

Phytotoxicity of New Furan-derived Aminophosphonic Acids, *N*-Aryl Furaldimines and 5-Nitrofuraldimine

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S Supporting Information

ABSTRACT: The aim of this work was to synthesize selected furaldimines and their aminophosphonic derivatives and evaluation the phytotoxicity of new obtained products according to OECD 208 Guideline. Four Schiff bases, *N*-furfurylidene-*p*-anisidine (**1a**), *N*-furfurylidene-*p*-toluidine (**1b**), *N*-furfurylidene-benzhydramine (**1c**), and *N*-(2-nitrofurfurylidene)-*p*-toluidine (**1d**) were synthesized and three new furan-derived *N*-substituted aminomethylphosphonic acids, namely: 2-furyl *N*-(*p*-methoxyphenyl)-aminomethylphosphonic acid (**2a**), 2-furyl *N*-(*p*-methylphenyl)-aminomethylphosphonic acid (**2b**) and 2-furyl *N*-(diphenylmethyl)-aminomethylphosphonic acid (**2c**) were synthesized by the addition of *in situ* generated bis-(trimethylsilyl) phosphite to azomethine bond of corresponding Schiff bases **1a–c**. Three Schiff bases **1a–b** and **1d** as well as all three aminophosphonic acids **2a–c** were analyzed in regard with their phytotoxicity toward two plants, radish (*Raphanus sativus*) and oat (*Avena sativa*).

It has been found that tested *N*-furfurylidene-*p*-anisidine (**1a**), *N*-(2-nitrofurfurylidene)-*p*-toluidine (**1d**) and aminophosphonic acids **2a–c** are toxic for selected plants. *N*-furfurylidene-*p*-toluidine (**1b**) did not show any ecotoxicological impact in used plant growth test.

KEYWORDS: furaldimines, 5-nitrofuraldimine, Schiff bases, aminophosphonic acids, phytotoxicity test, environmental protection, ecotoxicology, OECD standard

INTRODUCTION

It is well-known that aminophosphonic systems are highly biologically active compounds especially being inhibitors of various enzymes.^{1,2} Aminophosphonates has been also attracted much as pesticides with potential application in agrochemistry and their synthesis and assessment of aminophosphates bioactivity in plants has been already reported.^{3–5}

Nowadays there is a great problem associated with the application of conventional pesticides or fertilizers causing undesirable side effect after application. The presence of pesticides soil, water, and air has raised concerns for the protection of the natural environment. Unfortunately, only a part of the applied amount of pesticide reach their targets, therefore, the excess amount of these agrochemicals is distributed into the environment. Therefore, over the last decades, much effort has been put into the research of novel, intelligent crop protection chemicals in order to reduce their environmental impact.

On the other hand, furans play their important biological function and it does not seem strange that there were many successful attempts to construct drugs bearing furyl moiety. It is to mention here cefuroxime, the cephalosporin antibiotics or a histamine H₂-receptor antagonist-ranitidine. But the largest and most common group of drugs bearing a furan moiety derives from 5-nitrofurfural. Their action is based on the redox enzymatic reaction of nitrofurfural, which is reduced by nitroreductase enzymes to an active radical reacting with nucleic acids and proteins.^{6–8} Although nitrofurfural-deriving antimicrobial chemotherapeutics (nitrofurantoin, nifuroxazide

etc.) became generally less popular due to adverse effects, it is to stress that nifurtimox, apart from benznidazole is a second effective drug for treatment of *Trypanosoma cruzi* or *Trypanosoma brucei* infections (Chagas disease and sleeping sickness).⁹ It is then obvious that studies on antimicrobial properties on 5-nitrofurfural Schiff bases are being performed, as this group is structurally similar to microbiologically active 5-nitrofurfural hydrazones.

Recently, aminophosphonates bearing furan and *N*-methoxy- or *N*-methylphenyl moieties have been found to have important *in vitro* action toward various types of cancer such as human leukemia cells or squamous esophageal cancer cells.^{10,11} These reports demonstrated also cytotoxic properties of their parent 2-furaldimines.

