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Synthesis of bioactive benzopyridazine derivatives as antiproliferative agents against different cancer cell lines

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Abstract

New bioactive benzopyridazine derivatives **5a-d** and **7a,b** were successfully synthesized as antiproliferative agents via nucleophilic substitution reaction of 1-chloro-4-phenyl benzopyridazine **1** with the aromatic diamines **2a,b** respectively to form the free amines **3a,b**, The free amines **3a,b** coupled with phenyl isocyanates **4a,** phenyl isothiocyanate **4b** or 2-naphthalene sulfonyl chloride **6** to form **5a-d** and **7a,b** derivatives. The establishment of the synthesized benzopyridazine derivatives was affirmed *via* different spectroscopic methods. Moreover, the antiproliferative activity of the synthesized derivatives were investigated against different human cancer cell lines such as HCT-116, HepG-2, MCF-7. The new synthesized benzopyridazine derivatives were found to have promising antiproliferative agents against the three cancer cell lines compared to the reference drug doxorubicin. Notably, **5b-d** were found to be more selective towards HepG-2 cancer cell line with IC_{50} :1.6, 1.5, 1.6 µM as well as **7a, b** with IC_{50}: 1.6, 1.8 µM respectively higher than doxorubicin with IC_{50}: 3.8 µM. The selective toxicity of the synthesized derivatives was evaluated using BJ-1 normal cell showing low toxicity towards the healthy normal cells compared to the reference drug.

Keywords: Benzopyridazine, , Urea derivatives, Tiourea derivatives, Sulfonamide, Antiproliferative

1. Introduction

The worsening status of the uncontrolled growth of cancer makes the researchers to find and optimize new and safe anticancer drugs [1]. Even though the numerous discovered drugs as anticancer, cancer is one of the most causes of mortality according to WHO [2]. Furthermore, the non-selectivity of cancer chemotherapy agents makes a challenge to find new strategy including synthesis and development of small molecules with selective anticancer agents [3]. Naturally and synthetic based pharmaceutical natural products have a significant role in discovering and developing several drugs for curing from different diseases such as malaria, cancer and bacteria [4, 5]. It is worth remembering that heterocyclic compounds exhibited vital importance in medicinal chemistry as precursors or scaffolds in drug development for different diseases [6]. Notably, structural modification on fused rings bearing *N*-heterocyclic scaffolds demonstrated significant activities to be used as promising drugs such as quinoline, benzo[b]quinoline, and indoloquinoline [7-11]. benzopyridazine (Phthalazine) is one of the promising two fused rings containing two adjacent nitrogen atoms with a plethora of biological properties such as antimicrobial, anticancer, antidiabetic, and anti-inflammatory agents [12-15]. It also acts as DNA intercalation and topoisomerases inhibition [16]. It is worthwhile to note that Azelastin, Vatalanib and Hydralazine represented the pivotal presence of the pharmacophoric benzopyridazine derivatives in several commercially available drugs [17]. Several studies demonstrated the biological impact of sulfonamide as one of the most important classes in medicinal chemistry. It acts as antimicrobial, anticancer as well as anti-inflammatory agents [18]. Furthermore, the urea and thiourea moieties incorporated with small molecules have a widespread use in drug discovery regarding diverse biological activities such as anticancer, antimalarial, and antimicrobial [19]. Encouraged by the studies above, this work underscores newly synthesized hybrids including benzopyridazine precursor incorporated with urea, thiourea, or sulfonamide derivatives with evaluation of these derivatives as antiproliferative agents against different human cancer cell lines in addition to study their cytotoxicity against normal cell.

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1. Experimental

1.1. General

NMR spectroscopic analysis was operated with Bruker magnet system 400 Ascend/R (Huston, TX, USA) in DMSO d_6, with (400 MHz and 100MHz) for ^{1}H NMR and ^{13}C NMR, respectively at the Applied Nucleic Acid Research Center, Faculty of Science, Zagazig University. For the FTIR spectroscopy was performed in KBr with Alpha Bruker ATR mode (San Jose, CA, USA) at Faculty of Science, Menoufia University. The EIMS was carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University Direct Inlet part to mass analyzer in GCMS model with ISQ single quadrupole Thermoscientific Electron Impact mode (UK). Starting materials such as 1-chloro-4-phenyl benzopyridazine**1**, 1.4 phenylene diamine **2a**, and 4,4'-methylenedianiline **2b** were purchased from fisher scientific, UK. It is worthy note that 1-chloro-4-phenyl benzopyridazine**1** can be prepared according to literature [20]. The *in vitro* antiproliferative activity of the synthesized derivatives was screened against HCT-116, HepG-2, and MCF-7 cancer cell line as well as BJ-1 cell line.

