H), 6.89 (d, *J* = 11.6 Hz, 2H, Ar-H), 7.08 (s, 1H, Ar-H), 7.28 (t, *J* = 10.4 Hz, 1H, Ar-H), 7.34 – 7.37 (m, 3H, Ar-H), 7.44 – 7.47 (m, 3H, Ar-H), 7.56 – 7.64 (m, 2H, Ar-H), 7.94 (s, 2H, Ar-H); ¹³C NMR (DMSO-*d*₆ + CDCl₃; δ ppm): 22.01, 47.75, 60.04, 68.81, 110.36, 113.34, 116.32, 118.84, 126.47, 128.34, 130.95, 132.87, 133.75, 134.71, 137.74, 148.59, 160.23, 164.00, 168.67; MS (ESI *m/z*): 549.10 [M+H]⁺.

Results And Discussion

Chemistry:

The required starting material chalcones (3a-k) for the preparation of target compounds (4a-k), were obtained by reacting 2-Acetylfuran / 2-Acetylthiophene / 2-Acetylbenzofuran

with various substituted aldehydes in the presence of NaOH in ethanol at 0°C to room temperature as per the literature procedure [50, 51]. The title compounds dihydro pyrazolyl bithiazole derivatives (4a-k), were synthesized via multicomponent reaction approach by the condensation of an equimolar mixture of 2-bromo-1-(4-methyl-2-phenyl thiazol-5-yl)ethan-1-one (1), thiosemicarbazide (2) and substituted chalcones (3 a-k) in the presence of sodium hydroxide in ethanol under reflux with good yields (**Table 1**) as outlined in **Scheme 1**.

To know the feasibility of the reaction, initially we have carried out the reaction of 2-bromo-1-(4-methyl-2-phenyl thiazol-5-yl)ethan-1-one (1), thiosemicarbazide (2) and substituted chalcone (3a) in presence of absolute ethanol as a solvent for about 12-16 h reaction time at 70°C. The yield of the reaction was very poor and the reaction time was very high. In order to improve the yield of the reactions and to reduce the reaction time we tried the reaction by using Na₂CO₃, K₂CO₃, NaOH and KOH with different molar ratios (1 eq, 1.5 eq, 2 eq and 3 eq). Among these variations, 1.5 eq. NaOH at 70°C gave the product with good yield within shorter reaction time (4- 5 h). Here we reported reaction optimization conditions in **Table 2**.

A plausible reaction mechanism for target molecules 4(a-k) was shown in **Scheme 2**. Initially thiosemicarbazide (2) reacts with 2-bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethan-1-one (1) and forms Hantzsch thiazole by the elimination of HBr and H₂O. Subsequent condensation of α -hydrazinothiazole with α , β unsaturated ketones could result the formation of dihydropyrazole ring. Overall the three component domino transformation leads to three C-N and one C-S bonds. In this three component reaction two heterocyclic systems, thiazole and dihydropyrazoles were successfully synthesized.

The structures of the newly synthesized compounds were confirmed by IR, ¹H NMR, ¹³C NMR and Mass spectral data and described here in detail for a representative compound 4b. ¹H NMR spectrum of compound 4b, the three protons present at fourth and fifth position of pyrazoline ring exhibited characteristic ABX splitting pattern and displayed double doublets (dd). The chemical shift values for Ha proton appeared at δ 3.29 – 3.34 ppm (dd, J = 12.8 Hz, 6 Hz, 1H), for Hb proton appeared at δ 3.97- 4.04 ppm (dd, J = 16.8 Hz, 4.8 Hz, 1H), for Hx proton chemical shift value appeared at δ 5.61 – 5.66 ppm (dd, J = 11.2 Hz, 5.6 Hz, 1H) and the remaining aromatic protons appeared in the range of δ 7.07 – 7.90 ppm respectively. ¹³C NMR has been recorded for the compounds 4a-k and the characteristic peaks of dihydropyrazole C3, C4 and C5 are exhibited in the range of δ 163.5, 44.3 and 64.7 ppm respectively. The ESI-Mass spectra were recorded for the compounds 4 a-k and all the compounds exhibited (M+H)⁺ as base peak. All the spectral analysis data of the final compounds clearly indicates the formation of target compounds.