Boduszek's studies¹² revealed that the tripeptide bearing furylaminophosphonic moiety is the irreversible inhibitor of chymotrypsin, human neutrophilic elastase and pig pancreas elastase. Moreover, biological studies of diphenyl (2-furyl)-*N*-benzylaminomethyl-phosphonate demonstrated its herbicidal action inhibiting the root growth of *Lepidium sativum* or *Cumulus sativus*.¹²

Having in mind that furan-derived aminophosphonic compounds as well as their parent imines demonstrate the general biological activity, we decided to investigate, what will

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be their action on plant growth. Therefore, objectives of this study were to evaluate the phytotoxicity of three biologically active^{10,11} furaldimines: *N*-furfurylidene-*p*-anisidine (**1a**), *N*-furfurylidene-*p*-toluidine (**1b**), and *N*-(2-nitrofurfurylidene)-*p*-toluidine (**1d**) and three newly synthesized aminophosphonic acids bearing furan moiety, namely: 2-furyl *N*-(*p*-methoxyphenyl)aminomethylphosphonic acid (**2a**), 2-furyl *N*-(*p*-methylphenyl)aminomethylphosphonic acid (**2b**), and 2-furyl *N*-(diphenylmethyl)aminomethylphosphonic acid (**2c**) toward *Raphanus sativus* (radish) and *Avena sativa* (oat). The phytotoxicity evaluation was performed using OECD 208, Terrestrial Plant Growth Test, as its application to the polymeric degradation products or ionic liquids has been successfully reported in literature.^{13,14}

The study of phytotoxicity was not performed for the Schiff base **1c**, because this compound is not known to be biologically active and therefore it was out of our interest in the structural and functional point of view.

MATERIALS AND METHODS

Materials and Chemicals. All solvents (POCH, Poland) were routinely distilled and dried prior to use. Amines, diethyl phosphite, and furfural (Aldrich, Poland) were used as received. NMR spectra were recorded on a Bruker Avance III 600 MHz operating at 600 MHz (¹H NMR) and 243 MHz (³¹P NMR). TMS was used as the internal standard for ¹H NMR and phosphoric acid was used as the external standard for ³¹P NMR. Elemental analyses were carried out at the Centre for Molecular and Macromolecular Science of the Polish Academy of Science in Łódź, Poland.

Synthesis of Schiff Bases 1a–d. Furfural derivative (2.5 mmol) was dissolved in methanol (15 mL) and to this solution, amine (2.5 mmol) was added. The mixture was stirred at room temperature for 24 h, then solvent was evaporated and residue dried on vacuum to obtain pure Schiff base **1a–d**:

N-Furfurylidene-*p*-anisidine (**1a**). Quantitative yield (0.50 g), mp = 60–64 °C, 68–70 °C.¹⁵ ¹H NMR (CDCl₃, 600 MHz): δ 8.30 (s, CH=N, 1H), 7.59 (d, J = 1.8 Hz, H₅^{fur}, 1H), 7.25 (d, J = 9.0 Hz, *p*-C₆H₄, 2H), 6.92 (d, J = 9.0 Hz, *p*-C₆H₄, 2H), 6.91 (d, J = 3.6 Hz, H₃^{fur}, 1H), 6.54 (dd, J = 3.6 and 1.8 Hz, H₄^{fur}, 1H), 3.82 (s, OCH₃, 3H).

N-Furfurylidene-*p*-toluidine (**1b**). Quantitative yield (0.46 g), mp = 35–38 °C, 41–42 °C.¹⁰ ¹H NMR (CDCl₃, 600 MHz): δ 8.30 (s, CH=N, 1H), 7.59 (d, J = 1.8 Hz, H₅^{fur}, 1H), 7.19–7.15 (AA'BB' system, J = 9.0 Hz, *p*-C₆H₄, 4H), 6.92 (d, J = 3.6 Hz, H₃^{fur}, 1H), 6.54 (dd, J = 3.6 and 1.8 Hz, H₄^{fur}, 1H), 2.36 (s, CH₃, 3H).

