Asian Journal of Chemistry; Vol. 34, No. 10 (2022), 2717-2724

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2022.23984

An Efficient One Pot Multicomponent Synthesis of Dihydro Pyrazolyl Bisthiazole Derivatives as Potential Anticancer Agents and their Molecular Docking Studies

ANJANEYULU VARLUVOTHU[1], KRISHNAIAH VAARLA[2], RAJESH KUMAR KESHARWANI[3] and PANAGANTI LEELAVATHI[1,*]

[1]Department of Chemistry, Osmania University, Hyderabad-500007, India
[2]Department of Chemistry, National Institute of Technology, Warangal-506004, India
[3]Department of Computer Application, Nehru Gram Bharati (Deemed to be University), Prayagraj-221505, India

*Corresponding author: E-mail: leelaou@gmail.com; leelaou@osmania.ac.in

Received: 27 June 2022; *Accepted*: 4 August 2022; *Published online*: 19 September 2022; AJC-20979

A novel series of dihydropyrazolyl bisthiazoles were synthesized through simple reaction conditions *via* one pot multicomponent reaction of 2-bromo-1-(4-methyl-2-phenyl thiazol-5-yl)ethan-1-one, thiosemicarbazide and chalcones in the presence of sodium hydroxide in ethanol under reflux condition. The reaction generates two potential five membered heterocylic pharmacophores *i.e.* Hantzsch thiazole and dihydropyrazole in one step through simple operation, in shorter reaction time with high yields. The newly synthesized compounds were well characterized by IR, ^{1}H, ^{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. Some of the compounds exhibited excellent activity against MCF-7 cancer cell line and studied molecular interaction of probable target protein, human estrogen receptor alpha protein (3ert.pdb) using docking simulation.

Keywords: Multicomponent reaction, Dihydropyrazolyl bisthiazoles, Anticancer activity, Molecular docking studies.

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 a useful tool in 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 method 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. Improvement in quality of life is due to the contribution of heterocycles 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 area of medicinal chemistry and functional materials. Most of the thiazoles have been reported to exhibit antimicrobial [16,17], antiviral [18-20], antitubercular [21,22], anticancer [23-26], anti-inflammatory [27-29], antitrypanosomal [30,31] and antifungal activities *etc.* [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 [46,47].

In view of the biological significance of the thiazoles and dihydropyrazoles and our interest in construction of biologically active heterocyclic compounds [48,49], it is considered worthwhile to explore the synthesis and anticancer activity of thiazoles and dihydropyrazoles. Herein, we report the construction

of new heterocyclic compounds with thiazole and dihydropyrazole in a single molecular frame work through simple and efficient one pot multicomponent chemical transformation of 2-bromo-1-(4-methyl-2-phenyl thiazol-5-yl)ethan-1-one (**1**), thiosemicarbazide (**2**), various substituted chalcones (**3a-k**) and evaluation of their anticancer 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 context to its biological activity.

EXPERIMENTAL

Organic solvents and the reagents *e.g*. thiosemicarbazide and sodium hydroxide were procured from the commercial sources and used directly without any further purification. The reactions were screened 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. Infrared spectra data were recorded on Perkin-Elmer (Spectrum-100S) spectrophotometer. The ^{1}H and ^{13}C were analyzed by using Bruker spectrometer at 400 and 100 MHz, respectively using tetramethylsilane (TMS) as an internal standard. The mass spectra were recorded on Water micro-mass API instrument.

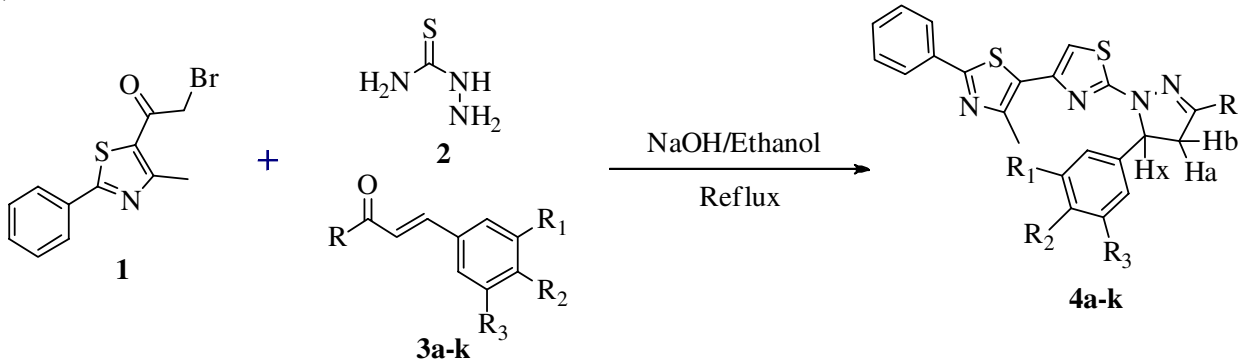
General Procedure for the synthesis of 2-(5-(aryl)-3-heteroaryl-4,5-dihydro-1*H*-pyrazol-1-yl)-4′-methyl-2′-phenyl-4,5′-bisthiazole derivatives (4a-k): A reaction mixture of 2-bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethan-1-one (**1**, 1 mmol), thiosemicarbazide (**2**, 1 mmol), chalcones (**3a-k**, 1 mmol) and NaOH (1.5 mmol) in ethanol (5 mL) were heated to 70 ºC 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 pure compounds (**Scheme-I**).

