

SYNTHESIS AND EVALUATION OF PYRAZOLINE-1- CARBOXAMIDE DERIVATIVES AS POTENTIAL ANTICANCER AGENTS

Hanmanthu Guguloth*

Department of Chemistry, University Arts & Sciences College, Kakatiya University, Warangal, Telangana

*Corresponding AuthorEmail: hanmanthu.guguloth@yahoo.com

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 (**5a-p**) were synthesized and 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 **5a-5p** showed different levels of anticancer inhibition with the IC_{50} ranges between 2.28-4.63 μ M. Among all, the unsubstituted, and electron donating group substituted derivatives exhibited good activity and within this ortho derivatives shown very potent than the respective meta and para derivatives.

KEY WORDS

synthesis of pyrazolines, Anti Cancer Agents

INTRODUCTION

Cancer is one of the dangerous and recognized to multifactorial diseases in humans. Cancer has no etiopathology and progresses in different organs, systems of body.¹ It cannot be definite as a single disease and defined as a broad group characterized as by cancer cells which are clearly differentiated from normal cells by an uncontrolled growth due to a serious disorder of the cell cycle controlling system.² It has been a major public health risk from the 21st century beginning. For the treatment of cancer different methods like surgery, radiotherapy and immunotherapy are used.³ Nowadays, chemotherapy is also additional method for the treatment of cancer. The cytotoxic and anti-hormonal drugs are the leading chemotherapeutics with significant adverse effects.⁴

Synthetic organic chemistry has always plays a vital role in combined and multidisciplinary practice for development of anticancer drugs. In

recent years, made efforts to synthesize potential anticancer drugs with chemical modifications of known classes of cancer therapeutic agents. Recently, pathogenesis of different types of cancer becomes clearer, more rational methods to the design of newer drugs with no or decreased side effects. However, the exact biology of cancer still remains enigmatic at large offering a lot of scope for the research to develop newer compounds to treat cancer cells.² 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 anticancer⁵, antioxidant⁶, antibacterial⁷, antifungal⁷,

antidepressant^{8,9}, antitubercular⁵, anti-inflammatory⁶, antimalarial¹⁰, anthelmintic¹¹, anticonvulsant⁹ 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 antitubercular¹², antibacterial¹², antifungal¹², antimalarial¹³, anticonvulsant¹⁴, anthelmintic¹⁵, analgesic¹⁶, anti-inflammatory¹⁶, antidiabetic¹⁷ and anticancer¹⁸ 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

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 (Shimadzu 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 $\pm 0.4\%$ 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 **5a-5p** are presented in **Table 1**.

General procedure for synthesis of 2-amino-benzothiazoles:

A solution of aniline (0.03 M) in 95% acetic acid (20 ml) was added to a solution of KSCN (0.12 M) in 95% acetic acid (20 ml). The reaction mixture was cooled to 0 °C and a solution of Br_2 (1.6 ml) in acetic acid (10 ml) was added over 90 minutes; during the addition, the temperature should not raise to 5 °C. After addition, continued the stirring for about 3 hr at 10-15 °C, and then poured into hot water (300 ml). Separated hydrogen bromide salt was filtered, washed with acetic acid and dried. It was dissolved in hot water and neutralizes with 26% ammonium hydroxide solution, filtered the solid product, washed with water and recrystallized from ethanol¹⁹.

General procedure for the synthesis of chalcones (**2a-2p**)

