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Cytotoxic and Anti-Oxidant Evaluation of Novel 4,4'-(4-Substituted Phenylpyridine-2,6-Diyl) Bis (N-Substituted Benzylidene Aniline) Derivatives

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ABSTRACT

In the present investigation, a series of newly reported Schiff bases of nitrogen containing heterocyclic nucleus evaluated for their possible Cytotoxic and Antioxidant activities. The synthesised compounds 4,4'-(4-phenylpyridine-2,6-diyl)bis (N-benzylideneaniline) derivatives (6a-o) were synthesized from an efficient condensation reaction of 4-Substituted Phenyl-2, 6-bis(4-Amino Phenyl) Pyridine, with Substituted Aromatic Aldehydes. The characterisation of the newly synthesized compounds have been confirmed from its elemental analysis and spectral studies (IR, ^{1}HNMR, ^{13}CNMR and Mass). The synthesized Schiff bases were evaluated for their cytotoxic activity and Antioxidant activity by MTT assay and Nitric oxide (NO) radical scavenging method respectively.

KEY WORDS: Cytotoxic activity, Antioxidant activity, Schiff base and free radical scavenging.

1. INTRODUCTION

Schiff base which contain an azomethane group attract much enthusiasm in synthetic chemistry. Schiff base with atoms (N,O,S) have structural similarities with natural biological systems and imports in explaining the component of changes and reseminations reactions in biological systems due to presence of azomethane (-N=CH-) group (Keskioglu, 2008). Schiff base are used as precursor in the preparation of a number of industrial and biologically active organic compounds via closure, cycloaddition and rearrangement reactions. Moreover, Schiff base are reported to have biological activities such as bactericidal (Sithambaram Karthikeyan, 2006; Singh, 2006), fungicidal (Sridhar, 2001; Pandeya, 1999), cytotoxic (Mladenora, 2002; Walsh, 1996) and free radical scavenging activities.

2. MATERIALS AND METHODS

Chemicals and cell culture the Dulbecco's modified Eagles medium (DMEM), 3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT), phosphate buffered saline (PBS), antibiotic/antimycotic solution, EDTA and trypsin were purchased from Sigma Chemicals Co. Fetal bovine serum (TMS-013-B) and 96 well plate were purchased from Merck. All other reagents were of analytical grade. MCF-7 (Breast adenocarcinoma cancer cell line), HT-29 cells (human colorectal cancer cell line) and HeLa (Human Cervical Carcinoma Cell line) were purchased from NCCS, Pune. Nitric Oxide, Griess reagent, Sodium nitro prusside, phosphate buffer pH (7.4)

Experimental Section:

Cytotoxic activity (Venkanna, 2014): The cell lines (MCF-7, HT-29 and HeLa) were kept up in culture with MEM supplemented with 10% Fetal bovine serum (FBS) and the antibiotics penicillin/streptomycin (0.5-1ml) in atmosphere of 95% air and 5% CO_2 at 37°C. Stock solutions of synthesized 4,4'-(4-phenylpyridine-2,6-diyl)bis(N-benzylideneaniline) derivatives (6a-o) were made in DMSO and kept in aliquots at -20°C for MTT assay, each test compound was weighed independently and dissolved in DMSO, made up the final concentration with media to 1 mg/ ml and the cells were treated with series of concentrations from 10 to 100µg/ml of test compounds (6a-o).

MTT assay: Inhibition of cell expansion by 4,4'-(4-phenylpyridine-2,6-diyl)bis (N-benzylideneaniline) derivatives (6a-o) were determined by using the methyl thiazolyltetrazolium (MTT) cell viability assay with three independent experiments with different concentrations of compounds in triplicates. MCF-7, HT-29 & HeLa cells were trypsinized and preformed the tryphan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 X 10^3 cells/well in 100µl media in 96 well plate culture medium and brooded overnight at 37°C. After incubation, the old media was taken off and added with new media 100µl with various concentrations of test compound in separate wells in 96 plates. After 48 hrs, the medication arrangement disposed of and the new media with MTT solution (0.5 mg/ml) was added to each well and plates were brooded at 37°C for 3 hrs. At the end of brooding time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was estimated at 570 nm on a microplate reader. The rate development inhibition was calculated utilising the following equation and concentration of test drug needed to inhibit cell development by 50% (IC_{50} values) is produced from the dose-response curves for each cell line.

