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In vitro Cytotoxic Evaluation of Some New Synthesized Pyridazine Derivatives

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ABSTRACT

A series of novel pyridazine, pyrazoles, pyrimidines derivatives have been synthesized through the reaction of Chloropyridazine1 with p-phenylenediamine to give Diazotization of 2 followed by coupling with active methylene compounds namely acetylacetone, ethylcyanoacetate and/or ethylacetoacetate afforded novel hydrazons derivatives of 4-6. The resulting hydrazons can have been cyclized using hydrazine hydrate and and guanidine gave the corresponding pyrazoles 7-9 and pyrimidine 10 derivatives. Reaction of 2 with acrylonitrile, aromatic aldehyde, p-chloroacetophenone and phenylisothiocyanate 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 tumor liver cancer cell line (HEPG2), human colon cancer cell line (HCT). Compounds 8 and 4 showed best activity against MCF-7 cell line, compounds 13a, 5 showed best activities against HePG2 cell line and compound 10 showed best activity against HCT cell lines.

Key words: Pyridazine, Pyrazoles, Pyrimidines, Hydrazones, Anti-cancer, Antitumor activity

INTRODUCTION

It is well known that pyridazine derivatives one of the most biologically active diazines heterocyclic compounds that shows high activity as inhibitors of protein tyrosine phosphatase 1bptp1b [1], anti-fungal [2], antimicrobial [3,4], anti-tuberculosis [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-phenylindole is a very 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 anti-cancer [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]. This promoted the author to incorporate 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 new synthesized pyridazines were screened against different cancer cell lines and showed high reactivity owing to the presence of the indolyl moiety in 4-postion.

MATERIAL AND METHODS

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-1H-indol-3-yl)pyridazin-3-yl)benzene-1, 4-diamine (2)

Refluxing a mixture of 1 (0.01 mol) and p-phenylenediamine (0.01 mol) in ethanol (30ml) for 6 hours. The solid obtained after concentration and cooling was crystallized from ethanol to give brown crystals **2** of 75% yield and m.p. 213˚C. Analysis of 2 $C_{32}H_{27}N_5$ (481) (%) calcd: C, 79.81; H, 5.65; N, 14.5. Found:, 79.79; H, 5.66; N, 14.7.

***Synthesis of N- (4- (chlorodiazenyl) phenyl)-6- (3, 4-dimethylphenyl)-4- (2-phenyl-1H-indol-3-yl)pyridazin-3-amine (*3)**

A mixture of **2** (0.01 mol) was dissolved in conc HCl (3ml) and stirred into ice this solution was diazotized with NaNO2 solution (prepared by dissolving 0.05 of$NaNO_2$ in 1ml H_2O). The addition was completed when a clear diazotized solution was obtained. Analysis of **3** $C_{32}H_{25}ClN_6$ (528) (%) calcd: C, 72.65; H, 4.76; Cl, 6.70; N, 15.89

Synthesis of 3- (2- (4- (6- (3, 4-dimethylphenyl)-4- (2-phenyl-1H-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-1H-indol-3-yl)pyridazin-3-ylamino)phenyl)hydrazono)acetate (5)andethyl 2- (2- (4- (6- (3, 4-dimethylphenyl)-4- (2-phenyl-1H-indol-3-yl)pyridazin-3-ylamino)phenyl)hydrazono)-3-oxobutanoate (6)

Add (0.01 mol) of acetyl acetone, ethyl cyanoacetate and/or ethyl acetoacetate to a solution of **2** (0.01 mol) in ethanol (50 ml) potion wise and the reaction mixture stirred in ice for 2 hr. The solid product obtained was filtered off and it was crystallized from ethanol to give **4-6**respectivelyas light brown, yellowish and orange crystals in 65, 78 and 88% yields and m.p. 228, 239 and 187˚C respectively. Analysis of **4**$C_{37}H_{32}N_6O_2$ (592) (%) calcd: C, 74.98; H, 5.44; N, 14.18 Found:C, 74.92; H, 5.52; N, 14.22.

Analysis of **5** $C_{37}H_{31}N_7O_2$ (605) (%) calcd:C, 73.37; H, 5.16; N, 16.19; O, 5.28. Found:C, 73.40; H, 5.14; N, 16.18; O, 5.27
Analysis of **6** $C_{38}H_{34}N_6O_3$ (622) (%) calcd: C, 73.29; H, 5.50; N, 13.50. Found: C, 73.25; H, 5.52; N, 13.52

Synthesis of N- (4- (3, 5-dimethyl-1H-pyrazol-4-yl)diazenyl)phenyl)-6- (3, 4-dimethylphenyl)-4- (2-phenyl-1H-indol-3-yl)pyridazin-3-amine (7), 3-amino-4- (4- (6- (3, 4-dimethylphenyl)-4- (2-phenyl-1H-indol-3-yl)pyridazin-3-ylamino)phenyl)diazenyl)-1H-pyrazol-5 (4H)-one (8), 4- (4- (6- (3, 4-dimethylphenyl)-4- (2-phenyl-1H-indol-3-yl)pyridazin-3-ylamino)phenyl)diazenyl)-3-methyl-1H-pyrazol-5 (4H)-one (9)

A mixture of **4**-**6** (0.01 mol) and hydrazine hydrate (0.01 mol) in ethanol (30 ml)was refluxed for 6 hrs. The solid obtained was crystalized after concentration and cooling from ethanol to give **7**-**9** as yellow, orange and yellowish crystals of 68, 71and 83% yield and m.p. were 275, 255 and 218˚C. Analysis of **7** $C_{37}H_{32}N_8$ (588) (%) calcd: C, 75.49; H, 5.48; N, 19.03. Found: C, 75.40; H, 5.50; N, 19.08 Analysis of **8** $C_{35}H_{29}N_9O$ (591) (%) calcd: C, 71.05; H, 4.94; N, 21.31. Found: C, 71.08; H, 4.92; N, 21.34. Analysis of **9** $C_{36}H_{30}N_8O$ (590) (%) calcd: C, 73.20; H, 5.12; N, 18.97; O, 2.71. Found:C, 73.23; H, 5.11; N, 18.96; O, 2.70

