Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(19):79-84
(http://www.derpharmachemica.com/archive.html)

Synthesis and *In Vitro* Anticancer Evaluation of some Acyclic *N*- and *S*-nucleosides of Pyridazine Derivatives

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ABSTRACT

Some new N-acyclic pyridazine nucleosides 2-5 and S-acyclic pyridazine nucleosides 8-10 were prepared from compounds 4,6-diphenyl-2H-pyridazin-3-one (1) and 4,6-diphenyl-2H-pyridazin-3-thione (7), respectively. Also, the hydrazino derivative 11 was prepared and used as a key compound for the preparation of some [1,2,4]triazolo[4,3-b]pyridazines 12-15. Moreover, the cytotoxicity and in vitro anticancer evaluation of some prepared compounds have also been assessed against different cancer cells including Lung (A594), Colon (HCT116), Breast (MCF-7), Liver (HepG2) and Prostate (PC3) as well as normal Melanocyte (HFB4). The results revealed that compounds 3 and 10 have moderate anticancer activities; while compounds 1, 2, 8, and 11 possess promising anticancer activities against breast MCF-7 and liver HepG2 cancer cell lines compared to the activity of the commonly used anticancer drug, doxorubicin. Otherwise, the results revealed that the previous compounds have no toxic effect against the normal HFB4 cells.

Keywords: Pyridazine, [1,2,4]triazolo[4,3-b]pyridazine, Acyclic N- and S-nucleosides, Anticancer potential, Cancer cell lines

INTRODUCTION

The chemistry of pyridazine and fused pyridazine derivatives has attracted attention during the last few decades because of their interesting pharmacological activities, especially as potential antiviral compounds with relatively higher HIV [1] and HAV inhibitory activity [2], antituberculosis [3], antimicrobial [4], and antioxidant [5] candidates. Recently, considerable attention has been devoted also to the synthesis of heterocyclic nucleosides because of their interesting reactivity and potential pharmaceutical applications as an antioxidant [6], antimicrobial [7], antiviral [8] and anticancer agents [9]. Moreover, synthetic compounds have been investigated to check their biological activity as anticancer agents, however many of them are not suitable for therapeutic use due to their toxic effect on the normal cells, carcinogenic and mutagenic properties. The use of chemotherapeutic drugs in cancer therapy involves the risk of life threatening host toxicity. The search, therefore, continues to develop drugs which selectively act on tumor cells. So, the discovery of the anticancer activity of the chemical synthetic compounds and its successful clinical use represents important progress for medicinal chemistry [10]. In the same direction and in continuing effort to discover more potent and selective anticancer compounds [11], herein, the synthesis of some acyclic nucleosides of pyridazine derivatives and their anticancer potential against different human cancer cell lines including Lung (A549), Colon (HCT116), Breast (MCF-7), Prostate (PC3) and Liver (HepG2) cancer cell lines will be evaluated as well as their toxic effect against the normal cells will be evaluated against normal Melanocyte (HFB4).

MATERIALS AND METHODS

Chemistry

All melting points are uncorrected and were measured using an Electrothermal IA 9100 apparatus, Shimadzu (Japan). Micro analytical data were performed by Vario El-Mentar apparatus, Organic Microanalysis Section, Micro Analytical center, Cairo University, Giza, Egypt. Their results were found to be in agreement with the calculated values (± 0.3). The IR spectra (KBr) were recorded on a Perkin-Elmer 1650 spectrophotometer, Micro Analytical center, Cairo University, Giza, Egypt. ^{1}H and ^{13}C-NMR spectra were determined on a Jeol 300 MHz in

deuterated dimethyl sulfoxide (DMSO-d_6), Micro Analytical center, Cairo University, Giza, Egypt, and the chemical shifts were expressed in ppm relative to Tetramethylsilane (TMS) as internal reference. Mass spectra were recorded on 70 eV EI Ms-QP 1000 EX (Shimadzu, Japan), Micro Analytical center, Cairo University, Giza, Egypt. Compounds 1 and 6 were prepared according to reported methods, compound 1 (m.p. 184-185°C and *lit.* m.p. 184°C [12]) and compound 6 (m.p. 100-101°C and *lit.* m.p 100°C [12]).

