Original Article

SYNTHESIS AND SCREENING OF NOVEL MANNICH AND SCHIFF BASE DERIVATIVES OF ISATINS FOR CYTOTOXIC AND ANTIOXIDANT ACTIVITY

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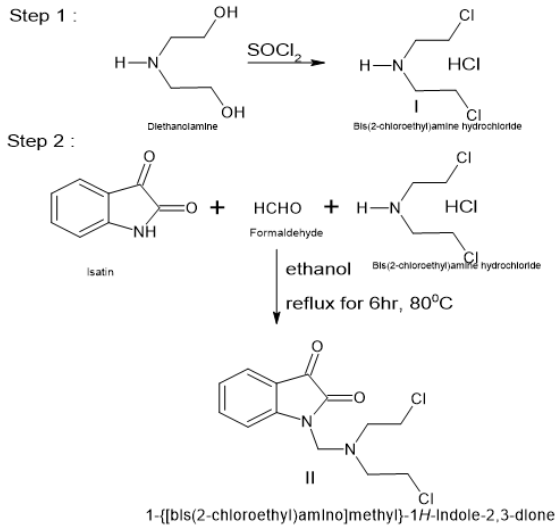
Abstract

A series of novel of 1-{[bis(2-chloroethyl)amino]methyl}-3-(phenylimino)1,3-dihydro-2H-indole-2-one derivatives(IIIa-j) were synthesized using appropriate methods. Structural confirmation for the synthesized compounds were done by spectral studies such as 1HNMR, MASS,IR etc. All the compounds were tested for cytotoxicity assay and antioxidant activity. All the compounds showed good cytotoxic activity. Of them, compound IIIi (R=4-F) was observed to possess more cytotoxic activity. All the derivatives were screened for antioxidant activity and the results were compared with the standard drug Ascorbic acid. All the series of the compounds showed antioxidant activity. Compound IIIi (R=4-F) was found to be more effective antioxidant.

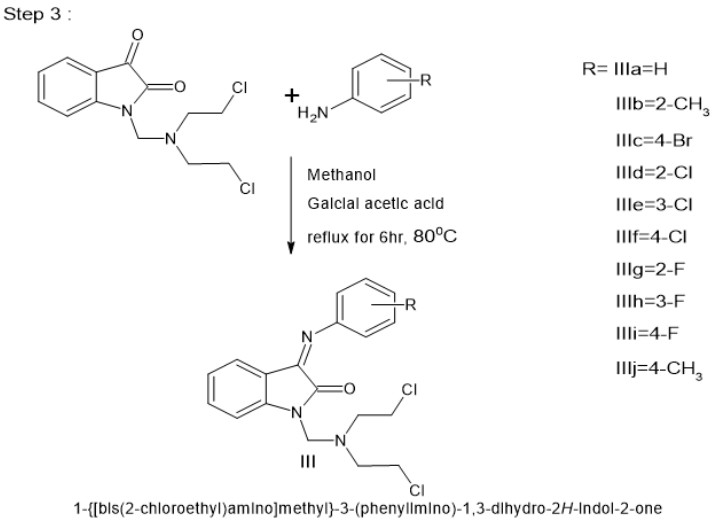
Keywords: Isatin, antioxidant activity, nucleophiles, NMR and MS, cytotoxic activity.

Introduction

Materials and Methods:



1-{[bis(2-chloroethyl)amino]methyl}-1*H*-indole-2,3-dione



Synthesis of bis(2-chloroethyl)amine hydrochloride (I):

To the excess of Thionylchloride, Diethanolamine (3:1) was added and stirred for 30min at 0^0 C. The reaction mixture was heated slowly to evaporate excess Thionylchloride. White colour solid was obtained.