Synthesis of N- (4- (2-amino-4, 6-dimethylpyrimidin-5-yl)diazenyl)phenyl)-6- (3, 4-dimethylphenyl)-4- (2-phenyl-1H-indol-3-yl)pyridazin-3-amine (10)

A mixture of **4** (0.01 mol) and guanidine HCl (0.01 mol) in ethanol (30 ml) was refluxed for 6 hrs. After cooling the separated solid is crystalized from ethanol to give **10** as yellow crystals of 78% yield and m.p. 285˚C. Analysis of **10** $C_{38}H_{33}N_9$ (615) (%) calcd: C, 74.12; H, 5.40; N, 20.47. Found:C, 74.10; H, 5.41; N, 20.48

Synthesis of 3- (4- (6- (3, 4-dimethylphenyl)-4- (2-phenyl-1H-indol-3-yl)pyridazin-3-lamino)phenylamino)propanenitrile (11)

Reflux (0.01 mol) of **2**, with (0.02 mol) of acrylonitrilein pyridine (20 ml) for 6 hours. Then pour into mixture of ice –HCl. Then filter and wash well with water and crystallized from ethanol to give **11** as brown crystals of 60% yield and m.p. 243˚C. Analysis of **11** $C_{35}H_{30}N_6$ (534) (%) calcd: C, 78.63; H, 5.66; N, 15.72 Found: C, 78.60; H, 5.67; N, 15.74

Synthesis N1- (6- (3, 4-dimethylphenyl)-4- (2-phenyl-1H-indol-3-yl)pyridazin-3-yl)-N3- (3-phenylallylidene)benzene-1, 3-diamine (12a), N1- (6- (3, 4-dimethylphenyl)-4- (2-phenyl-1H-indol-3-yl)pyridazin-3-yl)-N3- (2, 4, 6-trimethoxybenzylidene)benzene-1, 3-diamine (12b)

Stir **(**0.01 mol) of **2** and (0.01 mol) of cinnamaldehyde or 2, 4, 6trimethoxybenzaldehyde in (0.05 mol) of sodium ethoxide (prepared by dissolving (0.02 mol) of sodium metal in 20 ml of absolute ethanol) for 2 hours. Then filtered and recrystallized from ethanol as yellowish powder **12a, 12b** in 58, 60% yield and m.p. 244, 268˚C respectively. Analysis of **12a** $C_{41}H_{33}N_5$ (595.) (%) calcd: C, 82.66; H, 5.58; N, 11.76 Found:C, 82.65; H, 5.60; N, 11.75 Analysis of **12b** $C_{42}H_{37}N_5O_3$ (659) (%) calcd: C, 76.46; H, 5.65; N, 10.61; O, 7.27 Found: C, 76.50; H, 5.63; N, 10.60; O, 7.26.

Synthesis of 1- (4- (4- (6- (3, 4-dimethylphenyl)-4- (2-phenyl-1H-indol-3-yl)pyridazin-3-lamino)phenylamino)phenyl)ethanone (13)

Reflux in ethanol (50 ml) (0.01 mol) of **2** and p-chloroacetophenone (0.01 mol) for 6 hrs. Then crystalized cooling from the obtained solid feom ethanol to give **13** as yellow crystals in 65% yield and m.p. 239˚C. Analysis of **13** $C_{40}H_{33}N_5O$ (599) (%) calcd: C, 80.11; H, 5.55; N, 11.68. Found:C, 80.09; H, 5.57; N, 11.70.

Synthesis of 1- (4- (4- (6- (3, 4-dimethylphenyl)-4- (2-phenyl-1H-indol-3-yl)pyridazin-3-ylamino)phenylamino)phenyl)-3- (4-nitrophenyl)prop-2-en-1-one (14)

Stir for 2 hrs a mixture of **13** (0.01 mol) and p-nitro benzaldehyde (0.01 mol) in in absolute ethyl alcohol (30 ml) and 10% NaOH. The obtained precipitate filtered off and recrystallized from ethanol as yellow crystals **14** in 48% yield and m.p. 260˚C. Analysis of **14** $C_{47}H_{36}N_6O_3$ (732) (%) calcd: C, 77.03; H, 4.95; N, 11.47. Found:C, 77.01; H, 4.96; N, 11.48.

Synthesis of 4- (4- (4- (6- (3, 4-dimethylphenyl)-4- (2-phenyl-1H-indol-3-yl)pyridazin-3-ylamino)phenylamino)phenyl)-6- (4-nitrophenyl)-5, 6-dihydropyrimidin-2 (1H)-one (15)

Reflux **14** (0.01 mol) in ethanol (50 ml) and urea (0.01 mol) for 6 hours. The solid crystalized ethanol. to give **15** as yellow crystals in 70% yield and m.p. 283˚C. Analysis of **15** $C_{48}H_{38}N_8O_3$ (774) (%) calcd: C, 74.40; H, 4.94; N, 14.46. Found:C, 74.45; H, 4.92; N, 14.49.

Synthesis of N1- (6- (3, 4-dimethylphenyl)-4- (2-phenyl-1H-indol-3-yl)pyridazin-3-yl)-N4- (4- (5- (4-nitrophenyl)-4, 5-dihydro-1H-pyrazol-4-yl)phenyl)benzene-1, 4-diamine (16)

reflux **14** (0.01 mol) in ethanol (50 ml) with hydrazine hydrate (0.01 mol) for 6 hours. Crystal the solid obtained ethanol to give **16** as yellow crystals in 78% yield and m.p. 298˚C. Analysis of **16** $C_{47}H_{38}N_8O_2$ (746) (%) calcd: C, 75.58; H, 5.13; N, 15.00; O, 4.28. Found:C, 75.52; H, 5.15; N, 15.02; O, 4.30.