4′-Methyl-2′-phenyl-2-(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4,5′-bithiazole (4a): Yellow solid, yield: (406 mg, 84%); m.p.: 208-210 ºC; IR (KBr, ν_{max}, cm^{-1}): 1602 (C=N); ^{1}H 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); ^{13}C NMR (DMSO-d_6 + $CDCl_3$; 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-(5-(4-Fluorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4′-methyl -2′-phenyl-4,5′-bithiazole (4b): Orange solid, yield: (446 mg, 89%); m.p.: 185-187 ºC; IR (KBr, ν_{max}, cm^{-1}): 1612 (C=N); ^{1}H 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); ^{13}C NMR (DMSO-d_6 + $CDCl_3$; 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]$^+$.

2-(5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4′-methyl -2′-phenyl-4,5′-bithiazole (4c): Yellow solid, yield: (440 mg, 85%); m.p.: 256-258 ºC; IR (KBr, ν_{max}, cm^{-1}): 1610 (C=N), 569 (C-Cl); ^{1}H NMR (DMSO-d_6; δ ppm): 2.46 (s, 3H, -CH_3), 3.38-3.42 (dd, *J* = 14 Hz, 5.2 Hz, 1H, pyrazole-CH_2), 4.04-4.10 (dd, *J* = 14.4 Hz, 9.6 Hz, 1H, pyrazole-CH_2), 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); ^{13}C NMR (DMSO-d_6 + $CDCl_3$; 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]$^+$.

2-(5-(4-Bromophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4′-methyl -2′-phenyl-4,5′-bithiazole (4d): Orange solid, yield: (495 mg, 88%); m.p.: 296-298 ºC; IR (KBr, ν_{max}, cm^{-1}): 1615 (C=N), 598 (C-Br); ^{1}H NMR (DMSO-d_6; δ ppm): 2.51 (s, 3H, -CH_3), 3.33-3.41 (dd, *J* = 23.6 Hz, 10 Hz, 1H, pyrazole-CH_2), 3.91-4.01 (dd, *J* = 23.2 Hz, 15.6 Hz, 1H, pyrazole-CH_2), 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); ^{13}C NMR (DMSO-d_6 + $CDCl_3$; δ ppm): 22.01, 49.02, 60.71, 69.50, 110.08, 116.27, 123.69, 130.88, 132.51,



Scheme-I: One pot synthesis of novel dihydro pyrazolyl bithiazole derivatives (**4a-k**)

133.21, 133.69, 134.59, 138.26, 139.37, 148.27, 153.02, 169.19; MS (ESI *m/z*): 565.00 $[M+2H]^+$.

2-(5-(4-Methoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4′-methyl-2′-phenyl-4,5′-bithiazole (4e): Orange solid, yield: (406 mg, 79%); m.p.: 224-226 ºC; IR (KBr, ν_{max}, cm^{-1}): 1612 (C=N), 1265 (-OCH_3); ^1H NMR (DMSO-d_6; δ ppm): 2.52 (s, 3H, -CH_3), 3.32-3.38 (dd, *J* = 17.6 Hz, 6.8 Hz, 1H, pyrazole-H), 3.77 (s, 3H, -OCH_3), 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 $[M+H]^+$.

2-(5-(3,4-Dimethoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4′-methyl-2′-phenyl-4,5′-bithiazole (4f): Yellow solid, yield: (408 mg, 75%); m.p.: 232-234 ºC; IR (KBr, ν_{max}, cm^{-1}): 1625 (C=N), 1256 (-OCH_3); ^1H NMR (DMSO-d_6; δ ppm): 2.53 (s, 3H, -CH_3), 3.33-3.41 (dd, *J* = 23.6 Hz, 9.2 Hz, 1H, pyrazole-H), 3.85 (s, 3H, -OCH_3), 3.89 (s, 3H, -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 $[M+H]^+$.

4′-Methyl-2′-phenyl-2-(3-(thiophen-2-yl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4,5′-bithiazole (4g): Yellow solid, yield: (510 mg, 89%); m.p.: 250-252 ºC; IR (KBr, ν_{max}, cm^{-1}): 1612 (C=N), 1260 (-OCH_3); ^1H NMR (DMSO-d_6; δ ppm): 2.55 (s, 3H, -CH_3), 3.36-3.44 (dd, *J* = 23.6 Hz,10 Hz, 1H, pyrazole-CH_2), 3.78 (s, 3H, -OCH_3), 3.86 (s, 3H, -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 $[M+H]^+$.

2-(3-(Furan-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4′-methyl-2′-phenyl-4,5′-bithiazole (4h): Yellow solid, yield: (428 mg, 86%); m.p.: 218-220 ºC; IR (KBr, ν_{max}, cm^{-1}): 1610 (C=N), 1258 (-OCH_3); ^1H NMR (DMSO-d_6; δ ppm): 2.63 (s, 3H, -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, -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 $[M+H]^+$.

2-(5-(3,4-Dimethoxyphenyl)-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4′-methyl-2′-phenyl-4,5′-bithiazole (4i): Yellow solid, yield: (485 mg, 90%); m.p.: 262-264 ºC; IR (KBr, ν_{max}, cm^{-1}): 1622 (C=N), 1248 (-OCH_3); ^1H NMR (DMSO-d_6; δ ppm): 2.52 (s, 3H, -CH_3), 3.49-3.55 (dd, *J* = 18.4 Hz. 6.8 Hz, 1H, -Pyrazole-CH_2), 3.82 (s, 3H, -OCH_3), 3.87 (s, 3H, -OCH_3), 4.00-4.04 (dd, *J* = 12 Hz, 4 Hz, 1H, pyrazole–CH_2), 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) ^{13}C NMR (DMSO-d_6 + $CDCl_3$; 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 $[M+H]^+$.

