Chapter 1

Biological Activity of Novel Ureas and Thioureas Containing Bioactive Heterocycles

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Abstract

Heterocyclic compounds are widely distributed in nature and, along with their synthetic analogues, play an important role in agriculture and pharmacy. The general strategy of our studies was to design new bioactive compounds based on the combination of two or three types of active structural units: a 5-or 6-heterocyclic nucleus, a urea/thiourea bridge and alkyleneureido/thioureido groups. The plant growth regulatory and stress-protective activities of newly synthesized compounds were studied to determine how different structural combinations could influence the type of activity and its intensity. To obtain more detailed information about the specific effects of the tested compounds on the physiological, biochemical, cellular and genetic processes, different test-systems (plants and human lymphocytes) and experimental conditions were used.

Depending on the chemical structure, each class of compounds manifests important and multiple biological effects, such as reduction of the genotoxic effect of ultraviolet C (UV-C) and gamma-radiation; protective effect against herbicides and oxidative stress inductors; stimulating effect on the micro-propagation of higher plants; anti-senescence, anti-phytoviral and plant growth-regulating activities.

These beneficial biological effects give us the basis to recommend the newly synthesized bioactive heterocyclic ureas/thioureas for further testing and use in practice.

Keywords

Cytokinins, Herbicides, Heterocycles, Plant Growth Regulators, Phytophores, Stress-Protectors, (Thio)Ureas

Abbreviations

RHE = relative herbicidal efficiency; Chl = chlorophyll; FAAC = free amino acid content; AsPO = ascorbate peroxidase activity; CAT = catalase activity;

GPO = guaiacol peroxidase activity; RNase = total ribonuclease activity, MDA = malondialdehyde

1.1 Introduction

The search for and the extraction/synthesis of new substances with new chemical and biological properties is a problem that has received a lot of attention over the years and continues to be of present interest. The synthetic modification of bioactive natural products is one of the powerful tools to discover new biologically active compounds. The structure-bioactivity relationships determined for different series of compounds can serve as a starting point for the synthesis of new compounds with optimal biological activity. A classical approach is the purposeful synthesis through incorporation of active structural units from active biomolecules into a single structure resulting in compounds ("bioactive xenobiotics") which may possess unusually high activity.

Ureas and thioureas are very important compounds that show a wide spectrum of biological activities [1, 2]. It is well known that a number of nitrogen-containing heterocyclic compounds are widely distributed in nature and essential to life in various ways, which makes them important in pesticide and pharmacological chemistry [3, 4]. The presence of a 5-or 6-member heterocyclic nucleus in the urea and/or thiourea molecules confers important and multiple biological properties with potential applications in agriculture and medicine, and is the basis for target-oriented synthesis. A series of hybrid compounds containing both a heterocyclic ring and a (thio)urea bridge have been synthesized and their biological activities demonstrated, such as pesticidal [5, 6, 7, 8], antimalarial [9], antiviral [10, 14], anticancer [11, 12, 13], plant growth regulating [15, 16, 17, 18], stress-protecting [19, 20], antioxidant [21, 22], antimicrobial [23] activities, antifungal activity against plant pathogens [24] and antiamoebic activities [25] etc.

In view of the above-mentioned observations and in continuation of our search for biologically active compounds, we designed a strategy to develop agrochemicals of high potency taking into account the importance of the biological activity of compounds containing a N-heterocyclic ring connected to different functionalities, such as carboxamide, urea, thiourea. We synthesized a series of new compounds that incorporate two or three types of active phytophores: a 5-or 6-member heterocyclic nucleus, (thio)urea bridge, ethylene group, diamines, in the hope that they may be biologically active. The biological activity tested mainly included plant growth regulatory and stress-protective activities to prove how different structural combinations could influence the type of activity and its intensity. To obtain more detailed information about the specific effects of the compounds on the physiological, biochemical, cellular and genetic processes, various test-systems (unicellular algae, plants and human lymphocytes) and experimental conditions were used.

1.2 Biological Activity of Novel Ureas and Thioureas Containing Bioactive Heterocycles

All ureas and thioureas synthesized by us in which the structural modifications involve a combination of two or three active structural moieties could be conventionally divided into three series: 1,1'-hexamethylenebis (3-substituted)ureas; N-aryl and alkyl-N'-(heterocycle)ureas and thioureas; and 1-methyl and acetyl-4-substituted piperazines.

1.2.1 Series One: 1,1'-Hexamethylenebis(3-substituted)ureas

This series includes four new compounds which had not been previously described in the literature as protective plant growth regulators.

Het-NHCONH-$(CH_2)_6$-NHCONH-Het

Het = 2-thiazolyl (2-Ts, 1); 4-picolyl (4-Pic, 2); 4-pyridyl (4-Pyr, 3); 3,5-dichloro-4-pyridyl (3,5-Cl_2-4-Pyr, 4)

These compounds contain three bioactive structural units: a heterocyclic ring, carbamoyl groups and diamine. The synthesis of the heterocyclic bis-ureas (compounds 1-4), their physical properties and anti-senescence effect were described earlier [26, 27]. The structure and purity of compounds were elucidated by means of elemental carbon (C), hydrogen (H), and nitrogen (N) CHN-analysis, infrared (IR) and ultraviolet (UV) spectra, thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) characteristics.

Biological Effect of the Compounds

i) Anti-Senescence Effect

Active phenylurea cytokinins, derivatives of natural N, N'-diphenylurea and aliphatic di-and polyamines possess a common physiological property: senescence-retarding action. Cytokinins and polyamines have been regarded as the most potent senescence-retarding hormones in plants and they play a significant role in the regulation of leaf senescence [28]. A wide variety of studies have shown that exogenous cytokinin and polyamine applications lead to dramatic senescence retardation in the plant leaves. This provoked us to synthesize organic compounds possessing a diamine moiety as well as a urea moiety in their molecules and to investigate the effect of the obtained compounds on a process controlled well by both polyamines and cytokinins. One such process is leaf senescence. The anti-senescent effect of heterocyclic bis-ureas by dark-induced senescence of both barley (*Hordeum vulgare* L.) and radish (*Raphanus sativus* L.) leaf tissues was established for the first time [27, 29].

It was found that the investigated compounds have behaviour like putrescine [Put] rather than like N, N'-diphenylurea [DPU] (standards) in terms of the

determined parameters of leaf senescence in both plant systems. The phenylurea-containing cytokinin DPUprotected more strongly against Chl degradation by high levels of peroxidase and catalase activities, whereas diamine Put protected against proteolysis by an increase in both these activities only up to 48th h. 1, 1'-Hexamethylenebis(3-heterocycles)ureas (after primary chlorophyll defense) protected against proteolysis in dark-incubated leaves, more effectively than putrescine, by moderate increase in peroxidase and inhibition of catalase activities. And the halogen-substituted bis-phenylureas prevented the chlorophyll loss in dark-incubated leaves like DPU, by a strong increase in antioxidant enzyme activities [29].

The anti-senescence effect was elucidated based on the kinetics of senescence-dependent changes in some biochemical (chlorophyll, protein, free amino acids) and enzymatic (catalase EC 1.11.1.6; guaiacol peroxidase EC 1.11.1.7; ribonuclease EC 3.1.27.5) characteristics (endpoints) over a period of 72 h treatment. Chl degradation and protein hydrolysis in the aging leaf segments did not pass to the same rate. The tested compounds almost completely inhibited both these processes in the early stages of senescence and, as senescence advanced, provided stronger protection against proteolysis by a significant increase in peroxidase activity until day 2 and a decline in catalase activity after day 1 of senescence. All compounds manifested a more rapid and higher effect in the monocotyledonous plant system [29].

The heterocyclic bis-urea derivatives of 1,6-diaminohexane showed pronounced anti-senescence effect and it depended on the nature of the 3-substituent (ring type and ring's substituents) in the urea moiety. The integrity of the heterocyclic rings was an essential feature for providing high anti-senescence activity, particularly for compound 4 (shown above). This compound showed interesting behaviour in dark-incubated barley and radish

leaves. It delayed senescence in radish leaves during the whole senescence period, protecting more strongly (after a lag-time of 24 h) against the process of Chl degradation by a concomitant sharp decrease in antioxidant enzyme activities. However, the same compound protected against both processes, Chl degradation and proteolysis, in an almost similar manner and enhanced the stabilization of the Chl/protein ratio in senescing barley leaf segments by decline in peroxidase, catalase and RNase activities.

