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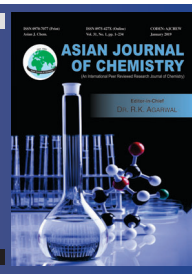

in vitro Cytotoxic Evaluation of Some New Synthesized Pyridazine Derivatives

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A series of novel pyridazine, pyrazoles, pyrimidines derivatives have been synthesized through the reaction of chloropyridazine (**1**) with *p*-phenylenediamine to give compound **2**. Diazotization of compound **2** followed by coupling with active methylene compounds namely acetylacetone, ethylcyanoacetate and/or ethylacetoacetate afforded novel hydrazons derivatives (**4-6**). The resulting hydrazons can have been cyclized using hydrazine hydrate and guanidine gave the corresponding pyrazoles (**7-9**) and pyimidine (**10**) derivatives. Reaction of compound **2** with acrylonitrile, aromatic aldehyde, *p*-chloroacetophenone and phenyl isothiocyanate gave compounds **11, 12a, 12b, 13** and **17**, respectively. The latter compounds have been used in synthesis of some heterocyclic compounds. The cytotoxic activity of the most active compounds was assessed *in vitro* against breast carcinoma cell line (MCF-7), human liver cancer cell line (HEPG2), human colon cancer cell line (HCT). Compounds **4, 8** showed best activity against MCF-7 cell line, compounds **5, 13a** showed best activity against HePG2 cell line and compound **10** showed best activity against HCT cell line.

Keywords: Pyridazine, Pyrazoles, Pyrimidines, Hydrazones, Anticancer, Antitumor activity.

INTRODUCTION

It is well known that pyridazine derivatives are one of the most biologically active diazines heterocyclic compounds that show high activity as inhibitors of protein tyrosine phosphatase 1BPTP1B [1], antifungal [2], antimicrobial [3,4], antituberculosis [5], herbicidal activities [6], antibacterial [7], anticonvulsant [8], anti-inflammatory [9], anticancer [10], antitumor [11,12], antiplatelet [13], as drugs acting on the cardiovascular system [14], antioxidant [15], analgesic [16], antiviral [17] and anti-HIV [18]. Also 2-phenyl indole is a reactive heterocyclic moiety. Indole is an important heterocyclic moiety because it provides the skeleton of indole alkaloids [19], also found to be a very potent anticancer [20], indolyl compounds are very efficient antioxidants, protecting both lipids and proteins from peroxidation. It is well known that the indole structure influences the antioxidant efficacy in biological systems [21]. Indole derivatives have been reported to possess a variety of physiological and pharmacological activities like antibacterial [22], antifungal [23], antioxidant [24], anticancer [25], analgesic [26], antiasthma mine [27] and antiviral [28] and to be effective in treatment of sexual dysfunction [29]. Similarly, a large number of pyrimidine derivatives are reported to exhibit antimicrobial [30], anticancer [31], antiangiogenic [32], antitumor [33], antiproliferative [34], antihistaminic [35], antiviral and antibacterial activities [36]. This prompted the authors to synthesize a new pyrimidine derivative through the reaction of compound **17** with malonic acid to give the corresponding thioxodihydropyrimidinedione (**19**).

This work is due to incorporation of the indole nucleus, a biologically accepted pharmacophore within pyridazine derivatives and as a continuation of our previous work hoping to prepare some new versatile heterocyclic possessing wide spectrum of biological activity. The newly synthesized pyridazines were screened against different cancer cell lines and showed high reactivity owing to the presence of the indolyl moiety in 4-postion.

EXPERIMENTAL

Melting points were measured using electro thermal digital melting points apparatus and are uncorrected. IR spectra were recorded on NICOLET (iS50 FT-IR) spectrometer. ^{1}H NMR was recorded on a Bruker AS 850 TM NMR and chemical shifts were given with respect to TMS. Mass spectra were

recorded on GC/MS with ionization by electron impact to (70 ev). Microanalysis was conducted using elemental analyzer 106.

Synthesis of N1-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)pyridazin-3-yl)benzene-1,4-diamine (2): Refluxing a mixture of compound **1** (0.01 mol) and *p*-phenylenediamine (0.01 mol) in ethanol (30 mL) for 6 h. The solid obtained after concentration and cooling was crystallized from ethanol to give brown crystals compound **2** of 75 % yield and m.p. 213 °C. Elemental analysis of $C_{32}H_{27}N_5$ (m.w. 481) calcd. (found) %: C, 79.81 (79.79); H, 5.65 (5.66); N, 14.5 (14.7).

Synthesis of N-(4-(chlorodiazenyl)phenyl)-6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)pyridazin-3-amine (3): A mixture of compound **2** (0.01 mol) was dissolved in conc. HCl (3 mL) and stirred into ice. This solution was diazotized with $NaNO_2$ solution (prepared by dissolving 0.05 of $NaNO_2$ in 1 mL H_2O). The addition was completed when a clear diazotized solution was obtained. Elemental analysis of $C_{32}H_{25}N_6Cl$ (m.w. 528) calcd. (found) %: C, 72.65 (72.64); H, 4.76 (4.75); N, 15.89 (15.90); Cl, 6.70 (6.72).

