# The Antioxidant Activity of Some Acylhydrazones with Dibenzo[a,d][7]annulene Moiety

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*Searching new antioxidants compounds to prevent or reduce the impact of oxidative stress on cells, we designed and synthesized several N-acylhydrazone derivatives with dibenzo[a,d][7]annulene moiety. In this work, a series of previously synthesized N-acylhydrazone derivatives bearing 5H-dibenzo[a,d][7]annulene moiety **7a-g** were evaluated for antioxidant activity effect, using the DPPH method. The highest antioxidant activity was exhibited by the compound **7f** containing in his molecule 4-hydroxy-3-methoxyphenyl fragment*

*Keywords: antioxidant activity, DPPH, acylhydrazone, 5H-dibenzo[a,d][7]annulene*

The presence of free radicals in biological materials is considered to be responsible for many undesired processes such as inflammation, ageing and diseases like Alzheimer, cancer, chronic hepatitis C and many others [1-5].

The balance between free radicals and the antioxidant system of the body has importance in maintaining the health of organism. The antioxidants compounds can reduce or neutralize free radicals, thus protecting the cells from oxidative injury. For this reason, scientists are interested in new synthetic antioxidants compounds to prevent or reduce the impact of oxidative stress on cells.

The hydrazones and their acylated derivatives constitute an important class of compounds for new drugs development, because these compounds are known for a wide range of pharmacological activities, such as antitubercular (isonicotinoyl hydrazones), intestinal antiseptic (Nifuroxazide), anti-inflammatory, antimicrobial, anticonvulsant, antiplatelet, analgesic, antiviral, vasodilatory, antioxidant, antidepressant and antitumoral [6-13].

These compounds have also shown potent antioxidant activities with regards to scavenging free radicals. It has been experimentally established that some acylhydrazone derivatives can function as free-radical scavengers [14-18]. Typically, the hydrazones are characterized by an imine (azomethine) group, which can explain their antioxidant activities [19-20].

The group -CO-NH-N=C of the acylhydrazone enables resonance to the adjacent aromatic rings, leading to multiple resonance structures, which allows this functional group to act as an electron donating group to enhance the radical scavenging activity. The imine group of the acylhydrazone that contains alone pair of electrons might be used to form a covalent bond with a biological target [21-22].

The 5H-dibenzo[a,d][7]annulene ring is present in the structure of some compounds used in therapeutics as antidepressant, antibacterial, anticonvulsivant, anti-cholinergic, miorelaxant, antihistaminic, and antimicrobial agents [23-25].

In our previous work [26], we presented the synthesis, spectral characterizations and cytotoxic activity of new acylhydrazones with dibenzo[a,d][7]annulene moieties. In continuation of our research [26], in this paper we present the determination of antioxidant activity of these compounds obtained by grafting on the dibenzo [a,d][7]annulene moieties of certain pharmacophore acylhydrazone groups.

## Experimental part

All reactants and solvents were obtained commercially with the highest purity and were used without further purification. The absorbance was measured on a SPECORD 40 Analytik Jena spectrophotometer.

The acylhydrazones **7a-g** (fig. 1) were previously synthesized [26] by reaction of 2-(5H-dibenzo[a,d] [7]annulene-5-yl)acetohydrazide with a variety of aromatic aldehydes. The 2-(5*H*-dibenzo[a,d][7]annulen-5-yl) acetohydrazide was obtained starting from dibenzo-suberenone according with literature. [27-31].

### *Antioxidant activity assay by DPPH method*

The antioxidant activity of all synthesized compounds was evaluated by DPPH method [32-34] and compared with standards (BHA and BHT). The free radical scavenging activity of acylhydrazone **7a-g** was carried out in the presence of stable free 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), using butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) antioxidant agents as positive control. Determination of the antioxidant activity using the DPPH method is very common and rapid and has been shown to be one of the most appropriate methods [32-35].

The DPPH solution has a deep purple color, with a strong absorption at 517 nm, and turns to yellow in the presence of antioxidants, which neutralizes free radicals by pairing DPPH odd electron with a hydrogen atom or by electron donation. Reduction of DPPH absorption at 517 nm represents the capacity of antioxidants to scavenge free radicals [35].

