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Synthesis of Novel Disperse Dyes based on Arylazophenols: Part 2. Anticancer Activities

Morsy Ahmed El-Apasery[1*], Mahmoud Elsayed Ahmed Abdellatif [2], Fathy Ahmed Yassin[2], Sara Morsy Ahmed[3]

[1]*Dyeing, Printing and Textile Auxiliaries Department, Textile Technology Research Institute, National Research Centre, 33 El Buhouth St., Cairo 12622, Egypt.*

[2]*Department of Chemistry, Faculty of Science, Zagazig University, Egypt.*

[3]*Applied Biosciences and Process Engineering Department 7, Anhalt University of Applied Sciences, Bernburger Street 55, P.O. Box 1458 06366 Köthen, Germany*

Abstract

As a continuation of our strategy towards synthesis new dyes, this has already been done in our previous study, and here we are presenting and studying the activity of these new dispersive dyes against two widespread types of cancer.

***Keywords*:** Disperse dyes, Anticancer activities, Arylazophenols.

1. Introduction

Enaminones have become widespread during the past ten years due to its effective effect in preparing some of main important organic compounds. It understood that enaminones are polydentate reactants that have large function in organic chemistry [1-4].One way of reusing the dye solution is to replenish the dye solution by adding the required amount of dye and chemicals to prevent and reduce contamination.

Enaminones have been utilized in the planning of different naturally dynamic compounds, just as dye intermediates [5]. In past investigations,

We have depicted the utility of β-enaminones as forerunners to poly functional organic chemistry [6].

According to our study we were able to synthesize anew series of enaminones were prepared according to the published procedures [7], mixtures of methyl ketones (acetophenone, p-methyl acetophenone, p-bromo acetophenone) (0.01 mol) and DMFDMA (1.19 g, 0.01 mol) was refluxed for 12-16 h. Completion of the reactions was monitored by TLC. The reaction mixture left to cool to room temperature and then treated with petroleum ether. The solid product, so formed, was collected by filtration and crystalized from a proper solvent to afford compounds 3-Dimethylamino-1-phenylpropenone, 3-Dimethylamino-1-p-tolyl-propenoneand 1-(4-Bromophenyl)-3-dimethylamino-propenone as yellow crystals with 60% yields.

Enaminones were exposed to coupling process with arylidene diazonium chloride to afford the disperse dyes 1(a-f).

The dyes 5(a-f) were reacted with ultra-pure acetone to create novel dispersion dyes 5(a-f), which possessed good results as cytotoxic compounds against different types of carcinoma cell line.

Cell cytotoxicity refers to the ability of certain chemicals or mediator cells to destroy living cells. By using a cytotoxic compound, healthy living cells can either be induced to undergo necrosis (accidental cell death) or apoptosis (programmed cell death).

Also, cytotoxicity is one of the most important indicators for biological evaluation in vitro studies. In vitro, chemicals such as drugs and pesticides have different cytotoxicity mechanisms such as destruction of cell membranes, prevention of protein . Synthesis, irreversible binding to receptors etc. In order to determine the cell death caused by these damages, there is a need for cheap, reliable and reproducible short-term cytotoxicity and cell viability assays. Cytotoxicity and cell viability assays are based on various cell functions.

A broad spectrum of cytotoxicity assays is currently used in the fields of toxicology and pharmacology [8]. There are different classifications for cytotoxicity and cell viability assays, these assays are classified according to measurement types of end points (color changes, fluorescence, luminescent etc.). Dye

*Corresponding author e-mail: elapaserym@yahoo.com.; (Morsy Ahmed Elapasery).

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exclusion: Trypan blue, eosin, Congo red, erythrosine B assays. Colorimetric assays: MTT assay, MTS assay, XTT assay, WST-1 assay, WST-8 assay, LDH assay, SRB assay, NRU assay and crystal violet assay. Fluor metric assays: Alamar Blue assay and CFDA-AM assay. Luminometric assays: ATP assay and real-time viability assay. In this study we will use crystal violet colorimetric assay to evaluate the cytotoxic effect of the synthesized dyes.

