

PHYTOBIOLOGICAL ASSESSMENT OF SOME NEW COMPOUNDS WITH DIBENZO[a,d][7]ANNULENE MOIETIES

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Manuscript received: June 2014

Abstract

5H-Dibenzo[a,d][7]annulene ring compounds get an increasing attention because of their multiple applications, especially in the medical field. For this reason, we have considered useful to apply phytobiological tests of some compounds having 5H-dibenzo[a,d][7]annulene ring, using the Constantinescu method, *Triticum* test. Synthesis of these compounds was described in our previous papers. The influence of the compounds tested on wheat embryonic root elongation depends both on chemical structure and on concentration, most compounds presenting mitoinhibitory activity, without presenting a cytotoxic effect. Hydrazinecarbothioamide substituted in position 4 with 4-fluorophenyl radical has a cytotoxic effect for the most concentrated tested solution.

Rezumat

Compușii cu nucleu 5H-dibenzo[a,d][7]anulenic atrag tot mai mult atenția ca urmare a numeroaselor lor aplicații, mai ales în domeniul medical. Din acest motiv, am considerat utilă testarea fitobiologică a unor compuși cu nucleu 5H-dibenzo[a,d][7]anulenic folosind metoda Constantinescu, testul *Triticum*. Sinteza acestor compuși a fost descrisă în lucrările noastre anterioare. S-a constatat că influența compușilor testați asupra alungirii radiculare la cariopsele de grâu depinde atât de structura chimică, cât și de concentrația compușilor testați cei mai mulți compuși manifestând efect mitoinhibitor, fără efect citotoxic. Hidrazincarbotoamida substituită în poziția 4 cu radicalul 4-fluorofenil manifestă efect citotoxic în cazul soluției celei mai concentrate.

Keywords: dibenzo[a,d][7]annulene, phytobiological testing, *Triticum* test

Introduction

The 5H-dibenzo[a,d][7]annulene ring is present in the structure of many compounds used in therapy as antibacterial, anticonvulsive, anticholinergic, miorelaxant, antihistaminic, antifungal, carbonic anhydrase inhibitors, anti-inflammatory, analgesic, anti-arrhythmia, antiparasitic agents, but mostly they are used as antidepressant drugs [1-6, 8, 12]. For this reason, we report in the present investigation phytobiological tests of some new compounds with 5H-dibenzo[a,d][7]annulene moieties, using Constantinescu method, *Triticum* test [7, 11, 12, 14]. Synthesis and characterization of these compounds were presented previously [3-5, 10].

We considered this method to be the most appropriate for preliminary toxicity testing, as wheat caryopses are very sensitive, and permit a rapid test.

The aim was to establish correlations between chemical structure of the synthesized compounds and the effects upon plant cells, considering macroscopic changes (aspect, number of radicles, lengths of epicotyl) at various concentrations and time of acting, as well, karyokinetic changes. In the same time, we determined the minimal active concentration of the tested compounds, which, depending on the time of acting, influences the process of root elongation.

Materials and Methods

The method consists in studying the influence of substances in various dilutions, on the root elongation and karyokinetic film, in well-defined experimental, laboratory conditions, depending on their acting time on the wheat (*Triticum vulgare* Mill, Dropia variety 2008) caryopses germinated in laboratory conditions [14].

The biologic material used was represented by embryonic roots of wheat caryopses (*Triticum vulgare* Mill, Dropia variety), germinated and tested in laboratory conditions. We tested the action of 15 compounds with 5H-dibenzo[a,d][7]annulene ring, conventionally noted from 1 to 15 (Table I).

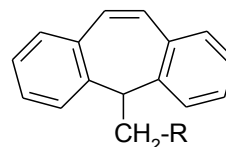
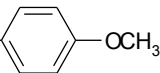
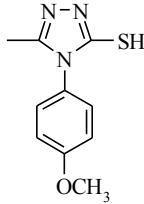
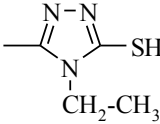
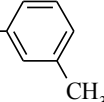
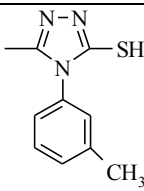
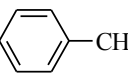
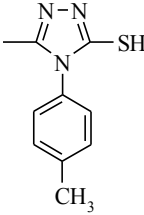
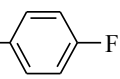
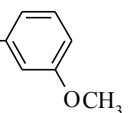
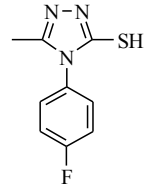
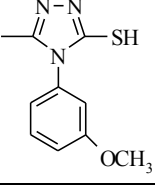
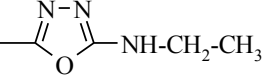
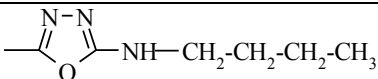


