PHYTOBIOLOGICAL TESTING OF SOME COMPOUNDS WITH 4(3H) - QUINAZOLONE STRUCTURE

MIHAELA BRATU[1], OCTAVIAN TUDOREL OLARU[2]*, ILEANA CORNELIA CHIRIŢĂ[1], MIHAELA DINU[2], ADRIANA IULIANA ANGHEL[2]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy, 6 Traian Vuia 020956, Bucharest, Romania
[2] Department of Pharmaceutical Botany, Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy, 6 Traian Vuia 020956, Bucharest, Romania
** corresponding author: octav_olaru2002@yahoo.com*

Abstract

This work aimed to study the changes of plant cells from radicular meristems belonging to *Triticum vulgare* Mill. species during cell division, in presence of 4(3H) - quinazolone synthetic derivatives solutions of various concentrations, at predefined time intervals.

The applied method (The Constantinescu D. Gr. Test) ensures the accuracy and the reproducibility of the results and allows for the surveys to be conducted in a short period of time.

Rezumat

Lucrarea a urmărit studierea modificărilor produse asupra celulelor vegetale aflate în diviziune din meristemele radiculare aparţinând speciei *Triticum vulgare* Mill. de către soluţiile de diferite concentraţii ale unor derivaţi de sinteză cu structură 4(3H)-chinazolonică, la diferite intervale de timp de acţiune asupra acestor meristeme.

Metoda utilizată (Testul Constantinescu D. Gr.) asigură exactitatea şi reproductibilitatea rezultatelor şi permite efectuarea cercetărilor într-un timp de lucru scurt.

Keywords: 4(3H)-quinazolones, phytobiologycal test.

Introduction

The main drug regulatory authorities officially adopted the An important place among quinazoline-structured derivatives is occupied by ceto-quinazolines, also known as quinazolinones. Among these, the 4(3H) – quinazolines or the 3,4-dyhydro-oxoquinazolines have been the raw materials for the synthesis of numerous compounds with potential therapeutic properties. In the literature there are mentioned quinazoline derivatives with antibacterial, antifungal [8, 12, 17], antimalarial [22],

analgesic, anti-inflammatory [1,4], antihypertensive [5], anticonvulsant [18], tuberculostatic [13], antiviral and antineoplasic actions [21].

Taking into consideration all of the above, through the present work we aimed to study the influence of some 4(3H)-quinazoline derivatives, which have a substitute represented by a heterocyclic structure, thiosemicarbazidic, acyl hydrazone or urea structures in the 3-position of the nucleus, upon plant cell, during mitotic division.

Materials and Methods

We underwent this phytobiological study because the vegetal material proved to be a useful "response agent/respondent" in evaluating the toxicity of potential therapeutically natural extracts and synthetic compounds [3, 15, 16].

The cytological experimental studies on plant cells are based on the structural and physiological similarity between the plant cell and the animal cell. Moreover, the tests conducted on plant cells are easily performed and they are more cost effective [9].

The advantages of the meristemic plant cells compared to the animal cells are:

- plant cells are less differentiated;
- plant cells function is less dependent on hormonal variations;
- plant cells membrane is more compliant to various substances being transported through it when coming into contact and this way observation of different cytological changes is more accurate.

The method consists in determining the most active dilution of the studied solutions, which influences the radicular elongation and the kariokinetic film depending on the period of action.

We observed the changes produced upon plant radicular meristemic cells of *Triticum vulgare* Mill. during division.

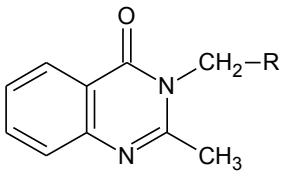
The testing method on wheat caryopses was developed by Prof. Constantinescu D. Gr. in the 1960's. This method is suitable for testing because the wheat kernels respond immediately to the action of substances, thereby allowing the research to be performed in a short period of time. The method ensures the accuracy and the reproductibility of the results and in the same time the diploid karyotype of 2n = 42 chromosomes allow an easy observation of alterations in the mitotic film [2, 6, 19, 20].

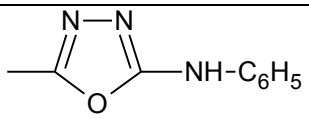
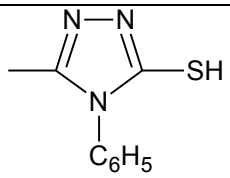
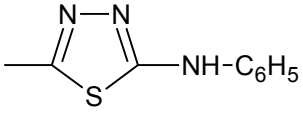
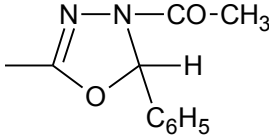
- *Chemical structures of the tested compounds*

The chemical structures of the 4(3H)-quinazolonic tested compounds are presented in Table I.

