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Evaluation of Anticancer Activities of Derivatives of 1,3,4-Thiadiazoles in Cell Line, Hep2 *In-vitro.*

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ABSTRACT

Present study was designed to evaluate anticancer Activities of Derivatives of 1,3,4-Thiadiazoles in vitro on cell line Hep2 using different concentrations(0.4 , 0.2, 0.1 and 0.05 µg/l), for an incubation period of 48 hrs. The results revealed a clear cytotoxic activity for all doses on growth of Hep2 cell line, and the effect was concentration-dependent. The results showed the best cytotoxic activity on Hep2 cell line, at the concentrations 0.2 µg/l, for C1 and C2 respectively. In contrast, the less cytotoxic activity on Hep2 cell line, were at the concentrations 0.1 µg/l, for C5 and C6 respectively

Keywords: 1,3,4-Thiadiazoles, Anticancer activity, Hep2, cytotoxicity

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INTRODUCTION

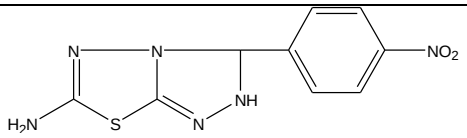
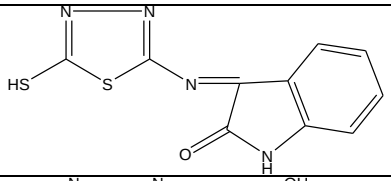
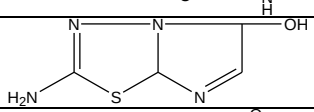
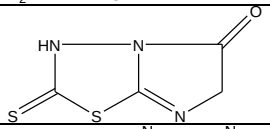
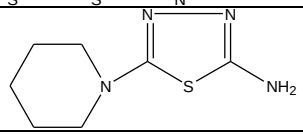
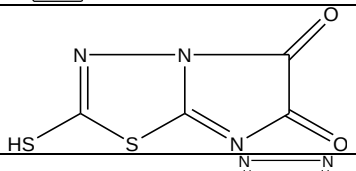
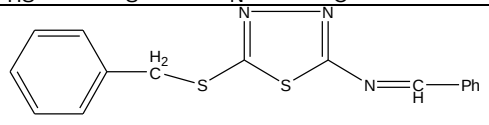
Thiadiazoles derivatives have a significant interest in medicinal.[1] A wide variety of 1,3,4-thiadiazoles derivatives have been found biologically active, e.g. they showed the anticancer activity [2, 3], antiviral, antibacterial [4,5], fungicidal [5] anti-inflammatory [7], anticonvulsant [8], carbonic-anhydrase inhibitory [9], and H-2-antagonist [10], activity. For these findings our work was study the anticancer activity of some new derivatives 1,3,4-thiadiazoles containing pyrimidino and Triazion Moieties that synthesized by Mohammad M. Saleh [11] and his group. The synthesized thiadiazoles derivatives were illustrated in (**Table 1**).

MATERIALS AND METHODS

Chemicals

The thiadiazoles derivatives used were synthesized (**Table 1**)by mohammad and his group in department of chemistry, college of science for women, university of Baghdad [11].

Table 1: Synthesized thiadiazoles derivatives

no	name	structure	symbol
1	3-(4-Nitro-phenyl)-2,3-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-ylamine		C1
2	3-(5-Mercapto-[1,3,4]thiadiazol-2-ylimino)-1,3-dihydro-indol-2-one		C2
3	2-Amino-5,7a-dihydro-imidazo[2,1-b][1,3,4]thiadiazol-5-ol		C3
4	2-Thioxo-2,3-dihydro-6H-imidazo[2,1-b][1,3,4]thiadiazol-5-one		C4
5	5-Piperidin-1-yl-[1,3,4]thiadiazol-2-ylamine		C5
6	2-Mercapto-imidazo[2,1-b][1,3,4]thiadiazole-5,6-dione		C6
7	Benzylidene-(5-benzylsulfanyl-[1,3,4]thiadiazol-2-yl)-amine		C7

Cell culture and Cytotoxicity

All the experiments of this study were carry out in the laboratories of the tissue culture unit of Biotechnology Research Center / Al-Nahrin University. Human epithelial type 2 (Hep-2) Cell Line was used. Cells were grown in RPMI-1640 medium containing 10% inactivated fetal calf serum (FCS) and penicillin (100U/ml)-streptomycin (100mg/ml) antibiotic. The cytotoxicity of 1,3,4-thiadiazoles derivatives was tested

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using the method of [12]. Each compound was dissolved in 0.1% dimethyl sulfoxide (DMSO) (Stock solution concentration 1mg/ml) and diluted with RPMI-1640 medium to give concentrations ranging from 0.4 – 0.05 µg / ml, the cells were grown in tissue culture flasks containing growth medium at 37ºC in an atmosphere of 5% CO_2 and 95% relative humidity in a CO_2 incubator.