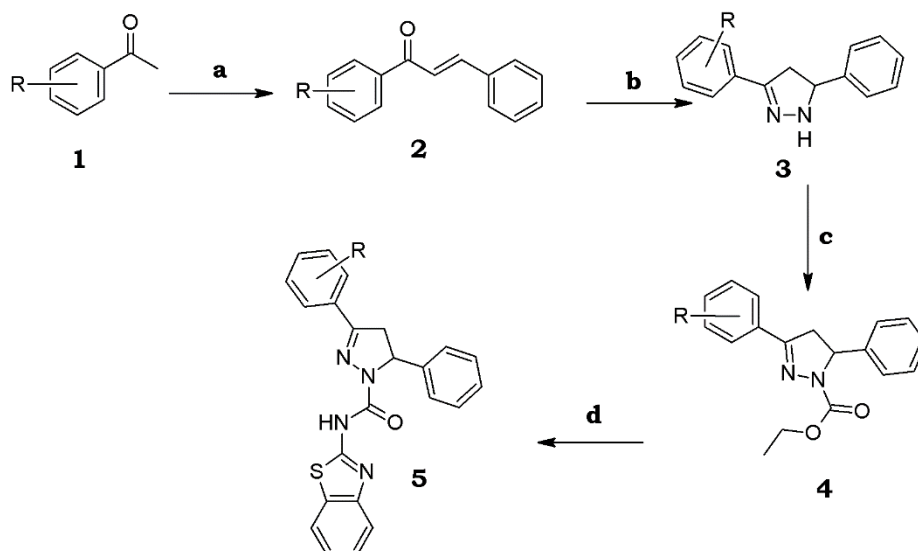
To a solution of suitably substituted acetophenone (0.01 M) and benzaldehyde (0.01 M) in ethanol (10 ml) was added aqueous solution of potassium hydroxide (60%) drop wise with continuous stirring at 0 °C over a period of 15 minutes. The reaction mixture was kept at room temperature for about 48 h with 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 **2a-2p** were obtained.

General procedure for the synthesis of 3,5-diaryl-4,5-dihydro-1H-pyrazole (**3a-3p**)

Scheme. 1



Reagents and condition: (a). Benzaldehyde, KOH (60%), stirring at 0 °C, 15 min, 48 hr, RT; (b) NH₂NH₂, ethanol, reflux 3–6 h; (c). Phenyl chloroformate, trimethylamine, THF, stirring at below 5 °C, 1 hr; (d). 2-amino benzothiazole, THF, stirring, RT, 3 hrs.

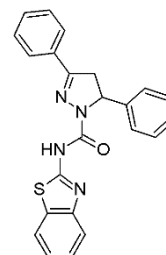
Appropriate chalcone (**1–2**) was treated with 10 times excess of hydrazine hydrate in dry ethanol and refluxed for 3–6 h. The hot reaction mixture was then poured into ice-cold water. The solid separated out was filtered, washed, dried and recrystallized from ethanol to afford respective pyrazoline (**3a-3p**).

General procedure for the synthesis of N-(1,3-benzothiazol-2-yl)-3-(substituted phenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5a-5p**):

Phenyl chloroformate (0.001 M) and triethylamine (0.001 M) were added to an ice-cooled solution of appropriate 3,5-diaryl-4,5-dihydro-1H-pyrazole derivative (**3a-3p**, 0.001 M) in dry THF and the mixture was stirred for 1 h. The solid obtained was filtered off and to the filtrate was added freshly prepared solution of 2-amino benzothiazole in THF. After stirring at room temperature for 3 h, the solid obtained was filtered, dried and recrystallized from suitable solvent to afford respective pyrazolines (**5a-5p**).

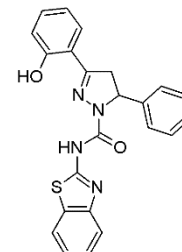
***N*-(1,3-benzothiazol-2-yl)-3,5-diphenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (5a):**

IR (KBr, cm^{-1}): 3148 (Ar-H), 3089, 2865 (C-H), 1616 (C=N), 1233 (C-N), 1264 (C-S); ^1H NMR (300 MHz, CDCl_3 , δ ppm): 3.36 (d, 2H, CH_2), 5.44 (t, 1H, CH_2), 6.78-7.36 (m, 14H, ArH), 9.02 (s, 1H, NH); FAB-MS (m/z): 399 [$M+1$] $^+$; Elemental analyses Found (Calcd.): C 69.12 (69.32) H 4.56 (4.55) N 14.03 (14.06)



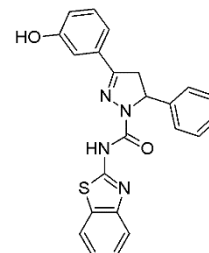
***N*-(1,3-benzothiazol-2-yl)-3-(2-hydroxyphenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (5b):**

IR (KBr, cm^{-1}): 3064 (Ar-H), 3012, 2818 (C-H), 1645 (C=N), 1496 (C-N), 1266 (C-S), 1315 (C-O), 2782 (O-H); ^1H NMR (300 MHz, CDCl_3 , δ ppm): 3.46 (d, 2H, CH_2), 5.88 (t, 1H, CH_2), 6.92-7.18 (m, 4H, ArH), 7.34-7.52 (m, 9H, ArH), 8.88 (Ar-OH) 9.56 (s, 1H, NH); FAB-MS (m/z): 416 [$M+1$] $^+$; Elemental analyses Found (Calcd.): C 66.40 (66.65) H 4.37(4.38) N 13.49 (13.52)



