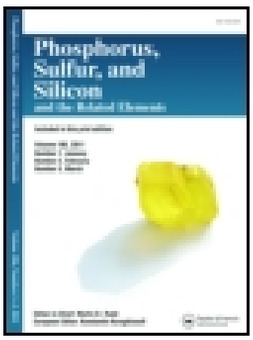

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Synthesis of novel *N*-(p-toluenesulfonyl)aminophosphonates and evaluation of their biological properties

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Abstract

The synthesis of a series of *N*-sulfonylaminophosphonates **2aA-2dC** and evaluation of their cytotoxicity and ecotoxicity is described.

N-sulfonylaminophsophonates **2aA-2dC** were obtained by addition of dialkyl phosphites to azomethine bonds of sulfonimines **1a-d** in dichloromethane, and the reaction was mediated by potassium carbonate. Sulfonimines **1a-d** were synthesized by direct condensation of selected aldehydes with *p*-toluenesulfonamide catalyzed by titanium(IV) ethoxide.

The cytotoxicity of compounds **2aB-2cB, 2aC-2cC, 2bA** and **2dA** against normal primary human lymphocytes, non-small cell lung cancer cells A549, promyelocytic leukemia cells HL-60 and glioblastoma astrocytoma cells U87 was found to be very low.

The phytotoxicity of **2dC** against monocotyledonous oat (*A. sativa*) and dicotyledonous radish (*R. sativus*) was measured and the compound was found to be practically not toxic for two model plants. Lack of toxicity for marine bacteria *A. fischeri* has been also detected. However, the compound **2dC** was found to be significantly toxic for freshwater, European crustaceans *H. incongruens* (ostracods).

Keywords: Sulfonamidomethylphosphonates, aza-Pudovik reaction, cytotoxicity, ecotoxicology, *A. fischeri* test, *H. incogruens* test

Introduction

Aminophosphonic derivatives are commonly known to have the potential biological activity and this fact has been reported many times.[1] Recently, several important papers have been published proving that aminophosphonic and aminophosphinic derivatives show interesting properties, which may be applied in medicine or pharmacy[2] or in agrochemistry and pesticide industry.[3,4]

A vast number of structure modifications has been made on aminophosphonates and among them, *N*-sulfonylaminophosphonates (or sulfonamidophosphonates) constitute an important group of compounds having biological potential. In 1977, Birum[5] patented the preparation of several of them and mentioned their biological activity. Much later, some authors reported their intriguing properties as inhibitors of matrix metalloproteinases (MMPs),[6,7] anti-HIV agents[8] or as agents inactivating the *E. cloacae* P99 β-lactamase.[9]

Nobody should be astonished then that a number of papers dealing with the preparation of sulfonylaminophosphonates has been published.[1,2,10,11] Das et al.[10] proposed the use of iodine

as a catalyst, while the Chinese team,[11] reported their synthesis in catalyst-free conditions. Sulfonylaminophosphonates have been applied to the synthesis of chiral thiourea derivatives bearing an α-aminophosphonic moiety having the antiviral properties,[12] or benzosultams bearing an α-triaryl-substituted stereogenic center.[13] Having that in mind, we performed synthesis of a series of novel α-sulfonylaminophosphonates **2aA-dC** and investigated some of their biological properties.

The REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) regulations point out to the necessity of evaluating the toxicological behavior of new compounds in order to predict their possible effect on environment and humans. Keeping in mind these recommendations, the potentially bioactive, newly synthesized compounds **2bA-dC** were then investigated in the aspect of their cytotoxicity on human lymphocytes. By this occasion, their cytotoxicity was also tested on several cancer cell lines. Finally, the selected, model compound **2dC** was the subject of ecotoxicological assessments, *i.e.* evaluation of its phytotoxicity against *Raphanus sativus* and *Avena sativa* as well as its ecotoxicity performed by means of commercial biotests: the Microtox® system (using *Aliivibrio fischeri* bacteria) and OSTRACODTOXKIT F (using *Heterocypris incogruens* crustaceans).

Results and Discussion

Preparation of N-sulfonylaminophosphonates 2bA-dC

Various methods for synthesis of sulfonamide phosphonates have been reported within last decade. Das et al.[10] reported their preparation by the addition of trimethylsilyldialkyl phosphite to sulfonimines using iodine as catalyst. Palacios et al.[14] performed the addition of dialkyl

phosphites to sulfonimines in the presence of triethylamine. Onysko et al.[15] suggested the different approach, i.e. the reaction of arenesulfonyl chloride with *N*-unsubstituted aminophosphonic acid esters. Pallikonda and Chakravarty[16] have proposed the reactions of sulfonamides with α-hydroxyphosphonates mediated by trifluoromethanesulfonic acid.

We decided to apply the addition of dialkyl phosphite to sulfonimines. The first step, however was to obtain sulfonimines **1a-d**. Numerous methods for preparation of these compounds have been published and in each paper, the necessity of using a catalyst is stressed. Love et al.[17] reported the use of tetraethyl orthosilicate, and later such catalysts as Montmorillonite K-10[18] or iron trichloride[19] were used. Ruano et al.[20] proposed to prepare first *N*-sulfinylimines by condensation of carbonyl compounds with sulfonamides catalyzed by titanium(IV) ethoxide[21] and their subsequent oxidation with *m*-CPBA.

To our knowledge, the direct preparation of sulfonimines from aldehydes catalyzed by titanium(IV) ethoxide was described only once,[22] where authors reported the synthesis of 4-methyl-*N*-(2-vinylbenzylidene)benzene-sulfonamide.

Basing on the above published methods, we performed the direct condensation of *p*-toluenesulfonamide with selected aldehydes catalyzed by titanium(IV) ethoxide. Reactions were carried out in dichloromethane. This method allowed to obtain the desired sulfonimines in good yields and the high degree of purity. (Fig. 1)

[Insert Figure 1]

Such obtained sulfonimines **1a-d** were used for the reaction with three model phosphites, namely diethyl, diisopropyl and dibenzyl phosphites. The addition of phosphites to sulfonimines

1a-d was mediated by potassium carbonate; reactions were carried out in dichloromethane. The use of potassium carbonate as a promoter for the addition of dialkyl phosphites to sulfonaldimines was described by Fan et al.[23] as an example of an α C-H oxidation and subsequent addition reactions of aryl sulfonamides. Such methodology allowed the desired *N*-sulfonyl aminophosphonates **2bA-dC** in a proper degree of purity and satisfactory yields to be obtained. (Fig. 1)

The obtained sulfinamidophosphonates **2bA-dC** were identified by the ^{1}H, ^{13}C and ^{31}P NMR as well as IR spectroscopy and ESI mass spectrometry. For all new compounds, elemental analysis was made, in a case of known compounds values of melting points were compared with literature data.

