Roumanian Biotechnological Letters *Vol. 13, No. 4, 2008, pp. 3837-3842*

ORIGINAL PAPER

Biological effect of some new pyridazine derivatives on wheat in germination experiments

Received for publication, June 5, 2008
Accepted, July 15, 2008

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Abstract

*Pyridazine compounds have an intense biological activity, being used as anticancer, antituberculosis, antihypertensive, antifungal, or antimicrobial agents. Nevertheless, some of them might be noxious persistent compounds to the environment. Therefore, several studies to test the biologic effect of some new pyridazine derivatives have been done using simple experiments of wheat germination. The germination percentage, shoot and root length, fresh weights, as well as free amino acid content varied as a function of concentration and structure of each investigated compound. Triplicate of 50-seed samples of wheat were treated with $5 \cdot 10^{-3}$ molar solutions of both pyridazinium bromides (**2a-c**) and the corresponding cycloadducts (**4-9**) for 1 hour. After a 7-day period of germination in the presence of the investigated compound, the wheat plantlets were cut from the seeds. The total height (**H**) of the lots and the weight (**W**) of the seedlings, as well as the free amino acids were measured. Among the pyridazine derivatives, the fluorine-containing **6a** proved to be the most toxic, whereas another fluorinated compound, **9a,** exhibited even a slight stimulatory action on the germination process. All the other tested compounds displayed a large variety of biological activities.*

Keywords: biological activity; pyridazine cycloadducts; monoquaternary salts; wheat germination; free amino acids, germination rate.

Introduction

Pyridazine compounds are commonly used as anticancer [1], antituberculosis [2], antihypertensive [3], antifungal [4,5], or antimicrobial [6-9] agents due to their intense

biological activity. They have a rapid systemic effect on the plants and are active at very low concentration. Some of the investigated pyridazine derivatives have chemical structures related to those of the phytohormones [5]. In addition, similar chemical structures occur in living cells, being involved in various biochemical reaction pathways. Some new synthesized pyridazine derivatives were used in many research fields due to their structure, stability and reactivity and their tendency to form stable ylides with important biological properties. On the other hand, as a rule, a cytotoxic effect of higher concentrated compounds is correlated with their stimulation effect at lower concentrations. We synthesized new pyridazine derivatives and cycloadducts derived from the pyridazine moiety in our group, still our concern is related to their possible toxicity on the environment. Therefore, this paper reports on the biological activity of some pyridazine derivatives on wheat germination and seedling growth. Higher plants are suitable for such research being recognized as excellent indicators of cytogenetic and mutagenic effects of some chemicals and are applicable for the detection of environmental noxious compounds [10-14]. The germination tests are very simple, little time consuming, cheap and, therefore, could be ideal methods for testing the biological activity of some new synthesized compounds. Because some of the investigated compounds might have a stimulatory action on germination, we searched for suitable agents among the pyridazine

structures to improve seed germination, that could be of paramount importance for genetic conservation work in the gene banks [15,16].

Materials and methods

Reagents. The pyridazine derivatives were synthesized both under classical and microwave heating. All other chemicals used were of analytical reagent grade and all solutions were prepared with redistilled water.

Apparatus. Germination test was performed in a growth chamber Conviron MP4030 model G30 with programmed temperature, humidity and light. The melting points were determined on a MELTEMP II apparatus and are uncorrected. ^{1}H-RMN and ^{13}C NMR spectra were performed using a Bruker Advance 400 DRX. Mass spectra were recorded with a Shimadzu QP 2010 mass spectrometer (EI).

Biological material. Seed samples of spring wheat (*Triticum aestivum*), Henika and Magistral varieties, were purchased from the Suceava Agricultural Research Station.

Synthesis and chemical characterization of the pyridazine compounds. A facile way to obtain condensed pyridazines is to use ylides as intermediates. This strategy assumes the obtaining of the corresponding cycloimmonium salts (**2**), in the first step, which, in the presence of Et_3N, generates the ylides *in situ* (**3**) (Figure 1). In the second step, symmetrical and non-symmetrical dipolarophiles (activated Z-alkene and Z-alkine) reacted with ylides (**3**) to afford the cycloadducts (**4-9**) (Figure 2).

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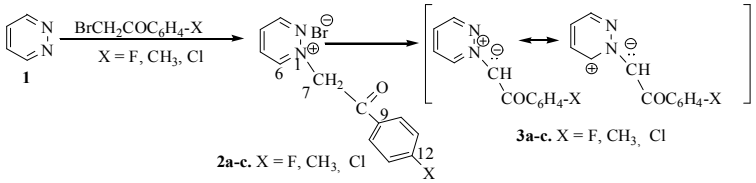

Figure 1. Cycloimmonium salts (**2**) used as precursors in the cycloaddition reaction.