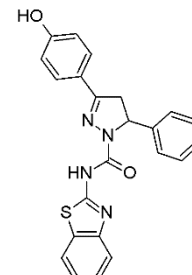
***N*-(1,3-benzothiazol-2-yl)-3-(3-hydroxyphenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (5c):**

IR (KBr, cm^{-1}): 3096 (Ar-H), 3064, 2851 (C-H), 1639 (C=N), 1460 (C-N), 1286 (C-S), 1308 (C-O), 2788 (O-H); ^1H NMR (300 MHz, CDCl_3 , δ ppm): 3.60 (d, 2H, CH_2), 5.78 (t, 1H, CH_2), 6.86-7.24 (m, 4H, ArH), 7.44-7.58 (m, 9H, ArH), 8.68 (Ar-OH), 9.22 (s, 1H, NH); FAB-MS (m/z): 416 [$M+1$] $^+$; Elemental analyses Found (Calcd.): C 66.52 (66.65) H 4.39 (4.38) N 13.48 (13.52)



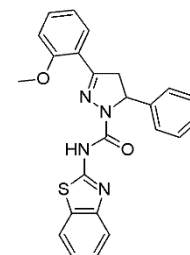
***N*-(1,3-benzothiazol-2-yl)-3-(4-hydroxyphenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (5d):**

IR (KBr, cm^{-1}): 2945 (Ar-H), 3068, 2845 (C-H), 1630 (C=N), 1440 (C-N), 1276 (C-S), 1298(C-O), 2788 (O-H); ^1H NMR (300 MHz, CDCl_3 , δ ppm): 3.62 (d, 2H, CH_2), 5.66 (t, 1H, CH_2), 6.94-7.28 (m, 4H, ArH), 7.36-7.48 (m, 9H, ArH), 8.86 (Ar-OH), 9.38 (s, 1H, NH); FAB-MS (m/z): 416 [$M+1$] $^+$; Elemental analyses Found (Calcd.): C 66.61 (66.65) H 4.37 (4.38) N 13.50 (13.52)

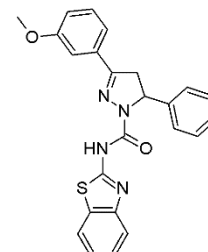


***N*-(1,3-benzothiazol-2-yl)-3-(2-methoxyphenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (5e):**

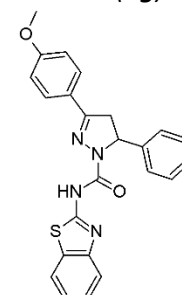
IR (KBr, cm^{-1}): 3121 (Ar-H), 3058, 2856 (C-H), 1622 (C=N), 1471 (C-N), 1281 (C-S), 1310 (C-O); ^1H NMR (300 MHz, CDCl_3 , δ ppm): 3.52 (s, 3H, -O-CH₃), 3.44 (d, 2H, CH₂), 5.82 (t, 1H, CH₂), 6.82-7.02 (m, 4H, ArH), 7.24-7.46 (m, 9H, ArH), 9.63 (s, 1H, NH); FAB-MS (m/z): 429 [$M+1$]⁺; Elemental analyses Found (Calcd.): C 67.19 (67.27) H 4.69 (4.70) N 13.04 (13.07)



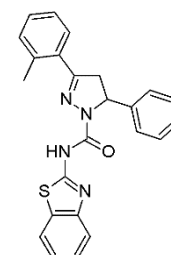
N-(1,3-benzothiazol-2-yl)-3-(3-methoxyphenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (5f):
IR (KBr, cm^{-1}): 3080 (Ar-H), 3122, 2860 (C-H), 1670 (C=N), 1264 (C-N), 1288 (C-S), 1352 (C-O); ^1H NMR (300 MHz, CDCl_3 , δ ppm): 3.58 (s, 3H, -O-CH₃), 3.50 (d, 2H, CH₂), 4.98 (t, 1H, CH₂), 6.92-7.14 (m, 4H, ArH), 7.32-7.42 (m, 9H, ArH), 9.46 (s, 1H, NH); FAB-MS (m/z): 429 [$M+1$]⁺; Elemental analyses Found (Calcd.): C 67.24 (67.27) H 4.68 (4.70) N 13.09 (13.07)