Table I

Chemical structures of the tested compounds



Compound	R	Chemical reading
C1	—COOH	2-(2-methyl-4-oxoquinazolin-3(4H)-yl)-acetic acid
C2	—C(=O)—O—CH_3	2-(2-methyl-4- oxoquinazolin-3(4H)-yl)-methyl acetate
C3	—C(=O)—NH-NH_2	2-(2-methyl-4- oxoquinazolin-3(4H)-yl)-acetohydrazide
C4		2-methyl-3-[(5-phenylamino-1,3,4-oxadiazol-2-yl)-methyl]-quinazolin-4(3H)-one
C5		2-methyl-3-[(5-mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)-methyl]-quinazolin-4(3H)-one
C6	—C(=O)—NH-N=CH-C_6H_5	N-benzilidene-2-(2-methyl-4-oxoquinazolin-3(4H)-yl)-acetohidrazide
C7		2-methyl-3-[(5-phenylamino-1,3,4-thyadiazol-2-yl)-methyl]-quinazolin-4(3H)-one
C8	—C(=O)—NH-NH-C(=S)—NH-C_6H_5	2-[2-(2-methyl-4-oxoquinazolin-3(4H)-yl)-acetyl]-N-phenyl-hidrazinocarbothioamide
C9	—NH-C(=O)—NH-C_6H_5	1-[(2-methyl-4-oxoquinazolin-3(4H)-yl)-methyl]-3-phenylurea
C10		3-[(4-acetyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)-methyl]-2-methylquinazolin-4(3H)-one

- *Samples preparation*

For each compound - C_1-C_{10}, a solution was prepared in chloroform in order to obtain 100mL of solution (in a volumetric flask) with 10^{-3} M concentration (SA). From this concentration, successive dilutions were prepared in order to obtain 10^{-4} (SB), 10^{-5} (SC) and 10^{-6} M (SD) concentrations.

For all tested compounds 15 mL from each solution containing 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} mol/L were brought to Petri dishes (d= 90mm) and maintained on water bath until solvent evaporation was complete. 15 mL of distilled water were added over the residue and the solutions prepared this way were noted with the compound abbreviation (C_1-C_{10}), followed by the concentration abbreviation (A for 10^{-3}, B for 10^{-4}, C for 10^{-5} and D for 10^{-6}, respectively). In parallel, a control sample (M) was prepared in the same way, without adding the test compounds.

- *Plant material preparation*

The biological material used was represented by wheat embryonic roots (*Triticum vulgare* Mill., *Boema* variety, obtained from SC Adaflor SRL – Tulcea County, Romania). The caryopses were soaked in distilled water for 24h, then on waterlogged filter papers in Linhart germination vessels until the radicle reached 1 cm length (after approximately 24h). The preparation of the biological material was performed under controlled conditions of temperature and humidity (25±1º C, 75% RH), in a Sanyo MLR 351-H (San Diego, CA, USA) germination room in the absence of light.

- *Cytotoxicity assay against Triticum vulgare Mill.*

Eleven caryopses with 1 cm root length were added to the previously prepared solutions. The samples were maintained in the same conditions as presented in *Plant material preparation* for 5 days (120h).

The assessment of root elongation was performed using the linear measurement technique at 24, 72 and 120 hours during the entire experiment.

After 24h, one caryopsis from each sample was taken; the embryonic root was removed and used for the preparation of microscopic slides. The embryonic roots were stained with acetic orcein solution and then squash and examined using a Labophot 2 Nikon microscope, ocular 10x, objectives 40x and 100x (cedar oil immersion) [14]. The presence or absence of mitotic divisions and the kariokinetic film alterations were investigated.

The kariokinetic film alterations were evaluated in comparison with the control (distilled water).

- *Data analysis*
  - *Data distribution analysis*

The analysis of the radicular elongation values distribution was performed by applying the D'Agostino & Pearson test ("omnibus K2", α = 0.05) [7].

- *The inhibitory effect analysis*

Taken into account the control root elongation values, the inhibitory effect ($Ef_{i\%}$) of the tested compounds was calculated using the following formula [7]:

$$Ef_{i\%} = 100 - \frac{P-1}{M-1}100$$

P – average value of root elongation for sample (cm)
M – average value of root elongation for control sample (cm)
1 – initial value of embryonic roots (cm)
100 – effect expressed as percentage

- *Statistical meaning of Efi%*

The comparison between the results of the testing compounds with the control sample was made in the cases of a normal distribution of values, by ANOVA test followed by Tukey post-test and, in the case of abnormal distribution of result, by Kruskal Wallis followed by Dunn's test. The results are more meaningful (statistically significant) when p value (α=0.05) is smaller. When p > 0.05, the results are insignificant from the statistic point of view [10, 11].