1.2. Chemistry

General procedure for synthesis of 1-aminoaryl amino benzopyridazine 3a, b:

Add (1mmol) of aromatic diamines **2a,b** to (1mmol) of 1-chloro-4-phenyl benzopyridazine **1** with the presence of triethyl amine (3mmol) and DMF as a solvent under reflux condition for 3h. After the consumption of the reactants, the mixture was poured into ice water then filtered off and dried. The observed products **3a, b** was recrystallized from ethanol with good yields.

N1-(4-(p-tolyl)phthalazin-1-yl)benzene-1,4-diamine (3a)

Yellow solid, yield: 80-82%, m.p = 198–200 °C, FTIR (KBr) cm^{-1}: 3417(NH), 3330(NH_2), 3134(CH_{Ar}), 2925(CH), 1661 (C=C_{Ar}), 1558 (C=N). ^{1}H NMR: 2,41(s, 3H, CH_3), 6.62(d, 2H, CH_{Ar}, *J*=8Hz), 7.23-7.93(br.m, 8H, CH_{Ar}), 8.32(br.m, 1H, CH_{Ar}), 8.57 (d, 1H, CH_{Ar}, *J*=8Hz), 8.89(br.s, 2H, NH_2), 12.80 (br.s, 1H, NH), ^{13}C NMR: 20.89, 113.46, 118.18, 123.82, 128.93, 129.48, 131.18, 131.69, 133.54, 137.65, 138.41, 1144.65, 146.41, 152.08, 159.23. EI Ms for $C_{21}H_{18}N_4$ calcd. 326.40, found. $[M-H]^+$ 325.68.

N-(4-(4-aminobenzyl)phenyl)-4-(p-tolyl)phthalazin-1-amine (3b)

Yellow solid, yield: 80-82%, m.p = 209–211 °C, FTIR(KBr) cm^{-1}: 3450(NH), 3384(NH_2), 3100(CH_{Ar}), 2923(CH), 1617(C=C_{Ar}), 1555(C=N).^{1}H NMR: 2,40(s, 3H, CH_3), 3.79(s, 2H, CH_2), 6.62(d, 4H, CH_{Ar}, *J*=8Hz), 6.89-7.88 (br.m, 8H, CH_{Ar}), 8.03(br.m, 2H, CH_{Ar}), 8.30 (d, 2H, CH_{Ar}, *J*=8Hz), 8.75(br.s, 2H, NH_2), 12.84 (br.s, 1H, NH). ^{13}C NMR: 20.94, 45.34, 117.08, 119.37, 122.26, 123.65, 126.49, 129.62, 127.91, 129.22, 131.57, 132.39, 133.56, 137.21, 138.42, 140.86, 146.41, 151.82, 152.80, 159.23. EI Ms for $C_{28}H_{24}N_4$ calcd. 416.53, found. $[M-H]^+$ 415.91.

General procedure for synthesis of benzopyridazine - phenyl urea/thiourea derivatives (5a-d)

To (1 mmol) of 1-aminoarylamino benzopyridazine **3a, b**, add (1,2 mmol) of phenyl iso/thio cyanate **4a, b** in presence of (3 mL) of methylene chloride under stirring condition at room temperature for 5h. The formed benzopyridazine derivatives **5a-d** were filtered off and dried and recrystallized from ethanol.