Table I
Chemical structure of the tested compounds

Compound	R	Name
1	CO-NH-NH ₂	2-(5H-Dibenzo[a,d][7]annulen-5-yl)acetohydrazide
2	CO-NH-NH-CS-NH- 	2-(5H-dibenzo[a,d][7]annulen-5-ylacetyl)-N-(4-methoxyphenyl)hydrazinecarbothioamide
3	CO-NH-NH-CS-NH-CH ₂ -CH ₃	2-(5H-dibenzo[a,d][7]annulen-5-ylacetyl)-N-ethylhydrazinecarbothioamide
4		5-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-4-(4-methoxyphenyl)-4H-1,2,4-triazole-3-thiol
5		5-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-4-ethyl-4H-1,2,4-triazole-3-thiol
6	CO-NH-NH-CS-NH- 	2-(5H-dibenzo[a,d][7]annulen-5-ylacetyl)-N-(3-methylphenyl)hydrazinecarbothioamide
7		5-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-4-(3-methylphenyl)-4H-1,2,4-triazole-3-thiol
8	CO-NH-NH-CS-NH- 	2-(5H-dibenzo[a,d][7]annulen-5-ylacetyl)-N-(4-methylphenyl)hydrazinecarbothioamide
9		5-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-4-(4-methylphenyl)-4H-1,2,4-triazole-3-thiol
10	CO-NH-NH-CS-NH- 	2-(5H-dibenzo[a,d][7]annulen-5-ylacetyl)-N-(4-fluorophenyl)hydrazinecarbothioamide
11	CO-NH-NH-CS-NH- 	2-(5H-dibenzo[a,d][7]annulen-5-ylacetyl)-N-(3-methoxyphenyl)hydrazinecarbothioamide

12		5-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-4-(4-fluorophenyl)-4H-1,2,4-triazole-3-thiol
13		5-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-4-(3-methoxyphenyl)-4H-1,2,4-triazole-3-thiol
14		2-(Aminoethyl)-5-[5H-dibenzo[a,d][7]annulen-5-ylmethyl]-1,3,4-oxadiazole
15		2-(Aminobutyl)-5-[5H-dibenzo[a,d][7]annulen-5-ylmethyl]-1,3,4-oxadiazole

Sample preparation. All the compounds were tested at 10^{-4} (C1), 6.6×10^{-5} (C2), 3.3×10^{-5} (C3) and 6.6×10^{-6} (C4) mol/L concentrations. The primary solutions were obtained by dissolving the substances in chloroform. Thus, the 10^{-4} M solutions were prepared by dissolving the substances in chloroform in volumetric flasks.

In Petri dishes with a diameter of 10 cm were added 15 mL, 10 mL, 5 mL and respectively 1 mL of the prepared chloroform solution of each compound. After evaporation to dryness on a water-bath, a volume of 15 mL of distilled water was added. In parallel, a control sample (M) was prepared, in which the test solution was replaced by distilled water. In each Petri box 10 caryopses of germinated wheat were placed, with the main root length of 1 cm. The dishes were covered with their lids and the caryopses were left in contact with the solutions for 5 days. Root elongation was evaluated at the same time for 5 days. There were performed 10 determinations for each case and in the tables there are presented the mean values. The experiments were performed in duplicate.

Macroscopic examination. Observations concerned the morphological changes, aspect, the number of radicles and the length of the main radicle. To assess the root growth in relation to the control sample, we used a linear measurement technique, estimating globally the axial growth of the main root, from the initial moment.

Microscopic examination. For the microscopic examination, after 24 h, the embryonic root of two

caryopses from each Petri dish was sectioned at a distance of 5 mm from the tip and stained with diluted acetic orcein, a dye with a great affinity for chromatin in acetic medium (an acid pH is required for the hydrolysis of the chromatin) which will be stained red. The stained sections were microscopically examined using a LaboPhot II Nikon microscope (ocular 10 $\times$, object-glass 100 $\times$) by immersion in cedar oil.

The observed changes were compared to a control sample, which presented the normal mitotic phases: prophase, metaphase, telophase, and anaphase.

The data represent the average values from two independent experiments and the results were processed statistically by the *Student's t-test* and *Fischer test* (Table II – for the first determination, Table III – for the second determination, Figures 1-4 for the first determination and Figures 5-8 for the second determination).

Results and Discussion

The effects of the tested compounds 1-15 on sprouting of wheat have been investigated. After treating with solutions 10^{-4} , 6.6×10^{-5} , 3.3×10^{-5} and 6.6×10^{-6} mol/L concentrations of compounds 1-15 for 5 days and comparing the length between the main root of caryopses treated with the tested compounds and those treated with distilled water, the plant growth regulating activities have been determined with the following formula:

$$\text{Effect (\%)} = 100 - \frac{\text{length of sample's radicle (mm)} - 10 \text{ mm}}{\text{length of reference's radicle (mm)} - 10 \text{ mm}} \times 100,$$

where 10 mm represents the initial length of the main root. A positive result represents an inhibition, whereas a negative result implies growth stimulation.

