



ANTIDOTES FOR REDUCTION OF PHYTOTOXICITY OF THE RESIDUES OF SULFONYLUREA HERBICIDES

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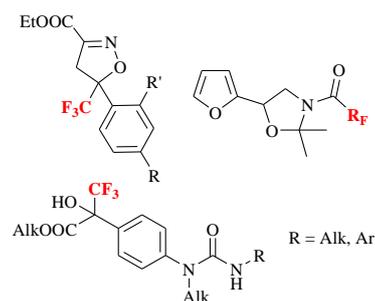
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Abstract

The present review considers the relationships between herbicides and antidotes (safeners, plant resistance inducers) in the context of the problem of a reduction in the crop yield loss associated with the residues of phytotoxic herbicides in soil. The main characteristics of sulfonylurea herbicides are outlined, which allows for the definition of the reasons for negative effects, arising upon their application, and evaluation of the possibility of their combined use with antidotes. The results of recent investigations on the functioning mechanisms of antidotes are summarized. The reports on syntheses of new efficient agents for crop protection from the sulfonylurea residues are discussed, and future outlook for the development of this field is provided.

Key words: herbicides, sulfonylureas, phytotoxicity, antidotes, safeners, organofluorine compounds, complex seed protectants.



antidotes to sulfonylurea herbicides

1. Introduction

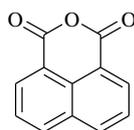
Modern industrial agriculture implies the extensive use of chemical crop protection agents. The global market of agrochemicals amounts 60 billion dollars annually and continues to grow. The largest market share is occupied by herbicides: 40–60% depending on the prevalence of different cultures in different parts of the world. Recent years have seen considerable growth of sales of biological crop protection products; however, their share comprises only 3–4% relative to that of chemical crop protection agents, and most frequently they are used in combination with chemicals [1, 2]. Hence, it is obvious that food production for the general public is connected with the wide use of crop protection chemicals and nowadays there is no alternative to this. Besides economic reasons, the necessity of application of herbicides is directly related to the social demands on reduction of hard unskilled manual labor. Among the factors that negatively affect the crop yield, in our country weeds head the list. Herbicides are the only real tool for protection from weeds at an industrial scale. However, it should be noted that herbicides do not display specificity towards weeds. Therefore, there is a problem of improving the selectivity of their action. One of the ways to reach this goal is to protect cultivated plants from the toxic effect of herbicides. Herbicide residues in soil can cause irreparable damages to the plant which will be grown on this field next year and, in some cases, even later. This limitation can be overcome by reducing the concentration of phytotoxic herbicide residues in soil (intensification of decomposition, sorption) and by application of antidotes

(safeners, resistance inducers) which can remove or reduce the toxic effect of a herbicide on a cultivated plant [3, 4]. In recent years, the problem of soil pollution with the residues of highly active herbicides has reached menacing proportions. The problem gets worse, since the yield depression occurs unnoticeably, without showing its real cause. We can cope with this situation by improving the culture of the application of herbicides and by using special techniques to protect crops from herbicide residues in soil. One of the key elements in these technologies is the application of antidotes that protect the crops from the effect of phytotoxicants. The functioning mechanisms of antidotes have been unknown until recently and their elucidation is connected with the recent advances in molecular biology. We believe that to date we have accumulated enough material to present a complex analysis of the problem of relationships between antidotes and popular herbicides such as sulfonylureas to a wide range of specialists in agrochemistry. In addition, the results of our own investigations devoted to the creation of new efficient antidotes to sulfonylureas are discussed.

