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Antioxidant activity evaluation of new compounds - hydrazones of bexarotene

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Retinoids are compounds related to vitamin A. The role of vitamin A as an antioxidant has long been known. Vitamin A and its natural and synthetic analogues play an important role in the human body and are implicated in several biological functions. Bexarotene is a third-generation synthetic retinoid used in the treatment of cutaneous T-cell lymphoma. The present paper reports the evaluation of new compounds – hydrazones of bexarotene – about their free radical scavenging activity. The tested substances were synthesized in our previous work and were analyzed by infrared spectroscopy. The antioxidant potential of hydrazones was determined by three different approaches. We used classical 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) tests. DPPH is a stable free radical which has an unpaired valence electron at one atom of the nitrogen bridge. Scavenging of DPPH radical is the basis of the popular DPPH antioxidant assay. Another approach by which we investigated the antioxidant properties of the new compounds was an electrochemical method. The experimental methodology involves the recording of a voltammogram of cathodes electro-oxygen reduction.

Keywords: retinoids, hydrazones, bexarotene, antioxidant activity

INTRODUCTION

The term retinoids describes the entire set of compounds including both natural and synthetic vitamin A analogs. The antioxidant effects of retinoids and carotenoids are known for decades. Their lipid nature and the localization within the lipophilic compartment of membranes and lipoproteins make retinoids effective in reducing lipid peroxidation by acting as chain-breaking antioxidants [1, 2].

Oxidation reactions are crucial for the maintenance of life. However, oxidative stress can also be damaging and cause different pathological states. Currently, scores antioxidants have been either synthesized or extracted from naturally occurring resources such as fruits, plants, and marine animals. Many of them exhibit good antioxidative activity against DPPH, ABTS, and hydroxyl radical [3].

A number of studies have examined the antioxidant potential of hydrazone derivatives. Hydrazone derivatives synthesized by Musad et al. (2011) are reported to have radical scavenging activity at the concentration of 10 μg/mL. Abdel-Wahab et al. (2011) evaluated imidazoline-based hydrazones 4 by 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay and reported them to have promising antioxidant activity [4, 5].

Bexarotene is a synthetic third-generation retinoid compound that exerts its biological action through selective binding and activation of the three retinoid X receptors (RXRs). When the retinoid receptors are activated, they function as transcription factors that regulate cellular differentiation and proliferation. *In vitro*, bexarotene inhibits the growth of tumor cell lines, *in vivo* it causes tumors regression in some animal models and prevents tumor induction in others. However, the exact mechanism of action of bexarotene in the treatment of cutaneous T-cell lymphoma (CTCL) is unknown [6].

There is evidence that some non-phenol hydrazones have the potential to maintain a moderate DPPH neutralization capacity that can be can be explained to the involvement of the -NH bond to antiradical capacity [7].

The present study aims to investigate the antioxidant potential of hydrazones of bexarotene by three different methods.

EXPERIMENTAL

Five newly synthesized bexarotene hydrazones derivatives were tested to determine their antioxidant potential.

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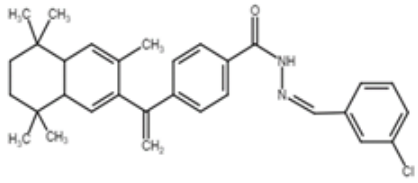
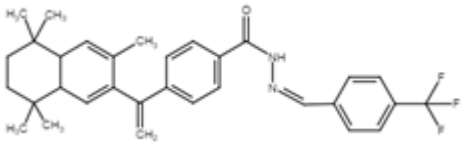
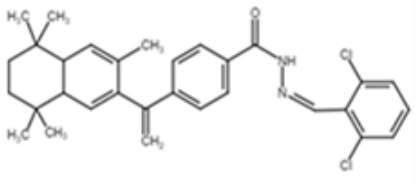
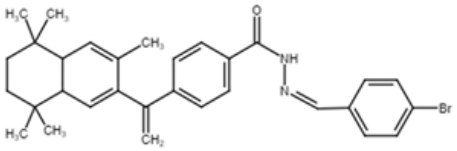
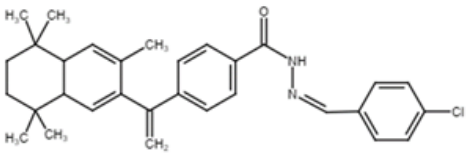
Table 1. Some characteristics of bexarotene hydrazones derivatives