2-(3-(Benzofuran-2-yl)-5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4′-methyl-2′-phenyl-4,5′-bithiazole (4j): Yellow solid, yield: (508 mg, 92%); m.p.: 236-238 ºC; IR (KBr, ν_{max}, cm^{-1}): 1612 (C=N), 725 (C-Cl); ^1H NMR (DMSO-d_6; δ ppm): 2.51 (s, 3H, -CH_3), 3.33-3.41 (dd, *J* = 23.6 Hz, 9.2 Hz, 1H, pyrazole-CH_2), 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-CH_2), 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); ^{13}C 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 $[M+H]^+$.

2-(3-(Benzofuran-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4′-methyl-2′-phenyl-4,5′-bithiazole (4k): Yellow solid, yield: (499 mg, 91%); m.p.: 287-289 ºC; IR (KBr, ν_{max}, cm^{-1}): 1630 (C=N), 1245 (C-OCH_3); ^1H NMR (DMSO-d_6; δ ppm): 2.58 (s, 3H, -CH_3), 3.36-3.44 (dd, 23.6 Hz, *J* = 8.8 Hz, 1H, pyrazole-CH_2), 3.78 (s, 3H, -OCH_3), 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); ^{13}C NMR (DMSO-d_6 + $CDCl_3$; 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 $[M+H]^+$.

Anticancer activity by using standard MTT assay: The synthesized 2-(5-(aryl)-3-heteryl-4,5-dihydro-1*H*-pyrazol-1-yl)-4′-methyl-2′-phenyl-4,5′-bithiazole derivatives (**4a-k**) were evaluated for their anticancer ativity 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 obtained from American Type Culture Collection (ATCC)

and cells were cultured in Dulbecco's modified Eagle medium (DMEM) with 10% fatal bovine serum (FBS), 1% antibiotics and incubated at 37 ºC in a humidified incubator with 5% CO_2. The synthesized compounds (**4a-k**) were dissolved in cell culture grade DMSO solvent. Four cancerous cell lines were taken in a 96-well tissue culture plate (10^4 cells/each well) and the cells were treated with various concentrations of newly 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 the 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 (Erα) protein as a probable target against breast cancer (MCF-7). The 3-D structure of human estrogen receptor alpha protein (3ERT.PDB) 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 bisthiazole derivatives) were drawn using Chem Sketch 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 and Molegro molecular viewer, respectively.

RESULTS AND DISCUSSION

The required starting material chalcones (**3a-k**) for the preparation of target compounds (**4a-k**), were obtained by reacting 2-acetylfuran/2-acetylthiphene/2-acetylbenzofuran with various substituted aldehydes in the presence of NaOH in ethanol at 0 ºC to room temperature as per the literature procedure [50,51]. The title compounds dihydro pyrazolyl bisthiazole derivatives (**4a-k**), were synthesized *via* multicomponent reaction method 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 (**3a-k**) in the presence of sodium hydroxide in ethanol under reflux with good yields (Table-1) as outlined in **Scheme-I**.

TABLE-1
STRUCTURES OF DIHYDRO PYRAZOLYL BITHIAZOLE DERIVATIVES (**4a-k**)

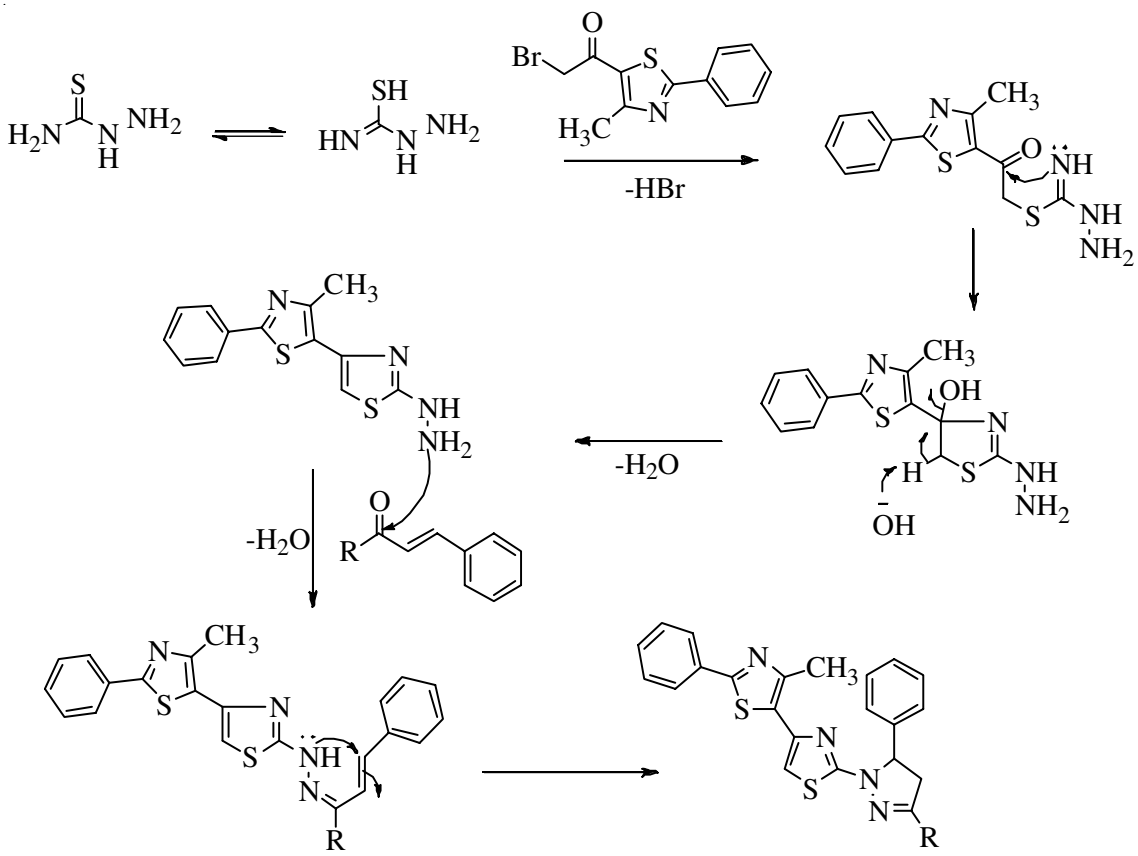
Product	R	R^1	R^2	R^3
4a	Thiophen-2-yl	H	H	H
4b	Thiophen-2-yl	H	F	H
4c	Thiophen-2-yl	H	Cl	H
4d	Thiophen-2-yl	H	Br	H
4e	Thiophen-2-yl	H	$-OCH_3$	H
4f	Thiophen-2-yl	$-OCH_3$	$-OCH_3$	H
4g	Thiophen-2-yl	$-OCH_3$	$-OCH_3$	$-OCH_3$
4h	Furan-2-yl	H	$-OCH_3$	H
4i	Pyridin-2-yl	$-OCH_3$	$-OCH_3$	H
4j	Benzofuran-2-yl	H	Cl	H
4k	Benzofuran-2-yl	H	$-OCH_3$	H

