New Heterocyclic Compounds from 1,2,4-triazoles Class with Potential Cytotoxic Activity

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Acylhydrazinecarbothioamides (2a,b) were synthesized by addition of 2-(5H-dibenzo[a,d][7]annulen-5-yl)acetohydrazide to different isothiocyanates. The new 1,2,4-triazol-3-thioles (3a,b) were synthesized by cyclization of new 2- acylhydrazinecarbothioamides (3a,b) in basic media. Alkylation of 1,2,4-triazole-3-thiols (3a-c) with ethyl bromide gave only S-substituted derivatives (4a-c). The structures of the synthesized compounds have been established on spectral data (IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy) and elemental analysis. The cytotoxic effect of new compounds was evaluated using two alternative models on plant and invertebrate organisms.

Keywords: acylhydrazinecarbothioamide; 1,2,4-triazol-3-thiole; dibenzo[a,d][7]annulene; alkylation; cytotoxicity

Malignant tumors represent one of the most serious threats against human health in the world, and the clinical prognosis remains relatively poor. Overall, cancer is the second leading cause of death following heart disease [1]. The researches on obtaining new antitumor drugs answer the needs of improving and developing new therapeutic strategies in diseases with elevated mortality, in order to improve the life quality of patients with cancer and to increase the survival rate [2]. The antitumor drugs used in therapy are generally systemic antiproliferative agents that preferentially kill the dividing cells. These cytotoxic agents may be antimetabolites, alkylating agents, complexing agents of DNA, mitosis inhibitors or hormones which exert their activity by affecting the DNA replication, the cellular transcription or the cellular division [3].

The study of literature highlights the fact that molecules containing a group of hydrazinecarbothioamide present a variety of biological actions among which the antitumor action [4-6].

Due to its synthetic and biological versatility, investigations on compounds containing hydrazincarbothioamide groups have become attractive, targeting compounds for new drug development, because of their potentially biological activities involving antiproliferative activities [7,8]. Siwek and his collaborators have studied the antitumor action upon certain MCF-7 cell lines involved in breast cancer, upon certain substituted hydrazinecarbothioamides [7]. This property is due to their inhibitory action upon II and IV topoisomerase, an enzyme involved in DNA replication and cell transcription [8]. Some hydrazinecarbothioamide derivatives exert an inhibitory action upon the ribonucleotide reductase, similar to the action of certain effective antitumor agents such as Triapine and Metisazone [8]. Gulea and collaborators studied the inhibitory effect on the proliferation of HL-60 cells involved in human leukemia, of certain compounds containing hydrazinecarbothioamide fragments [3].

1,2,4-Triazoles have attracted considerable attention in medicinal chemistry due to their pharmacological activities: tuberculostatic, analgesic, antioxidant, antiviral, antitumor, antibacterial, anti-inflammatory, carbonic anhydrase inhibitors [9-12]. Moreover, some drugs containing 1,2,4-triazole ring possess antitumoral effect: Vorozole (antineoplastic and imunnomodulatory activity) [13], Letrozole and Anastrozole (used for the treatment of cancer breast) [14].

Compounds with dibenzo[a,d][7]annulene moieties have many pharmacological applications: psychotropic action, antidepressant action, analgesic, anti-inflammatory, antihistamine action, anticonvulsant action, antimicrobial, antihypertensive, antiarrhythmic, antitumor, antiparasitic, inhibition of metallo-proteases activity etc [15-19].

Considering these aspects, this present work aims at making a synthesis between two different classes of compounds, hydrazinecarbothioamide derivatives and respectively 1,2,4-triazole derivatives with dibenzo [a,d][7]annulene moieties and evaluation of the cytotoxic effect of new compounds.

The synthesis of the new compounds was realized in several steps according to the literature method, starting from 2-(5H-dibenzo[a,d][7]annulen-5-yl)acetohydrazide (1) [18]. Acylhydrazinecarbothioamides (2a,c) were synthesized by nucleophilic addition of 2-(5H-dibenzo[a,d][7]annulen-5-yl)acetohydrazide (1) to different isothiocyanates. Cyclization of acylhydrazinecarbothioamides in NaOH solution produced the corresponding 1,2,4-triazole-3(4H)-thioles. The treatment of 1,2,4-triazole-3(4H)-thioles (3a-c) with alkyl bromide, in basic media, produced S-alkylated derivatives.