Compound	Chemical structure	Chemical name	Molecular mass	Physical properties
V_1		3-Chlorophenyl-methylidene-4- [1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphthalen-2-yl) ethenyl] benzohydrazide	485	Pale yellow crystalline substance, slightly soluble in water, soluble in methanol and DMSO
V_2		4- (3-fluoromethyl) phenyl-methylidene-4- [1- (3,5,5,8,8-pentamethyl-6,7-dihydronaphthalen-2-yl) ethenyl] benzohydrazide	519	Pale yellow crystalline substance, slightly soluble in water, soluble in methanol and DMSO
V_3		2,6-Dichlorophenyl-methylidene-4- [1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphthalen-2-yl) ethenyl] benzohydrazide	519	Pale yellow crystalline substance, slightly soluble in water, soluble in methanol and DMSO
V_4		4-Bromophenyl-methylidene-4- [1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphthalen-2-yl) ethenyl] benzohydrazide	529	Pale yellow crystalline substance, slightly soluble in water, soluble in methanol and DMSO
V_5		4-Chlorophenyl-methylidene-4- [1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphthalen-2-yl) ethenyl] benzohydrazide	485	Yellow crystalline substance, slightly soluble in water, soluble in methanol and DMSO

For the synthesis of the target hydrazones, bexarotene hydrazide reacts with aldehydes, forming the hydrazones presented in Table 1.

Three different approaches were used to determine the antioxidant potential of the compounds.

DPPH analysis is an approach for evaluating the antioxidant potential of compounds, plant extracts, etc. DPPH assay is characterized a as rapid and accessible method in determining the antioxidant activity of various compounds. The antioxidant activity (AOA) of hydrazone compounds was also determined by the ABTS [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] cation radical decolorization method with modifications.

These assays (ABTS • + and DPPH) are widely used for evaluating the antioxidant capacity of natural products. Both approaches are spectrophotometric techniques based on the extinction of stable color radicals. They exhibit the ability to detect antioxidants even when present in complex biological mixtures such as plant or food extracts.

In addition to the two approaches mentioned above, an electrochemical method was used to determine the antioxidant capacity.

DPPH assay

DPPH assay is a rapid and accessible method in determining the antioxidant activity of various compounds.

The activity rate is calculated by the formula:

$$Ab - Aa \times 100 \quad (1)$$

where: Ab is the absorption of DPPH, Aa- absorption of each sample.

The result is expressed as a percent inhibition of DPPH radical.

To determine the antioxidant potential of our test substances, 4 mg of DPPH dissolved in 1 ml of methanol were used. Transfer the resulting solution quantitatively into a 50 ml round-bottom flask and make up to the mark with methanol. As a result, a solution with a concentration of 1 mM is obtained.

The DPPH concentration of the radical was determined using Trolox as a standard. For this purpose, standard Trolox solutions in methanol were prepared at concentrations of 50, 25, 12.5, 6.25 and 3.125 μM. The solutions thus prepared were stirred on a vortex mixer for 1 min and then incubated at room temperature for 30 min in the dark. Absorption was measured at 517 nm on a Synergy 2 multifunction reader (BioTek). Based on the reported results, the calibration graph presented in Figure 1 was constructed.