2. Materials and Methods

The disperse dyes were prepared according to the procedures that we published in our previous research and checked by mass spectroscopy, essential examination, FT-IR, and 1H-NMR spectroscopy [9].

Cytotoxicity evaluation using viability assay:

The cells were seeded in 96-well plate at a cell concentration of 1×104 cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37ºC in a humidified incubator with 5% CO2 for a period of 24 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO.

The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for at 37°C, for 24 h, the viable cells yield was determined by a colorimetric method.

In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed.

Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Micro plate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain.

Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated. The optical density was measured with the micro plate reader (Sun Rise, TECAN, Inc., USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)] x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells.

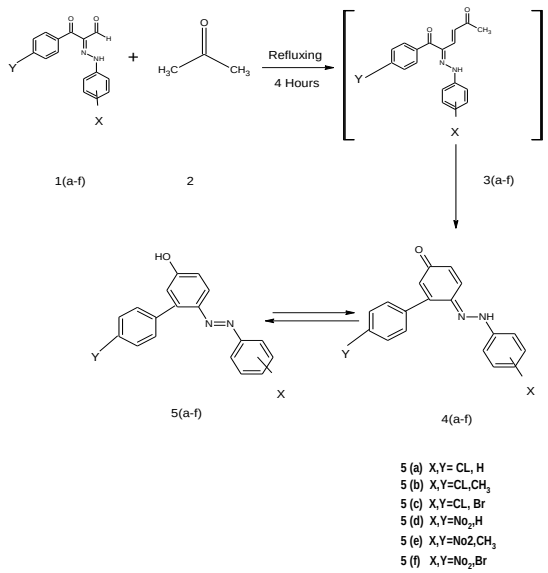
The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graph pad Prism software (San Diego, CA. USA) [10], [11].

3. Result and discussion

Anti-tumer activity:-

Cytotoxic effect was carried out at the regional center for mycology andBiotechnology at Al-Azhar University against Hepatocellularcarcinoma cells and lung cellular carcinoma. (IC_{50}) isthe concentration required to cause toxic effects in 50% of intact cells.

In vitro cytotoxicity assays should have some of advantages, such as speed, reduced cost and potential for automation, and tests using human cells may be more relevant than some in vivo animal tests. However, they have some disadvantages because theyare not technically advanced enough yet, to replace animal tests.



Scheme 1: Structures of new disperse dyes

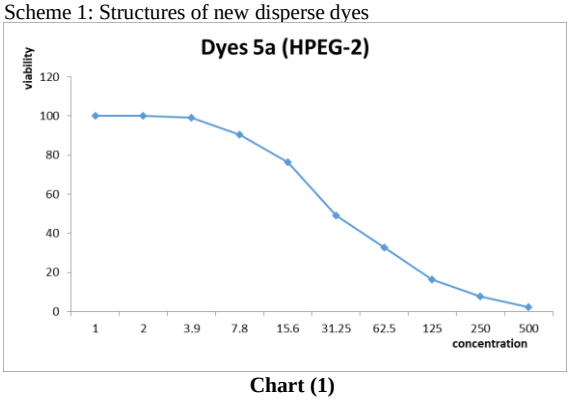


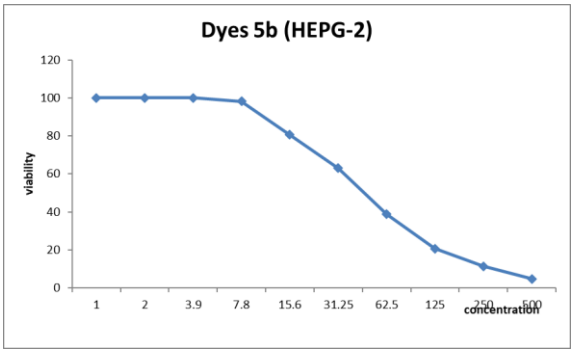
Chart (1)

