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A Study on Antitumor Effect of 1,3,4-Thiadiazole Derivatives in Prostate and Breast Cancer Cell Lines (*In Vitro*)

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Abstract

5-(4-aminophenyl)-2-amino -1,3,4-thiadiazole was prepared by reaction of Thiosemicarbazide with 4-amino benzoic acid under reflux condition for 7 hours. The compound which has been synthesized successfully was subjected to addition reaction with 4-(Dimethylamino) benzaldehyde under reflux condition for 6 hours to synthesize Schiff bases. These compounds was characterized by using FTIR) and evaluated for their anticancer activity. The effect of (1, 3, 4-thiadiazole derivative) on the activity of malignant cells was studied by using different types of cell lines [Breast cancer, and human prostate cancer]. And was used the Electron microscope to show that the effect of the derivative on the cancer cells before and after 3 days of the injection time. It was found that the Schiff base of thiadiazole-1,3,4-(Dimethylamino)benzylidineamino]- [4-2-phenyl]amino was effective in reducing the size and density of malignant cells. That of 46.7 while in breast) 145(DUprostate for growth inhibition produce of equal 85.9 µg/ml.

Keywords: 1,3,4-Thiadiazole; 1,2,4-Triazole; synthesis; MTT assay; Cytotoxicity of Schiff base

Introduction

Cyclic compounds having as ring members atoms of at least two different elements, e.g. quinoline,1,2-thiazole, bicyclo[3.3.1]-tetrasiloxane . Thiadiazole contains the five-membered di-unsaturated ring structure having molecular structure formula $C_2H_3N_3S$ containing a two carbon atom, three hydrogen, three nitrogen and one sulphur. Thiadiazole and its derivatives are used for biological activities such as antiviral, antibacterial, antifungal and antitubercular. It is clear to yellowish liquid with a pyridine like odor and soluble in alcohol [1]. In the last few years, heterocyclic compounds were not only used for development the heterocyclic derivatives, but also argumentation of the application in pharmaceutical and chemical field. Till date various heterocyclic compounds had been synthesized and evaluated for their significance. Firstly, Fischer was introduced 1,3,4-thiadiazole in 1882, whereas Freund and Kuh were described the true nature of the ring. In addition, thiadiazole is a widespread and important five-member heterocyclic system which contains two nitrogen atoms and a sulfur atom. 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, and 1,3,4-thiadiazole isomer of thiadiazole was discovered and evaluated for the biological activity[2].

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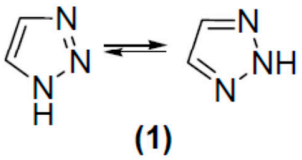
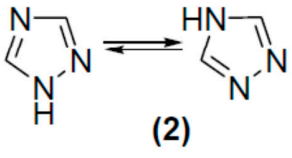


(1)



(2)

1,2,3-Triazoles are an important class of heterocycles due to their wide range of applications as synthetic intermediates and pharmaceuticals [3]. Several therapeutically interesting 1,2,3-triazoles have been reported, including anti-HIV agents [4], antimicrobial compounds [5], □□-selective adrenergic receptor agonists [6], kinase inhibitors [7] and other enzyme inhibitors [8]. The 1,2,3-triazole moiety is also present in a number of drugs, for example, the β-lactam antibiotic tazobactam [9] and the cephalosporin cefatrizine [10]. The presence of three nitrogen hetero-atoms in five-membered ring systems defines the triazole as an interesting class of compounds. This may be of two types, the 1,2,3-triazoles (1) and the 1,2,4-triazoles (2).

Chemotherapy is the use of any drug (such as aspirin or penicillin) to treat any disease. But for most people, chemotherapy refers as drugs used for cancer treatment. It's often shortened to "chemo." Chemotherapy is used to treat many cancers. More than 100 chemotherapy drugs are used today – either alone or in combination with other drugs or treatments. These drugs vary widely in their chemical composition, how they are taken, their usefulness in treating specific forms of cancer, and their side effects [11].