^{1}H NMR spectra revealed the diagnostic doublet at over 4 ppm assigned to the proton at alpha carbon atom with a characteristic coupling of a proton with a phosphorus nucleus. In some cases, this proton coupled additionally to a proton at nitrogen atom and then , this signal appeared as a doublet of doublets.

At ^{13}C NMR spectra, a carbon nucleus directly linked to phosphorus gave a doublet at around 40 ppm and of a coupling constant over 150 Hz, which is also characteristic for aminophosphonic systems.

N-sulfonylaminophosphonates **2bA-dC** obtained in this way were subsequently investigated in aspect of their biological properties.

Cytotoxic activity of 2aB-2cB, 2aC-2cC, 2bA and 2dA

The synthesized aminophosphonates **2aB-2cB, 2aC-2cC, 2bA** and **2dA** were then tested in aspect of their cytotoxicity. Compounds **2dB**, **2dC** and **2cA** were not considered because of their very low solubility in the medium used for cytotoxicity evaluation. The cytotoxic effect of newly synthesized phosphonates **2aB-2cB, 2aC-2cC, 2bA** and **2dA** was investigated against normal primary human lymphocytes (PBMCs) and three human cancer cell lines, namely the non-small cell lung cancer cells A549, the promyelocytic leukemia cells HL-60 and the glioblastoma astrocytoma cells U87 using standard resazurin reduction assays. This assay is based on the reduction of resazurin to resorufin catalyzed by cellular mitochondrial dehydrogenases.[24] The amount of fluorescent resorufin obtained directly correlates with the number of live cells in a culture. (Fig. 2)

[Insert Figure 2]

The activity of the tested compounds was studied in the concentration range from 1 nM to 10 µM. The compounds were prepared in relatively low concentrations due to their low solubility in culture medium. Analysis carried out for eight *N*-sulfonylaminophosphonates (**2aB-2cB, 2aC-2cC, 2bA** and **2dA**) demonstrated no cytotoxic effect against PBMCs, A549, HL-60 and U87 cells up to 1 µM and low cytotoxic effect at the concentration 10 µM – cell viability decreased approximately to 80%. It would allow us to suggest that the studied *N*-sulfonylaminophosphonates **2aB-2cB, 2aC-2cC, 2bA** and **2dA** are non-toxic probably due to their low solubility in aqueous solutions. (Fig. 2)

Evaluation of ecotoxicity of dibenzyl (ferrocenyl)(4-methylphenylsulfonamido)-methylphosphonate (2dC)

Plant Growth Test – Germination, Growth Inhibition, Dry and Fresh Matter

Phytotoxicity of dibenzyl (ferrocenyl)(4-methylphenylsulfonamido)-methylphosphonate **(2dC)** was tested on two model plants: monocotyledonous oat (*Avena sativa*) and dicotyledonous radish (*Raphanus sativus*) according to OECD/OCDE. Terrestrial plant test: Seedling emergence and seedling growth test.[25]

The aminophosphonate **2dC** was selected because of its structural features, *i.e.* dibenzyl, *N*-substituted ferrocene-derived aminophosphonate, as it was described in one of our previous papers.[26]

The percentage growth inhibition (GI%) values of shoot height, root length and fresh matter of both plants exposed on sulfonylaminophosphonate **2dC** are presented in Table 1. Growth inhibition of shoot height of both plants treated with dibenzyl (ferrocenyl)(4-methylphenylsulfonamido)-methylphosphonate (**2dC**) is not very high. Regardless the kind of examined plants, up to concentration 100 mg/kg of soil dry weight (s.d.w.), shoot growth inhibition did not exceed 10%. At 100 mg/kg s.d.w., stronger impact of the compound **2dC** on oat was observed (8.5% vs. 5.2% respectively). Further increasing of aminophosphonate concentration up to 200 mg/kg s.d.w. revealed larger difference in growth inhibition% of oat, which increased almost twice (16.14 mg/kg) as compared to radish, the growth of which inhibited slightly only (6.74 mg/kg).

The increase of concentration of **2dC** up to 400 mg/kg s.d.w. caused the reversal of tendency, the significant rise of growth inhibition value of radish shoot height (34.5%) was observed with simultaneous slight increase of oat shoot growth inhibition (18.2%). At higher concentrations (800 and 1000 mg/kg s.d.w.), sulfonylaminophosphonate **2dC** was distinctly much more harmful for radish than to oat, as shoot growth inhibition for the former were 39.4 and 43.4% respectively, while for oat – 21.5 and 27.6% respectively.

But it is to state that even at 1000 mg/kg s.d.w., dibenzyl (ferrocenyl)(4-methylphenyl-sulfonamido)methylphosphonate (**2dC**) caused the shoot growth inhibition in less than 50% for both plants. But it is worth mentioning that the higher negative impact on dicotyledonous radish vs. monocotyledonous oat was observed. (Table 1)

[Insert Table 1]

Percentage changes of growth inhibition of roots was similar to GI% of shoot height (Table 1) but the described above tendencies were not observed. Except for the 1-mg concentration of the compound **2dC**, it was definitely more harmful for radish than for oat regardless of the concentration of **2dC**. However, considering that, at 1000 mg/kg s.d.w., the studied compound **2dC** caused the radish root growth inhibition in 32.4% only, it is to state that the evaluated compound **2dC** is very moderately toxic for roots of both plants.

The tendency of changes of fresh matter for both plants was similar to growth inhibition of shoots of both tested plants (Table 1). Up to the concentration of 400 mg/kg s.d.w., the compound 2dC was evidently more harmful for oat that for radish. The most significant difference was observed at 100 mg/kg s.d.w. (16.5% for oat and 4% for radish fresh matter

inhibition). Further increase of the concentration resulted in smaller and smaller difference in toxicity between both plants and finally, at 800 mg/kg s.d.w., the tendency reversal was observed. At 800 and 1000 mg/kg s.d.w., the evaluated aminophosphonate **2dC** turned out to be significantly more toxic for radish fresh matter than for oat one (53.2 and 63.2% inhibition for radish and 29.1 and 35% for oat respectively). It requires to be stressed that the compound 2dC is seriously harmful for radish at 800 and 1000 mg/kg s.d.w.

The changes of percentage germination of tested plants are presented in Table 2. Comparing the germination of both examined plants, it has been noticed that the sulfonyl-aminophosphonate **2dC** did not stopped germination and that germination inhibition was extremely mere even at the highest tested concentration 1000 mg/kg of s.d.w.