Table (1)

concentration	5a		5b		5c		5d		5e		5f	
	Viability HePG 2	Viability A-549	Viability HePG 2	Viability A-549	Viability HePG 2	Viability A-549	Viability HePG 2	Viability A-549	Viability HePG 2	Viability A-549	Viability HePG 2	Viability A-549
0	100	100	100	100	100	100	100	100	100	100	100	100
1	100	100	100	100	100	100	100	100	100	100	100	100
2	100	100	100	100	100	100	100	100	100	100	97.14	100
3.9	99.52	100	100	100	98.86	100	98.42	100	100	100	88.46	98.61
7.8	90.48	98.34	98.15	99.23	93.72	97.28	85.40	99.20	100	100	70.49	83.75
15.6	76.31	81.29	80.63	89.54	78.94	84.16	67.18	80.23	100	100	46.32	67.24
31.25	49.02	56.68	62.94	78.48	57.13	62.95	45.23	57.14	98.06	99.65	35.18	45.93
62.5	32.68	37.14	38.71	46.32	39.08	43.16	31.06	36.21	87.49	92.71	23.47	31.79
125	16.49	20.61	20.64	29.58	18.27	25.03	14.98	18.65	48.17	61.42	10.85	16.52
250	7.54	9.82	11.39	18.76	9.76	13.48	6.23	9.42	32.06	38.27	5.19	7.26
500	2.35	3.94	4.78	6.41	4.37	5.29	1.96	2.73	14.93	19.46	1.65	2.34

Table (2)

Dye Number	Cytotoxic activity (IC_{50} µg/ml) HepG-2	Cytotoxic activity (IC_{50} µg/ml) A-549
Dye 5a	30.63 ± 1.23µg/ml.	41.93 ± 2.91 µg/ml.
Dye 5b	47.93 ± 3.57 µg/ml.	58.92 ± 4.12 µg/ml.
Dye 5c	43.59 ± 2.06 µg/ml.	51.69 ± 3.86 µg/ml.
Dye 5 d	27.80 ± 0.96 µg/ml.	41.91 ± 2.65 µg/ml.
Dye 5 e	122.09 ± 4.71 µg/ml.	186.65 ± 6.73 µg/ml.
Dye 5 f	14.41 ± 0.58 µg/ml.	28.22 ± 1.68 µg/ml.
cisplatin	3.67 ± 0.15 µg/ml.	7.53 □ 0.51 µg/ml.
imatinib	18.9 µg/ml.	



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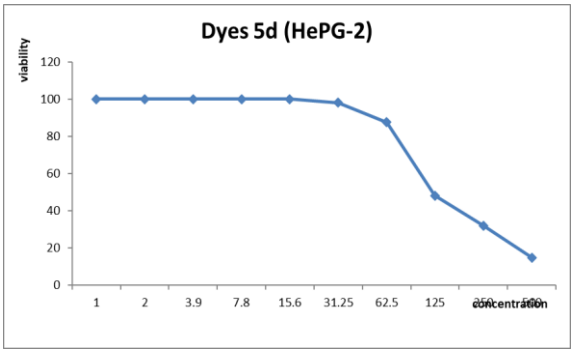

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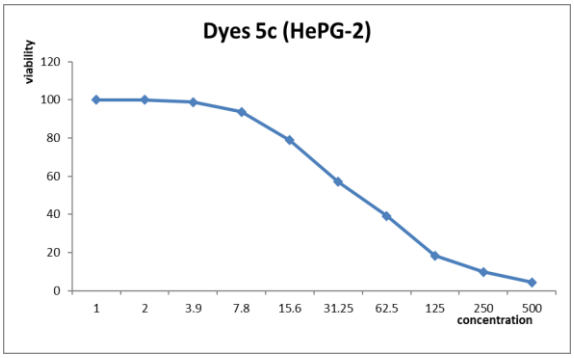

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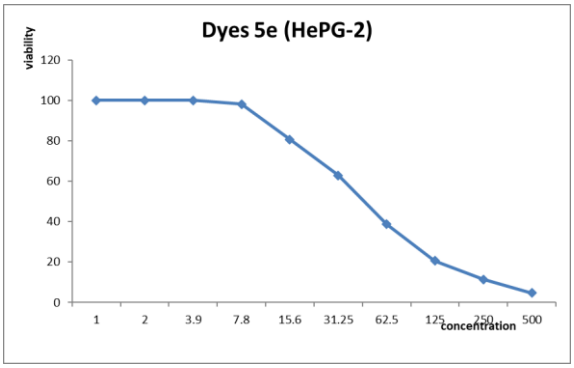

