Sydnone imines as a new class of promising plant growth modulators and potential drought-protective phytoeffectors – a first experimental structure-activity-analysis overview

Maria Cherevatskaya,[1*†] Ilia Cherepanov,[2] Natalia Kalganova, [2] Andrej Frolov,[3*] Tatiana Bilova,[1] Ludger A. Wessjohann,[3] Sergey Moiseev [2*†]

[1] Department of Plant Physiology and Biochemistry, St. Petersburg State University, St. Petersburg 199034, Russian Federation,

[2] Laboratory for Fine Organic Synthesis, A.N. Nesmeyanov Institute of Organoelement Compounds of Russian Academy of Sciences, ul. Vavilova 28, bldg. 1, Moscow 119334, Russian Federation,

[3] Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Weinberg 3, 06120 Halle/Saale, Germany

†These authors contributed equally to the manuscript

*Corresponding authors:

Dr. Sergey Moiseev
A.N. Nesmeyanov Institute of Organoelement Compounds of Russian Academy of Sciences
Laboratory for Fine Organic Synthesis
ul. Vavilova 28, bldg. 1 Moscow 119334, Russian Federation
Tel. +7 (499) 135 93 14
Email: skm@ineos.ac.ru

Dr. Maria Cherevatskaya
St. Petersburg State University
Department of Plant Physiology and Biochemistry
Universitetskaya emb. 7/9
St. Petersburg 199034, Russian Federation
Tel. +7 981 192 80 84
Email:maria.cherevatskaya@gmail.com

Abstract

Due to the oncoming climate changes, drought is the factor dramatically affecting crop yields and quality of produced foods. Therefore, to meet the growing food demand of the human population, improvement of drought tolerance of the currently cultured crops is required. The knowledge of the molecular mechanisms behind, e.g., drought tolerance provides a versatile instrument to correct the plant metabolism by chemical tools and to increase, thereby, their adaptive potential. This will preserve crop productivity and quality under water deficit conditions. Endogenously produced nitric oxide (NO) is one of the key signaling factors activating drought tolerance mechanisms in plants. Thus, application of synthetic NO donors as drought-protective phytoeffectors might support maintaining plant growth and productivity under dehydration conditions. Sydnone imines (Sydnonimines) are a class of clinically established mesoionic heterocyclic NO donors which represent a promising candidate group for such phytoeffectors. Therefore, here we provide an overview of the current progress in application of sydnone imines as exogenous NO donors in plants with a special emphasis on their potential as herbicides as well as herbicide antidotes, growth stimulants and suggest a deeper look into them as drought protectors, applicable to different plant species. We specifically address the structure-activity relationships in the context of the growth modulating activity of sydnone imines. According to the actual state of the art, growth-stimulating or antidote effects sre typical for 4-(α-hydroxybenzyl) derivatives of sydnone imines containing an alkyl substituent in position N-3. The nature of the substituent of the N-6 atom has a significant influence on the activity profile and the intensity of the effect. Nevertheless, further investigations are necessary to establish reliable structure-activity relationships (SAR). Consequently, sydnone imines might be considered as promising phytoeffector candidates, which are expected to exert either protective effects on plants growing under unfavorable conditions, or herbicidal ones, depending on the exact structure.

Abbreviations

ABA: Abscisic acid; AGE: Advanced glycation end products; ALEs: Advanced lipoxydation end products; APX: Ascorbate peroxidase; CAT: Catalase; cGMP: Cyclic guanosine monophosphate; CK: Cytokinins; Cys: cysteine; ET: Ethylene; GA: Gibberellic acid; GR: Glutathione reductase; GSNO: *S*-Nitrosoglutathione; g/t: grams of compound per ton of seeds (± ppm based on weight); GTP: Guanosine triphosphate; H_2O_2: Hydrogen peroxide; IAA: Indole-acetic-acid; JA: Jasmonic acid;Met: methionine; MDA: Malondialdehyde; NO: Nitric oxide; NOS: Nitric oxide synthase; NR: Nitrate reductase; $ONOO^-$: Peroxynitrite; PAs: Polyamines; PARP: Poly-(ADP-ribose)-polymerase; PTMs: Posttranslational modifications; RNS: Reactive nitrogen species; ROS: Reactive oxygen species; SA: Salicylic acid; SAR: structure-activity-relationship; sGC: Soluble guanylate cyclase; SOD: Superoxide dismutase; StDev: standard deviation; Tyr: Tyrosine; WP: wettable powder.

Key words: drought, drought tolerance, growth modulators, nitric oxide, phytoeffectors, plant protectant, herbicide, sydnone imines

1. Introduction

In nature, plants are often subjected to weak or moderate drought for relatively long periods of time. Due to the oncoming global climate changes, the impact of drought on plants is continuously increasing, and this ultimately results in a decrease of crop productivity. Thereby, drought is usually referred to as a period of below-normal rainfall which leads to deficiency of water available for plants [1]. Obviously, dehydration associated with severe long-term persisting drought cannot be sustained by the organism and ultimately leads to viability loss and death. On the other hand, transient dehydration triggers adaptive stress responses which ensure plant survival during the unfavorable period and help to sustain or regain its productivity [2]. Therefore, development of new strategies to enhance plant tolerance to short- and middle-term water deficit is the central problem of modern agricultural research.

To prevent water losses, plants respond to water deficit by stomata closure, growth retardation and accumulation of osmoprotectors [3]. Despite its vital importance for the plant survival, this adaptive strategy causes clearly adverse effects to plant physiology, which are manifested by earlier entrance into the reproductive phase, formation of low quality seeds and accelerated senescence [4-7]. These events dramatically affect nutritional value of crop plants and need to be avoided in the every day agricultural practice.

The drought-related damage of valuable crop nutrients (proteins, lipids and carbohydrates) is tightly associated with drought stress responses, especially its redox component [8, 9]. Thus, stomata closure results in imbalance in CO_2/O_2 exchange and triggers overproduction of ROS and RNS in photosynthetically active tissues [10]. The accumulation of drought-related sugar osmolytes in parallel to developing oxidative stress might enhance production of advanced glycation end products (AGE)-modified proteins. The protein glycation processes are a known factor of protein damage [11]. Additionally, due to increased production of ROS, lipid peroxidation is stimulated and results in the generation of reactive intermediates (α-dicarbonyls, hydroxyaldehydes and dialdehydes) and in formation of advanced lipoxydation end products [12]. Both glycated proteins and ALEs are known to be toxic due to their pro-inflammatory properties, clearly exposed in mammals [13]. Enhanced RNS generation leads to posttranslational modifications such as oxidation, nitration, nitrosation and metal- and *S*-nitrosylation using so-called nitrosative stress. Indeed, glycation and *S*-nitrosylation of plant proteins were promoted under drought conditions [14, 15]**.** Thus, drought-induced oxidative, carbonyl and nitrosative stresses might have a potential for a relevant impact on nutritional properties of plant proteins, a topic under current research [16-18]. In this context, preserving not only yield but quality of crops is also important aspect which, unfortunately, is often underestimated when designing new strategies to improve the drought tolerance of crop plants.

On the other hand, ROS and RNS when produced at relatively low levels are not considered as toxic but rather act as key signaling molecules that modify various physiological processes and activate different developmental programs. They stimulate the development of adaptive responses and coordinate them at the systemic level for acclimatization of the whole plant to the unfavorable environment [19]. The signaling role of ROS and RNS is accomplished via modification of the oxidative status of the targeted proteins by direct oxidation of Cys and Met residues [19]. The proteins subjected to such oxidation often belong to regulatory enzymes involved in phosphorylation/dephosphorylation-based signal transduction cascades and Ca^{2+} signaling, receptors and transcriptional factors [20]. ROS and RNS might impact on the drought-related signaling via interplay with hormone-dependent pathways [21, 22]. Thus, understanding of the molecular mechanisms underlying the ability of crop plants to withstand drought and to fine-tune stress-related production of ROS and RNS might give access to new cultivars with improved drought tolerance. This could improve survival times of agriculturally valuable crops and would allow sustaining their nutritional properties during and recovery after application of stress conditions.

One straightforward and fast way to achieve the required plant stress tolerance is manipulation of the plant genome. However, this is then limited to a certain crop type or clone, and temporal and spatial control of induction is difficult, also so far such GMOs often have proven less productive. Most importantly, in Europe the practical use of transgenic plants in routine agriculture is strictly regulated by legislation, and requires special approval [23, 24]. Because of these reasons, the alternative approaches for improvement of crop traits, not relying on genetic engineering but chemical intervention have regained importance, but recently have been boosted by exploiting informational resources of functional genetics (metabolomics, proteomics and transcriptomics). Based on the information on a plants transcriptome, proteome and metabolome, enzymes or regulatory proteins critically important for the manifestation of the deleterious effects associated with the drought stress response, can be identified. For these targets, appropriate inhibitors (i.e. phytoeffectors acting as drought-protective agrochemicals) can be selected and synthetically optimized. This strategy, known as the phytoeffector approach, was established among others by our group[25, 26]. In general, this strategy is analogous to the pharmaceutical approach for medicines that modify metabolic processes, e.g. by inhibition of specific enzymes, change in hormone levels and signaling or similar interventions of processes underlying the pathogenesis of human disorders – now transferred to plants.

Therefore, for an adequate targeting of plant drought tolerance, a comprehensive understanding of the underlying molecular mechanisms is absolutely mandatory. The knowledge of these mechanisms will give access to identify enzymes involved in undesired manifestations of the natural drought stress response. Thus, by affecting selected enzymes, the phytoeffectors might suppress the development of oxidative stress and stress-related metabolic adjustment, and reduce thereby deleterious effects of drought on crop productivity. Ideally the drought-protective phytoeffectors can be applied to various crops at the time and constriction in the area required. To date, this approach has been established for PARP inhibitors, which proved to be efficient drought-protective phytoeffectors [25, 26].

Recently, NO was shown to be a potent modulator of drought stress response [27-29]. The critical evaluation of NO release characteristics and some metabolic responses induced by the donors are given in the works of Ederli et al. [28] and Murgia et al. [29] which showed involvement of NO in ferritin regulation in plants. This fact attracted attention to NO donors, for example sodium nitroprusside, as potential drought-protective phytoeffectors.[30] Another novel type of NO-donors of heterocyclic nature such as sydnone imines (sydnonimines) have emerged in recent years as significant alternatives due to their increased hydrolytic stability and low toxicity confirmed in pharmacokinetic studies [31]. Sydnone imines represent a class of mesoionic heterocyclic compounds [32] exhibiting a broad range of biological activities and in this regard they are successfully applicable in medicine (cf. cpds **1-3** in figures below for typical structures of sydnone imines). Recently sydnone imines were addressed as the plant phytoeffectors and reported to be potent growth modulators [33]. As these compounds are prodrugs which after enzymatic activation and rearrangement can act as potent NO donors, and NO is involved in the modulation of drought response, this class of compounds can be considered as a source of prospective stress tolerance-enhancers and drought-protective agents as well. Therefore, in this review, we summarized the research progress related to (*i*) NO signaling role in biological systems, (*ii*) application of sydnone imines in pharma as NO donors, and (*iii*) use of different synthetically available sydnone imine derivatives and their role as herbicides or plant growth stimulators.