Reaction of 7c with phenyl isothiocyanate: Formation of 1- (4- (6- (3, 4-dimethylphenyl)-4- (2-phenyl-1H-indol-3-yl)pyridazin-3-ylamino)phenyl)-3-phenylthiourea (17)

Reflux a mixture of **2** (0.01 mol) and phenyl isothiocyanate (0.013 mol) in pyridine (20 ml) for 6 hours, then poured into ice – HCl mixture. The filtered solid washed well with water and recrystallized from ethanol to give **17** as yellow crystals in 80% yield and m.p. 250˚C. Analysis of **17** $C_{39}H_{32}N_6S$ (616) (%) calcd: C, 75.95; H, 5.23; N, 13.63; S, 5.20:. Found: C, 75.90; H, 5.25; N, 13.65; S, 5.21

Reaction of 16 with chloroacetic acid: Formation of 3- (4- (6- (3, 4-dimethylphenyl)-4- (2-phenyl-1H-indol-3-yl)pyridazin-3-ylamino)phenylimino)-2-phenylisothiazolidin-4-one (18)

In dry acetone reflux **17** (0.01 mol) and chloroacetic acid (0.03 mol) and anhydrous potassium carbonate for 24 hours on water bath. while hot filter off the solvent and evaporate it to obtain solid product which recrystallized from ethanol to give **18** as

yellow crystals in 87% yield and m.p. 289˚C. Analysis of **18** $C_{41}H_{32}N_6O_S$ (656) (%) calcd: C, 74.98; H, 4.91; N, 12.80; O, 2.44; S, 4.88. Found: C, 75.01; H, 4.90; N, 12.79; O, 2.43; S, 4.88

Reaction of 10 with malonic acid: Formation of1- (4- (6- (3, 4-dimethylphenyl)-4- (2-phenyl-1H-indol-3-yl)pyridazin-3-ylamino)phenyl)-3-phenyl-2-thioxodihydropyrimidine-4, 6 (1H, 5H)-dione (19)

Reflux on water bath a mixture of **17** (0.01 mol) and malonic acid (0.01 mol) for 2 hours then pour into ice. The collected product was recrystallized from ethanol as white crystals **19** of 60% yield and m.p. 278˚C. Analysis of **19** $C_{42}H_{32}N_6O_2S$ (684) (%) calcd:C, 73.66; H, 4.71; N, 12.27; O, 4.67; S, 4.68. Found:C, 73.60; H, 4.73; N, 12.29; O, 4.68; S, 4.69.

RESULTS AND DISCUSSION

The new derivatives were prepared following the reaction sequences depicted in Scheme **1** and **2.** The starting material pyridazine **2** was synthesized via the reaction of 3- (3-chloro-6- (3, 4-dimethylphenyl)pyridazin-4-yl)-2-phenyl-1H-indole prepared in our previous publication with p-phenylenediamine. The IR spectrum of **2** showed absorption bands at 1601 cm^{-1} and 3442/3345 cm^{-1} attributable to √ C=N and √ NH_2. The mass spectrum showed the molecular ion peak at m/z 480 (15.3%) and the ^{1}H-NMR (DMSO-d6) spectrum showed signals δppm at 11.32 (s, 1H, NH), 6.34-7.78 (m, 17H, Ar-H), 5.20 (s, 2H, NH2) and 1.13 (s, 6H, 2XCH_3). ^{13}C-NMR (DMSO-d6)

Novel hydrazons derivatives 4-6 have been obtanied via the diazoniumcation **3** resuting from the interaction of nitrite with compound **2** followed by coupling with active methylene compounds namely acetyl acetone, ethylcyanoacetate and/or ethylacetoacetate. The IR spectrum of **4** showed absorption bands at 1640 cm^{-1}, 1564 cm^{-1} and 3434 cm^{-1} attributable to √C=O, √ C=N and √ NH. The mass spectrum of compound **2** showed the molecular ion peak at m/z 593 (9.3%). The ^{1}H-NMR (DMSO-d6) spectrum showed signals δppm at 11, 67 (s, 1H, NHN) and 11.38 (s, 1H, NH), 6.90-7.98 (m, 17H, Ar-H), 2.34 (S, 6H, 2XCH3-CO) and 1.13 (s, 6H, 2X CH3).

The IR spectrum of **5** showed absorption bands at1745 cm^{-1}, 1605 cm^{-1}, cm^{-1}, 2320 cm^{-1} and 3434 cm^{-1} attributable to √C=O, √ C=N, √ CN, and √ NH. The mass spectrum showed the molecular ion peak at m/z 605 (3.5%). The ^{1}H-NMR (DMSO-D6) spectrum showed signals δppm 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, CH2CH3), 1.23 (s, 6H, 2XCH_3). The IR of compound **6** showed absorption bands at 1725 cm^{-1}, 1710 cm^{-1}, 1570 cm^{-1}, 3349 cm^{-1} attributable to√C=O (ester), √C=O, √C=Nand √ NH. The mass spectrum showed the molecular ion peak at m/z 622 (20.45%). The 1H-NMR (DMSO-d6) spectrum showed signals δppm 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, 2XCH_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 **7** was devoid of √C=O and showed absorption bands at 1628 cm^{-1}, 3434 cm^{-1} attributable to √ C=N and √ NH and. The mass spectrum showed the molecular ion peak at m/z 590 (8.15%). The ^{1}H-NMR (DMSO-d6) showed signal bands δppm at 11.48 (s, 2H, 2XNH), 6.57-8.17 (m, 17H, Ar-H), 2.34 (s, 12H, 4XCH_3).

