# Synthesis and Cytotoxicity Evaluation of new 5H-dibenzo[a,d][7]annulen-5-yl acetylhydrazones

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*Hydrazones with an azomethine –NH-N=CH- group constitute an important class of compounds for new drug development. In this work a series of new N-acylhydrazone derivatives bearing 5H-dibenzo[a,d][7]annulene moiety **7a-g** were synthesized in good yields through the reactions of 2-(5H-dibenzo[a,d][7]annulen-5-yl)acetohydrazide **5** with a variety of aromatic aldehydes **6a-g**. $^{1}$H-NMR analysis indicated the existence of two conformational isomers, a major axial (about 75%) and a minor equatorial one (25%) which are interconvertible by middle ring inversion. All the new compounds were characterized by elemental analysis, IR-, UV-, $^{1}$H-NMR and $^{13}$C-NMR spectroscopy and evaluated for cytotoxic effect using two alternative methods on invertebrate organisms.*

*Keywords: acylhydrazone, 5H-dibenzo[a,d][7]annulene, cytotoxicity, Artemia salina and Daphnia magna bioassays*

Hydrazones have attracted considerable attention in medicinal chemistry due to their distinctive structural features and a wide range of pharmacological activities: antimicrobial, anticonvulsant, analgesic, antiinflammatory, antiplatelet, antitubercular and antitumor activities.[1]

Acylhydrazones are organic compounds characterized by the presence of a -CONHN=CH- group in their molecule. In recent years, the *N*-acylhydrazone moiety has proved to be an important pharmacophore structure in pharmaceutical research. These structures have received much attention due to their chemotherapeutic potential in the development of novel antimicrobial agents[2,3]. In addition, many *N*-acylhydrazone derivatives have been reported to exhibit an array of biological activities such as intestinal antiseptic (nifuroxazide), analgesic, antiinflammatory, antimicrobial, anticonvulsant, antiplatelet, antitubercular (isonicotinoyl hydrazones), antiviral, schistomiasis, antitumor, vasodilatatory, antioxidant and antidepressant [4-19]. Furthermore, acylhydrazones are used as precursors and intermediates of many important organic molecules such as heterocycles, pharmaceuticals, polymers, dyestuffs and photographic products as possible ligands for metal complexes and organocatalysis [20,21].

The tricyclic framework of 5H-dibenzo[a,d][7]annulene constitutes an integral part of the structure of molecules that are known to be effective for the treatment of depressive disorders (Protriptyline, Demexiptyline). The dibenzo[a,d][7]annulene moieties is incorporated in biologically active compounds, which exhibit muscarinic receptor antagonist properties, antiallergic, antidiabetic, antiartherosclerotic, antiparasitic, metalloprotease inhibitors antimicrobial and antitumoral activity [22-27].

Considering these data, we proposed to attach the acylhydrazone fragments to dibenzo[a,d][7]annulene nucleus.

In this work, we reported the synthesis of new acylhydrazones bearing 5H-dibenzo[a,d][7]annulene moiety and we evaluated their cytotoxic activity.

The structures of these new compounds were elucidated by elemental analysis, IR, UV, $^{1}$H-NMR and $^{13}$C-NMR spectroscopy. The newly synthesized compounds were tested for potential cytotoxic activity using two alternative methods on invertebrate organisms. The cytotoxicity was assessed by *Artemia salina* (brine shrimps) and *Daphnia magna* bioassays.

## Experimental part

All reactants and solvents were obtained commercially with the highest purity and were used without further purification. Melting points were determined on a Boetius apparatus and are uncorrected. The UV-Vis spectra were recorded on a SPECORD 40 Analytik Jena spectrometer, in methanol ($2.5x10^{-5}$ M) in the wave length range 200–600 nm. The IR spectra were recorded in KBr pellets using a Vertex 70 Bruker spectrometer. Elemental analyses were performed on an ECS-40-10-Costeh micro-dosimeter (and are within ±0.4% of the theoretical values). The NMR spectra were recorded on a Varian Gemini 300 BB instrument operating at 300 MHz for a $^{1}$H and 75 MHz for $^{13}$C, using $CDCl_3$ as solvent. Chemical shifts (δ, ppm) were assigned according to the internal standard signal of tetramethylsilane in DMSO-$d_6$ (δ = 0 ppm). Coupling constants, *J*, are expressed in Hertz (Hz).