- *The calculation of the inhibitory concentration 50% (IC50)*

IC50 was calculated by linear regression interpolation based on logarithm of concentration and $Ef_{i\%}$. For each regression analysis there were calculated the regression line, the correlation coefficient (r^2) and the confidence interval (α = 0.05).

The results were analysed using Microsoft Office Excel 2003 and GraphPad Prism v.5.0 softwares.

Results and Discussion

- *Data distribution analysis*

The results for the distribution of root elongation values obtained for the tested compounds and control sample during the experiment are shown in Table II. An abnormal distribution of radicular elongation was registered for batches tested with C3 at 10^{-6} mol/L (day 1), 10^{-4} mol/L (day 3) and 10^{-5} mol/L (day 5), with C5 at 10^{-3} – 10^{-5} mol/L (day 5), with C8 at 10^{-6} mol/L (day 5) and for those treated with C10 at 10^{-4} mol/L (day 5). All the other values registered were normally distributed. Examples of frequency -

response histograms are presented for batches treated with the compounds C4, C6, C9 and C10 in Figure 1.

Table II

Results of D'Agostino and Pearson normality test

Compound	Passed normality test (α=0.05) p value											
	Day 1				Day 3				Day 5			
	Concentration (mol/L)											
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
M	Yes 0.4411				Yes 0.0840				Yes 0.0492			
C1	Yes 0.5144	Yes 0.4547	Yes 0.7278	Yes 0.5044	Yes 0.5567	Yes 0.0995	Yes 0.3205	Yes 0.3852	Yes 0.9057	Yes 0.6448	Yes 0.5760	Yes 0.4961
C2	Yes 0.2732	Yes 0.8551	Yes 0.6099	Yes 0.6530	Yes 0.2542	Yes 0.8606	Yes 0.6093	Yes 0.4175	Yes 0.5555	Yes 0.5602	Yes 0.6031	Yes 0.5500
C3	Yes 0.9911	Yes 0.9472	Yes 0.0875	No 0.0151	Yes 0.8864	No 0.0391	Yes 0.2537	Yes 0.9195	Yes 0.2611	Yes 0.7538	No 0.0194	Yes 0.6451
C4	Yes 0.5625	Yes 0.3754	Yes 0.3941	Yes 0.8804	Yes 0.8859	Yes 0.9813	Yes 0.5924	Yes 0.6096	Yes 0.4022	Yes 0.0600	Yes 0.2853	Yes 0.6319
C5	Yes 0.2944	Yes 0.4908	Yes 0.6592	Yes 0.8855	Yes 0.5073	Yes 0.4182	Yes 0.4871	Yes 0.4479	No < 0.0001	No 0.0010	No 0.0205	Yes 0.1234
C6	Yes 0.9748	Yes 0.5768	Yes 0.3467	Yes 0.1496	Yes 0.4248	Yes 0.8313	Yes 0.7982	Yes 0.4782	Yes 0.6548	Yes 0.8757	Yes 0.8087	Yes 0.3016
C7	Yes 0.8434	Yes 0.1958	Yes 0.5579	Yes 0.5215	Yes 0.6636	Yes 0.4828	Yes 0.5920	Yes 0.8222	Yes 0.9327	Yes 0.2976	Yes 0.4475	Yes 0.3494
C8	Yes 0.5314	Yes 0.7810	Yes 0.5385	Yes 0.9840	Yes 0.5587	Yes 0.5400	Yes 0.6481	Yes 0.1835	Yes 0.8802	Yes 0.4555	Yes 0.2321	No <0.0001
C9	Yes 0.3212	Yes 0.4518	Yes 0.4787	Yes 0.3781	Yes 0.6603	Yes 0.2427	Yes 0.3560	Yes 0.6869	Yes 0.4649	Yes 0.7227	Yes 0.5585	Yes 0.9482
C10	Yes 0.7178	Yes 0.3230	Yes 0.9823	Yes 0.8021	Yes 0.8236	Yes 0.0874	Yes 0.2195	Yes 0.4888	Yes 0.9314	No 0.0165	Yes 0.3536	Yes 0.6462