The compound 1,1'-hexamethylenebis[3-(3,5-dichloro-4-pyridyl)]urea (5, 3 and 1 mM) was the most effective in arresting chlorophyll loss and protein breakdown in dark-incubated barley and radish leaves, possibly by controlling senescence-linked events which occur in darkness and inactivation of the relevant enzyme activities (see Table 1).

Table 1. *Effect of 1,1'-hexamethylenebis(3-heterocycle)ureas [compounds 1 - 4] on the time-dependent changes in Chl (a+b), soluble protein, free amino acid contents, specific catalase and guaiacol peroxidase activities and total ribonuclease activity in barley leaf segments induced to senescence in darkness.*

Compound / Concentration	**Chl (a+b) %**			**Soluble protein %**			**Free amino acid %**		
	24h	**48h**	**72h**	**24h**	**48h**	**72h**	**24h**	**48h**	**72h**
Control	66	55	42	85	80	79	156	257	316
1 - 1.0 mM	79	73	54	97	136	174	140	167	242
1 - 0.1 mM	84	69	58	102	157	188	142	190	216
2 - 1.0 mM	82	67	50	74	97	176	147	186	248
3 - 1.0 mM	80	70	49	82	117	174	137	157	226
4 - 1.0 mM	91	95	85	184	181	180	83	63	24
4 - 3.0 mM	91	90	89	168	187	184	99	50	27
4 - 5.0 mM	92	88	88	157	146	173	106	67	24
Standards									
DPU-1 mM	75	62	44	68	63	71	109	133	194
Put - 5 mM	88	69	48	130	137	103	161	276	334

Table 1. *Continued.*

Compound / Concentration	C A T %			G P O %			RNase %		
	24h	**48h**	**72h**	**24h**	**48h**	**72h**	**24h**	**48h**	**72h**
Control	176	133	123	403	408	597	112	123	133
1 - 1.0 mM	20	22	8	293	502	373	-	-	-
1 - 0.1 mM	15	14	13	407	438	372	-	-	-
2 - 1.0 mM	116	113	31	370	455	371	-	-	-
3 - 1.0 mM	216	96	77	403	348	368	-	-	-
4 - 1.0 mM	80	37	36	104	96	64	150	70	124
4 - 3.0 mM	90	31	34	98	89	54	149	141	109
4 - 5.0 mM	123	93	71	97	119	63	119	130	102
Standards									
DPU-1 mM	57	301	59	383	751	957	-	-	-
Put - 5 mM	47	34	35	248	359	451	86	139	107

Segments were floated on phosphate buffer (control) or on test solutions for 24, 48 and 72 h.

Data are expressed as % of the initial values: Chl (a+b) = 127±2.10 µg/segment; Protein = 318±2 µg/segment; Free amino acid content = 2.237±0.26 µmol leucine eqv/segment; CAT = 0.295±0.0072 µmol destr. H_2O_2 mg^{-1} protein min^{-1}; GPO = 2.503±0.35 µmol GDHP mg^{-1} protein min^{-1}; RNase = 18.610 segment$^{-1}h^{-1}$.

ii) Antioxidant, Anti-Cytotoxic and Anti-Genotoxic Potential of HMPU against Chemical Mutagens

Among the first series of compounds, 1,1'-hexamethylenebis [3-(3,5-dichloro-4-pyridyl)]urea (5, 3 and 1 mM) / HMPU / manifests multiple biological properties in the highest grade. Together with the high senescence-retarding effect, this compound also exhibits a potential to modify the clastogenic effect of some chemical mutagens.

Some aspects of the response to the diversity of biotic and abiotic stresses have been highly conserved from bacteria to humans. The signaling pathways involved in the initiation and maintenance of the hypersensitive response (HR) and

systemic acquired resistance (SAR) are still poorly understood. Rapid recognition of a potential invader is a prerequisite for the initiation of an efficient defense response. On the other hand, it is found that the pre-treatment of eukaryotic cells with some chemical compounds leads to increased cell tolerance to subsequent oxidative challenges [30]. Nicotinamide (NIC) and its structural analogue isonicotinamide (IND) are reported as stress-associated compounds that can induce and regulate the defensive and secondary metabolism in plants [31, 32]. Our research also showed that the treatment with HMPU increases greatly the antioxidant capacity of algal cells. The most rapidly appearing defense response was the strongly reduced MDA content in cells. The activities of H_2O_2-scavenging enzymes CAT and GPO increased and H_2O_2 accumulation decreased with increasing the post-treatment time. Therefore, the defense mechanisms in the treated cells are expressed at different times [33].

The problem of chemical protection of plants against the injuries induced by different environmental factors has been gaining importance over the past four decades. A large number of chemicals, mainly synthetic ones, used singly or in combination have been evaluated. The process of experimental search of effective compounds which can trigger a defense response can be significantly accelerated by the use of compounds that show protective action against the natural mutagenic process such as senescence. For that reason, the investigation on the senescence-retarding effect of the newly synthesized compounds is an important part and a prerequisite for success in our subsequent studies on their protective potential against some genotoxins in different test-systems.

Human lymphocytes are more sensitive than *Hordeum vulgare* root tip meristem cells. The genotoxic/clastogenic effect of 1,1'-hexamethylenebis [3-(3,5-dichloro-4-pyridyl)]-urea (HMPU) depended on the susceptibility of the test-systems used. *Hordeum vulgare* root tip meristem cells and human

lymphocytes showed different susceptibility. Taking into account the chemical structure of HMPU, we tested its potential to modulate the cytotoxic and genotoxic effects of two well-known experimental mutagens in various experimental schemes. Hydroxyurea (HU, an antimetabolite, anti-cancer agent used in chemotherapy) and N-methyl-N-nitroso-N′-nitroguanidine (MNNG, a carcinogen and mutagen which acts by adding alkyl groups to the O^6 of guanine and O^4 of thymine) were used [34]. HMPU triggered adaptive response (AR) in both test-systems assessed as a decrease in the induction of chromosome aberrations. The optimal effect was observed at 6-hour inter-treatment time between HMPU conditioning treatment and challenge application of the mutagen. The chromosome injuries were reduced 3-fold in barley and 2-fold in human lymphocytes (Figure 1a and Figure 1b)

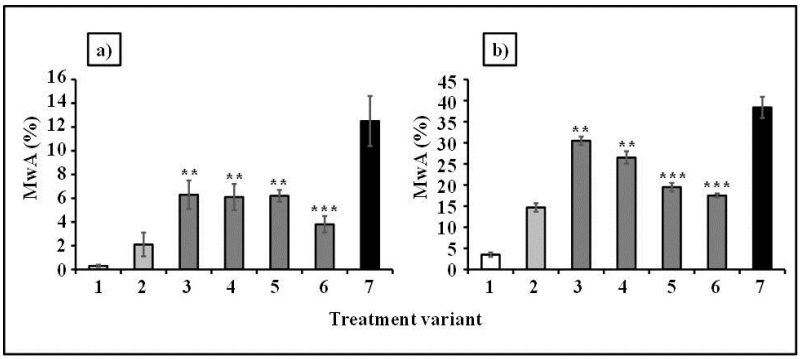


Figure 1. *Impact of HMPU conditioning treatment: (a) in barley root tip meristem cells prior to HU exposure; (b) in human lymphocytes in vitro prior to MNNG with different inter-treatment time (IT) detected as induction of chromosome aberrations.*
*** $p < 0.01$; *** $p < 0.001$.*

1- control
2- HMPU 7.5 x 10^{-3} M
3- HMPU 7.5 x 10^{-3} M - 2h IT - HU 3x10^{-2} M
4- HMPU 7.5 x 10^{-3} M - 3h IT - HU 3x10^{-2} M
5- HMPU 7.5 x 10^{-3} M - 4h IT - HU 3x10^{-2} M
6- HMPU 7.5 x 10^{-3} M - 6h IT - HU 3x10^{-2} M
7- HU 3x10^{-2} M

1- control
2- HMPU 10^{-5} M
3- HMPU 10^{-5} M - 1½h IT - MNNG 10^{-5} M
4- HMPU 10^{-5} M - 2½h IT - MNNG 10^{-5} M
5- HMPU 10^{-5} M - 4h IT - MNNG 10^{-5} M
6- HMPU 10^{-5} M - 6h IT - MNNG 10^{-5} M
7- MNNG 10^{-5} M

1.2.2 Series Two: N-aryl and alkyl-N'-(heterocycle)ureas and Thioureas

This series includes three subgroups with a total of 91 compounds (70 of which are new chemical substances) which had not been previously described in the literature as protective plant growth regulators.