The 400 μM solution of DPPH (2 mL) in ethanol was added to test sample solutions (2 mL) of different

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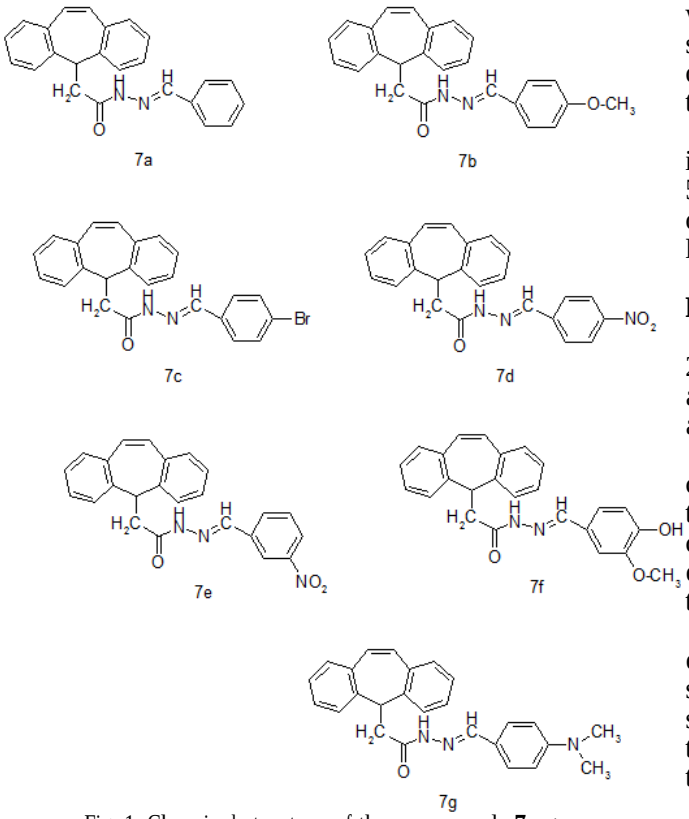


Fig. 1. Chemical structure of the compounds **7a-g**

concentrations (50, 100, 125, 200, 250 and 500 μM) in DMSO - ethanol 4:96 v/v. The samples were kept in the dark, at room temperature. After 30 min, the absorbance values were measured at 517 nm and convert into the percentage antioxidant activity (AA) using the formula [36], $AA\% = \{1-[(A_{sample}-A_{sampleblank})/A_{control}] \cdot 100\}$, where $A_{control}$ was the absorbance of DPPH solution without sample, $A_{sample}$ was the absorbance of sample solution with DPPH, $A_{sampleblank}$ was the absorbance of the sample solution without the DPPH. All analyses were undertaken on three replicates and the results averaged. The $IC_{50}$ values were calculated by linear regression plots, where the abscissa was represented by the concentration of tested compound solution (50, 100, 125, 200, 250 and 500 μM) and the ordinate - the average percent of antioxidant activity from three separate tests.

The antioxidant activity is expressed in terms of % inhibition and $IC_{50}$ (effective concentration for scavenging 50% of the initial DPPH) value (μM). The inhibitory effects of different concentrations of synthesized compounds on DPPH radical are presented in table 1.

## Results and discussions

The acylhydrazones **7a**–**g** obtained by condensation of 2-(5*H*-dibenzo[a,d][7]annulen-5-yl)acetohydrazide to aromatic aldehydes [26] were tested for the antioxidant activity using the DPPH method.

The results obtained by antioxidant screening of compounds **7a-g** indicated that the antioxidant activity of these acylhydrazone derivatives is low, except for compounds **7f** and **7g**. The highest antioxidant activity was exhibited by the compound **7f** containing in their molecule the 4-hydroxy-3-methoxyphenyl fragment (fig. 2).

All tested compounds exhibited a lower antioxidant effect than standard BHA (table 2). However, the scavenging effect of compound **7f** was higher than standard BHT. Also, the $IC_{50}$ value (125.32μM) obtained for this compound indicates a better antioxidant activity than the BHT standard (231.84μM).