Table 1. Synthesis of dihydro pyrazolyl bithiazole derivatives 4a-k:

S.No.	Product	R	R ¹	R ²	R ³
1	4a	<i>thiophen-2-yl</i>	H	H	H
2	4b	<i>thiophen-2-yl</i>	H	F	H
3	4c	<i>thiophen-2-yl</i>	H	Cl	H
4	4d	<i>thiophen-2-yl</i>	H	Br	H
5	4e	<i>thiophen-2-yl</i>	H	-OCH ₃	H
6	4f	<i>thiophen-2-yl</i>	-OCH ₃	-OCH ₃	H
7	4g	<i>thiophen-2-yl</i>	-OCH ₃	-OCH ₃	-OCH ₃
8	4h	<i>furan-2-yl</i>	H	-OCH ₃	H
9	4i	<i>pyridin-2-yl</i>	-OCH ₃	-OCH ₃	H
10	4j	<i>benzofuran-2-yl</i>	H	Cl	H
11	4k	<i>benzofuran-2-yl</i>	H	-OCH ₃	H

Table 2. Reaction optimization conditions for the synthesis of dihydro pyrazolyl bithiazole derivatives:

Anticancer activity application of 2-(5-(Aryl)-3-heteryl-4,5-dihydro-1H-pyrazol-1-yl)-4'-methyl-2'-phenyl-4,5'-bithiazole derivatives (4a-k):

The newly synthesized 2-(5-(Aryl)-3-heteryl-4,5-dihydro-1H-pyrazol-1-yl)-4'-methyl-2'-phenyl-4,5'-bithiazole derivatives (4a-k) were evaluated for anticancer activity application by using standard in vitro MTT assay [52] with four different human cancer cell lines like Non-small-cell lung cancer cell lines (A549), prostate cancer (DU-145), breast cancer (MCF-7), and Human neuroblastoma (SKNSH). The preliminary investigation of anticancer properties of the novel compounds (4a-K) were done at 50 and 100 μ M concentrations and percentage cell line growth inhibition was measured. From the preliminary anticancer activity results, majority of the compounds exhibited profound cell growth inhibition. All the newly synthesized compounds were further screened at various concentrations (1-100 μ M) and IC₅₀ values were calculated with respect to standard Doxorubicin, by repeating the experiments in thrice and the average values were shown in **Table 3**.

Table 3. Anticancer activity evaluation (IC₅₀ values) of novel 2-(5-(Aryl)-3-heteryl-4,5-dihydro-1H-pyrazol-1-yl)-4'-methyl-2'-phenyl-4,5'-bithiazole derivatives (4a-k):

Entry	Solvent	Base	Temperature (° C)	Time (h)	Yield (%)
1	Methanol	-	reflux	16	20
		Na ₂ CO ₃		8	45
		K ₂ CO ₃		8	50
		NaOH		6	65
		KOH		6	60
2	Ethanol	-	reflux	16	45
		Na ₂ CO ₃		12	50
		K ₂ CO ₃		10	55
		NaOH		4	85
		KOH		6	80
3	Acetonitrile	-	reflux	16	25
4	Dimethylformamide	-	120	16	40
5	Dimethylsulphoxide	-	120	16	35
6	Acetic acid	-	110	16	50

