ANTICANCER AND ANTIOXIDANT ACTIVITIES OF 2-AMINOBENZOIC ACID (2-OXO-1, 2-DIHYDRO-INDOL-3-YLIDENE) HYDRAZIDES

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ABSTRACT

In this communication, the anticancer and antioxidant activities of some new 2-aminobenzoic acid (2-oxo-1, 2-dihydro-indol-3-ylidene) hydrazides are presented. The new compounds were prepared by the condensation of indole-2, 3-diones with 2-aminobenzoic acid hydrazide. All the compounds were evaluated for anticancer activity (against HeLa cell lines, IMR-32 cell lines) and antioxidant activity. Some of the compounds exhibited moderate anticancer activity against the cell lines employed and promising antioxidant activity.

Key words: 2-Aminobezoic acid (2-oxo-1, 2-dihydro-indol-3-ylidene) hydrazides, Anticancer and Antioxidant.

INTRODUCTION

Cancer is a complex disease that involves multiple mechanisms; it results from a breakdown of the regulatory mechanism that governs normal cell behavior. The proliferation and survival of cells in multicellular organisms are carefully regulated to meet the needs of the organisms as a whole. This regulation is lost in cancer cells, which grow and divide in an uncontrolled manner, ultimately spreading throughout the body and interfering with the function of normal tissues and organs.

Types of cancer

Cancer can result from abnormal proliferation of any of the different kinds of cells in body and therefore, there are more than hundred distinct types of cancers, which

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can vary substantially in their behavior and response to treatment. The most important issue in cancer pathology is the distinction between benign and malignant tumors. A tumor is any abnormal proliferation of cells, which may be either benign or malignant. A benign tumor such as a common skin wart, remains confined to its original location, neither invading surrounding normal tissue nor spreading to distant body sites, a malignant tumor, however is capable of both invading surrounding normal tissue and spreading throughout the body via the circulatory or lymphatic systems (metastasis). Only malignant tumors are properly referred to as cancer and it is their ability to invade and metastasize that makes cancer so dangerous, whereas benign tumors can usually be removed surgically. The spread of malignant tumors to distinct body sites frequently makes them resist to such localized treatment. Recent literature reveals that several indole derivatives are associated with anticancer\textsuperscript{4,5} antioxidant activities. In view of present need for the developing new anticancer agents, some 2-aminobenzoic acid (2-oxo-1,2-dihydro-indol-3-ylidene) hydrazides (\textit{a-g}) have been screened for anticancer and antioxidant activities.

**EXPERIMENTAL**

**Anticancer activity**

Microculture tetrazolium (MTT) assay\textsuperscript{6,7}

**Materials and methods**

RPMI-1640 (Himedia, Mumbai, India), trypsin 0.25\% (Gibcous, USA), FBS (Fetal bovine serium) (Gibcous USA), MTT 4 mg/mL (Himedia), DMSO (Merck, India) and lysis buffer (15% SLS in 1:1 DMF and water).

Composition of RPMI: 9.54 g/L, 10\% FBS, 2000 mg sodium bicarbonate, 250 \textmu L each of penicillin (60 mg/mL), streptomycin (100 mg/mL), amphotericin (200 mg/mL).

**Principle:** Microculture tetrazolium assay (MTT) is based on the metabolic reduction of 3-(4,5-dimethylthiazol-2,5-diphenyl) tetrazolium bromide (MTT) to water insoluble formazan crystals with mitochondrial dehydrogenase enzyme, which gives direct correlation of viable cells.

**Method:** Cell suspension (0.1 mL containing 5 x 10\textsuperscript{5} cells / 100 \textmu L), 0.1 mL of the compound solution (10, 20, 50, 100, 150 and 200 \textmu g in DMSO such that the final concentration of DMSO in media is less than 1\%) were added to the 96 well plates and kept in carbon dioxide incubator with 5\% CO\textsubscript{2} at 37\textdegree C for 72 hours. Blank contains only
cell suspension and control wells contain 1% DMSO and cell suspension.