N-Furfurylidene-benzhydrylamine (**1c**). Quantitative yield (0.65 g), mp = 104–105 °C, 105–106 °C.¹⁶ ¹H NMR (CDCl₃, 600 MHz): δ 8.19 (s, CH=N, 1H), 7.53 (d, J = 1.8 Hz, H₅^{fur}, 1H), 7.36–7.34 (m, C₆H₅, 4H), 7.32–7.29 (m, C₆H₅, 4H), 7.24–7.22 (m, C₆H₅, 2H), 6.81 (dd, J = 3.6 and 0.6 Hz, H₃^{fur}, 1H), 6.47 (dd, J = 3.6 and 1.8 Hz, H₄^{fur}, 1H), 5.58 (s, CH, 1H).

N-(2-Nitrofurfurylidene)-*p*-toluidine (**1d**). Quantitative yield (0.57 g), mp = 131–133 °C, 130–130.5 °C.¹⁷ ¹H NMR (CDCl₃, 600 MHz): δ 8.41 (s, CH=N, 1H); 7.42 (dd, J = 4.2 and 0.6 Hz, H₃^{fur}, 1H); 7.24–7.20 (m, AA'BB' system, *p*-C₆H₄, 4H); 7.17 (dd, J = 4.2 and 0.6 Hz, H₄^{fur}, 1H); 2.39 (s, CH₃, 3H).

General Procedure for Preparation of Aminophosphonic Acids 2a–c. Furfural (2.5 mmol, 0.24 g) was dissolved in methanol (15 mL) and to this solution, amine (2.5 mmol) was added. The mixture was stirred at room temperature for 24 h, and obtained imine was used for further conversion without being isolated. To confirm completion of the reaction, 1 mL sample was taken, evaporated and dissolved in CDCl₃ and ¹H NMR spectrum was recorded.

Diethyl phosphite (2.5 mmol) was dissolved in dry dichloromethane (10 mL) and to this solution bromotrimethylsilane (6.8 mmol, 0.9 mL) was added dropwise for 10–15 min. The mixture was stirred for 1 h at room temperature, a solution of an appropriate imine (2.5 mmol) in dry dichloromethane (10 mL) was added and the mixture was refluxed for 4 h. Then, the reaction mixture was

concentrated under reduced pressure and the residue was dissolved in dry methanol and it was stirred for 30–45 min until precipitation of a solid, which was filtered off and collected. In a case, if the solid did not precipitated, 10–20 mL of propylene oxide was added and the mixture was refrigerated for 3–7 days and the solid precipitate was collected by filtration.

(2-Furyl)-*N*-(4-methoxyphenyl)aminomethylphosphonic Acid (**2a**). Yield: 0.49 g (69%); Mp: 177–180 °C. ¹H NMR (600 MHz, DMSO-*d*₆): δ 7.52 (d, J = 1.8 Hz, H₅^{fur}, 1H); 6.74 (d, J = 9.0 Hz, *p*-C₆H₄, 2H); 6.68 (d, J = 9.0 Hz, *p*-C₆H₄, 2H); 6.35 (dd, J = 1.8 and 3.2 Hz, H₄^{fur}, 1H); 6.32 (d, J = 3.2 Hz, H₃^{fur}, 1H); 4.63 (d, ²J_{PH} = 20.0 Hz, CHP, 1H); 3.62 (s, OCH₃, 3H). ¹³C NMR (250 MHz, DMSO-*d*₆): δ 152.67 (C_{arom}); 151.96 (C_{arom}); 142.32 (d, ³J_{CP} = 3.5 Hz, C_{fur}); 141.95 (d, ²J_{CP} = 23.0 Hz, C_{fur}); 114.95 (C_{arom}); 114.83 (C_{arom}); 110.84 (d, ⁵J_{CP} = 2.9 Hz, C_{fur}); 108.01 (d, ⁴J_{CP} = 9.7 Hz, C_{fur}); 55.73 (OCH₃); 51.69 (d, ¹J_{CP} = 153.5 Hz, C–P). ³¹P NMR (243 MHz, DMSO-*d*₆): δ 15.85. *Elem. anal.* Calcd. for C₁₂H₁₄NO₅P: C, 50.89; H, 4.98; N, 4.95. Found: C, 50.69; H, 4.97; N, 5.07.