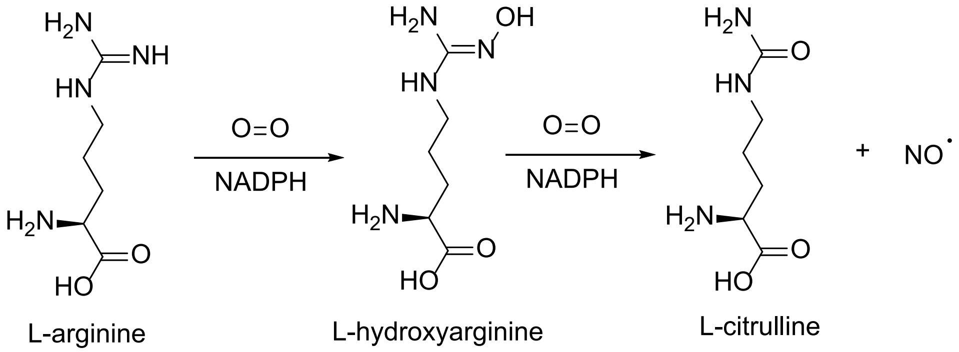
2. NO signaling in biological systems

Nitric oxide (NO) is a well-known toxic environmental pollutant, where enhanced anthropogenic production is associated with process of fuel combustion in furnaces in manufacturing industries, as well as in the operation of catalyst-deficient cars and internal combustion engines [34]. NO is also known to react with oxygen to form brown NO_x gases that are very dangerous if inhaled, causing lung irritation, airway inflammation, and react in the blood to form methemoglobin [35]. In living organisms, NO is produced in very low, highly controlled quantities both in animals and plants, where it acts as an important signaling molecule involved in a broad array of molecular events generally referred to as NO signaling [27, 36, 37].

To date, the signaling role of NO is better studied in animals than in plants. Thus, in mammals, NO is directly involved in the regulation of ion channels and modulation of enzyme activities in mitochondria. Moreover, NO is involved in the homeostasis of several PTMs of proteins – *S*-nitrosylation, *S*-glutathionylation, tyrosine nitration, and *S*-guanylation.

Obviously, the fact that NO reacts with thiols, proteins, sugars, metal ions, protein-bound hems, and other compounds localized in different tissues assumes its presence (or rapid generation from precursors) in the physiological liquids and cytoplasm of cells. Thus, NO can be treated as an intra- and inter-cellular regulator and a paracrine (i.e., influencing the functions of neighboring cells) compound that regulates a number of physiological processes: host defense mechanism, nerve impulses, vasodilation and transmission, blood pressure regulation, smooth muscle relaxation and many others [31, 38]. Historically, the discovery of the fact that NO binds the heme of soluble guanylate cyclase (sGC) to promote the conversion of GTP to cGMP for the downstream activation of protein kinase G was the start point for the recognition of NO importance in physiology.

The cGMP signaling is fundamental in cardiovascular physiology [39] and in neurotransmission. However, this physiological role of NO in living organisms remained undiscovered until the mid1980s. One of the first significant steps to elucidate the biological role of NO was done by Murad et al. [40]. They showed in 1978 that nitroglycerine is able to elevate cGMP via activation of the enzyme sGC. Further as shown by Böhme et al. in 1979 [41] the sydnone imine based drug molsidomine (Mols), an antianginal drug, also was able to enhance cGMP levels. In 1980 Furchgott [42] discovered an endothelial derived relaxing factor (EDRF), a relaxing substance released by the endothelium after stimulation with acetylcholine (Ach), that in parallel to the nitrovasodilators elevates cGMP. Finally in 1987 Palmer et al. [43] came to the surprising conclusion that this factor is the simple molecule NO. His group and others showed in 1988 that the physiological source of NO is the amino acid L-arginine and the enzyme performing the conversion of L-arginine to NO they called NO synthase. The chemistry of this enzyme system is rather complicated and not yet fully understood. The following **Scheme 1** simplifies the process.



Scheme 1 NO biosynthesis from arginine catalyzed by NO synthase

Disruption of NO production causes a broad range of vascular diseases as well as various immune and neurodegenerative disorders in humans. Probably, no other small endogenous compound performs such a versatile role of individual intra- and intercellular regulations in the organism. This fact stimulated exploration for novel NO-donors with improved pharmacokinetic profiles and prolonged action.

The history of the study of NO effects in plants is much shorter. Thus in late 90-ties NO was described, to the best of our knowledge, for the first time as a molecule involved in plant tolerant mechanism against pathogen attack [44, 45]. It looks likely, that the biological effects of NO in plants are mediated by mechanisms analogous to those in animals. Thus, most of the effects of NO in plants are related to defense and in responses to environmental stress as well as in symbiosis.

During evolution, plants have evolved a broad range of adaptive features and defense mechanisms against abiotic stresses, like a water-resistant cuticle on the plant surface, scavengers of ROS, UV-absorbers, molecular chaperones, and compatible solutes which can accumulate in cells in response to stress. Indeed, strong generation of ROS and RNS is the common feature of all plant defense responses to abiotic stress. Overproduction and accumulation of ROS and RNS ultimately result in development of oxidative stress and oxidative damage of cellular molecules, especially polymers. When produced at low levels, NO performs as a plant homeostasis regulator and can act as both negative and positive regulator depending on its location and concentration [46, 47]. NO is involved in a wide range of development and physiological responses. Thus, its *in vivo* generation was confirmed starting from pollination and pollen tube growth [48-50], during seed germination[51] and root development [52, 53]. Importantly, NO also is involved in the control of stomatal aperture [54, 55]. Nitric oxide plays an important role in responses to stressors [56], including heavy metals [57], salt [58], extreme temperature [59], high intense light [60] and pathogens [61].

Our special interest is focused on modifying drought effects in plants as drought is considered the by far most relevant factor contributing to crop yield losses. One has to take into account, however, that the mechanism of NO regulation under drought conditions is complex and its fine details are still unclear (Fig. 1), and tuning the wrong effects will be detrimental.

For the plant, it is critically important to sustain "high" NO levels in order to prevent possible drought related damages. High NO tissue contents correspond to a “primed” state of the plant (i.e. triggered stress defense mechanisms) that makes its chances to survive under drought conditions higher. One of the ways to supplement plants with enough amount of NO is to support its formation by application of phytoeffectors which can serve as exogenous NO donors or NO pro-phytoeffectors ("pro-drugs"). Relying on the fact that the majority of the fundamental principles of functioning of all living systems are common: cellular structure, existence of signaling and feedback systems, ligand-receptor interactions, one might expect that some of the substances that are physiologically active in humans and animals might be also active in plants. In other words, the search for new classes of compounds promising for use in the field of chemical plant protection agents is most productive among those classes of compounds whose representatives reliably possess physiological activity with respect to humans and animals, and vice versa.

The correctness of the logic of this assumption was illustrated by the investigation Garcia-Mata et al [62]. The authors showed that NO, when applied exogenously in form of the inorganic NO donor sodium nitroprusside and the organic *S*-nitroso-*N*-acetylpenicillamine, help to tolerate water stress conditions in wheat seeds. Later in 2019 [63], exogenous NO, also from the sodium nitroprusside, showed good results in plant drought tolerance enhancement when applied to *Medicago sativa* L. Following the exogenous NO donor approach here we focus on sydnone imines that are as well capable to release NO. They were found to be extremely valuable and effective in pharmaceutical applications.

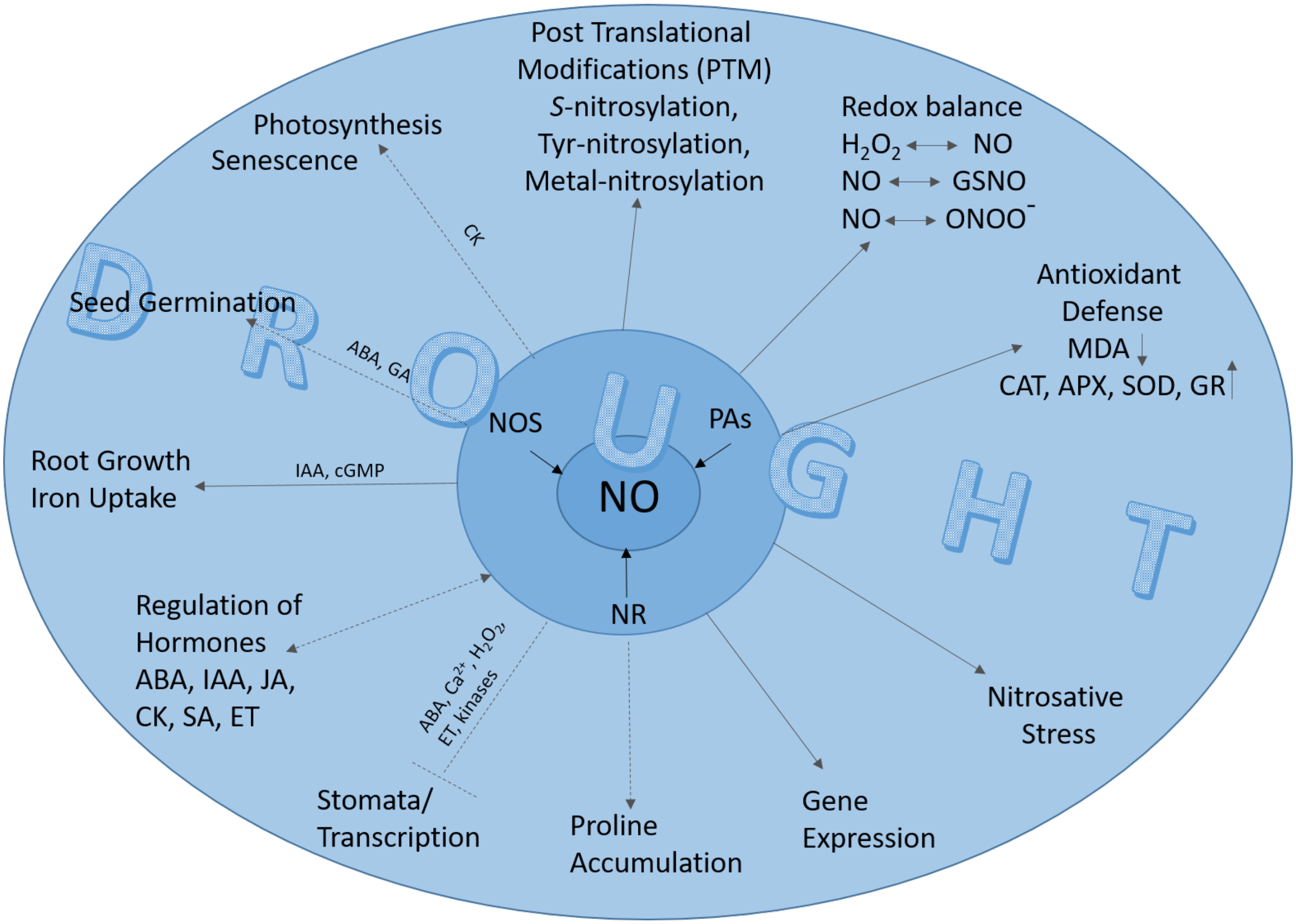
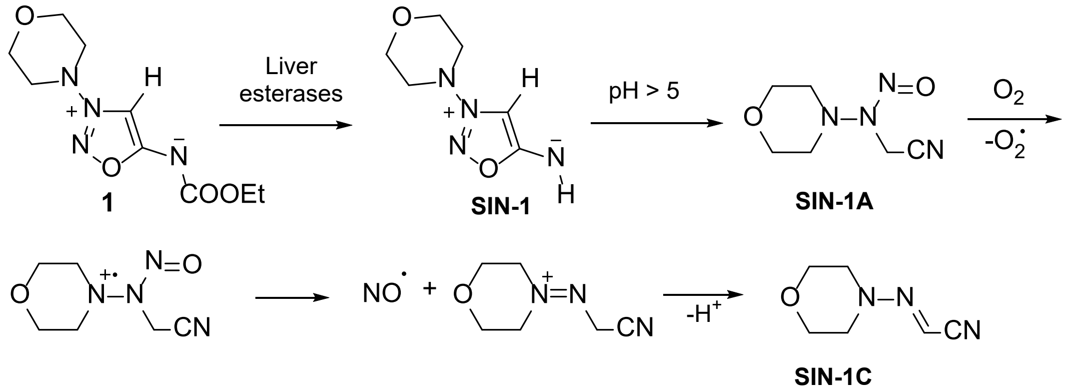


Figure 1 NO contribution in regulating various drought stress responses in plants. NO either stimulates (normal end arrow) or inhibits (blunt end arrow) processes in plants together with various plant hormones under drought conditions. The dotted line shows the pathways not studied clearly yet. The double-headed arrow shows the reciprocal regulation of molecules. With modifications adapted from Santisree et al. [64].