Synthesis of 3-(2-(4-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)pyridazin-3-ylamino)phenyl)hydrazono)-pentane-2,4-dione (4), ethyl 2-cyano-2-(2-(4-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)pyridazin-3-ylamino)-phenyl)hydrazono)acetate (5) and ethyl 2-(2-(4-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)pyridazin-3-ylamino)phenyl)hydrazono)-3-oxobutanoate (6): Added (0.01 mol) of acetyl acetone, ethyl cyanoacetate and/or ethyl aceto-acetate to a solution of compound **2** (0.01 mol) in ethanol (50 mL) potion wise and the reaction mixture stirred in ice for 2 h. The solid product obtained was filtered off and crystallized from ethanol to give compounds **4-6** respectively as light brown, yellowish and orange crystals in 65, 78 and 88 % yields and m.p. 228, 239 and 187 °C respectively. Elemental analysis of $C_{37}H_{32}N_6O_2$ (**4**) (m.w. 592) calcd. (found) %: C, 74.98 (74.92); H, 5.44 (5.52); N, 4.18 (14.22). Compound **5** $C_{37}H_{31}N_7O_2$ (m.w. 605) calcd. (found) %: C, 73.37 (73.40); H, 5.16 (5.14); N, 16.19 (16.18); O, 5.28 (5.27). Compound **6** $C_{38}H_{34}N_6O_3$ (m.w. 622) calcd. (found) %: C, 73.29 (73.25); H, 5.50 (5.52); N, 13.50 (13.52).

Synthesis of N-(4-((3,5-dimethyl-1*H*-pyrazol-4-yl)-diazenyl)phenyl)-6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)pyridazin-3-amine (7), 3-amino-4-((4-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)pyridazin-3-ylamino)phenyl)diazenyl)-1*H*-pyrazol-5(4*H*)-one (8), 4-((4-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)-pyridazin-3-ylamino)phenyl)diazenyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one (9): A mixture of corresponding compound **4-6** (0.01 mol) and hydrazine hydrate (0.01 mol) in ethanol (30 mL) was refluxed for 6 h. The solid obtained was crystalized after concentration and cooling from ethanol to give compounds **7-9** as yellow, orange and yellowish crystals of 68, 71 and 83 % yield and m.p. were 275, 255 and 218 °C. Elemental analysis of compound **7** $C_{37}H_{32}N_8$ (m.w. 588) calcd. (found) %: C, 75.49 (75.40); H, 5.48 (5.50); N, 19.03 (19.08). Compound **8** $C_{35}H_{29}N_9O$ (m.w. 591) (%) calcd. (found) %: C, 71.05 (71.08); H, 4.94 (4.92); N, 21.31 (21.34). Compound **9** $C_{36}H_{30}N_8O$ (m.w. 590) calcd. (found) %: C, 73.20 (73.23); H, 5.12 (5.11); N, 18.97 (18.96); O, 2.71 (2.70).

Synthesis of N-(4-((2-amino-4,6-dimethylpyrimidin-5-yl)diazenyl)phenyl)-6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)pyridazin-3-amine (10): A mixture of compound **4** (0.01 mol) and guanidine HCl (0.01 mol) in ethanol (30 mL) was refluxed for 6 h. After cooling the separated solid is crystallized from ethanol to give compound **10** as yellow crystals of 78 % yield and m.p. 285 °C. Elemental analysis of $C_{38}H_{33}N_9$ (**10**) (m.w. 615) calcd. (found) %: C, 74.12 (74.10); H, 5.40 (5.41); N, 20.47 (20.48).

Synthesis of 3-(4-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)pyridazin-3-ylamino)phenylamino)propane-nitrile (11): Refluxed (0.01 mol) of compound **2**, with (0.02 mol) of acrylonitrile in pyridine (20 mL) for 6 h. Then pour into mixture of ice –HCl. Then filter and wash well with water and crystallized from ethanol to give compound **11** as brown crystals of 60 % yield and m.p. 243 °C. Elemental analysis of $C_{35}H_{30}N_6$ (**11**) (m.w. 534) calcd. (found) %: C, 78.63 (78.60); H, 5.66 (5.67); N, 15.72 (15.74).

Synthesis N1-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)pyridazin-3-yl)-N3-(3-phenylallylidene)benzene-1,3-diamine (12a), N1-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)pyridazin-3-yl)-N3-(2,4,6-trimethoxybenzylidene)benzene-1,3-diamine (12b): Stirred 0.01 mol of compound **2** and 0.01 mol of cinnamaldehyde or 2,4,6-trimethoxybenzaldehyde (0.05 mol) in sodium ethoxide (prepared by dissolving (0.02 mol) of sodium metal in 20 mL of absolute ethanol) for 2 h. Then filtered and recrystallized from ethanol as yellowish powder compounds **12a, 12b** in 58,60 % yield and m.p. 244, 268 °C respectively. Elemental analysis of compound **12a** $C_{41}H_{33}N_5$ (m.w. 595) calcd. (found) %: C, 82.66 (82.65); H, 5.58 (5.60); N, 11.76 (11.75). Compound **12b** $C_{42}H_{37}N_5O_3$ (m.w. 659) calcd. (found) %: C, 76.46 (76.50); H, 5.65 (5.63); N, 10.61 (10.60); O, 7.27 (7.26).