(2-Furyl)-*N*-(4-methylphenyl)aminomethylphosphonic Acid (**2b**). Yield: 0.47 g (70%); Mp: 120–125 °C. ¹H NMR (600 MHz, DMSO-*d*₆): δ 7.52 (m, H₅^{fur}, 1H); 6.86 and 6.62 (AA'XX' system, ³J = 8.4, ⁴J = 1.8 Hz, *p*-C₆H₄, 2H); 6.35 (m, H₄^{fur}, 1H); 6.32 (m, H₃^{fur}, 1H); 4.35 (d, ²J_{PH} = 24.0 Hz, CHP, 1H); 2.15 (s, CH₃, 3H). ¹³C NMR (250 MHz, DMSO-*d*₆): δ 152.52 (C_{arom}); 145.61 (d, ²J_{CP} = 21.6 Hz, C_{fur}); 142.35 (d, ³J_{CP} = 3.5 Hz, C_{fur}); 129.61 (C_{arom}); 125.86 (C_{arom}); 113.89 (C_{arom}); 110.84 (d, ⁵J_{CP} = 2.9 Hz, C_{fur}); 108.03 (d, ⁴J_{CP} = 9.6 Hz, C_{fur}); 51.07 (d, ¹J_{CP} = 153.5 Hz, C–P); 20.50 (CH₃). ³¹P NMR (243 MHz, DMSO-*d*₆): δ 15.76. *Elem. anal.* Calcd. for C₁₂H₁₄NO₄P•²/₃H₂O: C, 51.62; H, 5.53; N, 5.02. Found: C, 51.59; H, 5.49; N, 4.96.

(2-Furyl)-*N*-(diphenylmethyl)aminomethylphosphonic Acid (**2c**). Yield: 0.55 g (64%); Mp: 138–140 °C. ¹H NMR (600 MHz, DMSO-*d*₆): δ 7.61–7.59 (d, J = 1.8 Hz, H₅^{fur}, 1H); 7.45–7.41 (m, PhH, 2H); 7.33–7.31 (m, PhH, 4H); 7.30–7.27 (m, PhH, 2H); 7.25–7.19 (m, PhH, 2H); 6.44 (dd, J = 1.8 and 3.6 Hz, H₄^{fur}, 1H); 6.29–6.28 (m, H₃^{fur}, 1H); 4.75 (s, CH, 1H); 3.73 (d, ²J_{PH} = 20.0 Hz, CHP, 1H). ¹³C NMR (250 MHz, DMSO-*d*₆): δ 152.34 (C_{fur}); 144.62 (C_{arom}); 142.81 (d, ³J_{CP} = 39.8 Hz, C_{fur}); 128.90 (d, ²J_{CP} = 15.0 Hz, C_{arom}); 127.78 (C_{arom}); 127.52 (d, ⁶J_{CP} = 4.5 Hz, C_{arom}); 110.96 (C_{fur}); 108.41 (d, ⁴J_{CP} = 6.0 Hz, C_{fur}); 64.42 (d, ³J_{CP} = 14.1 Hz, CH); 53.35 (d, ¹J_{CP} = 154.5 Hz, C–P). ³¹P NMR (243 MHz, DMSO-*d*₆): δ 16.40. *Elem. anal.* Calcd. for C₁₈H₁₈NO₄P: C, 62.97; H, 5.28; N, 4.08. Found: C, 62.82; H, 5.39; N, 4.33.

Evaluation of Potential Toxicity of New Synthesized Compounds. The plant growth test was performed under laboratory conditions by adapting the OECD 208 Terrestrial Plants Growth Test and the PN-ISO 11269–2:2001 International Standard for furan-derived aminophosphonic acids **2a–c**, *N*-aryl furaldimines **1a–b** and 5-nitrofuraldimine **1d**, using oat (*Avena sativa*) as a monocotyledonous plant and common radish (*Raphanus sativus* L. subvar. *radicula* Pers.), a dicotyledonous plant.^{18,19}

According to mentioned above standards the plant growth test of synthesized samples was carried out in sandy soil characterized with parameters as follows: granulometric composition of soil –80% sand, 12% dust and loam, organic carbon content of approximately 0.9%, pH(KCl) equal to 5.8 and moisture ranged from 15 to 18%.

