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Cytotoxic Activity and Molecular Docking Studies of Novel 1,3,5-Substituted 2-Pyrazoline Derivatives

PERLA SWATHI and RAJA SUNDARARAJAN*

Department of Pharmaceutical Chemistry, GITAM School of Pharmacy, GITAM (Deemed to be University), Gandhi Nagar, Rushikonda, Visakhapatnam-530045, India

*Corresponding author: E-mail: sraja61@gmail.com

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A series of novel 1,3,5-substituted 2-pyrazoline derivatives (**IIIa-j**) comprising benzo[7]annulene moiety and aromatic substitutions were synthesized *via* the reaction of (*E*)-3-(9-chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]annulen-8-yl)-1-(substituted phenyl)prop-2-en-1-one with phenylhydrazine. Using spectroscopic methods, the structures of the synthesized derivatives were confirmed. The newly synthesized pyrazoline derivatives were put to the test *in vitro* on two cancer cell lines (HT-29 and MCF-7). After 72 h of incubation, the results showed that compounds **IIId** and **IIIb** altered the morphology of treated cell lines more than untreated or vehicle-treated cells, possibly as a result of the presence of halogen groups (chloro and fluoro) at the *para* position of the phenyl ring. The nature of the interaction between the synthesized pyrazoline derivatives and the quinoxaline inhibitor bound to the JAK2 enzyme crystal structure were investigated using the molecular docking studies. These substances displayed the least amount of binding energy to the enzyme, according to the *in silico* investigations. As a result, the synthesized pyrazoline derivatives might be used as lead molecules for the development of new anticancer drugs.

Keywords: Benzo[7]annulene, Pyrazolines, Molecular docking, Anticancer activity, MTT assay.

INTRODUCTION

Much interest in the chemistry of the compounds with ring structures have also been generated by the biological and medicinal qualities of pyranes, pyrimidines, pyrazoles and thioles [1]. A number of unique substituted benzo[7]annulene compounds used in the treatment of cancer, including anticancer, antimicrobial, anti-inflammatory and others [2,3]. Benzosuberone (benzo-cycloheptenone) nucleus contains a 7-membered ring that is fused with an aromatic ring; this is where benzo[7]-annulenes are synthesized from benzosberones [4]. Its core structure has been found in many natural products, such as colchicines, theaflavin [5], bussealine, *etc*. Its primary ingredient is thiamine and the plant *Calchicum autumnal* has a significant amount of calcicine as well as strong anticancer properties [6,7].

Benzosuberone demonstrated antitumor activity, which forms the fundamental basis of calcineurin [8]. Conversely, the synthetic benzosuberone derivatives also demonstrated a number of biological activities, including anti-inflammatory, anticancer, antibacterial, antioxidant and antimalarial activities [9]. Using the MTT assay, benzo[7]annulene ring fused with the thiazolidine ring and its scaffolds showed promise results for the breast cancer cell line (MCF-7) and the lung cancer cell line (A-549) [10,11].

According to Behbehani *et al.* [12] benzosberone containing spiropyranzole derivatives are utilized in a variety of pharmaceutical products, such as those that inhibit acetyl-CoA carboxylase and have antiviral, antibacterial and anticancer effects. In light of the reported findings and interest in the synthesis of novel heterocycles of biological significance, Salam *et al.* [13] synthesized some new fused pyrazole, isoxazole and triazole moieties with benzosuberone and 3-nitrobenzosuberone in order to evaluate their predicted antibacterial properties.

Additionally, several reports of pyrazoline compounds with various synthetic techniques have been reported. The α,β-unsaturated carbonyl compounds or chalcones, were the starting compounds in the most widely used pyrazoline synthesis method. Using various acids and hydrazine hydrates, the chalcones were cyclized in an organic solvent to produce the pyrazolines [14]. Chalcone's ring-closing creates pyrazoline

[15] compounds, which are also present in many medications with pharmacological characteristics like anti-inflammatory [16], antimicrobial activity [17-19], analgesic, antidepresent, antipyretic, anticancer [20] and hypoglycemic effects. Many study groups have reported adding various structural moieties to the pyrazoline and isoxazoline framework in an effort to increase their biological activity [21]. Based on the biological significance of pyrazoline and benzocycloheptenone moieties, an attempt was made to design combination of two biologically versatile heterocyclic scaffolds like benzosuberone and pyrazoline in single molecular platform and synthesis of new molecules with better anti-proliferative properties.

EXPERIMENTAL

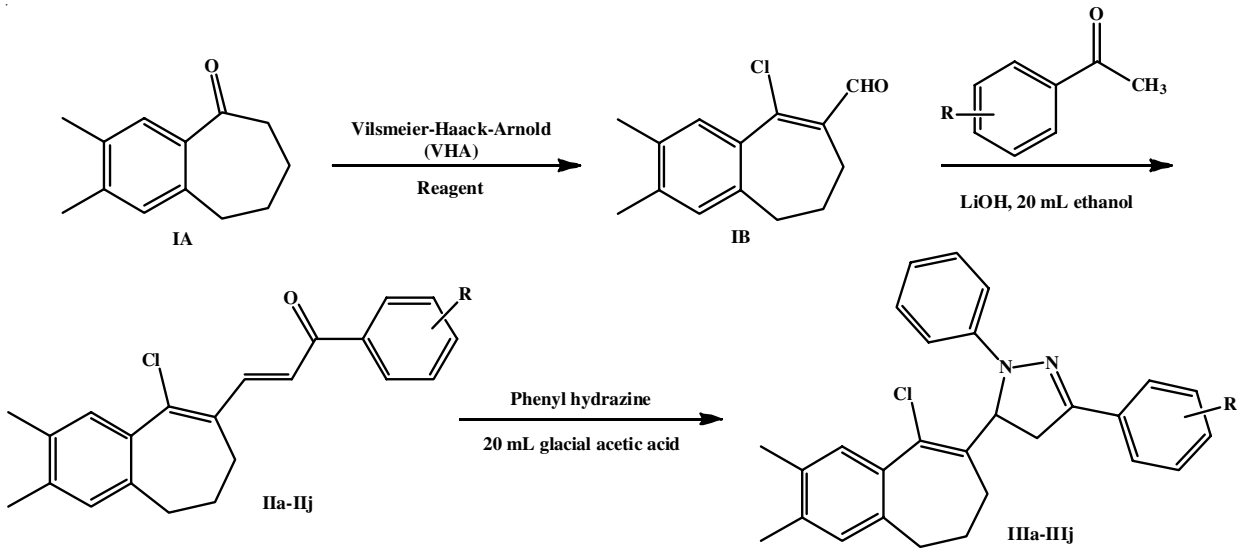
Without additional purification, the chemicals procured from Sigma-Aldrich, USA were utilized. The melting points of the synthesized compounds were measured using a Mettler Toledo FP 62 melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded using a 500 MHz Bruker Avance DPX 200 NMR system in DMSO-d_6 solvent using TMS as the internal standard. At 70 eV, mass spectra were recorded using a Shimadzu QP2010 spectrometer with a direct intake probe. Silica gel GF 254 underwent analytical TLC.

General procedure for synthesis of (*E*)-3-(9-chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]annulen-8-yl)-1-phenyl-prop-2-en-1-one (IIa-j): The Friedel-Craft acylation of aromatic hydrocarbons with glutaric anhydride produced aryl butyric acids, which Clemmenson then reduced and cyclized with excess polyphosphoric acid to obtain the required starting substituted benzosuberones (**IA**) [7]. The Vilsmeier-Haack-Arnold (VHA) reaction results in the formation of (*Z*)-8-carbaldehyde-9-chloro-6,7-dihydro-5*H*-benzo[7]annulene (**IB**) [8] by treating the substituted benzosuberones with $POCl_3$, DMF and 4 h between 0 and 60 ºC.