The cytotoxicity of new compounds was assessed against *Triticum aestivum* L. (Poaceae) and *Daphnia magna* Straus (Daphniidae). These tests are used for the preliminary screening of toxicity, anticancer and analgesic activities of natural and synthesis compounds and plant extracts.

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Experiemental part

Materials and methods

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 wavelength range 200–600 nm. IR spectra were recorded on a FT-IR-100 Perkin Elmer spectrometer, in solid phase by ATR and frequencies are expressed in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra are recorded on Varian Gemini 300 BB spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C) using DMSO-d_6 as solvent for hydrazinecarbothioamides and $CDCl_3$ for 1,2,4-triazole compounds, chemical shifts are given in ppm relative to TMS as internal standard. Complementary spectra: 2D-NMR and decoupling. Coupling constants, *J*, are expressed in Hertz (Hz). The content of C, H, and N was assayed using an ECS-40-10-Costeh microdosimeter. Biological determinations were performed under constant temperature and light conditions using a Sanyo MLR-351 H, USA climatic chamber (25 ± 1 °C, in the dark) [20,21].

Synthesis and characterization of compounds

2-(5*H*-Dibenzo[a,d][7]annulen-5-yl)acetohydrazide (1), was synthesized according to the previously reported method [18]. The newly 2-(5H-dibenzo[a,d][7]annulen-5-ylacetyl)-N-arylhydrazinecarbothioamides (2a,b) were obtained by addition of 2-(5*H*-dibenzo[a,d][7]annulen-5-yl)acetohydrazide (1) to 3-iodophenyl or 2-(4-morpholino) ethyl isothiocyanates [18,22-24]. Cyclization of acylhydrazinecarbothioamides in NaOH solution produced the corresponding 1,2,4-triazole-3(4H)-thioles. The treatment of 1,2,4-triazol-3-thioles (3a-b) with ethyl bromide, in basic media, produced only the new S-alkylated 1,2,4-triazoles (4a-c).

Synthesis of 2-(5H-dibenzo[a,d][7]annulen-5-ylacetyl)-N-R-hydrazinecarbothioamides (2a,b)

A mixture of 2-(5H-dibenzo[a,d][7]annulen-5-yl)acetohydrazide (1) (0.002 mol) and appropriate isothiocyanate (3-iodophenyl or 2-(4-morpholino)ethyl) (0.002 mol) in 20 mL anhydrous ethanol was refluxed for 6 h. Then the reaction mixture after cooling at room temperature, was filtered off and recrystallized from ethanol yielding (2a,b).

2-(5H-dibenzo[a,d][7]annulen-5-ylacetyl)-N-(3-iodophenyl)hydrazinecarbothioamide (2a): Yield: 88.6%; m.p.: 147-149°C; elemental analysis: anal. calcd. for $C_{24}H_{20}IN_3OS$ (525.40 g/mol): C, 54.86; H, 3.84; N, 8.00; found: C, 54.87; H, 3.82; N, 8.01 %; Spectral data (table 1).

2-(5H-dibenzo[a,d][7]annulen-5-ylacetyl)-N-(2-(4-morpholino)ethyl)hydrazinecarbothio-amide (2b): Yield: 83.5%; m.p.: 148-150°C; elemental analysis: anal. calcd. for $C_{24}H_{28}N_4O_2S$ (436.56 g/mol): C, 66.03; H, 6.46; N, 12.83; found: C, 66.03; H, 6.44; N, 12.82 %; Spectral data (table 1).

Synthesis of 5-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-4-(R)-4H-1,2,4-triazole-3-thioles (3a,b)

A mixture of the corresponding hydrazine-carbothioamide (2a,b) (1 mmole) and 10 mL of 8% NaOH solution was refluxed for 6-9 h, then filtered. Afterwards the reaction mixture was cooled, filtered and the filtrate was treated with acetic acid. The obtained white precipitate was filtered and recrystallized from $CHCl_3$: petroleum ether (1:2/v:v).