General procedure for the synthesis of compounds (2-5)

To a solution of compound 1 (0. 248 g, 1 mmol) in dry dimethylformamide (50 ml), 50% oil-immersed sodium hydride (0.20 g) was added. The reaction mixture was stirred at room temperature for 1 h, then ethyl iodide, 2-chloroacetaldehyde dimethyl-acetal, 2-chloroethanol or 2-(2-chloroethoxy)-ethanol (1 mmol) was added, and the reaction mixtures were stirred at 70°C for 5 h. After evaporation under reduced pressure, the residues were purified on silica gel column using chloroform:methanol (9:1) as an eluent to give compounds 2-5, respectively.

2-Ethyl-4,6-diphenyl-2*H*-pyridazin-3-one

Yield 72%; m.p. 122-123°C, IR (KBr), *v*=1636 (CO), ^{1}H-NMR (DMSO-d_6, ppm), δ=1.36 (t, 3H, J=7.50 Hz, NCH_2CH_3), 4.25 (q, 2H, J=7.20 Hz, N-CH_2CH_3), 7.42-7.53 (m, 6H, Ar-H), 7.90-7.99 (m, 4H, Ar-H), 8.10 (s, 1H, C5-H), ^{13}C-NMR (DMSO-d_6, ppm), δ=14.26 (CH_3), 34.12 (NCH_2), 125.85-129.98 (Ar-C), 136.62 (C-5), 144.55 (C-6), 149.54 (C-4), 159.76 (C=O). Anal. calcd for $C_{18}H_{16}N_2O$ (276.33): C, 78.24; H, 5.84; N, 10.14; found: C, 78.29; H, 5.81; N, 10.10.

2-(2,2-Dimethoxy-ethyl)-4,6-diphenyl-2*H*-pyridazin-3-one (3)

Yield 70%; m.p. 142-143°C, IR (KBr), *v*=1639 (CO), ^{1}H-NMR (DMSO-d_6, ppm), δ=4.30-4.37 (m, 8H, $2OCH_3$+NCH_2), 4.95 (t, 1H, J=2.4 Hz, CH), 7.42-7.53 (m, 6H, Ar-H), 7.92-8.00 (m, 4H, Ar-H), 8.13 (s, 1H, C5-H). Anal. calcd for $C_{20}H_{20}N_2O_3$ (336.38): C, 71.41; H, 5.99; N, 8.33; found: C, 71.39; H, 5.95; N, 8.37.

2-(2-Hydroxy-ethyl)-4,6-diphenyl-2H-pyridazin-3-one (4)

Yield 69%; m.p. 106-107°C, IR (KBr), *v*=3350-3300 (OH), 1640 (CO), ^{1}H-NMR (DMSO-d_6, ppm), δ=3.8-4.3 (m, 4H, CH_2CH_2), 4.87 (bs, 1H, OH, D_2O exchangeable), 7.43-7.53 (m, 6H, Ar-H), 7.93-7.99 (m, 4H, Ar-H), 8.14 (s, 1H, C5-H). Anal. calcd for $C_{18}H_{16}N_2O_2$ (292.33), C, 73.95; H, 5.52; N, 9.58; found: C, 73.90; H, 5.56; N, 9.56.

2-[2-(2-Hydroxy-ethoxy)-ethyl]-4,6-diphenyl-2H-pyridazin-3-one (5)

Yield 67%; m.p. 103-104°C, IR (KBr), *v*=3500-3290 (OH), 1640 (CO), ^{1}H-NMR (DMSO-d_6), δ=3.90 (m, 4H, $2CH_2$), 4.40 (m, 4H, $2CH_2$), 4.60 (bs, 1H, OH, D_2O exchangeable), 7.44-7.53 (m, 6H, Ar-H), 7.93-8.01 (m, 4H, Ar-H), 8.12 (s, 1H, C5-H). Anal. calcd for $C_{20}H_{20}N_2O_3$ (336.38): C, 71.41; H, 5.99; N, 8.33; found: C, 71.46; H, 5.95; N, 8.36.