Before being synthesized, intermediate (*E*)-3-(9-chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]annulen-8-yl)-1-phenyl-prop-2-en-1-one (**IIa-j**). Compound **IB** was reacted to aromatic aldehyde reaction (2 mmol) in the presence of LiOH as catalyst and 20 mL of ethanol as solvent results in the formation of intermediate **IIa-j**. During the course of reaction, the magnetic stirrer was used continuously to agitate the reaction mixture at a formadible temperature until the solution turned turbid. The progress of the reaction was observed through TLC using *n*-hexane:acetone (7:3) solvent mixture. The reaction mixture was added to the crushed ice and dil. HCl was used to neutralize. After filtering under a hover, the precipitate was rinsed with ice-cold ethanol, distilled water and then recrystallized with hot ethanol.

General procedure for the synthesis of 5-(9-chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]annulen-8-yl)-3-(substituted phenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (IIIa-j): By condensation with phenylhydrazine in the presence of 20 mL of glacial acetic acid, chalcone derivatives (*E*)-3-(9-chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]annulen-8-yl)-1-phenyl prop-2-en-1-one (**IIIa-j**) were synthesized. The solution was refluxed over an oil bath for 6-8 h while the reaction was being tracked using thin-layer chromatography (TLC). The mixture solution was poured into crushed ice to obtain the precipitate which was then dried at room temperature (**Scheme-I**). The resultant product was dried and washed with double-distilled water to obtain the chalcone derivatives [12].

5-(9-Chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]-annulen-8-yl)-3-(2-chloro phenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (IIIa): Colour: pale yellow solid; yield: 71%, m.p.: 182-184 ºC. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.56 (dddt, J = 7.1, 1.6 Hz, 2 Ar-H), 7.48 (ddt, J = 8.3, 1.2, 0.5 Hz, 2 Ar-H), 7.39 (td, J = 7.8, 1.6 Hz, 2 Ar-H), 7.36-7.28 (m, 6 Ar-H), 7.26-7.20 (m, 4 Ar-H), 7.11 (s, 2 Ar-H), 7.01 (d, J = 0.9 Hz, 1 Ar-H), 7.01-6.95 (m, 3 Ar-H), 4.73 (t, J = 6.8 Hz, 1 CH_2-HC-N), 3.52 (dd, J = 13.5, 6.6 Hz, HC-CH_2-C=N), 3.27 (dd, J = 13.5, 6.9 Hz, 1 HC-CH_2-C=N), 2.89-2.82 (m, 2 Ar-cyclic CH_2-CH_2), 2.58-2.45 (m, Ar-cyclic CH_2-CH_2), 2.28 (s, 6 CH_3), 2.01-1.84 (m, 4 Ar-cyclic CH_2-CH_2). ^{13}C NMR (300



Scheme-I: Synthesis of 5-(9-chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]annulen-8-yl)-3-(substituted phenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole

MHz, $CDCl_3$) δ ppm: 19.9-20.1 (20.0, 2C (**CH_3**), 26.1 (1C, HC-**CH_2**-CH), 30.4 (1C, HC-**CH_2**-CH), 34.8 (1C, C=C-**CH_2**-CH_2), 46.1 (1C, -HC-**CH_2**-C=N), 56.6 (1C, N-**CH**-CH_2), 122.8 (2C, Ar-CH=CH), 127.3 (2C, N-Ar-CH=CH), 127.4-127.5 (127.4, 2C (Ar-CH=CH, 127.8 (1C, Ar-CH=CH), 128.0 (1C, Ar-**C**=C-CH), 128.1-128.3 (128.2 3C (Ar-CH=CH), 128.3 (1C, Ar-CH= CH), 128.4 (1C, Ar-CH=CH)), 128.7 (1C, Ar-CH=CH)), 131.5 (1C, Ar-CH=CH), 132.0 (1C, Ar-CH=CH-Cl), 134.5-134.6 (134.6, 2C (CH_3-**CH**=CH), 134.9 (1C, Ar-C=**C**-CH_2 137.9 (1C, s), 140.4 (1C, N-Ar-CH=CH), 140.9 (1C, C-C=CH), 152.4 (1C, N=C-Ar). ESI-MS: *m/z* Anal. calcd. for $C_{28}H_{26}N_2Cl_2$ ([M + H]$^+$): 461.12; found: 462.

5-(9-Chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]-annulen-8-yl)-3-(4-chloro phenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (IIIb): Colour: pale yellow solid; yield: 79%, m.p.: 184-186 ºC. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.55-7.63 (m, 2 Ar-H), 7.43-7.46 (m, 2 Ar-H), 7.34 (tt, *J* = 7.3, 1.4 Hz, 2 Ar-H), 7.26-7.20 (m, 2 Ar-H), 7.11 (tt, 1 Ar-H), 7.03-6.95 (m, 2 Ar-H), 4.74 (dd, *J* = 11.5, 4.3 Hz, CH_2-HC-N), 2.70 (ddd, *J* = 13.7, 4.7 Hz, HC-CH_2-C=N), 2.68 (d d, *J* = 11.7, 8.1 Hz, HC-CH_2-C=N), 2.65 (ddd, *J* = 13.3, 9.6 Hz, 2 Ar-cyclic CH_2-CH_2), 2.22 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 2.35 (ddd Ar-cyclic CH_2-CH_2, *J* = 14.1, 4.6 Hz), 1.80-2.09 (2 Ar-cyclic CH_2-CH_2), 1.89-2.0 (ddt, d, *J* =13.5, 9.6, 4.6 Hz, Ar-cyclic CH_2-CH_2). ^{13}C NMR (300 MHz, DMSO-d_6) δ ppm: 19.9-20.1 (20.0, 2C (**CH_3**), 26.1 (1C, HC-**CH_2**-CH), 30.4 (1C, HC-**CH_2**-CH), 34.8 (1C, C=C-**CH_2**-CH_2), 46.1 (1C, -HC-**CH_2**-C=N), 56.6 (1C, N-**CH**-CH_2), 122.8 (2C, Ar-CH=CH), 127.3 (2C, N-Ar-CH= CH), 127.4-127.5 (127.4, 2C (Ar-CH=CH, 127.8 (1C, Ar-CH=CH), 128.0 (1C, Ar-**C**=C-CH), 128.2 (2C, Ar-CH=CH), 128.5 (2C, Ar-CH=CH), 128.7 (2C, CH=CH-Cl), 133.7 (1C, C=**C**-Cl), 134.5-134.6 (134.6, 2C (CH_3-**CH**=CH), 134.9 (1C, Ar-C=**C**-CH_2), 140.4 (1C, N-Ar-CH=CH), 140.9 (1C, (C-C=CH), 152.5 (1C, N=C-Ar). ESI-MS: *m/z* Anal. calcd. for $C_{28}H_{26}N_2Cl_2$ ([M + H]$^+$): 461.12; found: 462.