Changes of effective concentration EC_{50}, no observed effect concentration NOEC and lowest observed effect concentration LOEC values are presented in Figure 3.

[Insert Figure 3]

[Insert Table 2]

The values of effective concentration EC_{50} for the evaluated compound **2dC** have been found to be much lower for radish, which clearly demonstrates that the studied sulfonylaminophosphonate is significantly more toxic for radish than to oat. But this tendency alters depending on the given inhibition factor.

Comparing the impact of **2dC** on shoots and roots of both plants, it should be noted that the tested compound is more harmful for shoots than for roots. EC_{50} values of the examined compound **2dC** for oat and radish shoots are 6755 and 1172 mg/kg s.d.w. respectively, while for

roots: 16674 and 2971 mg/kg s.d.w. respectively. It clearly indicates that shoot of both plants is more susceptible for the tested compound than root. Nevertheless, it is to stress that the ratio EC_{50} for oat to EC_{50} for radish is in both cases similar (5.6 up to 5.8).

The fresh matter is significantly more susceptible for the compound **2dC** than shoots and roots. EC_{50} values are 4806 mg/kg s.d.w. for oat and 740 mg/kg s.d.w. for radish but their ratio is ca. 6.5, which pointed out to the selective action of **2dC** on radish fresh matter.

Determined values of NOEC (no effect concentration) and LOEC (lowest effect concentration) for the tested compound **2dC** showed different tendency. Its NOEC and LOEC values for oat are 10 and 100 mg/kg of soil dry weight, while for radish, NOEC is 100 and LOEC – 200 mg/kg s.d.w. This values show that less concentration (i.e. less amount) of dibenzyl (ferrocenyl)(4-methylphenylsulfonamido)-methylphosphonate (**2dC**) is harmful for monocotyledonous oat than for dicotyledonous radish. These data coincide well with above observation concerning growth inhibition factors – at smaller concentration values of **2dC**, inhibition of shoot and fresh matter growth was observed to be greater for oat. At higher concentrations, the compound **2dC** was found to be more effective (harmful) for radish.

Growing concentration of applied sulfonylaminophosphonate **2dC** in soil caused the gradual increase of dry weight value of both tested plants (Fig. 4). This phenomenon was described previously by us[26-28] and is caused by absorbing nutrients and water by plants through their roots. In result, plant growth and development are strongly dependent on the concentration of mineral nutrients available in the soil. At the beginning of plant growth, roots uptake water and absorb the nutrients very intensively being simultaneously exposed on toxic agents in soil. In

the measure of the exposition time, roots stop to develop and grow, the water absorption become more and more difficult and the level of dry matter in a plant starts to increase. Such a pattern during the exposure process of plants on contaminants was already described.[29,30]

[Insert Figure 4]

Changes of pigment levels

Growing concentration of the investigated sulfonamidophosphonate **2dC** in soil resulted in the decrease of the level of total chlorophyll in green parts of both tested plants (Fig. 5). The level of carotenoids in oat seedlings treated with the sulfonylaminophosphonate **2dCa** was very symptomatic. For both tested plants, the growing concentration of the investigated compound in soil provoked the constant decrease of the carotenoid amount in green parts of radish. Any symptoms of chlorosis and necrosis of treated plants have been observed in digital photographs (Fig. 6)

[Insert Figure 5]

[Insert Figure 6]

The results coincide with the determined level of plant pigments in stress conditions.[31,32] Symptoms of chlorosis appearing on plant leaves (yellowing or whitening of normally green plant tissue) indicate a decrease of the chlorophyll amount and are symptoms of disease (chlorosis) or nutrient deficiency.

The regular ratio of chlorophyll level to carotenoid level is a crucial factor reflecting the proper functionality of the photosynthetic system. Carotenoid pigments are critical for plant survival,

therefore their composition and amount strongly depends on the physiological and pathological conditions of living.[33,34]

It is well known that carotenoids play a distinct role in photoprotection of plants against exposure on biotic or abiotic factors, however correlation of carotenoid level with stress conditions is ambiguous.[35,36] As far as in a case of (5-nitrofurfuryl)-derived aminophosphonic esters, the level of carotenoids of oat seedlings has decreased,[28] while pyrrole-derived aminophosphonates caused an increase of the level of pigments.[27]

Microtox® assay

Results of the toxicity assessment using *Aliivibrio fischeri* Gram-negative bacteria as examined organisms demonstrated a rather low toxicity of the tested compound **2dC**. (Table 3, Fig. 7). In comparison with the previously published toxicities of ferrocene-derived aminophosphonates against *A. fischeri*,[26] where EC_{50} values varied from 48 up to 235 mg/kg s.d.w, the toxicity of sulfonylaminophosphonate **2dC** characterised by EC_{50} = 767.5 mg/kg s.d.w. is significantly smaller.

According to Hernando et al.,[37] the toxicity categories based on the EC_{50} values are as follows: "*very toxic to aquatic organisms*" ($EC_{50} \leq 1$ mg/L), "*toxic*" (EC_{50} in the range of 1–10 mg/L), and "*harmful*" (EC_{50} in the range of 10–100 mg/L), which are established in the Directive 93/67/EEC. Following these regulations, it is to stress that toxicity of the studied aminophosphonate **2dC** equaling 581.0 mg/L is outside the upper limit of harmfulness.

[Insert Table 3]

[Insert Figure 7]

Ostracodtoxkit® Test

The changes of crustacean *Heterocypris incongruens* mortality are presented in Fig. 8. The impact of sulfonamidophosphonate **2dC** on ostracods at concentrations 10, 50, 100 and 250 mg/kg s.d.w. indicate in an evident way that the investigated compound is significantly toxic against ostracods. This toxicity seems to be impressively high when compared with ostracod mortality of previously published ferrocene-derived aminophosphonates.[26] While the mortality (%) of the latter do not exceed 10% at 10 mg/kg s.d.w. (at 50 mg/kg, mortality% oscillates around 20%).[26] The mortality of ostracod after treating them with **2dC** is 35% at 10 mg/kg s.d.w. and 65% at 50 mg/kg. It is to state that sulfonamidophosphonate **2dC** is very toxic for H. incongruens crustaceans.

[Insert Figure 8]

Growth inhibition is the second criteria of the toxic effect indicated by the Ostracodtoxkit F™ microbiotest. This parameter allows for the evaluation of the sub-lethal toxicity of sediments (soil in this case). As usually, the growth inhibition is determined by comparing the size of surviving ostracods living in the test sediment with the size of ostracods living in the non treated sediment at the end of the test. Determination of the sub-lethal impact of sediment toxicants is justified only for sediments, which do not cause a high ostracod mortality. According to manual of Ostracodtoxkit® test, the growth inhibition should only be determined for sediments, which mortality was found to be less than 30%. Therefore, the growth inhibition was not chosen as a criterion of sub-lethal effects to determine toxicity not inducing substantial

mortality in the test organisms, because the mortality, for each studied concentration was higher than 30%.