The results of the *Triticum* bioassay - Constantinescu method (the root elongation values for each sample tested, in the day) are presented in Table II, III and Figures 1-8.

Table II

The influence of the compounds tested on wheat embryonic root elongation- first determination

Compound	Conc.* mol/L	Time 24h	Time 48h	Time 72h	Time 96h	Time 120h	Inhibition index %	Stimulation index %	Fisher Test $F_{calc.}^{**}$	Student's $t\text{-test } t_{calc.}^{***}$
M	-	2.10	3.93	4.72	5.88	7.12	-	-		
1	C1	3.61	6.35	8.02	10.92	12.18	-	82.67	1.711	-9.050
	C2	3.56	5.98	7.92	10.11	11.43	-	70.64	1.850	-7.147
	C3	3.31	5.86	7.51	9.55	10.86	-	61.11	1.962	-2.176
	C4	3.22	5.46	7.21	9.32	10.58	-	56.53	1.078	-2.403
2	C1	1.77	3.56	4.40	5.53	6.62	8.16	-	1.266	-0.333
	C2	1.85	3.58	4.48	5.70	7.05	1.14	-	2.465	0.033
	C3	1.78	3.75	4.61	5.86	7.10	0.32	-	1.677	0.012
	C4	2.15	4.07	4.93	6.01	7.20	-	1.3	1.332	-0.052
3	C1	2.92	4.92	6.22	9.47	11.56	-	72.54	1.335	-2.909
	C2	2.96	4.90	5.33	7.46	9.31	-	35.78	1.046	-1.533
	C3	2.33	4.60	5.18	6.30	7.48	-	5.88	1.737	-0.218
	C4	2.15	4.51	4.76	5.96	7.40	-	4.57	1.018	-0.222
4	C1	1.48	3.08	4.52	5.23	6.53	9.64	-	1.647	0.363
	C2	1.63	3.27	4.62	5.45	6.77	5.71	-	3.277	-
	C3	1.62	3.36	4.72	5.63	6.91	3.43	-	1.496	0.133
	C4	1.73	3.52	4.78	5.72	6.95	2.77	-	1.806	0.162
5	C1	1.31	2.77	2.97	4.57	5.67	23.69	-	1.192	0.981
	C2	1.37	2.91	3.73	5.12	5.92	19.60	-	1.256	0.897
	C3	1.75	3.18	3.97	5.40	6.48	10.45	-	2.019	0.369
	C4	1.92	3.83	5.28	7.10	8.08	-	15.68	2.113	0.545
6	C1	1.75	3.07	4.47	5.45	6.55	9.31	-	1.410	0.437
	C2	1.87	3.31	4.65	5.65	6.95	2.77	-	1.547	0.133
	C3	1.98	3.52	4.66	5.53	7.08	0.65	-	1.627	0.025
	C4	2.31	4.15	5.38	6.31	7.42	-	4.90	1.729	0.239
7	C1	1.89	3.60	4.32	5.52	6.90	3.59	-	5.567	-
	C2	2.01	3.77	4.61	5.72	7.15	-	0.49	3.18	-
	C3	2.61	4.08	5.22	6.21	7.35	-	3.75	4.016	-
	C4	2.72	4.51	5.58	6.70	7.78	-	10.78	1.361	0.430
8	C1	1.30	2.55	3.50	4.43	5.27	30.22	-	1.806	1.106
	C2	1.38	3.01	3.92	4.81	5.63	24.34	-	1.677	1.181
	C3	1.51	3.63	4.32	5.01	5.78	21.89	-	2.317	0.737
	C4	1.60	3.75	4.53	5.32	5.92	19.60	-	1.204	0.809
9	C1	1.22	1.81	2.36	2.66	2.86	69.61	-	1.727	2.583
	C2	1.42	2.02	2.57	2.96	3.43	60.29	-	3.527	-
	C3	1.55	2.83	3.01	3.55	4.26	46.73	-	3.781	-
	C4	1.63	3.41	3.91	4.21	4.91	36.11	-	1.539	1.723
10	C1	1.01	1.02	1.05	1.13	1.25	95.91	-	4.815	-
	C2	1.02	1.08	1.30	1.72	2.41	76.96	-	1.214	3.170
	C3	1.04	1.16	1.42	1.87	2.82	70.26	-	1.047	3.010
	C4	1.04	1.16	1.42	2.28	3.08	66.01	-	2.225	3.376
11	C1	2.17	3.21	4.31	5.45	6.78	5.55	-	1.282	0.225
	C2	2.02	3.21	4.46	5.61	6.92	3.26	-	1.393	0.153
	C3	2.08	3.85	4.57	5.76	7.05	1.14	-	1.822	0.042
	C4	2.10	3.90	4.71	5.80	7.07	0.81	-	6.348	-
12	C1	2.63	4.53	5.78	7.10	8.00	-	14.47	1.176	-0.597
	C2	2.52	4.51	5.68	6.78	7.91	-	12.90	1.496	-0.501
	C3	2.25	4.41	4.86	5.96	7.30	-	2.94	1.679	-0.143
	C4	2.10	3.46	4.43	5.71	6.92	3.26	-	2.466	0.108
13	C1	1.57	2.42	3.11	4.53	6.20	15.03	-	1.484	0.712
	C2	1.88	2.80	3.47	4.87	6.38	12.09	-	4.041	-
	C3	1.97	2.91	3.55	4.93	6.43	11.27	-	1.535	0.434
	C4	2.06	3.16	3.95	5.22	6.57	8.98	-	1.568	0.344

