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SYNTHESIS AND EVALUATION OF BENZOTHIAZOLYL-PYRAZOLINE DERIVATIVES AS POTENTIAL ANTICANCER AGENTS

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ABSTRACT

*The present study aims at the synthesis of pyrazolines bearing benzothiazole and their evaluation as anticancer agents. A new series of benzothiazole-pyrazoline hybrids (**4a-p**) were synthesized from condensation reaction of intermediate chalcones by cyclizing with phenyl hydrazine. The structures of these compounds were confirmed by IR, NMR, and mass spectroscopy. The compounds were tested for their antitumor activity against HBL-100 cell lines. The compound **4a–4p** showed different levels of anticancer inhibition with the IC_{50} ranges between 0.19-2.47 µM. Among all the derivatives, the unsubstituted, -OH and $–OCH_3$ derivatives exhibited good activity and within this ortho hydroxyl (**4b**) and ortho methoxy (**4e**) derivatives shown very potent than the respective meta (**4c & 4f**) and para (**4d & 4g**) derivatives.*

KEY WORDS

synthesis of pyrazolines, benzothiazole.

Introduction

Cancer is one of the dangerous and recognized to multifactorial diseases in humans. These factors may interact with each other, hence inducing biological changes in a single cell over a period of time. The interaction of the multifactorial properties amplifies the cancer. According to WHO, cancer is one of the leading causes of death worldwide, which accounted for 8.2 million deaths (around 13%) of the world's population in 2016. Furthermore, WHO estimated that the worldwide deaths are likely to rise to over 11 million in 2030[1]. The common cancers in worldwide are, breast cancer, lung cancer, large intestine cancer, stomach cancer and prostate cancer. Incidence and mortality rates for most cancers are increasing in several countries because of adoption of unhealthy lifestyles, such as smoking, physical inactivity and consumption of high calorie food[2].

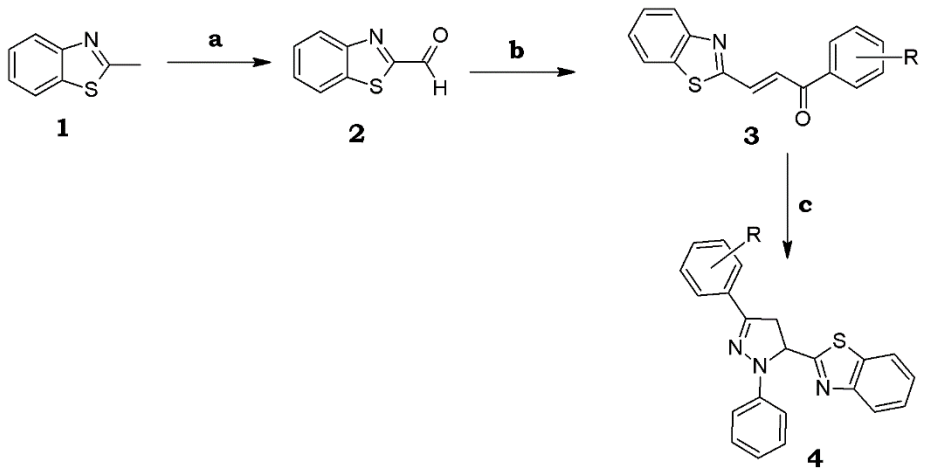
In the exertions to discovery of new drugs with these abilities, scientists have focused on many altered features of cancer biology during their research. To treat the cancer provide outstanding therapeutic drugs, numerous researchers have focused their work on discovering new multitarget drugs which are able to interact with multiple altered pathways[3,4].

Pyrazoline is a five-membered heterocyclic compound containing two nitrogen atoms in adjacent position and contains two endocyclic double bonds. It is dihydropyrazoline possessing only one endocyclic double bond and unique in their chemical behaviour. Among a wide range of heterocyclic compounds that have been explored for the development new molecules, pyrazolines constitute an interesting class of heterocycles due to their synthetic flexibility and effective biological activities such as anticancer[5], antioxidant[6], antibacterial[7], antifungal[7], antidepressant[8,9], antitubercular[10], anti-inflammatory[6,11], antimalarial[12], anthelmintic[13], anticonvulsant [9] properties and etc. Benzothiazole belongs to the family of bicyclic heterocyclic compounds having benzene nucleus fused with five-membered ring containing nitrogen and sulfur atoms. Benzothiazole consist of wide variety of biological activities and therapeutic functions including antitubercular[14], antibacterial[14], antifungal[14], antimalarial[15], anticonvulsant[16], anthelmintic[17],