The IR spectrum of **8** showed absorption bands at, 1656 cm^{-1}, 1616 cm^{-1}, 3224 cm^{-1} attributable to√C=O, √ C=N and √ NH. The mass spectrum showed the molecular ion peak at m/z 590 (18.25%). The ^{1}H-NMR (DMSO-d6) showed signal bands δppm at 11.48 (s, 2H, 2XNH), 6.97-7.87 (m, 17H, Ar-H), 5.44 (S, 1H, NH), 3.11 (s, 1H, CHN), 1.42 (s, 9H, $3XCH_3$).

The IR spectrum of **9** showed absorption bands at, 1658 cm^{-1}, 1564 cm^{-1} and 3230 cm^{-1} attributable to √C=O, √ C=N and √ NH. The mass spectrum showed the molecular ion peak at m/z 590 (7.23%). The ^{1}H-NMR (DMSO-d6) showed signal bands δppm at 11.48 (s, 3H, 2XNH), 6.63-7.96 (m, 17H, Ar-H), 4.45 (s, 2H, NH_2), 3.01 (s, 1H, CHN), 2.28 (s, 9H, $3XCH_3$).

On the other hand, reaction of **4** with guanidine HCl results in formation of the amino pyrimidine derivative **10,** its IR spectrum was devoid of √C=O and showed absorption bands at showed absorption bands at 1595 cm^{-1} and 3334 cm^{-1} attributable to√ C=Nand √ NH and devoid of C=O. The mass spectrum showed the molecular ion peak at m/z 620 (9.02%). The ^{1}H-NMR (DMSO-d6) spectrum showed signals δppm at 11.11 (s, 2H, 2XNH), 7.03-7.98 (m, 17H, Ar-H), 5.14 (s, 2H, NH_2), 2.23 (s, 12H, $4XCH_3$). The synthesized compound **2** can be used as key intermediate in preparation of some new compounds thus treatment of **2** with acrylonitrile in boiling pyridine afforded the adduct**11**. ItsIR spectrum of **11** showed absorption bands at 1609 cm^{-1}, 2261 cm^{-1}, 3160 cm^{-1} attributable to √ C=N, √ CN and √ NH and devoid of √NH_2 The mass spectrum showed the molecular ion peak at m/z 543 (2.45%). The ^{1}H-NMR (DMSO-d6) showed signal bands δppm at 11.5 (s, 2H, 2XNH), 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, $2XCH_3$)

Condensation of **2** with aromatic aldehydes, namely cinnamaldehyde and/ or 2, 4, 6trimethoxybenzaldehyde in ethanol in presence of sodium ethoxide afforded compounds **12a and 12b**. The IR spectrum of **12a** showed absorption bands at1591 cm^{-1}, and 3396 cm^{-1} attributable to √ C=N and √ NH. The mass spectrum showed the molecular ion peak at m/z 600 (13.32%). The^{1}H-NMR (DMSO-d6) showed signal bands δppm at 11.45 (s, 2H, 2xNH), 6.42-8.50 (m, 25H, Ar-H), 2.08 (s, 6H, 2XCH3)

While that for **12b**showed absorption bands at1599 cm^{-1}, and 3323 cm^{-1} attributable to √ C=N and √ NH. The mass spectrum showed the molecular ion peak at m/z 660 (52.12%). The^{1}H-NMR (DMSO-d6) showed signal bands δppm at 11.23 (s, 2H, 2xNH), 6.42-8.50 (m, 21H, Ar-H), 2.34 (S, 6H, $2XOCH_3$), 2.18 (s, 6H, $2XCH_3$).

However, reaction of **2** with p-chloroacetophenone gave compound **13,** which on condensation with p-nitrobenzaldehyde gave the α- enone **14**. The IR spectrum of **13** showed absorption bands at1671 cm^{-1}, 1588 cm^{-1}, and 3248 cm^{-1} attributable to √C=O, √ C=N and √ NH. The mass spectrum showed the molecular ion peak at m/z 600 (33.06%). The^{1}H-NMR (DMSO-d6) showed signal bands δppm at 11.45 (s, 2H, 2xNH), 6.42-8.50 (m, 21H, Ar-H), 5.06 (S, 1H, NH), 3.02 (s, 3H, OCH_3), 2.08 (s, 6H, $2XCH_3$).

The IR spectrum of **14** showed absorption bands at1650 cm^{-1}, 1580 cm^{-1} and 3230 cm^{-1} attributable to √C=O, √ C=N and √ NH. The mass spectrum showed the molecular ion peak at m/z 732 (43.12%). The^{1}H-NMR (DMSO-d6) showed signal bands δppm at11.32 (s, 2H, 2xNH), 7.10-8.05 (m, 27H, Ar-H), 5.21 (s, 1H, NH), 1.11 (s, 6H, 2XCH3). The olefinic double bond of **14** is activated by the ketone groups there for the β-carbon atom will accept nucleophils. Thus, reaction of **14** with urea and hydrazine hydrate afforded the 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 **15** showed absorption bands at1671 cm^{-1}, 1587 cm^{-1}, and 3430 cm^{-1} attributable to √C=O, √ C=N and √ NH. The mass spectrum showed the molecular ion peak at m/z 774 (1.14%). The ^{1}H-NMR (DMSO-d6) showed signal bands δppm at 11.64 (s, 2H, 2xNH), 7.10-8.05 (m, 26H, Ar-H), 5.42 (S, 2H, 2xNH), 2.16 (s, 6H, 2XCH3), 1.23 (s, 2H, CH2).

The IR spectrumof **16**was devoid of √C=O and showed absorption bands at1583 cm^{-1}, and 3494 cm^{-1} attributable √ C=N and √ NH. The mass spectrum showed the molecular ion peak at m/z 746 (10.10%). The ^{1}H-NMR (DMSO-d6) showed signal bands δppm at 11.81 (s, 2H, 2xNH), 7.10-8.05 (m, 26H, Ar-H), 4.89 (s, 2H, 2xNH), 2.26 (s, 2H, CH2), 1.18 (s, 6H, 2XCH3).