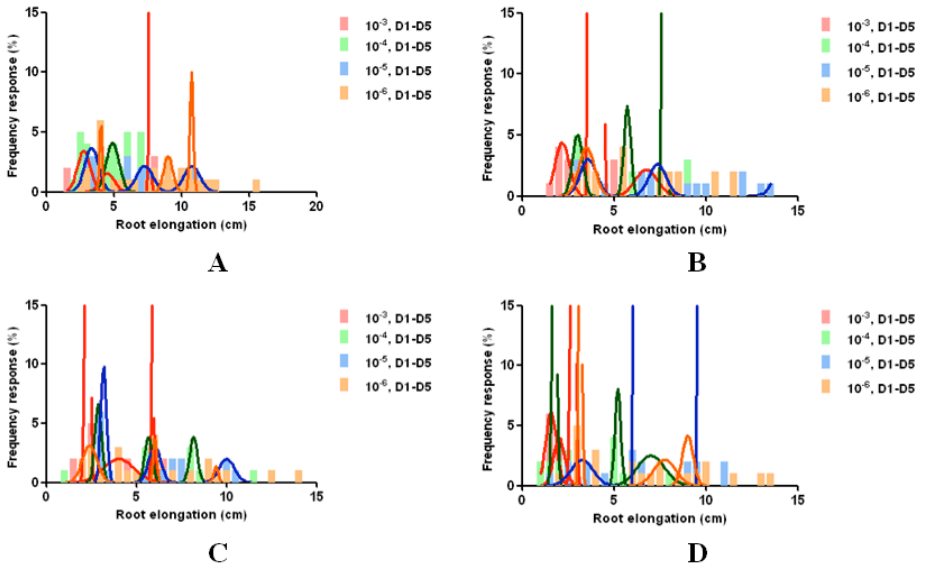


Figure 1.

Frequency response – root elongation histograms of root elongation for plants treated with C4 (A), C6 (B), C9 (C) and C10 (D)

- *Calculation and statistical meaning of $Ef_{i\%}$*

The Efi% and the statistical meaning are presented in Tabel III. For illustration, the results are graphically represented for batches treated with C4, C6, C9 and C10 in days 1, 3 and 5 in Figure 2.

Table III

Inhibitory effect (Efi%) and statistical meaning of the results in comparison with the control

Compound	Day	Efi%				Statistical test (ANOVA/ Kruskal Wallis)	Post test (Dunn / Tukey)			
Conc. (mol/L)		10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3} – 10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
C1	1	21.64	-36.43	-13.75	-12.71	***, R^2=0.4215	ns	**	ns	ns
	3	40.12	-4.60	8.94	18.63	***, R^2=0.4848	***	ns	ns	ns
	5	38.48	-4.08	7.20	13.65	***, R^2=0.3813	***	ns	ns	ns
C2	1	25.74	-1.29	-3.00	-14.98	*, R^2=0.2257	ns	ns	ns	ns
	3	41.28	24.28	28.25	16.70	**, R^2=0.2880	**	ns	ns	ns
	5	41.33	21.81	21.90	7.14	***, R^2=0.3470	***	ns	ns	ns
C3	1	-5.40	-4.03	8.29	-12.24	ns, KW=5.104	ns	ns	ns	ns
	3	18.57	7.77	30.98	-1.42	**, KW=16.03	ns	ns	*	ns
	5	14.38	9.62	19.90	-10.19	*, KW=11.62	ns	ns	ns	ns
C4	1	48.33	43.88	18.21	5.21	***, R^2=0.4832	***	***	ns	ns
	3	54.19	52.58	23.90	9.01	***, R^2=0.6068	***	***	ns	ns
	5	42.57	47.43	20.86	8.76	***, R^2=0.3813	***	***	ns	ns
C5	1	-18.40	9.32	-12.93	12.40	**, R^2=0.2590	ns	ns	ns	ns
	3	13.97	24.40	3.05	20.18	ns, R^2=0.1802	ns	ns	ns	ns
	5	0.86	22.19	-0.95	11.14	ns, KW=7.319	ns	ns	ns	ns
C6	1	57.91	25.74	14.11	18.56	***, R^2=0.5508	***	*	ns	ns
	3	62.76	41.03	24.90	36.07	***, R^2=0.6229	***	***	*	***
	5	51.62	31.90	10.67	29.71	***, R^2=0.4939	***	**	ns	**
C7	1	39.43	18.21	12.40	21.63	**, R^2=0.2603	**	ns	ns	ns
	3	47.12	37.31	23.28	28.62	***, R^2=0.4352	***	***	ns	*
	5	44.76	32.76	17.62	22.67	***, R^2=0.4334	***	**	ns	ns
C8	1	39.77	24.03	21.98	7.60	***, R^2=0.3386	***	ns	ns	ns
	3	66.36	46.99	28.87	10.00	***, R^2=0.7411	***	***	***	ns
	5	68.10	50.86	22.29	23.24	***, KW=34.77	***	***	ns	ns
C9	1	64.07	47.98	36.01	36.01	***, R^2=0.4783	***	***	*	*
	3	67.85	51.96	40.41	34.33	***, R^2=0.6007	***	***	***	**
	5	60.29	40.00	25.81	21.71	***, R^2=0.5462	***	***	*	ns
C10	1	81.18	71.94	28.14	25.06	***, R^2=0.6753	***	***	ns	ns
	3	85.48	61.89	22.79	20.43	***, R^2=0.7736	***	***	*	ns
	5	85.43	51.05	15.33	11.90	***, KW=36.40	***	**	ns	ns

Legend: ns – statistically insignificant ns >0,05 ** 0.001-0.01
*, **, *** - statistically significant * 0,01-0,05 *** <0.001
R^2 – assessment coeficient
K-W_S = H –Kruskal Wallis test coeficient

The maximum inhibitory effect was registered for batches treated with compound C10 at 10^{-4} mol/L, followed by C9 at the same

concentration. Efi% induced by C10 remained over 50% even for 10^{-4} mol/L, while for batches treated with C9 the value it was approximately 50% (47.98% in day 1, 51.96% in day 3 and 40.00% in day 5). The results were statistically significant at 10^{-4} – 10^{-5} mol/L for C10 and at 10^{-3} – 10^{-6} mol/L for C9 (Table III).