N-(1,3-benzothiazol-2-yl)-3-(4-methoxyphenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (5g):
IR (KBr, cm^{-1}): 3094 (Ar-H), 3086, 2842 (C-H), 1674 (C=N), 1460 (C-N), 1266 (C-S), 1246 (C-O); ^1H NMR (300 MHz, CDCl_3 , δ ppm): 3.60 (s, 3H, -OCH₃), 3.55 (d, 2H, CH₂), 5.72 (t, 1H, CH₂), 6.84-7.08 (m, 4H, ArH), 7.36-7.48 (m, 9H, ArH), 9.35 (s, 1H, NH); FAB-MS (m/z): 429 [$M+1$]⁺; Elemental analyses Found (Calcd.): C 67.34(67.27) H 4.71(4.70) N 13.10(13.07)

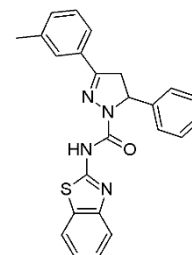


N-(1,3-benzothiazol-2-yl)-3-(2-methylphenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (5h):
IR (KBr, cm^{-1}): 3123 (Ar-H), 3048, 2910 (C-H), 1676 (C=N), 1348 (C-N), 1260 (C-S); ^1H NMR (300 MHz, CDCl_3 , δ ppm): 2.24 (s, 3H, -CH₃), 3.26 (d, 2H, CH₂), 5.23 (t, 1H, -CH), 6.52-6.82 (m, 4H, ArH), 7.12-7.44 (m, 9H, ArH), 9.36 (s, 1H, NH); FAB-MS (m/z): 413 [$M+1$]⁺; Elemental analyses Found (Calcd.): C 69.75(69.88) H 4.88(4.89) N 13.55(13.58)

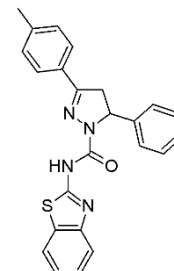


N-(1,3-benzothiazol-2-yl)-3-(3-methylphenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (5i):

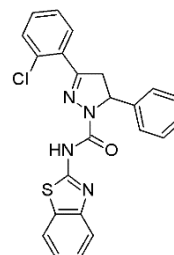
IR (KBr, cm^{-1}): 3084 (Ar-H), 3010, 2931 (C-H), 1667 (C=N), 1464 (C-N), 1284 (C-S); ^1H NMR (300 MHz, CDCl_3 , δ ppm): 2.28 (s, 3H, $-\text{CH}_3$), 3.24 (d, 2H, CH_2), 5.44 (t, 1H, -CH), 6.60-6.96 (m, 4H, ArH), 7.10-7.20 (m, 9H, ArH), 9.37 (s, 1H, NH); FAB-MS (m/z): 413 $[\text{M}+1]^+$; Elemental analyses Found (Calcd.): C 69.70(69.88) H 4.91(4.89) N 13.59(13.58)



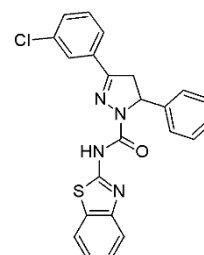
N-(1,3-benzothiazol-2-yl)-3-(4-methylphenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5j**): IR (KBr, cm^{-1}): 3064 (Ar-H), 3024, 2934 (C-H), 1676 (C=N), 1462 (C-N), 1274 (C-S); ^1H NMR (300 MHz, CDCl_3 , δ ppm): 2.24 (s, 3H, $-\text{CH}_3$), 3.32 (d, 2H, CH_2), 5.30 (t, 1H, -CH), 6.52-6.78 (m, 4H, ArH), 7.00-7.26 (m, 9H, ArH), 9.32 (s, 1H, NH); FAB-MS (m/z): 413 $[\text{M}+1]^+$; Elemental analyses Found (Calcd.): C 69.80(69.88) H 4.90(4.89) N 13.60(13.58)