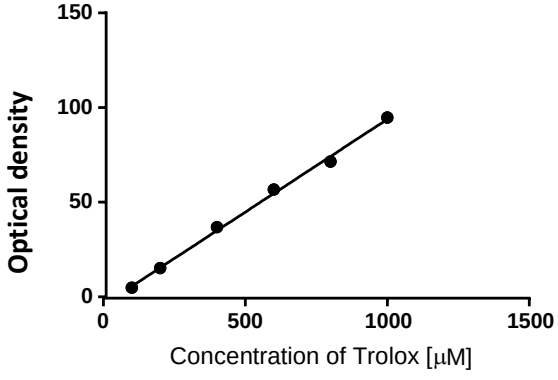


Fig. 1. Calibration graph of Trolox

To prepare the working solutions of the analytes, methanol was again used as the solvent. Solutions with appropriate concentrations were obtained: 1 mg / ml, 0.50 mg / ml, 0.250 mg / ml, 0.125 mg / ml.

The calibration graph was constructed using the obtained data, with the abscissa showing the concentration of the standard in μM and the ordinate the optical density. The resulting graph is presented in Figure 2.

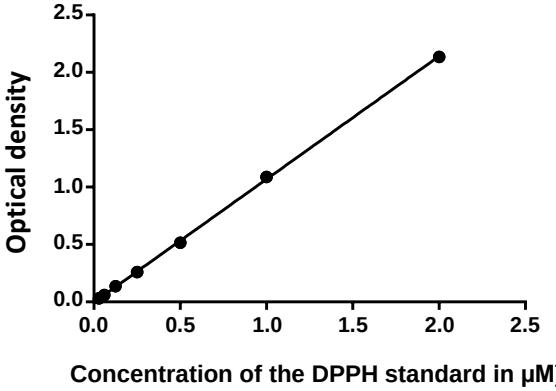


Fig. 2. Concentration of the DPPH standard graph

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

$$\% \text{ DPPH} = 1 - (\text{Sample} - \text{Blank} / \text{Control} - \text{Blank}) \times 100, \quad (2)$$

where: Sample - sample absorption (sample + DPPH), Blank - Blank Absorption (Methanol + DPPH), Control - the absorption of pure methanol.

ABTS test

To conduct the ABTS test, 10 μl of the analyte was added to 1000 μl of ABTS + solution in phosphate buffer (pH 7.4). The absorbance of the solution was previously adjusted to values of 0.700 ± 0.02 and read immediately before (0 min) and 6 min after the addition of the sample. Phosphate buffer (pH 7.4) was used as a blank.

Sampling absorption was calculated by the following formula:

$$A = (A \text{ sample } 0 \text{ min} - A \text{ sample } 6 \text{ min}) - (A \text{ blank sample } 0 \text{ min} - A \text{ blank sample } 6 \text{ min}) \quad (2)$$

where: A sample 0 min - the reported absorbance of the sample at the start of analysis; A sample for 6 min - the reported absorption of the sample after 6 min; A blank 0 min - the recorded blank absorbance at the start of the analysis; A blank for 6 min - reported blank absorption 6 min after the start of the analysis.

The AOA of the solution was determined by the calibration line constructed from the absorbance values of the standard solutions presented in Figure 3.

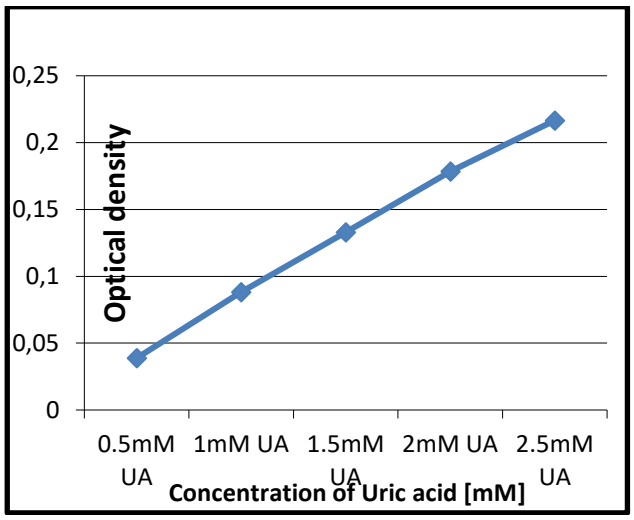


Fig. 3. Calibration line constructed from the absorbance values of the standard solutions