Considering the contents of the above mentioned instruction, and the results of mortality of *H. incongruens* on contact with the compound **2dC**, the growth inhibition tests was not performed as even 10-mg doses gave the mortality of 35%.

Summing up, ecotoxicological nature of dibenzyl (ferrocenyl)(4-methylphenylsulfonamido)methylphosphonate (**2dC**) is ambiguous. The compound **2dC** is not in fact toxic for two tested terrestrial higher plants: monocotyledonous oat and dicotyledonous radish. It is completely not toxic for *Aliivibrio fischeri* bacteria tested in a Microtox® system using the MSPT method.

However, its toxicity against crustaceans *Heterocypris incongruens* (ostracods) is surprisingly elevated in comparison with aminophosphonates bearing a ferrocene moiety.[26] The compound **2dC**, even in concentration of 10 mg/kg s.d.w. caused the 35%-mortality of these invertebrates; increasing the concentration up to 50 mg/kg s.d.w. the compound resulted in 65%-mortality.

Conclusions

In this work, the synthesis of a series of *N*-sulfonylaminophsophonates **2bA-2dC** and evaluation of some of their biological properties, *i.e.* cytotoxicity and ecotoxicity is described. In the two-step synthesis, *i.e.* preparation of sulfonimines **1a-d** followed by addition of phosphites, imines were synthesized by the direct condensation of *p*-toluenesulfonamide with model aldehydes catalyzed by titanium(IV) tetraethoxide. To our knowledge, this approach has been used only

once,[22] where authors described exclusively one case of preparation of 4-methyl-N-(2-vinylbenzylidene)benzene-sulfonamide.[22]

Imines **1a-d** were subsequently converted to *N*-sulfonylaminophsophonates **2bA-2dC** by addition of model dialkyl phosphites to their azomethine bonds mediated by potassium carbonate; reactions were carried out in dichloromethane. Potassium carbonate was applied only once as a promoter for the addition of dialkyl phosphites to sulfonaldimines as an example of an α C-H oxidation and subsequent addition reactions of aryl sulfonamides.[23]

The *N*-sulfonylaminophsophonates **2bA-2dC** were investigated in terms of their cytotoxic properties against PBMCs and three human cancer cell lines A549, HL-60 and U87. The evaluation revealed that no cytotoxic effect against PBMCs, A549, HL-60 and U87 cells up to 1 μM was observed. At the concentration 10 μM low cytotoxic effect was noticed, as cell viability decreased approximately to 80%. Higher concentrations could not be tested due to the low solubility of tested compounds in aqueous solutions.

Ecotoxicological evaluation of the model dibenzyl (ferrocenyl)(4-methylphenylsulfonamido)methyl-phosphonate (**2dC**) was performed in three aspects. Phytotoxicity against two model plants: monocotyledonous oat (*Avena sativa*) and dicotyledonous radish (*Raphanus sativus*) was performed according to the OECD/OCDE procedure. Tests revealed that the investigated compound was nearly completely not toxic for tested plants (EC_{50} = 4806 mg/kg s.d.w for oat and 740 mg/kg s.d.w for radish fresh matter). It is however to emphasize that the lowest effective concentration for oat was found to be 100 mg/kg s.d.w.

Toxicity of the sulfonamidophosphonate (**2dC**) for bacteria was performed on marine bacteria *Aliivibrio fischeri*, using the Microtox® measuring system. The compound (**2dC**) was found to be practically not toxic for these microorganisms (EC_{50} = 581.0 mg/L = 767.5 mg/kg s.d.w).

Dibenzyl (ferrocenyl)(4-methylphenylsulfonamido)methylphosphonate (**2dC**) was found to be toxic for freshwater crustaceans *Heterocypris incongruens*. The mortality% for the lowest applied concentration (10 mg/kg s.d.w.) was 35%, while at 100 mg/kg s.d.w., mortality reached 90%. In comparison with *N*-arylaminophsophonates bearing ferrocene moiety, which even at 250 mg/kg s.d.w., mortality did not exceed 75%, it is to state that the tested compound **2dC** is toxic for ostracods.

To conclude, *N*-sulfonylaminophsophonates from the investigated series could be claimed to be toxic neither for cell lines (*in vitro* tests), nor for prokaryotes. However, it is toxic for higher organisms such as crustaceans and therefore, one should handle this type of compounds with care.

Experimental

Chemical synthesis

All solvents (POCh, Gliwice, Poland) were routinely distilled and dried prior to use. Amines, diphenyl phosphite, as well as pyrrole-2-carboxaldehyde (Aldrich, Poznań, Poland) were used as received. Melting points were measured on a MelTemp II apparatus and were not corrected. NMR spectra were recorded on a Bruker Avance III 600 MHz (Billerica, MA, USA) operating at 600 MHz (^{1}H NMR), 150 MHz (^{13}C NMR) and 243 MHz (^{31}P NMR). FT-IR spectra were recorded on a Nexus FT-IR Thermo Nicolet. ESI-MS were recorded on a Varian 500-MS LC Ion Trap mass

spectrometer (Palo Alto, CA, USA). Elemental analyses were carried out at the Laboratory of Microanalysis, Faculty of Chemistry, University of Łódź, Poland.

Preparation of p-toluenesulfonimines 1a-d. General procedure.

To an appropriate aldehyde (2 mmol) dissolved in dichloromethane, *p*-toluenesulfonamide (0.34 g, 2 mmol) and titanium(IV) ethoxide (0.9 g, 4 mmol) were added. A mixture was stirred at room temperature for 48 hours then water (15 mL) was added and stirred for 5 min. Titanium oxide precipitated, which was washed 15 times with small portions of dichloromethane and finally with a small amount of water. The combined filtrates were placed in a separating funnel, an organic layer was separated, dried with $MgSO_4$ and evaporated. Obtained crude solids were crystallized with hexane-ethyl acetate (6:2) to give pure imines **1a-d**.

N-(4-methoxybenzylidene)-4-methylbenzenesulfonamide (1a).