Biological determinations were performed under constant temperature and light conditions using a Sanyo

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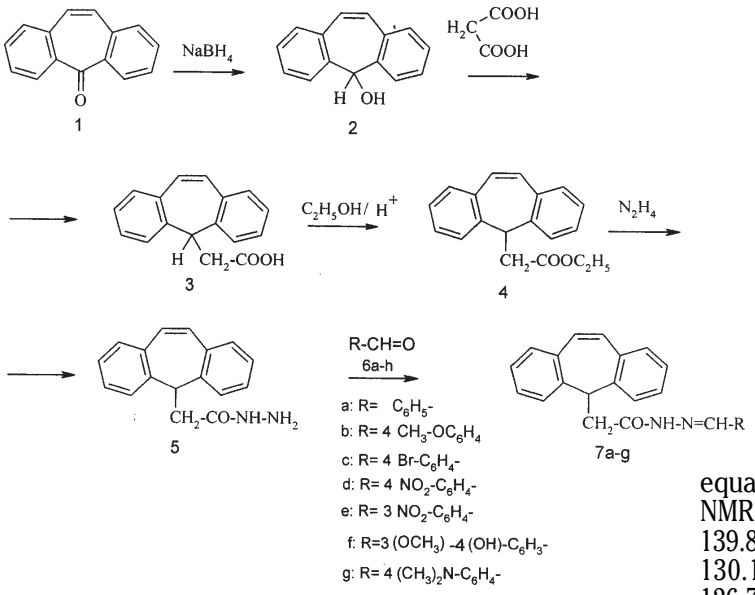



Scheme 1. Reaction pathway to the target compound **7a-g**

MLR-351 H, USA climatic chamber (25 ± 1°C, a 16 h photoperiod and 8 h of darkness).

The synthesis pathway used for the preparation of the title compounds is shown in scheme 1.

The synthesis of the new compounds was realized in several steps according to the literature method, starting from the dibenzosuberenone **1** which was transformed into its corresponding alcohol **2** [26-29]. By reaction of 5H-dibenzo[a,d][7]annulen-5-ol **2** with malonic acid to obtain 5H-dibenzo[a,d][7]annulen-5-ylacetic acid **3.** 5H-Dibenzo[a,d][7]annulen-5-ylacetic acid **3** was transformed into its corresponding ester **4**. Ethyl 5*H*-dibenzo[a,d][7]annulen-5-ylacetate **2** reacted with the hydrazine hydrate leading to a **5**. The key intermediate 2-(5*H*-dibenzo[a,d][7]annulen-5-yl)acetohydrazide **5** was synthesized by hydrazination of ethyl ester of the corresponding carboxylic acids **4**. Then the 2-(5*H*-dibenzo[a,d][7]annulen-5-yl)acetohydrazide **5** was condensed with different substituted aromatic aldehydes in refluxing ethanol to afford the corresponding *N*-acylhydrazones **7a-g** in good yield [30-34].

*General procedure for the preparation of 2-(5H-dibenzo[a,d][7]annulen-5-yl)-N'-[(R)methylidene] acetohydrazide*

The mixture of 2-(5*H*-dibenzo[a,d][7]annulen-5-yl)acetohydrazide **5** (0.004 mol) preparate according to the literature method and the corresponding aromatic aldehyde **6a-g** (0.004 mol) in absolute ethanol (30-50 mL) was refluxed for 6-12 h (scheme 1). On cooling the reaction content to room temperature, a solid appeared. This was filtered off and recrystallized from ethanol to obtain the desired compound.

*2-(5H-dibenzo[a,d][7]annulen-5-yl)-N'-[phenylmethylidene]acetohydrazide (7a):* Yield: 90.7%; *m.p.* 222-223 C° (dec); elemental analysis: anal. calcd. for $C_{24}H_{20}N_2O$ (352.42 g/mol): C, 81.79; H, 5.72; N 7.95; found: C, 81.80; H, 5.70; N, 7.95; UV-Vis($CH_3OH$) (λ max): 218.5, 223.8, 288.1; IR (KBr, $cm^{-1}$): 3445, 3184 (N–H stretching), 3065, 3023 (C–H stretching of aromatic ring), 2972, 2863 ($CH_2$ stretching), 1668 (C=O stretching), 1607 (C=N stretching), 1571, 1520 (C=C stretching); $^1$H-NMR (300 MHz, $CDCl_3$, δ, ppm): 9.60 (s, NH, equatorial isomer); 9.17 (s, NH, axial isomer); 7.86 (1H, s, CH=N); 7.80-7.05 ($13H_{ar}$, m); 6.95 (2H, s, $H^{10'-11'}$); 4.65 ($H^{5'}$, t, 7.8, axial isomer); 4.06 ($H^{5'}$, t, 7.8, equatorial isomer); 3.85 (2H, $H^{12'}$, d, 7.8, equatorial isomer); 3.16 (2H, $H^{12'}$, d, 7.8, axial isomer); $^{13}$C-NMR (75 MHz, $CDCl_3$, δ/ ppm): 174.25 ($C^{13'}$); 143.24 ($C^3$); 139.82 (2Cq); 134.44 (2Cq); 133.98 (Cq); 131.23 ($C^{10'-11'}$); 130.19 (CH); 129.83 (CH); 128.87 (CH); 127.24 (CH); 126.79 (CH); 50.53 ($C^{5'}$); 33.70 ($C^{12'}$);