Determination of antioxidant activity by an electrochemical method

The methodology of the experiment is to register a voltammogram of cathodes oxygen reduction using an AOA Analyzer (RU.C.31.113.A N28715) connected to a personal computer. The electrochemical cell is a glass cup with a background electrolyte solution and a working glass carbon electrode and a comparative silver chloride electrode immersed therein. A 0.1 M ethanol solution of $NaClO_4$ with a volume of 10 ml was selected as the background solution.

The antioxidant activity of the samples tested was estimated by the kinetic criterion K (μmol / l.min) which reflects the amount of oxygen reacted with the sample over time and is determined by the following formula:

$$K = \frac{Co_2}{t}\left(1 - \frac{I_i}{I_0}\right) \quad (4)$$

where: CO_2 is the concentration of oxygen in the stock solution, μmol/l; Ii - magnitude of the current of oxygen reduction, μA; Io is the magnitude of the current of electro-reduction of oxygen in the absence of a substance in solution, μA; t - process time, min.

The antioxidant activity of the sample is compared to the antioxidant activity of Trolox by the following formula:

$$AOA = Trial / Krolox \quad (5)$$

where: Sample - the activity of the sample according to the kinetic criterion calculated by the formula, Krolox - Trolox activity according to the kinetic criterion calculated by the formula.

RESULTS AND DISCUSSION

Three different methods for the determination of free radicals scavenging activity were applied in this paper as an attempt to establish the possible antioxidant effects demonstrated by the newly synthesized hydrazones.

All five newly synthesized compounds were tested for their interaction with the stable free radical DPPH and this interaction, in turn, indicated their radical scavenging activity.

Based on the obtained results, we can conclude that the chemical structural features of the hydrazone derivatives of bexarotene do not lead to a significant antioxidant potential.

These results can be explained by the lack of free hydroxyl groups in the skeleton of the compounds obtained. It is apparent that structural change against retinol leads to a change in their potential for action as antioxidants. Structural modification of the molecules does not lead to the formation of antioxidant potential, and this is without exception manifested in all newly synthesized hydrazones.

The results obtained at the DPPH assay of the antioxidant potential of hydrazones are presented in table 2.

Table 2. DPPH assay of antioxidant potential of hydrazones

Compounds	% DPPH neutralization
Bexarotene	0
Bexarotene methyl ester	12
V_1	2
V_2	0
V_3	0
V_4	0
V_5	4

Table 3. ABTS - test to evaluate the antioxidant potential of bexarotenes hydrazone derivatives.

Compounds	C mM/UA
Bexarotene	0
Bexarotene methyl ester	0
V_1	0
V_2	0
V_3	0
V_4	0
V_5	0

Table 4. Antioxidant potential of bexarotenes hydrazone derivatives determined by electrochemical method

Compounds	C, μg/ml	K, μmol/l.min±SD	AOA
Bexarotene	100	17.817±0.993	1.729
Bexarotene methyl ester	100	13.625±0.823	1.322
V1	100	12.053±0.642	1.169
V2	100	11.791±0.322	1.144
V3	100	10.328±0.226	1.002
V4	100	13.014±0.774	1.263
V5	100	12.994±0.628	1.261
Trolox	100	10.306±0.113	1.000

The data obtained from the ABTS test for bexarotene hydrazone derivatives are presented in Table 3. The electrochemical method allows us to determine the overall antioxidant potential of our samples. The analysis shows that the highest antioxidant potential is displayed by bexarotene and subsequent modifications in its structure has led to a decrease in the antioxidant effect. Thus, for compound V3, the value calculated for the antioxidant effect by an electrochemical method of analysis almost coincides with the value of Trolox, namely 1.002 AOA for V3 and 1.000 for Trolox.

The data obtained on the antioxidant potential of bexarotene hydrazone derivatives are presented in Table 4.

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