4,6-Diphenyl-2*H*-pyridazine-3-thione (7)

Compound 6 (0.268 g, 1 mmol) and (0.70 g, 1 mmol) of thiourea in dioxane (40 ml) was heated at reflux for 4 h. The precipitate that formed was collected and dissolved in sodium hydroxide (20 ml, 10%). The mixture was filtered off and the filtrate was precipitated with hydrochloric acid (5 ml, 34%). The solid product was collected and recrystallized from ethanol to give compound 7 in 75% yield; m.p. 174-175°C, ^{1}H-NMR (DMSO-d_6, ppm), δ=7.44-7.53 (m, 6H, Ar-H), 7.70-7.95 (m, 4H, Ar-H), 7.91 (s, 1H, C5-H), 14.90 (s, 1H, NH, exchangeable with D_2O), ^{13}C-NMR (DMSO-d_6, ppm), δ=125.85-129.98 (Ar-C), 136.62 (C-5), 144.55 (C-6), 149.54 (C-4), 178.41 (C=S). MS, *m/z* (%): 264 (M+, 100). Anal. calcd for $C_{16}H_{12}N_2S$ (264.35): C, 72.70; H, 4.58; N, 10.60; S, 12.13; found: C, 72.75; H, 4.60; N, 10.55; S, 12.09.

General procedure for the synthesis of compounds (8-10)

To a solution of sodium hydroxide (0.04 g, 1 mmol) in ethanol (50 ml), compound 6 (0.01 mol) was added, and then the reaction mixture was stirred at room temperature for about 1 h. Methyl iodide, 2-chloroethanol, 2-(2-chloroethoxy)-ethanol (0.01 mol) was added and the reaction mixture was stirred at 70°C for 3 h. The reaction mixtures were evaporated under reduced pressure and the residue were crystallized using ethanol to give the title compounds 8-10 respectively.

3-Methylsulfanyl-4,6-diphenyl-pyridazine (8)

Yield 75%; m.p. 144-145°C, ^{1}H-NMR (DMSO-d_6, ppm), δ=2.35 (s, 3H, CH_3), 7.50-7.57 (m, 4H, Ar-H), 7.89 (s, 1H, C5-H), 8.14-8.17 (m, 6H, Ar-H), ^{13}C-NMR (DMSO-d_6, ppm), δ=14.26 (CH_3), 125.85-129.98 (Ar-C), 136.62 (C-5), 144.55 (C-6), 149.54 (C-4). Anal. calcd for $C_{17}H_{14}N_2S$ (278.37), C, 73.35; H, 5.07; N, 10.06; S, 11.52; found: C, 73.30; H, 5.12; N, 10.10; S, 11.49.

2-(4,6-Diphenyl-pyridazin-3-ylsulfanyl)-ethanol (9)

Yield 79%; m.p. 155-156°C, IR (KBr), *v*=3351-3300 (OH), ^{1}H-NMR (DMSO-d_6, ppm), δ=3.42-3.70 (m, 4H, CH_2CH_2), 4.99 (bs, 1H, OH, D_2O exchangeable), 7.51-7.62 (m, 6H, Ar-H), 7.94 (s, 1H, C5-H), 8.18-8.21 (m, 4H, Ar-H). Anal. calcd for $C_{18}H_{16}N_2OS$ (308.40): C, 70.10; H, 5.23; N, 9.08; S, 10.40; found: C, 70.14; H, 5.20; N, 9.04; S, 10.43.