Material and Methods

Synthesis of 5-(4-aminophenyl)-2-amino -1,3,4-thiadiazole from Thiosemicarbozide[12]

Thiosemicarbazide (0.911g ,0.01mol) and 4-amino benzoic acid (0.137g , 0.01mol) was dissolved in 10 ml of abs. ethanol after mixing in around bottom flask, drop of hydro sulfuric acid was added to mixture and refluxed for 7 hours. The resulting solution was cooled to room temperature and the precipitated solid was filtered under suction, washed with cold ethanol and recrystallized with hot ethanol. m.p (213- 215)C. yelid 68% deep yellow.

Synthesis of 5-[4-aminophenyl]-2-[4\-(Dimethylamino) benzylidineamino]-1, 3, 4-thiadiazole

5-(4-aminophenyl)-2-amino-1,3,4thiadiazole(1.92g,0.01mol)and4(Dimethylamino)benzaldehyde (0.1.49 g, 0.01mol) was dissolved in 20 ml of abs. ethanol after mixing in around bottom flask,and refluxed for 6 hours . The resulting solution was cooled to room temperature and the precipitated solid was filtered under suction, washed with cold ethanol and recrystallized with hot ethanol. m.p(288 - 290)C. yield 68% deep brown.

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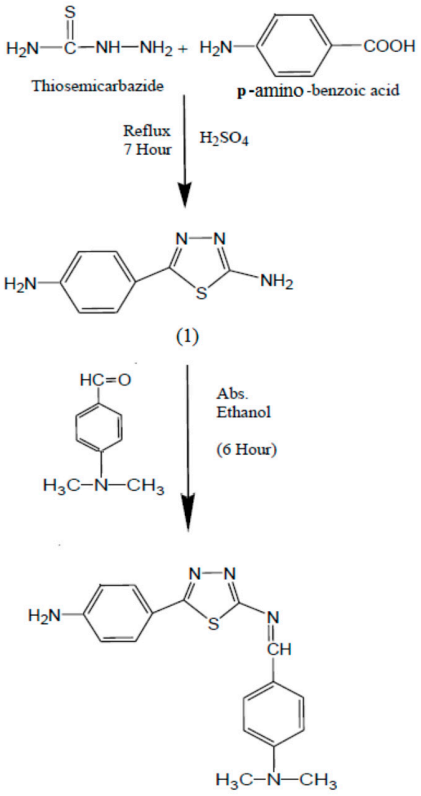

Sechem (1) Synthesis of shiff base of 5-[4-aminophenyl]-2-[4\-(Dimethylamino) benzylidineamino]-1,3,4-thiadiazole

MTT Cell Assay Protocol [13]

Day 1: Set up

Cell Dilution

1. Harvest cell, either by centrifugation (if suspension) or by trypsin

1. Stain 90ul cell suspension with 10ul of Trypan blue.

2. Determine whether the cells have greater than 90% viability.

3. Determine a cell count.

4. resuspend cells at the density needed for the MTT assay:

a. Density should be determined by a density experiment in general for leukemic cells, a density of 0.5-1.0x103 cells ml works for primary leukemic samples (which do not divide, plate at 1x106 cells ml. For solid tumors, the density varies from 1x104 to 1.5x105 s, so you will need to determine the appropriate density for each cell line.

b. For two plates of cells, you will need a total of 25ml of cells suspended in media.

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5. Calculate the proper number of cells needed for assay:

ie. 23/2 (23 alive cells/2 dead); cells needed: 1.5x105

(1.5x105 cells) (25ml)

10.8 ml cell suspension to be diluted to 25ml with media

2.3x105cells

Machine and Plate Set-up

Label your plates with tumor type, passage, drug, date, and name on cover.

Mark plate along the side so that you know which top belongs to which plate.

l. Turn on machine/ computer button by left foot)

2. Run method

3. Choose program CellPZA. Press "enter" 0 times to begin

4. Incubate your plates in 37C, 5% CO2 overnight. (Suspension cells can be

plated the same day and incubated for several hours, but it's best to do all the

plating the day before drug is added to decrease variability.