Synthesis of 1-(4-(4-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)pyridazin-3-ylamino)phenylamino)-phenyl)ethanone (13): Refluxed in ethanol (50 mL) (0.01 mol) of compound **2** and *p*-chloroacetophenone (0.01 mol) for 6 h. Then crystallized after cooling to obtain solid compound **13** from ethanol as yellow crystals in 65 % yield and m.p. 239 °C. Elemental analysis of compound **13** $C_{40}H_{33}N_5O$ (m.w. 599) calcd. (found) %: C, 80.11 (80.09); H, 5.55 (5.57); N, 11.68 (11.70).

Synthesis of 1-(4-(4-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)pyridazin-3-ylamino)phenylamino)-phenyl)-3-(4-nitrophenyl)prop-2-en-1-one (14): Stir for 2 h a mixture of compound **13** (0.01 mol) and *p*-nitro benzaldehyde (0.01 mol) in absolute ethyl alcohol (30 mL) and 10 % NaOH. The obtained precipitate filtered off and recrystallized from ethanol as yellow crystals compound **14** in 48 % yield and m.p. 260 °C. Elemental analysis of compound **14** $C_{47}H_{36}N_6O_3$ (m.w. 732) calcd. (found) %: C, 77.03 (77.01); H, 4.95 (4.96); N, 11.47 (11.48).

Synthesis of 4-(4-(4-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)pyridazin-3-ylamino)phenylamino)-phenyl)-6-(4-nitrophenyl)-5,6-dihydropyrimidin-2(1*H*)-one (15): Refluxed compound **14** (0.01 mol) in ethanol (50 mL) and urea (0.01 mol) for 6 h. The solid crystallized ethanol to give compound **15** as yellow crystals in 70 % yield and m.p.

283 °C. Elemental analysis of compound **15** $C_{48}H_{38}N_8O_3$ (m.w. 774) calcd. (found) %: C, 74.40 (74.45); H, 4.94 (4.92); N, 14.46 (14.49).

Synthesis of N1-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)pyridazin-3-yl)-N4-(4-(5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazol-4-yl)phenyl)benzene-1,4-diamine (16): Refluxed compound **14** (0.01 mol) in ethanol (50 mL) with hydrazine hydrate (0.01 mol) for 6 h. Crystal the solid obtained ethanol to give compound **16** as yellow crystals in 78 % yield and m.p. 298 °C. Elemental analysis of compound **16** $C_{47}H_{38}N_8O_2$ (m.w. 746) calcd. (found) %: C, 75.58 (75.52); H, 5.13 (5.15); N, 15.00 (15.02); O, 4.28 (4.30).

Reaction of 7c with phenyl isothiocyanate: Formation of 1-(4-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)pyridazin-3-ylamino)phenyl)-3-phenylthiourea (17): Refluxed a mixture of compound **2** (0.01 mol) and phenyl isothio-cyanate (0.013 mol) in pyridine (20 mL) for 6 h, then poured into ice-HCl mixture. The filtered solid washed well with water and recrystallized from ethanol to give compound **17** as yellow crystals in 80 % yield and m.p. 250 °C. Elemental analysis of compound **17** $C_{39}H_{32}N_6S$ (m.w. 616) calcd. (found) %: C, 75.95 (75.90); H, 5.23 (5.25); N, 13.63 (13.65); S, 5.20 (5.21).

Reaction of compound 16 with chloroacetic acid: Formation of 3-(4-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)-pyridazin-3-ylamino)phenylimino)-2-phenylisothiazolidin-4-one (18): In dry acetone reflux compound **17** (0.01 mol) and chloroacetic acid (0.03 mol) and anhydrous potassium carbonate for 24 h on water bath, while hot filter off the solvent and evaporate it to obtain solid product, which recrystallized from ethanol to give compound **18** as yellow crystals in 87 % yield and m.p. 289 °C. Elemental analysis of compound **18** $C_{41}H_{32}N_6O_S$ (m.w. 656) calcd. (found) %: C, 74.98 (75.01); H, 4.91 (4.90); N, 12.80 (12.79); O, 2.44 (2.43); S, 4.88 (4.88).

Reaction of compound 10 with malonic acid: Formation of 1-(4-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1*H*-indol-3-yl)pyridazin-3-ylamino)phenyl)-3-phenyl-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (19): Reflux on water bath a mixture of **17** (0.01 mol) and malonic acid (0.01 mol) for 2 h then pour into ice. The collected product was recrystallized from ethanol and collected as white crystals having 60 % yield and m.p. 278 ºC. Elemental analysis of compound **19** $C_{42}H_{32}N_6O_2S$ (m.w. 684) calcd. (found) %: C, 73.66 (73.60); H, 4.71 (4.73); N, 12.27 (12.29); O, 4.67 (4.68); S, 4.68 (4.69).