Initially, the reaction of 2-bromo-1-(4-methyl-2-phenyl thiazol-5-yl)ethan-1-one (**1**), thiosemicarbazide (**2**) and substituted chalcone (**3a**) in absolute ethanol for about 12-16 h reaction time at 70 ºC was carried out. The yield of the reaction was very poor and the reaction time was very high. To improve the yield of the reactions and to reduce the reaction time we tried the reaction by using Na_2CO_3, K_2CO_3, NaOH and KOH with different molar equivalents (1, 1.5, 2 and 3 equiv.). Among these variations, 1.5 equiv. NaOH at 70 ºC gave good yield with shorter reaction time (4-5 h). The optimization data is presented in Table-2.

TABLE-2
REACTION OPTIMIZATION CONDITIONS FOR THE SYNTHESIS OF DIHYDRO PYRAZOLYL BITHIAZOLE DERIVATIVES (**4a-k**)

Entry	Solvent	Base	Temp. (°C)	Time (h)	Yield (%)
1	Methanol	–	Reflux	16	20
		Na_2CO_3		8	45
		K_2CO_3		8	50
		NaOH		6	65
		KOH		6	60
2	Ethanol	–	Reflux	16	45
		Na_2CO_3		12	50
		K_2CO_3		10	55
		NaOH		4	85
		KOH		6	80
3	Acetonitrile	–	Reflux	16	25
4	DMF	–	120	16	40
5	DMSO	–	120	16	35
6	Acetic acid	–	110	16	50

A plausible reaction mechanism for target molecules (**4a-k**) is shown in **Scheme-II**. 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 H_2O. Subsequent condensation of α-hydrazinothiazole with α,β-unsaturated ketones results in the formation of dihydropyrazole ring. Overall the three component domino transfor-



Scheme-II: Plausible reaction mechanism for the synthesis of target molecules

mation leads to three C-N and one C-S bonds. In this three component reaction, two heterocyclic systems, thiazole and dihydropyrazoles were synthesized successfully.

The structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis and discussed here for the representative compound **4c**. ^{1}H NMR spectrum of compound **4c**, exhibited characteristic ABX splitting pattern *i.e.* displayed double doublets (dd) for the three protons present at fourth and fifth position of pyrazoline ring. The chemical shift values for H_a proton appeared at δ 3.38-3.42 (dd, J = 14 Hz, 5.2 Hz), for H_b proton appeared at δ 4.04-4.10 (dd, J = 14.4 Hz, 9.6 Hz), for H_x proton chemical shift value appeared at δ 5.64-5.68 (dd, J = 9.6 Hz, 5.2 Hz) and the remaining aromatic protons appeared at δ 7.07-7.90 ppm. ^{13}C NMR has been recorded for the compounds **4a-k** and the characteristic peaks of dihydropyrazole C_3, C_4 and C_5 displayed at δ 48.3, 61.1 and 169.2 ppm, respectively. The ESI-Mass spectra were recorded for the compounds **4a-k** and all these compounds exhibited $(M+H)^+$ as base peak. All the spectral analysis of the final compounds clearly indicates the formation of target compounds.