*2-(5H-dibenzo[a,d][7]annulen-5-yl)-N'-[(4-methoxi)phenylmethylidene]-acetohydra-zide (7b):* Yield: 93.1%; *m.p.* 213-215 C°; elemental analysis: anal. calcd. for $C_{25}H_{22}N_2O_2$ (382.45 g/mol): C, 78.51; H, 5.80; N 7.32; found: C, 78.50; H, 5.82; N, 7.94; UV-Vis($CH_3OH$) (λ max): 223.8, 290.7; IR (KBr, $cm^{-1}$): 3467, 3182 (NH stretching), 3064, 3023 (C–H stretching of aromatic ring), 2956, 2933, 2899, 2833 ($CH_2$ + $CH_3$ stretching), 1661 (C=O stretching), 1612 (C=N stretching), 1521, 1505 (C=C stretching), 1254, 1034 (C-O-C stretching); $^1$H-NMR (300 MHz, $CDCl_3$, δ, ppm, *J*, Hz): 9.59 (s, NH, equatorial isomer); 9.15 (s, NH, axial isomer); 7.68 (2H, d, 8.7, $H^5$, $H^9$, equatorial isomer); 7.52 (2H, d, 8.7, $H^5$, $H^9$, axial isomer); 7.39 (1H, s, $H^3$); 7.50-7.05 ($8H_{ar}$, m); 6.94 (2H s, $H^{10'-11'}$); 6.93 (2H, d, 8.7, $H^6$, $H^8$, equatorial isomer); 6.90 (2H, d, 8.7, $H^6$, $H^8$, axial isomer); 4.64 ($H^{5'}$, t, 7.8, axial isomer); 4.08 ($H^{5'}$, t, 7.8, equatorial isomer); 3.82 (3H, s, $OCH_3$, axial isomer) 3.76 (3H, s, $OCH_3$, equatorial isomer); 3.14 (2H, $H^{12'}$, d, 7.8); $^{13}$C-NMR (75 MHz, $CDCl_3$, δ, ppm): 174.09 ($C^{13'}$); 161.27 ($C^7$), 143.13 ($C^3$); 139.03 (2Cq); 134.46 (2Cq); 131.23 ($C^{10'-11'}$), 130.19 (CH); 129.83 (CH, axial isomer); 128.87 (CH, axial isomer); 128.75 (CH, axial isomer); 126.76 (CH, equatorial isomer); 125.68 (CH, equatorial isomer); 123.01 (CH, equatorial isomer); 114.33 (2CH, axial isomer); 55.55 ($OCH_3$); 50.56 ($C^{5'}$); 33.74 ($C^{12'}$);

*2-(5H-dibenzo[a,d][7]annulen-5-yl)-N'-[(4-bromo)phenylmethylidene]acetohydrazide (7c):* Yield: 90.9%; *m.p.* 215-217 C°; elemental analysis: anal. calcd. for $C_{24}H_{19}N_2O$ (431.32 g/mol): C, 66.83; H, 4.44; N 6.49; found: C, 66.84; H, 4.45; N, 6.46; UV-Vis($CH_3OH$) (λ max): 222.0, 289.0; IR (KBr, $cm^{-1}$): 3248 (N–H stretching), 3064, 3020 (C–H stretching of aromatic ring), 2968, 2883 ($CH_2$ stretching), 1664 (C=O stretching), 1606 (C=N stretching), 1591, 1550 (C=C stretching), 622 (C-Br); $^1$H-NMR (300 MHz, $CDCl_3$, δ, ppm, *J*, Hz): 10.72 (s, NH, equatorial isomer); 10.53 (s, NH, axial isomer); 7.97 (1H, s, $H^3$); 7.90-7.40 ($12H_{ar}$, m); 7.31 (2H, s, $H^{10'-11'}$); 5.02 ($H^{5'}$, t, 7.8, axial isomer), 4.20 ($H^{5'}$, t, 7.8, equatorial isomer); 3.49 (2H, $H^{12'}$, d, 7.8); $^{13}$C-NMR (75 MHz, $CDCl_3$, δ, ppm): 173.65 ($C^{13'}$); 141.50 ($C^3$); 139.95 (2Cq); 134.32 (2Cq); 133.92 (Cq); 131.81 ($C^{10'-11'}$); 131.81 (CH); 131.07 (CH); 129.68 (CH, axial isomer); 129.12 (CH, equatorial isomer); 128.33 (CH); 126.58 (CH, axial isomer); 126.72 (CH, axial isomer); 50.45 ($C^{5'}$); 33.55 ($C^{12'}$);