Cytotoxic assay: The antitumor activity of all the synthesized compounds has been evaluated against three cell lines HepG-2 cells (human hepatocellular cancer cell line), HCT-116 (colon cancer cell line) and MCF-7 (breast carcinoma). The inhibitory activity was detected by using different concentrations of the tested samples (500, 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, 2 and 1 µg/mL) and cell viability (%) was determined by colorimetric method. Doxorubicin was used as a reference and it is one of the most effective anticancer agents. The relationship between drug concentration and cell viability was plotted to obtain the survival curve of breast cancer cell line MCF-7, HCT-116 colon cancer cell line and hepatocellular carcinoma cell line HePG2. IC_{50} values were determined as the drug and sample concentrations at 50 % inhibition of cell growth.

RESULTS AND DISCUSSION

The new derivatives were prepared by the following the reaction sequences depicted in **Schemes I** and **II**. The starting material pyridazine (**2**) was synthesized *via* the reaction of 3-(3-chloro-6-(3,4-dimethylphenyl)pyridazin-4-yl)-2-phenyl-1*H*-indole. The IR spectrum of compound **2** showed absorption bands at 1601 cm^{-1} and 3442/3345 cm^{-1} which is attributed due to ν(C=N) and ν(NH_2). The mass spectrum showed the molecular ion peak at *m/z* 480 (15.3 %) and the ^{1}H NMR (DMSO-d_6) spectrum showed signals δ ppm at 11.32 (s, 1H, NH), 6.34-7.78 (m, 17H, Ar-H), 5.20 (s, 2H, NH_2) and 1.13 (s, 6H, 2×CH_3).

Novel hydrazons derivatives (**4-6**) have been obtained *via* the diazonium cation (**3**) resulting from the interaction of nitrite with compound **2** followed by coupling with active methylene compounds namely acetylacetone, ethylcyanoacetate and/or ethylacetoacetate. The IR spectrum of compound **4** showed absorption bands at1640, 1564 and 3434 cm^{-1} due to ν(C=O), ν(C=N) and ν(NH) respectively. The mass spectrum of compound **2** showed the molecular ion peak at *m/z* 593 (9.3 %). The ^{1}H NMR (DMSO-d_6) spectrum showed signals δ ppm at 11.67 (s, 1H, NHN) and 11.38 (s, 1H, NH), 6.90-7.98 (m, 17H, Ar-H), 2.34 (S, 6H, 2×CH_3-CO) and 1.13 (s, 6H, 2×CH_3).