1-phenyl-3-(4-((4-(p-tolyl)phthalazin-1-yl)amino)phenyl)urea (5a)

brown solid,, yield: 80-82%, m.p = 231–233 °C, FTIR (KBr) cm^{-1}: 3445(NH) 3257 (NH_2), 3055 (CH_{Ar}), 2925 (CH), 1631 (C=C_{Ar}), 1554 (C=N).^{1}H NMR: 2,42 (s, 3H, CH_3), 6.54 (br.s, 1H, NH), 7.28-7.99 (m, 12H, CH_{Ar}), 8.66 (br.m, 4H, CH_{Ar}), 9.19 (br.s, 2H, 2NH), 12.81 (br.s, 1H, NH). ^{13}C NMR: 20.92, 113.89, 114.20, 115.56, 118.19, 118.53. 119.02, 120.90, 121.81, 121.98, 125.79, 128.42, 128.79, 129.00, 129.55, 131.45, 132.00, 134.10, 134.56, 137.39, 139.72, 152.67. EI Ms for $C_{28}H_{23}N_5O$ calcd. 445.19 found. $[M]^+$ 445.47.

1-phenyl-3-(4-((4-(p-tolyl)phthalazin-1-yl)amino)phenyl)thiourea (5b)

Yellowish brown solid, yield: 80-82%, m.p = 243–245 °C, FTIR (KBr) cm^{-1}: 3445 (NH), 3218 (NH_2), 3025 (CH_{Ar}), 2925 (CH), 1600 (C=C_{Ar}), 1539 (C=N).^{1}H NMR: 2,33 (s, 3H, CH_3), 7.10-8.03 (m, 12H, CH_{Ar}), 8.67 (br.m, 4H, CH_{Ar}), 9.31 (br.s, 1H, NH), 9.82 (br.s, 1H, NH), 12.81 (br.s, 1H, NH). ^{13}C NMR: 20.90, 121.05, 122.71, 123.62, 124.31, 125.82, 125.90, 128.37, 128.40, 128.99, 129.55, 131.51, 133.76, 138.02, 138.40, 146.39, 159.21, 179.58. EI Ms for $C_{28}H_{23}N_5S$ calcd. 461.17 found. $[M]^+$ 461.06.

1-phenyl-3-(4-(4-((4-(p-tolyl)phthalazin-1-yl)amino)benzyl)phenyl)urea (5c)

Yellow solid, yield: 80-82%, m.p = 264–266 °C, FTIR (KBr) cm^{-1}: 3450 (NH), 3309 (NH_2), 3038 (CH_{Ar}), 2924 (CH), 1643 (C=C_{Ar}), 1554 (C=N).^{1}H NMR: 2,43 (s, 3H, CH_3), 3.80 (s, 2H, CH_2), 6.93-7.98 (m, 26H, CH_{Ar}), 8.68 (s, 2H, 2NH), 8.72 (br.s, 1H, NH). ^{13}C NMR: 21.06, 45.78, 118.20, 118.25, 118.48, 118.55, 121.90, 128.86, 128.88, 129.02, 129.15, 129.23, 129.31, 129.40, 129.44, 129.52, 135.13, 137.87, 139.57, 139.89, 152.75. EI Ms for $C_{35}H_{29}N_5O$ calcd. 535.35, found. $[M]^+$ 535.28.

1-phenyl-3-(4-(4-((4-(p-tolyl)phthalazin-1-yl)amino)benzyl)phenyl)thiourea (5d)

Yellow solid, yield: 80-82%, m.p = 272–274 °C, FTIR (KBr) cm^{-1}: 3446 (NH), 3210 (NH_2), 3008 (CH_{Ar}), 2926 (CH), 1614 (C=C_{Ar}), 1539 (C=N). ^{1}H NMR: 2,44 (s, 3H, CH_3), 3.88 (s, 2H, CH_2), 7.10-7.70 (m, 15H, CH_{Ar}), 7.97 (s, 2H, CH_{Ar}), 8.11 (d, 2H, CHAr, J=8Hz), 8.19 (d, 2H, CH_{Ar},*J*=8Hz), 8.95 (br.m, 1H, NH), 10.07(br.s, 1H, NH), 10.21(br.s, 1H, NH).^{13}C

NMR: 21.00, 45.51, 123.30, 123.39, 123.66, 124.22, 127.91, 128.34, 128.62, 129.74, 137.43, 139.61, 146.44, 152.05, 159.24, 179.45. EI Ms for $C_{35}H_{29}N_5S$ calcd. 551.21, found. $[M]^+$ 551.66.