3-(2-Bromophenyl)-5-(9-chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]annulen-8-yl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (IIIc): Colour: pale yellow solid; yield: 75%, m.p.: 190-192 ºC. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.69 (ddd, *J* = 7.9, 1.6 Hz, Ar-H), 7.62 (ddd, *J* = 7.0, 1.7 Hz, Ar-H), 7.56 (ddd, *J* = 7.9, 1.3 Hz Ar-H), 7.43 (dddd, *J* = 8.2, 1.6 Hz), 7.40-7.28 (m, 4H), 7.26-7.20 (m, 2 H), 7.11 (s, Ar-H), 7.01-6.95 (m, 2 Ar-H), 4.73 (t, *J* = 6.8 Hz, CH_2-HC-N), 3.49 (dd, *J* = 13.5, 6.6 Hz, HC-CH_2-C=N), 3.24 (d d, *J* = 13.5, 6.9 Hz, HC-CH_2-C=N), 2.89-2.82 (m, 2 Ar-cyclic CH_2-CH_2), 2.58-2.45 (m, 2 Ar-cyclic CH_2-CH_2), 2.34 (s, 3H, CH_3), 2.28 (s, 3H CH_3), 2.01-1.84 (m, 2 Ar-cyclic CH_2-CH_2). ^{13}C NMR (300 MHz, DMSO-d_6) δ ppm: 19.9-20.1 (20.0, 2C (**CH_3**), 26.1 (1C, HC-**CH_2**-CH), 30.4 (1C, HC-**CH_2**-CH), 34.8 (1C, C=C-**CH_2**-CH_2), 46.1 (1C, -HC-**CH_2**-C=N), 56.6 (1C, N-**CH**-CH_2), 122.0 (1C, (Br-Ar-C=CH), 122.8 (2C, (Ar-CH=C-N), 127.3 (2C, N-Ar-CH=CH), 127.4-127.5 (127.4, 2C (Ar-CH=CH), 127.8-127.8 (2C, 127.8 (Ar-CH=CH), 128.0-128.1 (2C, 128.0 (1C, Ar-**C**=C-CH), 128.1-128.3 (3C, 128.2 (Ar-CH=CH), 128.4 (1C, Ar-CH=CH), 132.6 (1C, Ar-**CH**=C-Br), 134.5-134.6 (134.6, 2C (CH_3-**CH**=CH), 134.9 (1C, Ar-C=**C**-CH_2), 140.4 (1C, N-Ar-CH=CH), 140.9 (1C, (C-C=CH), 152.5 (1C, N=C-Ar). ESI-MS: *m/z* Anal. calcd. for $C_{28}H_{26}N_2ClBr$ ([M + H]$^+$): 505.5; found: 506.

5-(9-Chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]-annulen-8-yl)-3-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (IIId): Colour: pale yellow solid; yield: 69%, m.p.: 182-184 ºC. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.56 (ddd, J =8.2, 12.6Hz, 1 Ar-H), 7.5(d, *J* = 0.4 Hz, Ar-H), 7.82-7.75 (m, 2 Ar-H), 7.34 (tt, *J* = 7.3, 1.4 Hz, 2 Ar-H), 7.30-7.20 (m, 4 Ar-H), 7.11 (s, Ar-H), 7.03-6.95 (m, 2 Ar-H), 4.74 (t, *J* = 6.6 Hz, CH_2-HC-N), 3.44 (dd, *J* = 13.7, 6.6 Hz, HC-CH_2-C=N), 3.19 (dd, *J* = 13.7, 6.9 Hz, HC-CH_2-C=N), 2.89-2.82 (m, 2 Ar-cyclic CH_2-CH_2), 2.51 (dt, *J* = 8.5, 4.2 Hz, 2 Ar-cyclic CH_2-CH_2), 2.34 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.01-1.84 (m, 2 Ar-cyclic CH_2-CH_2). ^{13}C NMR (300 MHz, DMSO-d_6) δ ppm: 19.9-20.1 (20.0, 2C (**CH_3**), 26.1 (1C, HC-**CH_2**-CH), 30.4 (1C, HC-**CH_2**-CH), 34.8 (1C, C=C-**CH_2**-CH_2), 46.1 (1C, -HC-**CH_2**-C=N), 56.6 (1C, N-**CH**-CH_2), 115.4 (2C, HC-CH=CF) 122.8 (2C, Ar-CH=CH), 127.3 (2C, N-Ar-CH=CH), 127.4-127.5 (127.4, 2C (Ar-CH=CH), 127.8 (1C, Ar-CH=CH), 128.0 (1C, Ar-**C**=C-CH), 128.2 (2C, Ar-CH=CH), 128.6 (2C, Ar-CH= CH), 134.5-134.6 (134.6, 2C (CH_3-**CH**=CH), 134.9 (1C, Ar-C=**C**-CH_2), 140.4 (1C, N-Ar-CH=CH), 140.9 (1C, (C-C=CH), 152.5 (1C, N=C-Ar). ESI-MS: *m/z* Anal. calcd. for $C_{28}H_{26}N_2FCl$ ([M + H]$^+$): 444.5; found: 445.

5-(9-Chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]-annulen-8-yl)-3-(3,5-dimethoxyphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (IIIe): Colour: pale yellow solid; yield: 76%, m.p.: 192-194 ºC. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.34 (t-t, *J* = 7.3, 1.4 Hz, 2Ar-H), 7.26-7.20 (m, 2Ar-H), 7.11 (s, Ar-H), 7.03-6.95 (m, 2 Ar-H), 6.77 (d, *J* = 2.2 Hz, 2 Ar-H), 6.65 (t, *J* = 2.3 Hz, 1Ar-H), 4.74 (t, *J* = 6.6 Hz, CH_2-HC-N), 3.82 (s, 6H -OCH_3), 3.47 (dd, *J* = 13.7, 6.9 Hz, HC-CH_2-C=N), 3.22 (dd, *J* = 13.7, 6.6 Hz, 1 HC-CH_2-C=N), 2.89-2.82 (m, 2 Ar-cyclic CH_2-CH_2), 2.58-2.45 (m, 2 Ar-cyclic CH_2-CH_2), 2.34 (s, 3H, CH_3), 2.28 (s, 3H CH_3), 2.01-1.84 (m, 2 cyclic CH_2-CH_2). ^{13}C NMR (300 MHz, DMSO-d_6) δ ppm: 19.9-20.1 (20.0, 2C (**CH_3**), 26.1 (1C, HC-**CH_2**-CH), 30.4 (1C, HC-**CH_2**-CH), 34.8 (1C, C=C **CH_2**-CH_2), 46.1 (1C, -HC-**CH_2**-C=N), 56.0 (2C, O-**C**H_3), 56.6 (1C, N-**CH**-CH_2), 101.7 (1C, Ar-C=**CH**-C-OCH_3), 110.6 (2C, N=C-**C**=C-OCH_3), 122.8 (2C, Ar-CH=CH), 127.3 (2C, N-Ar-CH=CH), 127.4-127.5 (127.4, 2C (Ar-CH=CH, 127.8 (1C, Ar-CH=CH), 128.0 (1C, Ar-**C**=C-CH), 128.2 (2C, Ar-CH=CH), 132.1 (1C, N=C-**C**=CH), 134.5-134.6 (134.6, 2C (CH_3-**CH**=CH), 134.9 (1C, Ar-C=**C**-CH_2), 140.4 (1C, N-Ar-CH=CH), 140.9 (1C, (C-C=CH), 152.5 (1C, N=Ar), 160.9 (2C, Ar-CH=C-OCH_3). ESI-MS: *m/z* Anal. calcd. for $C_{30}H_{31}N_2O_2Cl$ ([M + H]$^+$): 486.5; found: 487.