2. Antidotes to herbicides: creation history, functioning mechanisms, and application scope

The effect of plant protection from herbicides was observed for the first time by Otto Hoffman in 1947 who found that the tomato plants treated with 2,4,6-T (2,4,6-trichlorophenoxyacetic acid) acquired resistance to herbicide 2,4-D (2,4-

dichlorophenoxyacetic acid) [5]. Further investigations showed that the processing of leaves with 2,4-D protected the plants during further treatment with a herbicide of a carbamate series, namely, barban [6]. Unfortunately, this effect cannot be used in practice, since the treatment of seeds with 2,4-D led to plant death, while crop spraying afforded a reduction in the herbicide activity towards target weeds [7, 8]. At the same time, understanding the problem promoted target search for the compounds with antidotal activities. As a result of the screening of chemical compounds, 1,8-naphthalic anhydride (NA) was patented in 1971 as means for corn protection from thiocarbamate herbicides during presowing treatment (Scheme 1) [9]. NA did not enter the market, since it was displaced by the more recent antidote dichlorimid (dichloroacetic acid diallylamide), which application in a mixture with herbicides upon plant spraying appeared to be more effective from the economic point of view [10, 11]. Another reason is some phytotoxicity of NA, which is manifested upon the storage of the processed seeds [12, 13]. Nevertheless, the discovery of NA promoted an intensive search for the compounds that can improve the selectivity of herbicides. As a result, such preparations were found. Interestingly, they referred to different classes of chemical compounds that do not have structural similarities. Generally, the compounds were selected that protect cereal crop cultures: corn, rice, and sorghum. Whereas at the first step the preparations assigned for presowing treatment were preferred, later the priority was given to the compounds suitable for combined application for spraying of plants with highly active herbicides, such as aryloxyphenoxypropionates, sulfonyleureas, imidazolinones, isoxazolinones, cyclohexanediones, and isoxazolidinones [11, 13–15]. It is important to note that the same antidotes can protect from herbicides with different mechanisms of action. Nowadays, it is generally accepted that antidotes activate the expression of genes which are responsible for the protection from xenobiotics, *i.e.*, organic compounds foreign to biological organisms. As a rule, plants feature three phases of detoxification from herbicides: the first one (I) is the oxidative dehydration of the initial molecule followed by the conjugation with endogenous substrates at the second stage (phase II). In some cases, the initial molecule can pass phase I and immediately enter the second one. During the third phase (III), the resulting metabolites can undergo further conjugation, affording insoluble residues, which then are isolated in vacuoles or associate into lignin biopolymers [16].



1,8-naphthalic anhydride (NA)

Scheme 1

The reactions that occur during phase I are aimed to form the reactive functional groups in the structure of a xenobiotic, which can undergo further metabolism and detoxification. Most of the reactions are catalyzed by cytochrome P450-dependent monooxygenases, which are used for oxidation of molecular oxygen. The most popular cytochrome P450-dependent

reactions are hydroxylations of aromatic cores or alkyl groups, including oxidation of the carbon atoms attached to the oxygen or nitrogen ones, which eventually lead to dealkylation. It is natural to assume that the same enzymes can be used for the normal metabolism of plants. Indeed, there are about 50 similar oxidizing systems that are utilized by plants for the metabolism of styrene, terpenes, isoflavonoids, gibberellins, abscisic acid, cytokinins, and lignin. Some reactions of phase I are catalyzed by peroxidases, which utilize hydrogen peroxide for oxidation. For example, this route is used to oxidize indoleacetic acid. Furthermore, the important processes of the first stage are reduction and hydrolysis by carboxylesterases [16]. The functionalization of a xenobiotic allows for passing to phase II—the conjugation reactions, during which the created functional group is used for conjugation with other molecules, such as monosaccharides or glutathione (GSH) tripeptide, the most important thiol in a cell. The glycosylation is accomplished by uridine diphosphate-dependent glycosyltransferases (UDP-dependent glycosyltransferases, UGTs). This family of enzymes plays a key role in the biosynthesis of plant hormones and detoxification of toxins produced by pathogens. The most studied group of enzymes, which are involved in the metabolism of herbicides, are glutathione-S-transferases (GSTs). They catalyze the conjugation of the thiol with an electrophilic site of a substrate. The catalytic mechanism of these transformations includes the substitution of the halogen atom of a phenol group of the substrate by the thiolate anion. The resulting conjugates are transported into vacuoles (phase III) by the so-called multidrug resistance-associated proteins (MRPs), which include a subfamily of ABC transporters; the transport function of the latter has been studied recently [17]. The transformations of the conjugates involve reactions such as partial decomposition, secondary conjugation, and incorporation into a cell wall (phase IV). The presented scheme of detoxification of xenobiotics in plants describes only the main and most explored processes. The less popular biotransformations of herbicides include the oxidation of the nitrogen and sulfur atoms, the reduction of the nitro group, aldehydes, ketones, the reduction of unsaturated carbonyl compounds, as well as different isomerizations [16, 18]. Nowadays it is obvious that safeners activate the enzymatic systems which are responsible for the detoxification and protection of plants, such as P450s, UGTs, GSTs, ABC transporters. At the same time, they do not display significant toxicity. The activation is accomplished through an increase in the expression of genes which are included in herbicide metabolism. The understanding of the occurring processes is facilitated by intensive studies on the identification of genomes of the most important agricultural plants, which appeared since full genetic sequencing (establishing the whole linear sequence of a genome nucleotides) of *Arabidopsis thaliana* [19]. Another important trend in modern molecular biology studies is the elucidation of signaling mechanisms that cause an increase in the expression of these genes. Over recent years, considerable experimental materials have been accumulated to confirm the hypothesis about the connection to the signaling system in plants which controls the adaptation to changing biotic and abiotic factors. This protecting system is formed by the derivatives of oxidized fatty acids, oxylipins, which are involved in the processes of growth and development of all aerobic organisms, as well as into the formation of responses of

