Study of antioxidant activity of selected monocarbohydrazone derivatives

ABSTRACT

Many of carbohydrazone derivatives have shown a wide range of biological activities. In previous studies, these compounds have proven to be excellent anti-cancer, antitumor, antibacterial, antimicrobial and antioxidant agents. Monocarbohydrazones, as one of the carbohydrazone derivatives, have been slightly explored. Due to that reason, in this study, eight newly synthesized monocarbohydrazones were subjected to examinations by the use of three antioxidant tests. To determine the effectiveness of monocarbohydrazone as potential radical scavengers, DPPH (2,2-Diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and FRAP (Ferric Reducing Antioxidant Power) assays were used. The obtained results could open the door for further studies of these compounds as biologically active substances.

Keywords: antioxidant, ABTS, biological activity, DPPH, FRAP, monocarbohydrazones

1. INTRODUCTION

Carbohydrazones are compounds obtained by condensation of carbohydrazide with carbonyl compounds, aldehydes and/or ketones. So far, a large number of these derivatives have been tested and they have proved to be excellent anti-cancer[1] antitumor [2], antimicrobial[3-5] and antioxidant[6] agents. Monocarbohydrazones, tested in this study, were obtained under strictly controlled reaction conditions. Inspired by previous results and the fact that monocarbohydrazones, as one of the carbohydrazone derivatives, have been poorly studied so far, several derivatives have been picked out in order to investigate their antioxidant activity using three different assays [7–9] DPPH, ABTS and FRAP.

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is the most common and accepted model for evaluating the free radical scavenging capacity. When antioxidants react with DPPH (purple colored), which is a stable free radical, it becomes paired off in the presence of a hydrogen donor and it is reduced to the DPPHH form (yellow colored) with respect to the number of electrons captured. More the decolorization stronger is the reducing ability of compound tested. ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay is based on production of the blue/green ABTS** chromophore through the reaction between ABTS and potassium persulfate. Addition of antioxidants to the pre-formed radical cation reduces it back to its colorless neutral form, ABTS.

FRAP (Ferric Reducing Antioxidant Power) assay relies on the reduction of the complex ferrion – TPTZ (2,4,6-tri(2-pyridyl)- 1,3,5-triazine). The binding of Fe** to the ligand creates a very intense navy-blue color. The absorbance is measured to test the amount of iron reduced and it can be correlated with the amount of antioxidants.

Trolox or ascorbic acid can be used as reference antioxidant. These assays were performed using a spectrophotometric method.

2. EXPERIMENTAL PART

Eight monocarbohydrazone derivatives were synthesized and characterized in the Department of Chemistry, Biochemistry and Environmental Protection (Faculty of Sciences, University of Novi Sad). The structure of these analysed compounds is shown in Table 1.
Table 1. Structure of analysed monocarbohydrazones

Table 1. Struktura i oznaka ispitivanih monokarbohidrazona

<table>
<thead>
<tr>
<th>Structure</th>
<th>No</th>
<th>Substituent (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>1</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4-OH</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4-CH3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4-NO2</td>
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<td></td>
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<td>4-OCH3</td>
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<tr>
<td></td>
<td>6</td>
<td>4-Cl</td>
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<tr>
<td></td>
<td>7</td>
<td>4-Br</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4-F</td>
</tr>
</tbody>
</table>

Solutions of all analysed derivatives were made in absolute dimethyl–sulfoxide (Sigma Aldrich), at a concentration of 5 mg/mL.

**DPPH assay**

Prepared solutions of monocarbohydrazones were tested for their scavenging effect on the DPPH radical according to a known procedure [10]. Results were expressed as milligrams of Trolox equivalents (TE) per gram of dry weight (mg TE/g d.w.) of sample calculated according to the standard calibration curve.

**ABTS assay**

The ABTS assay was performed by a modified previously described procedure [11]. The results were expressed as Trolox equivalents per g of dry weight of sample (TEAC/g dw).