analgesic[18], anti-inflammatory[11,19], antidiabetic[20] and antitumor[21] activities and etc. In an attempt, to identify new and potent anticancer agents, tried benzothiazole-pyrazole hybrid motif, thus may be exhibit synergistic anticancer effect here to generate new benzothiazolyl-pyrazoline derivatives as anticancer agents using simple methods.

Experimental

Scheme. 1



Reagents and condition: (a). Trifluroacetic acid, I_2, EtOAc, Flurescent lamp, at 70 °C; (b). Appropriate acetophenone, KOH (60%), stirring at 0 °C, 15 min, 48 hr, RT; (c) $PhNHNH_2$, ethanol, reflux 3–6 h.

Chemistry

Melting points were determined using Thermonik Melting Point Apparatus (Campbell electronics, India) by capillary method and are uncorrected. Infrared (IR) spectra were taken on a Fourier Transform Infrared Spectrophotometer IR-Prestige 21 (Shimatzu Corporation, Japan) from 4000-400 cm^{-1} using KBr discs. ^1H NMR spectra were recorded at 400 MHz in DMSO-d_6 using a Bruker Avance 400 instrument (Bruker Instruments Inc., USA). Chemical shifts were measured at δ units (ppm) relative to tetramethylsilane (TMS). Fast-atom bombardment (FAB) mass spectra were recorded on a Jeol SX 102/DA-6000 mass spectrometer (Jeol Ltd Akishima, Tokyo, Japan) using argon/xenon (6 kV, 10 mA) as FAB gas, m-nitrobenzyl alcohol as matrix, and 10 kV as accelerating voltage at room temperature. Elemental analysis was performed on a Vario EL III Elemental Analyser (Elementar, Germany) using sulfanilamide as standard. All chemicals were purchased from Merck, Spectrochem or CDH, India. Solvents were of reagent grade and were purified and dried by standard procedure. Reactions were monitored by thin-layer chromatography on silica gel plates in either iodine or UV chambers. Intermediates were characterized by IR spectroscopic analysis and elemental analysis for CHN. In the elemental analysis, the observed values were within ±0.4% of the calculated values. Final compounds were characterized by ^1H NMR and FAB mass spectrometry (MS). The final yields and the physicochemical data of the compounds **4a-4p** are presented in **Table 1.**

Synthesis of benzothiazole-2-carboxaldehyde[22]:

A solution of 2-methylbenzothiazole (0.3 mmol), iodine (0.06 mmol), and TFA (0.3 mmol) in EtOAc (5 mL) was stirred, heated at 70 °C, and irradiated using a fluorescent lamp under an O2 atmosphere for 20 h. The reaction mixture was treated with saturated aqueous $Na_2S_2O_3$ (30 mL) and the aqueous layer was extracted three times with EtOAc (10 mL). Then, the organic layer was dried over magnesium sulfate and filtered. The solvent was removed by rotary evaporation and the residue purified by column to furnish benzothiazole-2-carboxaldehyde.

*General procedure for the synthesis of chalcones (**3a-3p**)*

To a solution of suitably substituted acetophenone (0.01 M) and benzothiazole-2-carboxaldehyde (**2,** 0.01 M) in ethanol (10 ml) was added aqueous solution of

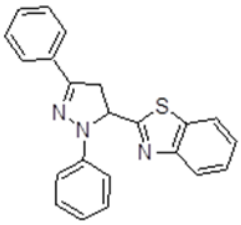
potassium hydroxide (60%) drop wise with continuous stirring at 0 °C over a period of 15 minutes. The reaction mixture was kept at room temperature for about 48 h with occasional shaking. After 48 h it was poured into ice-cold water, and then neutralized to pH 2 using 6 N hydrochloric acid. The yellow precipitate obtained was filtered, washed, dried, and recrystallized from dry methanol. The intermediates 1–2 were obtained.