Anticancer activity: The newly synthesized 2-(5-(aryl)-3-heteroaryl-4,5-dihydro-1*H*-pyrazol-1-yl)-4′-methyl-2′-phenyl-4,5′-bisthiazole 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 μM concentrations and percentage cell line growth inhibition was measured. The preliminary anticancer activity results, in majority of the compounds revealed profound inhibition of cell growth. The newly synthesized compounds were further screened at different concentrations (1-100 μM) and IC_{50} values were calculated with respect to standard doxorubicin, by repeating the experiments in thrice and the average values are shown in Table-3. It is concluded that all these 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 IC_{50} value of 1.36 ± 0.74 μM. Among the tested eleven compounds, compound **4a** exhibited distinguishing activity against DU-145, MCF-7and SKNSH cancer cell lines with IC_{50} 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 IC_{50} values of 9.21 ± 0.14 and 8.36 ± 0.32 μM concentrations. The compounds like **4d**, **4f**, **4h** and **4j** have shown the promising anticancer property against MCF-7 cell line with IC_{50} values of 7.17 ± 0.33, 3.47 ± 0.28, 3.76 ± 0.35 and 4.14 ± 0.26 μM concentrations. Whereas compounds **4f** and **4i** exhibited good anticancer property against SKNSH cancer cell line with IC_{50} values of 9.14 ± 0.12 and 5.36 ± 0.23 μM concentrations, respectively.

***In silico* studies:** The *in vitro* results suggest that breast cancer cell line is more effected by the synthesized 2-(5-(aryl)-

TABLE-3
ANTICANCER ACTIVITY EVALUATION (IC_{50} VALUES) OF NOVEL 2-(5-(ARYL)-3-HETEROARYL-4,5-DIHYDRO-1*H*-PYRAZOL-1-YL)-4'-METHYL-2'-PHENYL-4,5'-BITHIAZOLE DERIVATIVES (**4a-k**)

Compd.	IC_{50} (μM)			
	A549	DU145	MCF7	SKNSH
4a	13.88±0.21	9.41±0.32	4.62±0.34	8.17±0.31
4b	19.68±0.11	11.31±0.12	14.92±0.14	17.57±0.11
4c	47.71±0.28	9.21±0.14	14.43±0.62	19.42±0.34
4d	75.26±0.38	16.43±0.16	7.17±0.33	18.23±0.34
4e	20.86±0.34	14.98±0.21	11.64±0.24	13.2±0.42
4f	20.54±0.42	20.42±0.72	3.47±0.28	9.14±0.12
4g	24.93±0.19	12.83±0.37	1.36±0.74	11.44±0.73
4h	17.92±0.32	22.63±0.18	3.76±0.35	16.67±0.12
4i	21.64±0.36	8.36±0.32	15.65±0.28	5.36±0.23
4j	24.27±0.16	15.51±0.34	4.14±0.26	12.52±0.24
4k	25.84±0.17	20.38±0.24	13.51±0.15	18.6±0.48
Doxorubicin	1.37±0.11	3.72±0.14	1.29±0.16	2.18±0.32

3-heteroaryl-4,5-dihydro-1*H*-pyrazol-1-yl)-4′-methyl-2′-phenyl-4,5′-bsithiazole 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 studies explain the importance of various types of interactions to inhibit the functioning of probable target for breast cancer lines, human estrogen receptor alpha protein (3ert.pdb) [54]. In the present study only three molecules*viz.* **4f**, **4g** and **4i** with the residue Cys 530, Meth 522 and Thr 347 form hydrogen binding, respectively.

The binding energies for all the new derivatives has found to be stronger 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-crystalized bound ligand OHT. The pattern of cytotoxic activity of derivatives is matched more than 75% of *in silico* results. The binding affinity order of the 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 kcal/mol (Table-4).

The comparative analysis of active site residues interaction 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 occupy the same binding cavity and shown in Fig. 1 with secondary structure of receptor (3ert.pdb). The 2-D & 3-D representations are shown in Fig. 2a-c for molecule **4g**.

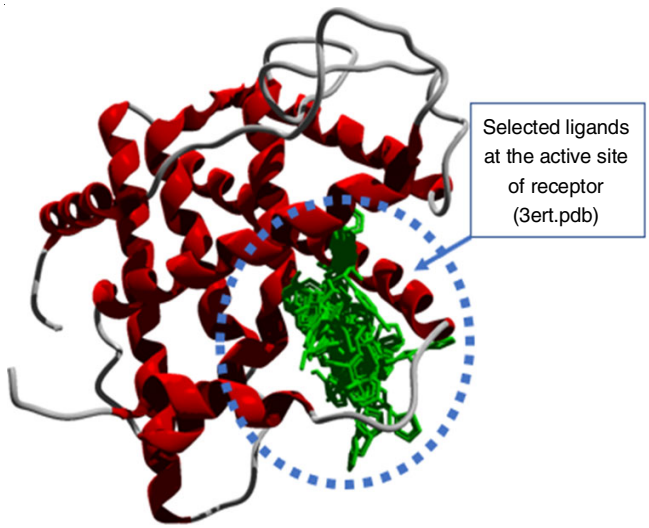


Fig. 1. Representation of secondary structure of human estrogen alpha protein (3ert.pdb) and bound ligands at the active/binding site cavity

Conclusion

In summary, a series of 2-(5-(aryl)-3-heteroaryl-4,5-dihydro-1*H*-pyrazol-1-yl)-4′-methyl-2′-phenyl-4,5′-bisthiazole derivatives (**4a-k**) were synthesized efficiently through a 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 showed promising anticancer activity against breast cancer (MCF-7) cell line.

