Synthesis and Pharmacological Characterization of Some 2- and 1,2-substituted Benzimidazoles

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Taking into account the important role of ultraviolet radiation in premature skin aging and cancer, 2- and 1,2-substituted benzimidazoles were synthesized, whole molecules contain conjugated chromophores and may produce strong absorption at wavelengths convenient for being photo protectors. 4 different benzimidazoles were used, of which 2 were never mentioned in the literature. The four compounds are characterized from the physical point of view, especially from a spectral perspective. To assess the acute toxicity of the four compounds (symbolized as S1, S2, S3 and S4), white male mice from the NMRJ strain were used, grouped in 4 groups, of 10 individuals each. The highest lethality was induced by the S3 compound (20%). To assess motor activity, 6 groups of 10 white NMRJ mice were used. Compounds S2, S3 and S4 display a central stimulating effect, highlighted by the evolution of vertical motor activity. Compound S3 caused the greatest percentage increase in horizontal and vertical motor activity compared to the reference. The toxicity of synthetized compounds was tested phytobiologically on Triticum aestivum caryopses (Triticum test). As a result of testing, a decreasing order of toxicity was noted: S3 , S2, S1 and S4.

Keywords: Benzimidazoles, 1,2- phenylenediamine, aldehydes, toxicity

Although they have been known for a long time, mono- or disubstituted benzimidazole derivatives [1], have found uses only in recent years.

Taking into account the similarity between the molecular structure of benzimidazole and purine bases in the DNA and the presence of such a ring in the molecule of Vitamin B_{12} [2] drug researchers have found applications of benzimidazole as inhibitors of human cytomegalovirus (HCMV) [3], in the treatment of ulcers [4] and antihistaminic agents [5], against herpes [6], topo-isomerase inhibitors [7], against flu [8], agents with an antitumoral potential [9, 10], angiotensin inhibitors [11], blood pressure medications [12] antibacterial and antifungal compounds [13, 14].

As photo protectors were also used 2-phenyl-benzimodazole-5-sulphonic acid and its potassium and triethanolamine salts with a UV absorption maximum at 310 nm.

We believe that one field in which benzimidazole derivatives may have applications is photo protection against solar radiation, taking into account the fact that benzimidazole rings contain strong aromatic conjugation, which gives rise to a wide absorption of UV radiation.

Today, one is familiar with the important role ultraviolet (UV) radiation plays in the emergence of various types of skin cancer and premature aging. UV radiation emitted by the sun is divided into three groups: UVC (200-290 nm) absorbed by the atmosphere, short wave UVB (290-320 nm) and long wave UVA (320-400 nm). The most dangerous radiation is UVA, richer in energy.

Since numerous organic photo protectors already in use have displayed side effects as well [15-17], such as photosensitization effects, estrogenic effects, mutagenic effects, the introduction of new photo protectors into therapy requires an assessment of the risk-benefit ratio.

Experimental part

Synthesis and characterization of benzimidazoles

To confirm the working hypothesis we selected four compounds out of benzimidazole derivatives:

S1 1-bezyl-2-phenyl-1*H*-1,3-benzimidazole

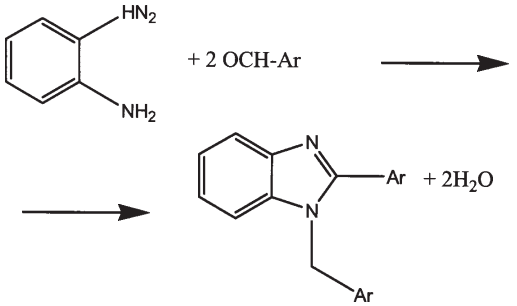
S2 1-(4-methyl-benzyl)-2-(4-methyl-phenyl)-*H*-1,3 benzimidazole

S3 1-(4-carboxy)phenyl-2-(4-metoxy-phenyl)-*H*-1,3-benzimidazole

S41-(4-dimethyl-amino)benzyl-2 -(4-dimethylamino-phenyl)-*H*-1,3-benzimidazole

The syntheses of the benzimidazoles were carried out using commercial reagents (Flucka , Merck and Aldrich). The progress of the synthesis reactions was monitored using HPLC.

Compounds S1 and S2 were obtained through the condensation of phenylenediamine with benzaldehyde and toluenealdehyde, respectively.



1. 0.54 g phenylenediamine (0.5 mmole) 1.06 g (10 mmole) benzaldehyde and 0.1 g Amberlit IR -120 were added in a ground neck flask. The solvent used was an ethanol-water mixture, 2:1 v/v, 10 ml. The content of the flask was isolated using a ground lid (greased). The mixture was subject to magnetic agitation at room temperature.