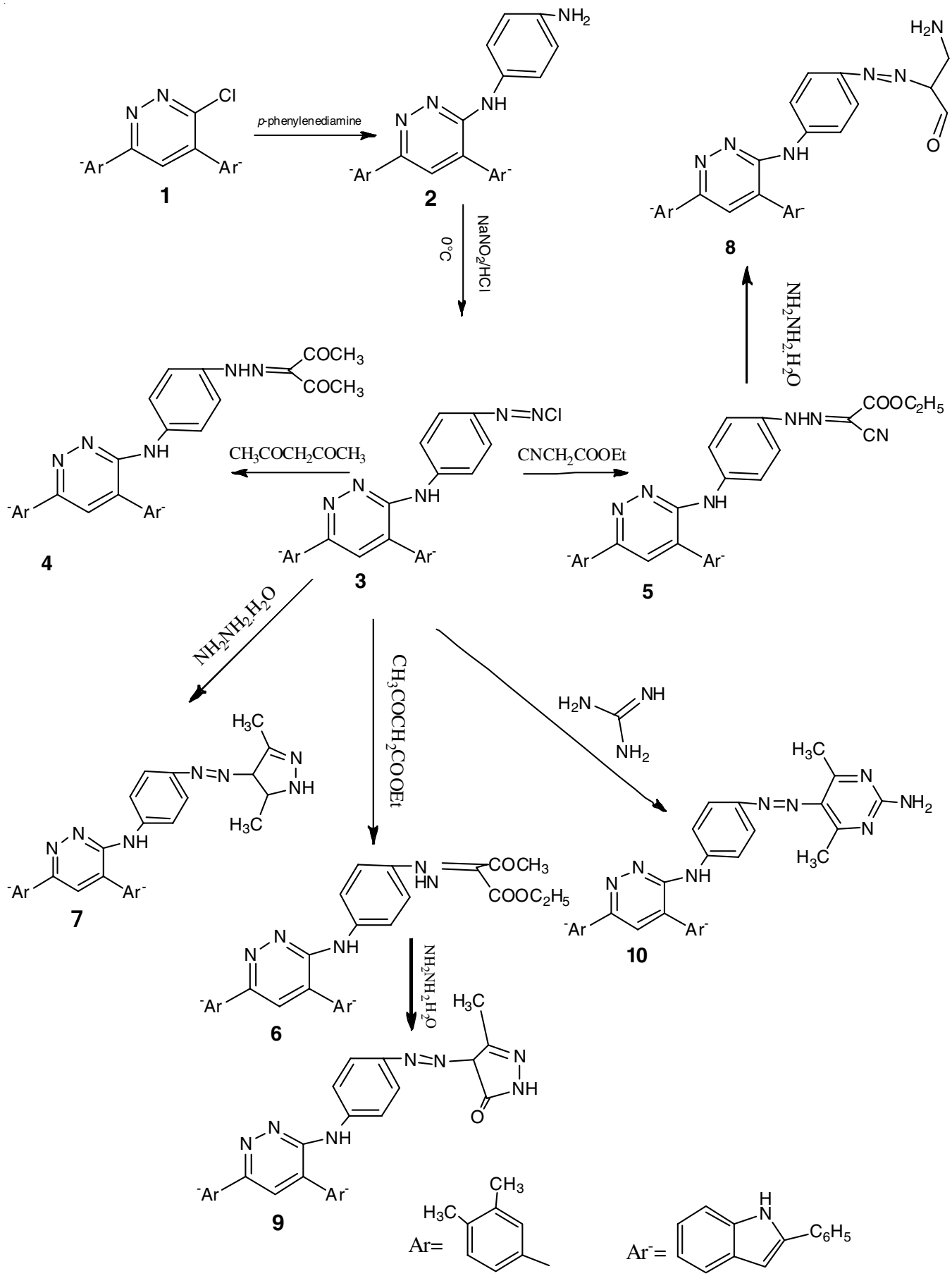
The IR spectrum of compound **5** showed absorption bands at 1745, 1605, 2320 and 3434 cm^{-1} due to ν(C=O), ν(C=N), ν(CN) and ν(NH) respectively. The mass spectrum showed the molecular ion peak at *m/z* 605 (3.5 %). The ^{1}H NMR (DMSO-d_6) spectrum showed signals δ ppm at11.32 (s, 1H, NHN) and 11.14 (s, 1H, NH) 7.02-7.99 (m, 17H, Ar-H, 2.45 (q, 2H, CH_2CH_3), 2.65 (t, 3H, CH_2CH_3), 1.23 (s, 6H, 2×CH_3). The IR of compound **6** showed absorption bands at 1725, 1710, 1570 and 3349 cm^{-1} due to ν(C=O) (ester), ν(C=O), ν(C=N) and ν(NH). The mass spectrum showed the molecular ion peak at *m/z* 622 (20.45 %). The ^{1}H NMR (DMSO-d_6) spectrum showed signals δ ppm at 11.52 (s, 1H, NHN) and 11.22 (s, 1H, NH), 6.99-7.75 (m, 17H, Ar-H), 2.68 (t, 3H, CH_2CH_3), 2.54 (q, 2H, CH_2CH_3), 2.35 (s, 3H, CH_3-CO) and 1.23 (s, 6H, 2×CH_3).

Structure of compounds **4-6** was further established by their reactions with hydrazine hydrate to afford the corresponding pyrazole derivatives (**7-9**), respectively.

