SAT-ONLINE-P-2-BFT(R)-04

EVALUATION OF ANTIOXIDANT ACTIVITY OF HYDRAZONE

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Abstract: *Antioxidants are molecules that can prevent or slow damage to cells caused by free radicals. It is therefore important to determine the content and effectiveness of antioxidants in various chemical compounds and foods. This necessitates the development of a rapid method for determining the potential antioxidant effect. In the present study, we investigate the antioxidant potential of newly synthesized hydrazone of the antineoplastic drug bexarotene. The analyzes used (ABTS • + and DPPH) are widely used methods for assessing the antioxidant capacity of natural products. Both approaches are spectrophotometric techniques based on the quenching of stable color radicals. The DPPH method allows to determine the antioxidant activity by using a stable free radical - 1,1-diphenyl-2-picrylhydrazyl. The study is based on measuring the suppression capacity of antioxidants to it. The antioxidant activity of the newly synthesized compound was also determined by ABTS [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] cation radical decolorization method.*

Keywords: *Antioxidant activity, hydrazine, antineoplastic drug, bexarotene*

INTRODUCTION

Free radicals are highly unstable molecules that are naturally formed or formed from a variety of environmental sources, such as cigarette smoke, air pollution, and sunlight. Free radicals can cause “oxidative stress,” a process that can trigger cell damage. Oxidative stress is thought to play a role in a variety of diseases including cancer, cardiovascular diseases, diabetes, Alzheimer’s disease, Parkinson’s disease, and eye diseases such as cataracts and age-related macular degeneration. Prolonged oxidative stress can damage DNA and other important molecules in the body. Sometimes it even leads to cell death.

Antioxidants are molecules that can prevent or slow damage to cells caused by free radicals. Antioxidant molecules have been shown to counteract oxidative stress in laboratory experiments.

The antioxidant properties of vitamin A were first discussed in studies by Monaghan and Schmitt (1932), who studied the effect of the vitamin on the oxidation of linoleic acid. This antioxidant activity is based on the hydrophobic chain of polyene units that can trap singlet oxygen. The action of retinoids as antioxidants is largely determined by their lipid nature. As lipophilic compounds, they are localized in the lipophilic regions of membranes and lipoproteins. This makes vitamin A effective in reducing lipid peroxidation.

EXPOSITION

There are a number of *in-vitro* methods for determining the antioxidant activity of various substances. They act through different mechanisms and represent different aspects of the antioxidant properties of substances. For the purposes of the present study, the antioxidant potential of the newly synthesized hydrazone structure was determined by two different methods. The analyzed hydrazone has the following structure presented in Figure 1.

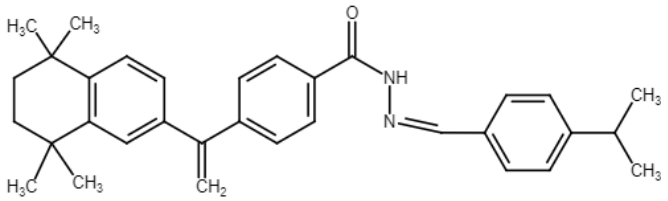

Fig. 1 Structure oh hydrazine of bexarotenen

The hydrazones are a group of compounds that contain the azomethine linkage. They are a part of the Schiff´s base family and are usually synthesized via condensation of primary amines with active carbonyl groups.

DPPH

DPPH free radical method is based on an electron-transfer. In a result that produces a violet solution in ethanol. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry. Thus it can be useful to assess various substances. DPPH analysis is characterized as fast and accessible in determining antioxidant activity.

The percentage of activity is calculated by the formula:

Ab - Aa x 100 (1)

Where:

Ab is the absorption of DPPH;

Aa- the absorption of each sample.

The result is resented as the percentage of inhibition of the DPPH radical.

To determine the antioxidant potential of the substance we studied, 4 mg of DPPH dissolved in 1 ml of methanol was used. The concentration of the DPPH radical was determined using Trolox as a standard. For this purpose, standard solutions of Trolox in methanol with the respective concentrations - 50, 25, 12.5, 6.25, and 3.125 μM were prepared. The absorbance was read at 517 nm on a Synergy 2 multifunction reader (BioTek). Based on the reported results, a calibration graph is constructed and presented in Figure 2.

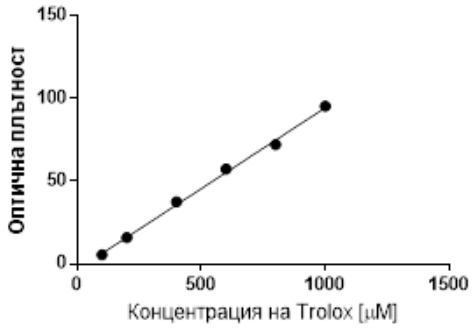

Fig. 2 Calibration graph of Trolox

Methanol was used as a solvent to prepare the working solutions of the analyte. Solutions were obtained with the following concentrations: 1mg/ml, 0.50mg/ml, 0.250mg/ml, 0.125mg/ml.

The decrease in absorption is linearly dependent on the antioxidant concentration. The percentage inhibition of the DPPH radical is determined according to the following formula:

% DPPH = 1 - (Sample - Blank Sample / Control - Blank Sample) x 100, (2)

Where:

Sample - sample absorption (sample + DPPH)

Blank sample - absorbance of the blank (methanol + DPPH)

Control - absorption of pure methanol

ABTS

The antioxidant activity (AOA) of the newly synthesized compound was also determined by ABTS [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] cation radical decolorization method with modifications. To perform the ABTS test, 10 µl of the analyte is added to a 1000 µl solution of ABTS + in phosphate buffer (pH 7.4). Phosphate buffer (pH 7.4) was used as the blank. The measurements were performed spectrophotometrically on a Camspec M501 apparatus.

CONCLUSION

Based on the obtained results, we can conclude that the chemical structural features of the newly synthesized hydrazone derivate of bexarotene do not lead to the manifestation of significant antioxidant potential. The data show that the newly synthesized derivative is not able to influence free radical processes related to the mechanisms of the defiance of oxidative stress in the body**.**

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