5-(9-Chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]-annulen-8-yl)-3-(2-nitrophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (IIIf): Colour: pale yellow solid; yield: 63%, m.p.: 186-188 ºC. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.08 (dd, *J* = 8.9, 1.5 Hz, 1Ar-H), 7.79 (dd, *J* = 6.9, 1.5 Hz, 1 Ar-H), 7.74-7.66 (m, Ar-H), 7.62-7.55 (m, Ar-H), 7.34 (tt, *J* = 7.3, 1.4 Hz, 2 Ar-H), 7.26-7.20 (m, 2 Ar-H), 7.11 (s, Ar-H), 7.02-6.95 (m, 2 Ar-H), 4.77 (t, *J* = 6.8 Hz, CH_2-HC-N), 3.61 (dd, *J* = 13.5, 6.9 Hz, HC-CH_2-C=N), 3.36 (dd, *J* = 13.5, 6.6 Hz, HC-CH_2-C=N), 2.89-2.82 (m, 2 cyclic CH_2-CH_2), 2.58-2.45 (m, 2 cyclic

CH_2-CH_2), 2.34 (s, 3H CH_3), 2.28 (s, 3H CH_3), 2.01-1.84 (m, 2 cyclic CH_2-CH_2). ^{13}C NMR (300 MHz, DMSO-d_6) δ ppm: 19.9-20.1 (20.0, 2C (**CH_3**), 26.1 (1C, HC-**CH_2**-CH), 30.4 (1C, HC-**CH_2**-CH), 34.8 (1C, C=C-**CH_2**-CH_2), 46.1 (1C, -HC-**CH_2**-C=N), 56.6 (1C, N-**CH**-CH_2), 117.7 (1C, Ar-CH=C-NO_2), 122.8 (2C, Ar-CH=CH), 124.7 (1C, Ar-CH=C), 127.0 (1C, N=C-**C**=CH-Ar), 127.3 (1C, N-Ar-CH=CH), 127.4-127.5 (2C, 127.4 (Ar-CH=CH), 127.8 (1C, Ar-CH=CH), 128.0 (1C, Ar-**C**=C-CH), 128.1-128.3 (3C, 128.2 (Ar-CH=CH), 128.4 (1C, Ar-CH=CH), 134.5-134.6 (2C, 134.6 (CH_3-**CH**=CH), 134.9 (1C, Ar-C=**C**-CH_2), 139.5 (1C, NO_2-C=CH-Ar), 140.4 (1C, N-Ar-CH=CH), 140.9 (1C(C-C=CH), 152.4 (1C, N=C-Ar). ESI-MS: *m/z* Anal. calcd. for $C_{28}H_{26}N_3O_2Cl$ ([M + H]$^+$): 471.15; found: 472.

4-(5-(9-Chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]-annulen-8-yl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)aniline (IIIg): Colour: pale yellow solid; yield: 73%, m.p.: 186-188 ºC. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.63-7.57 (m, 2 Ar-H), 7.34 (t-t, *J* = 7.3, 1.4 Hz, 2 Ar-H), 7.26-7.20 (m, 2 Ar-H), 7.11 (s, Ar-H), 7.02-6.95 (m, 2 Ar-H), 6.70-6.64 (m, 2 Ar-H), 5.35 (s, 2-NH_2), 4.74 (t, *J* = 6.6 Hz, CH_2-HC-N), 3.44 (dd, *J* = 13.7, 6.6 Hz, HC-CH_2-C=N), 3.19 (dd, *J* = 13.7, 6.9 Hz, HC-CH_2-C=N), 2.89-2.82 (m, 2 cyclic CH_2-CH_2), 2.51 (d t, *J* = 8.5, 4.2 Hz, 2 cyclic CH_2-CH_2), 2.34 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.01-1.84 (m, 2 cyclic CH_2-CH_2). ^{13}C NMR (300 MHz, DMSO-d_6) δ ppm: 19.9-20.1 (20.0, 2C (**CH_3**), 26.1 (1C, HC-**CH_2**-CH), 30.4 (1C, HC-**CH_2**-CH), 34.8 (1C, C=C-**CH_2**-CH_2), 46.1 (1C, -HC-**CH_2**-C=N), 56.6 (1C, N-**CH**-CH_2), 114.3 (2C (C=CH-N), 122.8 (2C, Ar-CH=CH), 127.3-127.3 ((2C, N-Ar-CH=CH), 127.4-127.5 (127.4, 2C (Ar-CH=CH, 127.8 (1C, Ar-CH=CH), 128.0 (1C, Ar-**C**=C-CH), 128.1-128.3 (128.2 4C(Ar-CH=CH), 134.5-134.6 (134.6, 2C (CH_3-**CH**=CH), 134.9 (1C, Ar-C=**C**-CH_2), 140.4 (1C, N-Ar-CH=CH), 140.9 (1C, C-C=CH), 152.5 (1C, N=C-Ar). ESI-MS: *m/z* Anal. calcd. for $C_{28}H_{28}N_3Cl$ ([M + H]$^+$): 441.17; found: 442.

5-(9-Chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]-annulen-8-yl)-3-(2-meth oxyphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (IIIh): Colour: pale yellow solid; yield: 68%, m.p.: 178-180 ºC. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.46-7.38 (m, 2 Ar-H), 7.34 (t, *J* = 7.3, 1.4 Hz, 2 Ar-H), 7.26-7.20 (m, 2 Ar-H), 7.19-7.10 (m, 2Ar-H), 7.05-6.95 (m, 3Ar-H), 4.74 (t, *J* = 6.8 Hz, CH_2-HC-N), 3.87 (s, 3 -OCH_3), 3.42 (d d, *J* = 13.5, 6.6 Hz, HC-CH_2-C=N), 3.17 (dd, *J* = 13.5, 6.6 Hz, HC-CH_2-C=N), 2.89-2.82 (m, 2 cyclic CH_2-CH_2), 2.51 (d t, *J* = 8.5, 4.2 Hz, 2 cyclic CH_2-CH_2), 2.34 (s, 3H CH_3), 2.28 (s, CH_3), 2.01-1.84 (m, 2 cyclic CH_2-CH_2). ^{13}C NMR (300 MHz, DMSO-d_6) δ ppm: 19.9-20.1 (20.0, 2C (**CH_3**), 26.1 (1C, HC-**CH_2**-CH), 30.4 (1C, HC-**CH_2**-CH), 34.8 (1C, C=C-**CH_2**-CH_2), 46.1 (1C, -HC-**CH_2**-C=N), 56.0 (1C, OCH_3), 56.6 (1C, N-**CH**-CH_2), 115.8 (1C, Ar-C-OCH_3)121.3 (1C, Ar-C=C-O), 122.8 (2C, Ar-CH=CH), 127.3 (2C, N-Ar-CH=CH), 127.4-127.5 (127.4, 2C (Ar-CH=CH, 127.8 (1C, Ar-CH=CH), 128.0 (1C, Ar-**C**=C-CH), 128.1-128.3 (128.2 4C (Ar-CH=CH), 134.5-134.6 (134.6, 2C (CH_3-**CH**=CH), 134.9 (1C, Ar-C=**C**-CH_2), 140.4 (1C, N-Ar-CH=CH), 140.9 (1C, C-C=CH), 152.4 (1C(N=C-Ar), 156.3, 1C(Ar-C=C-OCH_3). ESI-MS: *m/z* Anal. calcd. for $C_{29}H_{29}N_2OCl$ ([M + H]$^+$): 456.15, found 457. 76.22 (C), 6.40 (H)6.13 (N); Found: 76.19 (C), 6.38 (H), 6.10 (N).