3.1 Sydnone imines as NO donors in pharmaceutical studies.

Sydnone imines represent a class of mesoionic heterocyclic compounds [32] exhibiting a broad range of biological activities and in this regard they are successfully applicable in medicine.

For example, as is noted above it has been found that the biological action of the antianginal drug molsidomine (**1**) is related to its ability to release nitrogen oxide (**Scheme 2**). Decomposition of **SIN_1A** (unlike that of **SIN_1**) is a pH_independent process and involves oxygen. Oxygen consumption in buffered solutions of **SIN_1** is correlated with the formation of nitrogen oxide. It is significant that NO is formed from **SIN_1A** together with **SIN_1C**. Compound **SIN_1A** is the activator of soluble guanylate cyclase; it increases the intracellular concentration of cGMP and, correspondingly, enhances its effects (vasodilation, inhibition of thrombocyte aggregation, neurotransmission, immune response regulation) typical of nitrogen oxide donors [65].



Scheme 2 Nitrogen oxide release pathway from the drug molsidomine.

The capability of sydnone imines to function as nitrogen oxide donors is described in detail in a number of papers [66-68] and a review on their synthesis and properties has been published [32]. Sydnone imines are sensitive to oxygen and light and it decomposes -- in addition to nitric oxide – to form a super oxide radical anion, peroxynitrite and a hydroxyl radical [69].

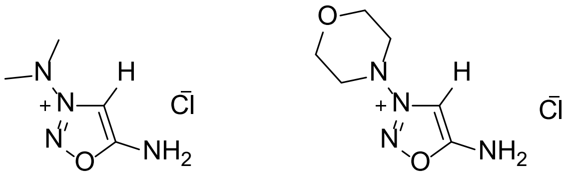
In other words, sydnone imines are essentially metabolotropic drugs (prodrugs) whose biotransformation results in the formation of NO molecules and anions and radicals, which are responsible for the physiological effects. In a similar way, for example, the growth regulator ethephon acts, which, upon entering the plant, slowly degrades under release of ethylene, thus affecting the ethylene status [70].

Consequently, it was shown that sydnone imine derivatives can lower blood pressure (hypotensive effect) [71], expand blood vessels, relax muscles [72], normalize the blood circulation system [73], and counteract platelet aggregation [74, 75]. It is known that the toxicity of sydnone imines and their derivatives is significantly lower than that of other substances with similar pharmacological activity. Sidnofen is used in medicine as a psychostimulant [76] and sidnocarb [77], also a vasodilating drug like molsidomine, is used for stenocardia treatment [78]. All of them are sydnone imine derivatives. However, the ability of sydnone imines and their derivatives to stimulate plant growth has not been known until very recently.

3.2 Sydnone imines as phytoeffectors in plants

The hypothesis of using sydnone imines as plant growth regulators was suggested only quite recently and proposed in the recent work of Olshevskaya et al. [33]. The authors aimed at finding chemical plant protection agents among new classes of chemical compounds with herbicidal, antidotal or growth regulatory activity. The compounds tested for activity were the representatives of chemical classes demonstrating pronounced physiological activity against humans and animals. Due to their high pharmacological activity as exogenous NO donors this list included sydnone imine derivatives. The authors addressed the effect of the tested compounds on **Figure 2** on germination rates of sunflower seeds. As a result, they could estimate the order of probability of detection among the selected classes of compounds with the types of activity of interest. The tested sydnone imines differed in stability in aqueous aerobic media, availability of a large variety of derivatives, and the ability to participate in redox processes. These characteristics make these compounds particularly attractive targets for screening studies. In addition, compounds unusual chemical moieties may turn out to be unique objects of research, since there are no enzyme systems in living organisms natively evolved or focused on the metabolism of such xenobiotics with a bioorthogonal chemistry. The mesoionic sydnone imines belong to this group.

The results of the tests of sydnone imine derivatives **2** and **3** in **Figure 2** showed that both of them in doses of 1 g/t (1 ppm) and 10 g/t (10 ppm) of seeds had a negative effect on the germination of sunflower seeds. The reduction of germination in relation to water control for both compounds was dose-dependent with 36 to 86%. Sydnone imine derivatives **2** and **3** also possess neither growth stimulating, nor antidote activity against metsulfuron-methyl, a sulfonylurea class herbicide (Zinger, WP), for sunflower seeds and has a negative effect on the seed germination rate itself. The germination inhibitory or herbicidal effect of compounds **2** and **3** was at the same level or exceeded that of the reference herbicide metsulfuron-methyl (Zinger, WP). Thus, as can be seen in **Table 1**, the herbicidal or germination inhibitory effects of the sydnone imines **2** and **3** and simultaneously used metsulfuron-methyl is complex (not additive) and dose-dependent. The fact that both sydnone imines studied have activity is a hint that the ability to exert an influence on plant organisms is not a random result attributed to an individual compound but is related to the structural type. The herbicidal/germination inhibitory effect was very pronounced even at very low doses (1-10 g/t seeds), which indicates the ability of these compounds to penetrate into the plant tissues and influence physiological processes at the molecular or cellular level.



2 **3**

Figure 2 Sydnone imine derivatives tested on sunflower seeds.

Table 1 Germination inhibition[a] effect of the sydnone imines **2** and **3** tested on sunflower seeds [33].

Compound	Compound dose, g/t seeds (ppm by weight)	Number of seeds germinated after 3 days				Seed germination, % of control,
		Replicate[b]			Average,	
		1	*2*	*3*		
2	1	7	9	10	9	64
	10	2	2	2	2	14
2 + Zinger, WP	1 + 20	6	8	7	7	50
	10 + 20	5	4	6	5	36
3	1	5	8	7	7	50
	10	3	5	4	4	28
3 + Zinger, WP	1 + 20	5	4	5	5	36
	10 + 20	3	2	3	3	21
Zinger, WP	20	6	5	6	6	43
Control		15	13	14	14	100

[a] pure and in combination with a herbicide. The germination inhibition may or may not be based on herbicidal properties of the sydnone imines.

[b] Each replicate included 15 seeds, each tested seed germinated for 3 days in wet paper in a Petri dish.

The results described above stimulated more detailed investigations of sydnone imine derivative influence on plant growth and development. Moiseev et al. [79] in 2018 investigated the growth-stimulating activity dependence on different structures of C(4)-unsubstituted sydnone imine derivatives **4-14** shown in **Figure 3**. They looked at the effect of the acyl substituent at N-6 and N-3 on the corn (maize) growth-stimulating activity, and the possibility to intensify early stages of plant development to accelerate the increase in mass of both the above-ground parts and the root system. The compounds were applying as seed pretreatment in doses of 1 and 10 g/t seeds. Since N-6-unsubstituted sydnone imines are stable only in form of their salts, the sydnone imine derivatives under study were salts, hydrochlorides were used (compounds **4-8)** as well as N-6-acylated derivatives (compounds **9-14**). N-6 Substituted sydnone imines are stable compounds, and the use of sydnone imine salts provides good solubility in water and, as assumed, easier penetration into the seeds coat. N-6-acylated compounds **9-14** are more lipophilic, which can lead to a significant change in the distribution of the substance in the plant and, accordingly, to a change in the profile of the manifested activity. Considering this, compounds **12** and **13** are derivatives of the compounds **2** and **3** (**Figure 2)** with herbicidal or germination inhibition effects on sunflower seeds, where N-6 is protected by a *tert*-butoxycarbonyl (Boc) group. Unlike the other acyl substituents **9-11, 14**, the Boc-group is relatively easily and traceless removed from the molecule under acidic conditions. It was assumed in the paper [79] that the lipophilic Boc-group is able to influence the molecules capability to penetrate through the cell membrane and accordingly influence compound distribution in the plant. Eventually, will result in N-6-unsubstituted sydnone imine but due to a different tissue distribution and BOC-removal kinetics nevertheless can have different effects than the parent compound.

Experiments on the growth-regulating activity of sydnone imine derivatives (**4-14**) were performed under thermostatic conditions using corn seeds of Krasnodarskaya 12 cultivar. It was found that all sydnone imine derivatives studied, except for one, showed a statistically significant effect on seed germination and seedling development of corn (**Table 2**). In contrast to N-3-aminosubstituted compounds **2** and **3** (**Figure 2)** which suppressed the sunflower seeds germination[33], the N-6-Boc-derivative **12** tested turned out to be ineffective on corn seeds. Furthermore, compound **13** not only failed to suppress plant development but, vice versa, showed a growth-stimulating effect on seedlings and their root system, especially at a dose of 10 g/t of seeds. The N-6-unsubstituted sydnone imines **4-8** were utilized in order to evaluate the influence of the substituent at N-3 position on compound activity in plants as part of a basic SAR approach. Compound **4** with N-3-isopropyl substituent shows only weak herbicidal properties, whereas the N-3-alkylated derivatives **5-7** proved to be growth stimulators, interestingly stronger at the dose of 1 g/t than 10 g/t. This effect was seen for both seedlings and roots, and tended to decrease in action as the alkyl chain length increased. At a dose of 1 g/t, the methyl derivative **5** was a strong growth simulant (seedling stimulation 43% and root stimulation 66%), whereas the corresponding values of the ethyl (40% and 47%) and *n*-butyl (26% and 11%) derivatives were markedly lower. Replacing one methylene link in the *n*-butyl group by oxygen (compound **8**) markedly increased the growth-stimulating effect, and especially so with respect to the root system (up from 11% to 71%). While alkyl chain branching in the case of isopropyl derivative **4** led, as noted above, inverted the activity profile to herbicidal.

Acylation of the exocyclic nitrogen atom N-6 in the N-3)-isopropyl derivative by acetyl and *para*-tolyl sulfonyl groups (compounds **9**, **10**, and **11**, respectively) also appeared to significantly affect the activity profile of the compounds. In this series, compound **9** was an effective stimulator of seedling growth (26-46%, depending on the dose) and especially of the root system (33-57%). Compound **10**, while continuing to maintain its stimulating effect on seedlings at the same level as compound **9**, lost it completely with respect to the root system. Tosyl derivative **11** exhibited a weaker stimulating effect in relation to seedlings, in relation to the root system it even acted as a moderate herbicide.

