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EVALUATION OF CYTOTOXIC PROPERTIES OF N,N′-BIS[(1-ARYL-3-HETEROARYL)PROPYLIDENE]-HYDRAZINE DIHYDROCHLORIDES

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N,N′-bis[(1-aryl-3-heteroaryl)propylidene]hydrazine dihydrochlorides, **P1, P4 – P8,** and **R1 – R7,** were assayed against human oral squamous cell carcinoma (HSC-2, HSC-3, HSC-4), human promyelocytic leukemia cell line (HL-60), and human normal oral cells (HGF, HPC, and HPLF) as non-tumor cells to evaluate their cytotoxic properties. Peplomycin was used as a reference compound. It was found that **P-** and **R-**series hydrazone compounds exhibited cytotoxicity in a range of 11 ± 0.68 – 300 ± 1.0 μM. Compound **P1** which is a non-substituted hydrazone containing piperidine ring and compound **R2** which is a 4-methyl hydrazone derivative containing pyrrolidine ring showed the most potent cytotoxic activity. These hydrazone compounds may serve as promising candidates for further studies.

Keywords: cytotoxic activity, hydrazone, Mannich base, cancer, selectivity, anticancer drugs.

1. INTRODUCTION

Hydrazones having the chemical structure of $R_1R_2C{=}NNH_2$ can be derived from a ketone or an aldehyde. If both R1 and R2 are aryl or alkyl, it indicates that hydrazone was derived from a ketone. Hydrazones are synthesized by the reaction of hydrazine with a ketone or an aldehyde. The oxygen atom of ketone or aldehyde is replaced with NNH_2 functional group as a result of synthesis reaction [1, 2]. α-Hydrogen atom in hydrazone is 10 times more acidic than in ketone, therefore, this hydrogen atom is more nucleophilic [3, 4]. The reactivity of hydrazones comes from nucleophilicity of hydrogen carbon atom [5]. Furthermore, two connected nitrogen atoms in hydrazone compounds have different natures. Both nitrogen atoms of the hydrazone compounds have nucleophilic character and the amino type nitrogen is more reactive. The carbon atom in hydrazone compounds shows electrophilic and nucleophilic properties [6]. Hydrazones have been one of the most studied functional groups because of diverse pharmacological activities including anti-inflammatory [7], anti-HIV [8], antituberculosis [9], antibacterial [10], monoamine oxidase inhibitory [11], antiplatelet aggregation [12], and antiproliferative or anticancer [13 – 18] properties.

Substrates having at least one active hydrogen atom, an aldehyde component (generally formaldehyde/paraformaldehyde), and an amine reagent react to form compounds generally known as Mannich bases. Mannich reactions are also known as aminoalkylation reactions since the obtained product is derivative of the substrate with an aminoalkyl moiety [19, 20]. Mannich bases are important pharmacophores and it is known that some compounds which are used today for clinical treatment of various diseases and disorders have this versatile scaffold, such as cocaine, fluoxetine, atropine, ethacrynic acid, procyclidine, trihexyphenidyl, ranitidine, and biperiden [21 – 23]. Furthermore, Mannich bases have different biological activities including antifungal [24 – 26], antimicrobial [27 – 29], anti-inflammatory [30 – 32], anti-HIV [33], carbonic anhydrase inhibition [34 – 40], cytotoxic and anticancer [18, 37, 40 – 50].