4-(5-(9-Chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]-annulen-8-yl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenol (IIIi): Colour: pale yellow solid; yield: 65%, m.p.: 182-184 ºC. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 9.86 (s, 1H), 7.63-7.57 (m, 2 Ar-H), 7.34 (tt, *J* = 7.3, 1.4 Hz, 2 Ar-H), 7.26-7.20 (m, 2 Ar-H), 7.11 (s, Ar-H), 7.03-6.95 (m, 2 Ar-H), 6.89-6.83 (m, 2 Ar-H), 4.74 (t, *J* = 6.6 Hz, 1 CH_2-HC-N)), 3.44 (dd, *J* = 13.7, 6.6 Hz, HC-CH_2-C=N), 3.19 (dd, *J* = 13.7, 6.9 Hz, HC-CH_2-C=N), 2.89-2.82 (m, 2 Ar-C_7H_{15}-H), 2.58-2.53 (m, 2 cyclic CH_2-CH_2), 2.51 (dd, *J* = 8.4, 4.3 Hz, 2 cyclic CH_2-CH_2), 2.34 (s, 3H, CH_3), 2.28 (s, 3 H CH_3), 2.01-1.84 (m, 2 cyclic CH_2-CH_2). ^{13}C NMR (300 MHz, DMSO-d_6) δ ppm: 19.9-20.1 (20.0, 2C (**CH_3**), 26.1 (1C, HC-**CH_2**-CH), 30.4 (1C, HC-**CH_2**-CH), 34.8 (1C, C=C-**CH_2**-CH_2), 46.1 (1C, -HC-**CH_2**-C=N), 56.6 (1C, N-**CH**-CH_2), 115.7 (2C, CH=C-OH), 122.8 (2C, Ar-CH=CH), 127.3 (2C, N-Ar-CH=CH), 127.4-127.5 (127.4, 2C (Ar-CH=CH), 127.8 (1C, Ar-CH=CH), 128.0 (1C, Ar-**C**=C-CH), 128.2 (2C, Ar-CH=CH), 128.9 (2C, Ar-CH=CH), 134.5-134.6 (134.6, 2C, CH_3-**CH**=CH), 134.9 (1C, Ar-C=**C**-CH_2), 140.4 (1C, N-Ar-CH=CH), 140.9 (1C, C-C=CH), 152.5 (1C, N=C-Ar).157.4 (1C, C=C-OH). ESI-MS: *m/z* Anal. calcd. for $C_{28}H_{27}N_2OCl$ ([M + H]$^+$): 442.1; found: 443.5.

3-(3-Bromo-4-nitrophenyl)-5-(9-chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]annulen-8-yl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (IIIj): Colour: pale yellow solid; yield: 62%, m.p.: 198-200 ºC. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.11 (d, *J* = 7.9 Hz, Ar-H), 8.03 (d, *J* = 2.0 Hz, Ar-H), 7.85 (dd, *J* = 8.0, 1.8 Hz, Ar-H), 7.34 (tt, *J* = 7.3, 1.4 Hz, 2 Ar-H), 7.26-7.20 (m, 2Ar-H), 7.11 (s, 1Ar-H), 7.02-6.95 (m, 2Ar-H), 4.74 (t, *J* = 6.6 Hz, CH_2-HC-N), 3.44 (d d, *J* = 13.7, 6.6 Hz, 1 HC-CH_2-C=N), 3.19 (d d, *J* = 13.7, 6.6 Hz, 1 HC-CH_2-C=N), 2.89-2.82 (m, 2 cyclic CH_2-CH_2), 2.51 (dt, *J* = 8.5, 4.2 Hz, 2 cyclic CH_2-CH_2), 2.34 (s, 3H, CH_3), 2.28 (s, 3H CH_3), 2.01-1.84 (m, 2 cyclic CH_2-CH_2). ^{13}C NMR (300 MHz, DMSO-d_6) δ ppm: 19.9-20.1 (2C, 20.0 (**CH_3**), 26.1 (1C, HC-**CH_2**-CH), 30.4 (1C, HC-**CH_2**-CH), 34.8 (1C, C=C-**CH_2**-CH_2), 46.1 (1C, -HC-**CH_2**-C=N), 56.6 (1C, N-**CH**-CH_2), 116.4 (1C, Ar-CH=C-NO_2), 120.0 (1C, Ar-CH=C-Br), 122.8 (2C, Ar-CH=CH), 127.0 (1C, N=C-C=CH-Ar), 127.3 (1C, 2C, 128.2 (2C, Ar-CH=CH N-Ar-CH=CH), 127.4-127.5 (127.4, 2C (Ar-CH=CH), 127.8-127.8 (2C, Ar-CH=CH), 128.0 (1C, Ar-**C**=C-CH), 128.2 (2C, Ar-CH=CH), 131.7 (1C, Ar-CH=C-Br), 134.5-134.6 (2C, 134.6), 2C (CH_3-**CH**=CH), 134.9 (1C, Ar-C=**C**-CH_2), 139.5 (1C, NO_2-C=CH-Ar), 140.4 (1C, N-Ar-CH=CH), 140.9 (1C, (C-C=CH), 152.4 (1C, N=C-Ar). ESI-MS: *m/z* Anal. calcd. for $C_{28}H_{25}N_3O_2ClBr$ ([M + H]$^+$): 550.16; found: 551.

Anticancer activity

Culture of cells: The subcultured cancer cell lines (HT 29 and MCF-7) were collected from the Lifesenz lab, Mumbai, India. The Dulbecco's modified eagles medium (Gibco Invitrogen, Paisley, UK) was used to cultivate the cells in 75 mL corning bottles, along with 10% foetal bovine serum, 1% non-essential amino acids, 1% penicillin (1000 U/mL), 1% streptomycin (1000 g/mL) and 1% amphotericin (250 U/mL). Using 0.25% trypsin-1 mM EDTA, subculturing was carried out at a density of 2.2×10^4 cells/cm^2. To prevent cell differentiation,

studies were carried out after 24 h of seeding and the culture media was changed after every 2 days.

The anticancer evaluation of the synthesized derivatives (**IIIa-j**) was performed by assessing their impact on the morphological behaviour of HT-29 and MCF-7 cell lines. Each compound was administered to the cell lines at a concentration of 100 μM. The cells were monitored for a period of 72 h following the application of the test compounds.

Cytotoxic studies: Utilizing the colorimetric MTT cell proliferation assay, the cell proliferation may be observed and quantified. In order to identify the cells, a cellular reductase converts the yellow tetrazole MTT form into the purple formazan form in living cells. A decrease in the cell proliferation and signal strength may be an indication of toxicity or unfavourable culture conditions, whereas the signal strength grows as cell proliferation grows. The experiment was performed in triplicate with doxorubicin serving as a positive control [14]. The percentage of growth inhibition was calculated with respect to vehicle control using the follwing formula:

$$\text{Inhibition } (\%) = \frac{\text{Conrol}_{\text{Abs}} - \text{Test}_{\text{Abs}}}{\text{Control}_{\text{Abs}}} \times 100$$

Docking studies: The current study conducted a rigid receptor docking of synthesized pyrazoline derivatives (**IIIa-j**) using the quinoxaline inhibitor interacts with the X-ray crystal structure of JAK2 (PDB: 3KRR, 1.8 resolution). The quinoxaline inhibitor interacts with the X-ray crystal structure of JAK2 (PDB: 3KRR, 1.8 resolution). For the docking simulations, Autodock 4.2 software was employed. These simulations included the addition of polar hydrogens, the addition of AD4-type atoms and the removal of water molecules and heteroatoms during the enzyme manufacturing process. The binding site was determined based on the location of the initial ligand's contact. For stimulation runs, the ligands were kept flexible while the amino acid residues in the active site were kept rigid with default values. The docking results showed the energy required for the formation of the intermolecular interactions and hydrogen-bonded connections between the amino acid residues and functional groups of substances (affinity in kcal/mol).