R - NHCYNH - Het

Y = O or S;

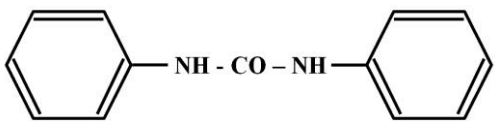
Het = 2-thiazolyl, 2-furfuryl; 2-, 3-, 4-pyridyl and substituted 2-pyridyl;

R = phenyl, 3- and 4-fluorophenyl, 3- and 4-chlorophenyl, 2,4-dichloro -phenyl, 4-bromophenyl, 2-chloroethyl, cyclohexyl, benzyl, 4-tolyl, 1-and 2-naphthyl; methyl, ethyl, n-butyl.

These compounds contained two bioactive structural units - a (thio)urea bridge and a 5-or 6-membered heterocyclic ring.

The synthesis of the different sets of compounds as well as their analytical characteristics and biological activity (herbicidal, growth inhibitory and stimulatory, anti-senescence and cytokinin-like activities) were earlier reported in a great number of articles [37, 39, 41, 42, 44, 45, 47, 48, etc]. The structure and purity of compounds were confirmed by means of elemental CHN-analysis, 1H NMR, 13C NMR, IR and UV spectra, TLC and HPLC characteristics.

The structural modifications of the parent molecule N, N'-diphenylurea (DPU) have different effects on the derivatives.



DPU

[Formula 1.1]

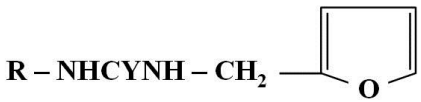
The substitution of one benzene ring with a heterocyclic ring (pyridyl, pyrazole, thiazole, thiadiazole and imidazole) on the one hand, and the presence of electron-acceptor substituents in the cycles, on the other hand, leads to increased biological activity. The five-membered heterocycles possessing adjacent nitrogen and sulfur atoms within the ring have received considerable attention in the fields of agricultural and medicinal chemistry.

Two basic groups of structurally different chemical substances show cytokinin effects; they are purine and non-purine compounds. The aromatic ureas, derivatives of N-phenyl-N'-(5-or 6-member heterocycle)ureas are the most significant group of non-purine cytokinins. Among them, there have been discovered compounds which manifest higher cytokinin activity than the classical purine cytokinins [15, 35, 36].

I. Biological Activity of N-aryl and alkyl-N'-(2-furfuryl and 2-thiazolyl)ureas and thioureas

The biological activity of two sets of N-aryl and alkyl-N'-(2-furfuryl and 2-thiazolyl)ureas and thioureas included herbicidal, root growth inhibitory and stimulatory activities (by root growth assay with wheat and cucumber seedlings) and cytokinin-like activity (by betacyanin and cotyledon enlargement assays). The structure-bioactivity relationships of the new compounds were also studied, focusing mainly on the substituent ® effect and the type of 5-member heterocycle [37]. The generally acknowledged high cytokinin activity of kinetin led us to synthesize the furfurylureas.

1- aryl and alkyl -3 - (2 – thiazolyl)ureas and thioureas



1- aryl and alkyl -3 - (2 – furfuryl)ureas and thioureas

[Formula 1.2]

In general, aryl substituents contributed greater activity than alkyl, 2-chloroethyl, or cyclohexyl substituents. The optimization in the benzene ring showed that compounds with an electronegative nonpolar substituent (F, Cl, Br) at the *meta* or *para* positions had the highest activity. In a series of halogenophenyl-ureas and thioureas, *meta*-and *para*-fluorophenyl derivatives had the highest herbicidal activity. Among fluorophenyl(thiazolyl and furfuryl)ureas, those having a fluorine in the *meta*-position of the aromatic ring tended to exhibit high cytokinin-like activity. The *para*-fluorophenyl substituent in the thiazolylthioureas resulted in a high cytokinin-like activity while in the furfurylthioureas, in a high herbicidal activity. Therefore, the 4-fluorophenyl substituent selectively influenced the herbicidal or cytokinin-like activities depending on the type of heterocyclic ring. The presence of two chlorine atoms on the benzene ring, of 4-tolyl and benzyl substituents did not significantly affect the herbicidal and cytokinin-like activities of the thiazolyl-and furfurylthioureas.

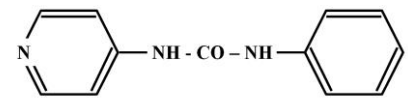
Alkyl (methyl, ethyl, n-butyl) derivatives of the thiazolylthioureas were at least 5-times more active herbicides than the aryl derivatives. The relationships between thiazolyl-and furfuryl-derivatives and their activity showed that the thiazol ring provided significantly enhanced activity, whereas the furan ring had

little effect, probably due to the separation of a furan ring from the urea/thiourea bridge by one methylene group.

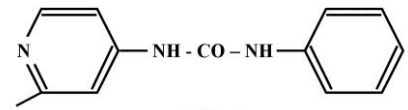
The ureas and thioureas containing thiazole nuclei were found to have high biological activity. The presence of an unsubstituted or *meta*-substituted (F or Cl) phenyl ring in these compounds increased the cytokinin-like activity, and alkyl groups markedly increased the herbicidal activity. The furfurylureas had only moderate cytokinin-like activity and the aryl(furfuryl)thioureas were completely inactive [37].

II. Biological Potential of N-(2-chloroethyl)-N'-(pyridyl)ureas

Among the aromatic ureas tested, N-phenyl-N'-(4-pyridyl)urea [4PU] exhibits strikingly high cytokinin activity comparable to N^6-benzylaminopurine [BAP]. Moreover, an electronegative chlorine atom introduced at the 2nd position of the pyridyl ring increases strongly the activity [4PU-30] and this activity is 10 times higher compared to that of purine cytokinins [36].



4PU



4PU – 30

[Formula 1.3]

It is well known that the 2-chloroethyl group included as a substituent to biologically active substrates (carriers) attributes different physiological action [38]. Thus, our following idea for target-oriented synthesis was to obtain novel N, N'-disubstituted ureas containing a pyridyl or a substituted-pyridyl ring connected to the one of the nitrogen atoms and a 2-chloroethyl group at the

other nitrogen atom, as well as to investigate the herbicidal and growth-regulating activities of the new compounds. In these urea derivatives, the chlorine atom introduced through the 2-chloroethyl group is at a stereochemical distance from the urea bridge (-NHCONH-) comparable to that in the N-phenyl-N'-[4-(2-chloro)pyridyl]urea (4PU-30).

[Formula 1.4]

At R = H, the amino group of the pyridyl nucleus is in 2nd, 3rd and 4th position;

At R = CH_3 or Cl, the amino group of the pyridyl nucleus is in 2nd position, and the CH_3 group is in 3rd, 4th, 5th and 6th position; di-CH_3 groups - in 4th and 6th positions; Cl - in 5th position; di-Cl - in 3rd and 5th positions [39, 41, 42].