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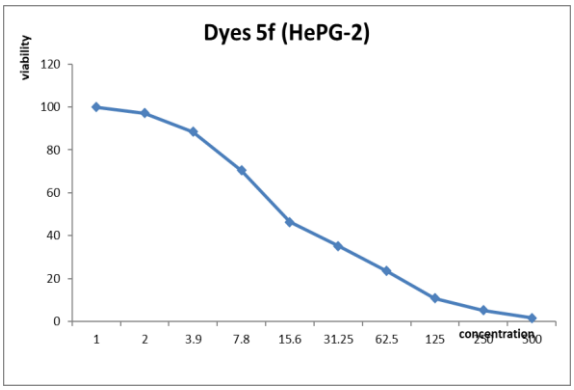

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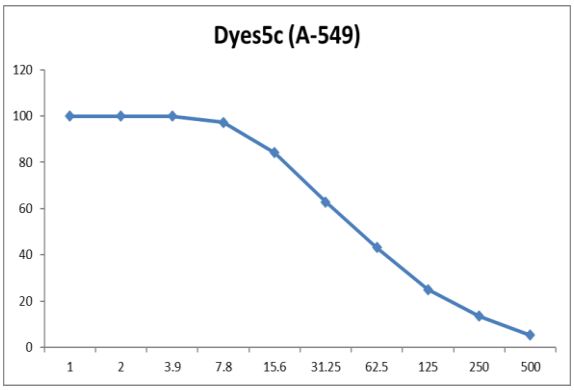

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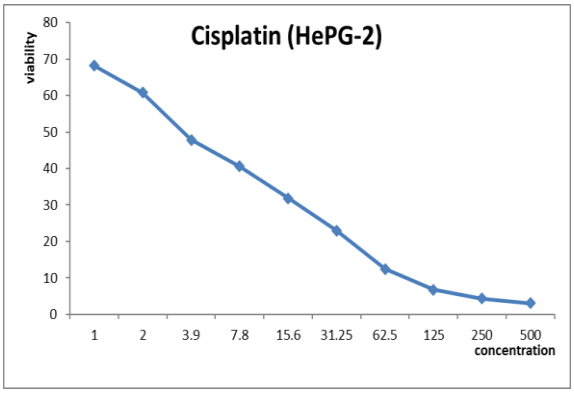

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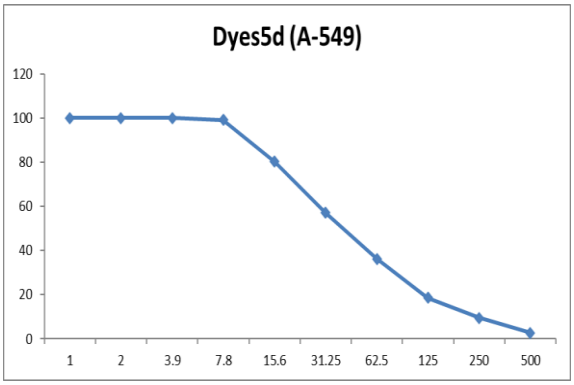

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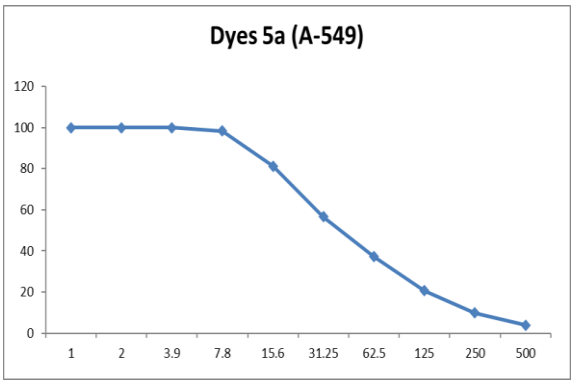