To prepare the test, pots made from polypropylene (diameter of 90 mm and capacity 300 cm³) were filled, with the control soil and with the soil with the test furaldimines and their aminophosphonic derivatives added at a specific concentration. Twenty identical seeds of each of the selected plant species, originating from the same source, were sown into the soil. Plants were grown for 14 days under controlled conditions maintaining constant humidity, light intensity (7000 lx), and temperature, 20 ± 2 °C. After that time, seedlings were counted and the dry and fresh weight of the plants above the soil surface was determined. After that time, seedlings were counted and the dry and fresh parts of the plants above the soil was weight.

The performed plant growth test comprised two testing steps: a preliminary test and a final, particular test. According to PN-ISO 11269–2:2001 standard, the preliminary test was carried out to determine the range of concentrations of compounds affecting the soil

Scheme 1. Synthesis of Schiff Bases 1a–d and Acids 2a–c

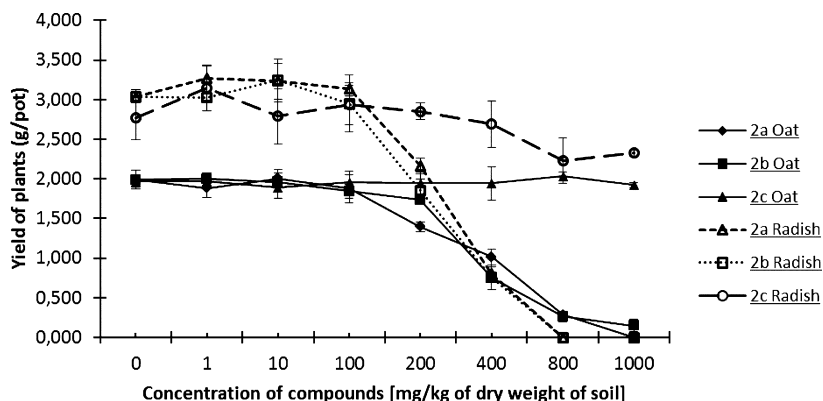
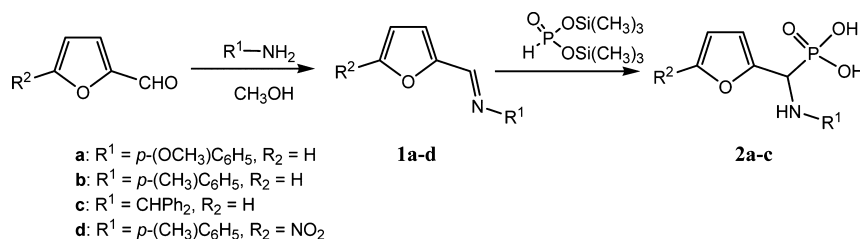


Figure 1. Changes of fresh weight value of oat and radish sprouts in toxicity test for aminophosphonic derivatives (aminophosphonic acids 2a–c).

quality, therefore prepared samples were introduced to the soil at the following concentrations: 0 mg (control), and 1 mg, 10 mg, 100 mg, and 1000 mg/kg of soil dry weight.

In the final test, concentrations were arranged in a geometric progression with a factor of 2, starting from the lowest concentration that reduces the germination and growth of plants.

In the experiment for aminophosphonic derivatives (2a–c), this concentration was found to be 1000 mg of the tested compound per 1 kg of soil; therefore, concentrations of 200 mg, 400 mg, and 800 mg/kg of soil dry weight were used in the final tests.

In preliminary test, the toxic concentration of compound 1a was found to be 100 mg per kg of dry weight of soil, with this respect, the concentrations 20 mg, 40 mg, and 80 mg/kg of soil dry weight, respectively, were used in the final tests.

In case of the compound 1d, the mentioned concentration equals 10 mg/kg of soil, hence the concentrations 2, 4, and 6 mg/kg of soil dry weight has been prepared.

The aminophosphonic derivatives (2a–c) were introduced to the soil in the form of water solutions. The compounds (1a–b, 1d) were dissolved in acetone, poured into the pots filled with 70 g of sand and mixed together. After total evaporation of used solvent, the sand with a tested compound was thoroughly mixed with 180g of soil. The final test for compound 1b was not necessary to be made, because no toxicity in preliminary test has been demonstrated.