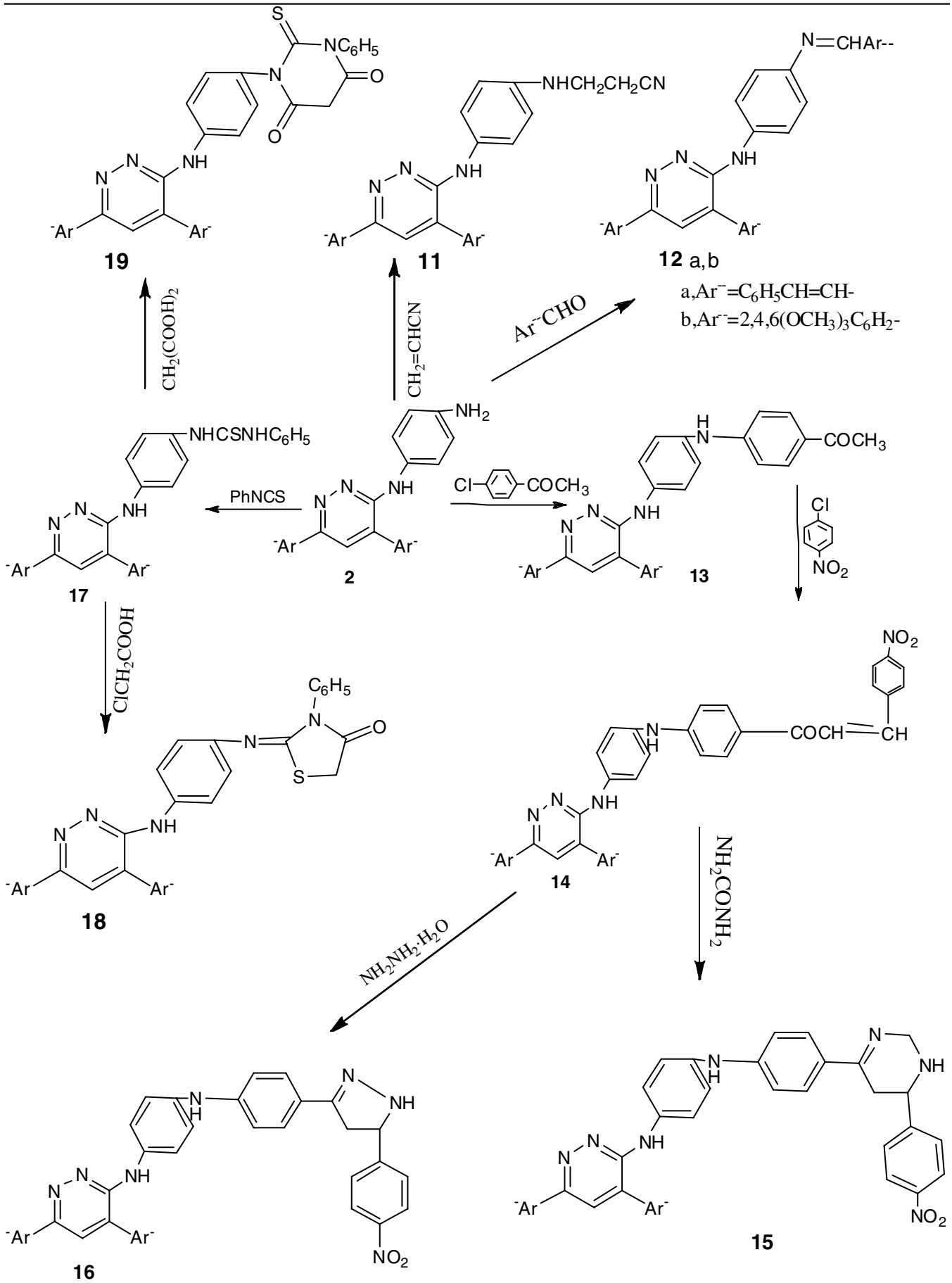
The IR spectrum of compound **7** was devoid of ν(C=O) and showed absorption bands at 1628 cm^{-1}, 3434 cm^{-1} due to ν(C=N) and ν(NH) respectively. The mass spectrum showed the molecular ion peak at *m/z* 590 (8.15 %). The ^{1}H NMR (DMSO-d_6) showed signal bands δ ppm at 11.48 (s, 2H, 2×NH), 6.57-8.17 (m, 17H, Ar-H), 2.34 (s, 12H, 4×CH_3).

The IR spectrum of compound **8** showed absorption bands at 1656, 1616 and 3224 cm^{-1} due to ν(C=O), ν(C=N) and ν(NH) respectively. The mass spectrum showed the molecular ion peak at *m/z* 590 (18.25 %). The ^{1}H NMR (DMSO-d_6) showed signal bands δ ppm at 11.48 (s, 2H, 2×NH), 6.97-7.87 (m, 17H, Ar-H), 5.44 (s, 1H, NH), 3.11(s, 1H, CHN), 1.42 (s, 9H, 3×CH_3).

The IR spectrum of compound **9** showed absorption bands at, 1658, 1564 and 3230 cm^{-1} due to ν(C=O), ν(C=N) and ν(NH) respectively. The mass spectrum showed the molecular ion peak at *m/z* 590 (7.23 %). The ^{1}H NMR (DMSO-d_6) showed signal bands δ ppm at 11.48 (s, 3H, 2×NH), 6.63-7.96 (m,


p-phenylenediamine
0°C
NaNO2/HCl
NH2NH2.H2O
CH3COCH2COCH3
CNCH2COOEt
CH3COCH2COOEt
Ar= Ar-=

Scheme-I


19
11
12 a,b
a,Ar⁻=C6H5CH=CH-
b,Ar⁻=2,4,6(OCH3)3C6H2-
CH2(COOH)2
CH2=CHCN
Ar⁻CHO
17
2
13
PhNCS
ClCH2COOH
18
14
NH2CONH2
NH2NH2.H2O
16
15

Scheme-II

17H, Ar-H), 4.45 (s, 2H, NH_2), 3.01 (s, 1H, CHN), 2.28 (s, 9H, 3×CH_3).