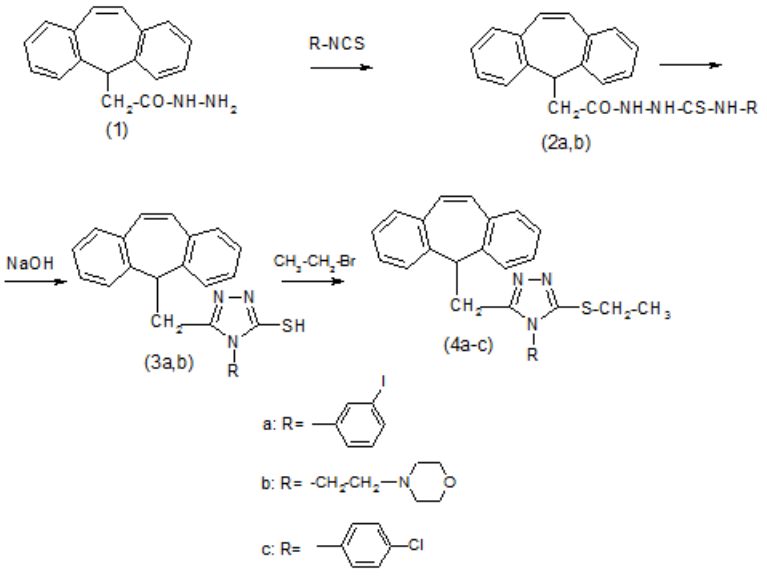
5-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-4-(3-iodophenyl)-4H-1,2,4-triazole-3-thiol (3a): Yield: 76.5%; m.p.: 200-202°C; Anal. calcd. for $C_{24}H_{18}IN_3S$ (FW: 507.39 g/mol): C, 56.81; H, 3.58; N, 8.28; Found: C, 56.80; H, 3.58; N, 8.26 %; spectral data (table 2).

5-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-4-(2-(4-morpholino))-4H-1,2,4-triazole-3-thiol (3b): Yield: 66.3%; m.p.: 281-283°C; Anal. calcd. for $C_{24}H_{26}N_4SO$ (FW: 418.55 g/mol): C, 68.87; H, 6.26; N, 13.39; Found: C, 68.85; H, 6.27; N, 13.41; spectral data (table 2).

General procedure for the preparation of 3-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-5-(ethylsulfanyl)-4-(R)-4H-1,2,4-triazoles (4a-c)

To a solution of sodium ethoxide (1mmol of sodium in 10 mL of absolute ethanol) was added the corresponding triazole (4a-c) (1 mmol). The reaction mixture was stirred at room temperature until a solution was obtained. To this solution was added ethyl bromide (1 mmol) and stirring was continued for 10 h. The reaction mixture was poured into ice water and the precipitate was filtered off, washed with water and recrystallized from ethanol.

3-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-5-(ethylsulfanyl)-4-(3-iodophenyl)-4H-1,2,4-triazole (4a): Yield: 55.2%; m.p. 166-167 °C; Anal. calcd. for $C_{26}H_{22}IN_3S$ (535.44g/mol): C, 58.32; H, 4.14; N, 7.85; Found: C, 58.33; H, 4.14; N, 7.83 %; spectral data, (table 3).



Scheme 1. Synthesis of the new compounds

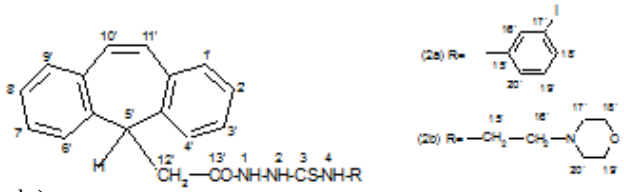


Table 1
SPECTRAL DATA FOR (2a,b)