*General procedure for the synthesis of 2-[3-(substituted phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl]-1,3-benzothiazole (**4a-4p**)*

Appropriate chalcone (**3a-3p**) was treated with 10 times excess of phenyl hydrazine in dry ethanol and refluxed for 3–6 h. The hot reaction mixture was then poured into ice-cold water. The solid separated out was filtered, washed, dried and recrystallized from ethanol to afford respective pyrazoline (**4a-4p**).

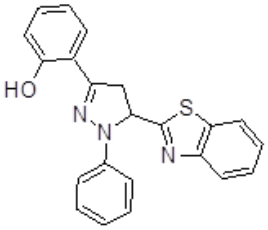
2-(1,3-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)-1,3-benzothiazole (**4a**):

IR (KBr, cm^{-1}): 3088 (Ar-H), 3056, 2865 (C-H), 1616 (C=N), 1240 (C-N), 1274 (C-S); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 3.34 (d, 2H, CH_2), 5.40 (t, 1H, CH_2), 6.78-7.28 (m, 14H, ArH); FAB-MS (m/z): 356 $[m+1]^+$; Elemental analyses Found (Calcd.): C 74.46 (74.34); H 4.81 (4.82); N 11.80 (11.82);



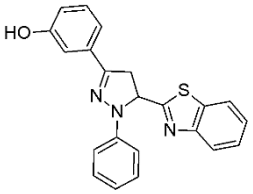
2-[5-(1,3-benzothiazol-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]phenol (**4b**):

IR (KBr, cm^{-1}): 3079 (Ar-H), 3056, 2918 (C-H), 1620 (C=N), 1463 (C-N), 1256 (C-S), 1315 (C-O), 2794 (O-H); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 3.56 (d, 2H, CH_2), 5.88 (t, 1H, CH_2), 6.92-7.18 (m, 4H, ArH), 7.34-7.52 (m, 9H, ArH), 8.82 (Ar-OH); FAB-MS (m/z): 372 $[M+1]^+$; Elemental analyses Found (Calcd.): C 70.88 (71.14); H 4.62 (4.61); N 11.28 (11.31)



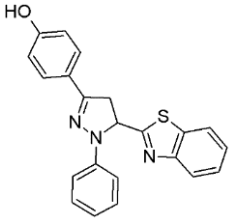
3-[5-(1,3-benzothiazol-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]phenol (**4c):**

IR (KBr, cm^{-1}): 3082 (Ar-H), 3056, 2851 (C-H), 1629 (C=N), 1460 (C-N), 1286 (C-S), 1308 (C-O), 2796 (O-H); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 3.58 (d, 2H, CH_2), 5.72 (t, 1H, CH_2), 6.96-7.16 (m, 4H, ArH), 7.38-7.44 (m, 9H, ArH), 8.88 (Ar-OH); FAB-MS (m/z): 372 $[M+1]^+$; Elemental analyses Found (Calcd.): C 71.02 (71.14); H 4.60 (4.61); N 11.28 (11.31)



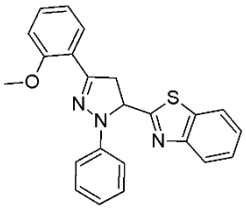
4-[5-(1,3-benzothiazol-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]phenol (**4d**):

IR (KBr, cm^{-1}): 2925 (Ar-H), 3080, 2865 (C-H), 1630 (C=N), 1440 (C-N), 1276 (C-S), 1298(C-O), 2790 (O-H); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 3.60 (d, 2H, CH_2), 5.64 (t, 1H, CH_2), 6.94-7.18 (m, 4H, ArH), 7.36-7.48 (m, 9H, ArH), 8.76 (Ar-OH); FAB-MS (m/z): 372 $[M+1]^+$; Elemental analyses Found (Calcd.): C 71.28 (71.14); H 4.59 (4.61); N 11.32 (11.31)

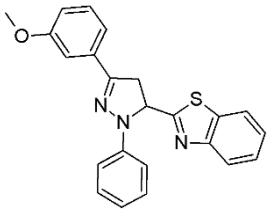


2-[3-(2-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl]-1,3-benzothiazole (**4e**)