The herbicidal activity of N-2-chloroethyl-N'-(pyridyl and methylpyridyl) ureas against both test plants (wheat and cucumber) was attributed to the aromatic (heterocyclic) unit rather than to the aliphatic unit (chloroethyl), which is in accordance with the conclusion that there is a direct proportion between the herbicidal activity and the lipophilic character of the compounds [40]. The N-2-chloroethyl-N'-4-pyridylurea [RHEx100=424] and N-2-chloroethyl -N'-[2-(6-methyl)pyridyl]urea [RHEx100=533] exerted the highest selective herbicidal activity towards wheat at the 1000 μM concentration and were substantially more herbicidally active than the standard Diuron [RHEx100=243]. However, the introduction of a 2-chloroethyl group at the first nitrogen atom leads to obtaining new highly active substances with cytokinin-like activity. We investigated the effect of all N-2-chloroethyl-N'-(pyridyland methylpyridyl) ureas on the betacyanin synthesis by Amaranthus bioassay [37]. The screening results showed that the above substances manifest considerably higher

cytokinin-like activity than the standard N, N'-diphenylurea (168% at 1000 μM, 151% at 100 μM and 133% at 10 μM) but are less active than the other standard, N-phenyl-N'-(4-pyridyl)urea (157% at 100 μM, 192% at 10 μM and 200% at 1 μM). Most active among the pyridyl isomers is the 2-isomer, N-2-chloroethyl-N'-2-pyridylurea - 210% at 1000 μM, 177% at 100 μM and 130% at 10 μM. Among the methylpyridyl derivatives, the compounds that displayed the highest cytokinin-like activity were N-2-chloroethy l-N'-2-(4-methyl)pyridylurea (240% at 100 μM and 186% at 10 μM) and N-2-chloroethyl-N'-2-(5-methyl)pyridylurea (182% at 100 μM and 133% at 10 μM).

i) Biological Activity of (2-pyridyl)-imidazolidine-2-one Derivatives

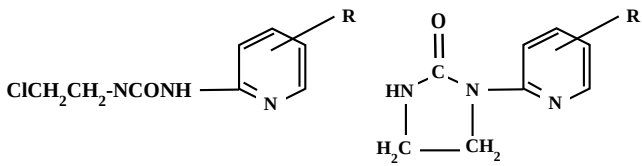
Two (2-pyridyl)-imidazolidine-2-one derivatives [B-1, B-2] comprising a cyclic ureido group were obtained for evaluation as plant growth regulators and were compared to the activity of (2-pyridyl)ureas possessing an acyclic ureido group. The literature survey revealed that linked bi-heterocyclic compounds containing hydrogenated NH-heterocycles are seldom reported to possess biological activity. In continuation of our studies on the synthesis of biologically active substances containing nitrogen heterocycles, we carried out synthesis of pyridines linked to a hydrogenated 5-member heterocycle (imidazolidine) as a result of the intramolecular cyclization of two (2-pyridyl)ureas: N-2-chloroethyl-N'-2-pyridylurea [R_1-Cl] and N-2-chloroethyl-N'-2-(4-methyl)pyridylurea [R_2-Cl] [41].

Both types of compounds, with and without a cyclic ureido group, had different behaviour in the bioassay systems employed for investigation of the herbicidal, root growth-regulating and cytokinin-like activities. The biheterocyclic compounds B-1 and B-2 did not have herbicidal activity towards either wheat or cucumber, but showed pronounced root growth stimulatory activity which well

correlated with their cytokinin-like activity at an optimal concentration of 10 μM. However, B-1 (153%) and B-2 (134%) are less active cytokinins than the corresponding R_1-Cl (130%) and R_2-Cl (186%) at 10 μM [41].

ii) Anti-senescence Effect of 2-pyridylureas with un-and/or cyclic-ureido Group

Dark-induced senescence of detached leaves or leaf segments is well suited to examine the effect of synthetic compounds on senescence as well as to study various physiological and biochemical changes associated with senescence. The potential anti-senescence effect of eight (2-pyridyl)ureas with an un-cyclic and cyclic ureido group was studied in excised barley (*Hordeum vulgare* L.) leaves which were induced to senescence by incubation in complete darkness [42].



1 – R = H (R_1 – Cl)
2 – R = 4 – Me (R_2 – Cl)
3 – R = 5 – Me
4 – R = 4,6 – Me_2
5 – R = 5 – Cl
6 – R = 3,5 – Cl_2

7 – R = H (B–1)
8 – R = 4 – Me (B-2)

[Formula 1.5]

The compounds possessing an uncyclic ureido group showed higher chlorophyll retention activity than those with a cyclic ureido group but this activity was lower compared to that of the standard 4PU at the end of the third day of aging. Treatment of leaf segments with R_1-Cl and B-2 led to increased carotenoids content after 48 h and it was higher than that in the 4PU-treated leaf tissues. The increased carotenoids content is an important response of cells to senescence or stress conditions. These effects of the compounds tested were

mediated by strongly increased H_2O_2-scavenging enzyme activities, the peroxisomal catalase activity being mainly affected. Among the compounds possessing one or two CH_3 groups, the most active member of this series was the 4-methyl isomer (R_2-Cl). Both compounds containing one or two Cl atoms on the pyridine ring were the most active compounds among all tested ones. The presence of two Cl atoms contributed to a long-term protective effect on chlorophyll degradation, while a single Cl atom induced a short-term effect. The highly activated state of the H_2O_2-scavenging enzymes contributed to the elimination of the consequences of the high extent of lipid peroxidation occurring in the tissues during day 1 of senescence [42].

Table 2. *Ratios of the activity of SOD relative to that of H_2O_2-scavenging enzyme during dark-induced senescence of barley leaf segments, 1 day, 2 days and 3 days after incubation.*

		SOD/CAT			SOD/AsPO			SOD/GPO		
Variants	**Concentration [(a)]**	**1day**	**2day**	**3day**	**1day**	**2day**	**3day**	**1day**	**2day**	**3day**
1	0.1	1.26	0.67	0.72	1.09	0.7	1.35	1.08	1.33	1.5
	1	0.9	0.47	0.65	0.71	0.74	1.48	1.02	1.5	1.55
2	0.1	0.7	0.62	0.6	0.49	0.89	1.03	1.1	1.11	1.13
	1	0.71	0.31	0.51	0.96	1.28	1.08	1.27	1.4	1.2
3	0.1	0.56	0.48	1.03	0.89	0.73	1.16	0.99	0.89	1.25
	1	0.41	0.27	0.85	0.75	0.75	1.64	0.99	0.88	1.41
4	0.1	0.76	0	0.71	0.83	0	1.34	1.07	0	1.27
	1	0.69	0	0.42	0.92	0	0.83	1.51	0	1
5	0.1	1.126	0.85	0.72	1.55	0.83	0.91	1.14	1.07	0.91
	1	0.36	0.48	0.46	0.59	0.62	0.56	0.51	0.88	0.73
6	0.1	1.04	0.61	0.69	1.28	1.21	1.17	1.52	1.22	0.92
	1	0.63	0.32	0.44	1.06	1.09	1.29	1.13	0.82	1.18
7	0.1	1.82	0.83	0.69	1.27	0.75	0.69	1.97	0.95	0.85
	1	1.8	0.6	0.51	1.23	0.49	0.45	2.16	0.59	0.63
8	0.1	0.82	0.85	0.91	0.68	0.76	0.84	1	1.08	1.16
	1	1.06	0.81	0.87	0.9	0.58	0.89	1.57	1.05	1.56
4PU[(b)]	0.1	1.22	0.83	0.85	1.07	0.85	0.87	1.34	0.73	0.92

a) concentration of test-compounds and 4PU in mM

Kanazawa et al. (2000) showed that the SOD/CAT ratio increased in the late stages of both natural and artificial senescence, whereas the SOD/AsPO and SOD/GPO ratios increased during artificial senescence but decreased during natural senescence [43]. We demonstrated that the activities of the antioxidant enzymes and the balance between them are important factors for the senescence-retarding effect of the tested urea compounds (see Table 2) [42]. The most active compound, 4PU, a well-known cytokinin used as a standard, was responsible for the balance between the H_2O_2-generating enzyme and H_2O_2-scavenging enzymes in the senescing segments, since the values of the three activity ratios were ~ 0.85 during the second and the third day of aging. The high anti-senescence effect of 4PU could be due to its cytokinin activity. The exogenous cytokinin can probably compensate the declined levels of the endogenous physiologically active cytokinins in senescing tissues. The cytokinin-like activity demonstrated by compound R_1-Cl in this and other cytokinin bioassays suggests that compounds 1-8 represent a new class of cytokinin mimics. In general, our findings showed that the cyclization of an ureido group in the imidasolidinone ring resulted in decreased chlorophyll retention activity, cytokinin-like activity, modified the level and mode of anti-senescence action compared to the compounds possessing anuncyclic ureido group. [42]

iii) Biological Properties of N – halogenophenyl - N'-(pyridyl)ureas and thioureas

The following group among the N-(pyridyl)ureas and thioureas contained halogenophenyl ring connected to the other nitrogen atom and the herbicidal and growth-regulating activities of the new compounds were investigated [44, 45, 46, 47, 48, 50, etc].