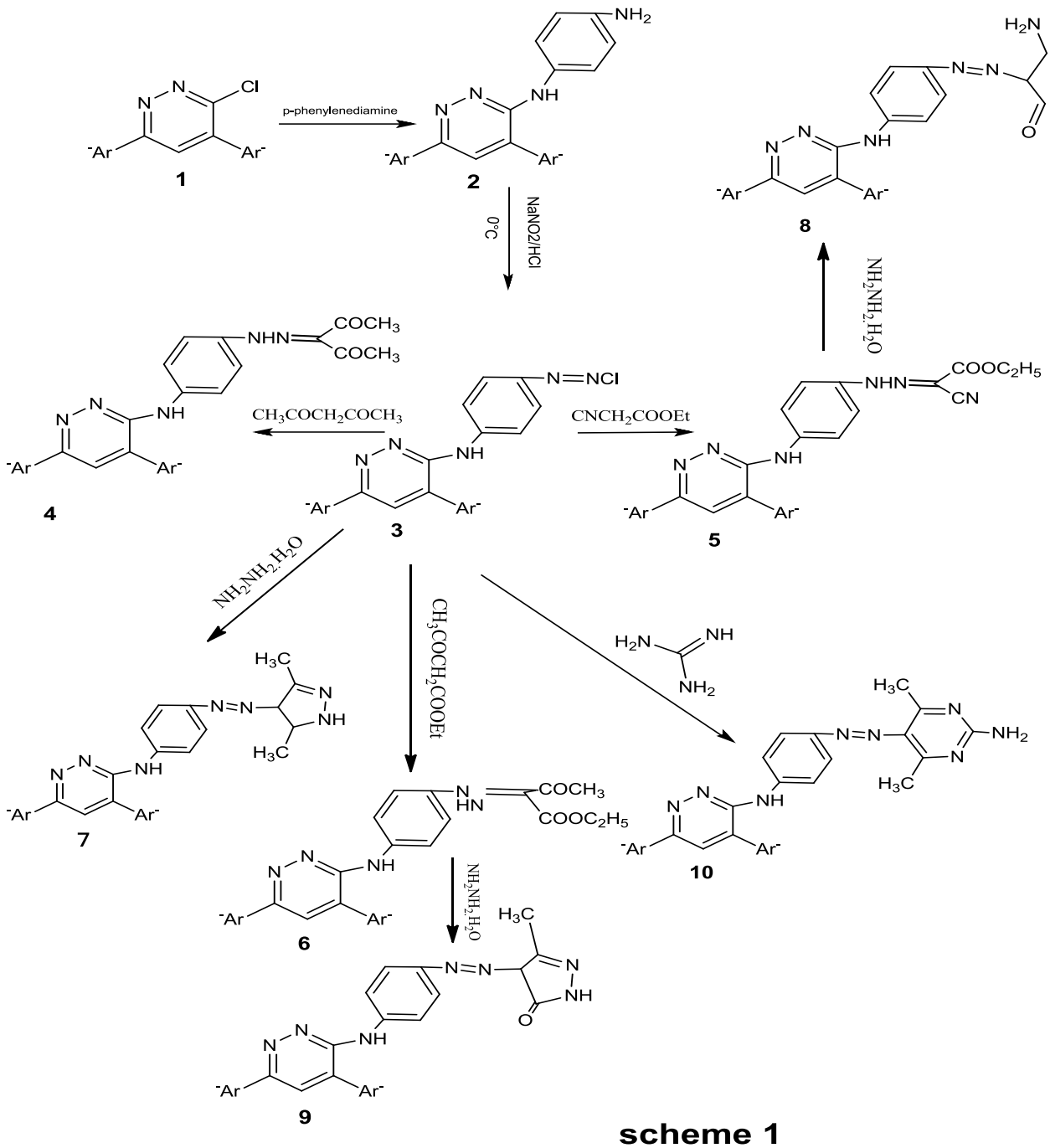
Treatment of **2** with phenylisothiocyanate afforded the corresponding adduct pyridazinyl-3-phenylthiourea derivative **17**. Its IR spectrum showed absorption bands at 1594 cm^{-1}, 1260 cm^{-1} and 3209 cm^{-1} attributable √ C=N, √ C=Sand √ NH. The mass spectrum showed the molecular ion peak at m/z 619 (17.21%). The ^{1}H-NMR (DMSO-d6) showed signal bands δppm at 11.61 (s, 1H, NH), 7.22-8.14 (m, 21H, Ar-H), 5.12 (s, 1H, NH), 4.89 (S, 2H, 2xNH), 2.13 (s, 6H, 2XCH3).

Treatment of **17** with chloroacetic acid in dry acetone and anhydrous potassium carbonate afforded the corresponding oxothiazolidin derivative **18**. Its IR spectrums howed absorption bands at 1680 cm^{-1}, 1594 cm^{-1}, 1441 cm^{-1} and 3211 cm^{-1} attributable √ C=O, √ C=N, √ C-S-C and √ NH. The mass spectrum showed the molecular ion peak at m/z 656 (20.34%). The ^{1}H-NMR (DMSO-D6) showed signal bands δppm 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, 2XCH_3).