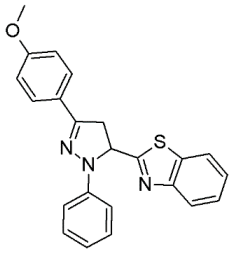
IR (KBr, cm^{-1}): 3121 (Ar-H), 3051, 2851 (C-H), 1632 (C=N), 1471 (C-N), 1281 (C-S), 1310 (C-O); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 3.50 (s, 3H, -O-CH_3, 3.42 (d, 2H, CH_2), 5.80 (t, 1H, CH_2), 6.82-7.02 (m, 4H, ArH), 7.24-7.36 (m, 9H, ArH); FAB-MS (m/z): 386 $[M+1]^+$; Elemental analyses Found (Calcd.): C 71.86 (71.66); H 4.95 (4.97); N 10.88 (10.90)



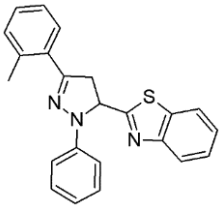
2-[3-(3-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl]-1,3-benzothiazole (**4f**): IR (KBr, cm^{-1}): 2998 (Ar-H), 3062, 2870 (C-H), 1670 (C=N), 1264 (C-N), 1298 (C-S), 1346(C-O); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 3.55 (s, 3H, -O-CH_3, 3.48 (d, 2H, CH_2), 5.88 (t, 1H, CH_2), 6.90-7.14 (m, 4H, ArH), 7.32-7.42 (m, 9H, ArH) ; FAB-MS (m/z): 386 $[M+1]^+$; Elemental analyses Found (Calcd.): C 71.58 (71.66); H 4.96 (4.97); N 10.92 (10.90)



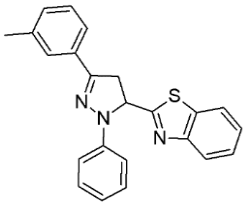
2-[3-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl]-1,3-benzothiazole (**4g**): IR (KBr, cm^{-1}): 3084 (Ar-H), 3066, 2842 (C-H), 1674 (C=N), 1460 (C-N), 1256 (C-S), 1286 (C-O); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 3.60 (s, 3H, -OCH_3, 3.50 (d, 2H, CH_2), 5.62 (t, 1H, CH_2), 6.84-7.08 (m, 4H, ArH), 7.26-7.38 (m, 9H, ArH); FAB-MS (m/z): 386 $[M+1]^+$; Elemental analyses Found (Calcd.): C 71.78 (71.66); H 4.96 (4.97); N 10.88 (10.90)



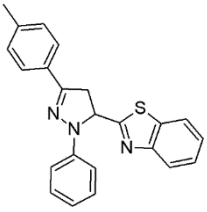
2-[3-(2-methylphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl]-1,3-benzothiazole (**4h**): IR (KBr, cm^{-1}): 3120 (Ar-H), 3048, 2910 (C-H), 1636 (C=N), 1398 (C-N), 1260 (C-S); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 2.2 (s, 3H, -CH_3, 3.24 (d, 2H, CH_2) , 5.26 (t, 1H, -CH), 6.52-6.74 (m, 4H, ArH), 6.96-7.14 (m, 9h, ArH); FAB-MS (m/z): 370$[M+1]^+$; Elemental analyses Found (Calcd.): C 75.02 (74.77); H 5.20 (5.18); N 11.38 (11.37)



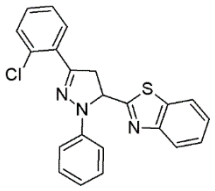
2-[3-(3-methylphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl]-1,3-benzothiazole (**4i**): IR (KBr, cm^{-1}): 3094 (Ar-H), 3050, 2951 (C-H), 1664 (C=N), 1468 (C-N), 1270 (C-S); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 2.18 (s, 3H, -CH_3, 3.20 (d, 2H, CH_2) , 5.22 (t, 1H, -CH), 6.50-6.76 (m, 4H, ArH), 6.90-7.10 (m, 9h, ArH) ; FAB-MS (m/z): 370 $[M+1]^+$; Elemental analyses Found (Calcd.): C 74.66 (74.77); H 5.16 (5.18); N 11.38 (11.37)