*2-(5H-dibenzo[a,d][7]annulen-5-yl)-N'-[(4-nitro)phenylmethylidene]acetohydrazide (7d):* Yield: 93.3%; *m.p.* 270-272 C° (dec); elemental analysis: anal. calcd. for $C_{24}H_{19}N_3O_3$ (397.42 g/mol): C, 72.53; H, 4.82; N 10.57; found: C, 72.53; H, 4.80; N 10.58; UV-Vis($CH_3OH$) (λ max): 226.4, 293.4; IR (KBr, $cm^{-1}$): 3437, 3175 (N–H stretching), 3071, 3022 (C–H stretching of aromatic ring), 2958, 2854 ($CH_2$ stretching), 1666 (C=O stretching), 1614 (C=N stretching), 1516 (C=C stretching); 1583, 1340 ($NO_2$ stretching), $^1$H-NMR (300 MHz, $CDCl_3$, δ, ppm, *J*, Hz): 10.97 (s, NH, equatorial isomer); 10.62 (s, NH, axial isomer); 7.75 (2H, d, 8.6, $H^6$, $H^8$); 7.32 (1H, s, $H^3$); 7.31 (2H, d, 8.6, $H^5$, $H^9$); 6.98-6.88 ($8H_{ar}$, m); 6.50 (2H, s, H $^{10'-11'}$); 4.17 ($H^{5'}$, t, 7.8, axial isomer); 4.25 (2H, $H^{12'}$, d, 7.8, equatorial isomer); 3.36 (2H, $H^{12'}$, d, 7.8, axial isomer); $^{13}$C-NMR (75 MHz, $CDCl_3$, δ, ppm): 172.75 ($C^{13'}$); 147.73 ($C^3$); 143.39 (Cq); 140.63 (2Cq); 140.07 (Cq); 139.71 (2Cq); 138.86 (CH); 133.25 ($Cq_n$); 130. 86($C^{10'-11'}$); 128.73 (CH); 127.84 (CH); 126.40 (CH); 125.71 (CH); 122.99 (2CH); 49.38 ($C^{5'}$, axial isomer); 48.82 ($C^{5'}$, equatorial isomer); 33.41 ($C^{12'}$);

*2-(5H-dibenzo[a,d][7]annulen-5-yl)-N'-[(3-nitro)phenylmethylidene]acetohydrazide (7e):* Yield: 94.0%; *m.p.* 211-213 C° (dec); elemental analysis: anal. calcd. for $C_{24}H_{19}N_3O_3$ (397.42 g/mol): C, 72.53; H, 4.82; N 10.57; found: C, 72.54; H, 4.83; N 10.58; UV-Vis($CH_3OH$) (λ max): 225.6, 282.8; IR (KBr, $cm^{-1}$): 3312, 3165 (N–H stretching), 3066, 3019 (C–H stretching of aromatic ring), 2958, 2854 ($CH_2$ stretching), 1667 (C=O stretching), 1619 (C=N stretching), 1532 (C=C stretching); 1560, 1350 ($NO_2$ stretching); $^1$H-NMR (300 MHz, $CDCl_3$, δ, ppm, *J*, Hz): 9.50 (s, NH); 8.49 (1H, t, 1.9, $H^5$); 8.19 (ddd, 8.2, 1.9, 1.1, $H^7$); 7.80 (1H, d, 8.2, $H^9$); 7.54 (1H, t, 8.2, $H^8$); 7.49 (1H, s, $H^3$); 7.32-7.10 ($8H_{ar}$, m); 6.99 (2H, s, H $^{10'-11'}$, axial isomer); 6.92 (2H, s, $H^{10'-11'}$, equatorial isomer); 4.60 ($H^{5'}$, t, 7.8, axial isomer), 4.07 ($H^{5'}$, t, 7.8, equatorial isomer); 3.84 (2H, $H^{12'}$, d, 7.8, equatorial isomer); 3.17 (2H, $H^{12'}$, d, 7.8, axial isomer); $^{13}$C-NMR (75 MHz, $CDCl_3$, δ, ppm): 174.70 ($C^{13'}$); 140.26 ($C^3$); 139.58 ($2C_q$); 135.87 ($2C_q$); 134.50 ($C_q$); 132.84 (CH); 131.26 ($C^{10'-11'}$); 129.73 (CH); 128.97 (CH); 126.90 (CH); 124.40 (CH); 121.51 (CH); 50.96 ($C^{5'}$); 33.65 ($C^{12'}$);