Evaluation of phytotoxicity of tested substances at applied concentrations was performed by comparison of the germination and (dry and fresh) weight of control plant sprouts (seedlings) with germination and (dry and fresh) of plants sprouts grown in the soil contaminated with appropriate amounts of the tested compounds.

The dry weight of the tested plants was determined after drying at 75 °C until constant weight was achieved.

The visual assessment was carried out by digital photography; pictures were analyzed in the point of view of any type of damage to the test species, such as growth inhibition, chlorosis and necrosis of growing plants. Tests for each sample were carried out in triplicate.

Evaluation of the significance of the obtained results was performed using an analysis of variance (Fisher-Snedecor's F test), whereas the least significant difference ($\text{LSD}_{0.95}$) values were calculated using Tukey's test.

Taking into account obtained results, the magnitudes of the LOEC (the lowest observed effect concentration)—the lowest concentration causing observable effects in the form of a reduction in growth and germination compared with the control—and the NOEC (no observed effect concentration)—the highest concentration not causing observable, toxic effects—were also determined.

RESULTS AND DISCUSSION

Synthesis of Studied Schiff Bases 1a–d and Aminophosphonic Acids 2a–c. Schiff bases 1a–d were synthesized following the previously published procedure by simple mixing furfural with appropriate amine in methanol and stirring them at room temperature for 24 h.²⁰ This procedure gave imines 1a–d in quantitative yield. Their identity was confirmed by melting point measurement and ^1H NMR spectral data, which were compared with literature data.^{10,15–17} The ^1H NMR spectra showed the diagnostic singlet of a proton of the azomethine group ($-\text{CH}=\text{N}-$) and signals of furan protons.

Aminophosphonic acids 2a–c were synthesized by the addition of bis-(trimethylsilyl) phosphite to the azomethine bond of corresponding Schiff bases 1a–c. Bis-(trimethylsilyl) phosphite has been prepared in situ by the reaction of diethyl phosphite with bromotrimethylsilane in dichloromethane,²¹ and its reaction with previously prepared Schiff bases 1a–c was carried out in dichloromethane. The conversion was monitored by means of ^{31}P NMR and when the reaction was complete, it was quenched by methanolysis. After addition of propylene oxide, resulting acids 2a–c were obtained in 60–70% yields. (Scheme 1) Their identity has been verified by means of the ^1H , ^{13}C , and ^{31}P NMR spectroscopy. The ^1H NMR spectra of acids 2a–c showed diagnostic signals typical for aminophosphonic acids, such as a doublet of a HCP group and typical for 2-substituted furans, that is, two doublets of H^5 and H^3 and a doublet of doublets of H^4 . ^{13}C NMR spectra of acids 2a–c showed a diagnostic doublet at around 50 ppm having a coupling constant oscillating around 150 Hz corresponding to a CP bond, which is also typical for aminophosphonic acids.

Their purity was confirmed by the elemental analysis and melting point measurements.

Preliminary Test of Phytotoxicity Evaluation for Compounds 1a–b, 1d and 2a–c. Schiff bases were reported to be investigated in soil, for example, for their effect on nitrification inhibition²² and applied conditions were similar to conditions used for our studies. Authors²² did not report any symptom of problems with their stability, therefore the tested Schiff bases were a priori considered to be stable under conditions of the described experiment.

Preliminary tests for aminophosphonic acids 2a–b, in concentrations up to 100 mg/kg of dry weight soil did not reveal any significant, negative effect on the germination and growth of tested plants. The first negative effect of used aminophosphonic acids 2a–b was noticed at the concentration 1000 mg/kg of dry weight of soil (Figure 1) and, in the highest applied concentration (1000 mg/kg), they were totally toxic for both radish and oat seeds, and no germination have been observed (except for the very slight germination of oat in a test with compound 2b).

Assessment of the toxicity of aminophosphonic acid 2c for oat have shown statistically insignificant influence on the germination and growth of this plant. It is slightly toxic for radish causing a drop in the fresh weight of sprouts below 90% (Figure 1).

Values obtained for the preliminary plant growth test for the furaldimine 1b and the visual examination of tested plants (see Supporting Information (SI) Figure S1) have not shown any significant, negative effect on the germination and growth of both tested plants even in concentration 1000 mg/kg of dry weight soil, so the Schiff base 1b is not toxic for any of them.