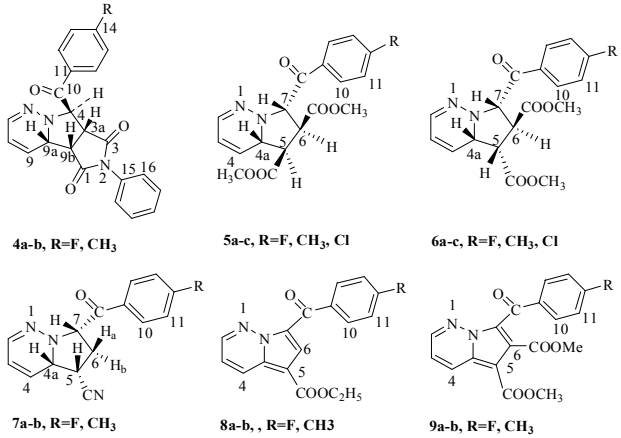

Figure 2. Pyridazine derivatives used in this work

The reaction pathways for synthesis of the investigated cycloadducts were presented previously in details [1,17]. The separation and purification of the compounds obtained were carried out using thin layer chromatography on silica gel and on silica gel column. The structure of the investigated chemical compounds was proved by elemental and spectral

analysis (FT-IR, MS, ^{1}H NMR, ^{13}C NMR, and two-dimensional experiments 2D-COSY, 2D-HETCOR (HMQC), long-range 2D-HETCOR (HMBC) [1,17].

Procedure. After performing the germination measurements in the Conviron MP4030 growth chamber, some additional experiments were carried out in Petri dishes, on double Watmann no. 1 filter paper at room temperature, as previously stated [5,12,14]. Young wheat plants were harvested from their seeds, measured (**H**, expressed as cm) and weighed (**W**, expressed as grams). The content of free amino acids in leaves were spectrophotometrically determined with ninhydrin [5,18].

Statistics. The data were validated using the Tukey test [19].

Results and discussion

The substituents were related to the alteration of host metabolism, which resulted in various germination rates and number of seedlings, as show in Table 1.

Table 1. The effect of some new pyridazine derivatives on wheat germination.

Comp.	Germination Rate (GR, %)	Number of plantlets in the lot	Comp.	Germination Rate (GR, %)	Number of plantlets in the lot
2a	64 ± 5	26 ± 1	**6b**	79 ± 4	26 ± 6
2b	38 ± 4	14 ± 5	**6c**	40 ± 5	28 ± 5
2c	29 ± 4	9 ± 2	**7a**	61 ± 4	26 ± 4
4a	72 ± 4	34 ±3	**7b**	54 ± 5	20 ± 3
4b	72 ± 4	25 ± 1	**8a**	81 ± 3	34 ± 4
5a	73 ± 5	30 ± 3	**8b**	54 ± 4	20 ± 4
5b	61± 5	17 ± 2	**9a**	73 ± 5	32 ± 2
5c	59 ± 6	22 ± 1	**9b**	80 ± 4	24 ± 3
6a	0 ± 0	0 ±0	**Blank**	88 ± 5	39 ± 2

All the pyridazine derivatives influenced dramatically the germination process of the wheat seeds. Thus, the germination rate ranged between 0% in the case of compound **6a** and 81 ± 3% for **8a** (**GR** of the blank was 88 ± 5%). Low germination rates characterized also the compounds **2c** (29 ± 4%) and **2b** (38 ± 4%). However, some pyridazine derivatives presented relatively higher values for **GR** (**9b** - 80 ± 4% and **6b** – 79 ± 4%).

The pyridazine derivatives investigated here proved to affect the number of seedlings. Thus, the treatments with **4a, 8a** and **9a** slowly reduced the number of plantlets obtained from the 50-seed lots (34 and 32 seedlings, respectively). In contrast, the compound **6a** completely inhibited the wheat germination, whereas **2c** and **5b** proved to be also very noxious, killing more than 50% of the seeds (9 ± 2% and 17 ± 2%, respectively, whereas the blank was 39 ± 2%).

Pyridazine salts **2a-c** manifested a more toxic effect on wheat germination (**GR** 44%; 16 plantlets in a lot) as compared to the cycloadducts, except the compound **6a**.

For salts **2a-c** we considered the free bromine ion interference, which resulted in the decreasing of the wheat germination, when the pyridazine salts were investigated. The most noxious effect was observed in the case of **2c** compound. Thus, the number of plantlets in the lot decreased from 39 ± 2 (blank) to 9 ± 2 in the case of the compound **2c**, and to 26 ± 1 seedlings for the compound **2a** (Table 1). In addition, both the height and the weight of the plantlets in the lots decreased accordingly (Table 2). The compound **2c** reduced the height of the lot from 204.5 cm (blank) to 19.0 cm, and the weight from 1.2 g to 0.16 g. The salt **2a** determined a height of 125.0 cm and a weight of 0.8 g and **2b** had an intermediary activity on the lots of seeds (50 cm for the height and 0.42 g for the weight of the plantlets in lot).