Y = 0.53 g (91%). m.p. = 132–134°C (white powder), lit.[38] 131–132°C or lit.[39] 117°C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.94 (s, CH=N, 1H); 7.88 (d, $^3J_{HH}$ = 8.7 Hz, *p*-C_6H_4, 2H); 7.87 (d, $^3J_{HH}$ = 8.0 Hz, *p*-C_6H_4, 2H); 7.33 (d, $^3J_{HH}$ = 8.2 Hz, *p*-C_6H_4, 2H); 6.97 (d, $^3J_{HH}$ = 8.8 Hz, *p*-C_6H_4, 2H); 3.88 (s, OCH_3, 3H); 2.43 (s, CH_3, 3H).

N-(2- methoxybenzylidene)-4-methylbenzenesulfonamide (1b).

Y = 0.50 g (86%). m.p. = 111–113°C (white powder), lit.[40] 110–111°C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.55 (s, CH=N, 1H); 8.05 (dd, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.7 Hz, *o*-C_6H_4, 1H); 7.88 (d, $^3J_{HH}$ = 8.3 Hz, *p*-C_6H_4, 2H); 7.55 (ddd, $^3J^{(1)}_{HH}$ = $^3J^{(2)}_{HH}$ = 8.7 Hz, $^4J_{HH}$ = 1,7 Hz, *o*-C_6H_4, 1H); 7.32 (d, $^3J_{HH}$ = 8,2 Hz, *p*-C_6H_4, 2H); 6.95-6.99 (m, *o*-C_6H_4, 2H); 3.92 (s, OCH_3, 3H); 2.43 (s, CH_3, 3H).

N-Thienylidene-4-methylbenzenesulfonamide (1c).

Y = 0.46 g (87%). m.p. = 125–127°C (yellow crystals), lit.[41] 99–100°C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.11 (s, CH=N, 1H); 7.87 (d, $^{3}J_{HH}$ = 8.3 Hz, *p*-C_6H_4, 2H); 7.77 (m, H_{thioph}, 2H); 7.33 (d, $^{3}J_{HH}$ = 8.1 Hz, *p*-C_6H_4, 2H); 7.20 (dd, $^{3}J_{HH}$ = 4.3 Hz, $^{3}J_{HH}$ = 8.7 Hz, H_{thioph}, 1H); 2.43 (s, CH_3, 3H).

N-Ferrocenylidene-4-methylbenzenesulfonamide (1d)[42-45]

Y = 0.68 g (92%). m.p. = 135–137°C (orange powder). ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.05 (s, CH=N, 1H); 7.86 (d, $^{3}J_{HH}$ = 8.1 Hz, *p*-C_6H_4, 2H); 7.32 (d, $^{3}J_{HH}$ = 8.0 Hz, *p*-C_6H_4, 2H); 4.83 (m, CH_{fer}, 2H); 4.71 (m, CH_{fer}, 2H); 4.19 (s, Cp, 5H), 2.42 (s, CH_3, 3H).

Elem. anal. Calcd for $C_{18}H_{17}FeNO_2S$: C 58.87; H 4.67; N 3.81. Found: C 58.89; H 4.72; N 3.79.

Preparation of N-(p-toluenosulfonamido)methylphosphonic Acids Esters 2bA-dC. General procedure.

To a corresponding sulfonimine (1 mmol) dissolved in dichloromethane, an appropriate phosphite (4 mmol) and potassium carbonate (5 mmol) were added. The obtained mixture was stirred at room temperature for 1 week (reaction progress was monitored by ^{31}P NMR) and after completion of the reaction, a small amount of saturated aqueous ammonium chloride was added. An organic layer was separated and washed 3 times with water (3x5 mL) and once with brine (5 mL), dried with $MgSO_4$ and evaporated. Solid residue was dissolved in a small portion of dichloromethane, and triple amount of hexane was added to precipitate pure aminophosphonates **2bA-dC**.

Diethyl (2-methoxyphenyl)(4-methylphenylsulfonamido)-methylphosphonate (2bA).

YIELDield = 0.17 g (40%). m.p. 175-177°C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.46 (d, $^{3}J_{HH}$ = 8.2 Hz, *p*-C_6H_4, 2H); 7.13-7.12 (m, *o*-C_6H_4, 1H); 7.10-7.07 (m, *o*-C_6H_4, 1H); 6.97 (d, $^{3}J_{HH}$ = 8.0 Hz, *p*-C_6H_4,

2H); 6.72 (t, $^{3}J_{HH}$ = 7.5Hz, *o*-C_6H_4, 1H); 6.61 (d, $^{3}J_{HH}$ = 8.2Hz, *o*-C_6H_4, 1H); 6.02 (dd, $^{3}J_{HH}$ = 3.3Hz, 3JHH = 10.2Hz, NH, 1H); 5.19 (dd, $^{3}J_{HH}$ = 10.3Hz, $^{2}J_{PH}$ = 24.5Hz, CHP, 1H), 4.22-4.17 (m, PO$\underline{CH_2}CH_3$, 2H); 3.92-3.85 (m, PO$\underline{CH_2}CH_3$, 1H); 3.74 (s, OCH_3, 3H); 3.68-3.64 (m, PO$\underline{CH_2}CH_3$, 1H); 2.27 (s, CH_3, 3H); 1.33 (t, $^{3}J_{HH}$ = 7.1Hz, $POCH_2\underline{CH_3}$, 3H); 1.03 (t, $^{3}J_{HH}$ = 7.1Hz, $POCH_2\underline{CH_3}$, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 156.54 (d, $^{2}J_{PC}$ = 5.8Hz, C_{arom}); 142.66 (C_{arom}); 137.53 (C_{arom}); 129.44 (s, $^{3}J_{PC}$ = 4.2Hz, C_{arom}); 128.98 (d, $^{3}J_{PC}$ = 2.7Hz, C_{arom}); 128.72 (C_{arom}); 127.01 (C_{arom}); 122.19 (C_{arom}); 120.58 (d, $^{4}J_{PC}$ = 1.9Hz, C_{arom}); 110.40 (C_{arom}); 63.78 (d, $^{2}J_{PC}$ = 6.8Hz, PO*C*); 63.32 (d, $^{2}J_{PC}$ = 6.8Hz, PO*C*); 55.50 (OCH_3); 49.31 (d, $^{1}J_{PC}$ = 163,9Hz, PC); 21.29 (CH_3); 16.43 (d, $^{3}J_{PC}$ = 5.7Hz, POC*C*); 16.01 (d, $^{3}J_{PC}$ = 5.6Hz, POC*C*). ^{31}P NMR (243 MHz, $CDCl_3$): δ 19.75. IR (KBr): 3432 (NH); 3155 (C-H_{arom}); 1061, 1497 (C=C_{arom}); 1336 (S=O); 1239 (P=O); 1161 (S=O); 1060, 1023 (P-O-C). ESI-MS m/z 428 ($M+H^+$); 450 (M+Na); 466 (M+K). Elem. anal. Calcd for $C_{19}H_{26}NO_6PS$: C 53.39; H 6.13; N 3.28. Found: C 53.32; H 6.05; N 3.16

Diethyl (2-thienyl)(4-methylphenylsulfonamido)-methylphosphonate (2cA).