Synthesis of 1-{[bis(2-chloroethyl)amino]methyl}-1H-indole-2,3-dione(II):

The mannich condensation was done by the following procedure. A mixture of equimolar concentration of Isatin (0.010 moles; 1.47g), Formaldehyde (0.010 moles; 0.3g, 1 mL), Bis(2-chloroethyl)aminehydrochloride (0.010 moles; 1.42g) was refluxed in ethanol (50 mL) for 6 hrs at 80^0 C. After filtering the filtrate was concentrated to one third its volume, dried over sodium sulfate. The residue was recrystallized from ethylacetate-petroleum ether gave pure material.

Synthesis of 1-{[bis(2-chloroethyl)amino]methyl}-3-(phenylimino)1,3-dihydro-2H-indole-2-one(III) :

1-{[bis(2-chloroethyl)amino]methyl}-1H-indole-2,3-dione(II) (0.0005moles; 0.150g) was condenced with Aniline derivatives (0.0005 moles) in methanol (20mL) and trace amount of glacial aceticacid for about 8 hrs at 80^0 C to get respective 1-{[bis(2-chloroethyl)amino]methyl}-3-(phenylimino)1,3-dihydro-2H-indole-2-one(III).

Cytotoxicity Assay:

Cell culture:HeLa- Human cervical carcinoma cell lines.

Cell number for subculture: one million cells for flask (30ml capacity).

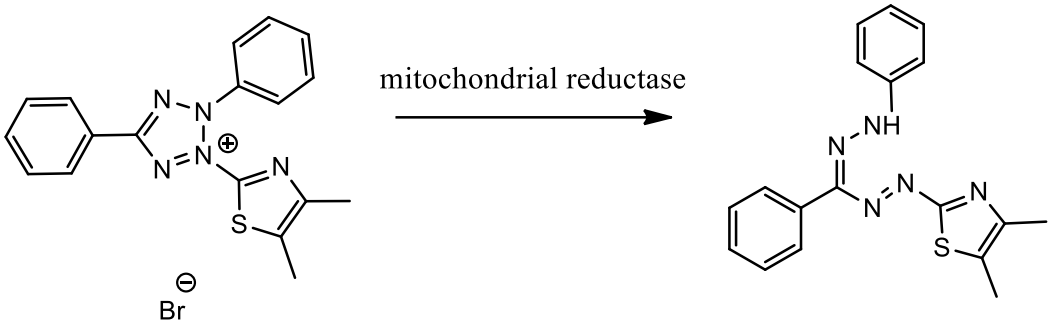
Cell loading into plate: 1000-2000 cells per well (96-well plate).

Drug solutions: 1 µg/ml to 100 µg/ml.

Principle:

MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) measures the metabolic activity of the viable cells. The assay is non-radioactive and can be performed entirely in a micro titer plate (MTP). It is suitable for measuring cell proliferation, cell viability or cytotoxicity. The reaction between MTT and 'mitochondrial dehydrogenase' produces water-insolule formazan salt. Procedure involves culturing the cells in a 96-well micro

titer plate and then incubating them with the MTT solution for approximately 2 hours. During incubation period, viable cells convert MTT to a water-insoluble formazan dye. The formazan dye in the MTP is solubilized and quantified with an ELISA plate reader. The absorbance directly correlates with the cell number. This is applicable for adherent cells cultured in MTP.



MTT Formazan

Procedure:

1. The adherent cells were trypsinized according to protocol and were resuspended infresh medium after centrifugation. Cell suspension was mixed thoroughly by pippettingseveral times to get a uniform single cell suspension[4-6].

2. 2. Different dilutions of drug solutions were made in media with final DMSO concentrationin the well to be less than 1%.

3. 3. 100µl of cell suspension was transferred aseptically to each well of a 96 well plate and toit 100µl of 1% media/ drug solution (in triplicate) in media was added.

4. 4. The plate was then incubated at 37^0C for 72 hours in CO_2 incubator.

5. 5. After 72 hours of incubation, 20µl of MTT was added to each well. The plate was again incubated for 2 hours.