The geometric isomers. Nevertheless, structural changes from the pyridazine salts to the corresponding cycloadducts might play an important role in the wheat germination.

Table 2. The effect of some pyridazine derivatives ($C = 5 \cdot 10^{-3}M$) on wheat germination and seedling growth (the total height of plantlets in the lot, **H,** the mean height of plantlets in the lot, **Hm**, the weight of plantlets in the lot, **W**, and the mean weight of plantlets in the lot, **Wm,** were determined).

Compound	H, cm	Hm, cm	W, g	Wm, mg	% Free amino acids
2a	121.3 ± 15.9	5.3 ± 1.1	0.89 ± 0.12	39.96 ± 5.49	0.610
2b	52.4 ± 13.5	4.6 ± 0.4	0.47 ± 0.19	41.96 ± 16.97	0.640
2c	52.4 ± 13.3	4.4 ± 0.9	0.47 ± 0,41	40.03 ± 2.67	0.647
4a	197.6 ± 7.2	5.3 ± 0.6	1.11 ± 0.15	28.52 ± 13.30	0.443
4b	148.7 ± 7.8	6.9 ± 0.3	0,95 ± 0,14	45.00 ± 4.35	0.611
5a	185.8± 32.5	5.6 ± 0.9	1.11 ± 0.19	31.66 ± 4.79	0.459
5b	77.3 ± 9.9	6.1 ± 0.7	0.64 ± 0.30	42.45 ± 2.14	0.571
5c	11.3 ± 0.3	0.55 ± 0.01	0.08 ± 0,01	39,84 ± 0,01	0.704
6a	0	0	0	0	-
6b	184.9 ± 19.2	8.1 ± 0.8	1.06 ± 0.19	47.44 ± 8.48	0.381
6c	136.4 ± 31.4	5.8 ± 1.3	0.98 ± 0.08	42.32 ± 3.36	0.609
7a	178.2 ± 17.2	5.9 ± 0.6	1.03 ± 0.12	34.32 ± 1.65	0.570
7b	120.4 ± 14.8	6.8 ± 0.8	0.80 ± 0,13	44.35 ± 0.07	0.551
8a	239.1 ± 17.2	6.4 ± 0.4	1.38 ± 0.16	35.32 ± 4.06	0.485
8b	117.3 ± 6.6	6.7 ± 0.4	0.75 ± 0,07	43,50 ± 3,90	0.555
9a	233.5 ± 25.9	6.6 ± 0.7	1.37 ± 0.14	37.05 ± 3.85	0.423
9b	133.1 ± 3.1	6.3 ± 0.1	0,87 ± 0,17	42.20 ± 8.11	0.543
B	223.7 ± 23.2	6.5 ± 0.7	1.42 ± 0.19	41.66 ± 5.93	0.406
D	42.2	1.3	0.42	13.23	0.121

In addition, some new behavioral patterns were observed in the case of cycloadducts as compared to the corresponding salts. Thus, although *cis*- **5a** and *trans*- **6a**, are isomers with the same composition, because of the different structure, they exhibited quite different biological actions: the adduct (**6a**) completely inhibited the wheat germination, whereas **5a**, slowed a slight decrease in the germination parameters (Table 1 and 2). The total height of the plantlets, **H**, was a more effective parameter to show the effect of the investigated compounds on wheat germination. Whereas, the **H** value for the blank was 223.7 cm, the solution of compound **6a** killed the seeds, which were not able to germinate. In contrast, the compounds **8a** and **9a** did not influence the height of the plantlets in the treated lots (Table 2). The treatment **5c** practically destroyed the biological material, resulting in a low H value, which was found to be around 11.2 cm (5% of blank total height). We grouped the treatment according to the ratio between the total height of the treated sample and that of the blank. Thus, **2a**, **4a**, **4b**, **5a**, **6b**, **6c**, **7a**, **7b**, **8b**, and **9b** had the total height of the lot more than 50% of the blank, even if values for **4a** and **5a** were 88% and 83%, respectively, whereas **2a** and **8b** only 54% and 52%.

The second group consists of very noxious compounds that reduced the total heights to more than 50% as compared to the blank (**2b**, **2c**, **5b**, **5c**, and **6a**).

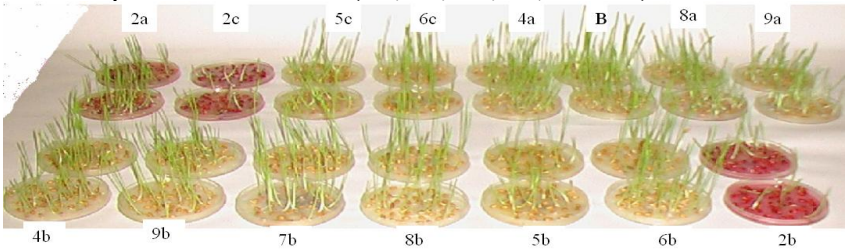

Figure 4. Representative samples of wheat treated with various pyridazine derivatives.