2-[2-(4,6-Diphenyl-pyridazin-3-ylsulfanyl)-ethoxy]-ethanol (10)

Yield 77%; m.p. 162-163°C; IR (KBr), *v*=3500-3290 (OH), ^{1}H-NMR (DMSO-d_6), δ=3.40-3.51 (m, 4H, $2CH_2$), 3.64-3.71 (m, 4H, $2CH_2$), 4.49 (bs, 1H, OH, D_2O exchangeable), 7.39-7.49 (m, 6H, Ar-H), 7.95 (s, 1H, C5-H), 8.04-8.18 (m, 4H, Ar-H). Anal. calcd for $C_{20}H_{20}N_2O_2S$ (352.45): C, 68.16; H, 5.72; N, 7.95; S, 9.10; found: C, 68.11; H, 5.77; N, 7.90; S, 9.14.

(4,6-Diphenyl-pyridazin-3-yl)-hydrazine (11)

To a solution of compound 6 (0.266 g, 1 mmol) in dry dioxane (30 ml), hydrazine hydrate (2 ml, 98%) was added and the mixture was heated at reflux for 4 h. The reaction mixture was cooled and poured into water and the separated solid was filtered off, dried, and recrystallize from dioxane to give compound 11 in 69% yield, m.p. 120-121°C, IR (KBr), *v*=3424, 3377 (NH_2) and 3309 (NH), ^{1}H-NMR (DMSO-d_6, ppm), δ=4.00 (brs, 1H, NH, exchangeable with D_2O), 7.42-7.58 (m, 8H, Ar-H+NH_2, exchangeable with D_2O), 7.80-8.32 (m, 5H, Ar-H+C5-H); MS, m/z (%): 262 (M+, 56). Anal. calcd for $C_{16}H_{14}N_4$ (262.31): required C, 73.26; H, 5.38; N, 21.36; found C, 73.21; H, 5.40; N, 21.34.

6,8-Diphenyl-[1,2,4]triazolo[4,3-*b*]pyridazine (12)

Compound 11 (0.262 g, 1 mmol) was heated under reflux temperature in 20 ml triethyl ortho formate for 15 h. The reaction mixture was evaporated till dryness and the remaining solid was recrystallized from dioxane to give compound 12 in 66% yield, m.p. 174-175°C, ^{1}H-NMR (DMSO-d_6, ppm), δ=7.41-7.54 (m, 6H, Ar-H), 7.70-7.93 (m, 4H, Ar-H), 8.11 (s, 1H, C7-H), 8.33 (s, 1H, C3-H); MS, m/z (%): 272 (M+, 100). Anal. calcd for $C_{17}H_{12}N_4$ (272.30): required C, 74.98; H, 4.44; N, 20.58; found C, 75.02; H, 4.39; N, 20.60.

3-Methyl-6,8-diphenyl-[1,2,4]triazolo[4,3-*b*]pyridazine (13)

Compound 11 (0.262 g, 1 mmol) was heated under reflux temperature in 20 ml triethyl orthoacetate for 15 h. The reaction mixture was evaporated till dryness and the remaining solid was recrystallized from dioxane to give compound 13 in 70% yield, m.p. 180-181°C, ^{1}H-NMR (DMSO-d_6, ppm), δ=2.30 (s, 3H, CH_3), 7.39-7.52 (m, 6H, Ar-H), 7.75-7.92 (m, 4H, Ar-H), 8.18 (s, 1H, C7-H); MS, m/z (%): 286 (M+, 100). Anal. calcd for $C_{18}H_{14}N_4$ (286.33): required C, 75.50; H, 4.93; N, 19.57; found C, 75.52; H, 4.89; N, 19.55.