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The progress of the was monitored using HPLC. At the end of the reaction, the mixture was filtered in order to separate the catalyst; the filtered solution was concentrated through evaporation and the product obtained was recrystallized from methanol. Mp 130^0 C, 93% yield.

2. **S2** was obtained under similar conditions. The reaction rate was slower. Mp 129^0 C, 90% yield.

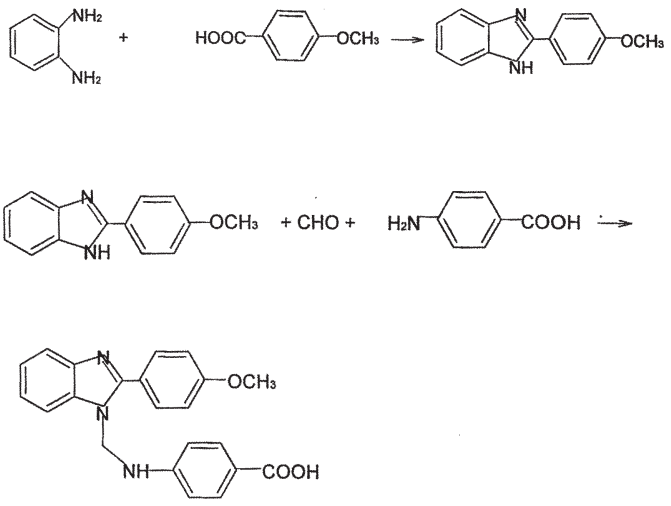
The condensation reaction between phenylenediamine and aromatic aldehydes also takes place in the absence of a catalyst. It is better to work in the absence of air, which favors the production of 2-substituted benzimidazoles.

3.To synthesize 1,2-disubstituted benzimidazoles we used a two-step working procedure:

a.Obtaining 2-substituted benzimidazoles followed by

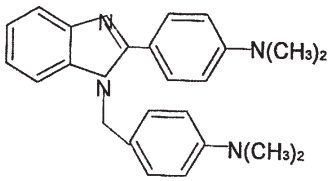
b.Obtaining Mannich bases at the nitrogen atom in position 1.

From among the 2-substituted benzimidazole derivatives we selected 4-metoxyphenyl-benzimidazole, when we obtained either through the condensation of phenylenediamine with metoxybenzaldehyde or through condensation with metoxybenzoic acid catalyzed by an acid. For example: 2.16 g phenylenediamine, 20 mmole; 3.04 g metoxybenzoic acid, 20 mmole and 4 mL acetic acid were mildly heated under reflux for 4 h. On cooling, a mass of needle-like crystals was formed, which were separated through filtration. The crystals were recrystallized from ethanol. Mp 208^{0}C, 77% yield. A Mannich base was obtained using 2-metoxyphenyl-benzimidazole, formic aldehyde and aminobenzoic acid.



In a 20 mL reaction vessel were added 2.24 g (10 mmole) 2-(4-metoxyphenyl)-benzimidazole, 1.37 g (10 mmole) para-aminobenzoic acid, 0.3 g (10 mmole) para-formaldehyde and 6 mL 96% ethanol. Agitation was initiated at room temperature and after 30 min the mixture was heated under reflux for two hours. A fine precipitate was formed. On the following day the mixture was filtered, the precipitate was washed on the filter with 96% alcohol and was dried on air. Melting point in a closed tube 167^{0}C. 92% yield. The resulting Mannich base is not described in the literature.

4.To synthesize compound S4, we used the condensation of phenylendiamine 5.40 g (50 mmole) and dimethyl-aminobenzaldehyde 14.91 g (100 mmole) in the presence of Amberlit IR-120 0.5 g in 80 mL ethanol:water 2:1. We found that the reaction progresses well even without a catalyst, but in the absence of air. The mixture was subject to magnetic agitation at room temperature. After 40 minuted it started turning yellow and a precipitate was formed. The precipitate was filtered after 12 hours (left overnight) and was recrystallized from ethyl acetate. Melting point in a closed capillary tube 135 ^{o}C, 88 % yield. The compound is not described in the literature, although many 1,2-disubstituted benzimidazoles were obtained.



Benzimidazoles synthesized

IR spectra

S1. 1-benzyl-2-phenyl-1H-benzimidazole. 3045 cm^{-1} (C-H stretching of aromatic ring), 2921 cm^{-1} (C-H stretching of methylene group), 1591 cm^{-1} (C=N stretching of imidazole ring) 1541 cm^{-1}, 1463 cm^{-1}, 1444 cm^{-1} (C=C stratching of aromatic ring), 1392 cm^{-1} (C-N stretching of imidazol ring).