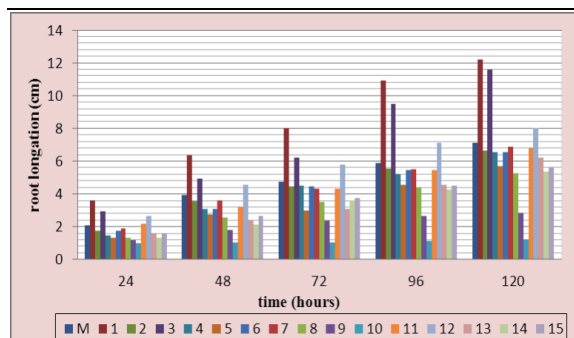
*C1 = 10^{-4} mol/L, C2 = 6.6×10^{-5} mol/L, C3 = 3.3×10^{-5} mol/L, C4 = 6.6×10^{-6} mol/L, **F = 3.18, ***t = 2.10

Table III

The influence of the compounds tested on wheat embryonic root elongation - second determination

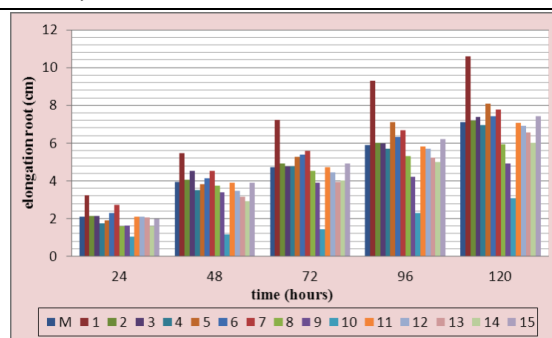
Compound	Conc. mol/L	Time 24h	Time 48h	Time 72h	Time 96h	Time 120h	Inhibition index %	Stimulation index %	Fisher test $F_{calc.}^*$	Student's t -test $t_{calc.}^{**}$
M	-	2.07	4.78	5.66	6.00	8.20	-	-	-	-
1	C1	3.67	6.52	8.23	9.72	12.13	-	54.58	1.161	-2.376
	C2	3.58	6.00	8.01	9.01	11.43	-	44.86	1.079	-2.068
	C3	3.37	5.72	7.72	8.51	11.01	-	39.02	1.020	-1.774
	C4	3.23	5.23	7.55	8.20	10.61	-	33.47	1.314	-1.408
2	C1	1.71	3.96	4.98	6.45	7.33	12.08	-	4.821	-
	C2	1.80	4.22	5.21	6.71	7.72	6.66	-	1.410	0.197
	C3	1.87	4.61	5.61	6.86	7.91	4.02	-	1.160	0.181
	C4	2.40	5.18	5.92	7.11	8.50	-	4.16	1.087	0.535
3	C1	2.78	5.40	6.66	9.62	11.42	-	44.72	1.002	-2.065
	C2	2.72	5.10	6.22	7.52	9.06	-	11.94	1.857	-0.147
	C3	2.23	4.92	5.93	7.05	8.48	-	3.88	1.069	-0.179
	C4	2.10	4.90	5.72	6.96	8.27	-	0.97	1.839	-0.050
4	C1	1.55	3.23	5.07	6.46	7.56	8.88	-	1.171	0.418
	C2	1.56	3.27	5.17	6.73	7.67	7.36	-	1.581	0.293
	C3	1.62	4.23	5.55	6.86	7.83	5.13	-	1.692	0.200
	C4	1.71	4.55	5.56	6.93	7.91	4.02	-	1.020	0.183
5	C1	1.36	3.70	4.00	5.45	6.67	21.25	-	1.156	0.996
	C2	1.56	4.06	4.63	6.20	7.16	14.44	-	1.582	0.723
	C3	1.83	4.45	5.13	6.40	7.53	9.30	-	1.286	0.447
	C4	2.23	4.83	5.83	7.40	8.75	-	7.63	2.719	0.253
6	C1	1.77	3.16	4.97	6.45	7.42	10.83	-	1.800	1.414
	C2	1.91	3.31	5.01	6.65	7.82	5.27	-	1.672	0.207
	C3	1.93	3.58	5.01	6.68	8.00	2.77	-	1.753	0.107
	C4	2.30	4.20	5.86	7.31	8.36	-	2.22	1.126	0.098
7	C1	1.77	4.48	5.40	6.36	7.88	4.44	-	1.015	0.202
	C2	2.18	4.90	5.73	7.13	7.90	4.16	-	1.052	0.191
	C3	2.41	5.01	5.86	7.21	8.47	-	-	2.680	-0.125