organisms to the environmental signals. Oxylinins are formed enzymatically or spontaneously from unsaturated fatty acids. In plants, the substrates for the formation of oxylinins are 16 and 18 carbon unsaturated fatty acids. The routes for biosynthesis of oxylinins are rather simple, but provide the formation of diverse biologically active compounds. Oxylinins take part in the transfer of stress signals, control the expression of stress-induced genes, as well as interact with multiple signaling ways in plant cells, including signal pathways of hormones—auxin, gibberellin, ethylene, and abscisic acid [16, 19]. Among all the oxylinins, a special group is formed by the so-called reactive electrophile species (RESs), which include α,β -unsaturated carbonyl compounds, such as 2(E)-alkenals, ketodienes, and ketotrienes formed from fatty acids. RESs attack nucleophilic sites of organic molecules, such as glutathione, proteins, and nucleic acids, changing their molecules and often leading to the damages in cell structures. At the same time, the role of these compounds in a cell is not restricted by destructive effects; RESs induce the expression of genes which are involved in regulation of protecting reactions of plants to the excess irradiation and the presence of xenobiotics, as well as the expression of genes that control a cell cycle [19, 20].

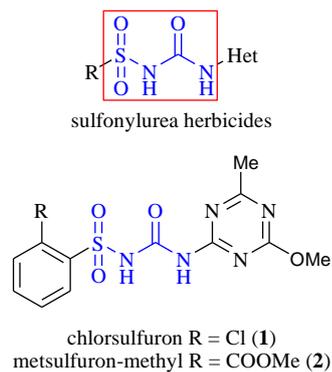
Turning to the problems of the practical application of antidotes, it should be mentioned that the potential of a herbicide–antidote pair is enormous. These combinations can be used for the following purposes [11]:

- crop protection from a damaging level of pesticide residues in soil, which allows for considerable flexibility in the selection of cultivated plants during crop rotation;
- application of herbicides with the preferred toxicological profile but not exhibiting high selectivity;
- control over weeds botanically related to the cultivated plant;
- protection from minor agricultural plants for which the development of selective herbicides is economically unreasonable.

The current review will focus on the first aspect of the mentioned application scope. Of particular importance as phytotoxicants are herbicides that display high activity and persistence, *i.e.*, the ability to remain in soil for a long time. This combination of the properties determines negative consequences, *i.e.*, suppression of crops by herbicide residues, which are planted in a year or even later after the use of the herbicide.