Values over 50% of Efi% were registered for other compounds too: C4, C6 and C8, but only at the highest concentration tested; the effects were decreasing below 50% (C4) or remained to close values (C6 and C8) at the next concentration. The results are statistically sustained at 10^{-3} and 10^{-4} mol/L for batches treated with C4 and those with C8 and at all concentrations for batches treated with C6. All other concentrations showed similar effects with the one induced by the control (from a statistical point of view, not regarding the biological effect obtained).

For batches treated with compounds C1, C2, C3, C5, and C7, the values of Efi% are below 50%. The difference is statistically sustained only at the highest concentration (C1, C2, C7) and insignificant for batches treated with compounds C3 and C5.

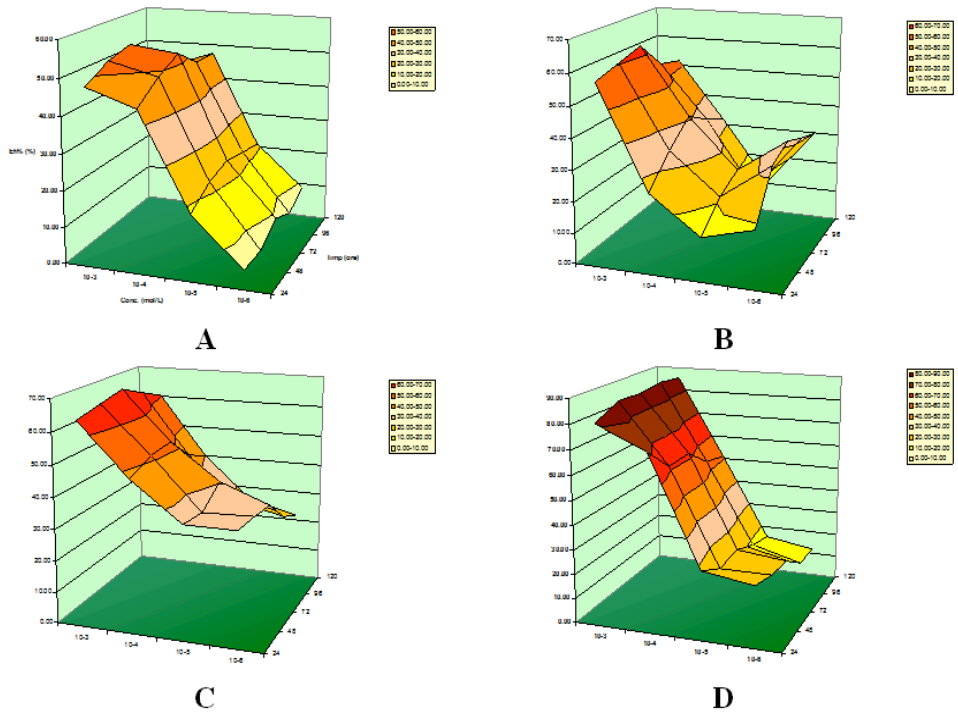


Figure 2.
The inhibitory effect plotted against time (hours) and concentrations (mol/L) for caryopses treated with compounds C4 (A), C6 (B), C9 (C) and C10 (D)

- *Estimation of the inhibitory concentration 50% (IC50)*

The values of IC50 were calculated only for batches treated with compounds C4, C6, C8, C9 and C10. As examples, for these compounds, a graphic representation of concentration-inhibitory effect regression curves is presented in Figure 3. The correlation of concentration with inhibitory or stimulatory effect was satisfactory for C2, C4, C6 (only in day 1), for C7 (only in day 3 and 5), for C8, C9 and C10. For batches treated with C1, C3 and C5, Efi% does not correlate with concentrations, and so, the effect is not being dependent on concentration (Table IV).