*2-(5H-dibenzo[a,d][7]annulen-5-yl)-N'-[3-metoxi-4-hydroxiphenylmethylidene]-acetohydrazide (7f):* Yield: 91.1%; *m.p.* 130-132 C°; elemental analysis: anal. calcd. for $C_{25}H_{22}N_2O_3$ (398.45 g/mol): C, 75.36; H, 5.57; N 7.03; found: C, 75.35; H, 5.58; N 7.04; UV-Vis($CH_3OH$) (λ max): 223.8, 292.5; IR (KBr, $cm^{-1}$): 3481, 3184 (N–H + O-H stretching), 3065, 3019 (C–H stretching of aromatic ring), 2966, 2941, 2899, ($CH_2$ + $CH_3$ stretching), 1663 (C=O stretching), 1590, 1562, 1517 (C=C stretching); 1254, 1036 (C-O-C stretching); $^1$H-NMR (300 MHz, $CDCl_3$, δ, ppm, *J*, Hz): 9.80 (s, NH); 7.92 (1H, s, $H^3$); 7.90-7.60 (8Har, m); 7.54 (1H, dd, 8.2, 1.9, $H^9$); 7.47 (2H, s, H $^{10'-11'}$); 7.33 (1H, s, $H^5$); 6.61 (1H, s, OH); 5.12 (1H, t, 7.7, $H^{5'}$); 4.50 (3H, s, $OCH_3$), 3.70 (2H, $H^{12'}$, d, 7.7); $^{13}$C-NMR (75 MHz, $CDCl_3$, δ, ppm): 173.92 ($C^{13'}$); 147.98 ($C_q$); 147.16 ($C_q$); 143.42 ($C^3$); 139.87 (2Cq) 134.64 ($2C_q$); 131.15 ($C^{10'-11'}$); 129.92 (CH); 129.85 (CH); 129.78 (CH); 128.89 (CH); 126.00 (CH); 122.55 (CH); 114.64 (CH); 107.93 (CH); 50.71 ($C^{5'}$); 58.57 ($CH_3$-O); 33.76 ($C^{12'}$);

*2-(5H-dibenzo[a,d][7]annulen-5-yl)-N'-[4-(N,N-dimethylamino)phenylmethylidene] acetohydrazide (7g):* Yield: 90.37%; *m.p.* 189-191 C°; elemental analysis: anal. calcd. for $C_{26}H_{25}N_3O$ (395.49 g/mol): C, 78.96; H, 6.37; N 10.62; found: C, 78.96; H, 6.38; N 10.63; UV-Vis($CH_3OH$) (λmax): 226.4, 297.8, 342.7; IR (KBr, $cm^{-1}$): 3252 (N–H stretching)3061, 3021 (C–H stretching of aromatic ring), 2968, 2883, 2811 ($CH_2$ + $CH_3$ stretching), 1664 (C=O stretching), 1609 (C=N stretching), 1552, 1525 (C=C stretching); $^1$H-NMR (300 MHz, $CDCl_3$, δ, ppm, *J*, Hz): 9.68 (s, NH, equatorial isomer); 9.32 (s, NH, axial isomer); 7.76 (1H, s, $H^3$); 7.80-7.70 ($8H_{ar}$, m); 7.50 (2H, s, H $^{10'-11'}$, axial isomer); 7.47 (2H, s, $H^{10'-11'}$, equatorial isomer); 5.22 ($H^{5'}$, t, 8.0, axial isomer), 4.65 ($H^{5'}$, t, 8.0, equatorial isomer); 4.39 (2H, $H^{12'}$, d, 8.0, equatorial isomer); 3.54 (6H, $(CH_3)_2N$, axial isomer); 3.68 (2H, $H^{12'}$, d, 8.0, axial isomer); 3.48 (6H, $(CH_3)_2N$, equatorial isomer); $^{13}$C-NMR (75 MHz, $CDCl_3$, δ, ppm): 173.57 ($C^{13'}$); 143.82 ($C^3$); 140.07 ($2C_q$); 134.53 ($2C_q$); 131.24 ($C^{10'-11'}$); 129.85 (CH); 129.80 (CH); 128.83 (CH); 128.60 (CH); 126.69 (CH); 111.98 (CH); 111.72 (CH); 50.58 ($C^{5'}$); 40.37 ($(CH_3)_2N$ ); 33.84 ($C^{12'}$);

*Cytoxicity evaluation*

The newly compounds were tested for potential cytotoxic activity using two alternative methods on invertebrate organisms: *Artemia salina* (brine shrimps) and *Daphnia magna*. These tests are simple, rapid and cost-efficiently and can predict several biological activities such as anticancer and analgesic activities [35-39].