Previously, we have designed and synthesized some hydrazone compounds, N,N′-bis[(1-aryl-3-heteroaryl)propylidene]hydrazine dihydrochlorides including **P-** and **R-**series *via* reaction of mono-Mannich bases, 1-aryl-3-heteroaryl-1-propanone, with hydrazine hydrate, evaluated their cytotoxic activity against human hepatoma (Huh7) and breast cancer (T47D) cell lines, and got some impressive results published in [16 – 18]. The present work was aimed to investigate the cytotoxic effect of synthesized compounds

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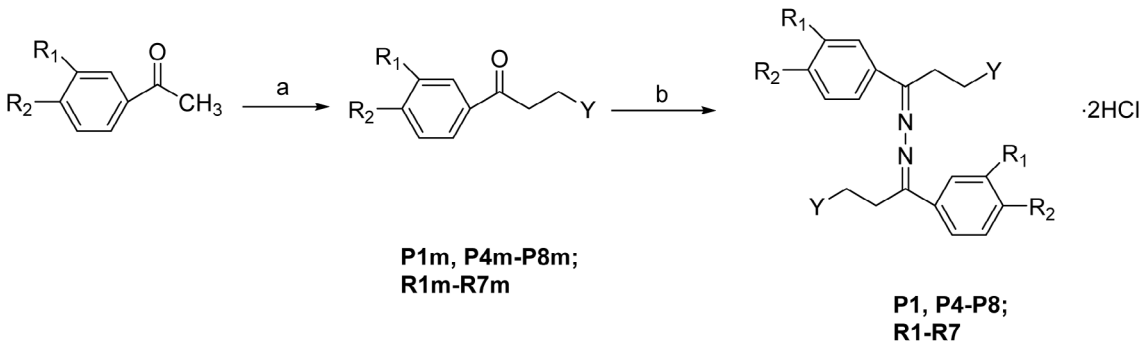

Y: Piperidine-1-yl for **P**-Series, Pyrrolidine-1-yl for **R**-Series

$R_1 = R_2 = H$ (**P1**); $R_1 = H$, $R_2 = OH$ (**P4**); $R_1 = H$, $R_2 = Cl$ (**P5**); $R_1 = OCH_3$, $R_2 = H$ (**P6**); $R_1 = H$, $R_2 = F$ (**P7**); $R_1 = H$, $R_2 = Br$ (**P8**); $R_1 = R_2 = H$ (**R1**); $R_1 = H$, $R_2 = CH_3$ (**R2**); $R_1 = H$, $R_2 = OCH_3$ (**R3**); $R_1 = H$, $R_2 = OH$ (**R4**); $R_1 = H$, $R_2 = Cl$ (**R5**); $R_1 = OCH_3$, $R_2 = H$ (**R6**); $R_1 = H$ $R_2 = F$ (**R7**)

Scheme 1. Synthesis of hydrazone compounds, N,N′-bis[(1-aryl-3-heteroaryl)propylidene]hydrazine dihydrochlorides **(P1, P4-P8;** and **R1-R7).** Reagents and conditions: (a) paraformaldehyde, piperidine HCl/pyrrolidine, HCl (37%) and EtOH, 4 – 9 h reflux for **P1m, P4m-P8m; R1m-R7m;** (b) ethanolic acetic acid (3% w/v), hydrazine hydrate stirring for 17 – 26 h for **P1, P4-P8; R2-R7** and 3 h reflux for **R1.**

(Scheme 1) against human oral squamous carcinoma cell lines (HSC-2, HSC-3, HSC-4) and human leukemia cell line (HL-60) as tumor cell lines and human promyelocytic normal oral cells (HGF, HPC, and HPLF) as non-tumor cells. It was expected to find out new lead compound(s) for treating dental cancer and leukemia problems.

2. EXPERIMENTAL CHEMICAL SECTION

2.1. Materials and Methods

All commercially available reagents used in the synthesis of **P-** and **R-**series hydrazones were purchased from Merck AG, Fluka AG, Acros Organics, Riedel-de Haën, J. T. Baker or Sigma-Aldrich Chemie and used without further purification. Melting points were measured on an Electrothermal 9100 melting point apparatus (IA9100, Electrothermal, Essex, UK). The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on Varian 400 MHz FT spectrometer (Danbury, USA) for **P**- and **R-**series hydrazone derivatives, while ^{1}H NMR (60 MHz) spectra were recorded on a Varian EM-360 spectrometer for **Pm** and **Rm** compounds (precursor mono-Mannich bases).