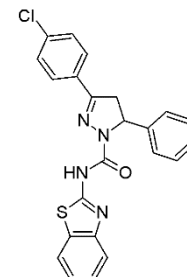
N-(1,3-benzothiazol-2-yl)-3-(2-chlorophenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5k**): IR (KBr, cm^{-1}): 3041 (Ar-H), 3023, 2832 (C-H), 1630 (C=N), 1274 (C-N), 1234 (C-S), 814 (C-Cl); ^1H NMR (300 MHz, CDCl_3 , δ ppm): 3.42 (d, 2H, CH_2), 5.98 (t, 1H, CH), 7.47-7.68 (m, 4H, ArH), 7.96-8.08 (m, 9H, ArH), 9.40 (s, 1H, NH); FAB-MS (m/z): 433 $[\text{M}+1]^+$; Elemental analyses Found (Calcd.): C 63.68(63.81) H 3.95(3.96) N 12.91(12.94)



N-(1,3-benzothiazol-2-yl)-3-(3-chlorophenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5l**): IR (KBr, cm^{-1}): 3058 (Ar-H), 30482, 2954 (C-H), 1625 (C=N), 1242 (C-N), 1208 (C-S), 834 (C-Cl); ^1H NMR (300 MHz, CDCl_3 , δ ppm): 3.37 (d, 2H, CH_2), 5.81 (t, 1H, CH), 7.26-7.60 (m, 4H, ArH), 7.90-8.28 (m, 9H, ArH), 9.24 (s, 1H, NH); FAB-MS (m/z): 433 $[\text{M}+1]^+$; Elemental analyses Found (Calcd.): C 63.72(63.81) H 3.97(3.96) N 12.93(12.94)



N-(1,3-benzothiazol-2-yl)-3-(4-chlorophenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5m**): IR (KBr, cm^{-1}): 3044 (Ar-H), 3089, 2846 (C-H), 1638 (C=N), 1286 (C-N), 1262 (C-S), 826 (C-Cl); ^1H NMR (300 MHz, CDCl_3 , δ ppm): 3.48 (d, 2H, CH_2), 6.08 (t, 1H, CH), 7.56-7.74 (m, 4H, ArH), 8.18-8.22 (m, 9H, ArH), 9.18 (s, 1H, NH); FAB-MS (m/z): 433 $[\text{M}+1]^+$; Elemental analyses Found (Calcd.): C 63.76(63.81) H 3.95(3.96) N 12.96(12.94)

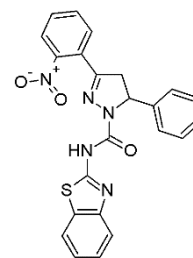


N-(1,3-benzothiazol-2-yl)-3-(2-nitrophenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5n**):

IR (KBr, cm^{-1}): 3086 (Ar-H), 3066, 2860 (C-H), 1620 (C=N), 1478 (C-N), 1244 (C-S);

^1H NMR (300 MHz, CDCl_3 , δ ppm): 3.30 (d, 2H, CH_2), 5.36 (t, 1H, -CH), 6.88-7.14 (m, 9h, ArH), 8.16-8.34 (m, 4H, ArH), 9.32 (s, 1H, NH); FAB-MS (m/z): 444 [$M+1$] $^+$;

Elemental analyses Found (Calcd.): C 62.20(62.29) H 3.85(3.86) N 15.75 (15.79)

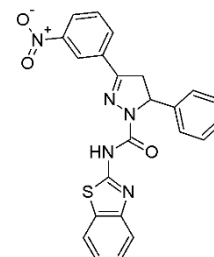


N-(1,3-benzothiazol-2-yl)-3-(3-nitrophenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5o**):

IR (KBr, cm^{-1}): 3121 (Ar-H), 3078, 2940 (C-H), 1625 (C=N), 1466 (C-N), 1234 (C-S);

^1H NMR (300 MHz, CDCl_3 , δ ppm): 3.33 (d, 2H, CH_2), 5.32 (t, 1H, -CH), 6.92-7.14 (m, 9h, ArH), 7.96-8.16 (m, 4H, ArH), 9.12 (s, 1H, NH); FAB-MS (m/z): 444 [$M+1$] $^+$;

Elemental analyses Found (Calcd.): C 62.27 (62.29) H 3.87 (3.86) N 15.78 (15.79)

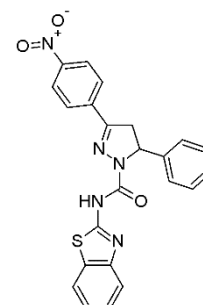


N-(1,3-benzothiazol-2-yl)-3-(4-nitrophenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5p**):

IR (KBr, cm^{-1}): 3082 (Ar-H), 3088, 2940 (C-H), 1624 (C=N), 1466 (C-N), 1260 (C-S);

^1H NMR (300 MHz, CDCl_3 , δ ppm): 3.32 (d, 2H, CH_2), 5.14 (t, 1H, -CH), 6.82-7.04 (m, 9h, ArH), 7.94-8.16 (m, 4H, ArH), 9.36 (s, 1H, NH); FAB-MS (m/z): 444 [$M+1$] $^+$;

Elemental analyses Found (Calcd.): C 62.31 (62.29) H 3.87 (3.86) N 15.81 (15.79).