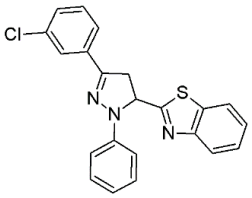
2-[3-(4-methylphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl]-1,3-benzothiazole (**4j**): IR (KBr, cm^{-1}): 3016 (Ar-H), 3074, 2954 (C-H), 1678 (C=N), 1462(C-N), 1274 (C-S); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 2.24 (s, 3H, -CH_3, 3.32 (d, 2H, CH_2) , 5.30 (t, 1H, -CH), 6.54-6.78 (m, 4H, ArH), 7.02-7.26 (m, 9h, ArH) ; FAB-MS (m/z): 370 $[M+1]^+$; Elemental analyses Found (Calcd.): C 74.64 (74.77); H 5.20 (5.18); N 11.40 (11.37)



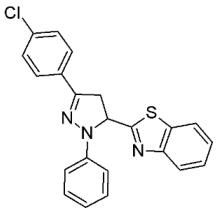
2-[3-(2-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl]-1,3-benzothiazole (**4k**): IR (KBr, cm-1): 3040 (Ar-H), 3048, 2842(C-H), 1620 (C=N), 1274(C-N), 1214 (C-S), 814 (C-Cl); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.42 (d, 2H, CH_2), 5.98 (t, 1H, CH), 7.33-7.48 (m, 4H, ArH), 7.96-8.08 (m, 9H, ArH); FAB-MS (m/z): 391 $[M+1]^+$; Elemental analyses Found (Calcd.): C 68.02 (67.77); H 4.12 (4.14); N 10.80 (10.78)



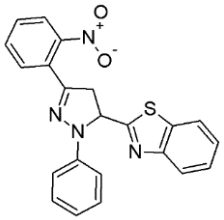
2-[3-(3-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl]-1,3-benzothiazole (**4l**): IR (KBr, cm^{-1}): 3058 (Ar-H), 3048, 2954 (C-H), 1625 (C=N), 1280 (C-N), 1218 (C-S), 818 (C-Cl); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.38 (d, 2H, CH_2), 5.84 (t, 1H, CH), 7.26-7.56 (m, 4H, ArH), 7.94-8.28 (m, 9H, ArH); FAB-MS (m/z): 391 $[M+1]^+$; Elemental analyses Found (Calcd.): C 67.90 (67.77); H 4.14 (4.14); N 10.80 (10.78)



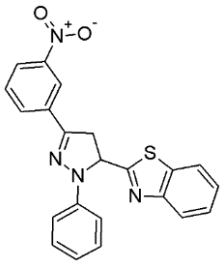
2-[3-(4-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl]-1,3-benzothiazole (**4m**): IR (KBr, cm^{-1}): 3041 (Ar-H), 3080, 2842 (C-H), 1632(C=N), 1276 (C-N), 1212 (C-S) 816 (C-Cl); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.44 (d, 2H, CH_2), 6.08 (t, 1H, CH), 7.46-7.68 (m, 4H, ArH), 8.08-8.12 (m, 9H, ArH);FAB-MS (m/z): 391 $[M+1]^+$; Elemental analyses Found (Calcd.): C 67. 94 (67.77); H 4.12 (4.14); N 10.82 (10.78)



2-[3-(2-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl]-1,3-benzothiazole (**4n**): MF: $C_{22}H_{16}N_4O_2S$; MW: 400.5; IR (KBr, cm^{-1}): 3088 (Ar-H), 3064, 2860 (C-H), 1630 (C=N), 1468 (C-N), 1240 (C-S); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.28 (d, 2H, CH_2) , 5.32 (t, 1H, -CH), 6.96-7.14 (m, 9h, ArH), 8.10-8.24 (m, 4H, ArH); FAB-MS (m/z): 400 $[M+1]^+$; Elemental analyses Found (Calcd.): C 66.14 (65.98); H 4.04 (4.03); N 14.00 (13.99)

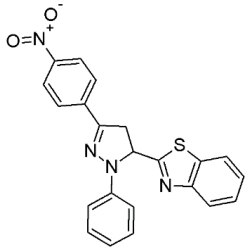


2-[3-(3-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl]-1,3-benzothiazole (**4o**): IR (KBr, cm^{-1}): 3121 (Ar-H), 3058, 2900 (C-H), 1625 (C=N), 1460 (C-N), 1244 (C-S); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.32 (d, 2H, CH_2) , 5.30 (t, 1H, -CH), 6.96-7.14 (m, 9h, ArH), 7.96-8.14 (m, 4H, ArH); FAB-MS (m/z): 400 $[M+1]^+$; Elemental analyses Found (Calcd.): C 66.04 (65.98) H 4.04 (4.03); N 13.96 (13.99)