2.2. Synthesis

Precursor mono-Mannich bases, 1-aryl-3-(heteroaryl)-1-propanone hydrochlorides (**P1m**, **P4m – P8m**, and **R1m – R7m**) and their hydrazones; N,N′-bis[(1-aryl-3-heteroaryl)propylidene]hydrazine dihydrochlorides (P1, P4 – P8, R1 – R7) were freshly synthesized according to Scheme 1 as described previously [16, 18].

3. EXPERIMENTAL BIOLOGICAL SECTION

The cytotoxicity of **P-** and **R-**series compounds was assayed against three human oral squamous carcinoma cell lines derived from tongue (HSC-2, HSC-3, HSC-4), human leukemia cell line (HL-60), and human normal oral cells (gingival fibroblasts, HGF; periodontal ligament fibroblasts, HPLF; and pulp cells, HPC) as described in our previous papers [37, 40 – 47, 49, 50, 52 – 60].

In brief, all cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Cells (2.5×10^3 cells/well) were inoculated and incubated for 48 h to achieve complete adherence. Near confluent cells were incubated for a further 48 h in the fresh culture medium containing each test compound (3.12, 6.25, 12.5, 25, 50, 100, 200, 400 μM) or peplomycin (positive control) (7.8, 15.6, 31.2, 62.5, 125, 250, 500, 1000 μM). The viable cell numbers were determined by the MTT method. Cytotoxicity induced by DMSO (0.0078, 0.0156, 0.03125, 0.0625, 0.125, 0.25, 0.5 or 1%) was subtracted from the value for each well. The CC_{50} values were determined from the dose-response curves. The tumor selectivity (TS) has been calculated using the following equation:

$$TS = \frac{\text{mean } CC_{50} \text{ against normal cells}}{\text{mean } CC_{50} \text{ against cancer cells}}$$

and is shown as B/A in Table 1.

4. RESULTS AND DISCUSSION

In this paper, cytotoxic activities of the hydrazone compounds; **P1, P4 – P8,** and **R1 – R7**, N,N′-bis[(1-aryl-3-hete-

roaryl)propylidene]hydrazine dihydrochlorides were investigated against HSC-2, HSC-3, HSC-4 human oral squamous carcinoma cells and HL-60 human leukemia cell line as tumor cell lines and HGF, HPC, and HPLF human normal oral cells as non-tumor cells since some compounds showed rather impressive results against human hepatoma (Huh7) and breast cancer (T47D) cell lines [30, 32] with hopes to find out new lead compounds for treating dental cancer and leukemia problems. The results are summarized in Table 1.

The results presented in Table 1 show that the cytotoxicities of **P-** and **R-**series compounds against tumor cell lines vary in the range of 11 ± 0.68 – 300 ± 1.0 μM. These data suggest that **P-** and **R-**series compounds possess cytotoxic properties. Peplomycin which is a semisynthetic analog of Bleomycin, a mixture of several basic glycopeptide antineoplastic antibiotics was used as a reference compound to compare with the cytotoxic properties of **P-** and **R-**series hydrazones. Compounds **P1** (3 times), **P5** (1.9 times), **P6** (1.6 times), **R2** (3.3 times), and **R6** (1.9 times) had 1.6 – 3.3 times higher cytotoxicity than peplomycin against HSC-4 cell line. Compound **R2** showed cytotoxicity (29 ± 9.0 μM) comparable with that of peplomycin (28.3 ± 5.9 μM) against HSC-2 cell line. A similar situation was observed for cytotoxic activity against HL-60 cell line, where compounds **P7**, **P8** and the reference compound peplomycin showed almost the same activity (28 μM).