In summary, it was found that sydnone imine derivatives have a pronounced effect on the growth of corn seedlings and root system. This effect is growth-stimulating in most cases and is characteristic of the overwhelming majority of the studied compounds, but can also turn into opposite effects. A first qualitative SAR for the different effects was devised for substituents at the N-3 and N-6 positions of the core compound.

There is a clear effect of the substituents on type and strength of germination, growth and herbicidal effects, but this may also be plant species dependent (v.i. for more data on species dependence). The growth-stimulating effect is promoted by the absence of substituents at position N-6 and the presence of an unbranched alkyl chain in position N-3. Moreover, the effect is more pronounced in cases of a shorter chain or the presence of a heteroatom (which makes it more hydrophilic). Also, a lower dose of the active substance (1 g/t seed) appears to be more beneficial than a dose of 10 g/t seed. Obviously – as in mammals – overdosing of NO is detrimental. In the case of N-6 -acylated derivatives, the type of acyl group appears to primarily affect the severity of the stimulating effect on the root system rather than on the upper parts of the seedlings. In the case of compounds with an N-3-isopropyl group, acylation of the N-6 position results in growth-stimulating properties of sydnone imine derivatives, regardless of the type of acyl group in the small group tested so far. For example, compound **14** showed that sydnone imine derivatives containing an additional substituent at the C-4 position can also exhibit growth-stimulating properties.

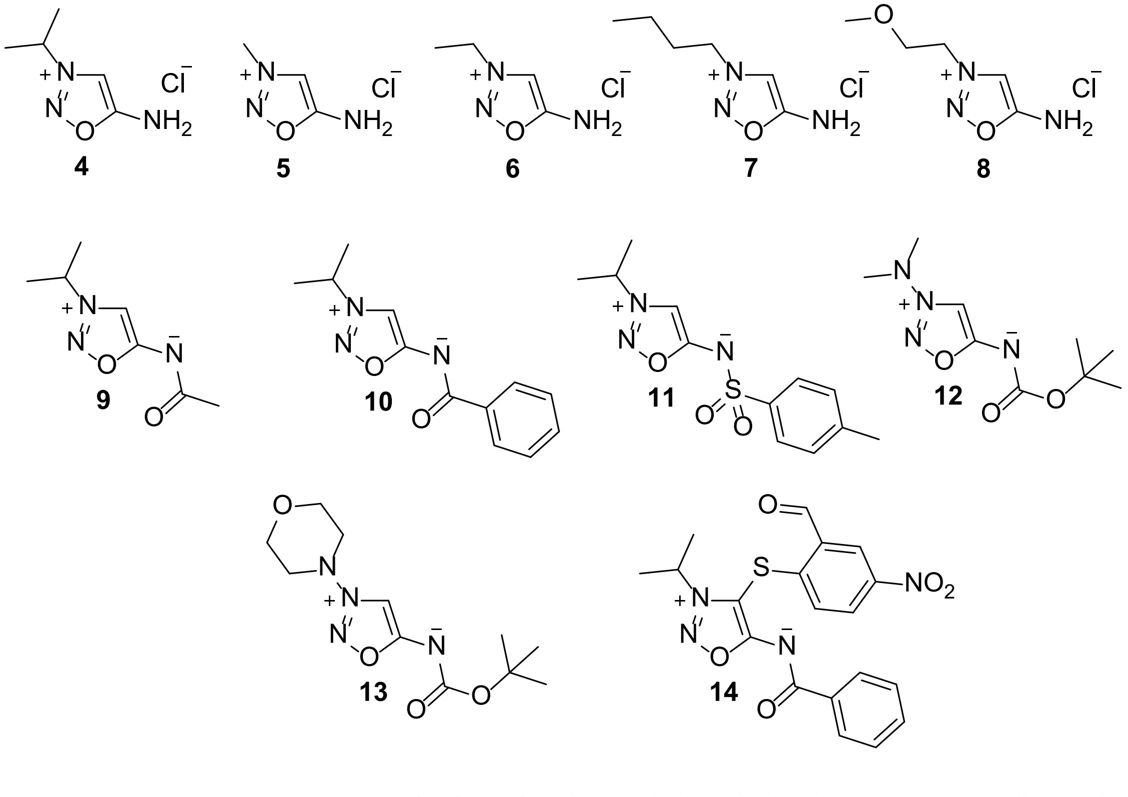


Figure 3 Structures of C-4-unsubstituted sydnone imine derivatives (except cpd. **14**) investigated for growth-stimulating activity using corn seeds of Krasnodarskaya 12 cultivar.

Table 2 Growth-stimulating activity of the sydnone imine derivatives **4-14** using the corn seeds of Krasnodarskaya 12 cultivar [79].

Compound	Compound dose, g/t seeds	Shoot length[a]		Root length[a]	
		Average[b], cm	% of control	Average[b], cm	% of control
4	1	3.8	109	5.3	77
	10	3.2	91	6.2	90
12	1	3.4	97	6.7	97
	10	3.3	94	6.9	100
13	1	4.1	117	6.6	96
	10	4.7	134	8.1	117
14	1	4.3	123	7.3	106
	10	4.0	114	7.3	106
Control		3.5	100	6.9	100
5	1	4.3	143	3.5	167
	10	3.1	103	2.2	105
6	1	4.2	140	3.1	148
	10	3.6	120	3.2	152
8	1	4.1	137	3.6	171
	10	4.3	143	3.4	162
9	1	3.8	127	3.3	157
	10	4.4	147	2.8	133
Control		3.0	100	2.1	100
7	1	6.2	127	10.2	111
	10	6.5	133	5.3	58
10	1	6.2	127	9.3	101
	10	7.1	145	8.1	88
11	1	5.9	120	7.4	82
	10	6.3	129	7.5	82
Control (distilled water)	10 liters per 1 ton of seeds	4.9	100	9.2	100

Note: Experiments were carried out in three parallels: compounds **4, 12,13,14** and control; compounds **5, 6, 8, 9** and control; compounds **7, 10, 11** and control.

[a] Length of shoots and roots of developed seedlings were measured at 7th day after beginning of tested seed germination; [b] Average of three replicates, each replicate included 15 tested seeds, each seed germinated in wet paper in a Petri dish.

Indeed, further synthetic studies on the chemistry of sydnone imines by Cherepanov [80] broadened the scope of available sydnone imine derivatives with plant growth regulating activity by further modification, especially regarding substituents at C-4 [80]. It was found that α-aryl substituted 4-hydroxymethylsydnone imines exhibit good plant growth regulating properties. Utilized as a pre-sowing treatment of corn seeds at very low doses (0.25-5 g per ton of seeds), most of the compounds shown in **Figure 4** showed a dose-dependant stimulation of the corn development, resulting in an increase of the shoots weight up to 64% compared to the control (**Table 3**). It was shown that the Boc-group at N-6 is able to influence the compound activity both in quantity and in quality terms, still this influence is not unequivocal. For example, *N-6*-unsubstituted compound **21** is more active at higher doses than its' Boc-protected analogue **15**, while the activity of derivative **16** decreases dramatically when the dose is decreased to 0.25 g/t of seeds. At the same time the effect of compound **21** seems to be largely independent of the dose (within the limits tested). The effect was dose dependent for compounds **16** and **22,** however, this dependence had opposite directions, improved growth for **16**, and in contrast, retarded growth for **22** with increasing dose. The plant growth stimulation effect also was dependent on substituent at N-3 position. Most active were the compounds with an N-3-isopropyl substituent. Aromatic substituents at a C-4 carbinol position (i.e. 1-hydroxybenzyl derivatives) also influenced the activity.

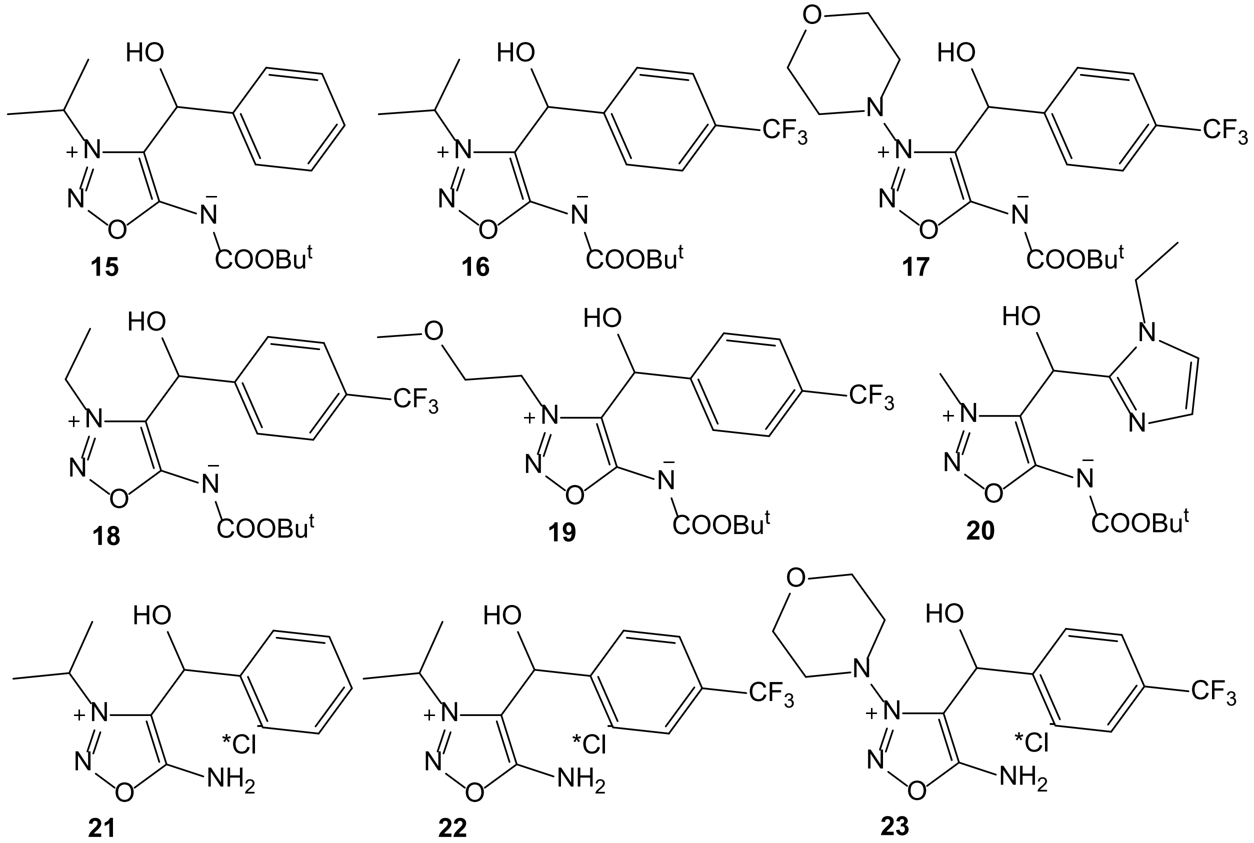


Figure 4 α-Aryl substituted 4-hydroxymethyl-sydnone imines with plant growth promoting properties on corn seeds (Krasnodarsky 291 AMV cultivar).

Table 3 Plant growth regulating activity of compounds **15 – 20** on corn (Krasnodarsky 291 AMV cultivar) [I. Cherepanov et al., unpublished data].