TABLE-4
In silico ACTIVITY EVALUATION OF LIGANDS (**4a-k**) USING ADT VINA AGAINST THE TARGET HUMAN ESTROGEN RECEPTOR ALPHA PROTEIN (3ert.pdb)

Name of ligands	Docking energy (kcal/mol)	Active site residues of receptor (3ert.pdb)
4a	-9.2	Leu 346, Thr 347, Ala 350, Asp 351, Trp 383, Leu 384, Met 522, Leu 525, Tyr 526. Leu 536
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
4c	-8.4	Met 343, Thr 347, Ala 350, Trp 383, Asn 519, Met 522, Glu 523, Leu 525, Try 526
4d	-8.8	Leu 346, Thr 347, Ala 350, Asp 351, Trp 383, Met 522, Leu 525, Tyr 526, Leu 536, Leu 539
4e	-7.8	Ala 350, Asp 351, Leu 354, Glu 380, Trp 383, Leu 525, Tyr 526, Lys 529, Cys 530
4f	-7.9	Met 522, Glu 523, Tyr 526, Leu 529, Cys 530, Val 533, Val 534, Leu 535, Leu 536
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
4h	-8.8	Leu 346, Thr 347, Ala 350, Asp 351, Trp 383, Leu 525, Val 533, Tyr 534, Leu 536, Leu 539
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
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
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
OHT	-7.8	Ala 350, Asp 351, Leu 354, Trp 383, Met 522, Tyr 526 Cys 530, Val 533, Leu 536

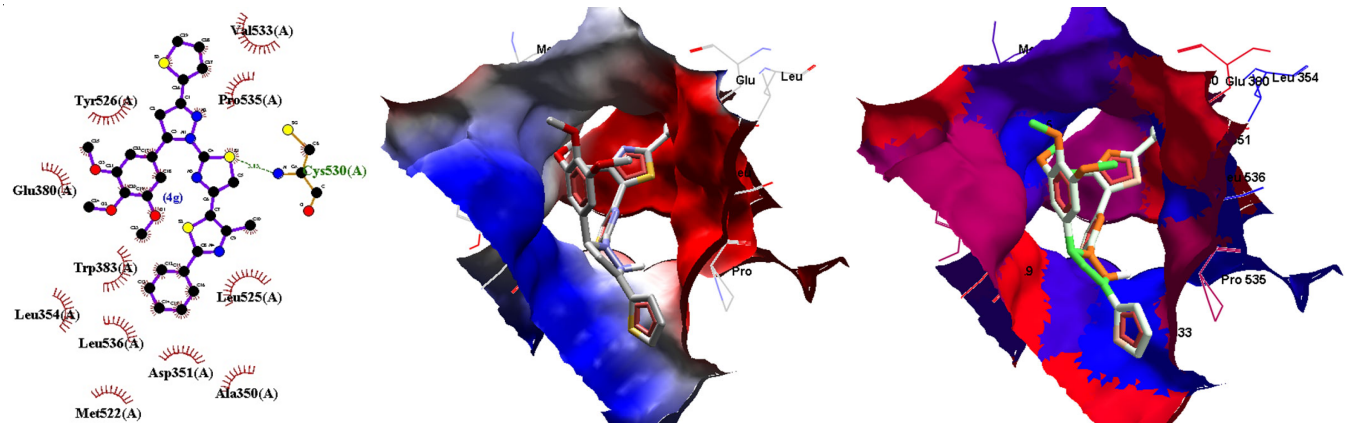


Fig. 2. (a) 2-D representation of ligand **4g** with hydrogen bonding view using LigPlus[55] (b) electrostatic and (c) hydrophobic interactions at the active site cavity of target protein (3ert.pdb) (hydrogen bonds- green colour dashed lines; hydrophobic as an arc)

ACKNOWLEDGEMENTS

The authors thank the Department of Chemistry, Osmania University, Hyderabad, India for the research laboratory infrastructure under SAP (DRS-I) and DST-FIST programmes.