3. Sulfonylureas: structures, properties, and the problem of residues in soil

The highest biological activity among herbicides is characteristic for sulfonylureas. These compounds have a sulfonylurea bridge between a substituted azine (*sym*-triazine, pyrimidine) and an *ortho*-substituted aryl or hetaryl residue. The first representative of this class of herbicides was chlorsulfuron **1**, introduced by DuPont in 1982 (Scheme 2). Nowadays, there are about 30 registered active substances of this series, which are used in more than 80 countries for weeding of the main cultivated plants [21]. In Russia, the major preparations of this class are registered and widely used [22]. Sulfonylureas are used in extremely low doses (10–100 g/ha), providing high selectivity



Scheme 2

of action and safety for humans and animals. This is connected with the fact that the mechanism of phytotoxic effects of sulfonylureas consists in the inhibition of acetolactate synthase (ALS, also called acetohydroxy acid synthase AHAS, EC 2.2.1.6), the first enzyme on the synthetic pathways of branched-chain amino acids (valine, leucine, and isoleucine). The inhibition of this enzyme terminates protein synthesis, which leads to the arrest of cell proliferation and the following death of an organism. This metabolic pathway exists only in fungi, bacteria, algae, and higher plants, which provides for the low toxicity for other organisms, including homeotherms [23].

Despite very favorable toxicological characteristics, the extremely high phytotoxicity of sulfonylureas towards many cultivated plants poses a potential hazard. The most important agricultural plants, such as cereal crops (wheat, rye, barley, and oat) are relatively stable to sulfonylureas, but there is a problem with plants that follow cereal crops upon rotation. In this context, of particular hazard are chlorsulfuron **1** and metsulfuron-methyl **2** which are widely used in agriculture (Scheme 2). The residual amounts of these herbicides can damage a range of crops that follow the cereals: sugar beet, soy, rape, corn, *etc.* [24, 25]. There are numerous examples of phytotoxic effects of sulfonylurea residues in soil. Thus, for example, the yields of flax, sugar beet, lentil, pea, and potato, obtained in a year after treatment of the area with chlorsulfuron in the dose of 10–40 g/ha, decreased in 2–9 times. The residues of metsulfuron-methyl in soil in a year after the use in the concentration of 4.5 g/ha reduced the yield of medick by 38% and that of potato by 28% [25]. There are a multitude of similar examples; however, it is obvious that the quantitative measures of crop losses of different plants depend on the persistence of sulfonylureas under different conditions.

The decomposition of sulfonylureas in soil proceeds as a result of hydrolysis, photolysis under exposure to light, and microbiological transformation [26]. It was reported that the main decomposition route of metsulfuron-methyl **2** in soil at pH below 6 is the chemical hydrolysis. At the higher values of pH, the microbiological degradation prevails. Weakly acidic conditions (pH 4.0–6.0) promote the hydrolysis of carbamide bonds in sulfonylureas with a relatively high rate. At the same time, the herbicides lose their phytotoxicity. Thus, the sulfonylurea residues are of certain hazard for seeds in neutral and alkali soils [27]. The decomposition rate is affected also by the soil characteristics such as humidity, temperature, and content of organic substances [28, 29]. Under conditions

unfavorable for the decomposition of sulfonylureas (alkali soil reaction, low temperatures, and insufficient humidity), the consequences of the use of chlorsulfuron **1** and metsulfuron-methyl **2** can be observed in 2 and even 3 years after their use [25]. The residues of chlorsulfuron and metsulfuron-methyl in soil even in the lower doses of 0.2 g/ha can negatively affect the growth and development of the plants such as sugar beet, buckwheat, and mustard [22]. A popular characteristic of the herbicide persistence is its half lifetime in soil [4, 30]. In the case of sulfonylureas, this parameter can lead to misinterpretation of the real hazard of negative consequences of the herbicide, since it was shown experimentally that the first 50% activity of the herbicide is lost rather rapidly, while remaining 50% can be retained 5–10 times longer [22].