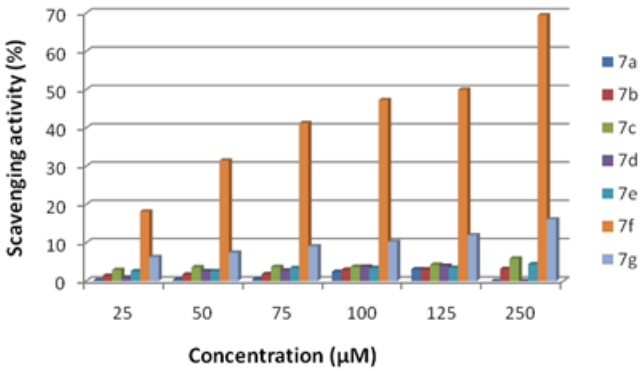


Fig. 2. Antioxidant activity of compounds **7a-g** assessed by DPPH method

| Comp. | Scavenging effect (%) | | | | | | $IC_{50}$ (μM) |
|---|---|---|---|---|---|---|---|
| | 25 μM | 50 μM | 75 μM | 100 μM | 125 μM | 250 μM | - |
| **7a** | 0.18 ± 0.27 | 0.34 ± 0.99 | 0.49 ± 1.30 | 2.38 ± 1.93 | 3.09 ± 0.64 | - | > 125 |
| **7b** | 1.37 ± 0.80 | 1.66 ± 1.14 | 1.77 ± 0.93 | 2.95 ± 0.40 | 3.00 ± 0.79 | 3.13 ± 0.88 | > 250 |
| **7c** | 2.87 ± 1.26 | 3.60 ± 1.18 | 3.69 ± 0.85 | 3.71 ± 1.43 | 4.25 ± 0.62 | 5.85 ± 0.48 | > 250 |
| **7d** | 0.90 ± 1.19 | 2.59 ± 0.86 | 2.76 ± 0.30 | 3.75 ± 1.24 | 3.99 ± 1.05 | - | > 125 |
| **7e** | 2.57 ± 0.82 | 2.61 ± 1.28 | 3.39 ± 1.03 | 3.40 ± 0.81 | 3.45 ± 0.74 | 4.42 ± 1.17 | > 250 |
| **7f** | 18.13 ± 1.14 | 31.45 ± 0.83 | 41.23 ± 0.97 | 47.24 ± 0.76 | 49.97 ± 1.27 | 69.41 ± 0.93 | 125.32 |
| **7g** | 6.26 ± 1.57 | 7.41 ± 0.97 | 9.08 ± 0.55 | 10.21 ± 0.52 | 11.97 ± 1.17 | 16.10 ± 0.91 | > 250 |
| **BHA** | 67.40 ± 0.96 | 88.66 ± 0.64 | 93.07 ± 0.81 | 94.03 ± 0.57 | 94.56 ± 0.99 | 95.45 ± 0.88 | 16.82 |
| **BHT** | 11.08 ± 1.16 | 16.46 ± 0.72 | 20.45 ± 1.15 | 24.20 ± 1.11 | 29.22 ± 1.29 | 53.96 ± 1.32 | 231.84 |

**Table 1**
ANTIOXIDANT ACTIVITY OF COMPOUNDS **7a-g** BY DPPH METHOD

The better antioxidant activity of the acylhydrazone **7f,** containing 4-hydroxy-3-methoxyphenyl fragment may be due of these three primary antioxidant groups (fig. 3). It is know that $OCH_3$ groups on aromatic systems have been extensively investigated for their well-known antioxidant effects and are important in cytotoxic and microtubule-binding agents used for cancer chemotherapy [38-40]. In addition, the antioxidant potential of phenolic compounds is attributed to their strong capability for electron transfer to ROS/free radicals, chelating metal ions, activating antioxidant enzymes and inhibitory oxidases [37].

So, the better antioxidant activity of the compound **7f** (obtained by condensation of 2-(5*H*-dibenzo[a,d][7]annulen-5-yl)acetohydrazide with vanillin) can be correlated with the presence of 4-hydroxy-3-methoxyphenyl fragment in their molecule (fig. 3) [15,16].

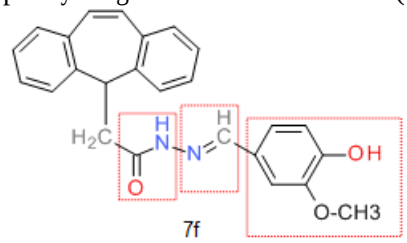


Fig. 3. Comparison between acylhydrazone **7f** and **7g**

The lower results of antioxidant activity of hydrazones **7a-e** are correlated with their stereochemistry which influence their interaction with DPPH.

## Conclusions

In conclusion, this study describes the antioxidant activities of a series of seven acylhydrazones with dibenzo[a,d]annulene moiety. The antioxidant activity of these acylhydrazone derivatives is low, except for compound **7f** which contains a 4-hydroxy-3-methoxyphenyl fragment. This compound indicates a better antioxidant activity than the BHT standard.