Day 2 Adding the Drug

l. Dry modules for drug dilutions. For each drug, you will need four-1/8

modules and one quarter-module for PBS

2. Determine amount of PBS and drug needed using the MTT single drug

assay sheet. Serial dilutions of 1:1o work well for initial experiments, I:3

or 1:4 work well when you have a ball- park idea of the IC50 for that drug in

your cells.

3. Set up wells for drug addition, Add PBS into each well.

4. Make drug dilutions starting with highest concentration and working down. Make sure to mix each module thoroughly before making the next dilution

5. Do a serial dilution of drug stock. Ex:

Drug Stock Conc.1 uM or 100 nM

Dilute to 50 nM

25 nM

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12.5 nM etc...

Put an additional 3ml PBS in the first well and add 6ul of 1 uM drug stock.

Mix and remove 3ml of the drug dilution to the next well, continuing the process from the right to the left until you end up with 6ml of diluted drug in the last well

6. Run program PBSA2-H3 to add 15ul PBS to columns 2 and 3

7. To add drug Run program ADDPZA2 for 2 plates ADDPZA for 1 Plate

8. After drug has been added, place the plates on the shaker for 3-5 min. on Setting 5.

9. Incubate plates for 72 hrs. 37C. cover with foil of light sensitive.

Day 3: Adding MTT and Reading Plates

Adding MTT

l. Fill a quarter module with a 2ml reservoir 2nml plate of MTT (ie. For 6 plates, need 10 ml MTT)

2. Place plates in on the left side of an empty cartridge) proper position

3. Run prgrm MTT- 15ul

4. Shake the plates for ~5min (a setting 6)

5. Incubate 4 hrs.

Preparing Plates for Reading

l. Spin the plates down for 5 min. A 1000rpm (for suspension cells)

2. Place half-module on the left side of an empty cartridge and select program: WASHI50 (this program takes out 150ul media out of each well

3. Meanwhile prepare a half module filled to the top with DMSO 4/5 you will need this amount or DMSO for every two plates, refill as you continue with more than 2 plates)

4. When each plate has undergone WASHI50 program, replace the module containing the supernatant with the module filled with DMSO select program: DMSO150 adds DMSO to each well Reading Plates

I. Turn on plate reader first, then the computer and printer

2. A the prompt type: mlabl /parcom

3. A Next prompt type: arcom30

4. Press enter a first choice in Main Menu

5. Type in your filename: ie. "JCos1205a" initials, date, plate Identification (ie.a-f) filter: 550

Press F1 to collect data

6. For next sample, rename your file and press F1 to collect data

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7. Press Escape, then FI to show data

8. Select plate to be printed and press F9

9. To copy the file: ESC out of program and type "copy JC041205a.msw A:" you have multiple files with the same shortcut, you can use as wildcard.

10. Run data through Excel program to calculate cytotoxicity.

Results and Discussion

Cytotoxicity of Schiff base

The Schiff base of 1,3,4-thiadiazol, shows breast cancer is high activity found in concentration 0.2µg/ml. When increase the concentration of 1,3,4-thiadiazol derivative, the growth inhibition on cell line of breast cancer (MCF7) was increased. In 0.01 µg/ml concentration of 1,3,4-thiadiazol, No effect on prostate cancer that differ in other conc. In 0.2 the growth inhibition of prostate equal 46.7 while in breast equal 85.9.[14-16]

Table 3:- Inhibition growth of 1,3,4-thiadiazole derivate for prostate (DU145) and breast cancer (MCF7) cell line concentration of this derivative

Cell lines / Conc.Mg/ml	Prostate DU_{145}	Breast (MCF_7)
0.01	0	2.1
0.05	7.9	39.6
0.1	25.3	65.8
0.2	46.7	85.9

Conclusion

The 1,3,4-thiadiazole derivative have the ability to reduce the proliferations activity of cell line tumor (prostate & breast cancer). Schiff base of 2-[4\-(Dimethylamino)benzylidine]amino-5-(4-aminophenyl)-1,2,3thiadiazole possess anticancer activity as described in percentage inhibition of tumor cell lines. In 0.2 the growth inhibition of prostate equals 46.7 while in breast equal 85.9. So that means effect of this thaidiazole derivative in breast cancer more than in prostate cell line

Acknowledgements

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