2-[3-(4-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl]-1,3-benzothiazole (**4p**):

IR (KBr, cm^{-1}): 3084 (Ar-H), 3078, 2940 (C-H), 1620 (C=N), 1466 (C-N), 1260 (C-S); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.30 (d, 2H, CH_2) , 5.32 (t, 1H, -CH), 6.92-7.04 (m, 9h, ArH), 7.98-8.16 (m, 4H, ArH); FAB-MS (m/z): 400 $[M+1]^+$; Elemental analyses Found (Calcd.): C 65.80 (65.98); H 4.02 (4.03); N 13.94 (13.99)



Pharmacology

Anticancer studies (MTT assay) [22–24] Compounds 4a–4l were evaluated for their anticancer activity on HT-29 cell lines using MTT assay by serial double dilution method in 96-well plate. Cells seeded in plate at 5000 cells/well. Different dilutions of test and standard (0.1–100 µM) were made with growth medium in such a way that the final DMSO concentration is around 0.5%. 100 mL of cell suspension and 100 mL of test and standard were transferred aseptically to each well. The plate was then incubated at 37 °C for 72 h in CO_2 incubator. After incubation, 20 mL of MTT was added to each well and plate was wrapped in aluminum foil to prevent the oxidation of the dye. The plate was again incubated for 2 h. 80 mL of lysis buffer was added to each well and the plate was placed on a shaker overnight. The absorbance was recorded on the ELISA reader at 562 nm wavelength. The absorbance of the test was compared with that of DMSO control to get the percentage inhibition and IC_{50} values are calculated by plotting a graph between log concentrations and percentage inhibition value. All the studies were performed in duplicate and results were presented in **Table 1.**

Results and Discussion:

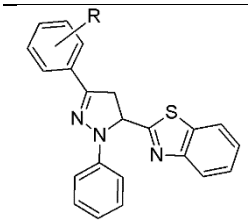
Chemistry

The compounds were synthesized as shown in **Scheme 1** according to previously reported method[23]. The transition metal-free, catalytic direct photo-oxidation of a methyl group on a 2-methyl benzothiazole (**1**) to form the benzothizole-2-carboxaldehyde (**2**). The catalytic oxidation of methyl group on benzothiazole via the iodinated intermediate could occur through the hemolytic cleavage of the C-I bond caused by irradiation with visible light using fluorescent lamp under an oxygen atmosphere in acidic medium (TFA) at 70 °C, giving the benzothizole-2-carboxaldehyde. The reaction gave in good yields with a high degree of selectivity[22]. The synthesis of chalcones (**3a-3p**) was carried out at room temperature by reacting with different substituted acetophenone in the presence of base by conventional Claisen–Schmidt condensation. These chalcones were then reacted with phenyl hydrazine in ethanol using catalytic amount of concentrated sulphuric acid offered **4a-4p.** The solid compound so obtained was filtered and purified by recrystallization from ethanol. The pyrazoline derivatives were characterized by their spectral studies using IR, ^{1}H NMR, and FAB-MS. All of the synthesized pyrazoline compounds gave satisfactory analytical and spectroscopic data, which were in full consistent with their depicted structures. The structures of pyrazolines were confirmed through the following spectral data. IR absorption peak at 32925-3121 cm^{-1} (Ar-H stretch), 3080-3048 & 2954-2842 cm^{-1} (C-H stretch), 1264-1471 cm^{-1} (C-N stretch), 1678-1616 cm^{-1} (C=N stretch) and 1298-1212 cm^{-1} (C-S stretch) in all pyrazolines (**4a-4p**). The IR absorption peak of C-O stretch were appeared at 1346-1286 cm^{-1} in spectrum of **4b-4f**, O-H stretch of **4b-4d** were appeare at 2796-2790 cm^{-1} and C-Cl peak of 4k-4m appeared at 818-814 cm^{-1}. ^{1}H NMR spectra revealed all the corresponding peaks of CH_2 appeared as doublet at δ 3.20-3.60 ppm which were assigned two protons, CH proton appeared at δ 5.22-6.08 ppm which is considered for one proton and the aromatic protons appeared as multiplets at δ 6.50-8.28 ppm. Along with above peaks, the reliable peaks appeared at δ 2.18-2.24 ppm as singlet for -CH_3 protons of **4h-4j**, δ 3.50-3.60 ppm as singlet for -OCH_3 protons of **4e-4g** and δ 8.76-8.88 ppm as singlet for Ar-OH proton of **4b-4d**. Further FAB-MS gave all the $[M+1]^+$ ion peaks corresponding to molecular weight of confirmed novel compounds. In the elemental analysis of CHN, the observed values were within ±0.4% of the calculated values.