S2.1-(4-methylbenzyl)-2-(4-methylphenyl)-1Hbenz-imidazole. 3026 cm^{-1} (C-H stretching of aromatic ring), 2917 cm^{-1} (C-H stretching of aliphatic), 1612 cm^{-1} (C=N stretching of imidazol ring), 1516 cm^{-1},1457 cm^{-1}, 1444 cm^{-1} (C=C stretching of aromatic ring), 1279 cm^{-1} (C-N stretching of imidazol ring) .

S3. 1-(4-methoxybenzyl)-2-(4-carboxyphenyl)-1H-benzimidazole. 3045 cm^{-1} (C-H stretching of aromatic), 2974 cm^{-1} (C-H sretching of aliphatic), 1675 cm^{-1}, (-COOH, stretching of CO from carboxylic groupe), 1602 cm^{-1}, 1532 cm^{-1}, 1422 cm^{-1} (C=C stretching of aromatic ring), 1260 cm^{-1}, 1043 cm^{-1} (C-O-C stretching of ether) .

S4.1-(4-dimethylaminobenzyl) -2-(4-dimethylamino-phenyl)benzimidazole. 3045 cm^{-1} (C-H of aromatic ring), 2974 cm^{-1}, 2883 cm^{-1} (stretching of aliphatic), 1611 cm^{-1} (C=N stretching of imidazol ring) ,1526 cm^{-1}, 1469 cm^{-1}, 1389 cm^{-1} (C=C stretching of aromatic ring), 1366 cm^{-1}.

NMR Spectra

The ^{1}HNMR spectra were recorded using a Bruker apparatus, at 400 MHz, using CO_3D solutions. Only

Symbol	Molecular Formula	Molecular Mass	Yield %	Melting Point (^{0}C)	Wavelenght λ(nm)
S1	$C_{20}H_{16}N_2$	284	93	130	286
S2	$C_{22}H_{20}N_2$	312	90	129	291,6
S3	$C_{22}H_{18}N_2O_3$	358	92	167	289
S4	$C_{24}H_{26}N_4$	370	88	135	377

Table 1

compound 4 required adding DMSO-d6. The ^{13}CNMR spectra were recorded at 125 MHz.

In the spectra there is a predominance of aromatic protons, present in large numbers. One can distinguish the signals δ= 5.49 ppm and δ = 5.41 ppm, corresponding to the nitrogen protons in compounds 1 and 2, absent in compounds 3 and 4. Also, signals are present corresponding to methyl protons in compounds 2, 3 and 4 δ= 2.97 - 2.40 ppm, which are absent in compound 1.

^{1}HNMR Spectra

S1, ppm,=7.93, 7.92 (d, 1H), 7.50, 7.48 (d, 1H), 7.50, 7.48 (d, 2H), 7.37, 7.35 (d, 3H), 7.32 (s, 3H), 7.28 (s, 1H), 7.15, 7.13 (d, 1H), 5.49 (s, 1H).

S2 = 7.87, 7.85 (d, 1H), 7.60, 7.58 (d, 1H), 7.20, 7.13 (m, 4H), 7.14, 7.12 (d, 1H), 7.01, 6.99 (d, 1H), 5.41 (s, 1H), 2.40, 2.33 (d, 3H).

S3 = 8.25, 8.23 (d, 2H), 7.60, 7.58 (c, 4H), 7.22, 7.20 (c, 4H), 6.84, 6.82 (d, 2H), 3.65 (s, 3H), 2.67 (s, 3H).

S4 = 7.33, 7.67 (t, 2H), 7.57, 7.55 (d, 1H), 7.20, 7.08 (m, 2H), 6.95, 6.91 (d, 2H), 6.67, 6.65 (d, 4H), 6.61, 6.59 (d, 1H), 5.30 (s, 1H), 2.97, 2.93 (d, 8H), 2.85 (s, 4H).

^{13}CNMR Spectra

S1, ppm,= 48.28, 76.69, 77.33, 110.47, 119.89, 122.61, 122.57, 125.89, 127.70, 128.68, 128.97, 129.18, 129.84, 136.31, 143.09, 154.10.

S2 = 21.04, 48.15, 77.01, 110.48, 119.76, 122.51, 122.80, 125.86, 127.12, 129.12, 129.20, 129.65, 133.41, 136.05, 137.39, 139.99, 143.11, 154.32.

S3 = 39.53, 55.11, 113.32, 114.17, 114.56, 125.17, 129.95, 131.10, 148.12, 163.11.

S4 = 39.95, 76.69, 77.01, 77.33, 112.22, 111.32, 111.56, 111.62, 112.53, 114.81, 116.91, 117.05, 118.91, 121.52, 121.59, 123.99, 124.53, 126.20, 130.02, 136.12, 138.07, 141.67, 143.03, 149.74, 150.97, 152.13, 154.79, 157.43.