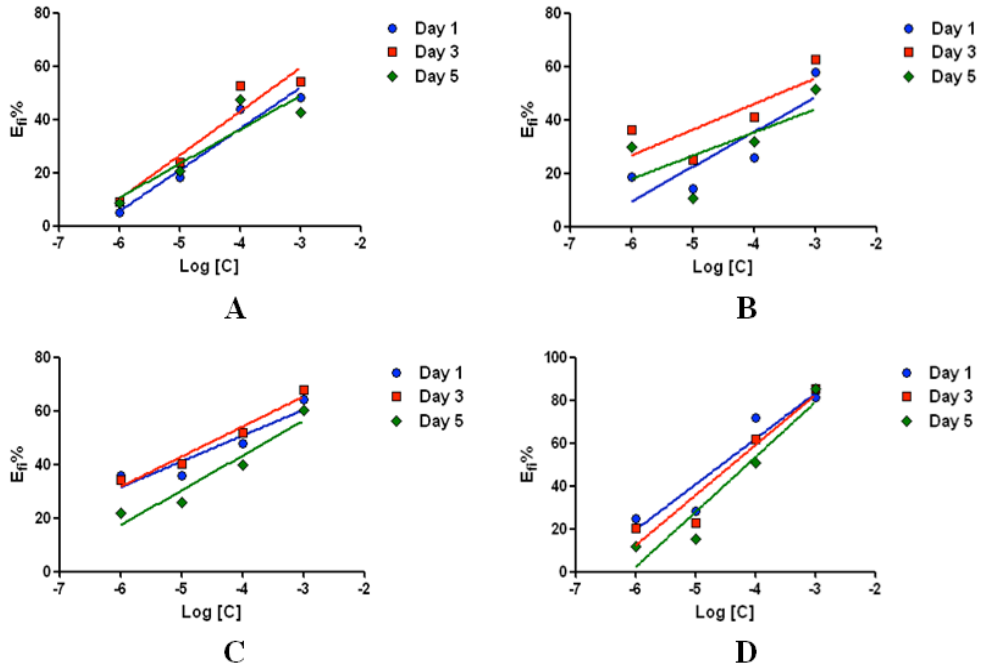


Figure 3.
The concentration-inhibitory effect curves for caryopses exposed to compounds C4 (A), C6 (B), C9 (C) and C10 (D)

In the first day of the experiment, the lowest value of IC50 was registered for C10, followed by C9, C4 and C6. The toxicity has decreased in the order: C10, C9, C8, C4 and C6. Compound C10 was approximately 3 times more toxic than C9 and over 10 times more toxic than other compounds: C8, C4 and C6. Compound C8 did not exhibit toxicity in the first day of the experiment, the value for IC50 being over 10^{-3} mol/L.

In the third day of the experiment, compounds C10 and C9 had approximately the same value of IC50, followed by C8, C4 and C6, which had registered a similar behaviour.

In the fifth day of the experiment, the toxicity varied in the following descending order: C10, C8, C9, C4 and C6. The compounds C1, C2, C3, C5 and C7 exhibited no toxicity at all, the values for IC50 being over the maximum concentration tested (10^{-3} mol/L), in all days of the experiment (Table IV).

Table IV

The IC50 values, regression equations and goodness of fit

Nr. crt.	Compound	Day	IC50 mol/L	Goodness of fit (r^2)
1	C1	1	$> 10^{-3}$	0.1878
		3	$> 10^{-3}$	0.1220
		5	$> 10^{-3}$	0.2055
2	C2	1	$> 10^{-3}$	0.8648
		3	$> 10^{-3}$	0.7668
		5	$> 10^{-3}$	0.8898
3	C3	1	$> 10^{-3}$	0.0153
		3	$> 10^{-3}$	0.1153
		5	$> 10^{-3}$	0.3906
4	C4	1	72.53×10^{-5}	0.9407
		3	26.20×10^{-5}	0.9136
		5	117.47×10^{-5}	0.8221
5	C5	1	$> 10^{-3}$	0.3402
		3	$> 10^{-3}$	0.0014
		5	$> 10^{-3}$	0.1018
6	C6	1	129.78×10^{-5}	0.7143
		3	26.05×10^{-5}	0.6114
		5	487.29×10^{-5}	0.4496
7	C7	1	$> 10^{-3}$	0.4306
		3	$> 10^{-3}$	0.7394
		5	$> 10^{-3}$	0.7684
8	C8	1	$> 10^{-3}$	0.9340
		3	13.74×10^{-5}	0.9999
		5	11.07×10^{-5}	0.8890
9	C9	1	8.21×10^{-5}	0.8721
		3	4.18×10^{-5}	0.9630
		5	31.93×10^{-5}	0.9271
10	C10	1	2.66×10^{-5}	0.8849
		3	3.98×10^{-5}	0.9167
		5	7.14×10^{-5}	0.9173

- *Assessment of changes on kariokinetic film (mitotic film) (Figure 4)*

Compound C10 presented cytotoxic action at the highest concentration tested (10^{-3} mol/L). Except for the absence of divisions

(mitoinhibitory effect), there were observed numerous areas with contracted nuclei and with waved cell walls. At 10^{-4} mol/L there were noticed both normal and abnormal divisions: telophases, anaphases and metaphases in tropokinesis, waved cell walls.