General procedure for synthesis of phthalazine- naphthalene sulfonamide derivatives (7a, b)

Equimolar ratio of 1-aminoarylamino phthalazine **3a,b** by 2-Naphthalenesulfonyl chloride 6 were added to (2 mL) of DMF and excessive amount of triethyl amine (3mL). The mixture was refluxed till the consumption of the starting compounds after 5h. The formed hybrids **7a, b** was filtered off and dried and recrystallized from ethanol.

N-(4-((4-(p-tolyl)phthalazin-1-yl)amino)phenyl)naphthalene-2-sulfonamide (7a)

Brown solid, yield: 80-82%, m.p = 241–243 °C, FTIR (KBr) cm^{-1}: 3450 (NH), 3379 (NH_2), 3067 (CH_{Ar}), 2926 (CH), 1654 (C=C_{Ar}), 1554(C=N), 1159(S=O). ^{1}H NMR: 2,40 (s,3H,CH_3), 7.09-8.59 (m,18H,CH_{Ar}), 9.12 (s,1H, NH), 10.11 (br.s,1H,NH), 12.78 (br.s, 1H, NH).^{13}C NMR: 21.04, 113.93, 118.54, 121.77, 121.80, 122.37, 122.70, 125.94,126.23, 126.75, 127.78, 127.99, 128.09, 128.16, 129.02, 129.12, 129.19, 129.26, 129.37, 129.48, 129.69, 129.90, 130.04, 130.10, 130.16, 131.62, 131.72, 134.05, 134.37, 136.79, 137.67, 138.08, 138.58, 146.56, 151.72, 153.42, 159.38. EI Ms for $C_{31}H_{24}N_4O_2S$ calcd. 516.16, found. $[M]^+$ 516.45.

N-(3-(4-((4-(p-tolyl)phthalazin-1-yl)amino)benzyl)phenyl)naphthalene-2-sulfonamide(7b)

Brown solid, yield: 80-82%, m.p = 258–260 °C, FTIR (KBr) cm^{-1}: 3446 (NH), 3423 (NH_2), 3055 (CH_{Ar}), 2925 (CH), 1620 (C=C_{Ar}), 1555 (C=N), 1157 (S=O).^{1}H NMR: 2,41 (s, 3H, CH_3), 3.61(s, 2H, CH_2), 6.42-8.17(m, 19H, CH_{Ar}), 8.32 (s, 2H, CH_{Ar}), 8.63 (s, 2H, CH_{Ar}), 10.18 (br.s, 1H, NH), 12.78 (br.s, 1H, NH).^{13}C NMR: 21.04, 45.88, 114.18, 120.77, 120.83, 122.19, 125.08, 125.54, 125.94, 126.23, 126.80, 127.04, 127.78, 127.95, 128.57, 128.68, 128.76, 129.05, 129.13, 129.23, 129.26, 129.31, 129.37, 129.46, 129.49, 129.71, 130.04, 131.61, 131.64, 131.72, 133.71, 134.33, 134.36, 134.51, 135.67, 136.74, 137.13, 139.54, 153.76, 160.22. EI Ms for $C_{38}H_{30}N_4O_2S$ calcd. 606.21, found. $[M]^+$ 606.91.

1.3. In vitro anticancer activity:

Cell culture

HepG-2 (Human liver carcinoma), HCT116 (human colorectal carcinoma), MCF-7 (human breast adenocarcinoma), and the normal human skin fibroblast (BJ-1) cell lines were purchased from the American Type Culture Collection (Rockville, MD, USA) and maintained in RPMI-1640 medium which was supplemented with 10% heat-inactivated FBS, 100U/ml penicillin and 100U/ml streptomycin. The cells were grown at 37°C in a humidified atmosphere of 5% CO_2. All experiments were conducted thrice in triplicate (n = 3). All the values were represented as means ± SD.