CONFLICT OF INTEREST

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

REFERENCES

1. L. Kong, R. Huang, H. He, Y. Fan, J. Lin and S. Yan, *Green Chem.*, **22**, 256 (2020); https://doi.org/10.1039/C9GC03692J
2. D.F.W. Victor Snieckus, *Synfacts*, **16**, 0020 (2020); https://doi.org/10.1055/s-0039-1691361
3. R.S. Mega, V.K. Duong, A. Noble and V.K. Aggarwal, *Angew. Chem. Int. Ed.*, **59**, 4375 (2020); https://doi.org/10.1002/anie.201916340
4. B. Paul, M. Maji, K. Chakrabarti and S. Kundu, *Org. Biomol. Chem.*, **18**, 2193 (2020); https://doi.org/10.1039/C9OB02760B
5. C. Kalinski, M. Umkehrer, L. Weber, J. Kolb, C. Burdack and G. Ross, *Mol. Divers.*, **14**, 513 (2010); https://doi.org/10.1007/s11030-010-9225-x
6. E. Ruijter and R.V.A. Orru, *Drug Discov. Today. Technol.*, **10**, e15 (2013); https://doi.org/10.1016/j.ddtec.2012.10.012
7. R.J. Capon, C. Peng and C. Dooms, *Org. Biomol. Chem.*, **6**, 2765 (2008); https://doi.org/10.1039/b803455a
8. R.J. Capon, R. Ratnayake, M. Stewart, E. Lacey, S. Tennant and J.H. Gill, *Org. Biomol. Chem.*, **3**, 123 (2005); https://doi.org/10.1039/B413440K
9. A.A. Watson, G.W.J. Fleet, N. Asano, R.J. Molyneux and R.J. Nash, *Phytochemistry*, **56**, 265 (2001); https://doi.org/10.1016/S0031-9422(00)00451-9
10. C. Lamberth, *Pest Manag. Sci.*, **69**, 1106 (2013); https://doi.org/10.1002/ps.3615
11. X. Hua, W. Liu, Y. Su, X. Liu, J. Liu, N. Liu, G. Wang, X. Jiao, X. Fan, C. Xue, Y. Liu and M. Liu, *Pest Manag. Sci.*, **76**, 2368 (2020); https://doi.org/10.1002/ps.5773
12. H. Dong, S. Xu, J. Wang, H. Zhang, Y. Chen, L. Bi and Z. Zhao, *New J. Chem.*, **44**, 8280 (2020); https://doi.org/10.1039/D0NJ00583E
13. V. Maffeis, L. Moni, D. Di Stefano, S. Giordani and R. Riva, *Front. Chem. Sci. Eng.*, **14**, 76 (2020); https://doi.org/10.1007/s11705-019-1833-0
14. M. Klimek-Szczykutowicz, A. Szopa and Ekiert, *Plants*, **9**, 119 (2020); https://doi.org/10.3390/plants9010119
15. M. Kreidl, M. Rainer, T. Jakschitz and G.K. Bonn, *Anal. Chim. Acta*, **1101**, 211 (2020); https://doi.org/10.1016/j.aca.2019.12.015
16. L.L. Wang, N. Battini, R.R.Y. Bheemanaboina, S.L. Zhang and C.H. Zhou, *Eur. J. Med. Chem.*, **167**, 105 (2019); https://doi.org/10.1016/j.ejmech.2019.01.072
17. E.O. Olawode, R. Tandlich, E. Prinsloo, M. Isaacs, H. Hoppe, R. Seldon, D.F. Warner, V. Steenkamp and P.T. Kaye, *Bioorg. Med. Chem. Lett.*, **29**, 1572 (2019); https://doi.org/10.1016/j.bmcl.2019.05.001
18. E. Gürsoy, E.D. Dincel, L. Naesens and N. Ulusoy Güzeldemirci, *Bioorg. Chem.*, **95**, 103496 (2020); https://doi.org/10.1016/j.bioorg.2019.103496
19. I.P. Singh, S. Gupta and S. Kumar, *Med. Chem.*, **16**, 4 (2020); https://doi.org/10.2174/1573406415666190614101253
20. A. Lamut, M. Gjorgjieva, L. Naesens, S. Liekens, K.E. Lillsunde, P. Tammela, D. Kikelj and T. Tomasiè, *Bioorg. Chem.*, **98**, 103733 (2020); https://doi.org/10.1016/j.bioorg.2020.103733
21. U.B. Karale, V.S. Krishna, E.V. Krishna, A.S. Choudhari, M. Shukla, V.R. Gaikwad, B. Mahizhaveni, S. Chopra, S. Misra, D. Sarkar, D. Sriram, V.N.A. Dusthackeer and H.B. Rode, *Eur. J. Med. Chem.*, **178**, 315 (2019); https://doi.org/10.1016/j.ejmech.2019.05.082
22. I. Mishra, R. Mishra, S. Mujwar, P. Chandra and N. Sachan, *J. Heterocycl. Chem.*, **57**, 2304 (2020); https://doi.org/10.1002/jhet.3970
23. M. Ansari, M. Shokrzadeh, S. Karima, S. Rajaei, N. Ghassemi-Barghi, M. Fallah, M. Ghasemian and S. Emami, *Eur. J. Med. Chem.*, **185**, 111784 (2020); https://doi.org/10.1016/j.ejmech.2019.111784
24. A.R. Sayed, S.M. Gomha, E.A. Taher, Z.A. Muhammad, H.R. El-Seedi, H.M. Gaber and M.M. Ahmed, *Drug Des. Devel. Ther.*, **14**, 1363 (2020); https://doi.org/10.2147/DDDT.S221263
25. T.A. Farghaly, G.S. Masaret, Z.A. Muhammad and M.F. Harras, *Bioorg. Chem.*, **98**, 103761 (2020); https://doi.org/10.1016/j.bioorg.2020.103761
26. L.R.P. de Siqueira, P.A.T. de Moraes Gomes, L.P. de Lima Ferreira, M.J.B. de Melo Rêgo and A.C.L. Leite, *Eur. J. Med. Chem.*, **170**, 237 (2019); https://doi.org/10.1016/j.ejmech.2019.03.024
27. J. Jacob P and S.L. Manju, *Bioorg. Chem.*, **100**, 103882 (2020); https://doi.org/10.1016/j.bioorg.2020.103882
28. A. Ahmed, K.I. Molvi, H.M. Patel, R. Ullah and A. Bari, *J. Infect. Public Health*, **13**, 472 (2020); https://doi.org/10.1016/j.jiph.2020.02.002
29. A.E. Ghonim, A. Ligresti, A. Rabbito, A.M. Mahmoud, V. Di Marzo, N.A. Osman and A.H. Abadi, *Eur. J. Med. Chem.*, **180**, 154 (2019); https://doi.org/10.1016/j.ejmech.2019.07.002