4. Progress in creation of antidote preparations for seed protection from the sulfonylurea residues in soil

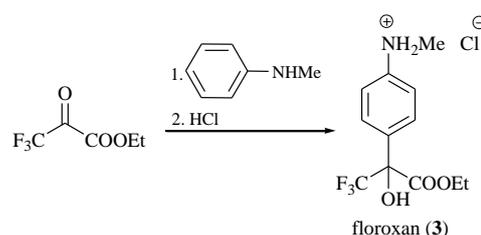
The protection of seeds from the sulfonylurea residues in soil is undoubtedly an urgent task. Due to the relatively low farming standards, especially towards the use of plant protection agents, in our country this problem is of special significance. The good results for the solution of this problem can be achieved by presowing treatment of seeds with antidotes. The available information indicates that the highest effect in this approach is demonstrated by 1,8-naphthalic anhydride (NA). The incrustation of seeds of corn, wheat, rice, or sorghum with naphthalic anhydride in the dose of 0.5 wt % relative to the seeds considerably enhanced the resistance of all the plants to chlorsulfuron **1**. For example, chlorsulfuron **1** in the concentration of 5 g/ha decreased a dry mass of corn shoots up to 15% compared to the untreated control. In the case when seeds were preliminarily treated with NA, the mass of shoots reduced only to 80% [31]. Chlorsulfuron **1** in the dose of 5 g/ha suppressed the growth of corn roots and shoots by 50–60%, but in the case when the seeds were preliminarily treated with NA, the same toxic effect required the use of chlorsulfuron dose of 40 g/ha, *i.e.*, ten times as high dose. This value is called a protective factor. Upon treatment of corn, sorghum, and rice with chlorsulfuron **1**, the protective factor provided by NA ranges from 4 to 8. In the case of wheat and barley, which are much more stable to the action of chlorsulfuron **1**, this value composes 2–4. The toxic dose of chlorsulfuron **1** for these crops increases up to 100–400 g/ha [32]. The high efficiency of NA for protection of corn from chlorsulfuron **1** was confirmed under different conditions in Moscow Oblast and Primorskii Krai (Moscow and Primorskii regions). It was shown that the expenditure rate of NA upon seed treatment can be essentially lower than the previously used concentration [33]. The mechanism of protective action of NA is connected with the induction of specific cytochrome P450-dependent monooxygenases, which catalyze the hydroxylation of the herbicide substrate, and with an increase in the activity of glutathione-S-transferases [25, 34, 35, 36].

A leading group of product engineers in the field of agrochemical preparations in USSR developed for the first time a series of combined NA-containing protectants for seeds of corn, flax, sugar beet, soy, and rape, which are broadly referred

to as koprang (complex protectant and antidote to herbicides). A combination of a protectant, an insecticide, and an antidote in a single preparation allows for the complex protection of plants from fungal diseases, soil-inhabiting pests, and damages from the residues of chlorsulfuron **1** in soil. An optimal method for the application of these preparations is the presowing incrustation of seeds of cultivated plants, which allows one to include an antidote into a unified system of seed treatment [25, 33]. A series of these preparations were registered by the State Committee of the Russian Federation; however, insufficient financial support hampered their implementation into manufacturing practice [33].

Our studies devoted to the creation of compositions for presowing treatment of seeds were concerned with NA as a reference, since up to date there is no data on the industrial preparations which excel NA as antidotes to sulfonylureas for presowing processing. Our original approach to the production of these preparations is based on the use of a plant growth regulator synthesized in the Laboratory of Physiologically Active Organofluorine Compounds at INEOS RAS, which received a commercial name of floroan and represents water-soluble 2-(4-methylaminophenyl)-3,3,3-trifluoro-2-hydroxypropionic acid hydrochloride (Scheme 3). This compound exhibits the properties of a plant growth stimulant during both presowing treatment and spraying of vegetative plants. In the dose of only 100 mg/t of seeds, the compound stimulated the growth of cotton, leading to a total increase in the raw cotton yield by 20% [37, 38].