Yield = 0.07 g (17%). m.p. 138-141°C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.58 (d, $^{3}J_{HH}$ = 8.2 Hz, p-C_6H_4, 2H); 7.13-7.12 (m, p-C_6H_4 and H_{thioph}, 3H); 6.94 (m, H_{thioph}, 1H); 6.80 (dd, $^{4}J_{HH}$ = 0.8Hz, $^{3}J_{HH}$ = 4.7Hz, H_{thioph}, 1H); 5.80 (dd, $^{3}J_{HH}$ = 4.6Hz, $^{3}J_{PH}$ = 9.7Hz, NH, 1H); 5.07 (dd, $^{2}J_{PH}$ 23.6Hz, $^{3}J_{HH}$ = 9.3Hz, CHP, 1H); 4.26-4.18 (m, PO$\underline{CH_2}CH_3$, 2H); 4.04-4.00 (m, PO$\underline{CH_2}CH_3$, 1H); 3.83-3.79 (m, PO$\underline{CH_2}CH_3$, 1H); 2.36 (s, CH_3, 3H); 1.35 (t, $^{3}J_{HH}$ = 7.1Hz, $POCH_2\underline{CH_3}$, 3H); 1.14 (t, $^{3}J_{HH}$ = 7.0Hz, $POCH_2\underline{CH_3}$, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 142.89 (C_{arom}); 138.05 (C_{thioph}); 136.16 (C_{arom}); 129.05 (C_{arom}); 127.69 (d, $^{2}J_{PC}$ = 7.4Hz, C_{thioph}); 127.04 (C_{arom}); 126.67 (d, $^{4}J_{PC}$ = 2Hz, C_{thioph}); 125.77 (d, $^{3}J_{PC}$ = 3.1Hz, C_{thioph}); 64.28 (d, $^{2}J_{PC}$ = 7.2Hz, POC); 63.81 (d, $^{2}J_{PC}$ = 7.0Hz, POC); 50.62 (d,

$^{1}J_{PC}$ = 162.9Hz, PCN); 21.39 (CH_3); 16.40 (d, $^{2}J_{PC}$ = 5.6Hz, POCC); 16.11 (d, $^{2}J_{PC}$ = 5.7Hz, POCC). ^{31}P NMR (243 MHz, $CDCl_3$): δ 18.01. IR (KBr): 3432 (NH); 3109 (C-H_{arom}), 1598, 1460 (C=C_{arom}); 1333 (S=O); 1234 (P=O); 1163 (S=O); 1055, 1020 (P-O-C). ESI-MS m/z 426 (M+Na); 442 (M+K). Elem. anal. Calcd for $C_{16}H_{22}NO_5PS_2$: C 47.63; H 5.50; N 3.47. Found: C 47.89; H 5.63; N 3.28

Diethyl (ferrocenyl)(4-methylphenylsulfonamido)-methylphosphonate (2dA).

Yield = 0.25 g (49%). m.p. 173-175°C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.84 (d, $^{3}J_{HH}$ = 8.2 Hz, p-C_6H_4, 2H); 7.31 (d, $^{3}J_{HH}$ = 8.0 Hz, p-C_6H_4, 2H); 5.10 (dd, $^{3}J_{HH}$ = 3.6Hz, $^{3}J_{PH}$ = 8.7Hz, NH, 1H); 4.62 (dd, $^{2}J_{PH}$ = 18.7Hz, $^{3}J_{HH}$ = 9.1Hz, CHP, 1H); 4.26 (s, CH_{fer}, 5H); 4.22 (m, CH_{fer}, 1H); 4.17 (m, CH_{fer}, 1H); 4.15-4.14 (m, CH_{fer}, 2H); 3.96-3.87 (m, PO$\underline{CH_2}CH_3$, 2H); 3.77-3.67 (m, PO$\underline{CH_2}CH_3$, 2H); 2.43 (s, CH_3, 3H); 1.14 (t, $^{3}J_{HH}$ = 7.1Hz, POCH$_2\underline{CH_3}$, 3H); 1.12 (t, $^{3}J_{HH}$ = 7.1Hz, POCH$_2\underline{CH_3}$, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 142.85 (C_{arom}); 139.37 (C_{arom}); 129.31 (2x C_{arom}); 127.00 (C_{arom}); 84.22 (d, $^{2}J_{PC}$ = 6.9Hz, C_{fer}); 69.24 (C_{fer}); 68.15 (d, $^{3}J_{PC}$ = 3.4Hz, C_{fer}); 67.90 (C_{fer}); 67.83 (C_{fer}); 67.06 (d, $^{3}J_{PC}$ = 3.6Hz, C_{fer}); 63.06 (d, $^{2}J_{PC}$ = 6.8Hz, POC); 62.80 (d, $^{2}J_{PC}$ = 7.5Hz, POC); 50.58 (d, $^{1}J_{PC}$ = 158.9Hz, PCN); 21.48 (CH_3); 16.28 (d, $^{3}J_{PC}$ = 5.5Hz, POCC); 16.23 (d, $^{3}J_{PC}$ = 5.5Hz, POCC). ^{31}P NMR (243 MHz, $CDCl_3$): δ 19.69. IR (KBr): 3432 (NH); 3118 (C-H_{arom}), 1598, 1476 (C=C_{arom}); 1325 (S=O); 1231 (P=O); 1141 (S=O); 1055, 1027 (P-O-C). ESI-MS m/z 528.1 (M+Na); 544.1 (M+K). Elem. anal. Calcd for $C_{22}H_{28}FeNO_5PS$: C 52.29; H 5.58; N 2.77. Found: C 52.88; H 5.85; N 2.55

Diisopropyl (4-methoxyphenyl)(4-methylphenylsulfonamido)-methylphosphonate (2aB).