6. 6. 80µl of lysis buffer was added to each well the plate was wrapped in aluminum foil toprevent the oxidation of the dye and the plate was placed on a shaker for overnight.

7. The absorbances were recorded on the ELISA reader at 562nm wavelength. The

absorbance of the test was compared with that of DMSO control to get the %inhibition.

Calculation of IC_{50} values:

Calculation of IC_{50} values for molecules (IIIa-IIIj) and standard drug (Cisplatin) on HeLa cell lines using MTT-assay are as follows.

Table 1: Cytotoxic activity of 1{[bis(2-chloroethyl)amino]methyl}-3-(phenylimino)1,3-dihydro-2H-indole-2-one(III a-j):

S.No	Compound	R	IC_{50} (µg/ml)
1	IIIa	H	37.48

2	IIIb	2-CH_3	24.23
3	IIIc	4-Br	21.84
4	IIId	2-Cl	23.50
5	IIIe	3-Cl	22.38
6	IIIf	4-Cl	22.25
7	IIIg	2-F	24.34
8	IIIh	3-F	23.70
9	IIIi	4-F	21.20
10	IIIj	4-CH_3	24.41
11	standard	Cisplatin	11.67

Antioxidant activity:

DPPH method:

A simple method that has been developed to determine the antioxidant activity of the drug utilizes the stable 2,2-diphenyl-1-picrylhydrazyl(DPPH) radical. The odd electron in the DPPH free radical gives a strong absorption maximum at 517nm and is purple in colour. The colour turns from purple to yellow as the molar absorptivity of the DPPH radical at 517nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH+H^+. The resulting decolourisation is stoichiometric with respect to the number of electrons captured. Antioxidant compounds may be water soluble, lipid soluble, insoluble or bound to cell walls[7-8]. Hence extraction efficiency is an important factor in quantification of antioxidant activity of foods. Ascorbic acid (as the reference standard) and the sample are reacted with DPPH solution in methanol/water for 30mins at 35^0C in a test tube and the absorbance changes are measured at 517nm.

Preparation of standard solution:

Ascorbic acid was used as standard for antioxidant activity. The weight equivalent to concentrations of 20, 40, 60, 80 and 100 µg/ml was weighed and dissolved in methanol.

Preparation of test solution:

- Stock solutions of samples were prepared by dissolving 10 mg of test sample in 9.5 ml of methanol and 0.5ml of DMSO to give concentration of 1000µg/ml.
- From the above stock solutions the concentrations of 20, 40, 60, 80 and 100 µg/ml were prepared by dissolving equivalent quantity in methanol.

Method:

- The method of Liyana-Pathiana and Shahidi was used for the determination of scavenging activity of DPPH free radical.
- To 1 ml of 0.135 mMDPPH prepared in methanol was added 1.0 ml of test compounds ranging from 20-100 µg/ml.
- The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 min.
- The absorbance was measured spectrophotometrically at 517 nm. The scavenging ability of the test compounds was calculated using the standard equation.

The IC_{50} values were given in table.

The amount of DPPH radical was calculated following this equation:

% inhibition of DPPH = $[A_0 - A_s]/A_0 \times 100$

Where A_0 is the absorbance of control and A_s is the absorbance of sample.

Standard drug is Ascorbic acid.

Table 2: Comparision of IC_{50} values of standard and synthesized molecules

S.No	**Compound**	**R**	**IC_{50} (µg/ml)**
1	IIIa	H	73.36
2	IIIb	2-CH_3	70.01
3	IIIc	4-Br	50.61
4	IIId	2-Cl	60.75
5	IIIe	3-Cl	54.80
6	IIIf	4-Cl	52.88
7	IIIg	2-F	69.91
8	IIIh	3-F	68.77
9	IIIi	4-F	41.22
10	IIIj	4-CH_3	70.16
11	standard	Ascorbic acid	5.84

Results and Discussion:

In this study, we have synthesized a new series isatin derivatives. Yields of all synthesized compounds were good. The structure of isatin has been the subject of numerous investigations. The presence of amine group at positon 1 in isatin and free amine group of bis(2-chloroethylamine and formaldehye reacts to give mannich base,1{[bis(2-chloroethyl)amino]methyl}-1H-indole-2,3-dione(II). The presence of carbonyl group at position 3 in 1-{[bis(2-chloroethyl)amino]methyl}-1H-indole-2,3-dione(II)and free amino group of the aniline derivatives furnishes reaction site at position 3 for condensation reaction[9-11]. The title compounds were synthesized by condensation reactions of II and aniline derivatives to give **1-{[bis(2-chloroethyl)amino]methyl}-3-(phenylimino)1,3-**

dihydro-2H-indole-2-one(III). The compounds were confirmed by spectral studies such as IR, ^{1}H NMR and MS. All the above reactions are briefly summarized in scheme. All the derivatives were subjected to cytotoxic activity (MTT assay method) and antioxidant activity (DPPH method).

Cytotoxic activity:

All the new isatin derivatives employed in the investigation have been found to have cytotoxic activity. All the compounds were tested at 1,3,10,30,100µg/ml concentrations and the results were compared with the standard drug (Cisplatin) at the same concentrations. Table 12 shows the cytotoxic activity data of 1-{[bis(2-chloroethyl)amino]methyl}-3-(phenylimino)1,3-dihydro-2H-indole-2-one..

- All the derivatives were having cytotoxic activity.
- Figure-12 shows bar graph between microgram per ml concentration of compounds required for 50% inhibition of standard and the test compounds.
- Amongst them, none of the compounds were found to be active than standard (cisplatin).
- The compound IIIi was found to be more active than all the compounds tested.
- The compound IIIa was found to be least active than all the compounds tested.
- All the compounds were having minute difference in their cytotoxic activity. This tells that there was no major effect of substituent on cytotoxic activity.

Antioxidant Activity:

All the new isatin derivatives employed in the investigation have been found to have antioxidant activity. All the compounds were tested at 20, 40,60,80,100µg/ml concentrations and the results were compared with the standard drug (Ascorbic acid) at the same concentrations[12-13].

- All the derivatives were having antioxidant activity.
- Figure-13 shows bar graph between microgram per ml concentration of compounds required for 50% inhibition of standard and the test compounds.
- Amongst them, none of the compounds were found to be active than standard (ascorbic acid).
- The compound IIIi was found to be more active than all the compounds tested.
- The compound IIIa was found to be least active than all the compounds tested.

Conclusion:

The study concluded that synthetic work of these studies have positively undergone as per the plan and as such in all the reactions carried, the compounds alone could be obtained. Ten title compounds were synthesized (IIIa-j) and were analyzed by physical and spectral data (FT-IR, NMR,Mass).Yield of the compounds synthesized by above method was good. Synthesized isatin derivatives gave satisfactory results for various evaluations like TLC, melting point, spectral data, cytotoxic and antioxidant activities. Completion of reactions was confirmed by TLC by using suitable solvent systems. Characterization of the synthesized compounds was done by FTIR,^{1}HNMR and Mass spectral data. The structures, functional groups and molecular weights of the compounds were confirmed[14]. All the compounds were screened for cytotoxic activity by MTT assay method. The results of derivatives were compared with standard drug cisplatin. All the compounds were less active than standard. Among all the

compounds, compound IIIi (R=4-F) was observed to possess more cytotoxic activity. All the derivatives were screened for antioxidant activity and the results were compared with the standard drug Ascorbic acid. All the series of the compounds showed antioxidant activity. Compound IIIi (R=4-F) was found to be more effective antioxidant. The results of the antioxidant activity revealed the compounds to possess good spectrum of antioxidant activity. Therefore, this study would be a fruitful matrix for the development of novel class of antioxidant and cytotoxic agents.

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