Experimental pharmacological researches regarding the toxicity and the motor behaviour induced by some new compounds

Materials and methods

Two communities of white mice, male, with masses as close (25 ± 3g), have been used in experimental research.

To assess acute toxicity, 4 groups of 10 mice each were used, treated with 2000 mg/kgb.w PO, in a unique dose of substances S1, S2, S3 and S4, respectively. The animals were monitored for 14 days regarding mortality, evolution of body weight and behavior. During the period of monitorization, the mice were kept under constant conditions of temperature (22C ± 1°C) and humidity (45 – 50%). Food was stopped one hour prior to administration and water was provided ad libitum.

To assess motor activity, 4 groups were used (10 animals/group), treated with the investigational substances (S1, S2, S3, S4) in a 200 mg/kgb.w dose, PO (1% suspension). In parallel, a reference group was used (10 animals/group), treated with distilled water 0.1 mL/10 g body weight, PO.

4 consecutive doses were administered for 4 days. Administration was done in the morning at 9 am. One hour after administration the motor activity was assessed after each dose administered. Each mouse was kept in an actometer for 5 min, during which time both the horizontal and the vertical motor activity was recorded. Activity Cage – Ugo Basile type of device, was used for determinations.

Statistical interpretation of the results was performed using GraphPad Prism 5. Distribution of normality population response was made using Kolmogorov–Smirnov. For the normal distribution, statistical calculation was performed using Student test (compared to the original) and ANOVA (compared to witness). For abnormal distribution, Statistical calculation was done using Mann – Whitney (compared to the original) and Kruskal – Wallis (compared to witness).

All studies were performed in compliance with Directive 86/609/EEC of November 24, 1986, on the protection of animals used for experimental and other scientific purposes.

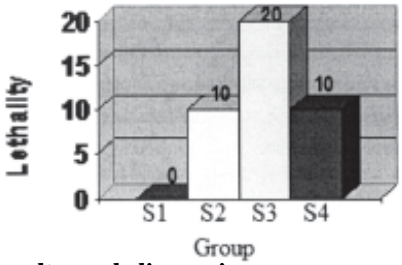


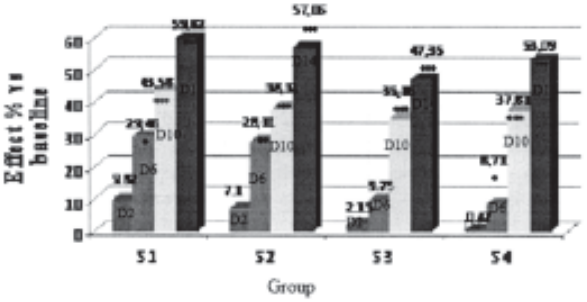
Fig. 1 - Lethality Evolution Induced by Substances S1, S2, S3, S4

Results and discussions

In assays of acute toxicity for substances S2 and S4 hyperreflexia was recorded to majority of animals in the lot.

For S3 substance, which was the highest mortality recorded, vasoconstriction was observed, cyanosis, apnea, followed by respiratory depression, muscle hypertonia, hyperreflexia and seizures.

The evolution of body weight during the surveillance period is shown in figure 2.



D2= day 2 , D6= day 6 , D10= day 10 , D14 = day 14

Fig. 2 Evolution of body weight of the animals treated with a single dose (2000 mg/kg) from substances S1, S2, S3, S4.

The test materials fall within the class of low toxicity, whereas a dose of 200 mg / kg of body weight to achieve up to 20% lethality for the substance S3. Survivors had growth rates of body mass similar to those of untreated body mass which confirms reduced toxicity.

Motor Activity

The influence of the investigated substances (expressed as the ratio of the average and lot) on the horizontal and vertical motor activity is shown in figure 3 and 4.

The experimental results showed that only substances S1 and S3 stimulates horizontal motor activity, at all times determinations. For substances S2 and S4, we notice a weak inhibitor effect (not statistically significant) of horizontal motor activity (fig. 5). Increasing vertical motor activity is recorded for all substances tested after two and 3 doses (fig. 6).

Among the compounds studied, S3 exhibits the highest rate of growth compared to control horizontal motor activity (+8.54% after 2 doses, +16.02% after 3 doses and +7.26% after 4 doses), followed by S1 (+7.74% după 2 doses, +12.55% 3 doses and +0.81% after 4 doses).