S.No.	Compound	IC ₅₀ (μM)			
		A549	DU145	MCF7	SKNSH
1	4a	13.88 ± 0.21	9.41 ± 0.32	4.62 ± 0.34	8.17 ± 0.31
2	4b	19.68 ± 0.11	11.31 ± 0.12	14.92 ± 0.14	17.57 ± 0.11
3	4c	47.71 ± 0.28	9.21 ± 0.14	14.43 ± 0.62	19.42 ± 0.34
4	4d	75.26 ± 0.38	16.43 ± 0.16	7.17 ± 0.33	18.23 ± 0.34
5	4e	20.86 ± 0.34	14.98 ± 0.21	11.64 ± 0.24	13.2 ± 0.42
6	4f	20.54 ± 0.42	20.42 ± 0.72	3.47 ± 0.28	9.14 ± 0.12
7	4g	24.93 ± 0.19	12.83 ± 0.37	1.36 ± 0.74	11.44 ± 0.73
8	4h	17.92 ± 0.32	22.63 ± 0.18	3.76 ± 0.35	16.67 ± 0.12
9	4i	21.64 ± 0.36	8.36 ± 0.32	15.65 ± 0.28	5.36 ± 0.23
10	4j	24.27 ± 0.16	15.51 ± 0.34	4.14 ± 0.26	12.52 ± 0.24
11	4k	25.84 ± 0.17	20.38 ± 0.24	13.51 ± 0.15	18.6 ± 0.48
12	Doxorubicin	1.37 ± 0.11	3.72 ± 0.14	1.29 ± 0.16	2.18 ± 0.32

From the anticancer activity evaluation results, all the compounds exhibited good to moderate anticancer activity against the four screened human cancer cell lines. Compound 4g exhibited excellent activity against MCF-7 cancer cell line with IC₅₀ value of 1.36 ± 0.74 μM. Among the tested eleven compounds, compound 4a exhibited promising activity against DU-145, MCF-7 and SKNSH cancer cell lines with IC₅₀ values of 9.41 ± 0.32, 4.62 ± 0.34 and 8.17 ± 0.31 μM concentrations. Compounds 4c and 4i were shown good activity against DU-145 cell line with IC₅₀ values of 9.21 ± 0.14 and 8.36 ± 0.32 μM concentrations. Majority of the compounds like 4d, 4f, 4h and 4j have shown promising anticancer property against MCF-7 cell line with IC₅₀ values of 7.17 ± 0.33, 3.47 ± 0.28, 3.76 ± 0.35 and 4.14 ± 0.26 μM concentrations. Whereas the compound 4f and 4i exhibited good anticancer property against SKNSH cancer cell line with IC₅₀ values of 9.14 ± 0.12 and 5.36 ± 0.23 μM concentrations respectively.

In silico studies of the 2-(5-(Aryl)-3-heteryl-4,5-dihydro-1H-pyrazol-1-yl)-4'-methyl-2'-phenyl-4,5'-bithiazole derivatives:

The in vitro results suggest that breast cancer cell line is more effected by the novel 2-(5-(Aryl)-3-heteryl-4,5-dihydro-1H-pyrazol-1-yl)-4'-methyl-2'-phenyl-4,5'-bithiazole derivatives (**4a-k**) than the other cell lines. The cytotoxicity result has been validated by docking simulation study using AutoDock Vina (ADT Vina) [53]. Docking results elucidate the importance of different types of interactions to inhibit the function of probable target for breast cancer lines, Human Estrogen Receptor Alpha protein (3ert.pdb) [54]. In the present study only three ligands, 4f, 4g and 4i with the residue Cys 530, Meth 522 and Thr 347 form hydrogen binding respectively.

The binding affinity of all the novel derivatives has stronger binding energies than that of the bound ligand OHT. The docking energy values of derivatives are given in Table 3 with their active site residues. Among all the ligands, compound 4j having a good binding affinity compared to co-crystallized bound ligand OHT. The pattern of cytotoxic activity of derivatives is matching more than 75% of in silico results. The order of binding affinity of selected ligands against the target is **4j > 4a > 4i > 4d > 4h > 4b > 4c > 4g > 4f > 4e > 4k** and OHT with binding energy range -7.8 to -10.1 k cal/mol (**Table 4**).