The attempts to establish the mechanism of action of this compound by conventional methods did not afford clear results. At the same time, the analysis of the literature data allows us to make some assumptions. In 2009 Hoffmann-La Roche Inc. issued a patent devoted to the control and prevention of central nervous system diseases, which protects the derivatives of 2-phenyl-3,3,3-trifluoro-2-hydroxypropionic acid as the compounds that interact with GABA_B receptors [39]. Hence, floroan **3**, containing the methylamino group in the phenyl core, is a compound which falls within the scope of this patent and is very likely to interact with GABA_B receptors. The hydrophilicity of floroan **3**, which successfully passed the toxicological tests, must hamper its action on the brain tissue of humans and vertebrates, which are protected by a blood-brain barrier. At the same time, recently it was shown that GABA_B is at the very beginning of a chain that causes the plant reaction to stress [40]. This implies that floroan **3** affects the growth and development of plants through the signaling system which regulates the metabolism of plant hormones and may participate in the transfer of signals regulating the expression of stress-induced genes. The confirmation of this assumption requires serious experimental studies. An argument for this work is the



Scheme 3

fact that a series of ureas derived from floroan and related compounds displayed high antidotal activity against sulfonylureas upon application in extremely low doses. Thus, for example, chlorine-substituted diarylurea **4**, obtained from floroan **3** (Scheme 4), effectively reduced the toxic effect of industrial herbicide metsulfuron-methyl **2** in soil upon treatment of seeds of spring rape already in the dose of 1g/t. An increment of the plant herbage composed 40% relative to the control. NA in the same dose did not provide significant protective action [41, 42]. This result is the first example of the effective protection of rape from sulfonylurea herbicides during presowing treatment in such a low preparation dose. The high protective efficiency of diarylurea **5** from chlorsulfuron was observed for flax [43].

The antidotal activities were also detected in the case of ureas **6** and **7** (Scheme 5) [44, 45].

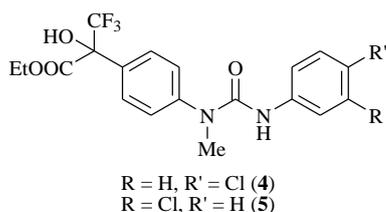
We continued also the development of complex seed protectants that exhibit the antidotal activities (koprans) [25, 33]. The initial multicomponent composition included tebuconazole (TBC) and tetramethylthiuram disulfide (TMTD) as fungicides, NA as an antidote, a sodium salt of carboxymethyl cellulose (Na-CMC) as an adhesive with the addition of form-building components (surfactant, emulsifier). The homogeneous suspensions were obtained in a ball mill [46]. The resulting preparations promoted the germinating capacity of seeds of various cultivated plants (spring wheat, spring rape, and corn) and stimulated their growth. The presowing treatment of rape seeds allowed for overcoming 30% inhibition of the plant development by soil-contained metsulfuron-methyl **2** [47, 48]. Further progress was connected with the investigation of the possibility of substitution of NA for another efficient antidote. This demand was caused by the above-mentioned intrinsic phytotoxicity of NA, which is manifested upon the storage of seeds [12, 13]. Furthermore, modern agrochemical preparations are used in the essentially lower doses than in the case of NA,

since a reduction in the expenditure rate of the active chemical compounds decreases the environmental impact on agrobiocenosis.

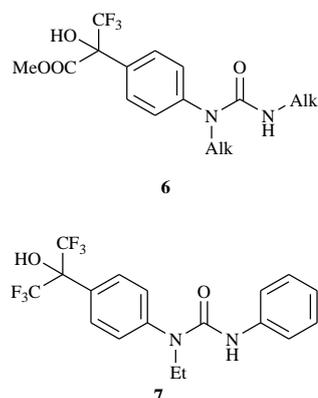
As a substitute for NA in the composition of the complex protectant with the antidotal activity, a representative of *N*-dichloroacetyl-1,3-oxazolidines, namely, industrial antidote furylazole

(*R,S*-3-dichloroacetyl-5-(2-furyl)-2,2-dimethyloxazolidine **8**) was tested (Scheme 6). This antidote