At the high concentrations (10^{-3} and 10^{-4} mol/L), compounds C9, C8 C4 and C6 induced the following changes at cellular level: metapahses, telophases and anaphases in tropokinesis and disorganized metaphases. Out of the compounds with IC50>10^{-3} mol/L, C1, C2 and C3 induced changes on kariokinetic film but only at the maximum concentration (metapahses, anaphases and telophases in tropokinesis for C1-C3, disorganized metaphases for C2 and bridged anaphases for C3).

The examination of microscopic slides from the batch treated with C7, at 10^{-3} mol/L revealed normal divisions, more frequently than for the control sample. Compound C5 did not induce any modifications on kariokinetic film at any of the tested concentrations.

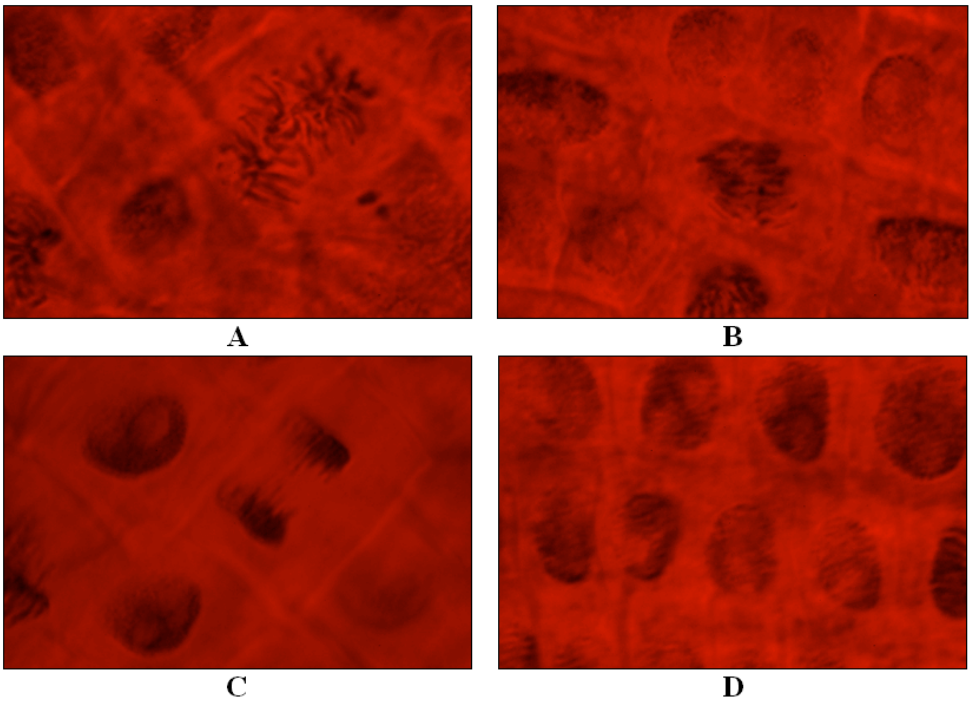


Figure 4.

Kariokinetic film modifications observed: A – disorganized metaphase induced by C2 at 10^{-3} mol/L; B – anaphase in tropokinesis induced by C4 at 10^{-4} mol/L; C – anaphase with late chromosomes induced by C9 at 10^{-3} mol/L; D – cytotoxicity induced by C10 at 10^{-3} mol/L

Conclusions

Compounds C1, C2 and C3 presented low toxicity; the genotoxic potential of these compounds was emphasised only at 10^{-3} mol/L. Compounds C10, C9, C8, C4 and C6 induced toxicity at all tested concentrations, this being proved by their influence on radicular elongation and by the changes produced on the kariokinetic film. Compounds C5 and C7 did not exhibit any toxic effect on radicular elongation nor on the mitotic film.

The research indicates a high risk of genotoxicity for compounds C4, C6, C8, C9 and C10, a low risk for C1, C2 and C3 and no risk at all for compounds C5 and C7.