Biological determinations were performed in duplicate. 5%DMSO was used as negative control. Positive control was represented by amitriptyline (**AMI**) and 2-(5*H*-dibenzo[a,d][7]annulen-5-yl)acetohydrazide **5** (**H**) at the same concentrations as compound samples. **AMI** was selected due to its structural similarities with the new synthesized compounds and available data concerning cytotoxicity effects on human cancer cells [39]. **H** was used in order to register the differences between the obtained compounds and the start material. Confidence intervals (CI95%) could not be calculated for some (e.g. AMI at 24h) of the determinations because of the results obtained and were noted with ND (not determined). The estimated value of LC50 of AMI at 24h is higher than 100 μmol/L, the highest tested concentration. Therefore, the value should be regarded >100 μmol/L, rathar than 684.0.

*Brine shrimp bioassay*

Brine shrimp (*Artemia salina* L.) lethality assay was performed using protocol described by Meyer (1982) [42] with some modifications [41]. Briefly, brine shrimp cysts obtained from a local aquarium shop (Bucharest, Romania) were incubated in artificial sea water (40 g/L salinity) for 48 h, under continuous aeration. 20 larvae were added in Petri dishes in a final volume of 4000μL containing serial dilutions of the compounds. Five concentrations ranging from $10^{-8}$ to $10^{-4}$ mol/L from each of the new synthesized compounds were tested. At 24 and 48h the number of survivors was counted and percentage of lethality was calculated. Naupli were considered dead if they did not move their appendages for 10 s during observation. The results of brine shrimps lethality test are presented in table 1.

*Daphnia magna bioassay*

*Daphnia magna* Straus have been maintained parthenogenetically in "Carol Davila" University (Department of Pharmaceutical Botany and Cell Biology), since 2012. The bioassay was performed according to the method described by Fan et al [44] with some modifications [43]. Young daphnids were sorted according to their size and, used in inserted (ten daphnids) in 10 mL test tubes with serial dilutions from each compound as described in A. salina bioassay. After 24h and 48h the lethality was calculated against the negative control.

| Compound | Moment of determination | LC50 (µmol/L) | CI95% of LC50 (µmol/L) | Std. error of Log[C] | ($r^2$) |
|---|---|---|---|---|---|
| **AMI** | 24 h | 684.0* | ND | 0.2406 | 0.8954 |
| | 48 h | 55.16 | 29.95 - 127.10 | 0.1571 | 0.9131 |
| **H** | 24 h | 1.50 | 0.69 - 3.29 | 0.1476 | 0.9359 |
| | 48 h | 0.42 | 0.17 - 1.04 | 0.1687 | 0.9125 |
| **7a** | 24 h | 17.32 | ND | 17.320 | 0.9941 |
| | 48 h | 15.72 | 7.31 - 33.82 | 15.720 | 0.9760 |
| **7b** | 24 h | 146.90 | 109.10 - 197.90 | 0.0561 | 0.9647 |
| | 48 h | 84.23 | 70.68 - 100.40 | 0.0330 | 0.9819 |
| **7c** | 24 h | 7.27 | 2.05 - 25.83 | 0.2388 | 0.8302 |
| | 48 h | 5.16 | 1.85 - 14.38 | 0.1930 | 0.8880 |
| **7d** | 24 h | 28.87 | 20.97 - 39.73 | 0.0601 | 0.9918 |
| | 48 h | 16.01 | ND | 1.3390 | 0.9891 |
| **7e** | 24 h | 2615.00* | ND | 0.2022 | 0.7167 |
| | 48 h | 1.73 | ND | 30.8800 | 0.9960 |
| **7f** | 24 h | 1263.00* | ND | 0.2476 | 0.9177 |
| | 48 h | 3030.00* | ND | 0.3706 | 0.8846 |
| **7g** | 24 h | 0.26 | 0.08 - 0.61 | 0.1862 | 0.8861 |
| | 48 h | 0.04 | 0.02 - 0.08 | 0.1302 | 0.9325 |

* - LC50 was estimated, but not included in the interval of determined concentrations; ND - not determined.