Anticancer activity

The *in vitro* anticancer screening of pyrazolines **4a–4p** was done by means of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay using HBL-100 cancer cell line. After 24 h incubation at 37 °C under a humidified 5% CO_2 to allow cell attachment, the cancer cells in the wells were, respectively, treated with target compounds at various concentrations for 48 h. The experiment was done in triplicate and the inhibitory concentration (IC_{50}) values were calculated from a dose response curve. IC_{50} is the concentration in 'µM' required for 50% inhibition of cell growth as compared to that of untreated control. The cell viability was measured with the purple formazan that was metabolized from MTT mitochondrial dehydrogenase, which is active only in live cells. The data reported in **Table 1** indicates that compound **4a–4p** showed different levels of anticancer inhibition with the IC_{50} ranges between 0.19-2.47 µM. Among all the derivatives, the unsubstituted, -OH and $–OCH_3$ derivatives exhibited good activity and within this 2-OH (**4b**; IC_{50} value = 0.26 µM) and 2-OCH_3 (**4e**; IC_{50} value = 0.38 µM) derivatives shown very potent than the respective meta (**4c & 4f**; IC_{50} value = 0.62 & 0.84 µM, respectively) and para (**4d & 4g**; IC_{50} value = 0.64 & 0.84 µM, respectively) derivatives. The unsubstituted and strong electron donating groups (OH & OCH_3) substituted derivatives were observed more potent than compounds substituted with electron withdrawing groups and mild electron donating groups.

Table. 1: Physical data of **4a-4p** and anticancer activity against HBL-100



Code	R	MF	MF	% Yeild	IC_{50} (µM)
4a	H	$C_{22}H_{17}N_3S$	355	62.46	0.86
4b	2-OH	$C_{22}H_{17}N_3OS$	371	64.21	0.26
4c	3-OH	$C_{22}H_{17}N_3OS$	371	63.84	0.62
4d	4-OH	$C_{22}H_{17}N_3OS$	371	64.46	0.64
4e	2-OCH_3	$C_{23}H_{19}N_3OS$	385	72.01	0.38
4f	3-OCH_3	$C_{23}H_{19}N_3OS$	385	73.41	0.84
4g	4-OCH_3	$C_{23}H_{19}N_3OS$	385	72.46	0.84
4h	2-CH_3	$C_{23}H_{19}N_3S$	369	65.84	1.08
4i	3-CH_3	$C_{23}H_{19}N_3S$	369	65.12	1.56
4j	4-CH_3	$C_{23}H_{19}N_3S$	369	68.74	2.14
4k	2-Cl	$C_{22}H_{16}ClN_3S$	389	80.02	1.24
4l	3-Cl	$C_{22}H_{16}ClN_3S$	389	81.96	2.28
4m	4-Cl	$C_{22}H_{16}ClN_3S$	389	82.74	2.36
4n	2-NO_2	$C_{22}H_{16}N_4O_2S$	400	78.63	1.62
4o	3-NO_2	$C_{22}H_{16}N_4O_2S$	400	82.21	1.98
4p	4-NO_2	$C_{22}H_{16}N_4O_2S$	400	74.62	2.47

Conclusion:

The present investigation synthesized 16 molecules (**4a–4p**) and characterized based on its physical and spectral data. The synthesized compounds were exhibited potent to moderate anticancer activity against HBL -100 cell line by MTT assay method. Among all, the derivatives were substituted with electron donating groups especially ortho derivatives were observed more potent than electron withdrawing substituted derivatives. Furthermore, our data suggest that generating hybrid compounds containing ortho hydroxy derivatives are a promising new approach of developing an effective anticancer agent.

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