Lactate dehydrogenase (LDH) assay

To determine the effect of each synthesized compound on membrane permeability in HepG2, MCF-7 and HCT-116 cancer cell lines as well as BJ-1 normal cell line, a lactate dehydrogenase (LDH) release assay was used [21-25]. The cells were seeded in 24-well culture plates at a density of 2 × 105 cells/well in 500 µL volume and allowed to grow for 18h before treatment. After treatment with a series of different concentrations of each compound or Doxorubicin® (positive control), the plates were incubated for 48h. Then, the supernatant (40 µL) was transferred to a new 96 well to determine LDH release and 6% triton X-100 (40 µL) was added to the original plate for determination of total LDH. An aliquot of 0.1 M potassium phosphate buffer (100 µL, pH 7.5) containing 4.6 mM pyruvic acid was mixed to the supernatant using repeated pipetting. Then, 0.1 M potassium phosphate buffer (100 µL, pH 7.5) containing 0.4 mg/mL reduced β-NADH was added to the wells. The kinetic changes were read for 1 min using ELISA microplate reader in absorbance at wavelength 340 nm. This procedure was repeated with 40 µL of the total cell lysate to determine total LDH. The percentage of LDH release was determined by dividing the LDH released into the media by the total LDH following cell lysis in the same well.

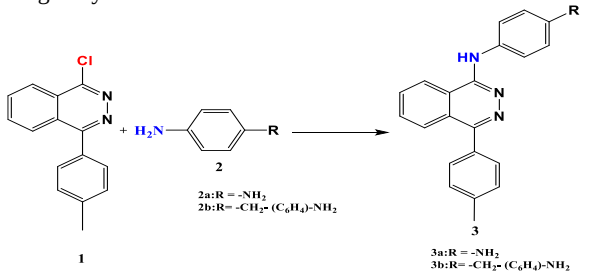
Statistical analysis

All experiments were conducted in triplicate (n = 3). All the values were represented as mean ± SD. Significant differences between the means of parameters as well as IC_{50} values were determined by probit analysis using SPSS software program (SPSS Inc., Chicago, IL).

3. Results and Discussion

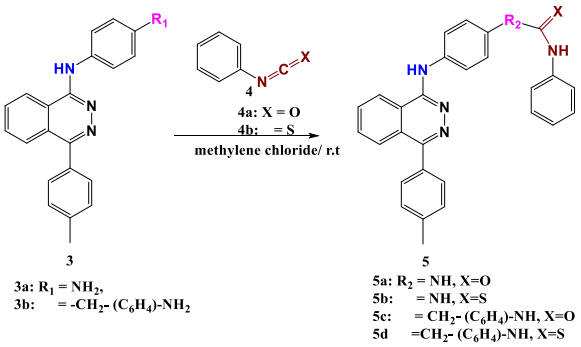
3.1. Chemistry

Two derivatives of amino aryl amino benzopyridazine were synthesized *via* nucleophilic substitution reaction (SN_{Ar}) of 1-chloro-4-phenyl benzopyridazine **1** with aromatic diamines such as 1,4-phenylene diamine **2a** or 4,4'-methylenedianiline **2b** in equimolar ratio with the presence of three times of triethylamine and DMF as a solvent to get the free amines of benzopyridazine derivatives **3a,b** in good yields as illustrated in **Scheme 1**.



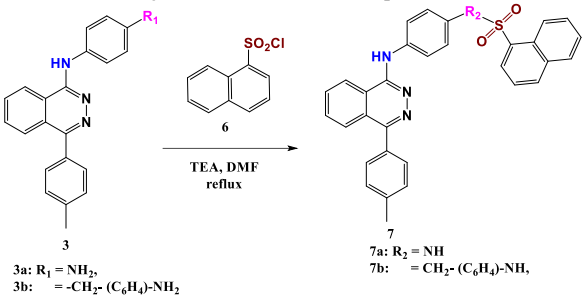
Scheme 1: Synthesis of amino aryl amino benzopyridazine derivatives.

Additionally, the installation of benzopyridazine derivatives bearing phenyl urea and thiourea moieties **5a-d** was accomplished by the reaction of (1mmol) of free amines of benzopyridazine derivatives with (1,2 mmol) of phenyl isocyanate **4a** or phenyl thiocyanate **4b** using methylene chloride as a solvent under room temperature to acquire the target derivatives with good yields as shown in **Scheme 2**.



Scheme 2: Synthesis of benzopyridazine derivatives containing phenyl urea and thiourea moieties.

Eventually, the establishment of benzopyridazine derivatives containing sulfonamide moiety **7a,b** were formed with good yields by coupling of free amine of benzopyridazine derivatives **3a, b** with 2-naphthalene sulphonyl chloride **6** in equimolar ratio with presence of excessive amount of triethyl amine and DMF as reported in **Scheme 3.**



Scheme 3: Synthesis of benzopyridazine derivatives containing sulfonamide moiety.