Compound	Compound dose, g/t	Weight of shoots, g						Weight increase relative to Control., %
		replicate No.					Average[a],	
		1	*2*	*3*	*4*	*5*		
15	0.25	6.9	6.3	6.6	6.5	6.5	6.6*	140
	0.5	6.0	6.5	5.9	6.2	8.0	6.5*	138
	1	6.4	6.0	6.3	7.0	6.2	6.4*	136
16	0.25	6.1	5.9	5.9	5.7	5.7	5.9*	126
	0.5	5.4	4.9	5.3	5.3	5.9	5.4*	115
	1	7.3	6.5	7.0	7.8	6.5	7.0*	149
21	0.25	5.6	6.2	5.3	6.0	5.7	5.8*	123
	0.5	7.4	7.4	8.0	7.2	6.8	7.4*	157
	1	7.3	7.6	8.0	7.2	8.2	7.7*	164
Control		4.7	4.7	4.2	5.0	4.7	4.7	100
17	0.5	8.0	9.3	8.1	8.8	9.9	8.8*	138
	1	7.8	5.5	7.1	7.3	8.7	7.3	114

	5	9.9	5.0	7.4	5.3	8.9	7.3	114
18	0.5	8.6	9.7	6.8	8.7	9.7	8.7*	136
	1	6.7	8.8	7.5	6.5	7.4	7.4*	116
	5	8.2	7.9	9.3	7.1	8.6	8.2*	128
19	0.5	7.3	6.4	7.8	6.4	6.7	6.9	108
	1	6.8	7.7	6.0	6.1	6.1	6.5	102
	5	6.9	5.5	6.5	8.1	6.8	6.8	106
20	0.5	6.1	5.1	6.5	6.7	6.3	6.1	95
	1	6.1	7.6	7.4	5.2	5.3	6.3	98
	5	6.2	5.5	6.2	8.0	5.2	6.2	97
22	0.5	9.8	10.2	9.1	7.0	9.4	9.1*	142
	1	9.8	8.7	9.5	9.0	9.3	9.3*	145
	5	9.8	7.3	8.0	7.9	7.0	8.0*	125
23	0.5	8.0	8.4	8.1	6.3	7.9	7.7*	120
	1	7.0	8.2	6.3	7.8	7.0	7.3*	114
Control		6.0	6.6	6.4	6.4	6.6	6.4*	100

Experiments were carried out in two parallels: compounds **15, 16, 21** and control; compounds 1**7, 18, 19, 20, 22, 23** and control

[a] Average of five replicates, each replicate included 15 tested seeds, each seed was germinated in wet paper in a Petri dish

* denotes significant difference (T-test, $p<0.05$) in shoot weight as compared with control

In paper [81] the effectiveness of sydnone imine derivatives as growth stimulators of plants belonging to different species was compared in pot experiments using the corn cultivars Krasnodar 291 AMV and winter wheat Moscow 56 (cereals, monocotyledons),and the Makhaon cultivar of sunflower (dicotyledon) as test plants. The tests were done as in previous pot experiments on corn [80], now with sydnone imine derivatives **17, 22, 23, 24 (Figure 5**) at C-4 position substituted with a (4'-trifluoromethyl-phenyl) hydroxymethyl substituent. These compounds were of both subgroups, N-6-unprotected sydnone imine salts (**22, 23**) and N-6-Boc-protected compounds (**17, 24**). They were used for a pre-treatment (seed-priming / Beize) of seeds at doses of 0.5, 1.0, 2.5 and 5.0 g/t of seeds. The growth-stimulating activity of the compounds was dose- and substituent-dependent, and the intensity of the effect (up to 37-53%) depended on the plant species too. Compounds **22, 17, 23** confirmed their growth-stimulating effect on corn (**Table 4**) as found earlier [80], with compound **22** being the most active one (growth stimulation up to 53%). Compound **24** proved to be inactive. No significant differences in the activity of N-6-unsubstituted derivative **23** and N-6-Boc-substituted compound **17** were found (in contrast to the germination differences of C-4-unsubstituted derivatives discussed above). Compound **22** showed a more pronounced growth stimulation in corn at higher doses (2.5 and 5.0 g/t seed), while derivatives **17** and **23** were more active at lower doses of 0.5 and 1.0 g/seed. Only compounds **22** (up to 37% at doses of 2.5 and 5.0 g/t seed) and **17** (up to 28% at a dose of 1.0 g/t seed) showed growth-stimulating activity with winter wheat (**Table 4**). Compounds **23** and **24** were inactive. all four compounds (**17, 22, 23, 24**) showed growth-stimulating activity on sunflower (**Table 4**). Compound **24** showed a more pronounced effect (up to 39%), and compounds **17, 22, 23** stimulated the growth of sunflowers by about 20%. The maximum growth stimulation exhibited by compounds **17** and **23** was similar (21-22%), but the N-6-unsubstituted derivative **23** stimulated sunflower growth in a broader range of doses than the N-6-Boc-substituted compound **17**. In terms of chemical structure, the activity of sydnone imine derivatives is significantly influenced by the substituent at the N-3 position. The presence of the protective Boc-group at the N-6-position can have a significant effect on the activity of the compound, but the nature of this effect cannot be predicted at present. All in all, the growth-regulating activity of sydnone imine derivatives has a pronounced dependence on the plant species, but needs a deeper look into with more derivatives and species and cultivars, and under different growth conditions.

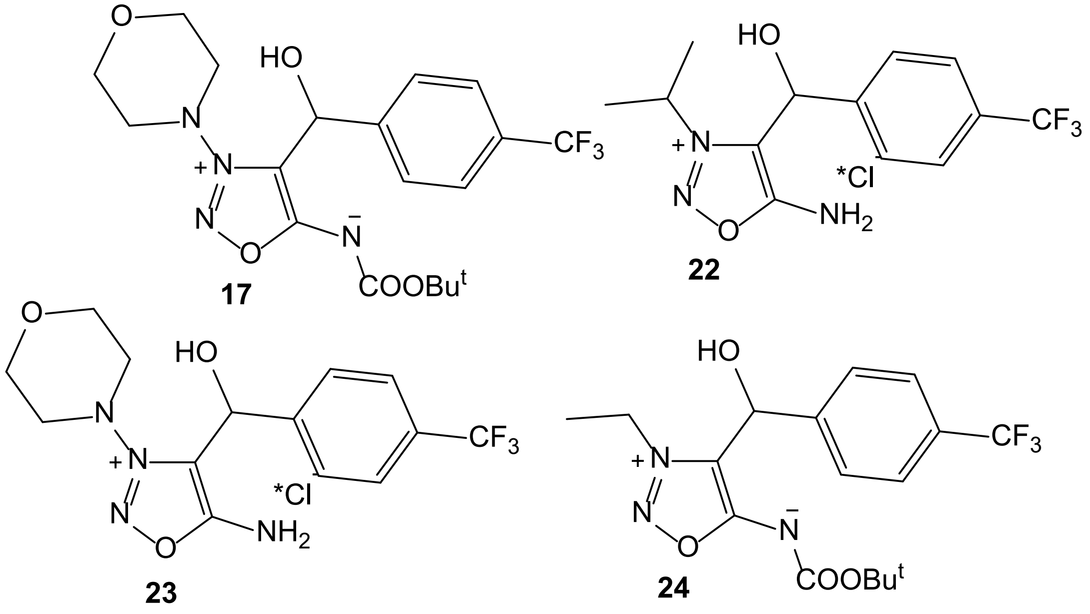


Figure 5 Sydnone imine derivatives containing in C(4) position the (4'-trifluoromethyl-phenyl-hydroxymethyl) substituent used for tests on different crop plant species.

Table 4 Growth regulating activity of the compounds **22, 17, 23, 24** on varied crop plants: corn Krasnodar 291 AMV cultivar and Moscow 56 winter wheat (cereals) cultivar and Makhaon cultivar sunflower [81].

Compound	Compound dose, g/t seeds	Above-ground test-plant weight corn Krasnodar 291 AMV		Above-ground test-plant weight, Moscow 56 winter wheat		Above-ground test-plant weight, Makhaon cultivar sunflower	
		% relative to control	Average[a], g	% relative to control	Average [a], g	% relative to control	Average [a], g
22	0.5	136	4.1	119	1.36	117	4.1
	1.0	120	3.6	126	1.44	112	3.9
	2.5	153	4.6	137	1.56	112	3.9
	5.0	151	4.5	137	1.56	87	3.0
17	0.5	133	4.0	118	1.34	100	3.5
	1.0	133	4.0	128	1.46	122	4.3
	2.5	109	3.3	105	1.2	92	3.2
	5.0	117	3.5	95	1.08	92	3.2
23	0.5	126	3.8	105	1.2	106	3.7
	1.0	137	4.1	104	1.18	112	3.9
	2.5	105	3.2	104	1.18	121	4.2
	5.0	89	2.7	88	1.0	111	3.9
24	0.5	110	3.3	95	1.08	130	4.6

	1.0	91	2.7	102	1.16	139	4.9
	2.5	100	3.0	104	1.18	131	4.3
	5.0	100	3.0	88	1.0	122	4.3
Control (distilled water)		100	3.0	100	1.14	100	3.5

^{a}Average of five replicates, each replicate included 5 tested plants which were developed from seeds germinated in paper beaker with sod-podzolic soil, after 12-17 days the above-ground parts of the test plants was cut off and weighed.

In addition to their growth regulatory effect, some sydnone imine derivatives have been shown other effects on plant physiology. Thus, it was shown that they can exhibit the properties of herbicide antidotes [81, 82]. Modern intensive agriculture is impossible without the use of herbicides in order to sufficiently control weeds. However, herbicide residues in the soil or, more problematic even, in the final food product can significantly reduce the yield of cultivated plants in crop rotations up to 35% [81], or forbid marketing at all, respectively. Herbicides of the sulfonylurea class, which are very widespread in agriculture, are particularly dangerous with respect to their negative effects on crops. They are able to exert their effect in small doses and are only slowly biodegraded. To reduce the phytotoxicity of herbicides or completely eliminate their negative effect, herbicide antidotes are used. Work [81] studied the ability of sydnone imines **15, 21, 22, 25-31** (**Figure 6)** to act as inducers of plant resistance to the phytotoxic effect of metsulfuron-methyl, one of the most important representatives of sulfonylurea herbicides.