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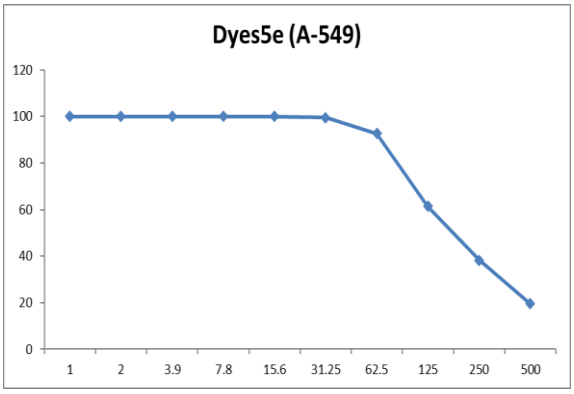

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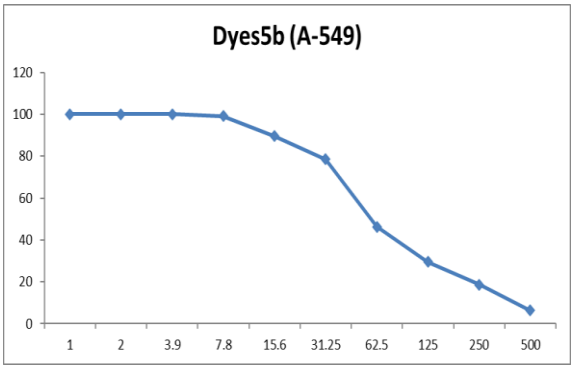

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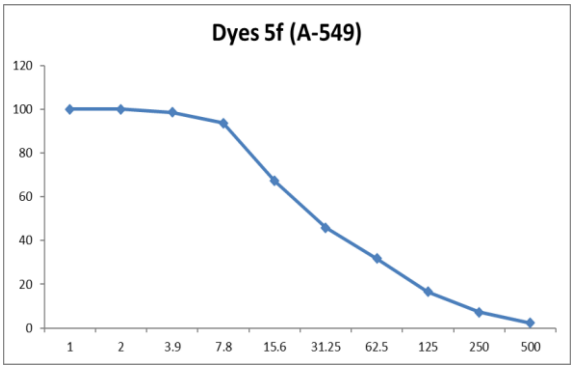

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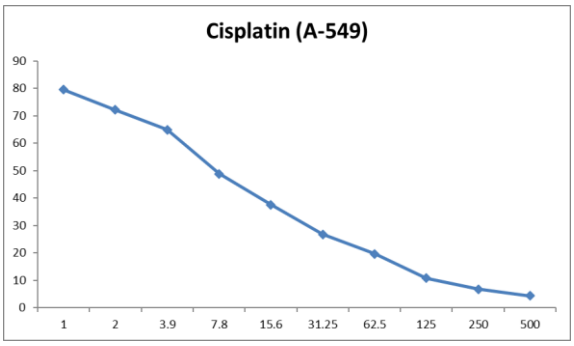

Chart 14

The cytotoxic effects observed for azo dyes might be due to the action of dyes on the cells or, especially, to the formation of metabolites resulting from the azo bond reduction.

Metabolites can react with the DNA molecule, damaging both its structure and function. Because of the significant increase in chemical compounds being discharged into the environment, bioassays have been carried out using different organisms in order to identify and evaluate the harmful effects of various agents at their different concentrations and exposure periods.

The in vitro inhibitory activities of tested compounds against the Hepatocellular carcinoma cell lines (HEPG-2) have the descending order as follow

Dye 21 (f)> Dye 21 (d)> Dye 21 (a)> Dye 21(c)> Dye 21 (b)> Dye 21 (e) according to the data listed in table (2).

The in vitro inhibitory activities of tested compounds against theLung carcinoma cell lines (A-549) have the descending order as follow

Dye 21 (f)> Dye 21 (d)> Dye 21 (a)> Dye 21(c)> Dye 21 (b)> Dye 21 (e) according to the data listed in table (2).

4. Conclusions

In this study, the activity of these dyes was reviewed for two important types of cancer, and these dyes gave promising results for the possibility of using them in various medical purposes

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