It is worth recalling that the formation of all synthesized free amine of benzopyridazine and their derivatives were affirmed across different spectroscopic methods such as NMR, FT IR and mass spectra. For the FT IR spectra, the absorption band of υ_{NH} of for the synthesized free amine of benzopyridazine **3a,b** at 3417 and 3450 cm^{-1} respectively whereas, the υ_{NH2} of **3a,b** showed at 3330 and 3384 cm^{-1} in succession. Moreover, the aromatic υ_{CHAr} absorption band for the free amines **3a,b** was recorded at 3134 and 3100 cm^{-1} meanwhile the aliphatic υ_{CH} was detected at 2925 and 2923 cm^{-1} consecutively. Furthermore, the C=N group of benzopyridazine core was shown at υ =1558 and 1555 cm-1 respectively for **3a, b**.

Regarding the FTIR spectra of the benzopyridazine derivatives **5a-d** as well as **7a,b**, the υ_{NH} absorption band showed at 3445 cm^{-1} for both derivatives **5a, b** as well as at 3450 and 3446 cm^{-1} for **5c** and **5d** respectively. In addition, the NH group of **7a** and **7b** showed at υ = 3450 and 3436 cm^{-1}. Meanwhile, the υ_{NH2} of **5a-d** showed their absorption bands at 3257, 3218, 3309, and 3210 cm^{-1} in succession. Additionally, the detected υ_{NH2} appeared at 3379 and 3423 cm^{-1} for **7a,b** respectively. Moreover, the characteristic (C=N) group of benzopyridazine core showed its absorption band at 1554 cm^{-1} for **5a, 5d** and **7a** as well as 1539 cm^{-1} for **5b** and 5d. While the $\upsilon_{C=N}$ absorption band for **7b** showed at 1557 cm^{-1}. Eventually, the (S=O) absorption band was detected at 1159 and 1157 cm^{-1} for **7a, b** respectively.

The establishment of the synthesized hybrids was affirmed via ^{1}H NMR spectra. It is noticeable that the protons (CH_3) group bearing benzopyridazine ring showed singlet peak at 2.41 and 2.40 ppm respectively for **3a** and **3b** as well as at δ: 2.33-2.44 ppm for hybrids **5a-d** and δ: 2.40, 2.41 ppm for **7a,b** respectively. Furthermore, the methylene protons (CH_2) bearing 4,4'-methylenedianiline reported at δ: 3.79, 3.80, 3.88, and 3.41 ppm for **3b, 5c, 5d**, and **7b** respectively. Moreover, the aromatic peaks for all synthesized derivatives were shown at their expected values. On the other hand, ^{13}C NMR spectra of the synthesized confirmed the formation of synthesized hybrids via the appearance of carbon of CH_3 benzopyridazine core of **3a,b** at δ: 20,89 and 20.94 ppm in succession. Additionally, the carbon CH_3 of benzopyridazine core for hybrids 5a-d showed at δ: 20.92, 20.90, 21.06, and 21.00 ppm as well as δ: 21.04 ppm for both derivatives **7a,b.** Whereas, the chemical shift for CH_2 carbon bearing 4,4'-methylenedianiline reported at δ: 45.34, 45.78, 45.51, and 45.88 ppm for **3b, 5c, 5d**, and **7b**. The mass spectra for all synthesized derivatives showed data consistency with their expected molecular ion peak values.

3.2. In vitro antiproliferative screening

Eight compounds were examined *in vitro* for their activity against HCT-116, HepG2, and MCF-7 human cancer cells and one human healthy cell line (BJ-1) using the LDH assay. The percentages of dead cells were calculated and compared to those of the control. Activities of these compounds against the three carcinoma cell lines were compared to the activity of doxorubicin.