The first negative symptom of *N*-furfurylidene-*p*-anisidine (1a) such as drop of crop fresh weight (g/pot), has been observed for both tested plants at the concentration 100 mg/kg of dry weight of soil (Table 1). At the highest concentration of

between *N*-furfurylidene-*p*-toluidine (1b) and *N*-furfurylidene-*p*-anisidine (1a) is the substituent of the phenyl moiety, that is, the methyl and methoxy group, respectively, which points out to the methoxy moiety to play a key role in toxicity effect against tested plants (see SI Figure S1).

Taking into account results obtained during plant growth test for the 5-nitrofuraldimine 1d (Table 1), the concentration of 10 mg/kg dry weight of soil was toxic for both plants decreasing a fresh weight of emerged sprouts, (see SI Tables S3 and S4). Visual evaluation of a plant growth test for compound 1d is presented in SI Figure S2.

Based on data collected in Table 1, inhibitory effect of compound 1a on oat fresh matter (crop) at concentration of 100 mg/kg soil was found to be 25%, while for higher concentration, (1000 mg/kg of soil) inhibition has reached 34%. Its inhibitory effect on radish at concentration level 70, 100, and 1000 mg per kg of soil reached 30, 48, and 100%, respectively. 5-Nitrofuraldimine 1d revealed to be even more toxic than the imine 1a for both tested plants. In case of oat, essential inhibition was observed at concentrations 100 and 1000 mg/kg of soil reaching 63 and 95%, respectively. 5-Nitrofuraldimine 1d was completely toxic for the radish at concentration of 100 and 1000 mg/kg of soil and inhibitory effect has reached 100%. From among both tested plants, the radish revealed to be more sensitive against tested compounds 1a and 1d.

Final Test of Phytotoxicity of Imines 1a, 1d and Aminophosphonic Acids 2a–c. Based on results obtained in a preliminary plant growth test for all used compounds, according to the PN-ISO 11269–2:2001 standard, only the imine 1b did not require any additional final test, because at the highest applied concentration, this compound was found to be toxic neither for monocotyledonous nor for dicotyledonous plants.

As mentioned in the Materials and Methods section, the concentrations at the final test were prepared in a geometric progression with a factor of 2, starting from the lowest concentration that reduces the crop, germination or plant growth. With this respect, for aminophosphonic acids 2a–c, this concentration was found to be 1000 mg of the test compound per 1 kg of soil; therefore, concentrations used in the final tests were 200 mg, 400 mg, and 800 mg/kg of soil dry weight. In case of the *N*-(2-nitrofurfurylidene)-*p*-toluidine (1d), the lowest toxic concentration was found to be 10 mg/kg of soil, hence, its concentrations used for a final test were 2, 4, and 8 mg/kg of soil dry weight.

Increasing the concentration of compounds 2a–c (only for radish) from 200 to 800 mg/kg of the soil dry weight resulted in a systematic decrease in the crop fresh weight of total sprouts and the crop fresh weight per plant as well as a large increase in dry weight, both for oat and for common radish (Figure 1).

Basing on the results obtained for compounds 2a and 2b in the final test, the NOEC, that is, the highest concentration of the tested compound causing no significant decrease in the plant germination and growth, was estimated to be 100 mg/kg of the soil dry weight, whereas the LOEC (the lowest concentration causing a reduction in plant growth/germination) was 200 mg of the respective substance per kilogram of the soil dry weight. NOEC and LOEC values for aminophosphonic acid 2c were determined only for radish and were 400 and 800 mg per kg soil dry weight. As for *N*-furfurylidene-*p*-anisidine (1a), the NOEC values for oat and radish were 80

Table 1. Mean Values of Crop of *N*-Furfurylidene-*p*-anisidine (1a) and *N*-(2-nitrofurfurylidene)-*p*-toluidine (1d) versus Control (%)^a

compound	tested plant	concentration (mg/kg d.m. of soil)						
		8	10	20	40	80	100	1000
1a	oat	nd	91	104	107	<u>27</u>	<u>25</u>	66
	SD		4.2	13.0	4.8	5.9	4.8	0.1
	radish	nd	110	115	<u>101</u>	<u>70</u>	52	
	SD		1.6	6.3	3.3	8.0	3.9	
1d	oat	<u>105</u>	<u>82</u>	nd	nd	nd	37	5
	SD	2.7	4.6				1.7	2.1
	radish	<u>107</u>	<u>77</u>	nd	nd	nd		
	SD	5.7	12.6					

^aSD, standard deviation; nd, not determined. Underlined data indicate values of NOEC and LOEC of tested compounds.