References

1. Alagarsamy V., Solomon V. R., Dhanabal K., Synthesis and pharmacological evaluation of some 3-phenyl-2-substituted-3H -quinazolin-4-one as analgesic, anti-inflammatory agents. *Bioorg. Med. Chem*., 2007; 7: 235–241.
2. Ancuceanu R., Istudor V., Dinu M., Codreanu M., Contribution to the study of some *Cuscuta* species. Note 2. Use of the Constantinescu bioassay in the study of the correlations between *Cuscuta sp*. and their hosts. *Farmacia*, 2005; 53(5): 63-75.
3. Anghel A.I., Olaru O.T., Gatea F., Dinu M., Ancuceanu R.V., Istudor V., Preliminary research on *Portulaca grandiflora* Hook. species (*Portulacaceae*) for therapeutic use. *Farmacia*, 2013; 61(4): 694-702.
4. Balakumar C., Lamba P., Pran Kishore D., Lakshmi Narayana B., Venkat Rao K., Rajwinder K., Raghuram Rao A., Shireesha B., Narsaiah B., Synthesis, anti-inflammatory evaluation and docking studies of some new fluorinated fused quinazolines. *European Journal of Medicinal Chemistry*, 2010; 45(11): 4904-4913.
5. Chaudhary M., Bhattacharya S., Ahmad Y., Synthesis of some novel triazolo-quinazolinone derivatives and investigation of their antihypertensive agents. *Der Pharmacia Sinica*, 2012; 3(4): 479-487.
6. Ciulei I., Istudor V., Palade M., Albulescu D., Gârd C., Analiza farmacognostică şi fitochimică a produselor vegetale, vol. I. Ed. Tehnoplast Company, Buc., 1995, 23 -141.
7. D´Agostino R. B., Test for Normal Distribution in Goodness of Fit Techniques, ed. by D´Agostino R. B. and Stepenes M. A., Decker M., 1986, 405-413.
8. Deshmukh M. B., Patil S., Patil S. S., Jadhav S. D., Synthesis and Antimicrobial Screening of Pyrazolo-3-Aryl Quinazolin-4(3H)ones. *Indian J. Pharm. Sci*., 2010; 72(4): 500–504.
9. Deysson G., Les effects des composes chimiques antimitotiques sur la cellule vegetale. *Chemotherapia*, 1961; 2: 138-162.
10. Glaser A. N., High Yield TM: Biostatistics. Lippincott Williams & Wilkins, Baltimore, 2001; 42-45, 52.
11. Govindarajulu Z., Statistical Techniques in Bioassay. S. Karger AG, Basel, 2001; 35, 36, 40-47.
12. Khodarahmi G. A., Khajouei M.R., Hakimelahi G.H., Abedi D., Jafari E., Hassanzadeh F., Antibacterial, antifungal and cytotoxic evaluation of some new 2,3-disubstituted 4(3H)-quinazolinone derivatives. *Res. Pharm. Sci*., 2012; 7(3): 151-158.
13. Kunes J., Bazant J., Pour M., Waisser K., Slosarek M., Janota J., Quinazoline derivatives with antitubercular activity. *Il Farmaco*, 2000; 55: 725–729.
14. La Cour L., Acetic-orcein: a new stain-fixative for chromosomes. Stain Technology, 1941; 16: 169-174.

15. Niţulescu G.M., Drăghici C., Olaru O.T., New Potential Antitumor Pyrazole Derivatives: Synthesis and Cytotoxic Evaluation. *Int. J. Mol. Sci*., 2013; 14(11): 21805-21818.
16. Olaru O.T., Anghel A.I., Istudor V., Ancuceanu R.V., Dinu M., Contributions to the pharmacognostical and phytobiological study of *Fallopia aubertii* (L. Henry) holub. (*Polygonaceae*). *Farmacia*, 2013; 61(5): 991-999.
17. Pandey S. K., Singh A., Antimicrobial studies of some novel quinazolinones fused with [1,2,4]-triazole, [1,2,4]-triazine and [1,2,4,5]-tetrazine rings. *Eur. J. Med. Chem*., 2009; 44: 1188–1197.
18. Paneersalvam P., Raj T., Ishar M.P.S., Singh B., Sharma V., Rather B.A., Anticonvulsant activity of Schiff bases of 3-amino-6,8-dibromo-2-phenyl-quinazolin-4(3H)-ones. *Indian J. Pharm. Sci*., 2010; 7: 375–378.
19. Pavel M., Voştinaru O., Mogoşan C., Shibu S., Phytochemical and Pharmacological Research on some extracts obtained from *Serpylli herba*. *Farmacia*, 2011; 59(1): 77-84.
20. Popescu M. L., Dinu M., Contribution to the pharmacognostical and phytobiological study of *Forsythia viridissima L*. (*Oleaceae*). *Farmacia*, 2008; 56(3): 267-274.
21. Selvam P., Murugesh N., Chandramohan M., Pannecouque C., De Clercq E., Synthesis, Antiviral and Cytotoxic Activities of 2-(2-Phenyl carboxylic acid)-3-Phenylquinazolin - 4(3H)-one Derivatives. *Indian J. Pharm. Sci*., 2010; 72(6): 806–809.
22. Zhu S., Wang J., Chandrashekar G., Smith E., Liu X., Zhang Y., Synthesis and Evaluation of 4-Quinazolinone Compounds as Potential Antimalarial Agents. *Eur. J. Med. Chem*., 2010; 45(9): 3864–3869.

Manuscript received: February 2014