On the other hand, reaction of compound **4** with guanidine HCl results in formation of the amino pyrimidine derivative (**10**) its IR spectrum was devoid of ν(C=O) and showed absorption bands at 1595 and 3334 cm^{-1} due to ν(C=N) and ν(NH). The mass spectrum showed the molecular ion peak at *m/z* 620 (9.02 %). The ^{1}H NMR (DMSO-d_6) spectrum showed signals δ ppm at 11.11 (s, 2H, 2×NH), 7.03-7.98 (m, 17H, Ar-H), 5.14 (s, 2H, NH_2), 2.23 (s, 12H, 4×CH_3). The compound **2** can be used as key intermediate in preparation of some new compounds thus treatment of compound **2** with acrylonitrile in boiling pyridine afforded the adduct (**11**). The IR spectrum of compound **11** showed absorption bands at 1609, 2261 and 3160 cm^{-1} due to ν(C=N), ν(CN) and ν(NH) respectively and devoid of ν(NH_2). The mass spectrum showed the molecular ion peak at *m/z* 543 (2.45 %). The ^{1}H NMR (DMSO-d_6) showed signal bands δ ppm at 11.5 (s, 2H, 2×NH), 6.91-8.01 (m, 17H, Ar-H), 4.84 (S, 1H, NH), 3.45 (t, 2H, $NHCH_2$), 3.31 (t, 2H, CH_2CN), 2.34 (S, 6H, 2×CH_3).

Condensation of compound **2** with aromatic aldehydes, namely cinnamaldehyde and/or 2,4,6-trimethoxybenzaldehyde in ethanol in presence of sodium ethoxide afforded compounds **12a** and **12b**. The IR spectrum of compound **12a** showed absorption bands at1591 and 3396 cm^{-1} due to ν(C=N) and ν(NH). The mass spectrum showed the molecular ion peak at *m/z* 600 (13.32 %). The ^{1}H NMR (DMSO-d_6) showed signal bands δ ppm at 11.45 (s, 2H, 2×NH), 6.42-8.50 (m, 25H, Ar-H), 2.08 (s, 6H, 2×CH_3)

While that for compound **12b** showed absorption bands at 1599 and 3323 cm^{-1} due to ν(C=N) and ν(NH). The mass spectrum showed the molecular ion peak at *m/z* 660 (52.12 %). The ^{1}H NMR (DMSO-d_6) showed signal bands δ ppm at 11.23 (s, 2H, 2×NH), 6.42-8.50 (m, 21H, Ar-H), 2.34 (s, 6H, 2×OCH_3), 2.18 (s, 6H, 2×CH_3).

However, reaction of compound **2** with *p*-chloroacetophenone gave compound **13**, which on condensation with *p*-nitrobenzaldehyde gave the α-enone (**14**). The IR spectrum of compound **13** showed absorption bands at1671, 1588 and 3248 cm^{-1} due to ν(C=O), ν(C=N) and ν(NH) respectively. The mass spectrum showed the molecular ion peak at *m/z* 600 (33.06 %). The^{1}H NMR (DMSO-d_6) showed signal bands δ ppm at 11.45 (s, 2H, 2×NH), 6.42-8.50 (m, 21H, Ar-H), 5.06 (s, 1H, NH), 3.02 (s, 3H, OCH_3), 2.08 (s, 6H, 2×CH_3).

The IR spectrum of compound **14** showed absorption bands at1650, 1580 and 3230 cm^{-1} due to ν(C=O), ν(C=N) and ν(NH) respectively. The mass spectrum showed the molecular ion peak at *m/z* 732 (43.12 %). The ^{1}H NMR (DMSO-d_6) showed signal bands δ ppm at 11.32 (s, 2H, 2×NH), 7.10-8.05 (m, 27H, Ar-H), 5.21 (s, 1H, NH), 1.11 (s, 6H, 2×CH_3). The olefinic double bond of compound **14** is activated by the ketone groups there for the β-carbon atom will accept nucleophils. Thus, reaction of compound **14** with urea and hydrazine hydrate afforded pyrimidinyl (**15**) and pyrazolyl (**16**), derivatives, which can be visualized on the bases of cyclocondensation of urea and hydrazine hydrate with enone (**14**).

The IR spectrum of compound **15** showed absorption bands at 1671, 1587 and 3430 cm^{-1} due to ν(C=O), ν(C=N) and ν(NH) respectively. The mass spectrum showed the molecular ion peak at *m/z* 774 (1.14 %). The ^{1}H NMR (DMSO-d_6) showed signal bands δ ppm at 11.64 (s, 2H, 2×NH), 7.10-8.05 (m, 26H, Ar-H), 5.42 (s, 2H, 2×NH), 2.16 (s, 6H, 2×CH_3), 1.23 (s, 2H, CH_2).

The IR spectrum of compound **16** was devoid of ν(C=O) and showed absorption bands at 1583 and 3494 cm^{-1} due to ν(C=N) and ν(NH) respectively. The mass spectrum showed the molecular ion peak at *m/z* 746 (10.10 %). The ^{1}H NMR (DMSO-d_6) showed signal bands δ ppm at 11.81 (s, 2H, 2×NH), 7.10-8.05 (m, 26H, Ar-H), 4.89 (s, 2H, 2×NH), 2.26 (s, 2H, CH_2), 1.18 (s, 6H, 2×CH_3).