Table 4. *In silico* activity evaluation ligands (**4a-k**) using ADT Vina against the target Human Estrogen Receptor Alpha protein (3ert.pdb):

S. No.	Name of Ligands	Docking Energy (kcal/mol)	Active site residues of receptor (3ert.pdb)
1.	4a	-9.2	Leu 346, Thr 347, Ala 350, Asp 351, Trp 383, Leu 384, Met 522, Leu 525, Tyr 526, Leu 536
2.	4b	-8.7	Met 343, Thr 347, Ala 350, Trp 383, Met 522, Leu 525, Try 526, Cys 530, Lys 531, Val 533, Pro 535
3.	4c	-8.4	Met 343, Thr 347, Ala 350, Trp 383, Asn 519, Met 522, Glu 523, Leu 525, Try 526
4.	4d	-8.8	Leu 346, Thr 347, Ala 350, Asp 351, Trp 383, Met 522, Leu 525, Tyr 526, Leu 536, Leu 539
5.	4e	-7.8	Ala 350, Asp 351, Leu 354, Glu 380, Trp 383, Leu 525, Tyr 526, Lys 529, Cys 530
6.	4f	-7.9	Met 522, Glu 523, Tyr 526, Leu 529, Cys 530, Val 533, Val 534, Leu 535, Leu 536
7.	4g	-8.2	Ala 350, Asp 351, Leu 354, Glu 380, Trp 383, Met 522, Leu 525, Tyr 526, Cys 530 , Val 533, Pro 535, Leu 536
8.	4h	-8.8	Leu 346, Thr 347, Ala 350, Asp 351, Trp 383, Leu 525, Val 533, Tyr 534, Leu 536, Leu 539
9.	4i	-8.9	Leu 346, Thr 347, Ala 350, Asp 351, Trp 383, Leu 525, Tyr 526, Met 533, Val 534, Leu 536, Met 543
10.	4j	-10.1	Leu 346, Thr 347, Ala 350, Asp 351, Trp 383, Leu 384, Leu 525, Met 528, Lys 529, Met 533, Pro 535, Leu 536, Leu 539
11.	4k	-7.8	Leu 346, Thr 347, Ala 350, Asp 351, Trp 383, Leu 384, Met 528, Lys 529, Met 533, Pro 535, Leu 536, Leu 539
12.	OHT	-7.8	Ala 350, Asp 351, Leu 354, Trp 383, Met 522, Tyr 526 Cys 530, Val 533, Leu 536

With the help of comparative analysis of active site residues interactions with the ligands shows that Thr 347, Asp 351, TYR 526, Leu 536, Leu 525, Ala 350, Trp 383 are key residue of catalytic site of Estrogen Receptor Alpha protein (3ert.pdb) and commonly occurs 8, 8, 8, 9, 10, 10 and 11 times respectively. The bound confirmation of docked ligands depicted that all are occupy the same binding cavity and shown in figure 2 with secondary structure of receptor (3ert.pdb). The 2-D & 3-D representations are shown in figure 3a-c for ligand 4g.

Conclusion

In summary, a series of 2-(5-(Aryl)-3-heteryl-4,5-dihydro-1*H*pyrazol-1-yl)-4'-methyl-2'-phenyl-4,5'-bithiazole derivatives (4a-k) were synthesized efficiently via one pot multicomponent reaction approach with good yields and evaluated for their anticancer activity properties. The method applied for the novel targets is simple and highly efficient in shorter reaction time. Most of the newly synthesized compounds were shown promising anticancer activity against breast cancer (MCF-7) cell line.

Declarations

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Conflict of Interest:

The authors declare no conflict of interest.

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Scheme

Scheme 1 and 2 is available in supplementary section.

Figures

Figure 1

Therapeutic active drugs possessing dihydropyrazole and thiazole scaffolds.

Figure 2

Secondary structure representation of Human Estrogen Alpha protein (3ert.pdb) and bound ligands at the active/binding site cavity.

Figure 3

(a) 2-D representation of ligand 4g with hydrogen bonding view using LigPlus and **(b)** Electrostatic and **(c)** hydrophobic interactions at the active site cavity of target protein (3ert.pdb) (Hydrogen bonds- Green color dashed lines; Hydrophobic as an arc) [55].

Supplementary Files

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