After 72 hours, 20 µL of MTT was added and kept in carbon dioxide incubator for 2 hours followed by 80 µL of lysis buffer (15% SLS in 1:1 DMF and water). The plate was covered with aluminium foil to protect from light and then the 96 well plates were kept on rotary shaker for 8 hours. After 8 hours, the 96 well plates were processed on ELISA reader for absorption at 562 nm. The readings were averaged and viability of the test samples was compared with DMSO control.

**Antioxidant activity**

**Materials and methods**

Ascorbic acid (Analytical grade, Merck India), methanol (HPLC grade, Merck India), DPPH (Sigma-Aldrich, USA), test compounds and double distilled water.

**Preparation of standard solutions of ascorbic acid:** Required amount of ascorbic acid was accurately weighed and dissolved in distilled water to prepare 1 mM stock solution. Solutions of different concentrations of ascorbic acid 10 nM, 30 nM, 100 nM, 300 nM, 1 µM, 3 µM, 100 µM, 300 µM and 1 mM were prepared from stock solution.

**Preparation of DPPH solution:** 0.05 mM of DPPH was prepared by dissolving 19.71 mg of DPPH in 100 mL of methanol. The solution was protected from sunlight to prevent the oxidation of DPPH.

**Preparation of test compounds:** Required amount of test compound was dissolved in methanol and 1mM stock solution was prepared. Solutions of concentrations ranging from 100 nM to 1 mM were prepared from the stock solution.

**Standard graph of ascorbic acid:** 0.2 mL of DPPH solution was added to 2.8 mL of ascorbic acid solution in a test tube wrapped with aluminium foil and its absorbance was read at 517 nm using UV-visible double beam spectrophotometer. The results were plotted on a graph and IC₅₀ value was determined.

The IC₅₀ values of the test compounds were determined by a procedure similar to the ascorbic acid determination.

**Principle:** The method is based on the principle described by Blois et al.⁸: The model of scavenging the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) radical is a widely
used method to evaluate antioxidant activity in a relatively shorter time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be their hydrogen donating ability\(^9\).

DPPH is a stable free radical and accepts an electron (or) hydrogen radical to become a stable diamagnetic molecule\(^{10}\). The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm.

The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidant activity\(^{11,12}\).

The reduction in absorbance is calculated as percentage inhibition as follows:

\[
\text{% Inhibition} = \frac{(\text{Absorbance of blank} - \text{Absorbance of test})}{\text{Absorbance of blank} \times 100}
\]

**RESULTS AND DISCUSSION**

Table 1 presents the results of anticancer activity of phenyl amino acetic acid (2-oxo-1,2-dihydro-indol-3-ylidene) hydrazides. Some of the compounds exhibited moderate activity against HeLa cell lines and IMR-32 cell lines. Among the test compounds, compound \(g\) (\(R = 5\-Br\)) and \(b\) (\(R = 5\-CH_3\)) showed comparatively more cytotoxic activity with \(IC_{50}\) values of 241.62\(\mu\text{M}\) and 259.51 \(\mu\text{M}\) against HeLa cell lines, respectively. Compounds \(e, i, k, m\) and \(n\) did not show any activity against both the cell lines employed.

All the compounds showed antioxidant activity in the range with \(IC_{50}\) values of 10.29 to 26.70. Most significant of them has been found to be the compound \(j\) (\(R = 6\-Br\)) with \(IC_{50}\) value of 10.29 \(\mu\text{M}\), which is most potent. Compounds \(h\) (\(R = 5\-Cl\)) and \(l\) (\(R = 7\-NO_2\)) are found to be next in the order of antioxidant activity with \(IC_{50}\) values 13.65 \(\mu\text{M}\) and 13.68 \(\mu\text{M}\), respectively whereas the \(IC_{50}\) values of rest of the compounds is in the range of 16.00 \(\mu\text{M}\) to 26.7 \(\mu\text{M}\).
Table 1. Anticancer and antioxidant activities of 2-amino benzoic acid (2-oxo-1,2-dihydro-indol-3-ylidene) hydrazides

<table>
<thead>
<tr>
<th>Code</th>
<th>Substituent</th>
<th>Molecular formula</th>
<th>HeLa cell lines</th>
<th>IMR-32 cell lines</th>
<th>Antioxidant activity</th>
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<td>IC&lt;sub&gt;50&lt;/sub&gt; value (μM)</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; value (mM)</td>
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