Comp.	IR (KBr, ν, cm^{-1})	^{1}H-NMR (300 MHz) (DMSO-d_6, δ / ppm, J Hz)	^{13}C-NMR (75 MHz) (DMSO-d_6, δ / ppm, J Hz)
(2a)	3291, 3131, 3066, 3019 2978, 2866, 1680, 1602, 1585, 1546, 1470, 1253, 493;	10.29 (1H, *s*, NH), 9.80 (1H, *s*, NH), 9.07 (1H, s, NH), 7.00-7.80 (12H, *m*, aromatic), 7.03 (2H, *s*, H$^{10'}$, H$^{11'}$), 4.64 (1H, *t*, H$^{5'}$)- axial isomers, 3.56 (1H, *t*, 7.0, H$^{5'}$)- equatorial isomers, 2.62 (2H, *d*, 7.0, H$^{12'}$);	180.53 (C=S), 170.10 (C=O), 140.48 (C$_q$), 137.78 (2C$_q$), 134.90 (C$_q$)133.76 (C$_q$), 130.85 (C$^{10'}$, C$^{11'}$) equatorial isomers, 131.33 (2CH), 130.81 (C$^{10'}$, C$^{11'}$), 129.68 (2CH), 129.56 (2CH), 128.81 (2CH), 128.57 (CH), 127.97 (CH), 127.63 (CH), 125.56 (CH), 93.37 (C-I), 48.48 (C$^{5'}$), 34.56 (C$^{12'}$)
(2b)	3227, 3121, 3044, 3014, 2956, 2909, 2861, 2818, 1679, 1541, 1492, 1434, 1248;	10.15 (H, *s*, NH), 9.56 (H, *s*, NH), 9.39 (1H, s, NH), 6.95-7.50 (8H, *m*, aromatic), 7.03 (2H, *s*, H$^{10'}$, H$^{11'}$), 4.61 (1H, *t*,7.0, H$^{5'}$)- axial isomers, 3.80 (1H, *t*, 7.0, H$^{5'}$)- equatorial isomers, 3.41 (m, 8H, H$^{17'}$, H$^{18'}$, H$^{19'}$, H$^{20'}$), 2.58 (2H, *d*, 7.0, H$^{12'}$);	181.70 (C=S), 170.07 (C=O), 139.91 (2C$_q$), 133.75 (2C$_q$), 130.79 (C$^{10'}$, C$^{11'}$), 129.79 (CH), , 129.56 (CH), 128.80 (CH), 126.57 (CH), 65.72 (C$^{18'}$, C$^{19'}$) , 63.02 (C$^{17'}$, C$^{20'}$), 56.30 (C$^{16'}$), 48.65 (C$^{5'}$), 40.33 (C$^{15'}$), 34.47 (C$^{12'}$)

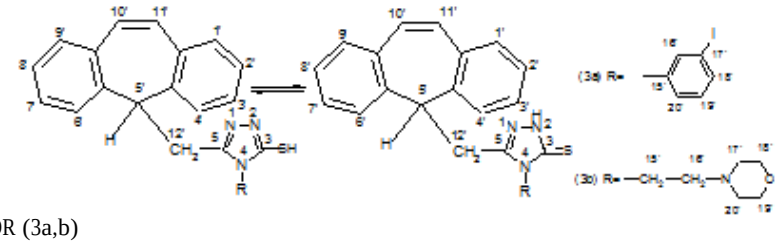


Table 2
^{1}H-NMRDATA FOR (3a,b)