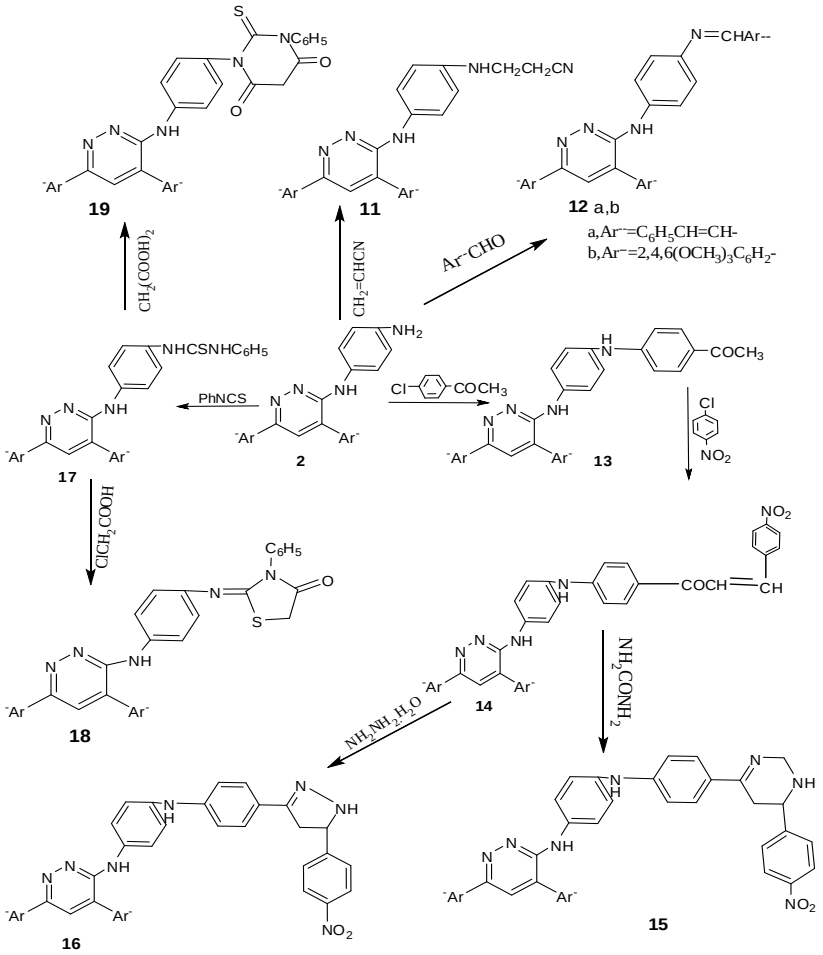
A large number of pyrimidine derivatives are reported to exhibit antimicrobial [30]**,** anti-cancer [31] anti-angiogenic [32], antitumor [33], anti-proliferative [34]**,** anti-histaminic [35], antiviral and antibacterial activities [36]. This prompted the author to synthesize a new pyrimidine derivative through the reaction of **17** with malonic acid to give the corresponding thioxodihydropyrimidinedione-**19**. Its IR spectrum showed absorption bands at1630 cm^{-1}, 1594 cm^{-1}, 1267 cm^{-1} and 33378 cm^{-1} attributable √ C=O, √ C=N, √ C=S and √ NH. The mass spectrum showed the molecular ion peak at m/z 685 (12.56%). The ^{1}H-NMR (DMSO-d6) showed signal bands δppm at 11.71 (s, 1H, NH), 7.12-8.24 (m, 22H, Ar-H),, 5.08 (s, 1H, NH), 3.35 (s, 2H, CH2), 2.15 (s, 6H, 2X CH3).

Cytotoxic assay

The anti-tumor activity of all synthesized compounds has been evaluated against two cell lines HepG-2 cells (human Hepatocellular 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 and hepatocellular carcinoma cell line HePG2 as shown in Tables 1 and 2 and Figure 1. IC50 values were determined as the drug and sample concentrations at 50% inhibition of cell growth (Figure 2).


p-phenylenediamine
NaNO2/HCl
0°C
CH3COCH2COCH3
CNCH2COOEt
NH2NH2.H2O
CH3COCH2COOEt
1
2
3
4
5
6
7
8
9
10
Ar=
Ar=

scheme 1



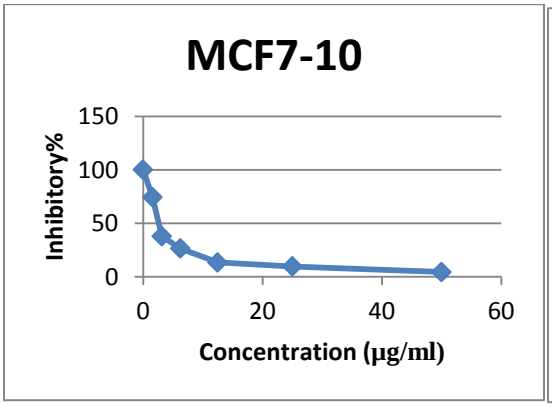
Scheme 2

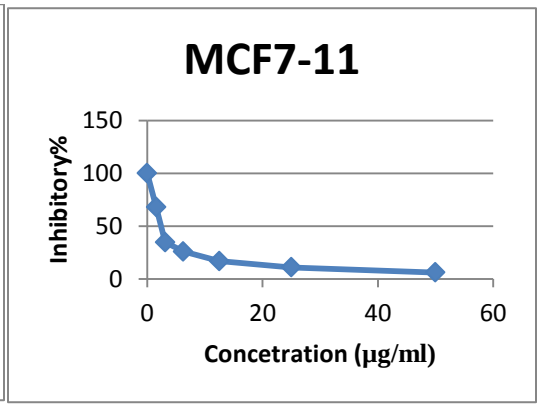
Table 1: Cytotoxic activity of 3 (2H)- pyrdazine derivatives (2, 4, 5, 6, 7, 10, 11, 13a, 16, 17, 19)

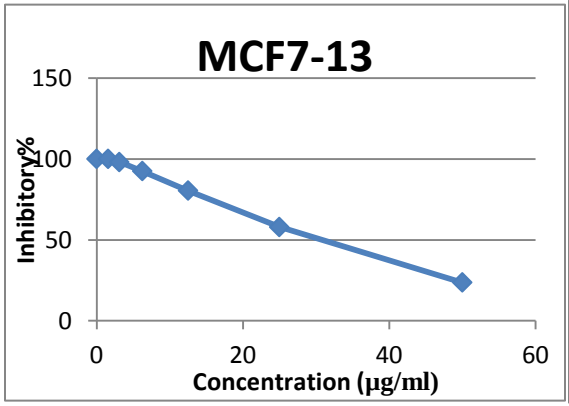
HCT116				**HEPG2**				**MCF7**				**Conc.µg/ml**
19	**17**	**10**	**7**	**16**	**13a**	**6**	**5**	**11**	**8**	**4**	**2**	
100	100	100	100	100	100	100	100	100	100	100	100	**0**
92.23	90.64	76.72	80.98	90.82	98.57	98.65	100	100	100	67.91	74.23	**1.56**
84.71	72.58	57.18	63.77	74.56	93.42	93.17	96.42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