**Table 1**
CYTOTOXICITY OF THE COMPOUNDS ON *A. SALINA*

| Compound | Moment of determination | LC50 (µmol/L) | CI95% of LC50 (µmol/L) | Std. error of Log[C] | ($r^2$) |
|---|---|---|---|---|---|
| **AMI** | 24 h | 9.25 | 5.71 - 15.01 | 0.0911 | 0.9646 |
| | 48 h | 2.74 | 1.65 - 4.55 | 0.0958 | 0.9709 |
| **H** | 24 h | 10.19 | ND | ND | 0.9968 |
| | 48 h | 4.14 | 2.92 - 5.79 | 0.0633 | 0.9852 |
| **7a** | 24 h | ND | ND | ND | ND |
| | 48 h | ND | ND | ND | ND |
| **7b** | 24 h | ND | ND | ND | ND |
| | 48 h | ND | ND | ND | ND |
| **7c** | 24 h | 133.00 | 65.42 - 270.30 | 0.1336 | 0.9349 |
| | 48 h | 12.03 | 7.25 - 19.97 | 0.0954 | 0.9656 |
| **7d** | 24 h | ND | ND | ND | ND |
| | 48 h | ND | ND | ND | ND |
| **7e** | 24 h | ND | ND | ND | ND |
| | 48 h | ND | ND | ND | ND |
| **7f** | 24 h | 62.36 | 32.03 - 121.40 | 0.1255 | 0.9240 |
| | 48 h | 21.38 | 10.81 - 42.28 | 0.1284 | 0.9303 |
| **7g** | 24 h | 130.00 | 83.70 - 201.90 | 0.0829 | 0.9636 |
| | 48 h | 44.61 | 32.97 - 60.35 | 0.0856 | 0.9772 |

ND - not determined

**Table 2**
CYTOTOXICITY OF THE COMPOUNDS ON *DAPHNIA MAGNA*

Daphnids were considered dead if they did not move their appendages for 30 s during observation. Assay results are shown in table 2. Several LC50 values and their confidence intervals could not be calculated and were noted with ND (not determined).

*Statistical analysis*

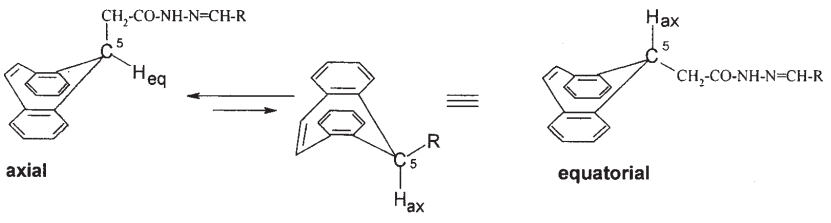
The lethal concentrations that kill 50% of organisms (LC50) were determined by interpolating on lethality - logarithm of concentration curves using the least squares fit method. 95% confidence interval, standard error of LC50 (CI95%) and the correlation coefficient ($r^2$) of the curves, were also calculated. All calculations were performed using GraphPad Prism version 5.0 software (USA).

## Results and discussions

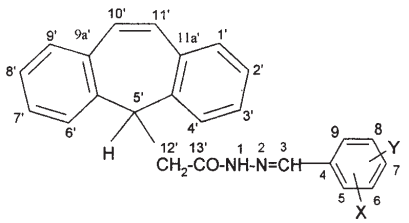
*Chemistry*

The nucleophilic addition of 2-(5*H*-dibenzo[a,d][7]annulen-5-yl)acetohydrazides to aromatic aldehydes is confirmed in the infrared spectra of the new acylhydrazones **7a–g** by the appearance of a new absorption bands due to stretching vibration of C=N group ( 1605-1620 $cm^{-1}$). The absorbtion in the 1686–1674 $cm^{-1}$ region corresponds to that of the amide group (-NH-CO-). The presence of NH group is indicated by absorbtion band at 3419–3184 $cm^{-1}$.

The $^1$H-NMR spectra of **7a-g** N-acylhydrazones indicated the presence of two isomers, 5'-axial and 5'-equatorial in about 3:1 ratio, interconvertible by middle ring inversion, except **7f** wich exist in a single conformational isomer, namely the axial one (scheme 2).