***N*-Benzylidene-*N'*-(4,6-diphenyl-pyridazin-3-yl)-hydrazine (14)**

A mixture of compound 11 (0.262 g, 1 mmol) and benzaldehyde (0.10 g, 1 mmol) in ethanol (30 ml) was heated for 2 h. The formed precipitate was filtered off, dried, and recrystallize from dioxane to give compound 14. Yield 81%, m.p. 217-218°C, IR (KBr), *v*=3442 (NH), ^{1}H-NMR (DMSO-d_6, ppm), δ=7.34-7.58 (m, 10H, Ar-H and CH=N), 7.69-7.89 (m, 6H, Ar-H), 8.14 (s, 1H, C5-H); 11.55 (s, 1H, NH, D_2O exchangeable); MS, m/z (%): 350 (M+, 89). Anal. calcd for $C_{23}H_{18}N_4$ (350.42): required C, 78.83; H, 5.18; N, 15.99; found C, 78.79; H, 5.14; N, 16.02.

3,6,8-Triphenyl-[1,2,4]triazolo[4,3-*b*]pyridazine (15)

To a solution of compound 14 (0.28 g, 1 mmol) and anhydrous sodium acetate (0.08 g, 1 mmol) in glacial acetic acid (20 ml), bromine (0.08 ml, 0.5 mmol) was added with stirring. The reaction mixture was refluxed for 1 h, then poured into water and the precipitated solid was filtered off, washed with water, and recrystallized from dioxane to give compound 15. Yield 75%, m.p. 230-228°C. IR showed absence of NH group. ^{1}H-NMR (DMSO-d_6, ppm), δ=7.43-7.56 (m, 9H, Ar-H), 7.72-7.88 (m, 6H, Ar-H), 8.08 (s, 1H, C7-H); MS, m/z (%): 348 (M+, 100). Anal. calcd for $C_{23}H_{16}N_4$ (348.40): required C, 79.29; H, 4.63; N, 16.08; found C, 79.32; H, 4.67; N, 16.05.

Anticancer activity

Methodology

Fetal Bovine Serum (FBS) and L-glutamine, were obtained from Gibco Invitrogen Company (Scotland, UK). Dulbecco's Modified Eagle's (DMEM) medium was provided from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and Sulfo-Rhodamine-B stain (SRB) (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were obtained from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade and purchased from Sigma-Aldrich chemical Co. (St. Louis, MO, USA).

For anticancer activity screening of the tested compounds, A549, HCT116, MCF-7, PC3 and HepG2 cancer cell lines as well as the normal cell line HFB4 were obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were maintained in DMEM supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37°C in humidified atmosphere containing 5% CO_2. Cells at a concentration of 0.50×10^6 were grown in a 25 cm^2 flask in 5 ml of culture medium.

In vitro cancer activity assay

The anticancer activity of the tested compounds was measured *in vitro* using the SRB assay according to Skehan et al. [13], modified by Ali et al. [14,15]. Briefly, cells were inoculated in 96-well microtiter plate (10^4 cells/well) for 24 h before treatment with the tested compounds to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO at 1 mg/ml immediately before use and diluted to the appropriate volume just before addition to the cell culture. Different concentration of tested compounds and doxorubicin were added to the cells. Three wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37°C and in atmosphere of 5% CO_2. After 48 h cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and attached stain was recovered with Ethylenediaminetetraacetic acid (Tris-EDTA) buffer. Color intensity was measured in an Enzyme-linked Immunosorbent Assay (ELISA) reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for each cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in Table 1. The results were compared to the effect of the reference drug, doxorubicin.