Yield = 0.29 g (64%). m.p. 139-140°C, lit[14] 130-131°C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.43 (d, $^{3}J_{HH}$ = 8.2Hz, p-C_6H_4, 2H); 7.10 (dd, $^{3}J_{HH}$ = 8.6Hz, $^{4}J_{HH}$ = 1.9Hz, p-C_6H_4, 2H); 6.94 (d, $^{3}J_{HH}$ = 7.9Hz, p-C_6H_4, 2H); 6.68-6.66 (m, NH, 1H); 6.57 (d, $^{3}J_{HH}$ = 8.5Hz, p-C_6H_4, 2H); 4.84-4.80 (m, OCH$(CH_3)_2$, 1H); 4.66

(dd, $^{3}J_{HH}$ = 9.5Hz, $^{2}J_{PH}$ = 24.2Hz, CHP, 1H); 4.38-4.34 (m, $OCH(CH_3)_2$, 1H); 3.71 (s, OCH_3, 3H); 2.26 (s, CH_3, 3H); 1.36 (m, CH_3, 6H); 1.21 (d, $^{3}J_{HH}$ = 6.2Hz, CH_3, 3H); 0.82 (d, $^{3}J_{HH}$ = 6.2Hz, CH_3, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 159.16 (d, $^{4}J_{PC}$ = 2.2Hz, C_{arom}); 142.30 (C_{arom}); 138.36 (C_{arom}); 129.75 (d, $^{3}J_{PC}$ = 5.8Hz, C_{arom}); 128.75 (C_{arom}); 127.09 (C_{arom}); 126.09 (C_{arom}); 113.34 (C_{arom}); 72.65 (d, $^{2}J_{PC}$ = 7.5Hz, POC); 72.38 (d, $^{2}J_{PC}$ = 7.1Hz, POC); 55.29 (d, $^{1}J_{PC}$ = 160.6Hz, PC); 55.22 (OCH_3); 24.34 (d, $^{3}J_{PC}$ = 3.1Hz, POCC); 24.16 (d, $^{3}J_{PC}$ = 3.0Hz, POCC); 23.80 (d, $^{3}J_{PC}$ = 5.6Hz, POCC); 23.02 (d, $^{3}J_{PC}$ = 6.1Hz, POCC); 21.27 (CH_3). ^{31}P NMR (243 MHz, $CDCl_3$): δ 18.09. IR (KBr): 3421 (NH); 3136 (C-H_{arom}), 1612, 1515 (C=C_{arom}); 1309 (S=O); 1235 (P=O); 1159 (S=O); 1037, 1007 (P-O-C). ESI-MS m/z 478 (M+Na); 494 (M+K).

Diisopropyl (2-methoxyphenyl)(4-methylphenylsulfonamido)-methylphosphonate (2bB).

Yield = 0.07 g (16%). m.p. 210-212°C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.44 (d, $^{3}J_{HH}$ = 8.1 Hz, *p*-C_6H_4, 2H); 7.12-7.10 (m, *o*-C_6H_4, 1H); 7.08-7.04 (m, *o*-C_6H_4, 1H); 6.94 (d, $^{3}J_{HH}$ = 8.1 Hz, *p*-C_6H_4, 2H); 6.69 (t, $^{3}J_{HH}$ = 7.5Hz, *o*-C_6H_4, 1H); 6.57 (d, $^{3}J_{HH}$ = 8.2Hz, *o*-C_6H_4, 1H); 5.89 (d, $^{3}J_{HH}$ = 6.4 Hz, NH, 1H); 5.14 (dd, $^{3}J_{HH}$ = 9.4Hz, $^{2}J_{PH}$ = 23.8Hz, CHP, 1H), 4.82-4.77 (m, PO<u>CH</u>$(CH_3)_2$, 1H); 3.38-3.33 (m, PO<u>CH</u>$(CH_3)_2$, 1H); 3.72 (s, OCH_3, 3H); 2.25 (s, CH_3, 3H); 1.36 (d, $^{3}J_{HH}$ = 6.2Hz, POCH(<u>CH_3</u>)$_2$, 3H); 1.35 (d, $^{3}J_{HH}$ = 6.2Hz, POCH(<u>CH_3</u>)$_2$, 3H); 1.18 (d, $^{3}J_{HH}$ = 6.2Hz, POCH(<u>CH_3</u>)$_2$, 3H); 0.80 (d, $^{3}J_{HH}$ = 6.2Hz, POCH(<u>CH_3</u>)$_2$, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 156.73 (d, $^{2}J_{PC}$ = 5.6 Hz, C_{arom}); 142.75 (C_{arom}); 137.36 (C_{arom}); 129.33 (s, $^{3}J_{PC}$ = 4.2Hz, C_{arom}); 128.88 (d, $^{3}J_{PC}$ = 2.9 Hz, C_{arom}); 128.76 (C_{arom}); 127.03 (C_{arom}); 122.44 (C_{arom}); 120.50 (d, $^{4}J_{PC}$ = 1.9 Hz, C_{arom}); 110.49 (C_{arom}); 72.35 (d, $^{2}J_{PC}$ = 7.4 Hz, PO*C*); 72.10 (d, $^{2}J_{PC}$ = 7.1 Hz, PO*C*); 55.42 (OCH_3); 50.30 (br s, PC); 24.26 (d, $^{3}J_{PC}$ = 3.2Hz, POC*C*); 24.14 (d, $^{3}J_{PC}$ = 3.2Hz, POC*C*); 23.77 (d, $^{3}J_{PC}$ = 5.5Hz, POC*C*); 22.91 (d, $^{3}J_{PC}$ = 6.1Hz, POC*C*);

21.29 (CH_3). ^{31}P NMR (243 MHz, $CDCl_3$): δ 18.21. IR (KBr): 3432 (NH); 3149 (C-Harom); 1059, 1497 (C=Carom); 1336 (S=O); 1239 (P=O); 1161 (S=O); 1025 (P-O-C). ESI-MS m/z 478 (M+Na); 494 (M+K). Elem. anal. Calcd for $C_{21}H_{30}NO_6PS$: C 55.37; H 6.64; N 3.07. Found: C 54.98; H 6.61 N 2.95.

Diisopropyl (2-thienyl)(4-methylphenylsulfonamido)-methylphosphonate (2cB).