Treatment of compound **2** with phenyl isothiocyanate afforded the corresponding adduct pyridazinyl-3-phenylthiourea derivative (**17**). Its IR spectrum showed absorption bands at1594, 1260 and 3209 cm^{-1} due to ν(C=N), ν(C=S) and ν(NH) respectively. The mass spectrum showed the molecular ion peak at *m/z* 619 (17.21 %). The ^{1}H NMR (DMSO-d_6) showed signal bands δ ppm at 11.61 (s, 1H, NH), 7.22-8.14 (m, 21H, Ar-H), 5.12 (s, 1H, NH), 4.89 (s, 2H, 2×NH), 2.13 (s, 6H, 2×CH_3).

Treatment of compound **17** with chloroacetic acid in dry acetone and anhydrous potassium carbonate afforded the corresponding oxothiazolidin derivative (**18**). Its IR spectrums showed absorption bands at 1680, 1594, 1441 and 3211 cm^{-1} due to ν(C=O), ν(C=N), ν(C-S-C) and ν(NH). The mass spectrum showed the molecular ion peak at *m/z* 656 (20.34 %). The ^{1}H NMR (DMSO-d_6) showed signal bands δ ppm at 11.58 (s, 1H, NH), 7.01-7.99 (m, 22H, Ar-H), 4.99 (s, 1H, NH), 3.24 (s, 2H, CH_2), 2.32 (s, 6H, 2×CH_3).

Its IR spectrum showed absorption bands at 1630, 1594, 1267 and 33378 cm^{-1} due to ν(C=O), ν(C=N), ν(C=S) and ν(NH) respectively. The mass spectrum showed the molecular ion peak at *m/z* 685 (12.56 %). The ^{1}H NMR (DMSO-d_6) showed signal bands δ ppm at 11.71 (s, 1H, NH), 7.12-8.24 (m, 22H, Ar-H), 5.08 (s, 1H, NH), 3.35 (s, 2H, CH_2), 2.15 (s, 6H, 2×CH_3).

TABLE-1
CYTOTOXIC ACTIVITY OF 3(2*H*)-PYRIDAZINE DERIVATIVES

Conc. (μg/mL)	MCF7				HEPG2				HCT116			
	2	**4**	**8**	**11**	**5**	**6**	**13a**	**16**	**7**	**10**	**17**	**19**
0	100	100	100	100	100	100	100	100	100	100	100	100
1.56	74.23	67.91	100	100	100	98.65	98.57	90.82	80.98	76.72	90.64	92.23
3.125	37.86	34.63	98.16	98.48	96.42	93.17	93.42	74.56	63.77	57.18	72.58	84.71
6.25	26.41	25.94	92.54	94.77	89.14	84.86	82.14	58.25	41.12	28.02	56.79	69.68
12.5	13.37	16.86	80.43	86.35	79.68	77.33	65.93	39.67	17.93	17.25	28.89	26.14
25	9.62	10.86	58.04	78.42	68.47	61.03	36.84	23.18	10.26	9.72	12.94	14.27
50	4.35	6.14	23.67	60.56	16.19	26.46	8.15	10.86	5.88	5.43	7.18	8.36

TABLE-2
IC_{50} OF SOME PYRIDAZINE DERIVATIVES

Cell lines	IC_{50} (µg/mL)												
	Doxrubcin	**2**	**4**	**5**	**6**	**7**	**8**	**10**	**11**	**13a**	**16**	**17**	**19**
Hep G-2	0.426	–	–	3.1	29	–	–	–	–	1.5	4	–	–
HCT-116	0.469	–	–	–	–	5	–	2.1	–	–	–	7.1	8.3
MCF-7	0.468	19	2.2	–	–	–	2.5	-	50	–	–	–	–

Cytotoxic activity: An examination of data revealed that most of the compounds showed good to moderate activity (Table-1). Compounds **5**, **13a** has the best activity against HePG2 cell line (IC_{50} = 3.1,2.1 µg/mL), compounds **4**, **8** have the best activity against MCF-7 cell line (IC_{50} = 2.2,2.5 µg/mL), compound **10** has the best activity, against HCT-116 cell line (IC_{50} = 2.1 µg/mL) (Table-2). Structure and biological activity relationship showed that the activity of compound **4** against breast cancer due to a high lipophilicity of dicarbonyl moiety which enhanced its absorption to the cancer cells. Similarly, the relative potency of compound **10** against HCT with the IC_{50} of 2.1 µg/mL may also be related to the presence of N=N group with its hydrophobic nature. Also, the activity of compound **5** against liver cancer due to the hydrophobic of sterically hindered NH moiety of pyridazine ring that enhances the penetration of compound **5** to the cancer cell (Table-2).

Conclusion

Conversion of amino pyridazine incorporating the indolyl moiety into various pyrazolo pyridazines derivatives *via* diazotization followed by coupling with active methylene results in formation of new heterocyclic compounds containing pyrazolone, isoxazole and pyrmidine derivatives all these derivatives had been proved chemically and by several spectral data and showed significant activity toward various human cell lines. Also pyridazine was converted into isoxazols and thioxodihydropyrimidinedione with high activity.

CONFLICT OF INTEREST

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

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