Scheme 2. The general structure of **7a-g** with atom numbering




7a: X= H, Y=H
7b: X= 4-$OCH_3$, Y= H
7c: X= 4-Br, Y= H
7d: X=4-$NO_2$ , Y= H
7e: X=3-$NO_2$ , Y= H
7f: X=4-OH, Y= 3-$OCH_3$
7g: X=4- $N(CH_3)_2$ , Y= H

In the spectrum of acylhydrazones **7a-g** the NH protons signals appear as singlets between 9.2-10.7ppm. The double bond protons $H^{10'}$ and $H^{11'}$ appear as singlets at 6.9-7.5ppm.

In the $^{13}$C-NMR spectrum of **7a-g** the dibenzo[*a,d*][7] annulene moiety appears in a narrow δ domain (122-140ppm). The signal at δ = 130.9-131.3ppm corresponds to the $C^{10'}$ and $C^{11'}$ atom.

It should be noted that in the $^{1}$H-NMR spectrum at **7a-g** the $H^{5'}$(eq) is deshielded, manifested as a triplet at 4.6-5.2 ppm, whereas the $CH_2^{12'}$ protons are shielded by the double bond, showing a doublet at 3.1-3.7 ppm (scheme 2). Duble bonds shield $H^{5'}$- axial, while aromatic rings deshield $H^{5'}$-equatorial, because of the current ring. The $H^{5'}$(ax) appears at δ=4.2-4.6 ppm (triplet).

In the $^{13}$C-NMR spectrum of **7a-g** the C=O signal appear at 173-174ppm and the -CH=N- signals appear at 141-147ppm.

*Cytoxicity evaluation*

Artemia salina bioassay

Compound **7g** induced the highest toxic effect on both moments of determination (24 h and 48 h), LC50 being about six-fold lower at 24 h and ten-fold lower at 48 h than LC50 of H and about 1380-fold lower than **AMI**. At 24 h LC50 ascending order of LC50 is: **7g**, **H**, **7e**, **7d**, **7b**, **AMI** and at 48h: **7g**, **H**, **7e**, **7a**, **7d**, **AMI** and **7b**. The 48 h LC50 were significant lower than 24 h for compounds **7g**, **H** and **AMI**, whereas for compounds **7a**, **7d** and **7b** were about two-fold lower. Compound **7f** did not induced lethality at tested concentrations on *A. salina* invertebrates. An unusual lethality was induced on brine shrimps by compound **7e**, which at 24 h did not exhibit any visible toxicity, whereas at 48 h being cytotoxic, with a LC50 of four-fold higher than **H** and about thirty-fold lower than **AMI**. The high difference of lethality of the tested compounds and **AMI** suggest that the new synthetized compounds induce cytotoxicity on *A. salina* through different mechanisms from those induced by **AMI**. Thus, the compounds should be tested in further studies on human cancerous cell lines in order to reveal the pathways involved in the biologic effect.

*Daphnia magna* bioassay

At 24 h the highest toxic effect was induced by compound **7f**, followed by **7g** and **7c**. The ascending order of toxicity at 48 h is **7c**, **7f** and **7g**. All other compounds did not induce any toxic effect on the tested concentrations, the highest toxicity being induced by **7a** and **7d** (20%). 48 h LC50 were about three-fold lower for **7a** and **7g** and 11-fold lower for **7c** than 24 h LC50 which can indicate an indirect mechanism of toxicity. Both positive controls showed good cytotoxicity at 24 and/or 48 h. The results obtained for **AMI** were close to those obtained by Calleja et al (1994) (LC50 at 24 h = 20 μmol/L) [39]. In comparison with positive controls, **AMI** and **H**, the cytotoxic effect was significant lower for all compounds. Thus, the LC50 of the most potent compound at 24 h – **7f** was about six-fold higher than both controls and at 48 h, **7c** induce a LC50 four-fold higher than **AMI** and three fold higher than **H**.

*D. magna* bioasay indicate that all tested compounds are less toxic than **AMI** and **H**.

## Conclusions

This paper presented synthesis and characterization of new acetyl hydrazones derivatives containing 5*H*-dibenzo[a,d][7]annulene moiety. The structures of compounds were confirmed by spectral data (IR-, UV-, $^{1}$H-NMR and $^{13}$C-NMR).

The $^{1}$H-NMR spectra of **7a-g** N-acylhydrazones indicated the presence of two isomers, 5'-axial and 5'-equatorial in about 3:1 ratio.

All the compounds have been investigated for their cytotoxic activity on aquatic invertebrates. The results of the two bioassays indicate that new compounds **7g** (with dimethylamino group) and **7c** (with bromine atom) induce cytotoxic effect at low concentrations and should be included in further screening tests in order to evaluate their mechanisms and to verify the data presented in *in vitro* models using cancerous human cells.

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