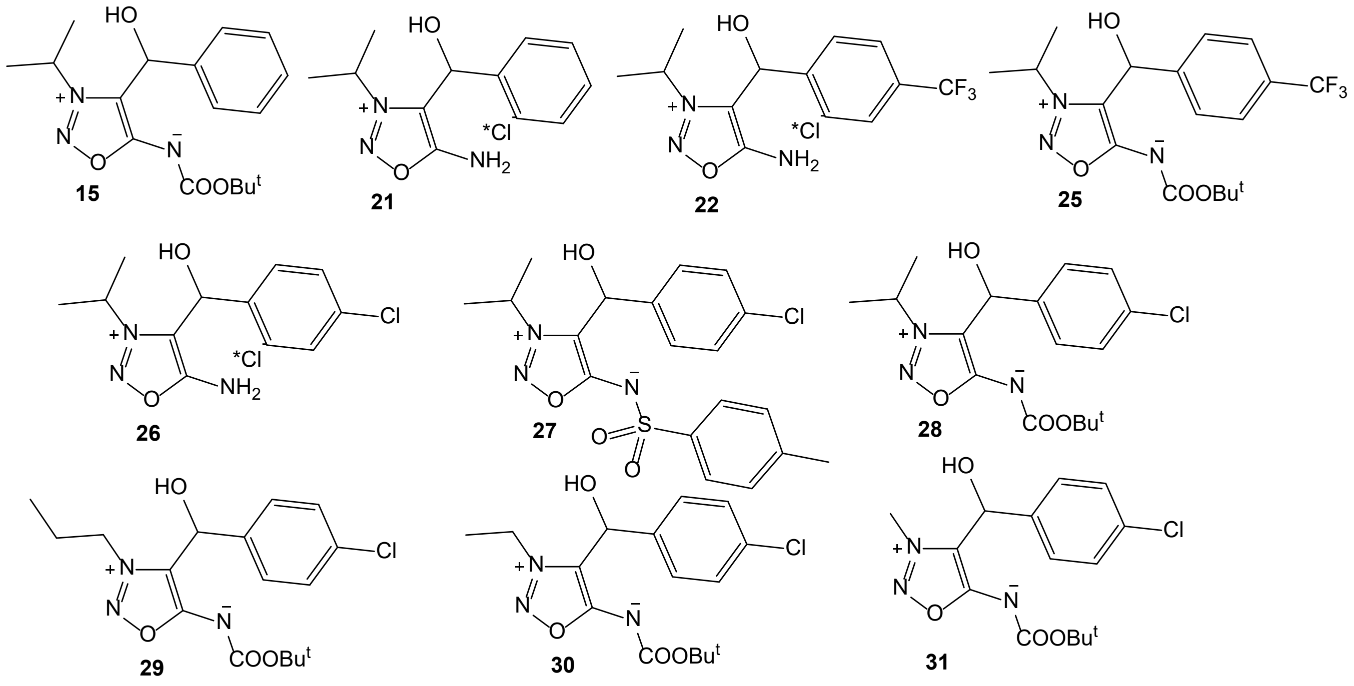


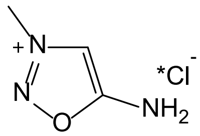
Figure 6 Structures of sydnone imines, tested as inducers of plant resistance to the phytotoxic effect of metsulfuron-methyl.

Corn of Krasnodar 291 AMV cultivar was used as test plant in the pot experiments. Compounds **15, 21, 22, 25-31** were used for seed pretreatment in doses of 0.25, 0.5 and 1.0 g/t seed. Metsulfuron-methyl herbicide (Zinger, WP) was pre-applied with a sprayer to sod-podzolic soil at a dose of 2 g/hectar. At the same time in the same climate chamber, parallel experiments without herbicide-pretreatment of the soil was performed. The data obtained show that the effect of the sydnone imine derivatives on corn growth was dose-dependent. Most of them acted as corn growth stimulators. Among them, sydnone imines **25, 26, 27** and **31**, reduced or completely eliminated the effect of the herbicide at the appropriate doses. Moreover, in these cases the result could not be explained by the simultaneous and oppositely directed growth-stimulating effect of the sydnone imine derivatives and the herbicidal effect of metsulfuron-methyl. That is, compounds **25, 26, 27** and **31** acted precisely as metsulfuron-methyl antagonists. Analysis of the structure-activity relationship showed that the growth-stimulating and antidote effects of the tested compounds were influenced by the structure of the alkyl substituent at position N-3, the nature of the substituent at position N-6 and the substituent in the *para*-position of the aryl substituent of the hydroxy-methyl at C-4.

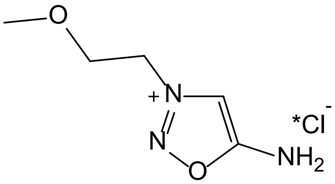
The effect of the concentration of aqueous solutions of sydnone imine derivatives on seed germination and growth of axial organs in wheat has been studied [83]. The experiments were conducted on the seeds of the winter wheat cultivar Moscow 39. Solutions of nine sydnone imine derivatives shown in **Figure 7** at concentrations of 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} mol/L were used for germination of wheat seeds. It was shown that in the indicated concentration range, various sydnone imine derivatives had both stimulating and inhibitory effects on seed germination and on root and seedling development. Sydnone imines **22, 33**, and **34 increased** seed germination up to 100%. At the same time, derivatives **8, 15,** and **32** showed inhibitory effects on seed germination (86-92%). In particular, compound **34** was found to stimulate root growth by 22–48% at concentrations of 10^{-7}–10^{-9} mol/L. Growth stimulation was also shown by derivatives **25** and **32 ,** as with substances **15** and **17** which inhibited this process at concentrations of 10^{-6}–10^{-9} mol/L with up to 72% more success compared to control. Stimulation of seedling growth by 30-51% was observed for two compounds, **22** and **34**, at concentrations of 10^{-6}-10^{-7} mol/L. Several of the compounds tested (**15** and **17**) inhibited seedling growth. As a result, the tests revealed one compound that stimulated both seed germination and axial growth (potential wheat growth stimulator) and two compounds that showed inhibitory effects on both seed germination and root and seedling growth (potential retardants).

The effect of sydnone imine derivatives on the growth and development of plants under various types of stress has been studied in a number of works.

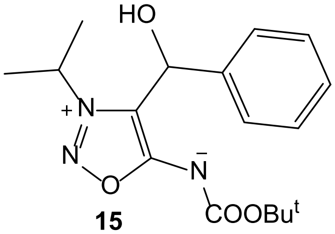
Unfavorable temperatures are among the common stressors which disturb physiological processes in agricultural plants and reduce their productivity. Treatment of plants with growth regulators is used to reduce the adverse effects of stressful temperatures. Since sydnone imine derivatives exhibit growth regulatory action, the same nine sydnone imine derivatives of **Figure 7** were studied for a possible protective effect against temperature stress[84]. The experiments were performed on winter wheat (*Triticum aestivum* L.) of cultivar Moscow 39 and corn (*Zea mays* L.) cultivar hybrid Voronezhsky 158 CB. At the first stage, the growth-regulating activity of sydnone imines was evaluated by germinating wheat and corn seeds using solutions of the tested compounds in concentrations ranging from 10^{-6} to 10^{-9} mol/L for 7 days. In the second step, the effect of seed priming was analyzed with the preparations that maximally stimulated growth on young plants when exposed to temperature stress caused by reduced or increased temperature (wheat 2°C and 38°C for 18 h; corn 3°C or 43°C for 24 h). The damaging effect of temperature stress conditions on plants was evaluated by electrolyte yield, chlorophyll fluorescence parameters, activity of plant antioxidant system components, and reduction of oxidative stress markers. Four of the nine sydnone imines studied (**15**, **25**, **32,** **34**) were found to stimulate the growth of wheat and corn plants and to reduce the damaging effects of elevated and reduced temperatures.



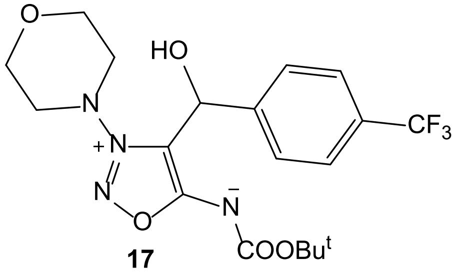
5



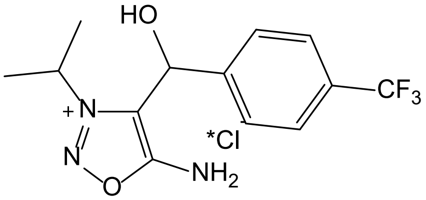
8



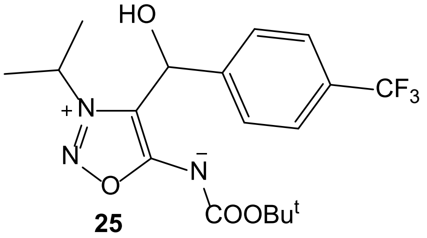
15



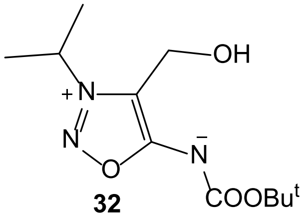
17



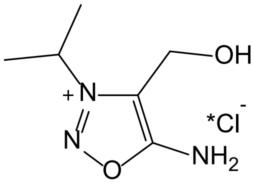
22



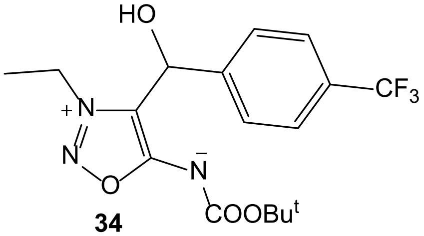
25



32



33



34

Figure 7 Structures of sydnone imine derivatives tested for germination of seeds under low and high temperature stress.

The positive effect of sydnone imine derivative **5 (Figure 7**) on corn plants under temperature stress (3°C or 38°C for 24 h) was shown in ref. [85]. Experiments were performed on corn (*Zea mays* L.) plants of the hybrid cultivar Cascade 195 CB using three sydnone imine derivatives at concentrations of 10^{-6}, 10^{-7} and 10^{-8} mol/L for seed priming. One day after temperature exposure, the degree of plant damage and leaf surface area as well as the intensity of lipid peroxidation in leaves by accumulation of malonicdialdehyde were evaluated. It was found that all three tested compounds in almost all concentrations under low-temperature stress reduced the MDA content in plant tissues compared to the control. In contrast, under high-temperature stress conditions, only one compound at a concentration of 10^{-8} mol/L reduced the intensity of lipid peroxidation in corn leaves.

The authors of ref. [86] demonstrated the positive effect of sydnone imine derivatives **22**, **32, 33** (**Figure 7**) on canola growth under stress conditions caused by Ni^{2+} presence. The effect of sydnone imine, used as seed pretreatment, on physiological and biochemical processes in rape seed seedlings against the background of heavy metals (HM) was studied for Ni^{2+}, Pb^{2+}, Zn^{2+}, and Cu^{2+} at concentrations from 10 to 1000 μM. HM ions induce oxidative stress in plant cells. Seed pretreatment with sydnone imine derivative showed the best protective effects for 1000 μM Ni^{2+} ions . At the same time, an increase in germination of rape seeds, stimulation of growth of above-ground and underground organs of seedlings, decrease of the $O_2^{\bullet-}$ radical generation rate, and lipid peroxidation intensity was observed.

4. Conclusion

Discovery of NO as a versatile signaling molecule both in animals and in plants revealed a plethora of physiological processes that are regulated by this small molecule. Some of these processes, which are critically important for the agricultural value of crops (e.g. germination, seed maturation, stress protection) can be affected by exogenous NO sources. Moreover, as the success of NO donors in human medical practice is obvious, something similar might be possible in plant and agricultural science as well. Therefore, NO donors which proved their efficiency in human organism, in modified form to meet the needs of agriculture and differentiate from human drugs, might serve as prospective phytoeffectors in plants. Sydnone imines, which represent one type of mesoionic heterocyclic compounds, are efficient exogenous NO donors. Until recently, there were no data on the use of sydnone imines as phytoeffectors available, and their affect on plant physiology. However, since 2017, several reports highlighted that members of this compound class can act as plant growth stimulants, herbicide antidotes, retardants and germination inhibitors (and eventually herbicides?), and inducers of plant tolerance to temperature and salt stress. This perspective attracts a special attention to sydnone imines as promising potential phytoeffectors which might also be capable of increasing plant drought tolerance. In order to establish structure – activity relationships a broader selection of sydnone imine derivatives needs to be explored in further series of experiments in adequate drought stress models in combination with functional genomics, i. e. with metabolomics, genomics and proteomics data taken into account. However, not only describing (as herein) SAR, but devising a predictive model for 2nd generation syntheses rounds in this case is especially challenging, since the differential action of the sydnone imines is not related to a specific (known) target, but rather by uptake, distribution and metabolic as well as non-biotic activation of the different derivatives – an unprecedented task in plant protectant and phytoeffector research. This special behavior also currently poses applicatin difficulties. The effects are very sensitive not only on the (controllable) substitution pattern but also very concentration and plant species dependent. New derivatives should have a larger application window ("therapetic selectivity index") and also show little or ideally no effects on men and mammals to protect the applicants and livestock from adverse effects.