On taking into consideration the salts of pyridazine, all of them powerfully reduced the height of the lots. Nevertheless, **2a** was found in the first group of toxicity, whereas **2b** and **2c** within the second one, both of them with similar pattern (H = 52.4 cm). When compared to the germination rate, the activity of **2b** and **2c** appeared much more interesting. Thus, the

presence of chlorine atom, in the salt **2c**, resulted in a drastically inhibition of the germination, however, once germinated, the seedlings were more vigorous. The presence of methyl group, in the salt **2b**, slower inhibited the germination, but its negative influence on seedling growth was more intense.

To understand the effect of fluorine atom, chlorine atom and the methyl group in the compounds, on the germination process, we compared the effects of various cycloadducts on the height of the seedlings in the lots. In general, the fluorine atom determined a low decrease in the height of the shoots, with the exception of **6a**. The replacing fluorine atom with a methyl group reduced the **H** value by almost 50% (for example, **8a-b** and **9a-b**, Table 2). The differences in the **H** parameter were not so high, when fluorine was replaced by methyl, probably due to the steric effects by the other groups and radicals in the molecule. The very complex structure may interfere itself with the enzyme sites to disturb various biochemical processes.

The activities of compounds **5a-c** were compared to those of **6a-c** in order to understand the effect of geometrical isomerism. The biological differences between the isomers were statistically demonstrated. Thus, **5a** determined a slow decrease (H = 185.8 cm), whereas **6a** completely inhibited the germination. Both **5b** and **5c** provoked a high decrease of the plantlet height (77.3 and 11.3 cm), whereas their isomers **6b** and **6c** had a more moderate action (184.9 and 136.4, respectively).

Because the number of plantlets was also influenced by the treatment compounds, **Hm** values varied much. Thus, **5a** and **6b**, although their similar total heights, the mean heights were completely different (5.6 cm and 8.1 cm).

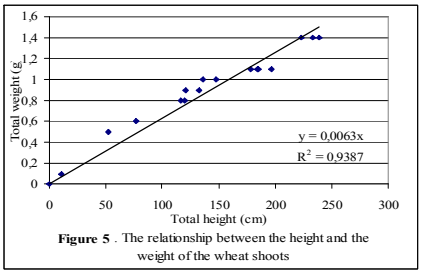

Figure 5 . The relationship between the height and the weight of the wheat shoots

In general, the weight of the shoots in a lot varied much with the treatments, being in the range from 0 to 1.42 g and the investigated compounds presented lower values as compared to the blank. We found a positive correlation between the height and the weight of the lots, with a correlation coefficient r = 0.969, and the regression equation, W = 0.0063 H.

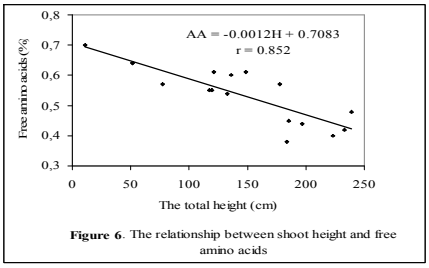

Figure 6. The relationship between shoot height and free amino acids

The content of total free amino acids within the seedlings treated with the investigated compounds was higher as compared to the blank, except for **6b**. A negative correlation between the total height of the seedlings and the free amino acids was found (r = 0.852; AA = –0.0012H + 0.7083, where, AA, % amino acids in fresh shoots and H, the height of the shoots).

The obtained results demonstrated that the $5 \cdot 10^{-3}$ molar concentrations of **2, 4-9** had a specific and effective activity within the germination experiments and that the germination tests might be used to characterize the biological effect of some new synthesized compounds. So far, we do not know the molecular mechanism, the biological activity is based on. A hypothesis was advanced to explain the observed phenomena based on the interaction between various enzymes and the investigated compounds. They could change the conformations of the involved enzymes or block the active enzymatic sites. Further investigation is still necessary to establish the correct mechanism of action of pyridazine derivatives studied here.

Conclusion

Pyridazine derivatives may influence germination rate, shoot and root length and fresh weights as a function of concentration and structure of each investigated compound. The

compound **6a** has the strongest inhibitory effect, whereas the compound **8a** presents a slight stimulatory effect. A negative correlation between the germination parameters and the content of free amino acids was calculated. The mechanisms promoting toxicity are yet to be determined although we have advanced some hypotheses here. Nevertheless, further research is required to assess the impact of pyridazine derivatives on living organisms and, especially, wheat germination

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