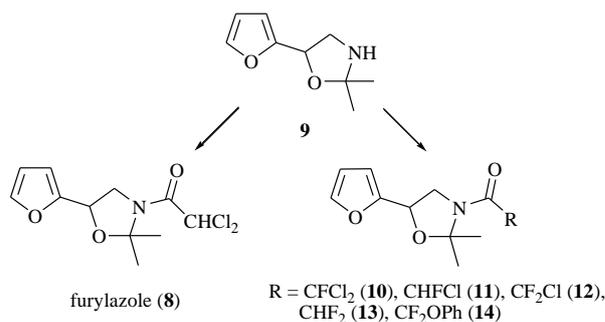
was developed by Monsanto. The preparation is used as a mixture with highly active herbicides, such as sulfonylureas and imidazolinones upon spraying of corn crops. There were no literature data on the presowing treatment of seeds with this antidote. The preliminary tests were carried out *in vitro* in Petri dishes with corn shoots. During germination of the seeds treated with furylazole **8** in the concentrations of 100 mg/t and 1 g/t of seeds, there was observed a considerable increase (up to 45%) of the lengths of roots and shoots. In the dose of 25 g/t, furylazole displays a marked antidotal activity relative to chlorsulfuron **1**. Upon inhibition of the root growth by the herbicide more than by 50%, the preparation not only removes this effect but also stimulates the growth of roots approximately by the same value [50]. Further tests were carried out in an artificial climate laboratory (ACL) chamber. To study the effect of furylazole on the protection of corn shoots from the residues of metsulfuron-methyl **2** in soil, the suspension compositions were prepared analogously to NA that involved furylazole instead of NA as an antidote in the doses of 12.5, 25.0, 50.0 and 100.0 g/t of seeds. A standard composition containing 50.0 g of NA per a tone of seeds was used for comparison. The experiment in which the herbicide, introduced into soil, reduced the mass of untreated three-week plants by 60% is very illustrative. All the methods of presowing treatment with the compositions containing furylazole **8** demonstrated partial protection from the herbicide. The optimal doses were 50–75 g/t of seeds, which provided a reduction in the plant mass of only 20%. A further increase in the antidote content did not lead to the improvement of the protective action and did not lead to the strengthening of the plant growth inhibition. The standard composition containing 50.0 g of NA per a tone of seeds did not exert any protective effect [51]. Hence, furylazole exhibits antidotal action in the much lower concentrations than NA, but without providing full protection of plants from the sulfonylurea effect. We suppose that furylazole **8** can be used in complex technologies for the protection of plants from the residues of phytotoxicants in soil, which we are going to further develop. We also attempted to modify the structure of furylazole **8** and study the biological



Scheme 4



Scheme 5

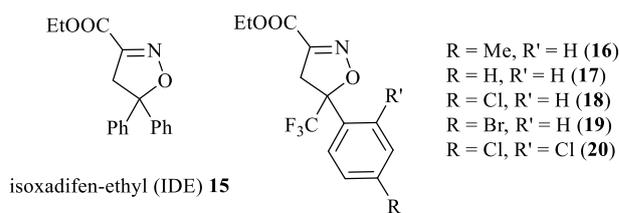


Scheme 6

activities of the resulting compounds. Furylazole **8** refers to one of the main classes of antidotes, which represent the derivatives of dichloroacetic acid amides. Amides of mono- and trichloroacetic acids exhibit lower activities than the dichloro-substituted derivatives [13]. The data on the derivatives of furylazole containing fluoroacetic acid residues were missing. These derivatives seemed to be interesting, first of all, because the introduction of fluorine atoms allows for efficient modification of the biological activities of different compounds [52]. The probability of the appearance of useful properties is so high that nowadays about 40% of pesticides contain fluorine atoms [53]. The synthesis of analogs of furylazole **10–14** was carried out by the acylation of 5-(2-furyl)-2,2-dimethyloxazolidine **9** with anhydrides or chlorides of dichlorofluoroacetic acid (for compound **10**), chlorofluoroacetic acid (for **11**), chlorodifluoroacetic acid (for **12**), difluoroacetic acid (for **13**), and phenoxydifluoroacetic acid (for **14**) in the presence of triethylamine (Scheme 6).