RESULTS AND DISCUSSION

The multistep synthetic route for obtaining 5-(9-chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]annulen-8-yl)-3-(substituted phenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (**IIIa-j**) was precisely discussed in this work. Using Vilsmeier-Haack-Arnold reaction reagent, 2,3-dimethyl-6,7,8,9-tetrahydro-5*H*-benzo[7]annulene-5-one (**1a**) was converted into 9-chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]annulene-8-carbaldehyde (**1b**). To synthesize (*E*)-3-(9-chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]annulene-8-yl)-1-(substituted phenyl)prop-2-en-1-one (**IIa-j**), compound **1b** was further reacted with various aromatic ketones. The phenyl hydrazine was used to cyclize compounds **IIa-j** in order to obtain desired compounds **IIIa-j**.

The NMR and Mass spectra used to confirm the structures of the synthesized compounds. A signal at δ 2.52 ppm was identified as the C4-H of the pyrazole ring. At around 2.52 ppm confirmed the existence of methyl group, whereas a triplet peak in the range of δ 4.72-4.83 ppm and a doublet peak in the range of δ 2.91-2.52 ppm caused by the proton at the fourth position were observed. A peak at δ 2.91 ppm confirmed that benzo[7]-annulene was present in the end product.

Morphological screening: Target compounds **IIIa-j** were administered to the human colon cancer cell line HT-29 and the human breast cancer cell line MCF-7 at a concentration of 2.5 μM to 20 μM. After 72 h of the incubation period, the morphological behaviour of these cells was examined using a phase contrast microscope. The molecules **IIIb** and **IIId** were discovered to elicit morphological changes in both HT-29 and MCF-7 cells when compared to the control or vehicle-treated cells. Similar to what was seen with DOX therapy, treatment with **IIIb** and **IIId** led to a marked shift in cellular morphology in which the shape of the cells was entirely transformed. The HT-29 and MCF-7 cells were polygonal in shape in the control cells, but following treatment, they took on a spherical shape.

Cytotoxic behaviour of title compounds (IIIa-j): The antiproliferative effects of the synthesized compounds **IIIa-j** were evaluated against HT-29 and MCF-7 cell lines by measured their IC_{50} values. The MTT assay indicated that compounds **IIIb** and **IIId** exhibited concentration dependent inhibition of cellular growth. The initial MTT assay revealed that these two compounds displayed the IC_{50} values of 2.62 and 2.73, respectively, against HT-29 cells and IC_{50} values of 2.82 and 4.45, respectively, against MCF-7 cells (Table-1). The time-dependent MTT assay was performed to assess the time-dependency of these compounds in inhibiting tumor cell growth by calculating their IC_{50} values. These results indicated that compounds **IIIb** and **IIId** reduced the viability of both HT-29 and MCF-7 cells in a dose- and time-dependent manner. Furthermore, the results demonstrated that compounds **IIIb** and **IIId** were significantly different from the control (0.1% DMSO) and positive control (doxorubicin) in terms of reducing cell viability.

Docking studies: In present investigation, docking studies of the synthesized compounds with JAK2 enzyme in combination with a powerful quinoxaline inhibitor (PDB: 3KRR) were carried out using Autodock 4.2 software. As shown in Table-2, the docking analysis findings revealed that all the compounds demonstrated energetically advantageous Auto-dock scores. The autodock findings were -10.31 and -10.61, respectively, for the two most powerful compounds, **IIIb** and **IIId**. In contrast, the ratings of the less energetic chemicals were higher than those of the energetic ones. This finding suggests that less energy is required for efficient chemical-receptor enzyme binding interactions. The *para*-substituted fluorine atom on the phenyl ring at position 5 of the pyrazoline ring and the nitrogen atom of the modified pyrazole ring were engaged in the binding interaction of the most active molecule, **IIId**.

With the amino acid residues in the target protein's active site, these groups created hydrogen bonds. Another active substance, **IIIb**, forming hydrogen bonds with the substituted groups on the phenyl ring connected to the pyrazoline ring in order to interact with the amino acid residues val863, pro1017 and leu983. Fig. 1 showed an illustration of every interaction between the active substances. The importance of the pyrazole nucleus having a substituted phenyl ring, which improves

TABLE-1
% CELL VIABILITY AGAINST HT-29 AND MCF-7

Compound	HT-29					MCF-7				
	2.5 µM	5 µM	10 µM	20 µM	IC_{50} (µM/mL)	2.5 µM	5 µM	10 µM	20 µM	IC_{50} (µM/mL)
IIIa	66.48	52.40	42.76	21.99	3.87	68.35	50.17	35.96	19.74	4.219053244
IIIb	86.67	77.49	65.77	33.38	2.62	67.67	50.43	31.32	18.48	4.458712324
IIIc	71.06	62.97	52.21	24.52	3.32	66.89	54.22	36.45	21.27	4.056795132
IIId	91.39	73.37	62.25	31.34	2.73	86.65	74.87	55.70	32.04	2.821351992
IIIe	74.27	66.14	48.33	24.81	3.33	68.73	49.03	37.90	13.54	4.482294935
IIIf	72.51	66.81	56.61	27.09	3.10	55.51	42.75	28.26	12.74	5.420054201
IIIg	63.15	56.49	37.23	19.72	4.09	66.58	50.71	34.64	20.96	4.216207100
IIIh	60.08	54.36	28.29	15.40	4.76	55.26	40.56	25.95	11.61	5.754402118
IIIi	57.60	63.73	46.04	23.73	3.59	56.85	48.61	29.73	12.90	5.087505088
IIIj	61.56	52.82	37.77	20.71	4.13	54.99	43.67	28.56	10.33	5.569789462
DOXO	92.64	82.03	73.52	35.60	2.42	92.64	82.03	73.52	35.60	2.420000000

Potent Moderate

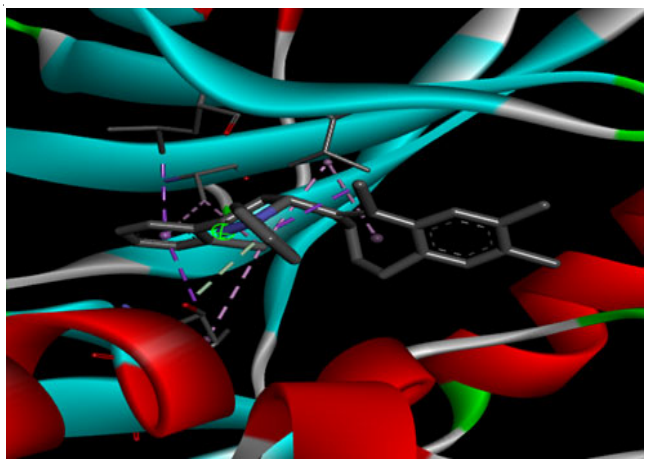
TABLE-2
FINDINGS OF THE SYNTHESIZED COMPOUNDS' DOCKING

Compound	Docked energy (kcal/mol)	Compound	Docked energy (kcal/mol)
IIIa	-9.35	**IIIg**	-8.33
IIIb	-10.37	**IIIh**	-8.87
IIIc	-9.76	**IIIi**	-9.62
IIId	-10.61	**IIIj**	-9.04
IIIe	-9.8	Doxorubicin	-11.32
IIIf	-8.82		

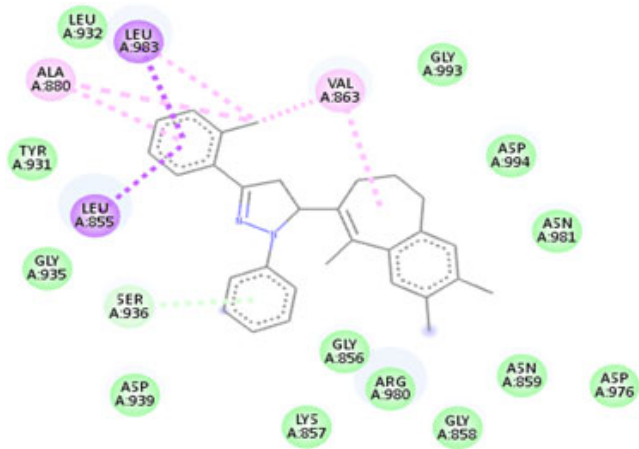
favorable binding interactions with the receptors and increases anticancer activity, is highlighted by these binding interactions.