All compounds suppressed three cancer cells (HCT-116, HepG2 and MCF-7) in a dose-dependent manner (Fig. 1 - 3). In case of HCT-116 human colorectal carcinoma cells: both Figure **1** and Table **1** show that two compounds (**3b** and **3a**, respectively) have comparable cytotoxic activities; the rest of the compounds have moderate cytotoxic activities against HCT-116 relative to that of doxorubicin. In case of MCF-7 human breast cancer cells: two compounds (**3a** and **3b** respectively) have superior cytotoxic activities; four compounds (**7b, 5a**, **7a** and **5c**, respectively) have comparable cytotoxic activities; two compounds (**5d** and **5b**) have moderate cytotoxic activities against MCF-7 relative to the reference drug (Figure 2 &Table 1). In the case of HepG2 human liver cancer cells: all compounds have superior cytotoxic activities against HepG2 relative to that of doxorubicin (Figure 3 &Table 1). In case of the non-tumor fibroblast-derived cell line (BJ-1): both Figure 4 and Table **1** show that two compounds (**5a** and **3a**) have superior cytotoxic activities; the rest of the compounds have moderate cytotoxic activities against the healthy cells relative to that of doxorubicin.

By comparing the cytotoxicity results on all cancer types relative to normal cell line, one can conclude that all compounds can be considered as good human liver and breast anticancer candidate drugs rather than on the human colon cancer type as their cytotoxic activities on both cancer types greater than their cytotoxic activities on the normal cells.

Table 1: The antiproliferative IC_{50} of the nine compounds against the four cell lines according to the LDH assay

Code	IC_{50} (µM) ± SD			
	HCT-116	**HepG-2**	**MCF-7**	**BJ-1**
3a	9.3 ± 1.9	1.5 ± 0.1	1.6 ± 0.2	6.8 ± 0.6
3b	8.9 ± 2.3	1.5 ± 0.2	1.7 ± 0.1	9.5 ± 0.9
5a	11.2 ± 2.1	1.5 ± 0.2	2.1 ± 0.1	6.8 ± 0.7
5b	14.4 ± 2.2	1.6 ± 0.3	3.5 ± 0.3	17.6 ± 2.9
5c	13.1 ± 1.7	1.5 ± 0.1	2.1 ± 0.2	14.2 ± 2.1
5d	12.9 ± 2.1	1.6 ± 0.2	4.1 ± 0.4	15.6 ± 3.1
7a	10.1 ± 1.7	1.6 ± 0.2	2.1 ± 0.2	13.3 ± 2.5
7b	11.1 ± 2.1	1.8 ± 0.3	1.9 ± 0.2	13.7 ± 3.1
Doxo.	8.1 ± 0.7	3.8 ± 0.3	1.8 ± 0.2	6.9 ± 0.5

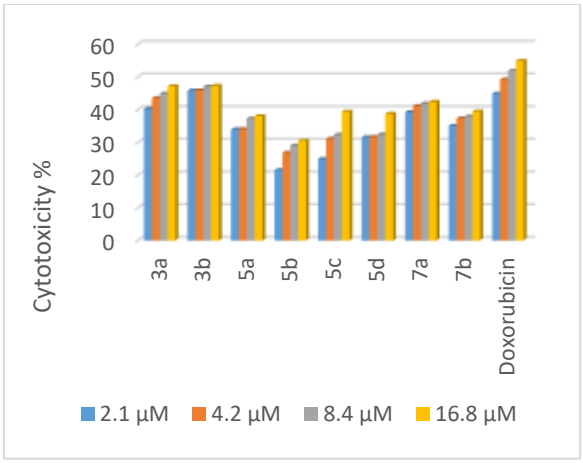


Figure 1: Dose dependent antiproliferative data of the nine compounds against HCT-116 cancer cells according to the LDH assay after 48 h of exposure.

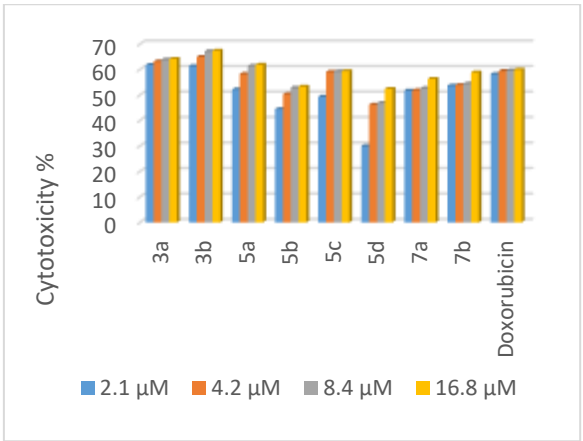


Figure 2: Dose dependent antiproliferative data of the nine compounds against MCF-7 cancer cells according to the LDH assay after 48h of exposure.