	C4	2.80	5.37	6.30	7.71	8.70	-	-	2.893	-0.383
8	C1	1.30	2.63	3.40	5.17	6.42	24.72	-	1.430	0.015
	C2	1.27	2.88	3.92	5.60	6.72	20.55	-	3.022	1.140
	C3	1.36	3.70	4.62	6.36	6.98	16.94	-	2.084	0.617
	C4	1.53	4.02	5.01	6.42	7.31	12.36	-	1.660	0.485
9	C1	1.32	1.83	2.67	3.07	3.68	62.77	-	1.294	2.652
	C2	1.53	2.06	2.75	3.46	3.92	59.44	-	1.800	2.273
	C3	1.71	3.08	3.01	3.55	4.57	49.58	-	2.297	1.777
	C4	1.75	3.30	4.80	5.31	5.87	32.36	-	2.994	1.793
10	C1	1.02	1.03	1.10	1.13	1.35	95.13	-	7.200	-
	C2	1.02	1.07	1.36	1.80	2.46	79.72	-	1.138	3.722
	C3	1.03	1.13	1.50	2.10	3.00	72.22	-	1.266	3.454
	C4	1.06	1.20	1.51	2.37	3.43	66.25	-	1.618	3.333
11	C1	1.97	3.71	5.27	6.66	7.78	5.83	-	1.278	0.272
	C2	2.02	4.43	5.37	6.72	7.80	5.55	-	1.473	0.275
	C3	2.08	4.65	5.57	6.83	8.05	2.08	-	1.410	0.086
	C4	2.10	4.90	5.73	7.16	8.33	-	1.80	1.305	-0.087
12	C1	2.55	4.93	5.93	7.56	9.00	-	11.11	1.929	0.577
	C2	2.35	4.92	5.83	7.46	8.81	-	8.47	1.030	0.380
	C3	2.22	4.86	5.68	7.21	8.47	-	3.75	2.247	0.200
	C4	2.11	4.46	5.57	6.78	8.22	-	0.27	1.183	-0.012
13	C1	1.63	3.01	4.15	5.51	6.46	24.16	-	1.523	1.201
	C2	1.86	3.28	4.53	5.93	6.70	20.83	-	3.296	-
	C3	2.02	3.60	4.71	6.00	6.88	19.44	-	1.442	0.751
	C4	2.16	3.67	4.85	6.27	6.97	17.08	-	1.327	0.825

*C1 = 10^{-4} mol/L, C2 = 6.6×10^{-5} mol/L, C3 = 3.3×10^{-5} mol/L, C4 = 6.6×10^{-6} mol/L, **F = 3.18, ***t = 2.10

**Figure 1.**

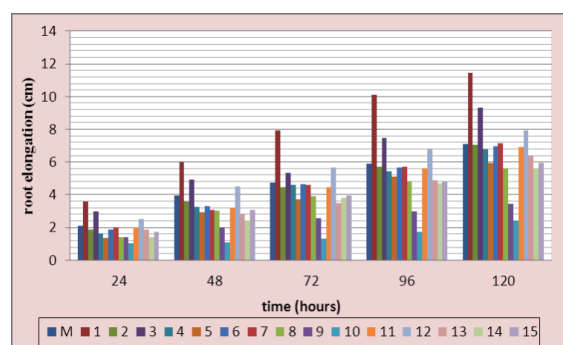
Daily root elongation in *Triticum* test under the treatment with 1-15 compounds at $C1 = 10^{-4}$ mol/L concentration (first determination)****

****M = control sample; 1-15 = tested compounds

**Figure 4.**

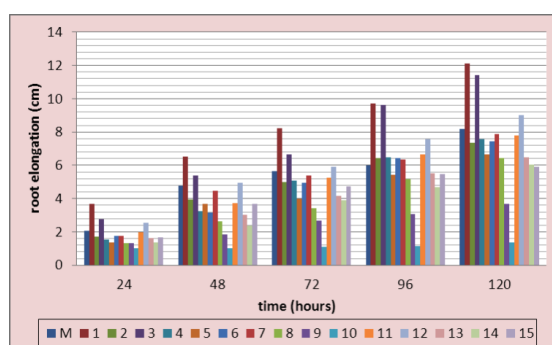
Daily root elongation in *Triticum* test under the treatment with 1-15 compounds at $C4 = 6.6 \times 10^{-6}$ mol/L concentration (first determination)****