Declaration of competing interest

The authors declare no conflicts of interests.

Funding

Funding for this study was from the Russian Scientific Foundation (grant # 22-26-00337)

References

[1] J.S. Boyer, Plant productivity and environment, Science (New York, N.Y.) 218(4571) (1982) 443-8.
[2] N. Osmolovskaya, J. Shumilina, A. Kim, A. Didio, T. Grishina, T. Bilova, O.A. Keltsieva, V. Zhukov, I. Tikhonovich, E. Tarakhovskaya, A. Frolov, L.A. Wessjohann, Methodology of Drought Stress Research: Experimental Setup and Physiological Characterization, International journal of molecular sciences 19(12) (2018).
[3] N. Tuteja, Abscisic Acid and abiotic stress signaling, Plant Signal Behav 2(3) (2007) 135-8.
[4] B. Barnabás, K. Jäger, A. Fehér, The effect of drought and heat stress on reproductive processes in cereals, Plant, cell & environment 31(1) (2008) 11-38.
[5] Y. Shavrukov, A. Kurishbayev, S. Jatayev, V. Shvidchenko, L. Zotova, F. Koekemoer, S. de Groot, K. Soole, P. Langridge, Early Flowering as a Drought Escape Mechanism in Plants: How Can It Aid Wheat Production?, Front Plant Sci 8 (2017) 1950.
[6] A. Sehgal, K. Sita, K.H.M. Siddique, R. Kumar, S. Bhogireddy, R.K. Varshney, B. HanumanthaRao, R.M. Nair, P.V.V. Prasad, H. Nayyar, Drought or/and Heat-Stress Effects on Seed Filling in Food Crops: Impacts on Functional Biochemistry, Seed Yields, and Nutritional Quality, Front Plant Sci 9 (2018) 1705.
[7] A.H. Ahkami, W. Wang, T.W. Wietsma, T. Winkler, I. Lange, C. Jansson, B.M. Lange, N.G. McDowell, Metabolic shifts associated with drought-induced senescence in Brachypodium, Plant science : an international journal of experimental plant biology 289 (2019) 110278.
[8] J. Shumilina, D. Gorbach, V. Popova, A. Tsarev, A. Kuznetsova, M. Grashina, M. Dorn, E. Lukasheva, N. Osmolovskaya, E. Romanovskaya, V. Zhukov, C. Ihling, T. Grishina, T. Bilova, A. Frolov, Protein glycation and drought response of pea root nodule proteome: a proteomics approach, Biological Communications 66(3) (2021) 210–224.
[9] M. Farooq, A. Wahid, N. Kobayashi, D. Fujita, S.M.A. Basra, Plant drought stress: effects, mechanisms and management, Agronomy for Sustainable Development 29(1) (2009) 185-212.
[10] P. Filippou, C. Antoniou, V. Fotopoulos, Effect of drought and rewatering on the cellular status and antioxidant response of Medicago truncatula plants, Plant Signal Behav 6(2) (2011) 270-7.
[11] J. Shumilina, A. Kusnetsova, A. Tsarev, H.C. Janse van Rensburg, S. Medvedev, V. Demidchik, W. Van den Ende, A. Frolov, Glycation of Plant Proteins: Regulatory Roles and Interplay with Sugar Signalling?, International journal of molecular sciences 20(9) (2019).
[12] G. Vistoli, D. De Maddis, A. Cipak, N. Zarkovic, M. Carini, G. Aldini, Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): an overview of their mechanisms of formation, Free Radical Research 47(sup1) (2013) 3-27.
[13] M.W. Poulsen, R.V. Hedegaard, J.M. Andersen, B. de Courten, S. Bügel, J. Nielsen, L.H. Skibsted, L.O. Dragsted, Advanced glycation endproducts in food and their effects on health, Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 60 (2013) 10-37.
[14] R. Valderrama, F.J. Corpas, A. Carreras, A. Fernández-Ocaña, M. Chaki, F. Luque, M.V. Gómez-Rodríguez, P. Colmenero-Varea, L.A. Del Río, J.B. Barroso, Nitrosative stress in plants, FEBS letters 581(3) (2007) 453-61.
[15] J. Astier, C. Lindermayr, Nitric oxide-dependent posttranslational modification in plants: an update, International journal of molecular sciences 13(11) (2012) 15193-208.
[16] A. Soboleva, N. Frolova, K. Bureiko, J. Shumilina, G.U. Balcke, V.A. Zhukov, I.A. Tikhonovich, A. Frolov, Dynamics of Reactive Carbonyl Species in Pea Root Nodules in Response to Polyethylene Glycol (PEG)-Induced Osmotic Stress, International journal of molecular sciences 23(5) (2022).
[17] T. Leonova, V. Popova, A. Tsarev, C. Henning, K. Antonova, N. Rogovskaya, M. Vikhnina, T. Baldensperger, A. Soboleva, E. Dinastia, M. Dorn, O. Shiroglasova, T. Grishina, G.U. Balcke, C. Ihling, G. Smolikova, S. Medvedev, V.A. Zhukov, V. Babakov, I.A. Tikhonovich, M.A. Glomb, T. Bilova, A. Frolov, Does Protein Glycation Impact on the Drought-Related Changes in Metabolism and Nutritional Properties of Mature Pea (Pisum sativum L.) Seeds?, International journal of molecular sciences 21(2) (2020) 567.

[18] I. Lounifi, E. Arc, A. Molassiotis, D. Job, L. Rajjou, G. Tanou, Interplay between protein carbonylation and nitrosylation in plants, Proteomics 13(3-4) (2013) 568-78.

[19] M. Mandal, M. Sarkar, A. Khan, M. Biswas, A. Masi, R. Rakwal, G.K. Agrawal, A. Srivastava, A. Sarkar, Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) in plants– maintenance of structural individuality and functional blend, Advances in Redox Research 5 (2022) 100039.

[20] M. Poteser, C. Romanin, W. Schreibmayer, B. Mayer, K. Groschner, S-Nitrosation Controls Gating and Conductance of the α1 Subunit of Class C L-type Ca2+ Channels*, Journal of Biological Chemistry 276(18) (2001) 14797-14803.

[21] L. Sanz, P. Albertos, I. Mateos, I. Sánchez-Vicente, T. Lechón, M. Fernández-Marcos, O. Lorenzo, Nitric oxide (NO) and phytohormones crosstalk during early plant development, Journal of Experimental Botany 66(10) (2015) 2857-2868.

[22] Y. Xu, P. Burgess, X. Zhang, B. Huang, Enhancing cytokinin synthesis by overexpressing ipt alleviated drought inhibition of root growth through activating ROS-scavenging systems in Agrostis stolonifera, J Exp Bot 67(6) (2016) 1979-92.

[23] Disharmonization in the Regulation of Transgenic Plants in Europe, Biotechnology Law Report 38(6) (2019) 350-375.

[24] C. Turnbull, M. Lillemo, T.A.K. Hvoslef-Eide, Global Regulation of Genetically Modified Crops Amid the Gene Edited Crop Boom - A Review, Front Plant Sci 12 (2021) 630396.

[25] M. De Block, C. Verduyn, D. De Brouwer, M. Cornelissen, Poly(ADP-ribose) polymerase in plants affects energy homeostasis, cell death and stress tolerance, The Plant journal : for cell and molecular biology 41(1) (2005) 95-106.

[26] T. Geissler, L.A. Wessjohann, A Whole-Plant Microtiter Plate Assay for Drought Stress Tolerance-Inducing Effects, Journal of Plant Growth Regulation 30(4) (2011) 504-511.

[27] A.A. Saddhe, M.R. Malvankar, S.B. Karle, K. Kumar, Reactive nitrogen species: Paradigms of cellular signaling and regulation of salt stress in plants, Environmental and Experimental Botany 161 (2019) 86-97.

[28] L. Ederli, L. Reale, L. Madeo, F. Ferranti, C. Gehring, M. Fornaciari, B. Romano, S. Pasqualini, NO release by nitric oxide donors in vitro and in planta, Plant Physiology and Biochemistry 47(1) (2009) 42-48.

[29] I. Murgia, M. Concetta de Pinto, M. Delledonne, C. Soave, L. De Gara, Comparative effects of various nitric oxide donors on ferritin regulation, programmed cell death, and cell redox state in plant cells, Journal of Plant Physiology 161(7) (2004) 777-783.

[30] V. Ziogas, G. Tanou, M. Belghazi, P. Filippou, V. Fotopoulos, D. Grigorios, A. Molassiotis, Roles of sodium hydrosulfide and sodium nitroprusside as priming molecules during drought acclimation in citrus plants, Plant molecular biology 89(4-5) (2015) 433-50.

[31] L.L. Fershtat, E.S. Zhilin, Recent Advances in the Synthesis and Biomedical Applications of Heterocyclic NO-Donors, Molecules 26(18) (2021) 5705.

[32] I.Y.A. Cherepanov, S.K. Moiseev, Chapter Two - Recent developments in the chemistry of sydnones and sydnone imines, in: E.F.V. Scriven, C.A. Ramsden (Eds.), Advances in Heterocyclic Chemistry, Academic Press (2020) 49-164.

[33] V. Ol'shevskaya, I. Cherepanov, S.G. Spiridonov YuA, A. Makarenkov, A. Samarskaya, Herbicidal activity of carboranes, sydnone imine and ferrocene derivatives, Agrokhimiya 4 (2017) 16-21.

[34] P. Francisc, I. Ioana, Anthropogenic Air Pollution Sources, (2010).

[35] B.J. Locey, Nitrites*, in: P. Wexler (Ed.), Encyclopedia of Toxicology (Second Edition), Elsevier, New York, 2005, 232-235.

[36] J.T. Hancock, Nitric Oxide Signaling in Plants, Plants (Basel, Switzerland) 9(11) (2020).

[37] E. Baudouin, J.T. Hancock, Nitric oxide signaling in plants, Front Plant Sci 4 (2013) 553.

[38] P.D. Lokhande, B. Kuchekar, A. Chabukswar, S. Jagdale, Nitric Oxide: Role in Biological System, Asian Journal of Biochemistry 1 (2006) 1-17.

[39] S.H. Francis, J.L. Busch, J.D. Corbin, cGMP-Dependent Protein Kinases and cGMP Phosphodiesterases in Nitric Oxide and cGMP Action, Pharmacological Reviews 62(3) (2010) 525-563.

[40] F. Murad, C.K. Mittal, W.P. Arnold, S. Katsuki, H. Kimura, Guanylate cyclase: activation by azide, nitro compounds, nitric oxide, and hydroxyl radical and inhibition by hemoglobin and myoglobin, Advances in cyclic nucleotide research 9 (1978) 145-58.