Comp.	IR (KBr, ν, cm^{-1})	^{1}H-NMR (CDCl$_3$, δ / ppm, J Hz)	^{13}C-NMR (CDCl$_3$, δ / ppm, J Hz)
(3a)	3100, 3071, 3037, 2940, 2835, 1579, 1564, 1494, 1438, 502;	10.99 (1H, *s*, SH), 7.87 (1H, *ddd*, *J*=8.2, 2.8, 1.4 Hz, H-iodophenyl), 6.80-7.40 (11H, *m*, aromatic), 6.64 (2H, s, H$^{10'}$, H$^{11'}$), 4.43 (1H, *t*, 8.1, H$^{5'}$), 2.93 (2H, *d*, 8.1, H$^{12'}$)	167.61 (triazole-C3), 151.42 (triazole-C5), 138.96 (C$^{18'}$), 138.22 (2C$_q$), 136.81 (C$^{16'}$), 133.85 (2C$_q$), 130.89 (2CH), 130.65 (C$^{10'}$,C$^{11'}$), 130.10 (2CH), 129.60(2CH), 129.25 (2CH), 94.23 (C-I), 53.43 (C$^{5'}$), 25.91 (C$^{12'}$)
(3b)	3100, 3071, 3037, 2940, 2835, 1579, 1564, 1494, 1438;	11.24 (1H, *s*, SH), 7.12-7.20 (8H, *m*, aromatic), 6.98 (2H, s, H$^{10'}$, H$^{11'}$), 4.57 (1H, *t*, 7.3, H$^{5'}$), 3.67 (2H, *t*, 6.3, H$^{15'}$, H$^{16'}$), 3.57 (4H, *t*, 4.7, H$^{18'}$, H$^{19'}$), 3.13 (2H, *d*, 7.3, H$^{12'}$), 2.44 (m, 4H, H$^{17'}$, H$^{20'}$),	166.38 (triazole-C3), 151.60 (triazole-C5), 138.89 (2C$_q$), 133.67 (2C$_q$), 131.01 (C$^{10'}$,C$^{11'}$), 130.01 (2CH), 129.83 (2CH), 129.33 (2CH), 127.24(2CH), 66.78 (C$^{18'}$, C$^{19'}$) , 56.11 (C$^{16'}$), 53.80 (C$^{17'}$, C$^{20'}$), 52.66 (C$^{5'}$), 40.82 (C$^{15'}$), 27.03 (C$^{12'}$)

3-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-5-(ethylsulfanyl)-4-(2-(4-morpholino))-4H-1,2,4-triazole (4b): Yield: 52.2%; m.p. 320-322 (dec.)°C; Anal. calcd. for $C_{26}H_{30}N_4OS$ (446.60g/mol): C, 69.92; H, 6.77; N, 12.54; Found: C, 69.93; H, 6.78; N, 12.53; spectral data (tabel 3).

3-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-5-(ethylsulfanyl)-4-(4-clorophenyl)-4H-1,2,4-triazole (4c): Yield: 67.1%; m.p. 102-104 °C; Anal. calcd. for $C_{26}H_{22}ClN_3S$ (443.9g/mol): C, 70.33; H, 4.99; N, 9.46; Found: C, 70.32; H, 4.98; N, 9.47 %; spectral data (tabel 3)

Cytoxicity evaluation

Root growth inhibition was tested according to the method described in the literature with some modifications [22,25,26]. The test was performed using caryopses from *Triticum vulgare Mill.* The caryopses were washed and then soaked for 24h to promote germination. Pre-germinated caryopses were placed in Petri dishes (d = 90 mm) and compounds solutions of concentrations in range of 0.1 to 500 μM were added. Each solution was prepared in water with 1% DMSO. The caryopses were maintained for 24h in a climatic room chamber (Sanyo MLR351H, Sanyo) under the conditions described above. Amitriptyline (AMI) and 2-(5*H*-dibenzo[a,d][7]annulen-5-yl)acetohydrazide (1) in the same concentrations as the samples, and 1% DMSO were used as positive and negative, respectively controls (table 1). AMI was selected because it's structural similarities with the new synthesized compounds and available data concerning cytotoxicity effects on human cancer cells [25]. The embryonic root elongation was monitored and the length was measured using the application Image J version 1.46r (Wayne Rasband National Institutes of Health, USA). The values of root elongation were expressed in mm. The inhibitory effect was calculated using the equation

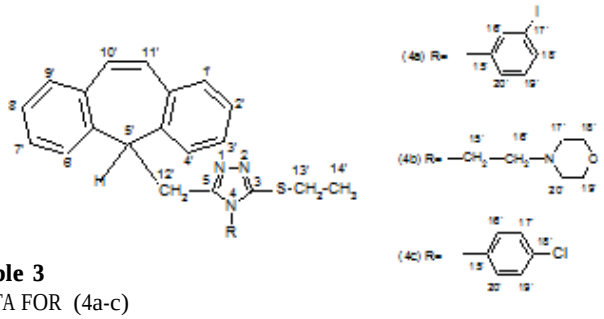


Table 3
^{1}H-NMR DATA FOR (4a-c)