[Formula 1.6]

Y = O, S;

X = 3-and 4-F; 3-and 4-Cl;

R = H, the amino group of the pyridyl nucleus is in 2nd, 3rd and 4th position;

R = CH_3 or Cl, the amino group of the pyridyl nucleus is in 2nd position, and 3-, 4-, 5- and 6-CH_3; 4,6-di-CH_3; 5-Cl; 3,5-di-Cl.

Biological Activity of N-(3-and 4-fluorophenyl)-N'-pyridylureas and thioureas

N-(3-and 4-fluorophenyl)-N'-pyridyl and methylpyridyl-ureas/thioureas did not show herbicidal and root growth stimulatory activities but possessed high cytokinin-like activity (by the Amaranthus bioassay) with an optimal concentration of 10 μM. The more active ureas and thioureas started to affect amaranthin synthesis at a concentration of 0.1 μM and 1.0 μM, respectively. N-(4-fluorophenyl)-N'-[2-(4-methyl)pyridyl]thiourea was an exception. This compound started the amaranthin synthesis at 0.001 μM.

The cytokinin activity of N-(3-and 4-fluorophenyl)-N'-2-,3-and 4-pyridylureas/thioureas increased with the removal of the 3-and 4-fluorophenyl(thio)ureido group [3/4-FPhNHCYNH-] away from the heteroatom of the pyridyl cycle and the relative activity order is 4>3>2. Substitution in the pyridyl cycle with one methyl group reduced the cytokinin activity, as the degree of reduction depended on the CH_3-position in the 2-pyridyl ring toward the heteroatom and toward the urea/thiourea bridge. The 3-and 6-methyl-isomers (ortho-position) are usually less active, whereas the 4-and 5-methyl-isomers are highly active compounds. Therefore, the movement

of the methyl group away from the heteroatom and from the urea/thiourea bridge favors the manifestation of high cytokinin activity [44, 45].

We proved the cytokinin activity of N-(3-fluorophenyl)-N'-(2-pyridyl)urea in tobacco (CMS/81) and lucerne (74RS2) callus biotests as well. This substance (0.025 mg/l) possessed a cytokinin activity close to that of kinetin (0.5mg/l in tobacco and 0.2mg/l in lucerne biotests). [46].

Biological Evaluation of N-(3-and 4-chlorophenyl)-N'-pyridylureas

Only two compounds, N-(3-and 4-chlorophenyl)-N'-(4-pyridyl)ureas, displayed higher selective herbicidal activity against wheat [RHEx100=408 and 260, respectively] compared to the diuron standard [RHEx100=243]. The tested urea's compounds manifested high cytokinin activity at low concentrations of 1.0 μM to 100 μM, whereas DPU showed the highest cytokinin activity at 1000 μM.e structure-activity relationships in this group of substances were similar to those in the set of fluorophenyl-pyridylureas discussed above.

In general: 1) ureas with unsubstituted pyridyl or with 4-CH_3 monosubstituted 2-pyridyl rings showed considerable cytokinin activity; 2) the effect of substitution in the phenyl ring with one chlorine atom depended on its position toward the urea bridge; the 3rd position of chlorine atom was more favorable for the display of high growth-regulating and cytokinin activities [47].

We established that the nature of the halogen atom (F or Cl) in the benzene ring influences the biological activity of the above two sets of compounds. The N-3-chlorophenyl-N'-(methyl)pyridylureas had higher selective herbicidal activity and lower cytokinin activity compared to the N-3-fluorophenyl-N'-(methyl)pyridylureas. However, the N-3-fluorophenyl -N'-(2-pyridyl) urea showed a much weaker cytokinin effect on the growth of tobacco (*Nicotiana tabacum* L. CMS/81) callus compared to its chlorine analogue N-3-chlorophenyl-N'-(2-pyridyl)urea at the same concentration of

0.5 mg/l. The effect of all compounds depended on the specificity and susceptibility of the used test-system [48].

Antiphytoviral Activity of N-(3- and 4-chlorophenyl)-N'-Pyridylureas

Cytokinins such as kinetin and N^6-benzyladenine influence the augmentation of plant viruses. Correlative relations between the cytokinin and the antiphytoviral activities of adenine-and (thio)carbamoyl-compounds were established [49].

The antiphytoviral activity of 14 synthetic N-(3-and 4-chlorophenyl) -N'-pyridylureas and of seven initial amines (at $5x10^{-3}$M) was investigated by using the following "virus-host plant" systems: potato virus X strain H19 (PVX)-tobacco leaves (*Nicotiana tabacum* L. cv. Samsun NN); red clover mottle virus (RCMV)-peas leaves (*Pisum sativum* L. cv. Nadja). All compounds tested had positive I %-values, i.e. they inhibited the virus replication to a more or less marked degree. The highest effect in reducing the PVX concentration showed N-(3-chlorophenyl)-N'-(4-pyridyl)urea (80%). The starting compound 4-aminopyridine also had high inhibiting activity (73%); it increased weakly in the urea containing a 3-chlorophenyl group, whereas in the urea with a 4-chlorophenyl group, it was reduced to 50%. Among the ureas containing a 3-chlorophenyl group, four derivatives displayed superior PVX-inhibiting activity (50-80%) and only one derivative had a good RCMV-inhibiting activity (43%). Among the ureas containing a 4-chlorophenyl group, two derivatives had a good PVX-inhibiting activity (~50%). The potent compounds were those that contained an unsubstituted 4-pyridyl residue and/or one CH_3 group at the pyridyl nucleus in the 4th or 5th positions. The ureas with a 3-chlorophenyl group were more active than those with a 4-chlorophenyl group [50]. The same structure-activity relationships for the antiphytoviral and cytokinin activities of these compounds were observed. With regard to the problem of the increase in

plant tolerance to phytopatogenic viruses, our investigations lead to the design of new compounds with high antiphytoviral activity.

A series of novel derivatives of N-phenylurea containing a pyrimidine ring with two nitrogen atoms instead of a pyridyl ring were synthesized and their antiviral activity was evaluated. All of the target compounds exhibited good anti-TMV (tobacco mosaic virus) activity and two compounds had higher activity to virazole at $5.0x10^{-4}$ g/ml [10].

In addition, a series of N, N'-dipyridylthioureas inhibit the replication of HIV and other related viruses *in vitro*. The most active compound is reported to be N-[2-(2-pyridyl)ethyl]-N'-(5-bromo-2-pyridyl)thiourea [14].

[Formula 1.7]

Effect of the Synthetic Cytokinins of the Urea Type on the Growth and Development of Cytokinin-Dependent Tissue Cultures

The formation of active oxygen species plays an important role in cell division and can inhibit the morphogenesis of plant cells and tissues *in vitro* culture. Cytokinins have been found as effective free radical scavengers. The N^6-substituted adenine derivatives are the classical cytokinins which can induce callus growth in tissue cultures. However, the cytokinin-active phenylureas could substitute the adenine cytokinins in inducing callus growth and organogenesis in different plant species.

Among the three compounds, N-3-chlorophenyl-N'-(2-,3-and 4-pyridyl)ureas, which are positional isomers in relation to the pyridyl nucleus, the 2-isomer

showed cytokinin activity higher than that of kinetin, whereas the activity of the 3-and 4-isomers was equal to that of kinetin in tobacco (*Nicotiana tabacum* L. CMS/81) callus assay. The 4-isomer also manifested organogenic effect on the meristemal explants from cork oak (*Quercus suber* L.) and ash-tree (*Flaxinus excelsior* L.) which was most marked in the ash-tree [48].

The effect of two cytokinin-active compounds, N-3-chlorophenyl-N'-[2-and (4-methyl)-pyridyl)ureas, on *in vitro* shoot cultures and the physiological state of the micropropagated *Gypsophila paniculata* L. was studied and compared to that of the conventional purine cytokinin kinetin. It was found that the highest rate of shoot multiplication was in the explants grown on media containing the tested substances as compared to that in explants grown on media with kinetin. Moreover, the produced explants preserved this potential for growth of *Gypsophila* shoots on cytokinin-free medium. The physiological state induced by the cytokinins tested was characterized by an increased content of pigments, proline and enhanced invertase activity ensuring energy for the growth process of the explants cultivated *in vitro* [51]. The active phenylurea cytokinins tested in this research could find application in the micropropagation of flower species.