These derivatives were tested for antidotal and growth-regulation activities, which were studied *in vitro* in Petri dishes on corn seeds in the concentrations of 100 mg/t, 1 g/t, and 10 g/t of seeds [50]. The concentrations were chosen so that the use of relatively expensive fluorine-containing analogs of furylazole in agricultural practice can be economically reasonable upon application of these preparations in the essentially lower concentrations than in the case of furylazole. The experiments showed that, at least, in the low concentrations compounds **10–14** did not exhibit antidotal activity towards chlorsulfuron **1**. At the same time, all the fluorine-containing analogs stimulated the growth of both roots and shoots of corn upon treatment of seeds with these compounds. The highest activity was detected in the case of a derivative of chlorodifluoroacetic acid **10**: the processing with this compound in the dose of 1g/t of seeds led to a double increase in the length of roots and almost 50% increase in the length of shoots, which significantly exceeded the growth-stimulating activity of furylazole **8**. Such a marked efficiency of stimulation of plant growth and development can be used in practice in multicomponent compositions for presowing treatment. The demand in stimulation of development of plants is caused mainly by the fact that fungicide compositions, as a rule, lead to some (and often significant) suppression of the plant growth. Floroxan **3** was successfully used in the composition of a fungicide protectant for cereals to overcome this [54–56]. At the same time, in the composition of our basic formulation for presowing treatment, containing TBC and TMTD as fungicides, floroxan **3** does not remove the inhibiting action of fungicides on corn, rape, and soy shoots. Its substitution for the fluorine-containing derivative of furylazole **10** in the concentration of 1 g/t of seeds allows one not only to remove the suppression of plant growth but also to stimulate it in the case of corn and rape, which is expressed in an increase of biomass compared to the untreated control. Hence, 2-chloro-2,2-difluoro-1-[(5-furan-2-yl)-2,2-dimethyloxazolidin-3-yl]ethanone **10** is of paramount interest as a potential component of fungicide compositions [57].

Another test object was industrial safener isoxadifen-ethyl (IDE)—ethyl 5,5-diphenyl-4,5-dihydroisoxazole-3-carboxylate **15** (Scheme 7). This modern safener of Bayer Crop Science production was used in a mixture with herbicides of



Scheme 7

sulfonylurea family for weeding of corn and rice crops. The investigation of IDE **15** in the standard composition, including TBC and TMTD, revealed a moderate antidotal activity towards metsulfuron-methyl **2** on corn and rape shoots. Thus, a reduction in the shoot mass in the tests on corn, which was caused by the presence of the herbicide in soil, was partially compensated from 67% to 37%. The antidote dose composed 100 g/t of seeds. An increase in the dose to 200 g/t did not afford the improvement of the protective effect [58]. Hence, in our assessment, isoxadifen-ethyl was inferior in the activity to furylazole. In order to increase the activity of IDE, we accomplished the synthesis of its fluorine-containing derivatives **16–20** (Scheme 7). One of the phenyl groups was substituted for the CF₃ moiety, and the second one was replaced for the following substituents: CH₃, Cl, or Br. *In vitro* studies in Petri dishes on corn seeds did not reveal the essential antidotal activity [59]. At the same time, IDE also did not display the activity under these conditions.

5. Conclusions

Therefore, the use of antidotes in complex preparations for presowing treatment allows one to overcome the inhibiting action of the residues of sulfonylurea herbicides in soil on plants to a large extent. The antidotes can be both new original compounds and industrial preparations, which are used as mixtures with herbicides for weeding of vegetative plants. A promising antidote is industrial preparation furylazole. This type of advances can be implemented into agriculture even today [60]. The application of these technologies does not require additional agronomical procedures and utilizes low doses of the already used antidotes. For a series of agricultural plants, these antidotal protectants can be used prophylactically. At the same time, the application of antidotes alone is likely not to fully address the problem of the herbicide residues in soil. Significant advances were achieved upon introduction of sorbents into soil, which bind the herbicide residues [61]. The combined use of sorbents and antidotes can solve the problem in complex cases of contamination with high doses of phytotoxicants. The development of new antidotes is important from the practical point of view and will facilitate their use as tools for investigation of the protective mechanisms of plant metabolism [14].

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