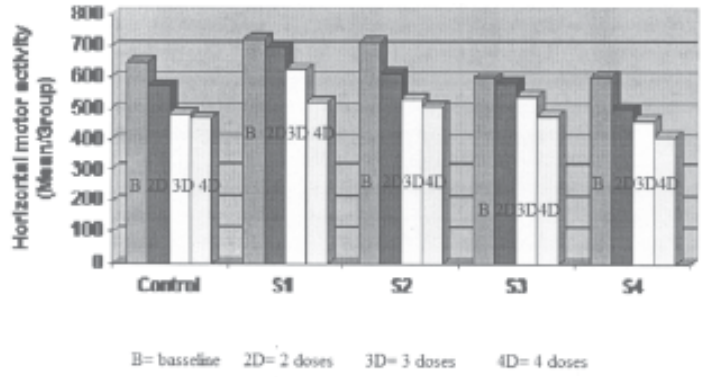


Fig. 3 Evolution of the horizontal motor activity for control group and for substances S1, S2, S3, S4.

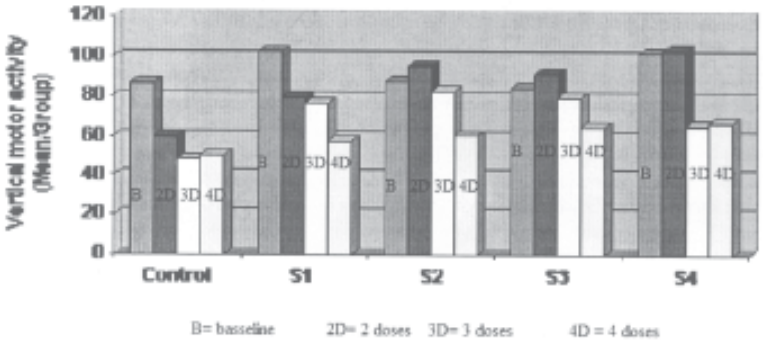


Fig. 4. Evolution of vertical motor activity for control group and for substances S1, S2, S3, S4

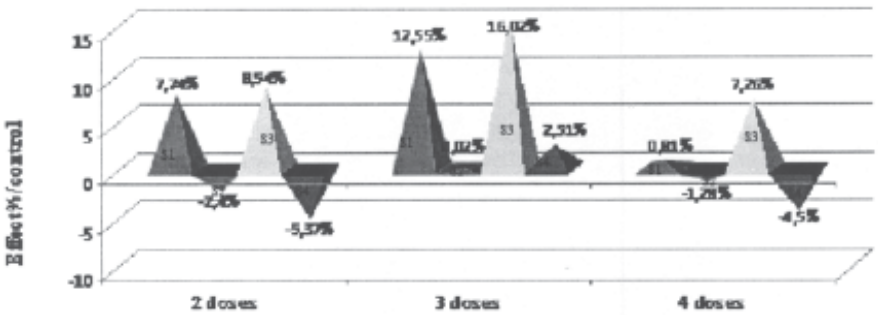


Fig. 5. Evolution of horizontal motor activity for control group and for substances, compared to control group

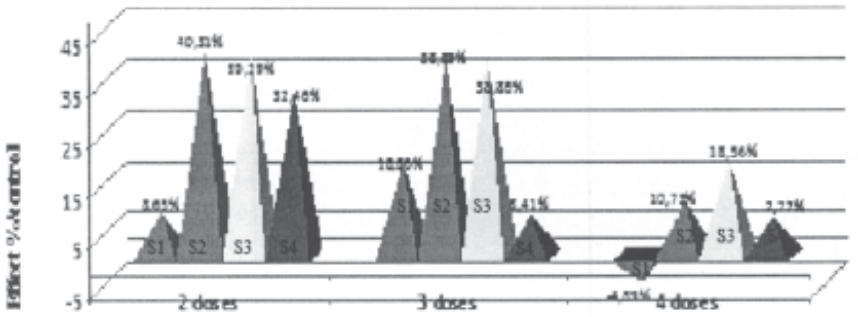


Fig. 6. Evolution of vertical motor activity for control group and for substances S1, S2, S3, S4, compared to control group

Vertical motor activity compared to the control was enhanced most by compounds S2, S3 and S4.

S3 caused an increase by +39.29% after 2 doses (not statistically significant), +38.86% after 3 doses (statistically significant) and +18.56% after 4 doses (not statistically significant).

S4 caused an increase by +32.46% after 2 doses(statistically significant), +8.41% after 3 doses (not statistically significant) and +7.77% after 4 doses (not statistically significant).

Experimental phytobiological researches regarding the toxicity induced by some new compounds

The bioassay was performed using procedures described by Popescu and Dinu (2008) with some modifications [18]. Phytobiological 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 [18].The bioassay is used for the assessment of the cytotoxic activity of natural extracts and synthetic compounds [19, 20, 21]. Additionally, the phytobiological tests can provide useful information about the genotoxicity of the new compounds [22].