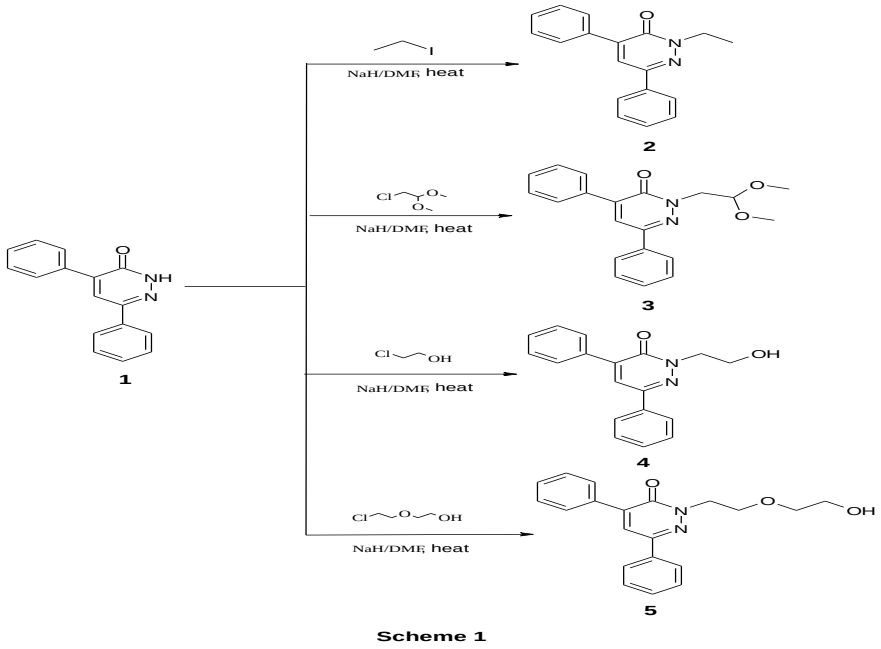
RESULTS AND DISCUSSION

Chemistry

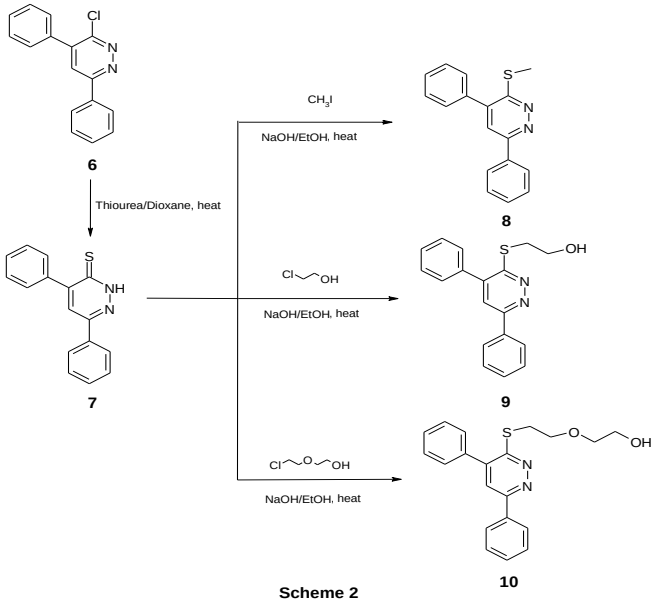
In this work some *N*- and *S*-acyclic nucleoside of pyridazine derivatives were prepared to start with 4,6-diphenyl-2*H*-pyridazin-3-one (1) [12] as the key compound for this study (Scheme 1). When the sodium salt of compound 1 was treated with ethyl iodide, 2-chloroacetaldehyde dimethyl-acetal, 2-chloroethanol or 2-(2-chloroethoxy)-ethanol, it gave the corresponding *N*-acyclic nucleosides 2-5, respectively (Scheme 1). The IR and ^{13}C-NMR spectra of the latter compounds revealed the presence of the C=O signal and the ^{1}H-NMR spectra indicated the absence of the NH group and the presence of ethyl, dimethoxyethyl, hydroxyethyl and hydroxyethoxy ethyl signals, respectively (cf. experimental).

Thionation of the chloro derivative 6 [12] with thiourea, in dioxane, afforded the corresponding pyridazine thione derivative 7 in a quantitative yield which used as a key compound for further synthesis. The ^{13}C-NMR spectrum of compound 7 showed a signal at δ=178 ppm characteristics for C=S group (cf. experimental). Moreover, alkaline treatment of compound 7 with methyl iodide in the presence of sodium hydroxide gave the corresponding *S*-alkyl derivative 3-methylsulfanyl-4,6-diphenyl-pyridazine (8). The latter compound showed a signal at δ=2.35 for the *S*-methyl group, in ^{1}H-NMR spectrum, while in ^{13}C-NMR spectrum, it showed a signal at δ=14.26 as well as the absence of C=S group, which indicated that the site of the attack is on the sulfur and not on the nitrogen. Similarly, treatment of the sodium salt of compound 7 with 2-chloroethanol or 2-(2-chloroethoxy)-ethanol, it afforded the corresponding *S*-acyclic nucleosides 9 and 10, respectively (Scheme 2). The IR spectra of the latter compounds revealed the presence of the OH absorption band and the ^{1}H-NMR spectra indicated the presence of hydroxyethyl and