The tumor selectivity (TS) of each hydrazone compound was previously calculated in [41 – 47, 49 – 59]. For this purpose, the average CC_{50} value toward a total of four cancer cell lines (TS = B/ A, Table 1) [37, 40, 42, 46, 59]. This calculation pointed out that compounds **P4** showed the highest TS value (1.6). The other hydrazone derivatives having relatively high TS values were compounds **P5** and **R3** with the TS values of 1.4.

Thus, compound **P1** which is a non-substituted hydrazone derivative containing piperidine, and compound **R2** which is a 4-methyl hydrazone derivative containing pyrrolidine can be chosen as lead compounds for the cancers mentioned above and used for further investigations. **P-** and **R-**series hydrazone compounds were tested against HSC-2, HSC-3, HSC-4, and HL-60 cancer cell lines, HGF, HPLF and HPC normal cells. Compounds **P1** and **R2** were the most potent cytotoxic agents with the highest tumor selectivity in **P**- and **R**-series. These compounds may serve as lead molecules in further search of new drugs for solving cancer problems under consideration.

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TABLE 1. Cytotoxic Evaluation of N,N′-bis[(1-aryl-3-heteroaryl)propylidene]hydrazine Dihydrochlorides **(P1, P4-P8;** and **R1-R7)** Hydrazone Compounds.

Compound	CC_{50} (μM)									
	Human oral squamous carcinoma cell lines			Human leukemia cell line		Human oral normal cells				Tumor-specificity index (TS)
	HSC-2	HSC-3	HSC-4	HL-60	mean (A)	HGF	HPLF	HPC	mean (B)	B/A
P1	121 ± 11	115 ± 40	12 ± 0.5	88 ± 26	84	79 ± 14	72 ± 6.0	84 ± 7.8	78	0.9
P4	226 ± 8.0	248 ± 7.5	78 ± 6.0	90 ± 9.1	161	156 ± 3.1	297 ± 32	338 ± 3.5	264	1.6
P5	71 ± 6.1	41 ± 2.6	19 ± 3.1	61 ± 23	48	72 ± 14	92 ± 4.4	44 ± 6.0	69	1.4
P6	283 ± 37	83 ± 54	23 ± 1.7	241 ± 87	158	94 ± 63	167 ± 145	56 ± 24	106	0.7
P7	40 ± 3.8	20 ± 1.0	114 ± 43	28 ± 7.2	51	20 ± 1.0	25 ± 7.2	13 ± 1.2	19	0.4
P8	60 ± 23	78 ± 8.8	300 ± 1.0	28 ± 4.0	117	138 ± 25	144 ± 41	60 ± 9.7	114	1.0
R1	125 ± 17	110 ± 8	92 ± 11	125 ± 13	113	158 ± 1.0	161 ± 5.7	84 ± 1.0	134	1.2
R2	29 ± 9.0	11 ± 6	11 ± 0.68	33 ± 5.0	21	17.3 ± 4.6	15.3 ± 3.5	6.2 ± 3.0	12.9	0.6
R3	231 ± 60	152 ± 20	163 ± 50	125 ± 21	168	292 ± 6	148 ± 5.0	264 ± 69	235	1.4
R4	291 ± 23	251 ± 11	274 ± 17	195 ± 26	253	145 ± 3.8	152 ± 1.7	151 ± 41	149	0.6
R5	72 ± 3.5	66 ± 4.2	64 ± 7.5	53 ± 13	64	99 ± 33	73 ± 4.6	58 ± 9.1	77	1.2
R6	74 ± 19	18 ± 10	19 ± 0.95	47 ± 0.95	40	10.3 ± 0.55	11 ± 1.0	8.5 ± 1.2	9.9	0.2
R7	126 ± 10	87 ± 8.1	128 ± 4.4	98 ± 15	110	152 ± 1.7	103 ± 46	129 ± 17	128	1.2
Peplomycin	28.3 ± 5.9	6.6 ± 0.80	36.2 ± 2.9	28.0 ± 87	25	>400	>400	332 ± 38	>377	15.1

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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