Comp	IR (KBr, ν, cm^{-1})	^{1}H-NMR (CDCl$_3$, δ / ppm, J Hz)	^{13}C-NMR (CDCl$_3$, δ / ppm, J Hz)
(4a)	3067, 3018, 2978, 2928, 2869, 1584, 1570, 1493, 1446, 797, 460;	7.82 (H, *dl*, *J*=8.0 Hz, C$^{18'}$), 7.25-7.15 (8H, *m*, aromatic), 7.11 (t, 8.0, 1H, H$^{19'}$), 6.76 (t, 8.0, 1H, H$^{16'}$), 6.66 (dl, H$^{20'}$) 6.53 (2H, s, H$^{10'}$, H$^{11'}$), 4.64 (1H, *t*, 7.9, H$^{5'}$), 3.08 (q, 7.4, S-CH$_2$); 3.06 (2H, *d*, 7.9, H$^{12'}$), 1.28 (3H, t, 7.4, CH$_3$);	154.60 (triazole-C^3), 150.26 (triazole-C^5), 139.10 (2C$_q$), 138.66 (CH), 136.09 (CH), 133.99 (2C$_q$), 130.80 (CH), 130.63 (C$^{10'}$, C$^{11'}$), 129.95 (CH), 129.10 (CH), 126.96 (CH), 126.70 (CH); 95.25 (C-I), 54.46 (C$^{5'}$), 27.30 (C$^{12'}$), 25.21 (S-CH2), 14.71 (CH$_3$);
(4b)	3060, 3039, 2970, 2955, 2922, 2893, 2875, 2855, 2828, 1569, 1517, 1493, 1446, 775;	7.20-7.35 (8H, *m*, aromatic), 6.98 (2H, s, H$^{10'}$, H$^{11'}$), 4.75 (1H, *t*, 7.8, H$^{5'}$), 3.58 (2H, *tl*, 4.7, H$^{18'}$, H$^{19'}$), 3.37 (2H, *t*, 6.5, H$^{15'}$), 3.17 (2H, *d*, 7.8, H$^{12'}$), 3.05 (2H, q, 7.3, S-CH$_2$); 2.32 (2H, *tl*, 4.7, H$^{17'}$, H$^{20'}$), 2.21 (t, 6.5, H$^{15'}$), 1.23 (3H, t, 7.3, CH$_3$)	158.63 (triazole-C^3), 145.67 (triazole-C^5), 139.69 (C$_q$), 138.48 (C$_q$), 133.97 (C$_q$), 131.16 (C$^{10'}$,C$^{11'}$), 130.43 (C$_q$), 129.98 (C$_q$), 129.44 (2CH), 128.80 (2CH), 129.33 (2CH), 127.14(2CH), 66.90 (C$^{18'}$, C$^{19'}$) , 57.95 (C$^{16'}$), 53.92 (C$^{17'}$, C$^{20'}$), 57.83 (C$^{5'}$), 40.75 (C$^{15'}$), 26.55 (C$^{12'}$), 28.58 (S-CH$_2$), 14.79 (CH$_3$);
(4c)	3044, 3017, 2965, 2909, 2870, 2835, 1493, 1443, 766;	7.35 (2H, *d*, *J*=8.5 Hz, C$^{17'}$, C$^{19'}$), 7.27-7.10 (8H, *m*, aromatic), 6.53 (2H, s, H$^{10'}$, H$^{11'}$), 6.48 (d, 8.5, 2H, H$^{16'}$, H$^{20'}$), 4.63 (1H, *t*, 7.7, H$^{5'}$), 3.08 (2H, *q*, 7.4, S-CH$_2$), 3.05 (2H, *d*, 7.7, H$^{12'}$), 1.27 (3H, *t*, 7.4, CH$_3$);	154.74 (triazole-C^3), 150.38 (triazole-C^5), 139.27 (2C$_q$), 135.65 (C$_q$), 131.41 (C$_q$), 130.68 (C$^{10'}$, C$^{11'}$), 129.07 (CH), 129.90 (CH), 54.22 (C$^{5'}$), 27.23 (C$^{12'}$), 25.35 (S-CH$_2$), 14.79 (CH$_3$);

described in the literature [27]. Distribution of the results (D'Agostino Pearson normality), statistical differences between replicates and samples (Kruskal Wallis and ANOVA tests) and inhibitory effect vs. logarithm of concentration (least square method) were analyzed using GraphPad Prism v.5.0 software (GraphPad Software, USA).