30. M. Georgiadis, V. Kourbeli, I.P. Papanastasiou, A. Tsotinis, M.C. Taylor and J.M. Kelly, *RSC Med. Chem.*, **11**, 72 (2020); https://doi.org/10.1039/C9MD00478E
31. A. Kryshchyshyn, D. Kaminskyy, O. Karpenko, A. Gzella, P. Grellier and R. Lesyk, *Eur. J. Med. Chem.*, **174**, 292 (2019); https://doi.org/10.1016/j.ejmech.2019.04.052
32. B. Yu, S. Zhou, L. Cao, Z. Hao, D. Yang, X. Guo, N. Zhang, V.A. Bakulev and Z. Fan, *J. Agric. Food Chem.*, **68**, 7093 (2020); https://doi.org/10.1021/acs.jafc.0c00062
33. Q. Wu, B. Zhao, Z. Fan, J. Zhao, X. Guo, D. Yang, N. Zhang, B. Yu, T. Kalinina and T. Glukhareva, *RSC Adv.*, **8**, 39593 (2018); https://doi.org/10.1039/C8RA07619G
34. T. Sato, K. Sekimata, N. Sakai, H. Watanabe, C. Mishima-Tsumagari, T. Taguri, T. Matsumoto, Y. Fujii, N. Handa, A. Tanaka, M. Shirouzu, S. Yokoyama, Y. Hashizume, K. Miyazono, H. Koyama and T. Honma, *ACS Omega*, **5**, 11411 (2020); https://doi.org/10.1021/acsomega.9b04245
35. O. Ebenezer, P. Awolade, N. Koorbanally and P. Singh, *Chem. Biol. Drug Des.*, **95**, 162 (2020); https://doi.org/10.1111/cbdd.13632
36. R. Sivaramakarthikeyan, S. Iniyaval, V. Saravanan, W. Lim, C. Mai and C. Ramalingan, *ACS Omega*, **5**, 10089 (2020); https://doi.org/10.1021/acsomega.0c00630
37. J. Xu, H. Tan, Y. Zhang, D. Tang, F. Zhan, H. Li, Z.Z. Chen and Z.G. Xu, *Sci. Rep.*, **10**, 9281 (2020); https://doi.org/10.1038/s41598-020-66137-z
38. M. Abdallah, S. Gomha, I. Abbas, M. Kazem, S. Alterary and Y. Mabkhot, *Appl. Sci.*, **7**, 785 (2017); https://doi.org/10.3390/app7080785
39. A. Secrieru, P.M. O'Neill and M.L.S. Cristiano, *Molecules*, **25**, 42 (2019); https://doi.org/10.3390/molecules25010042
40. F.K. Keter and J. Darkwa, *Biometals*, **25**, 9 (2012); https://doi.org/10.1007/s10534-011-9496-4
41. B. He, F.-X. Wu, L. Yu, L. Wu, Q. Chen, G.-F. Hao, W.-C. Yang, H. Lin and G. Yang, *J. Agric. Food Chem.*, **68**, 5059 (2020); https://doi.org/10.1021/acs.jafc.0c00051
42. W. Geng, Y. Jia, Y. Chen, Q. Ma, G. Fan and L. Liao, *CrystEngComm*, **22**, 3144 (2020); https://doi.org/10.1039/D0CE00278J
43. F. Lai, S. Yu, Y. Chang, T. Wu, K. Wu, Y. Chang, S. Ding, H. Chen and C. Lai, *J. Polym. Sci.*, **58**, 1400 (2020); https://doi.org/10.1002/pol.20190127
44. G. Brewer, R.J. Butcher and P. Zavalij, *Materials*, **13**, 1595 (2020); https://doi.org/10.3390/ma13071595
45. A. Pedrini, A. Maspero, S. Bracco, A. Comotti, S. Galli, L. Marchiò, L. Nardo, A. Penoni, L. Scapinello, P. Sozzani, G. Vesco and M. Mella, *New J. Chem.*, **44**, 6443 (2020); https://doi.org/10.1039/D0NJ00259C
46. S.J. Takate, A.D. Shinde, B.K. Karale, H. Akolkar, L. Nawale, D. Sarkar and P.C. Mhaske, *Bioorg. Med. Chem. Lett.*, **29**, 1199 (2019); https://doi.org/10.1016/j.bmcl.2019.03.020
47. B. Sever, M.D. Altýntop, M.O. Radwan, A. Özdemir, M. Otsuka, M. Fujita and H.I. Ciftci, *Eur. J. Med. Chem.*, **182**, 111648 (2019); https://doi.org/10.1016/j.ejmech.2019.111648
48. K. Mahesh, K. Ravi, P.K. Rathod and P. Leelavathi, *New J. Chem.*, **44**, 2367 (2020); https://doi.org/10.1039/C9NJ05254B
49. P. Bharath Kumar Reddy, K. Ravi, K. Mahesh and P. Leelavathi, *Tetrahedron Lett.*, **59**, 4039 (2018); https://doi.org/10.1016/j.tetlet.2018.09.068
50. T.V. Quoc, D.T.T. Thuy, T.D. Thanh, T.P. Ngoc, V.N. Thien, C.N. Thuy and L. Van Meervelt, *Acta Crystallogr. E Crystallogr. Commun.*, **75**, 957 (2019); https://doi.org/10.1107/S2056989019007503
51. R. Nagwanshi, M. Bakhru and S. Jain, *Med. Chem. Res.*, **21**, 1587 (2012); https://doi.org/10.1007/s00044-011-9667-4
52. M.A. Mujeeb, A. Vedamurthy, A.K. Shettar, S.I. Puranik, S. Ghagane and S.C. Thimmappa, *Int. J. Cancer Res.*, **16**, 1 (2019); https://doi.org/10.3923/ijcr.2020.1.9
53. A.K. Shiau, D. Barstad, P.M. Loria, L. Cheng, P.J. Kushner, D.A. Agard and G.L. Greene, *Cell*, **95**, 927 (1998); https://doi.org/10.1016/S0092-8674(00)81717-1
54. R.A. Laskowski and M.B. Swindells, *J. Chem. Inf. Model.*, **51**, 2778 (2011); https://doi.org/10.1021/ci200227u