N-furfurylidene-*p*-anisidine, radish was found to be more sensitive plant, as no germination of seedlings has been observed (see SI Tables S1 and S2, Figure S1), while a drop in the fresh weight of oat sprouts of approximately 35% as compared to control plants was observed. The toxicity impact was strictly dependent on concentration of applied compound, the higher concentration, the stronger inhibition of growth has occurred. It is to point out that the sole structural difference

and 40 mg/per kg of dry weight of soil, while the LOEC values, 100 and 80 mg/kg of dry weight of soil, respectively.

In the final test of nitrofuraldimine **1d**, for all used concentrations ranged between 2 and 8 mg per kilogram of the soil dry weight, the value of crop fresh and dry weight as well as germination of oat and radish was above 90% as compared to the control plant. NOEC and LOEC values of this compound were determined to be 8 and 10 mg/kg of soil dry weight, respectively, for both plants. They are presented in Table 2.

Table 2. Values of No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) for All Tested Compounds (in mg/kg of Dry Weight of soil)

compound	NOEC		LOEC	
	oat	radish	oat	radish
1a	80	40	100	80
1b				
1d	8	8	10	10
2a	100	100	200	200
2b	100	100	200	200
2c		400		800

Investigated compounds **1a**, **1d**, and **2a–c** were toxic toward selected plants and the results obtained from the preliminary plant growth tests were confirmed by independently performed final tests. The progressive inhibition of germination and reduction of fresh/dry weight of plants depended on the applied concentration and observed changes were greater when a higher concentration of the chemical compounds was applied. Analysis of results demonstrated that dicotyledonous radish is much more sensitive plant to tested compound **1a**, **1d**, and **2b** than monocotyledonous oat.

High phytotoxicity of nitrofuraldimine **1d** is slightly astonishing in reference to the structurally similar, nontoxic furaldimine **1b**. Taking into account that the only difference in structure between *N*-(2-nitrofurfurylidene)-*p*-toluidine **1d** and *N*-furfurylidene-*p*-toluidine **1b** is the presence of a nitro group in position “5”, this group seems to be responsible for the toxicological impact of an aldimine **1d** against the used plants. It can be supposed that the action of nitroreductases may play the key role in mechanism of its toxicity, as nitroreductases cause reduction of nitro groups into more active species. The case of the nitrofurfural derivative **1d** may be similar to the described fact of assimilation and nitroreductase conversion of 2,4,6-trinitrotoluene (TNT) in several agricultural plants.²³ Authors suggested that the nitroreductase enzyme participating in reduction of the TNT nitro group has also been found in algae, ferns, monocotyledonous and dicotyledonous plants, and even in trees,²³ and nitroreductase enzymes may act also on the nitro group linked to furfural.

To conclude, the collected data demonstrated that furan-derived aminophosphonic acids **2a–c** as well as the biologically active¹¹ imine **1a** show moderate but interesting phytotoxic properties against plants, which depended on the applied concentrations and type of plant. Because of this phytotoxicological impact, any application of the studied aminophosphonic systems **2a–c** and the Schiff base **1a** must be considered in aspect of the environmental protection. Our results should call attention to the necessity for further phytotoxicological

investigation of any new synthesized furaldimines and their aminophosphonic derivatives.

The case of *N*-(2-nitrofurfurylidene)-*p*-toluidine **1d** is of different significance, as this Schiff base **1d** is highly phytotoxic. Therefore, derivatives of 5-nitrofurfural, which are known to have antibacterial and antiprotozoal properties, can logically be suspected to be strongly phytotoxic too. Therefore, it is to stress that any wastes containing 5-nitrofurfural-deriving antibacterial and antiprotozoal drugs must be handled carefully because they can be very hazardous for plants. However, the problem is still on study and the further investigations of these family of compounds from the public health and environmental protection point of view are required.

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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