## References


1.SCHONEICH C., Exp. Gerontol., **34**, 1999, p.19
2.SAHINOGLU T., STEVENS C. R., BHATT B., BLAKE D. R., Methods, **9**, 1996, p. 628
3.SCHONEICH C., Biochim. Biophys. Acta Proteins Proteomics, **1703**, 2005, p. 111
4.OKA M., TACHIBANA M, NODA. K., INOUE N., TANAKA M., KUWABARA K., Phytomedicine, **14**, 2007, p. 465
5.DE MARIA N., COLANTONL A., FAGIUOLI S., LIU G. J., ROGERS B. K., FARINATI F., VAN THIEL D. H., FLOYD R. A., Free Radic. Biol. Med., **21**, 1996, p. 291
6. TODESCHINI, A. R.; MIRANDA, A. L.; SILVA C. M.; PARRINI, S. C.; BARREIRO, E. J., Eur. J. Med. Chem., 1998, **33** , p. 189
7. PANDEY J, PAL R, DWIVEDI A, HAJELA K., Arzneimittelforschung. 2002, **52**, 39
8. MOHAMMAD, A. , Int. J. Chem. Appl. Biol. Sci., **1**, 2014, p. 23
9. CUI, Z. N.; LI, Y.; LING, Y.; HUANG, J. A.; CUI, J. R.; WANG, R. Q.; YANG, X. L., Eur. J. Med. Chem., **45**, 2010, p. 5576
10.ALTINTOP, M. D.; KAPLANCIKL, Z. A.; CIFTCI, G. A.; DEMIREL, R., Eur. J. Med. Chem., **74**, 2014, p. 264
11.KUMMERLE A. E., RAIMUNDO J. M., LEAL C. M.,. DA SILVA G. S, BALLIANO T. L., PEREIRA M. A., DE SIMONE C. A.,. SUDO R. T, ZAPATA-SUDO G., FRAGA C. A.M., BARREIRO E. J., Eur. J. Med. Chem., **44**, 2009, p. 4004
12.TIAN, B. H.; HE, M. Z.; TAN, Z. W.; TANG, S. X.; HEWLETT, I.; CHEN, S. G.; JIN, Y. X.; YANG, M., Chem. Biol. Drug Des., **77**, 2011, p. 189
13.WANG Q., PANY., WANG J., PENG Q., LUO H., ZHENG J., Afr. J. Biotechnol., **10**, no. 78, 2011, p. 18013
14.NIKOLAEVSKII, A. N., KNIGA, O. P., KHIZHAN, E. I. , TIKHONOVA G. A., VINOGRADOV V. V., KHIZHAN A. I., Russ. J. Phys. Chem., **86**, 2012, p. 1816
15.DUARTE C. D, TRIBUTINO J. L., LACERDA D. I., MARTINS M. V., ALEXANDRE-MOREIRA M. S., DUTRA F., BECHARA E. J., DE-PAULA F. S., GOULART M. O., FERREIRA J, CALIXTO J. B, NUNES M. P., BERTHO A. L., MIRANDA A. L., BARREIRO E. J., FRAGA C. A., Bioorg Med Chem., **15**, no. 6, 2007, p. 2421
16.CONSTANTIN S., BURON F., ROUTIER S., CONFEDERAT LUMINITA, IACOB A.T., MIRON A, PROFIRE L, Farmacia, **64**, no. 4, 2016, p. 565
17.MOHAMMED KHAN K., SHAH Z., UDDIN AHMAD V., KHAN M., TAHA M., RAHIM F., ALI S., AMBREEN N., PERVEEN S., IQBAL CHOUDHARY M., Med. Chem., **8**, 2012, p. 452
18.MOHAMMED KHAN K., TAHA M., NAZ F., SIDDIQUI S., ALI S., RAHIM F., PERVEEN S., IQBAL CHOUDHARY M., Med. Chem., **8**, 2012, p. 705
19.BAKALBASSIS E. G., LITHOXOIDOU A. T., VAFIADIS A. P., J. Phys. Chem. A **110**, 2006, p. 11151
20.BELKHEIRI N., BOUGUERNE B., BEDOS-BELVAL F., DURAN H., BERNIS C., SALVAYRE R., NEGRE-SALVAYRE A., BALTAS M., Eur. J. Med. Chem. **45**, 2010, p. 3019