hydroxyethoxy ethyl signals, respectively (cf. experimental).



Scheme 1: Preparation of N-acyclic nucleosides (2-5) of pyridazine derivatives

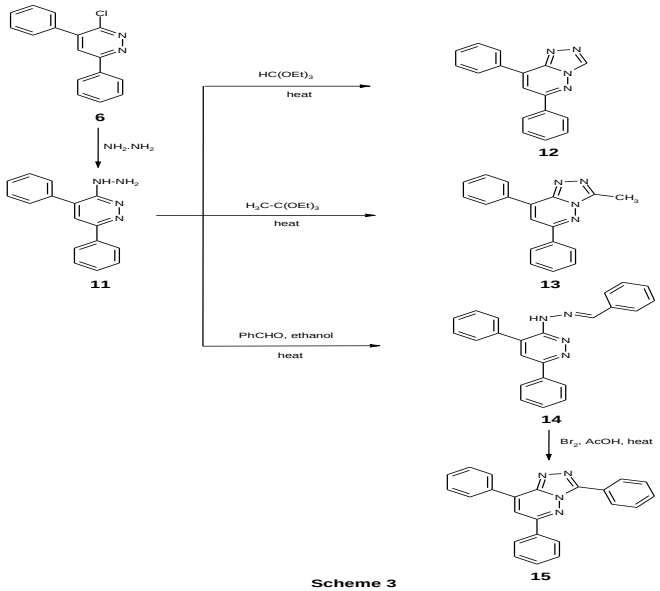


Scheme 2: Preparation of S-acyclic nucleosides (9-10) of pyridazine derivatives

On the other hand, when a solution of compound 6, in dry dioxane, was heated with hydrazine hydrate, it afforded (4,6-diphenyl-pyridazin-3-yl)-hydrazine 11 (Scheme 3). The ^{1}H-NMR spectrum of the latter compound revealed the presence of signals for NH_2 and NH (D_2O exchangeable). Heating of compound 11 with triethyl ortho formate or triethyl orthoacetate at reflux temperature, gave [1,2,4]triazolo[4,3-*b*]pyridazine 12 and 13, respectively. Condensation of compound 11 with benzaldehyde, in the presence of a few drops of piperidine, gave the corresponding hydrazone 14. The latter compound was annulated to its corresponding [1,2,4]triazolo[4,3-*b*]pyridazine derivative 15 by refluxing with bromine in glacial acetic acid (cf. experimental).

Anticancer activity

The *in vitro* antitumor activities of the synthesized compounds against five cancer cell lines, including Lung (A594), Colon (HCT116), Breast (MCF-7), Liver (HepG2) and Prostate (PC3) as well as normal Melanocyte (HFB4), were assayed by SRB method and the results expressed as IC_{50} are summarized in Table 1. Doxorubicin was used as a positive control. The results revealed that nearly all the tested compound have no toxic effect against the normal HFB4 cells. Otherwise, the results revealed that the tested compounds were exerted very weak anticancer activity against lung, colon, and prostate cancer cells. Compounds 3 and 10 revealed moderate anticancer activity comparing with the reference drug doxorubicin with IC_{50} values 9.60 ± 1.29 and 9.00 ± 1.21 µg/ml (versus 3.16 ± 0.38 µg/ml for doxorubicin) in case of breast cancer, as well as 10.63 ± 1.68 and 11.00 ± 1.30 µg/ml (versus 3.11 ± 0.36 µg/ml for doxorubicin) in case of liver cancer.