Pharmacology

Anticancer studies (MTT assay) Compounds **5a–5p** 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 $^{\circ}\text{C}$ 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:

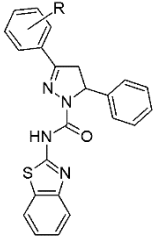
Chemistry

The compounds were synthesized as shown in **Scheme 1** according to previously reported method²⁰. The synthesis of chalcones (**2a-2p**) was carried out at room temperature by reacting with different substituted acetophenone and benzaldehyde in the presence of base by conventional Claisen-Schmidt condensation. These chalcones were then reacted with hydrazine in ethanol using catalytic amount of concentrated sulphuric acid offered **3a-3p**. The solid compound so obtained was filtered and purified by recrystallization from ethanol. The final pyrazoline derivatives **5a-5p** were obtained by the reaction of appropriate pyrazoline **3a-3p** with phenyl chloroformate followed by 2-amino benzothiazole in THF at room temperature. The pyrazoline derivatives were characterized by their spectral studies using IR, ¹H 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 (**5a-5p**) were confirmed through the IR, ¹H NMR, FAB-MS spectral data. In the elemental analysis of CHN, the observed values were within $\pm 0.4\%$ of the calculated values.

Anticancer activity

The in vitro anticancer screening of pyrazolines **5a-5p** 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% CO₂ 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 (IC₅₀) values were calculated from a dose response curve. IC₅₀ 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 IC₅₀ ranges between 2.28-4.63 μM. The unsubstituted and strong electron donating groups substituted derivatives were observed more potent than compounds substituted with electron withdrawing groups and mild electron donating groups.

Table. 1: Physical data of **5a-5p** and anticancer activity against HBL-100



Code	R	MF	MW	% Yield	IC ₅₀ (μM)
4a	H	C ₂₃ H ₁₈ N ₄ OS	398	66.40	2.50
4b	2-OH	C ₂₃ H ₁₈ N ₄ O ₂ S	414	67.22	2.28
4c	3-OH	C ₂₃ H ₁₈ N ₄ O ₂ S	414	69.83	2.79
4d	4-OH	C ₂₃ H ₁₈ N ₄ O ₂ S	414	67.66	2.62
4e	2-OCH ₃	C ₂₄ H ₂₀ N ₄ O ₂ S	428	76.16	3.16
4f	3-OCH ₃	C ₂₄ H ₂₀ N ₄ O ₂ S	428	74.48	3.58
4g	4-OCH ₃	C ₂₄ H ₂₀ N ₄ O ₂ S	428	75.89	3.64
4h	2-CH ₃	C ₂₄ H ₂₀ N ₄ OS	412	69.88	4.78

4i	3-CH ₃	C ₂₄ H ₂₀ N ₄ O ₅	412	68.84	4.42
4j	4-CH ₃	C ₂₄ H ₂₀ N ₄ O ₅	412	69.89	4.05
4k	2-Cl	C ₂₃ H ₁₇ ClN ₄ O ₅	432	82.84	4.10
4l	3-Cl	C ₂₃ H ₁₇ ClN ₄ O ₅	432	89.99	3.90
4m	4-Cl	C ₂₃ H ₁₇ ClN ₄ O ₅	432	86.81	3.94
4n	2-NO ₂	C ₂₃ H ₁₇ N ₅ O ₃ S	443	79.63	4.57
4o	3-NO ₂	C ₂₃ H ₁₇ N ₅ O ₃ S	443	84.28	4.63
4p	4-NO ₂	C ₂₃ H ₁₇ N ₅ O ₃ S	443	79.66	4.18

CONCLUSION:

The present investigation synthesized 16 molecules (**5a–5p**) 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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***Corresponding Author:**

Hanmanthu Guguloth

Department of Chemistry, University Arts & Sciences
College, Kakatiya University, Warangal, Telangana