****M = control sample; 1-15 = tested compounds

**Figure 2.**

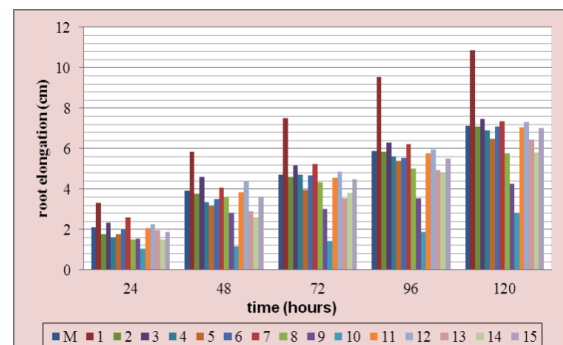
Daily root elongation in *Triticum* test under the treatment with 1-15 compounds at $C2 = 6.6 \times 10^{-5}$ mol/L concentration (first determination)****

****M = control sample; 1-15 = tested compounds

**Figure 5.**

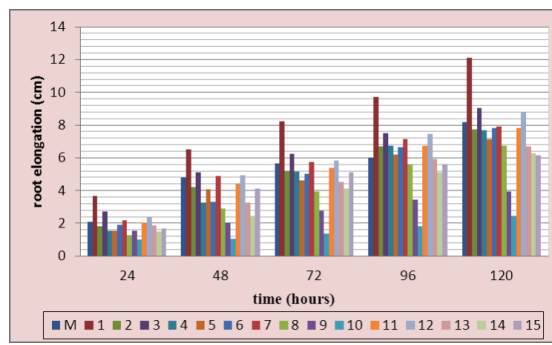
Daily root elongation in *Triticum* test under the treatment with 1-15 compounds at $C1 = 10^{-4}$ mol/L concentration (second determination)****

****M = control sample; 1-15 = tested compounds

**Figure 3.**

Daily root elongation in *Triticum* test under the treatment with 1-15 compounds at $C3 = 3.3 \times 10^{-5}$ mol/L concentration (first determination)****

****M = control sample; 1-15 = tested compounds

**Figure 6.**

Daily root elongation in *Triticum* test under the treatment with 1-15 compounds at $C2 = 6.6 \times 10^{-5}$ mol/L concentration (second determination)****

****M = control sample; 1-15 = tested compounds

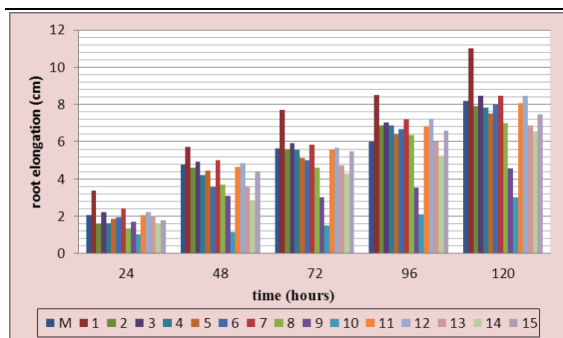


Figure 7.

Daily root elongation in *Triticum* test under the treatment with 1-15 compounds at $C3 = 3.3 \times 10^{-5}$ mol/L concentration (second determination)****
 ****M = control sample; 1-15 = tested compounds

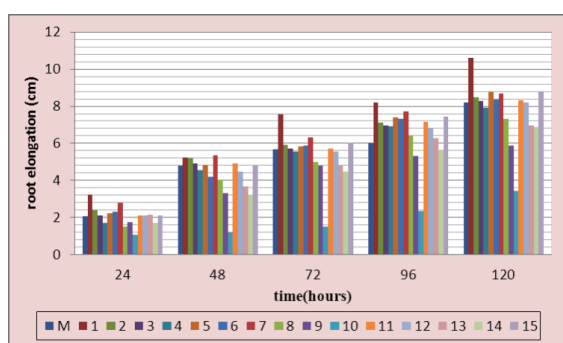


Figure 8.

Daily root elongation in *Triticum* test under the treatment with 1-15 compounds at $C4 = 6.6 \times 10^{-6}$ mol/L concentration (second determination)****
 ****M = control sample; 1-15 = tested compounds

We noticed that, for 10^{-4} mol/L concentration, there was registered a preponderant inhibitory effect, compared with control. Exceptions are represented by compounds 1, 3 and 12, which exhibited growth stimulatory effects, especially for (2-(5H-dibenzo[a,d][7]annulen-5-yl)acetohydrazide 1. At this concentration, 2-(5H-dibenzo[a,d][7]annulen-5-ylacetyl)-N-(4-fluorophenyl)hydrazinecarbothioamide 10, presented a cytotoxic effect.