Scheme 3: Preparation of (4,6-diphenyl-pyridazin-3-yl)-hydrazine 11

Table 1: The anticancer activity after 48 h exposure to the synthesized compounds on different cell lines

Compounds	IC_{50} (µg/ml)					
	A549	**HCT11**	**MCF-7**	**HepG2**	**PC3**	**HFB4**
Doxorubicin	3.70 ± 0.40	4.50 ± 0.67	3.16 ± 0.38	3.11 ± 0.36	5.16 ± 0.46	86.70 ± 9.90
1	39.40 ± 5.11	60.19 ± 7.80	3.40 ± 0.27	5.06 ± 0.47	72.00 ± 8.00	82.75 ± 10.65
2	60.73 ± 7.39	37.80 ± 5.11	3.80 ± 0.46	4.19 ± 0.61	33.82 ± 4.09	78.60 ± 9.77
3	71.00 ± 8.32	66.31 ± 7.82	9.60 ± 1.29	10.63 ± 1.68	38.75 ± 6.08	80.95 ± 11.36
4	38.16 ± 4.56	37.61 ± 4.86	32.60 ± 4.79	75.20 ± 8.71	21.95 ± 3.90	67.38 ± 8.20
5	60.12 ± 7.88	29.74 ± 4.11	20.65 ± 3.61	36.85 ± 5.07	41.80 ± 5.65	68.37 ± 8.42
6	35.65 ± 4.80	46.20 ± 6.23	45.00 ± 5.00	64.31 ± 7.50	56.50 ± 7.20	76.40 ± 9.11
7	60.33 ± 7.72	73.11 ± 6.98	33.60 ± 4.80	35.20 ± 3.60	46.95 ± 6.29	80.66 ± 10.00
8	64.05 ± 7.26	34.30 ± 4.60	3.10 ± 0.25	3.67 ± 0.52	40.00 ± 5.26	85.00 ± 10.48
9	40.87 ± 5.80	37.60 ± 4.80	17.75 ± 2.31	47.03 ± 6.04	28.14 ± 4.11	72.90 ± 9.06
10	38.62 ± 5.18	65.28 ± 7.85	9.00 ± 1.21	11.00 ± 1.30	39.90 ± 4.70	68.44 ± 8.90
11	31.66 ± 2.86	66.00 ± 8.10	3.11 ± 0.40	4.00 ± 0.55	43.33 ± 6.00	80.36 ± 9.66

The IC_{50} values represent the concentration resulting in a 50% decrease in cell growth after 48 h incubation, which were Mean ± Standard error (S.E.) of three repeated experiments

On the other hand, within the series of the synthesized compounds, it was found that compounds 1, 2, 8 and 11 displayed the most promising anticancer activities against the breast and liver cell lines. In case of breast cancer the IC_{50} values of the latter compounds, as compared to the standard drug doxorubicin, were 3.80 ± 0.46, 3.40 ± 0.27, 3.10 ± 0.25 and 3.11 ± 0.40 µg/ml respectively (versus 3.16 ± 0.38 µg/ml for doxorubicin). While, in liver cancer the IC_{50} values were 4.19 ± 0.61, 5.06 ± 0.47, 3.67 ± 0.52 and 4.00 ± 0.55 µg/ml respectively (versus 3.11 ± 0.36 µg/ml for doxorubicin).

CONCLUSION

Evaluation of some newly synthesized *N*-acyclic and *S*-acyclic pyridazine nucleosides were performed against five cancer cell lines, by SRB method. The results revealed that nearly all the tested compounds have no toxic effect against the normal HFB4 cells. Otherwise, the results revealed that the tested compounds were exerted very weak anticancer activity against lung, colon, and prostate cancer cells. While compounds 1, 2, 8, and 11 possess promising anticancer activities against the breast and liver cancer cell lines compared to doxorubicin.

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