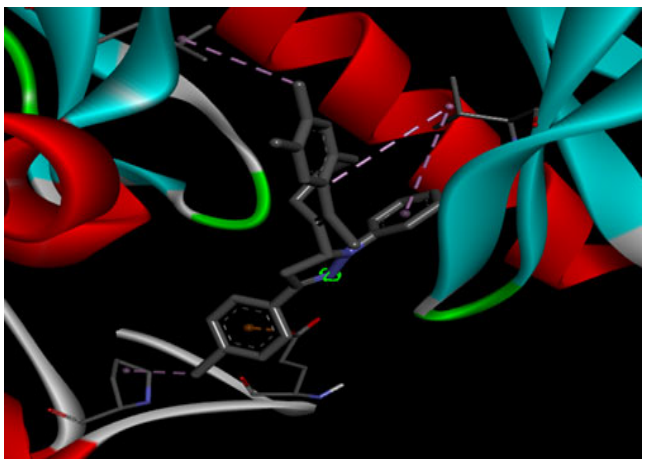
Conclusion

A series of novel 2-pyrazoline derivatives containing benzo-[7]annulene moiety and aromatic substitutions were synthesized and characterized. The potentiality of synthesized compounds of the anticancer action was tested *in vitro* on the cancer cell lines HT-29 and MCF-7. After 72 h of incubation, compounds **IIId** and **IIIb** caused the morphological changes in the