**FRAP assay**

To evaluate the reducing power of tested compounds, the ferric ion reducing antioxidant power (FRAP) assay [9] was undertaken with some modifications. Mean values of reducing power were expressed as milligrams equivalents ascorbic acid (AA) per gram of dry weight (mg eq AA/g dw) of sample calculated according to the standard calibration curve.

**3. RESULTS AND DISCUSSION**

Investigations on antioxidant activity of natural or synthetic molecules represent an assessment of their ability to neutralize free radical species which have harmful effects on different mammalian tissues.

Antioxidant potential of tested compounds presented in the Table 1 was determined using three in vitro assays: DPPH, ABTS and FRAP. The obtained results are presented in the Figures 1, 2 and 3 and Table 2.

Table 2. Results of three assays used

<table>
<thead>
<tr>
<th>Compound</th>
<th>DPPH</th>
<th>ABTS</th>
<th>FRAP</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>146.31</td>
<td>135.67</td>
<td>658.82</td>
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<tr>
<td>2</td>
<td>141.87</td>
<td>135.16</td>
<td>609.72</td>
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<tr>
<td>3</td>
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<td>4</td>
<td>131.39</td>
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<td>5</td>
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<td>645.75</td>
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<tr>
<td>6</td>
<td>141.47</td>
<td>134.28</td>
<td>615.06</td>
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<tr>
<td>7</td>
<td>136.23</td>
<td>134.41</td>
<td>636.48</td>
</tr>
<tr>
<td>8</td>
<td>110.42</td>
<td>135.55</td>
<td>663.99</td>
</tr>
</tbody>
</table>

![Figure 1. Assessment of antioxidant potential of tested compounds using DPPH assay](image)

**Figure 1. Assessment of antioxidant potential of tested compounds using DPPH assay**

Slika 1. Procena antioksidantnog potencijala ispitivanih jedinjenja pomoću DPPH testa

![Figure 2. Assessment of antioxidant potential of tested compounds using ABTS assay](image)

**Figure 2. Assessment of antioxidant potential of tested compounds using ABTS assay**

Slika 2. Procena antioksidantnog potencijala ispitivanih jedinjenja pomoću ABTS testa
4. CONCLUSIONS

In this study eight newly synthesized monocarbohydrazones were subjected to three different antioxidant assays: DPPH, ABTS and FRAP assay, in order to evaluate their antioxidant potential. Results obtained showed that all of the tested compounds have major antioxidant power.

The highest antioxidant potential in DPPH assay was shown by compound 1 (146.31 TEAC/g dw), while in FRAP it is compound 8 (663.99 mg eq AA/g dw). On the other hand, using ABTS assay, the most active compound cannot be clearly distinguished due to extremely close values. These results could be explained by the fact that not all assays are equally sensitive to the same compounds. It can be concluded that antioxidant potential depends on the structure of the compound, position and the nature of substituent present.

Obtained results of three used assays for monocarbohydrazones are predicting great biological activity. In further studies, more biological testing will be done.

Acknowledgments

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5. REFERENCES


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IZVOD

PROUČAVANJE ANTIOKSIDATIVNE AKTIVNOSTI ODABRANIH DERIVATA MONOKARBOHIDRAZONA

Mnogi derivati karbohidrazona pokazali su širok spektar bioloških aktivnosti. U prethodnim studijama ova jedinjenja su se pokazala kao odlični antikancerogeni, antitumorski, antibakterijski, antimikrobi i antioksidantni agensi. Monokarbohidrazoni, kao jedni od derivata karbohidrazona, su do sada malo istraženi. Iz tog razloga, u ovoj studiji, osam novosintetisanih monokarbohidrazona podvrgnuto je ispitivanju njihove antioksidativne aktivnosti primenom različitih testova. Da bi se utvrdila efikasnost monokarbohidrazona kao potencijalnih agenasa za uklanjanje radikala, korišćeni su DPPH, ABTS i FRAP testovi. Dobijeni rezultati predstavljaju osnovu za dalja ispitivanja ovih jedinjenja kao biološki aktivnih supstanci.

Ključne reči: antioksidant, ABTS, biološka aktivnost, DPPH, FRAP, monokarbohidrazoni

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