At 6.6×10^{-5} mol/L concentration, the effect upon the radicular elongation was preponderant mitoinhibitory, compared to control. On the contrary, compounds 1, 3 and 12 manifested a growth stimulatory effect on radicle elongation, especially for compound 1.

At 3.3×10^{-5} mol/L, the effect was inhibitory, compared to control, for compounds 4, 5, 8, 9, 10, 13, 14 and 15. Compounds 1, 3, 7 and 12 had a stimulatory effect on root elongation, especially for compound 1. Substances 2, 6 and 11 presented a similar effect to the control.

For 6.6×10^{-6} mol/L concentration, the effect upon radicle growth was mitoinhibitory, compared to control, for compounds 4, 8, 9, 10, 13 and 14. Substances 1, 3, 5, 6 and 7 manifested a growth

stimulatory effect on radicles, especially for compound 1 (56.53%). Compounds 3, 11, 12, 15 had a similar behaviour as the control.

The influence of the tested compounds on radicle elongation for wheat caryopses, germinated and treated in laboratory conditions, depends on the chemical structure and concentration of them.

2-(5H-Dibenzo[a,d][7]annulen-5-yl)acetohydrazide 1 exhibited a strong stimulatory effect on radicle elongation, this aspect being confirmed at both determinations. There is a linear dependence between the stimulatory effect and concentration.

Hydrazinecarbothioamides have different actions, depending on the radical bond to N^4 . If the radical is aliphatic (ethyl), the effect is stimulatory. Replacing aliphatic radical with an aromatic radical (3-anisyl, 4-anisyl, 4-tolyl, 3-tolyl, 4 fluorophenyl), a mitoinhibitory effect is observed, which is linear depending on concentration, except compound 6, which, at 6.6×10^{-6} mol/L manifested a slightly stimulatory effect. The 4-substituted hydrazinecarbothioamide with fluorophenyl presented a cytotoxic effect for the most concentrated tested solution.

The effect of 1,2,4-triazoles on radicle elongation depends on their chemical structure. Substituted derivatives in position 4 with 4-tolyl, 4-anisyl and 3-anisyl presented a mitoinhibitory effect regardless of concentration. The higher the concentration the stronger was the mitoinhibitory effect. The 1,2,4-triazole derivative substituted in position 4 with ethyl manifested a mitoinhibitory effect at 10^{-4} mol/L, 6.6×10^{-5} mol/L and 3.3×10^{-5} mol/L concentrations. The triazole derivative substituted in position 4 with 3-tolyl presented a mitoinhibitory effect at 10^{-4} mol/L, a neutral effect (compared to control) at 6.6×10^{-5} mol/L, and a stimulating effect at other tested concentrations. The 1,2,4-triazole derivative substituted in position 4 with fluorophenyl induced a weak mitoinhibitory effect, compared to the control, only for the most diluted tested solution.

The data represent the average values from two independent experiments and the results were processed statistically by the Student's *t*-test and Fischer test (Table II and III).

In order to proper use Student's *t*-test, it is important to establish whether groups had equal or unequal variance. F Fisher test is used to determine this aspect. Using Fisher test the variance was verified for both groups: wheat caryopses treated with tested substances, at certain concentration, and control ones (treated with distilled water).

Once the equality of variance was established, Student's *t*-test was applied. There were taken into consideration the same samples obtained for *Triticum* test.

The level of statistical significance is $\alpha = 0.05$ (5%), and depending on corresponding table value the equality hypothesis has been accepted or

rejected. If there were small differences obtained (the equality hypothesis has been accepted at a level of statistical significance $\alpha = 0.05$), that was the consequence of some factors, such as: low water solubility of analysed compounds, climatic conditions, biologic variability and so on.

For compounds 1, 3, 9, 10 (for certain or all concentrations used), we obtained statistically significant results (the equality hypothesis has been rejected).

Microscopic aspects (Figures 9-12) observed for radicle meristems after 24 hours treatment with the tested compounds were:

- prophase, metaphase, anaphase and telophase were normal while slight tropokinesis and slightly hypertrophied nuclei were seen for the compounds that presented a growth stimulation effect;
- zones of kinesis inhibition (compounds 9 and 10), zones of mitotic depression, rare divisions (compounds 8, 9, 10), zones with nuclei presenting hypertrophied nucleoli, anaphases and telophasic bridges (retard chromosomes - compound 5) corrugated cell walls were observed for the compounds that inhibit radicular elongation (Figures 9-11);
- for 10^{-4} mol/L concentration of 10, we noticed zones with no divisions, as well as zones presenting diffused nuclear material, this fact indicating the cytotoxicity of this compound (Figure 12).