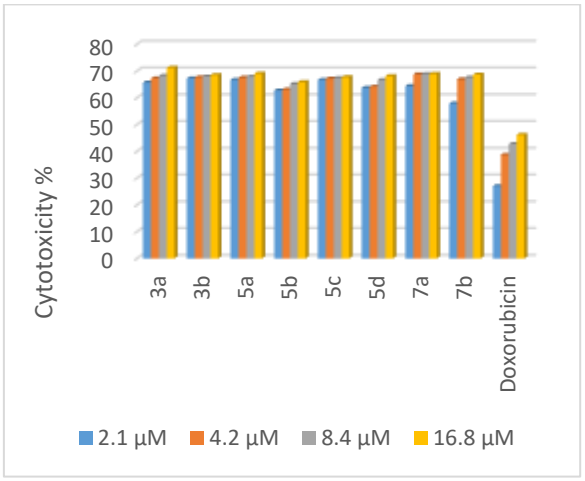


Figure 3: Dose dependent antiproliferative data of the nine compounds against HepG2 cancer cells according to the LDH assay after 48 h of exposure.

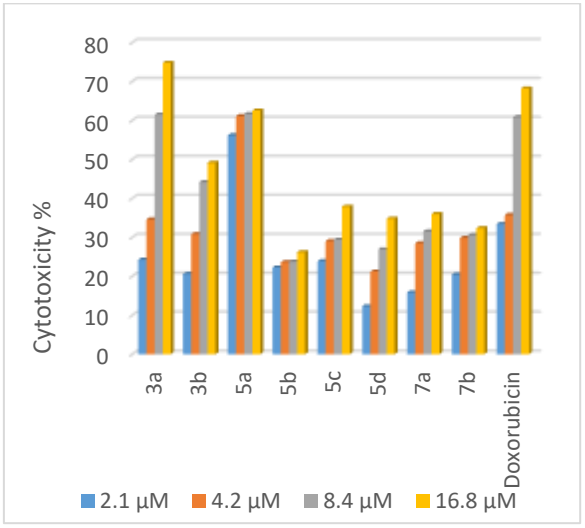


Figure 4: Dose dependent antiproliferative data of the nine compounds against BJ-1 normal cells according to the LDH assay after 48h of exposure.

3.3. Structure activity relationship of the synthesized benzopyridazine derivatives.

This study demonstrated structural modification of benzopyridazine *via* in cooperation with pharmaceutical moieties such as urea, thiourea, and sulfonamide derivatives. It is notable that the synthesized benzopyridazine derivatives have potency and selectivity against cancer cell lines emphasizing in the IC_{50} values of the synthesized derivatives comparing to their precursors of free amines **3a,b** as well as the reference drug against HepG-2 cancer cell line. The selectivity of the synthesized derivatives may be enhanced owing to the presence of urea, thiourea and sulfonamide moieties in the synthesized derivatives. From IC_{50} values of synthesized derivatives against HepG-2 cancer cell line and BJ-1 cell line, it is notable that the derivatives **5b** and **5d** containing thiourea scaffold were more selective towards HepG-2 comparing to their analogues **5a** and **5c** containing urea moiety as well as doxorubicin. On the other hand, **7a** containing one phenyl group between two amino groups and showed better activity than **7b** containing two phenyl groups with the same selectivity towards HepG-2 cancer cell line.

2. Conclusions

Newly synthesized benzopyridazine derivatives **5a-d** and **7a,b** with antiproliferative properties were established, characterized and evaluated against three human HCT-116, HepG-2, MCF-7 cancer cell lines and; BJ-1 normal cells. The hybrids showed potent activity against cancer cell lines, especially against HepG-2 Human cancer cell line.it is worth recalling that **5b-d**, as well as **7a,b** demonstrated their potency and selectivity towards HepG-2 cancer cell line with IC_{50s}: 1.6, 1.5, and 1.6 µM as compared with the reference drug doxorubicin IC_{50}: 3.8 µM.

Conflicts of interest

"There are no conflicts to declare".

3. References

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