D. magna test was performed according to the method described in the literature with some modifications [22,25,26]. Acute toxicity was performed in 4 mL 12-tissue culture wells, using 10 daphnids/well and each sample was tested in duplicate. The bioassay was performed using the same concentrations tested at *Triticum* bioassay, considering dead the organisms that did not move their appendages for 30 s. All experiments were conducted in dark, in the same conditions described above, using the same controls. The lethal concentration (LC50), which produces a 50% lethality, was determined by interpolation and 95% confidence interval (95% CI) were calculated. The statistical analysis was performed using GraphPad Prism version 5.01 software (GraphPad Software, USA). The cytotoxicity of new compounds was assessed using *Triticum aestivum* and *Daphnia magna* bioassays. LC50 and IC50 values and the statistics of the two bioassays are presented in table 4.

Table 4
RESULTS OF CYTOTOXICITY EVALUATION

Compound	*Triticum aestivum* bioassay			*Daphnia magna* bioassay		
	IC50 (μM)	95%CI (μM)	Goodness of fit (r^2)	LC50 (μM)	95%CI (μM)	Goodness of fit (r^2)
(2a)	ND	ND	NC	25.06	4.61 – 136.1	0.9305
(2b)	1114	46.53 - 2666	0.9005	24.02	6.76 – 85.28	0.9605
(3a)	ND	ND	NC	ND	ND	NC
(3b)	ND	ND	NC	70.05	45.24 – 108.5	0.9930
(4c)	ND	ND	NC	ND	ND	0.8120
(4a)	ND	ND	NC	ND	ND	0.6274
(1)	ND	ND	NC	11.74	5.51 – 95.48	0.9976
AMI	373.1	9.54 – 974.53	0.6411	16.28	0.48 – 541.4	0.9976

ND – not determined; NC – not correlated.

Results and discussions

The reaction sequences employed for synthesis of title compounds are showed in Scheme 1. In the present work, 2-acylhydrazinecarbothioamides (2a,b) were synthesized by reaction of 2-(5*H*-dibenzo[a,d][7]annulen-5-yl) acetohydrazide (1) with different isothiocyanate, in absolute ethanol, at reflux.

The synthetic pathway for the new *1,2,4-triazole-3-thioles* (3a,b) (that exist in equilibrium with their thione tautomer) consists of heterocyclization of acylhydrazine-carbothioamides (2a,b) in sodium hydroxide solution under reflux.

The treatment of 1,2,4-triazoles (3a-c) with ethyl bromide, in basic media, produced S-alkylated derivatives (4a-c) and not *N*-methylated derivatives.

The IR spectra of hydrazinecarbothioamide derivatives (2a,b) exhibit a new absorption band at 1248–1253 cm^{-1} corresponding to C=S stretching vibration. Also, in the IR spectra of these compounds (2a,b), a strong characteristic absorption band for carbonyl group at 1679–1680 cm^{-1} was present. The stretching bands corresponding to NH groups were observed in range 3121–3291 cm^{-1}.

Acylhydrazinecarbothioamides (2a,b) were present as two conformational isomers, 5'-axial and 5'-equatorial in about 3:1 ratio, interconvertible by middle ring inversion, confirmed by ^{1}H-NMR spectra), similar with our precedent work [18]. The $H^{5'}$(eq) appears at δ =4.61-4.64 ppm (triplet) and $H^{5'}$(ax) appears at δ=3.56-3.80 ppm (triplet). Double bonds shield $H^{5'}$- axial, while aromatic rings deshield $H^{5'}$- equatorial, because of the current cycle. The signals of NH protons appear as singlets between 9.07-10.29 ppm (tabel 1). The ^{13}C-NMR spectra of compounds (2a,b) showed a narrow δ domain (126-140 ppm) with $C^{10'}$ and $C^{11'}$ easily recognizable at δ=130-131 ppm, corresponding to dibenzo[*a,d*] [7]annulene moiety. C=S carbon atom is responsible for the appearance of a signal at δ=180-181 ppm.