Protective Effects of N-(3-chlorophenyl)-N'-Pyridylureas

The growth and development of plants are determined by the interactions between their genome and the environment. To date, there is scant evidence in the literature for modulation and improvement in some basic physiological processes, such as photosynthesis, plant resistance etc., responsible for plant productivity.

It has been suggested that yield increases in crops might be at least partly accounted for by delayed leaf senescence and higher leaf net photosynthetic rates during the mid-to late grain-filling period [52]. In this respect, we investigated the effects of two active phenylurea derivatives,

N-(3-chlorophenyl)-N'-(2-pyridyl)urea [3CP-2PU] and N-(3-chlorophenyl) -N'-[2-(4-methyl)pyridyl]urea [3CP-4MPU] at 100 μM, on some photosynthetic parameters, such as stomatal conductance (Gs), net photosynthetic rate (Pn), enzyme activities of carbonic anhydrase (CA) and phosphoenolpyruvate carboxylase (PEPC), and chlorophyll and protein contents in wheat flag leaves. Wheat plants (*Triticum aestivum* L. cv. Sadovo-1) were grown under field conditions and the studied parameters were measured on the 7th and 14th days after single foliar spraying.

To the best of our knowledge, we demonstrated for the first time the ability of two phenylurea compounds to increase the photosynthetic capacity in wheat flag leaves during the early grain-filling stage [53]. The fact that they cause the opening of stomata suggests that the effect of the compounds is due to their function as cytokinins. This was associated with an increase in flag leaf chlorophyll, soluble protein, net photosynthesis rate and grain dry matter (see Table 3).

Table 3. *Effect of 3-CP-2PU and 3-CP-4MPU treatments on wheat grain yield in relation to number of grains per plant and 1000-grains weight (percent of control).*

Treatment	No. grains per plant[a]	1000-grain weight[b] (g)
control	100.00	100.00
3-CP-2PU	111.27	112.46
3-CP-4MPU	118.35	120.21

[a] control value = 60.13
[b] control value = 43.8 g

The treatment with both substances, 3-CP-2PU and 3-CP-4MPU, caused changes in the polypeptide patterns of soluble proteins from wheat flag leaves. They increased differentially the quantity and composition most individual polypeptides identified compared to the non-treated leaves. While 3-CP-2PU

only increased the polypeptide quantity, 3-CP-4MPU led to the appearance of a new 51 kDa polypeptide [54].

A positive effect of both substances, 3-CP-2PU and 3-CP-4MPU, on the photosynthetic potential, transpiration and green biomass production was also found in maize and sunflower plants [55].

A crucial problem in agriculture is to improve the stress- and disease-tolerance of cultivated plants, thus increasing crop yield. According to many studies, treatment with various natural and synthetic cytokinins can significantly contribute to prevent and reduce the damaging effect of various unfavorable environmental conditions.

Another field of great theoretical and practical importance in the era of atomic energy is the search for new, more effective radio-protectors for radiation protection. We examined the potential radio-protective effect of two active phenylurea derivatives, 3-CP-2PU and 3-CP-4MPU, which were applied as post-radiation (6 Gy) treatment of pea seeds (50 Gy) and oat plants during the vegetation period. The compounds exhibited a considerable radio-protective effect. The treatments resulted in significant reduction in the radiation-induced damage to the primary development of pea seeds and the survival and productivity (30% increased yield) of oat plants. The data from the anaphase analysis of pea plants indicated that the application of the novel compounds with protective effect increased the recombinant potential of the irradiated cells [56, 57].

The phenylurea derivative N-(3-chlorophenyl)-N'-[2-(5-chloro)pyridyl]urea (showing high cytokinin-like activity) induced protective effect against injury induced by the herbicide chlorsulfuron in *Nicotiana tabacum* callus cultures and young plants [58]. The safener-mediated induction of the herbicide-detoxifying enzyme glutathione S-transferase appears to be a major component of the stress

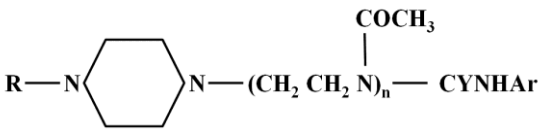
response. The treatment of young tobacco plants with the same compound resulted in enhanced tolerance to NaCl (85.5 mM). The protective effect was organ-specific, being more strongly pronounced on shoots than on roots (Yonova et al., unpublished data).

In addition, another one of our urea derivatives, N-(2-chloroethyl) - N'-[2-(4-methyl)pyridy]urea (with high cytokinin-like activity), manifested a protective effect against NaCl (up to 170 mM) in tobacco callus tissues. The protective potential was similar to that of 4PU-30 up to 85.5 mM of NaCl and equal to that of kinetin up to 126.5 mM of NaCl.

1.2.3 Series Three: 1-methyl and acetyl-4-substituted Piperazines

The series of 1-methyl and acetyl-4-substituted piperazines included 12 compounds (9 of which are novel chemical substances) which had not been previously described in the literature as protective plant growth regulators.

These compounds possess a hydrogenated NH-heterocycle [piperazine ring] and aryl(thio)carbamoyl groups connected directly or through an ethylene group and have the following general structure:

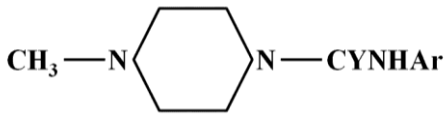


[Formula 1.8]

i) R = CH_3, n = 0, Ar = phenyl and halogenophenyl, Y = O, S [compounds 1 - 8]

ii) R = CH_3CO, n = 1, Ar = phenyl and halogenophenyl, Y = O, S [compounds 9 - 12]

Compounds 1-8 include a combination of two types of bioactive structural units (phytophores): aryl(thio)carbamoyl groups directly connected to the secondary nitrogen atom of the piperazine ring.



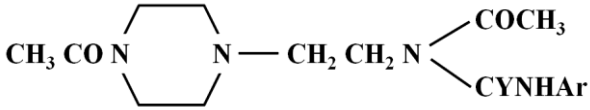
[Formula 1.9]

1-aryl(thio)carbamoyl-4-methyl-piperazines (i)

Y=O, Ar = Ph (1), 3-ClPh (2), 4-FPh (3), 4-ClPh (4);

Y=S, Ar = Ph (5), 4-FPh (6), 4-ClPh (7), 4-BrPh (8).

Compounds 9-12 combine three types of phytophores: aryl (thio)carbamoyl groups connected to the piperazine ring through an ethylene group.



[Formula 1.10]

1-[2-(acetylamino)ethyl]-4-acetyl-piperazines, N-aryl(thio)carbamoyl (ii)

Y = O, Ar = Ph (9), 4-FPh (10);

Y = S, Ar = Ph (11), 4-FPh (12).

The syntheses of all compounds as well as their analytical characteristics and biological activity (herbicidal, growth inhibitory, stimulatory and cytokinin-like activities) were reported by us [59]. The structure and purity of the compounds were confirmed by means of elemental CHN-analysis, 1H NMR, 13C NMR, IR and UV spectra, TLC and HPLC characteristics.

In spite of the scarce reports in the literature, it is interesting to note that some representatives of the groups of N-(phenyl or pyridyl)-N'-[(heterocycle or saturated NH-heterocycle)ethyl]-ureas and thioureas show important biological properties [14, 19, 60]. For instance, 1-phenyl-3-[2-(2-oxo-1-imidazolidinyl) ethyl]urea (EDU, ethylendiurea, containing an un-cyclic and a cyclic ureido group) displayed cytokinin-like activity; it is the most efficient synthetic protectant against acute ozone injury in a number of plant species, against UV-B radiation in soybean and also inhibits plant senescence.

EDU

[Formula 1.11]

I. Biological Activity of 1-methyl and acetyl-4-substituted Piperazines

The herbicidal, growth stimulatory and cytokinin-like activities of the two novel groups of aryl-ureas and thioureas possessing piperazine or 1-ethyl-piperazine ringswere studied [59].