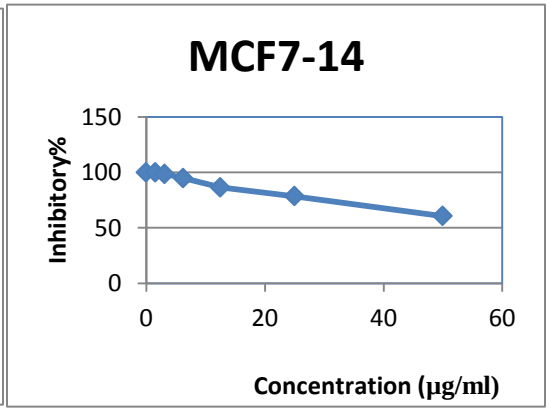
Table 2: Cytotoxic activity of some pyrdazine derivatives (2, 4, 5, 6, 7, 10, 11, 13a, 16, 17, 19)

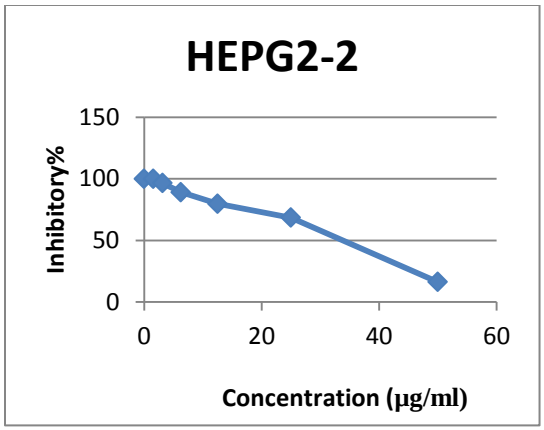
Cell lines	IC50 (µg/mL)												
	Doxrubcin	2	4	5	6	7	8	10	11	13a	16	17	19
Hep G-2	0.426	-	-	3.1	29	-	-	-	-	1.5	4	-	-
HCT-116	0.469	-	-	-	-	5	-	2.1	-	-	-	7.1	8.3
MCF-7	0.468	19	2.2	-	-	-	2.5	-	50	-	-	-	-