Preparation of Solutions

For each compound were prepared in chloroform five dilutions: 10^{-4}, 10^{-5}, 10^{-6}, $10^{-7}$and 10^{-8} mol/L. Appropriate volumes of each dilution were added in Petri dishes (d=90mm) and maintained on water bath until solvent evaporation was complete. Over the residue were added 15 mL of distilled water in each Petri dish. A negative control sample was prepared with distilled water.

Plant material preparation

Wheat embryonic roots (*Triticum vulgare* Mill., Boema variety, obtained from SC Adaflor SRL – Tulcea County, Romania) were used. The plant material was obtained by

soaking the caryopses in distilled water for 24h and allowed to germinate on moist filter paper until the main radicle attained 1 cm (approx. 24h). Eleven caryopses with 1 cm long embryonic roots were placed in Petri dishes with 15 mL of the tested compounds. The bioassay was conducted at 25 ± 1°C, 75% relative humidity and in the absence of light, in a plant growth chamber (Sanyo MLR-351 H, San Diego, CA, USA). The length of the embryonic root was measured at 24h during the exposure period (5 days). The karyokinetic film modifications were observed after 24h after staining the primary wheat root meristems with diluted acetic orcein solution [23], using a Labophot 2 Nikon microscope (ocular 10×, ob. 40×, 100×) (Nikon, Chiyoda-ku, Tokyo, Japan).

Data Analysis

Analysis of the distribution of values †of root elongation was achieved by using the D'Agostino & Pearson test ("omnibus K2", $\alpha = 0.05$) [24]. The inhibitory effect (Efi%) compared with the control was calculated using the formula [25]:

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

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 significance of $Ef_i\%$ was evaluated by comparing with control group of results, was performed by ANOVA test, followed by Bonferroni post-test if normal distribution and Kruskal Wallis followed by Dunn's in case of abnormal data distribution [26]. Tests were considered statistically significant for a $p<0.05$. The inhibitory concentration 50% (IC50) was calculated by interpolation on the regression line plotted against the logarithm of the concentration and Efi% [27]. For each regression analysis were regression equation and correlation coefficient (r^2)calculated. Results were analyzed using Microsoft Office Excel 2003 software and GraphPad Prism v.5.0 (San Diego, CA, USA, 2007).

Results and dscussions

Distribution of root elongation was abnormal for the groups exposed to S1(10^{-6} mol/L on 2-nd day, 10^{-7} mol/L and 10^{-8} mol/L on 5-th day), S2 (10^{-4} mol/L on 1-st day and 10^{-8} mol/ on 5-th day), S3 (10^{-8} mol/L on 2-nd and 5-th day) and S4 (10^{-8} mol/L on 2-nd and 5-th day, 10^{-6} mol/L on 1-5 days and 10^{-8} mol/L on 1-st day). All other root elongation valueswere normally (Gaussian) distributed.

Efi% values are shown in figure 7. It is noted that for all the compounds tested at all the concentrations and all the days of the determination, the application of the overall ANOVA and Kruskal Wallis test, p value less than 0.001, which indicates a statistically significant inhibitory effect.

On first day of determination, results of the post-tests (Bonferroni for the Kruskal Wallis ANOVA and Dunn) shows statistical significance to the first four concentrations (10^{-4} - 10^{-7} mol/L) for compounds S1 and S2. S3 Efi% statistical significant at all tested concentrations and compound S4 only at higher concentratiions (10^{-4} - 10^{-6} mol/L). During the exposure period *Efi%* decreased starting from the 2-nd day of determinations.