[41] E. Bohme, C. Spies, G. Grossmann, J. Herz, Stimulation of soluble guanylate cyclase by sydnone imines; relaxants of smooth muscle and inhibitors of platelet aggregation, Naunyn Schmiedebergs Arch Pharmacol [Suppl] 316 (1981) R26.

[42] R.F. Furchgott, J.V. Zawadzki, The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine, Nature 288(5789) (1980) 373-6.

[43] R.M. Palmer, A.G. Ferrige, S. Moncada, Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor, Nature 327(6122) (1987) 524-6.

[44] M. Delledonne, Y. Xia, R.A. Dixon, C. Lamb, Nitric oxide functions as a signal in plant disease resistance, Nature 394(6693) (1998) 585-8.

[45] J. Durner, D. Wendehenne, D.F. Klessig, Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose, Proceedings of the National Academy of Sciences of the United States of America 95(17) (1998) 10328-33.

[46] Y.A.Y. Leshem, E. Haramaty, The Characterization and Contrasting Effects of the Nitric Oxide Free Radical in Vegetative Stress and Senescence of Pisum sativum Linn. Foliage, Journal of Plant Physiology 148(3) (1996) 258-263.

[47] L.A.J. Mur, J. Mandon, S. Persijn, S.M. Cristescu, I.E. Moshkov, G.V. Novikova, M.A. Hall, F.J.M. Harren, K.H. Hebelstrup, K.J. Gupta, Nitric oxide in plants: an assessment of the current state of knowledge, AoB PLANTS 5 (2013).

[48] S. Hiscock, J. Bright, S.M. McInnis, R. Desikan, J.T. Hancock, Signaling on the Stigma, Plant Signaling & Behavior 2(1) (2007) 23-24.

[49] S.A. Reichler, J. Torres, A.L. Rivera, V.A. Cintolesi, G. Clark, S.J. Roux, Intersection of two signalling pathways: extracellular nucleotides regulate pollen germination and pollen tube growth via nitric oxide, Journal of Experimental Botany 60(7) (2009) 2129-2138.

[50] J. Šírová, M. Sedlářová, J. Piterková, L. Luhová, M. Petřivalský, The role of nitric oxide in the germination of plant seeds and pollen, Plant Science 181(5) (2011) 560-572.

[51] E. Arc, M. Galland, B. Godin, G. Cueff, L. Rajjou, Nitric oxide implication in the control of seed dormancy and germination, Front Plant Sci 4 (2013) 346.

[52] N. Correa-Aragunde, M. Graziano, L. Lamattina, Nitric oxide plays a central role in determining lateral root development in tomato, Planta 218(6) (2004) 900-5.

[53] M.C. Lombardo, M. Graziano, J.C. Polacco, L. Lamattina, Nitric oxide functions as a positive regulator of root hair development, Plant Signal Behav 1(1) (2006) 28-33.

[54] S. Neill, R. Barros, J. Bright, R. Desikan, J. Hancock, J. Harrison, P. Morris, D. Ribeiro, I. Wilson, Nitric oxide, stomatal closure, and abiotic stress, J Exp Bot 59(2) (2008) 165-76.

[55] G. Gayatri, S. Agurla, A.S. Raghavendra, Nitric oxide in guard cells as an important secondary messenger during stomatal closure, Front Plant Sci 4 (2013) 425.

[56] N.N. Fancy, A.K. Bahlmann, G.J. Loake, Nitric oxide function in plant abiotic stress, Plant, cell & environment 40(4) (2017) 462-472.

[57] S.S. Gill, M. Hasanuzzaman, K. Nahar, A. Macovei, N. Tuteja, Importance of nitric oxide in cadmium stress tolerance in crop plants, Plant physiology and biochemistry : PPB 63 (2013) 254-61.

[58] A. Tailor, R. Tandon, S.C. Bhatla, Nitric oxide modulates polyamine homeostasis in sunflower seedling cotyledons under salt stress, Plant Signal Behav 14(11) (2019) 1667730.

[59] K.K. Rai, N. Pandey, S.P. Rai, Salicylic acid and nitric oxide signaling in plant heat stress, Physiologia plantarum 168(2) (2020) 241-255.

[60] D.I. Lytvyn, C. Raynaud, A.I. Yemets, C. Bergounioux, Y.B. Blume, Involvement of Inositol Biosynthesis and Nitric Oxide in the Mediation of UV-B Induced Oxidative Stress, Front Plant Sci 7 (2016) 430.

[61] L.A.J. Mur, C. Simpson, A. Kumari, A.K. Gupta, K.J. Gupta, Moving nitrogen to the centre of plant defence against pathogens, Annals of botany 119(5) (2017) 703-709.

[62] C. García-Mata, L. Lamattina, Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress, Plant physiology 126(3) (2001) 1196-204.

[63] Y. Zhao, W. Ma, X. Wei, Y. Long, Y. Zhao, M. Su, Q. Luo, Identification of Exogenous Nitric Oxide-Responsive miRNAs from Alfalfa (Medicago sativa L.) under Drought Stress by High-Throughput Sequencing, Genes 11(1) (2019).

[64] P. Santisree, P. Bhatnagar-Mathur, K.K. Sharma, NO to drought-multifunctional role of nitric oxide in plant drought: Do we have all the answers?, Plant science : an international journal of experimental plant biology 239 (2015) 44-55.

[65] H. Kankaanranta, R.G. Knowles, P. Vuorinen, O. Kosonen, P. Holm, E. Moilanen, 3-Morpholino-Sydnonimine-Induced Suppression of Human Neutrophil Degranulation is Not Mediated by Cyclic GMP, Nitric Oxide or Peroxynitrite: Inhibition of the Increase in Intracellular Free Calcium Concentration by *N*-Morpholino-iminoacetonitrile, a Metabolite of 3-Morpholino-Sydnonimine, Molecular Pharmacology 51(5) (1997) 882-888.
[66] E.Y. Khmel nitskaya, V.I. Levina, L.A. Trukhacheva, N.B. Grigoriev, V.N. Kalinin, I.A. Cherepanov, S.N. Lebedev, V.G. Granik, Sydnonimines as exogenous NO donors, Russian Chemical Bulletin 53 (2004) 2840-2844.
[67] V.G. Yashunskii, L. Kholodov, The chemistry of sydnone imines, Russian Chemical Reviews 49(1) (1980) 28.
[68] C.G. Newton, C.A. Ramsden, Meso-ionic heterocycles (1976–1980), Tetrahedron 38(20) (1982) 2965-3011.
[69] E.Y. Khmel'nitskaya, V.I. Levina, L.A. Trukhacheva, N.B. Grigoriev, V.N. Kalinin, I.A. Cherepanov, S.N. Lebedev, V.G. Granik, Sydnonimines as exogenous NO donors, Russian Chemical Bulletin 53(12) (2004) 2840-2844.
[70] W. Rademacher, Plant Growth Regulators: Backgrounds and Uses in Plant Production, Journal of Plant Growth Regulation 34(4) (2015) 845-872.
[71] B.S. Ingelheim, Sydnonimine derivatives, GB 1219254A, 1971.
[72] M. Katsutada, I. Yoshio, Sydnonimine derivatives, US3312690A, 1967.
[73] Hiroyoshi Hidaka, Ikuo Matsumoto, Junji Yoshizawa, Shigenori Kotani, Syndonimine derivatives, process for production thereof, and use thereof, US4421754A, 1983.
[74] P. Provost, J. Tremblay, Y. Merhi, The Antiadhesive and Antithrombotic Effects of the Nitric Oxide Donor SIN-1 Are Combined With a Decreased Vasoconstriction in a Porcine Model of Balloon Angioplasty, Arteriosclerosis, Thrombosis, and Vascular Biology 17(9) (1997) 1806-1812.
[75] R. Gerzer, B. Karrenbrock, W. Siess, J.M. Heim, Direct comparison of the effects of nitroprusside, SIN 1, and various nitrates on platelet aggregation and soluble guanylate cyclase activity, Thrombosis Research 52(1) (1988) 11-21.
[76] L. D'Iakonova T, G.E. Samonina, The naloxone-dependent effects of the psychostimulant sidnofen: a study on identified neurons of the snail, Zhurnal vysshei nervnoi deiatelnosti imeni I P Pavlova 44(4-5) (1994) 786-95.
[77] M. D.Mashkovsky, V. G. Yashunsky, R. A. Altshuller, L. E. Knolodov, G. Y. Avrutsky, J. A. Alexandrovsky, A. B. Smulevich, Novel sydnonimine derivative, GB1262830A, 1972.
[78] B. Rosenkranz, B.R. Winkelmann, M.J. Parnham, Clinical Pharmacokinetics of Molsidomine, Clinical Pharmacokinetics 30(5) (1996) 372-384.
[79] Y.Y.Spiridonov. I. A. Cherepanov, O. A. Chichvarina, A. S. Samarskaya, S.K.Moiseev. A. B. Ponomaryov, Growth Stimulating Activity of Sydnonimine Derivatives, Agrochemistry 9 (2018) 50-55.
[80] I.A. Cherepanov, E.V. Shevaldina, D.A. Lapshin, Y.Y. Spiridonov, V.A. Abubikerov, S.K. Moiseev, 4-lithiosydnone imines: Generation and stability. Plant growth regulating activity of 4-hydroxymethyl derivatives of sydnone imines, Journal of Organometallic Chemistry 943 (2021) 1218-41.
[81] Y.Y.Spiridonov, I. A. Cherepanov, V.A. Abubikerov , Y.Y.Spiridonova , N. V. Kalganova, D. A. Lapshin , C. K. Moiseev, Comparative study of growth stimulating effects of sydnone imine derivatives on corn, sunflower and winter wheat, Agrochemistry (4) (2022) 36-45.
[82] Y.Y.Spiridonov, I. A. Cherepanov, S. K. Moiseev, N. G. Frolova, Substituted sydnone imines as herbicides antidotes, RU2772224C1, 2022.
[83] A.S. Lukatkin, A.S.Gurianov, I.A. Cherepanov, S.K. Moiseev., Evaluation of the potential use of a new class of regulators to stimulate early growth of wheat. , II Republican Scientific Conference "Adaptation of living organisms to changing environmental conditions", MN "Donish", Tajikistan, Dushanbe, 2021, pp. 21-25.
[84] A.S. Guryanova, A.S. Lukatkin, Galkina A.A., Kalganova N.V., Cherepanov I.A, Moiseev S.K. , Protective effect of oxadiazolonium derivatives on growth and temperature stress markers in Triticum aestivum L. and Zea mays L. plants III International Scientific Conference "Cell Biology and Plant Biotechnology", BSU, Republic Belarus, Minsk, 2022, p. 90.
[85] A.A. Galkina, O. V. Ignatieva, A.S. Lukatkin, N.V. Kalganova. , The effectiveness of oxadiazolonium derivatives under the effect of temperature stress on corn plants, Ecology of the native land:problems and solutions, Kirov, 2022, 228-231.
[86] A. S. Gurianova, A.S. Lukatkin, A. A. Galkina, N. V. Kalganova, I. A. Cherepanova, S. K. Moiseev, Modification of canola adaptive responses to stress by growth regulators in: BSU (Ed.) III International Scientific and Practical Conference "Cell Biology and Plant Biotechnology", Republic of Belarus, Minsk, 2022, 90-91.