21.AL-AMIERY A. A., AL-MAJEDY Y. K., IBRAHIM H. H., AL-TAMIMI A. A., Org. Med. Chem. Lett., **2**, 2012, p. 1
22.HUDA S. KAREEM, AZHAR ARIFFIN, NURDIANA NORDIN, THORSTEN HEIDELBERG, AZLINA ABDUL-AZIZ, KIN WENG KONG, WAGEEH A.YEHYE, Eur. J. Med. Chem., **103**, 2015, p. 497
23.SOCEA, L. I., SARAMET, G., DINU PARVU, C. E., DRAGHICI, C., SOCEA, B., Rev. Chim. (Bucharest), **65**, no.3,2014, p. 25
24.MARINDALE, M., The Extra Pharmacopoeia, 30th ed., Pharmaceutical Press, London, 1993, p. 932
25.MUNOZ-BELLIDO, J. L., MUNOZ-CRIDO, S., GARCIA RODRIGUEZ, J. A., Int. J. Antimicrobial Agents, **14**, 2000, p. 177
26.SOCEA, L. I., SOCEA, B., SARAMET, G., BARBUCEANU, S.F., DRAGHICI, C., CONSTANTIN, V.D., OLARU, O.T., Rev. Chim. (Bucharest), **66**, no.8, 2015, p. 1122
27.SOCEA, L.I., SARAMET, G., DRAGHICI, C., BARBUCEANU, S.F., ILIES, D.C., SOCEA, B., Rev. Chim. (Bucharest), **67**, no 1, 2016, p. 17
28.SOCEA, L. I., SARAMET, G., MIHALCEA, F., APOSTOL, T. V., ANDREESCU, C., DRAGHICI, C., SOCEA, B., Rev. Chim. (Bucharest), **65**, no.2, 2014, p. 156
29.SOCEA L. I., SARAMET, G., DRAGHICI, C., SOCEA, B., CONSTANTIN V. D., RADU-POPESCU M. A., J. Serb. Chem. Soc., **80**, no. 12, 2015, p. 1461
30.SOCEA, L. I., BARBUCEANU, S. F., DRAGHICI, C., NITULESCU, G. M., SARAMET, G., SOCEA, B., APOSTOL, T. V., Rev. Chim. (Bucharest), **68**, no.12, 2017, p. 2761
31.SOCEA, A.I., BARBUCEANU, S.F., SOCEA, B., DRAGHICI, C., APOSTOL, T.V., PAHONTU, E.M., OLARU, O.T., Rev. Chim. (Bucharest), **68**, no. 11, 2017, p. 2503
32. BARBUCEANU S. F, ILIES D. C., SARAMET G., UIVAROSI V., DRAGHICI C., RADULESCU V., Int. J. Mol. Sci., **15**, 2014, 10908
33. BARBUCEANU, S. F, ILIES, D. C., RADULESCU, V., SOCEA, L. I., DRAGHICI, C., SARAMET, G., Rev. Chim. (Bucharest), **65**, no.10, 2014, p. 1172
34.ILIES D. C., SHOVA S., RADULESCU V., PAHONTU E, ROSU T., Polyhedron, **97**, 2015, p. 157
35.ZHOU B., LI B., YI W., BU X., MA L., Bioorg Med Chem Lett., **23**, no. 13, 2013, 3759
36.XIAO-JUAN DUAN, WEI-WEI ZHANG, XIAO-MING LI, BIN-GUI WANG, Food. Chem., **95**, 2006, p. 37
37.HALL, C.A., CUPPETT, S.L., Antioxidant Methodology: In vivo and in vitro concepts, AOCS Press, Champaign, Illinois, 1997, p. 141
38.MARINHO J., PEDRO M., PINTO D. C., SILVA A., CAVALEIRO J. A., SUNKEL C. E., NASCIMENTO M.S.J., Biochem. Pharmacol., **75**, 2008, p. 826
39.ODLO K., FOURNIER-DIT-CHABERT J., DUCKI S., GANI O.A., SYLTE I., HANSEN T.V, Bioorg. Med. Chem., **18**, 2010, p. 6874
40.SALIH AGIRTAS M., CABIR B., OZDEMIR S., Dyes Pigments, **96**, 2013, p. 152


Manuscript received: 27.08.2017