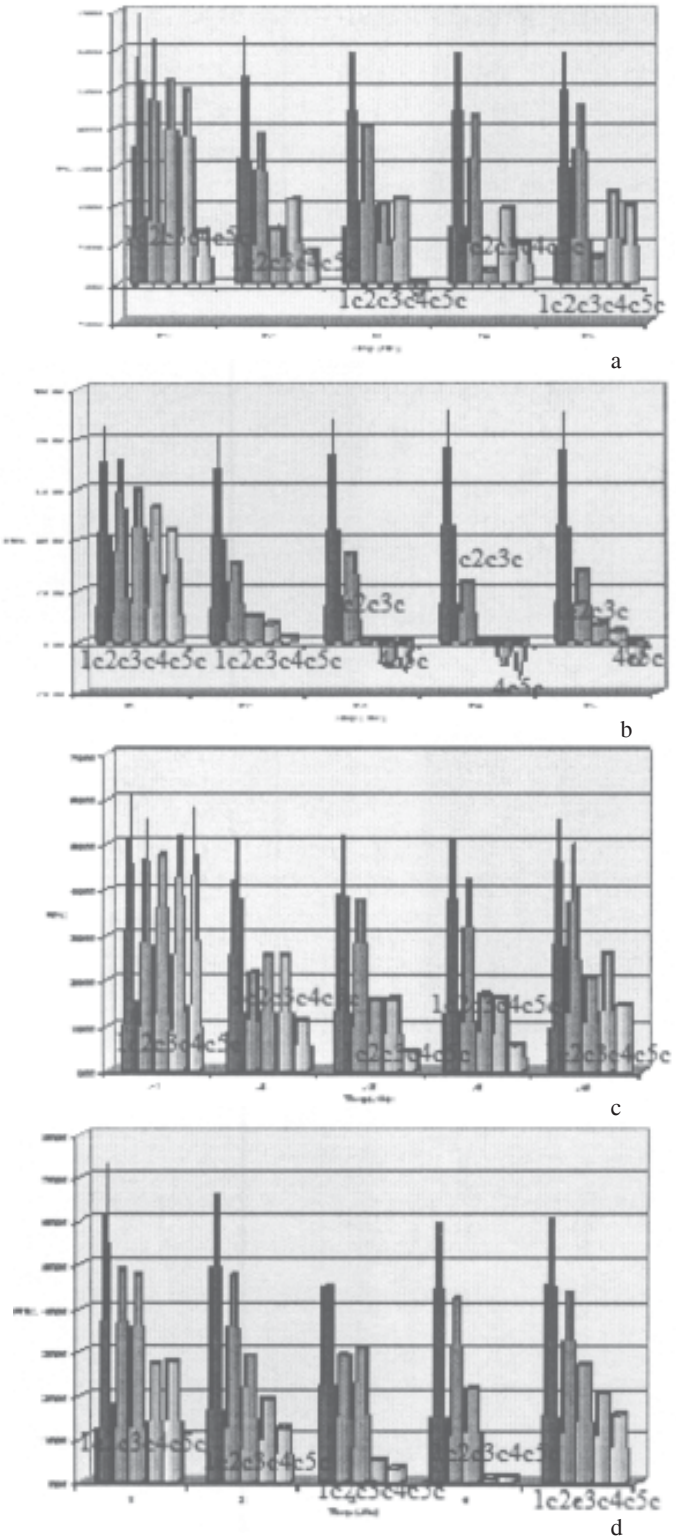


Fig. 7. The inhibitory effect induced by S1 (a), S2 (b), S3 (c), S4 (d) at concentrations of 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8}

The inhibitory concentrations 50% (IC50) are shown in table 2.

On the first day od exposure, the lowest IC50 was obtained for compound S2 (0.01×10^{-5} mol / L). This is about 10-fold lower than the IC50 of S1 and 28-fold lower than the IC50 of S4. The toxicity varies in the following decreasing order: day 2 - S2, S4, S1; day 3 - S4, S3, S1, S2; day 4 and 5 - S2, S4 and S3. The following IC50 values could not be calculated due to the lack of correlation between concentration and Efi%: S3 - days 1 and 2; S1 - days 4 and 5. During the experiment, IC50 tends to increase,

Compound	Day	Regression Equation	logIC50	IC50	Correlation Coefficient (r^2)
S1	1	Y=12.55X+62.09	-0.9636	0.11	0.8329
	2	Y=12.77X+42.04	0.6232	4.20	0.8170
	3	Y=14.52X+41.96	0.5540	3.58	0.9373
	4	Y=12.03X+39.09	0.9072	**	0.6718
	5	Y=10.16X+41.32	0.8544	**	0.5784
S2	1	Y=9.99X+72.55	-2.2549	0.01	0.9789
	2	Y=18.32X+44.85	0.2810	1.91	0.7836
	3	Y=24.55X+44.95	0.2057	1.61	0.8439
	4	Y=24.69X+42.41	0.3073	2.03	0.7940
	5	Y=22.46X+46.79	0.1427	1.39	0.8031
S3	1	*	*	*	*
	2	Y=7.50X+34.44	2.0735	**	0.6737
	3	Y=11.64X+36.85	1.1306	13.51	0.9243
	4	Y=11.56X+38.03	1.0356	10.86	0.9209
	5	Y=10.61X+44.10	0.5565	3.60	0.8444
S4	1	Y=11.21X+56.17	-0.5508	0.28	0.8842
	2	Y=13.50X+48.28	0.1277	1.34	0.9602
	3	Y=10.71X+33.40	1.5504	35.52	0.8953
	4	Y=15.61X+40.74	0.5932	3.92	0.9427
	5	Y=11.24X+44.79	0.4641	2.91	0.9372

Table 2

* IC50 could not be calculated because of Efi% between 57.52 to 69.11% at all concentrations

** The results do not correlate well (r2 <0.7000)

probably due to instability of the compounds in the aqueous medium. Although the correlation coefficient is satisfactory for most measurements, due to the lack of information on the stability of the compunds, we consider only the toxicity order at 24 h of exposure (decreasing order: S3, S2, S1 and S4).