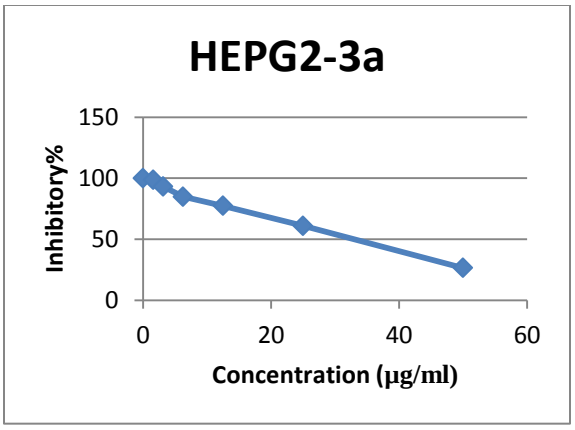


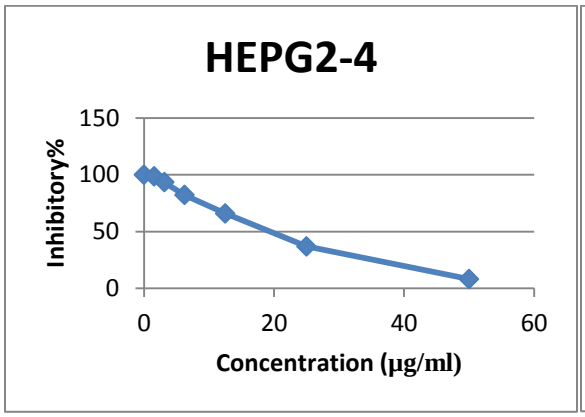


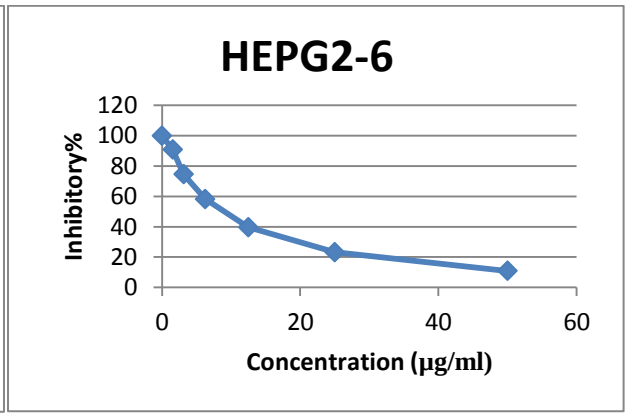


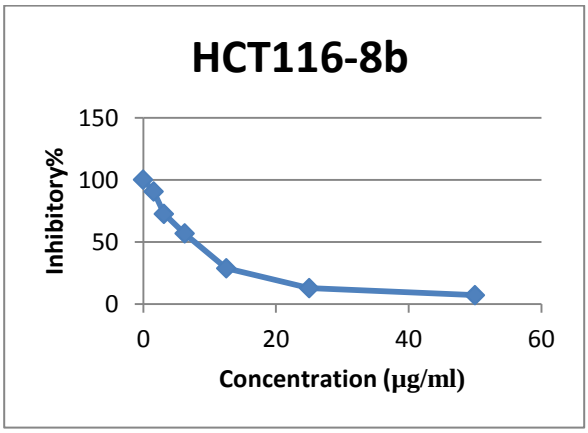


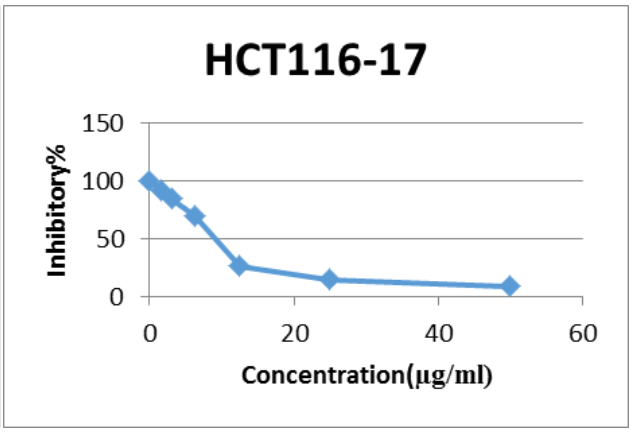

HEPG2-2
Inhibitory%
150
100
50
0
0
20
40
60
Concentration (µg/ml)


HEPG2-3a
Inhibitory%
150
100
50
0
0
20
40
60
Concentration (µg/ml)


HEPG2-4
Inhibitory%
150
100
50
0
0
20
40
60
Concentration (µg/ml)


HEPG2-6
Inhibitory%
120
100
80
60
40
20
0
0
20
40
60
Concentration (µg/ml)


HCT116-8b
Inhibitory%
150
100
50
0
0
20
40
60
Concentration (µg/ml)


HCT116-17
Inhibitory%
150
100
50
0
0
20
40
60
Concentration(µg/ml)

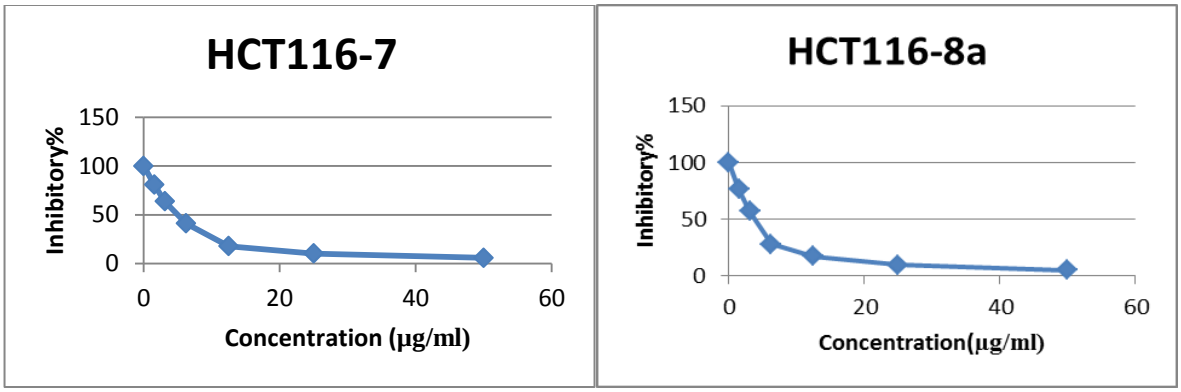


Figure 1: Cytotoxic activity of 3 (2H)- pyrdazine derivatives (2, 4, 5, 6, 7, 10, 11, 13a, 16, 17, 19).

Cytotoxic activity

An examination of the data reveled that most of compounds showed good to moderate activity. Compound 5, 13a has the best activity against HePG2 cell line (IC50 = 3.1, 2.1 1 µg/mL), compound 4, 8 have the best activity against MCF-7 cell line (IC50 = 2.2, 2.5 1 µg/mL), compound 10 has the best activity, against HCT-116 cell line (IC50 = 2.1 1 µg/mL). 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 IC50 of 21.6 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 the pyridazine ring that enhances the penetration of compound 5 to the cancer cell. The data are represented in Figure 2.

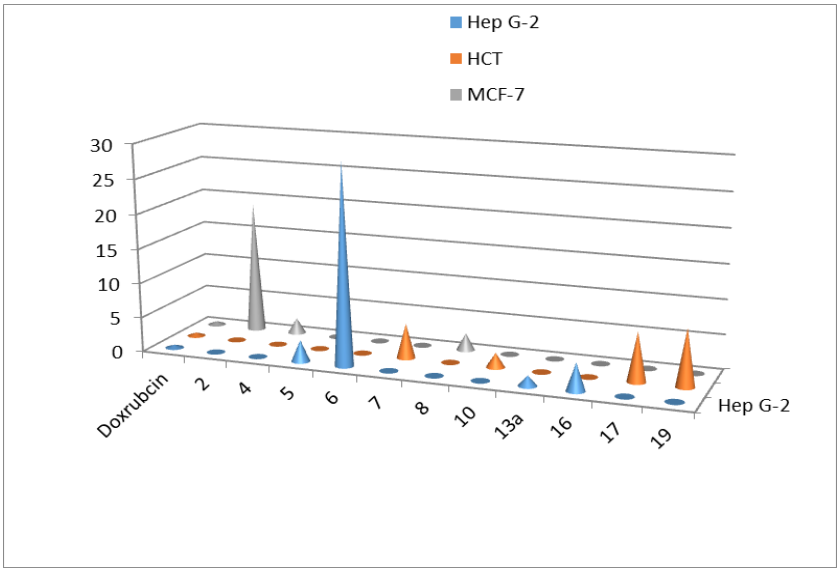


Figure 2: Cytotoxic activity of some pyrdazine derivatives.

CONCLUSION

Conversion of amino pyridazine incorrupt indolyl moiety into various pyrazolo pyridazines derivatives via diazotization followed by coupling with active methylene improved the activity of newly prepared compounds as anti-cancer agent. Also pyridazine was converted into isoxazols and thioxodihydropyrimidinedione with highly activity.

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