In the first group of compounds (1-8), compounds 7 and 8, which possess 4-chloro-and bromo-phenylthiocarbamoyl groups, showed the best herbicidal activity as well as high selective herbicidal activity against wheat (RHE=316 and 438, respectively; in relation to chlorsulfuron, RHE=397). Stimulation of root growth both in wheat and cucumber was observed within the whole studied concentration range of compounds 1 and 6. Compounds 3, 4, 7 and 8 also exhibited high levels of selective stimulatory activity, more pronounced against wheat at 100, 10 and 1 μM (129-146%). Our results from the betacyanin bioassay of 1-aryl(thio)carbamoyl-4-methyl-piperazines (compounds 1-8) showed that the

compounds lacking herbicidal activity exhibited good cytokinin-like activity. Compound 1, which possesses an unsubstituted phenylcarbamoyl group, manifested the highest cytokinin-like activity at 1000 μM (like DPU). The introduction of a halogen atom at positions 3 or 4 of the phenyl ring in the 1-arylcarbamoyl-4-methyl-piperazines (compounds 1-4) tended to reduce the overall activity, whereas in the 1-arylthiocarbamoyl-4-methyl-piperazines (5-8) this led to a complete loss of activity.

Among the second group of compounds (compounds 9-12), compound 10, which possesses a 4-fluorophenylcarbamoyl group connected to the piperazine ring through an ethylene group, was a highly active herbicide against both wheat and cucumber at 1000 μM. It showed very high selective herbicidal activity at 100 μM against wheat (RHE = 529), which exceeded that of chlorsulfuron (RHE=397 at 1000 μM). This group of compounds also produced a strong stimulating effect mostly on the wheat root growth at concentrations of 10^{-1} μM. However, this stimulating effect of compounds 9-12 did not correlate with their cytokinin-like activity. All compounds inhibited the pigment synthesis (7-24%) within the whole concentration range tested. Compound 10 was more active, showing ~ 40% inhibition at concentrations of 1 and 0.1 μM.

In general, our results showed that ureas in which the ethylene group was missing (compounds 1-4) exhibited relatively good cytokinin-like activity. Thioureas without the ethylene group possessing 4-chloro-and bromo-phenyl substituents (compounds 7, 8) manifested highly selective herbicidal activity against wheat. Among the ureas and thioureas possessing an ethylene group, compound 10 exhibited the highest herbicidal activity against *Triticum aestivum*. Whereas the presence of an ethylene group determined a different type of activity of the fluorinated urea derivatives 3 and 10, it did not affect any of the activities of the fluorinated thiourea derivatives 6 and 12. Thus, our results

complement the existing information about the structure-activity relationships of aryl-ureas and thioureas containing piperazine or 1-ethyl-piperazine rings.

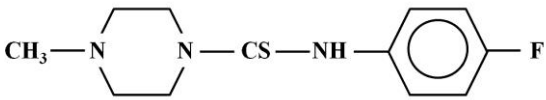
II. Protective Effect against Herbicides (Herbicide Antidotes)

A major problem associated with the use of herbicides for a long time is the emergence of herbicide-resistant weeds [61]. Consequently, there is a continuous need for development of new products with new modes of action. To stop the need for creation of an ever increasing number of chemicals, there are two alternative approaches: 1) to increase the herbicide selectivity, or 2) to increase the herbicide tolerance of sensitive crops. The latter may be achieved by using appropriate herbicide protectors (antidotes). Up to now, limited evidence has been reported on the effect of antidotes on the herbicidal activity of chlorsulfuron (CS) and paraquat (PQ).

We investigated the protective effect of two new compounds, urea and thiourea derivatives of 1-methylpiperazine (compounds 3 and 6), applied as pre-treatment against chlorsulfuron (10 μM) injury in the sensitive plant maize (*Zea mays* L.) and determined their effects (alone or in combination with the herbicide) on some parameters of the growth, the antioxidant system and the activity of acetolactate synthase and glutathione S-transferase [62, 63, 64]. The mode of action of chlorsulfuron appears to block the activity of acetolactate synthase (ALS, EC 4.1.3.18), a key enzyme in plants needed in the biosynthesis of the branched amino acids isoleucine, leucine and valine. The enzymatic conjugation of herbicide with glutathione and consequently its detoxification is mediated by glutathione S-transferases (GSTs, EC 2.5.1.18). These enzymes have the potential to decrease and/or eliminate the cytotoxic or genotoxic effects of compounds that can damage DNA, RNA and proteins.

To the best of our knowledge, we demonstrated for the first time the antidote activity of both the urea and thiourea derivatives of 1-methylpiperazine

(compounds 3 and 6) against chlorsulfuron in maize. It was demonstrated that the thiourea derivative showed higher protective effect against CS than the urea derivative at a concentration of 500 μM and IT=0 h (inter-treatment time). The amount of free amino acids in the combined variant was similar to that in the variant with the protector alone (38%), while this amount in the variant with chlorsulfuron alone was 63% over the control. Treatments with the herbicide and compounds 3 / 6 alone or in combination had no significant effect on the parameters of the antioxidative defense system tested.



FTMP

[Formula 1.12]

The antidote effect of the synthetic compound 6 (FTMP) (500 μM) against chlorsulfuron in maize plants was compared to the effect of the commercial herbicide safener 1,8-naphthalic anhydride (NA) (500 μM). Our results showed that both tested compounds manifested a specific effect on the changes in growth, in ALS and GST activities. The specificity of the action on ALS was supported by the lack of effect on the enzyme activity *in vitro*, i.e. we could suggested that the defense action of both compounds does not involve an effect on the enzyme synthesis. However, pre-treatment of seeds with both protectors overcame the chlorsulfuron-induced inhibition of ALS activity in leaves and roots, 8 and 12 days after treatment. The pre-treatment of seeds with NA and FTMP and following treatment with chlorsulfuron moderately increased the GST (CDNB) activity only in roots on the 8th day but the effect of FTMP was higher than that of NA on the 12^{th} day after the treatment.

We can conclude that FTMP was a more effective protector against CS injury in the roots, whereas NA, in aerial part of the maize plants.

The protective effect of FTMP was proved, to the best of our knowledge, for the first time against the herbicide paraquat in young barley (*Hordeum vulgare* L.) plants as well. Seeds with growing root meristems were used as an experimental material. Treatment with FTMP (5, 50, 500 and 1000 μM) prior to paraquat (10 μM) application was performed. Pre-treatment with FTMP reduced the inhibiting effect of PQ on the growth of shoots to 20% and of roots to 8.0% in 9-day-old barley plants, increased the chlorophyll content in leaves, decreased the level of oxidative stress (H_2O_2 and MDA) more greatly in leaves (except for 1000 μM) than in roots (only at 5 μM) compared to treatment with PQ only. A stimulation of both ascorbate-and guaiacol-peroxidases in the roots of plants pre-treated with PQ/FTMP along with an increase in the level of lipid peroxidation was found. The protective effect of FTMP against paraquat depended on the applied concentration. Among the four tested concentrations, the one that gave the best effect was 5 μM: it completely eliminated the paraquat-induced oxidative damages in leaves and roots of barley plants [65]. We suggest that the protective effect of FTMP against paraquat was mediated by the increased activities of antioxidant enzymes in the roots as an initial defense against PQ.

It is noteworthy that the tested compound FTMP may display antioxidant properties due to conversion of the thiourea [-N-C(=S)-NH-] group into an isothiourea group [-N-C(-SH)-N-]. Thus, we propose that FTMP contributes to the decrease in the oxidative potential in leaves and roots of PQ/FTMP pre-treated plants as a reducing agent.

In general, we showed that the newly synthesized compound FTMP is highly effective as a herbicide protector against chlorsulfuron in maize and against paraquat in barley plants. On the basis of the results obtained by us, it is difficult to explain the exact mechanism of the protective action of FTMP

against the herbicide-induced damage. Probably, the protective mechanism of FTMP depended on the mode of action of each herbicide.

III. Anti-cytotoxic and Anti-genotoxic Effects

The stress-induced cytotoxic and genotoxic effects in living cells continuously exposed to the action of mutagenic factors are mainly caused by the increased level of oxidative stress, leading to instability of the genome. The activity of the antioxidant defense machinery is often insufficient to neutralize the overproduced reactive oxygen species [66]. One of the modern approaches for reduction of the mutagenic burden in cells is the application of natural and synthetic compounds that have protective and anti-mutagenic potential.

In view of genome protection, we investigated the potential of some of our (thio)urea derivatives to mitigate the genotoxicity of "radiomimetics" (paraquat), UV-C and gamma-radiation.