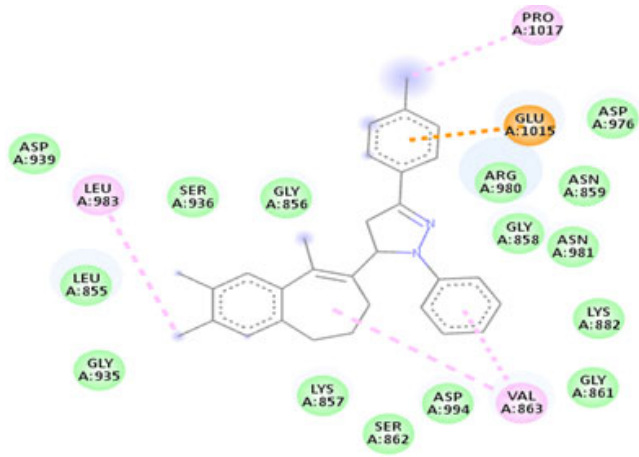


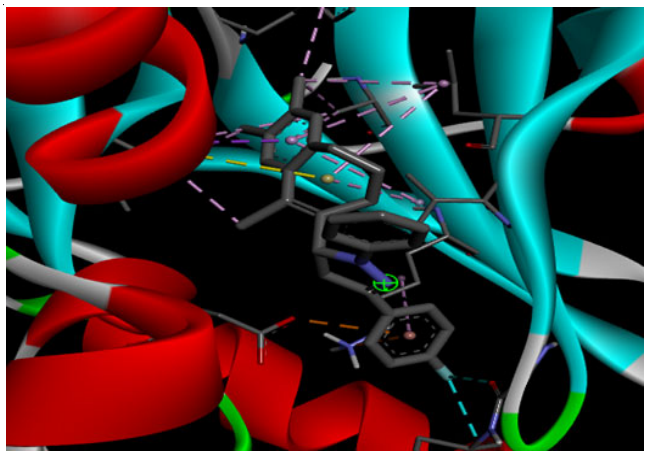
IIIa



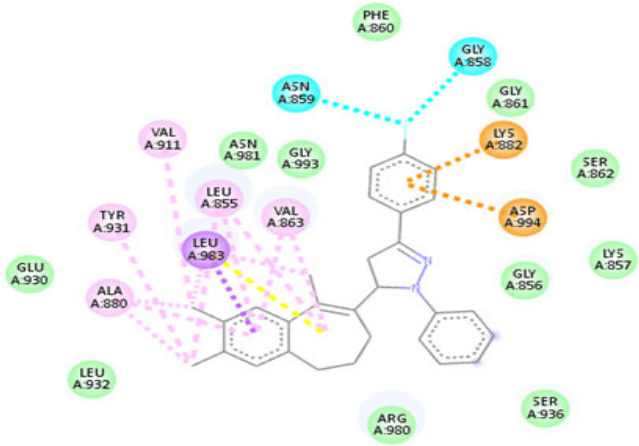


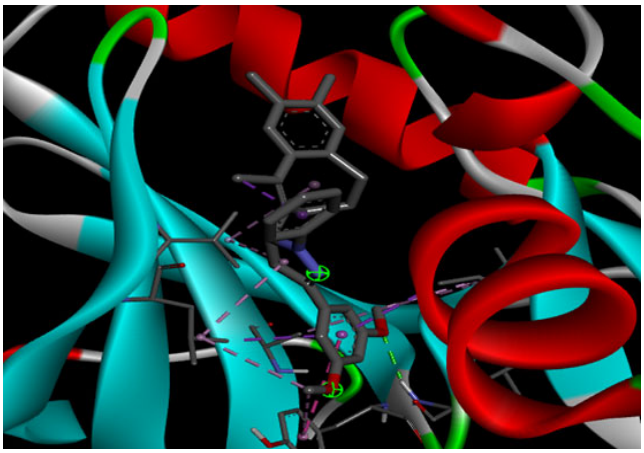
IIIb



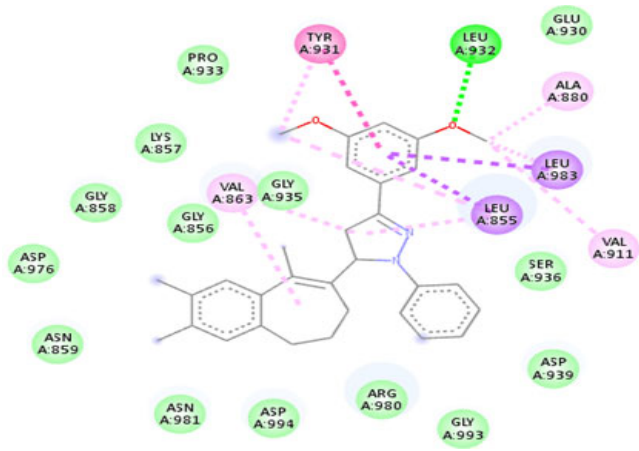


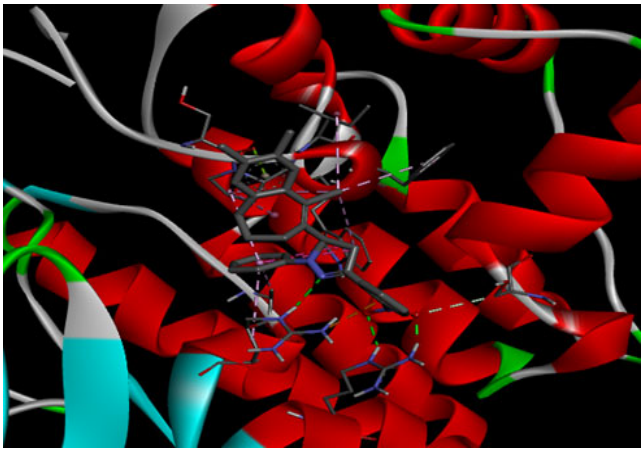
IIId



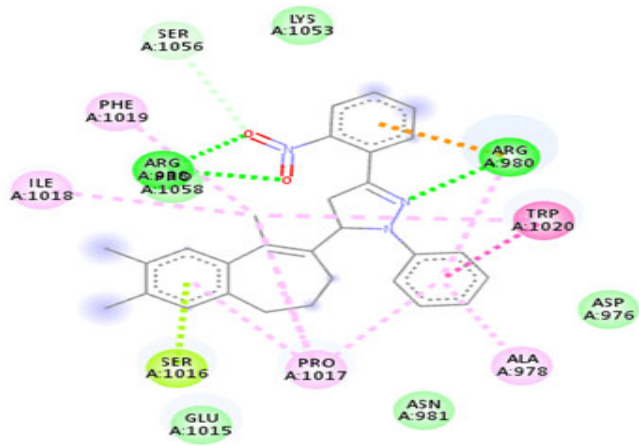


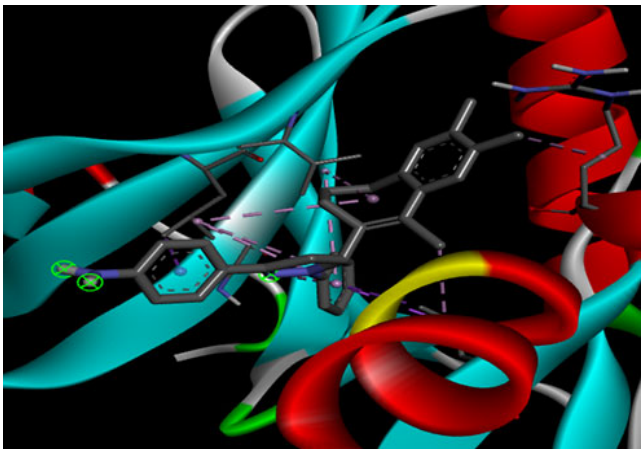
IIIe



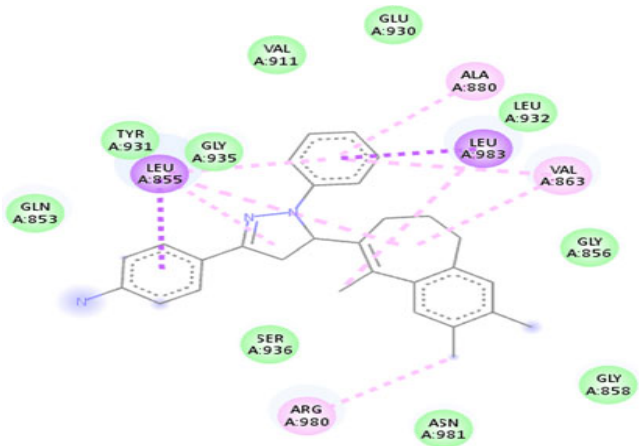


IIIf





IIIg



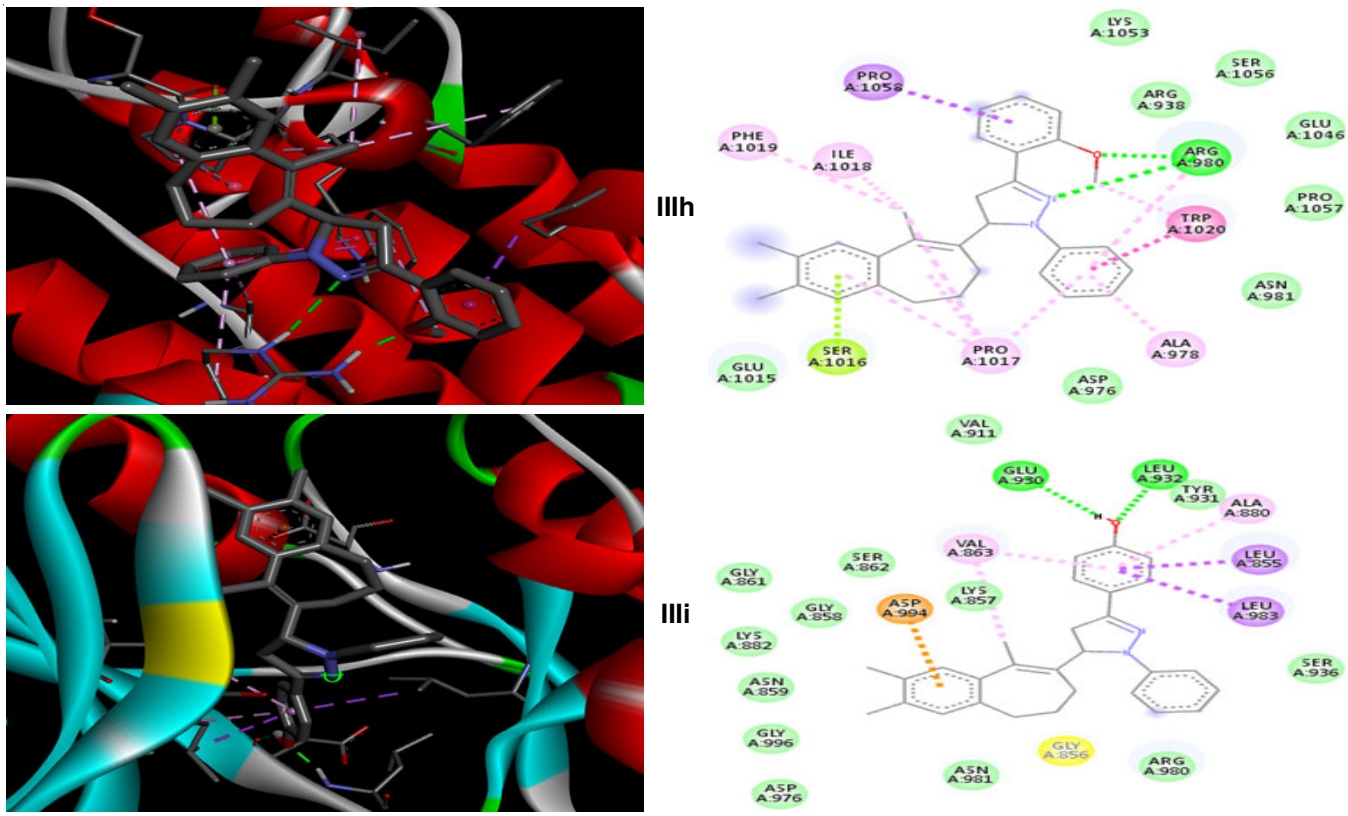


Fig. 1. A perspective of the active substances and enzyme in docked positions

treated cells compared to the control or vehicle-treated cells. The results of the cytotoxicity tests revealed that compounds **IIIb** and **IIId** reduced cell viability in a time- and dose-dependent manner, presumably as a result of the presence of halogen groups (chloro and fluoro) at the *para*-position of the phenyl ring. The synthesized compounds have low enzyme binding energies, thus molecular docking studies were performed using the crystal structure of the quinoxaline inhibitor bound to enzyme JAK2 (PDB: 3KRR). These findings indicated that the possibility for further investigation and advancement of novel anticancer drugs.

CONFLICT OF INTEREST

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

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