%Inhibition=100 (Control-Treatment)/Treatment

Antioxidant activity (Mohammad, 2010; Green, 1982): All the newly reported compounds were tested for their *in vitro* Nitric oxide (NO) free radical scavenging activity.

Nitric oxide (NO) Radical Scavenging activity: The Nitric Oxide radical scavenging activity was calculated by using Griess reagent. Each of synthesized derivatives (20-200 µg/mL) and ascorbic acid (standard) of various concentrations (20–200 µg/mL) were incubated with 5mL of sodium nitroprusside solution (5mM) in standard phosphate buffer (pH 7.4, 5 mL) at 25°C for 5 hours. Control was prepared without compound but with equal amount of buffer. After incubation, add 0.5mL of the incubation mixture was mixed with 0.5 mL of Griess reagent and the absorbance measured at 546 nm against blank (DMSO). From the absorbance the percent scavenging activity was calculated using the formula. The experiments were performed in triplicate.

$$\% \text{ scavenging} = Ac - As/Ac \times 100$$

Where, As = the absorbance of the test sample; Ac = the absorbance of the control.

3. RESULTS AND DISCUSSION

In this study, the newly synthesised new Schiff bases were screened for their cytotoxic activity and antioxidant activity.

The *in vitro* cytotoxic activity of all the synthesized (6a-o) compounds was carried out by MTT assay method. An observation of the results reveals that amongst the new synthesized compounds 6a-o, compound 6h and 6g exhibited excellent activity. Compounds 6l, 6b, 6m, 6k, 6f, 6o, 6c, 6n, 6i, 6j, 6e and 6d were found to exhibited significant biological activity as compared to standard Cytotoxic agent Cisplatin (Table.1).

All the synthesized compounds (6a-o) were screened for antioxidant activity using nitric oxide free radical scavenging method. The nitric oxide assay has been broadly used to assess the free radical scavenging effectiveness of different antioxidant substances. Nitric oxide created because of decay of sodium nitroprusside in aqueous medium, interacts with oxygen at physiological pH to generate nitrite ions, which are estimated by using Griess' reagent. The nitrite ions were subjected to diazotization reaction followed by azo-coupling reaction to yield an azo-dye, estimated by an absorption band at 546nm. The scavenging activity of the synthesized compounds was compared with ascorbic acid as a standard. Compounds 6h, 6m, 6g, 6c and 6b produced better scavenging ability (Table-1), remaining compounds showed moderate radical scavenging activity.

Table.1. IC_{50} (µM) values of Cytotoxic and Antioxidant activity of 4,4'-(4-phenylpyridine-2,6-diyl)bis(N-benzylideneaniline) derivatives (6a-o)

Compound	Substituent		Cytotoxic activity			Antioxidant activity
	(R)	(R')	MCF-7	HT-29	HeLa	
6a	H	H	75.36	73.23	72.15	54.09
6b	H	4-CN	35.23	33.56	36.15	34.73
6c	H	4-Cl	39.56	42.1	43.52	31.06
6d	H	2,3-OH	64.19	62.52	63.26	55.11
6e	H	2,4-OH	62.1	61.74	63.16	53.61
6f	4-CN	H	41.56	40.86	40.25	38.50
6g	4-CN	4-CN	29.16	27.34	26.29	30.12
6h	**4-CN**	**4-Cl**	**23.51**	**24.85**	**25.76**	**21.83**
6i	4-CN	2,3-OH	45.16	46.56	46.78	42.62
6j	4-CN	2,4-OH	49.95	51.46	53.29	43.82
6k	4-Cl	H	35.56	37.34	39.28	38.86
6l	4-Cl	4-CN	33.83	32.31	31.54	36.73
6m	4-Cl	4-Cl	37.75	38.65	38.43	24.86
6n	4-Cl	2,3-OH	47.28	45.48	43.67	39.03
6o	4-Cl	2,4-OH	45.27	43.29	40.68	38.42
Cisplatin	-	-	4.34	5.15	6.28	-
Ascorbic acid	-	-	-	-	-	8.96

4. CONCLUSION

The new synthesized title compounds (6a-o) are characterized by spectral data and evaluate for their Cytotoxic and Antioxidant activity. From the synthesized compounds 6h and 6b showing good cytotoxic activity and maximum free radical scavenging activity. Therefore, the series has created new interest for possible modifications of the pharmacophoric replacements of Cytotoxic, Antioxidant and future exploitation.

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