The radio-protective effect of 1-(phenylthiocarbamoyl)-4-methylpiperazine (compdound 5) was demonstrated in oat plants. Post-radiation (6 Gy) treatment of oat plants during the vegetation period with this compound (at 1000 μM) modified the damaging effect of gamma-irradiation upon oat plants, which resulted in increased plant productivity (by 13%).

The radio-protective effect of the other investigated compound, 3-CP-4MPU, was even higher (30% increased yield) in oat plants [57].

The ability of 1-(4-fluorophenylthiocarbamoyl)-4-methylpiperazine (FPTU) to reduce PQ-induced oxidative stress in *Hordeum vulgare* and human lymphocytes *in vitro* was proved by endpoints for cytoxicity (mitotic index, MI) and genotoxicity (induction of chromosome aberrations and micronuclei) [67]. The DNA protective potential of FTMP was manifested by decreasing both chromosome aberrations (MwA) and micronuclei (MN) in barley and human

lymphocytes (Figure 2). The obtained results are specific and they depend on the experimental test-system. The mitotic activity was not significantly influenced in barley, whereas in lymphocyte cultures *in vitro*, conditioning treatment with two different non-toxic FTMP concentrations prior to PQ challenge treatment significantly enhanced the mitotic activity ($p < 0.001$) (data are not shown).

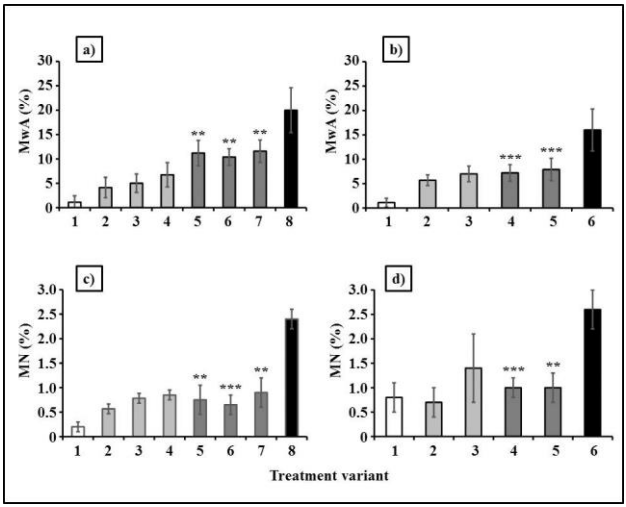


Figure 2. *Protective effect of FTMP against PQ applied as conditioning treatment at non-toxic concentrations, prior to experimental mutagen, assessed as induction of chromosome aberrations and micronuclei in barley root tip meristem cells (a, c) and in human lymphocytes in vitro (b, d). ** $p < 0.01$; *** $p < 0.001$*

1- Control
2- FTMP 10^{-6} M
3- FTMP 10^{-5} M
4- FTMP 10^{-4} M
5- FTMP 10^{-6} M - 4h IT - PQ 10^{-4} M
6- FTMP 10^{-5} M - 4h IT - PQ 10^{-4} M
7- FTMP 10^{-4} M - 4h IT - PQ 10^{-4} M
8- PQ 10^{-4} M

1- Control
2- FTMP 10^{-6} M
3- FTMP $5x10^{-6}$ M
4- FTMP 10^{-6} M - 4h IT - PQ 10^{-4} M
5- FTMP $5x10^{-6}$ M - 4h IT - PQ 10^{-4} M
6- PQ 10^{-4} M

In *Hordeum vulgare* root tip meristem cells, FTMP conditioning treatment prior to PQ resulted in a significantly decreased ($p < 0.01$) frequency of

chromosome aberrations in all effective concentrations of FTMP compared to the PQ-induced chromosome alterations (Figure 2a). Lymphocyte cultures were found to be more sensitive to FTMP than barley. Here, conditioning with FTMP prior to the PQ challenge resulted in a significant decrease ($p < 0.001$) in the structural chromosome disturbances, which were approximately three-fold lower compared to those induced by PQ alone (Figure 2b).

The other endpoint in our investigations, induced micronuclei, also revealed similarities in the trends for all exposure concentrations in both test-systems used in the study. In barley, conditioning treatment with FTMP and inter-treatment time of 4 h significantly reduced the yield of MN compared to the single PQ treatment ($p < 0.01$) (Figure 2c). The MN rate was significantly reduced in cultured lymphocytes when FTMP was applied in effective concentrations with an inter-treatment time of 4 h ($p < 0.001$), compared to the PQ treatment alone (Figure 2d).

The protective potential of FTMP was also examined against UV-C radiation. UV-C (200-280 nm), a component of sunlight, shows strong cytotoxic and genotoxic effects after irradiation in mammalian cells [68]. It induces photodimers in DNA, which, if not repaired, might cause DNA damage leading to skin photoaging and photocarcinogenesis [69]. FTMP applied to human lymphocytes *in vitro* as a conditioning treatment before UV-C (100-150 J/m^2) decreased slightly, but significantly, the harmful UV-C effect ($p < 0.05$) in the variants with 4-hour inter-treatment time, as assessed by mitotic activity and chromosome aberrations (Figure 3a, b). All these results demonstrate that the thiourea synthetic compound FTMP provide genomic protection against the oxidative stress inducer PQ as well as against UV-C radiation.

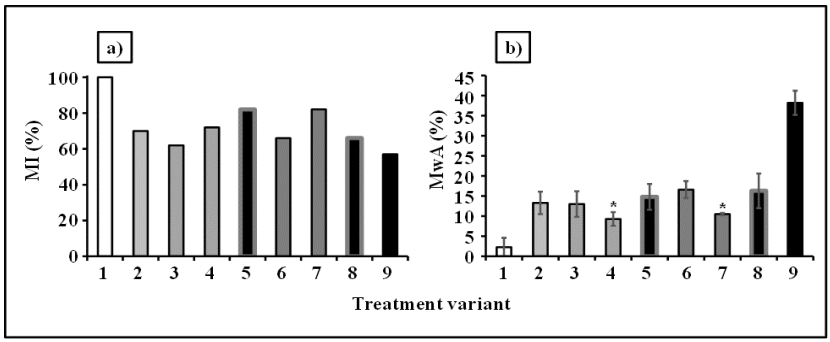


Figure 3. *Effect of FTMP conditioning treatment prior to UV-C exposure on mitotic activity (a) and chromosome aberration induction (b) in cultured human lymphocytes. * $p < 0.05$.*

1- Control
2- FTMP 10^{-6} M
3- FTMP 10^{-6} M - 1½h IT - UV-C 100 J/m^2
4- FTMP 10^{-6} M - 4h IT - UV-C 100 J/m^2
5- UV-C 100 J/m^2
6- FTMP 10^{-6} M - 1½h IT - UV-C 150 J/m^2
7- FTMP 10^{-6} M - 4h IT - UV-C 150 J/m^2
8- UV-C 150 J/m^2
9- MNNG 10^{-5} M

1.3 Conclusions

The novel effective ureas and thioureas containing bioactive heterocycles showed antisenescence effect, antioxidant and anti-genotoxic potential against various environmental genotoxins and stress factors.

The search for alternative protectants which would be safe is a rather urgent task. Such protectants could possibly be based on natural substances of plant origin. The structure of the series of synthetic compounds presented in this review includes structural units/groups from the natural phenylurea-type cytokinin N, N'-diphenylurea (DPU) and different N-containing heterocyclic compounds. The protective effect of our substances on the described plant species and stress factors was demonstrated in experimental studies on pre-treated seeds or vegetative propagules. This is technique, which does not have any impact on the environment, is highly effective and less costly than

post-treatment application. Besides the protective effects, the discussed substances also have other beneficial effects on sensitive crops, i.e. stimulate growth and development, enhance stress tolerance and have low toxicity to plants and human lymphocytes *in vitro*. It may be concluded that these synthetic compounds could be used for plant protection as well as for enhancement of plant productivity because they are considerably less expensive and toxic.

Thus, we developed new classes of agrochemicals that are promising and could be recommended for agricultural application as active ingredients of: 1) effective plant-growth regulators and pesticides; 2) means and ways for crop protection and creation of mutants with valuable agricultural qualities; 3) protocols for micropropagation of flower and woody species that are difficult to propagate.

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