The cells at subconfluent stage were gathered from the flask by handling with trypsin-versine solution (20 ml trypsin in 370 ml PBS containing 10 ml versine) and suspended in the medium. Cells with more than 97% viability (trypan blue exclusion) were used for determination of cytotoxicity. Cells were plated in 96 multi well plate for 24 hours in a CO_2 incubator at 37ºC. Different concentration of the tested substance (0.4, 0.2, 0.1,and 0.05 µg /ml) were added to the cells (three replicate wells were prepared for each individual concentration) and reincubated for the selected exposure time (48 hrs). Control cultures containing RPMI-1640 alone were tested for back ground cytotoxicity. After that, 50 µl of crystal violate stain were added to the wells, and the plates were incubated in a CO_2 incubator for 30 minutes at 37 ºC. The spot was washed gently with tap water for three times and reader at 492 nm. The inhibitory rate of cell growth was calculated as following formula [13]:

Inhibition (%)= [(Optical density of control wells - Optical density of test wells) / Optical density of control wells] * 100.

Death cell rate (%) = 100 - Inhibition (%)

Statistical analysis

The experimental data were analyzed using statistical software SPSS (SPSS 22 for windows, SPSS Ins. III., USA). Significant difference between control and sample means was assessed using Chi-Square and P values < 0.01 were considered significant.

RESULTS

The effect of treating Hep-2 cells by The thiadiazoles derivatives are shown in (**Figure 1**). Death cell rate in all doses (0.4 , 0.2, 0.1 and 0.05 µg / l) were observed in Hep-2 cell line in significant effect (p<0.01) on Hep-2 cell line.

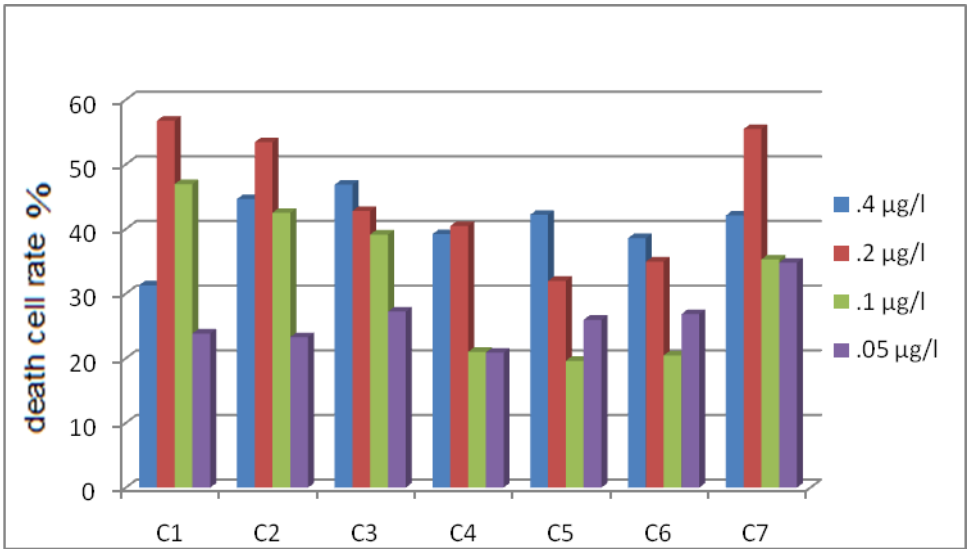

Figure 1: distribution of death cell rate according to compound used and their concentration.

The death cell rate percentage for C1, C2, C3, C4, C5, C6, and C7 on Hep-2 cell line at 0.4 µg/L were 31%, 45%, 47%, 39%, 42%, 39%, and 42% respectively. While for 0.2 µg/L were 57%, 53%, 43%, 40%, 32%, 35%, and 55% respectively, and 47%, 43%, 39%, 21%, 20%, 20%, and 35% for 0.1 µg/L concentration, While for 0.05 µg/L were 24%, 23%, 27%, 21%, 26%, 27%, and 35% respectively.

Based on the results of optical density the death cell rate effect was calculated, the results in table 2, revealed that the Hep-2 cell line was more sensitive to the concentration 0.2 µg / l in thiadiazoles derivatives (C1, C2, C4, and C7). Also, the results in table 1- showed that best concentration for thiadiazoles derivatives (C3, C5, and C6) was 0.4 µg / l than the other concentrations.

The best death cell rate percentage was for C1 on Hep-2 cell line at 0.2 µg/L concentration (57%), while the lower death cell rate percentage was for C5 and C6 on Hep-2 cell line at 0.1 µg/L concentration was (20%).

Table 2: Percentage of death cell rate of Hep-2 cells by using thiadiazoles derivatives at different concentration

µg / l	C1	C2	C3	C4	C5	C6	C7	Control
0.4	31	45	47	39	42	39	42	0
0.2	57	53	43	40	32	35	55	0
0.1	47	43	39	21	20	20	35	0
0.05	24	23	27	21	26	27	35	0

DISCUSSION

In this study, thiadiazoles derivatives demonstrated different cytotocicity in vitro toward Hep-2 cell line according to its concentration. The present results showed in (**Table 2**) indicate that increasing number of substituted groups effected on the death cell rate of Hep-2 cells. Nadia and Mona found that the presence of the 4-chlorophenyl and 4-bromophenyl substituent was essential for anticancer activity [14].

Zeyad F. and Anwar H. suggested that different properties of thiadiazoles depend on the electron charge density [15], and that may explain different toxicity between thiadiazoles derivatives

Comparison of compounds C1 with C6, C2 and C7 with C5, allows the conclusion that the Nitro-phenyl in C1 and phenyl in C7 was more active than Mercapto-imidazo derivatives in C6 against Hep-2 cells studied.

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