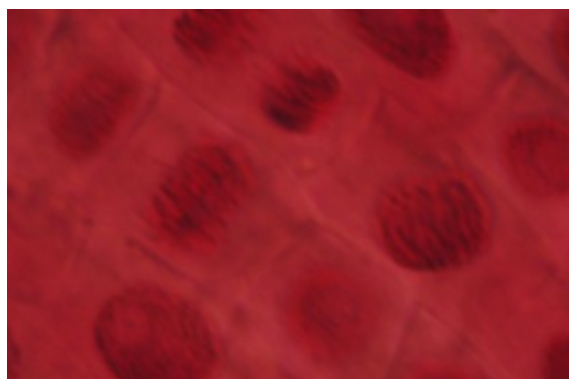


Figure 9.
Telophasic bridges

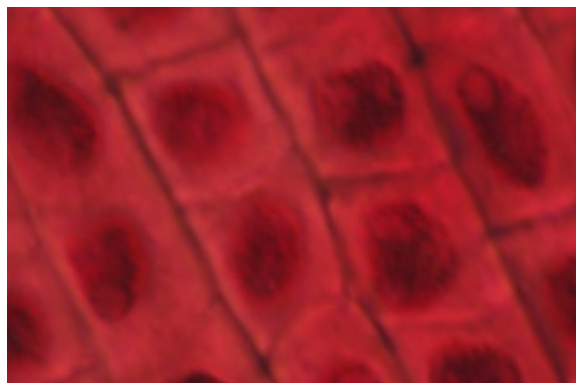


Figure 10.
Mitoinhibition

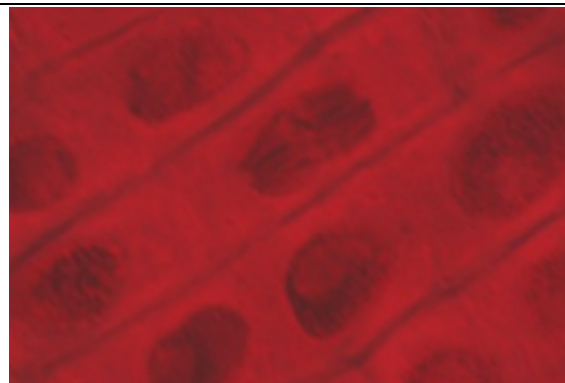


Figure 11.
Anaphases with bridges

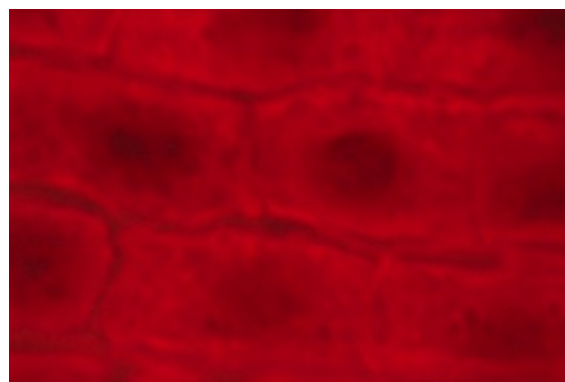


Figure 12.
Diffused nuclear material. Corrugated cell wall.
Citotoxicity activity

Conclusions

All the compounds have been investigated concerning regulation of growth for wheat, using the phytobiological method – the *Triticum* test.

The *Triticum* bioassay (Constantinescu method) revealed structure and concentration dependent effects of the mitotic film for the investigated substances.

Tests revealed that these compounds did not present cytotoxic effect, except compound 10, 2-(5H-dibenzo[a,d][7]annulen-5-ylacetyl)-N-(4-fluorophenyl) hydrazinecarbothioamide, which, at 10^{-4} mol/L concentration exhibited such an effect.

Qualitatively and quantitatively, macroscopically and microscopically, most of the substances analysed presented the same biologic effects on wheat embryonic roots. Some exceptions were also noticed. 2-(5H-Dibenzo[a,d][7]annulen-5-yl)acetohydrazide 1 presented statistically significant stimulatory effects, at all tested concentrations in the first determination and at the highest concentration in the second determination.

Statistically and biologic significant inhibitory effects were exhibited by 5-(5H-dibenzo[a,d][7]annulen-5-ylmethyl)-4-(4-methylphenyl)-4H-1,2,4-triazole-3-thiole 9 and 2-(5H-dibenzo[a,d]

[7]annulen-5-ylacetyl)-N-(4-fluorophenyl)hydrazine carbothioamide 10, which manifested this effect in both determinations and for all concentrations. Microscopic examinations confirmed the macroscopic test results. We observed numerous cells active divisions for compounds that stimulated root growth, and affected mitotic sequences for those with inhibitory effect.

Acknowledgements

This work was supported by University of Medicine and Pharmacy "Carol Davila" Bucharest, project number 28331/04.11.2013.

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