We previously found [18] that 1,2,4-triazole-3-thiols gave rise to thione–thiol tautomeric equilibrium. Cyclization of (2a,b) to (3a,b) produced a single conformational isomer (the axial one) of triazoles. $H^{5'}$(eq) appears at δ=4.43-4.57 ppm (triplet) and $CH_2^{12'}$ protons manifest as doublets at 2.98-3.13 ppm in (4a,b). NH signals of (2a,b) disappeared and were replaced by singlets atδ=10.99-11.24 ppm attributable to SH proton (tabel 2). Thus, in solution, the above equilibrium is shifted towards the thiolic form. In ^{13}C-NMR spectra for 1,2,4-triazol-3-thioles appears a new quaternary carbon signal (for C^3) at δ=166.38-167.61 ppm (scheme 3) and a signal for C^5 at δ ~151 ppm.

The alkylation may involve both endocyclic nitrogen atom and exocyclic sulfur atom with formation of N- and S-substituted isomers. We examined alkylation of 1,2,4-triazoles (3a-c) and the structure of compounds (4a-b) as S-substituted 1,2,4-triazole-3-thiol was unambiguously proved by spectral analysis. Some new bands in 2929–2983 cm^{-1} region, due to presence of ethyl group (νCH_3 and νCH_2) in IR spectra, confirmed the structures of compounds (4a-c), obtained by alkylation of triazoles (3a-c) with ethyl bromide. A proof of S-alkylation which leaded to the formation of compounds (4a-c) is represented by the disappearance of C=S stretching band in IR spectra.

The most significant proof of the alkylation of triazoles (3a-c) with ethyl bromide was the presence in ^{13}C-NMR spectra of compounds (4a-c) of two new signals at 25.21-26.55 and 14.69–14.71 ppm corresponding to S-CH_2 and CH_3 carbon atoms from a ethyl group. Moreover, the heterocyclic carbons C^3 and C^5 from these ethylated compounds resonate at 154.60–158.63 ppm (more shielded than the C^3 heterocyclic carbon from 1,2,4-triazoles (3a-c)) and 145.67–150,38 ppm, respectively (table 3).

The cytotoxicity of new compounds was assessed using *Triticum aestivum* and *Daphnia magna* bioassays. LC50 and IC50 values and the statistics of the two bioassays are presented in table 4. IC50 could be calculated only for compound (2b), and its value indicate a low cytotoxicity on *Triticum aestivum*. All other compounds induced a maximum inhibitory effect of 40% and no correlation was found between the biological effect and concentration. Amitriptyline induced a moderate cytotoxicity on wheat roots, whereas compound (1) induced no modifications compared to negative control. On *D. magna*, compound (2b) induced the highest lethality, followed by (2a) and (3b). The toxicity induced by these compounds was lower than both, AMI and (1), although their 95% CI are similar. However, data analysis revealed that between LC50 values of (2b), (2a) and (3b) is a low or no statistical difference. Compounds (3a), (4a) and (4c) did not induced a lethality higher than 20% on *D. magna*. The functionalization of (1) determined a decrease in toxicity to *D. magna*.

Conclusions

In this study, we present the design, synthesis and characterization of two new hydrazinecarbothioamides, two new 1,2,4-triazole-3-thioles and three new S-alkylated 1,2,4-triazole derivatives incorporating in their molecule dibenzo[a,d][7]annulene moieties. The structure of the newly compounds were confirmed by IR, ^{1}H-NMR and ^{13}C-NMR spectroscopic analysis.

The cytotoxicity of new compounds was assessed against *Triticum aestivum* L. (Poaceae) and *Daphnia magna* Straus (Daphniidae). Three of the new synthetized compounds showed promising cytotoxic potential, effect similar to that induced by amitriptyline.

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