Microscopic assessment

In the groups exposed to S1 .. S1 induced telophases in tropokinesis at 10^{-4} - 10^{-6} mol/L (fig. 8 b), whereas at 10^{-8} mol/L, all divisions were comparable with the control. Cell divisions were observed at all concentrations tested.

Table 2 50% inhibitory concentration equations regression lines and correlation coefficients for Efi% induced by compounds S1-S4

S2 blocked mitotic division at the highest concentration. At this concentration, only nuclei with 1-3 hypertrophy nucleoli were observed (fig.8a). Between 10^{-5} and 10^{-8} mol/L, S2 did not induce modifications on mitotic film (all phases of mitosis were observed). However, an effect of formation of "waves" on cell walls was observed. Compound S3 showed no mitoinhibitor effect at any of the 5 concentrations tested. Thus, numerous divisions have been observed. These were normal at concentrations 10^{-7}, 10^{-8} mol/L and presented modifications (disorganized metaphases and anaphases with retarded chromosomes, metaphases anaphase and telophase in tropokinesis - fig.8c) at concentrations 10^{-4}, 10^{-5} and 10^{-6} mol/L. S3 induced the same modifications as S2 at the cell wall level. S4 induced modifications in kariochinetic film (metaphases,

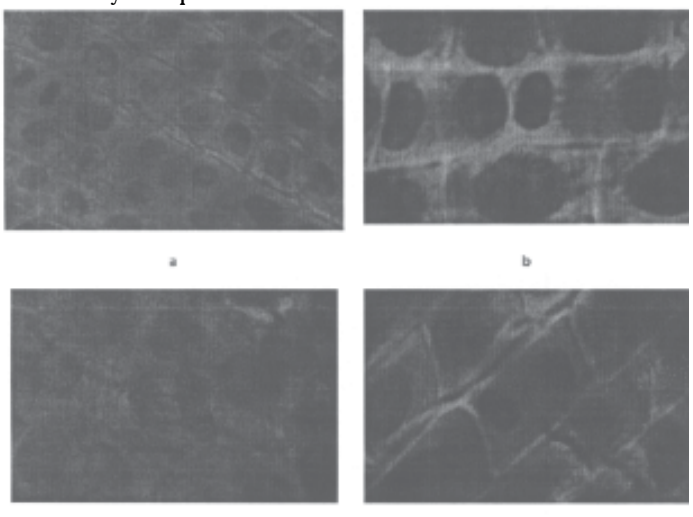


Fig. 8 Kariokinetic film modifications observed in *Triticum* test: a- interphase with nuclei with 1-3 hypertroffedd nucleoli induced by S2 at 10^{-4} mol/L; b - telophases in light tropokinesis induced by S1 at 10^{-4} mol/L; c – anaphases in tropokinesis induced by S3 at 10^{-5} mol/L; d - telophases in tropokinesis induced by S4 at 10^{-4} mol/L.

anaphases and telophases in tropokinesis) at all tested concentrations (fig. 8 d).

Of the synthesized compounds S1, 1-benzyl 2-phenyl - 1H, 1,3-benzimidazole absorbs at 286 nm, lower value to UVB range. Compounds S3 and S4 absorb in the UVB range and compound S4 in UVA range. Therefore they could be used in preparations for sunscreen protection. Are obtained with good yields. Their structural formulas deduced from molecular synthesis reactions, was confirmed by IR and NMR spectra. The absence of statistical significance for developments in horizontal motor activity, is explained by the normal downward trend of this activity, from one determination to another, as the mice are increasingly familiar with actometer enclosure and so it is reduced curiosity.

Enhancing statistical significance vertical motor activity of the compounds S2 and S4 after 2 doses, respectively S2 and S3 after 3 doses which confirms that these substances have CNS stimulant. S3 compound caused the largest percentage increase in motor activity both horizontal and vertical to control, which is closely correlated with the observed data in determining the acute toxicity (death of animals occurred in convulsions, typical manifestations of CNS stimulation). Compound S1 influenced the least motor activity, Work anticipated that the acute test, S1 proved to be the least toxic. At batches of extracts were treated with S1 order observed at all concentrations tested. At the highest concentration tested (10^{-4} mol/L), S2 blocked mitotic division. S3 influenced cytodieresis being observed numerous cells with undulated cell walls. In groups treated with S4 numorous divisions was observed at all concentrations tested. Highest toxicity presents S3 compound and compounds S1 and S4 are the least toxic.

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