Yield = 0.10 g (23%). m.p. 204-205°C. ^1H NMR (600 MHz, $CDCl_3$): δ 7.52 (d, $^3J_{HH}$ = 8.3 Hz, *p*-C_6H_4, 2H); 7.09-7.07 (m, *p*-C_6H_4 and H_{thioph}, 3H); 6.89-6.88 (m, H_{thioph}, 1H); 6.75 (dd, $^3J_{HH}$ = 4.0Hz, $^3J_{HH}$ = 4.8Hz, H_{thioph}, 1H); 5.60 (dd, $^3J_{HH}$ = 9.1Hz, $^3J_{PH}$ = 5.2Hz, NH, 1H); 4.99 (dd, $^2J_{PH}$ = 23.9Hz, $^3J_{HH}$ = 9.2Hz, CHP, 1H); 4.80-4.74 (m, $OCH(CH_3)_2$, 1H); 4.52-4.47 (m, $OCH(CH_3)_2$, 1H); 2.33 (s, CH_3, 3H); 1.35 (d, $^3J_{HH}$ = 6.2Hz, CH_3, 3H); 1.33 (d, $^3J_{HH}$ = 6.2Hz, CH_3, 3H); 1.24 (d, $^3J_{HH}$ = 6.1Hz, CH_3, 3H); 0.94 (d, $^3J_{HH}$ = 6.2Hz, CH_3, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 143.08 (C_{arom}); 137.79 (C_{arom}); 136.68 (C_{arom}); 129.18 (C_{arom}); 127.53 (d, $^2J_{PC}$ = 7.6Hz, C_{thioph}); 127.05 (C_{arom}); 126.51 (d, $^4J_{PC}$ = 1.9Hz, C_{thioph}); 125.84 (d, $^3J_{PC}$ = 3.0Hz, C_{thioph}); 72.97 (d, $^2J_{PC}$ = 8.3Hz, POC); 72.92 (d, $^2J_{PC}$ = 8.3Hz, POC); 51.15 (d, $^1J_{PC}$ = 162.6Hz, PC); 24.21 (d, $^3J_{PC}$ = 3.4Hz, POCC); 24.15 (d, $^3J_{PC}$ = 3.1Hz, POCC); 23.70 (d, $^3J_{PC}$ = 5.5Hz, POCC); 23.08 (d, $^3J_{PC}$ = 5.6Hz, POCC); 21.40 (CH_3). ^{31}P NMR (243 MHz, $CDCl_3$): δ 16.37. IR (KBr): 3424 (NH); 3131 (C-H_{arom}), 1600, 1453 (C=C_{arom}); 1335 (S=O); 1241 (P=O); 1163 (S=O); 1060, 992 (P-O-C). ESI-MS m/z 454 (M+Na); 470 (M+K). Elem. anal. Calcd for $C_{18}H_{26}NO_5PS_2$: C 50.10; H 6.07; N 3.25. Found: C 50.09; H 6.04 N 3.19.

Diisopropyl (ferrocenyl)(4-methylphenylsulfonamido)-methylphosphonate (2dB).

Yield = 0.19 g (36%). m.p. 182-183°C. ^1H NMR (600 MHz, $CDCl_3$): δ 7.85 (d, $^3J_{HH}$ = 7.2Hz, p-C_6H_4, 2H); 7.31 (d, $^3J_{HH}$ = 7.5Hz, p-C_6H_4, 2H); 5.08 (dd, $^3J_{HH}$ = 4.0Hz, $^3J_{PH}$ = 8.9Hz, NH, 1H); 4.64 (dd, $^2J_{PH}$

= 19.4Hz, $^{3}J_{HH}$ = 8.9Hz, CHP, 1H); 4.51-4.42 (m, $OCH(CH_3)_2$, 2H); 4.28 (m, CH_{fer}, 1H); 4.24 (s, CH_{fer}, 5H); 4.19 (m, CH_{fer}, 1H); 4.14-4.11 (m, CH_{fer}, 2H); 2.43 (s, CH_3, 3H); 1.20 (d, $^{3}J_{HH}$ = 5.8 Hz, CH_3, 3H); 1.17 (d, $^{3}J_{HH}$ = 5.8Hz, CH_3, 3H); 1.10 (d, $^{3}J_{HH}$ = 5.8Hz, CH_3, 3H); 1.06 (d, $^{3}J_{HH}$ = 5.8Hz, CH_3, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 143.16 (C_{arom}); 139.09 (C_{arom}); 129.44 (C_{arom}); 127.18 (C_{arom}); 84.61 (d, $^{2}J_{PC}$ = 6.5Hz, C_{fer}); 71.79 (d, $^{2}J_{PC}$ = 7.0Hz, POC); 71.76 (d, $^{2}J_{PC}$ = 7.2Hz, POC); 69.26 (C_{fer}); 68.25 (d, $^{3}J_{PC}$ = 4.4Hz, C_{fer}); 67.92 (C_{fer}); 67.72 (C_{fer}); 67.01 (d, $^{3}J_{PC}$ = 4.2Hz, C_{fer}); 51.26 (d, $^{1}J_{PC}$ = 158.6Hz, CHP); 24.02 (d, $^{3}J_{PC}$ = 3.3Hz, POCC); 23.93 (d, $^{3}J_{PC}$ = 3.2Hz, POCC); 23.59 (d, $^{3}J_{PC}$ = 2.41Hz, POCC); 21.48 (CH_3). ^{31}P NMR (243 MHz, $CDCl_3$): δ 17.66. IR (KBr): 3439 (NH); 3092 (C-H_{arom}), 1630, 1598 (C=C_{arom}); 1332 (S=O); 1237 (P=O); 1161 (S=O); 1047, 1036 (P-O-C). ESI-MS m/z 556 (M+Na); 572 (M+K). Elem. anal. Calcd for $C_{24}H_{32}NO_5PSFe$: 54.04; H 6.05; N 2.63. Found: C 53.85; H 5.99; N 2.44.

Dibenzyl (4-methoxyphenyl)(4-methylphenylsulfonamido)-methylphosphonate (2aC).

Yield = 1.20 g (74%). m.p. 159-161°C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.47 (d, $^{3}J_{HH}$ = 8.2Hz, ArH, 2H); 7.33-7.29 (m, ArH, 5H); 7.26-7.22 (m, ArH, 3H); 7.11 (dd, $^{4}J_{HH}$ = 2.0 Hz, $^{3}J_{HH}$ = 8.7 Hz, ArH, 2H); 7.104-7.03 (m, ArH, 2H); 6.96 (d, $^{3}J_{HH}$ = 8.1 Hz, ArH, 2H); 6.62 (d, $^{3}J_{HH}$ = 8.6 Hz, ArH, 2H); 6.31 (dd, $^{3}J_{PH}$ = 5.5Hz, $^{3}J_{HH}$ = 9.1Hz, NH, 1H); 5.08 and 5.01 (Part of ABX system, $^{2}J_{HH}$ = 11.8 Hz, $^{3}J_{PH}$ = 7.2 and 9.1 Hz, POCH, 1H); 4.82 (dd, $^{3}J_{HH}$ = 9.4 Hz, $^{2}J_{PH}$ = 23.7 Hz, CHP, 1H); 4.77 (Part of ABX system, $^{3}J_{PH}$ = 7.4 Hz, $^{2}J_{HH}$ = 11.8 Hz, POCH, 1H); 4.52 (Part of ABX system, $^{3}J_{PH}$ = 8.8Hz, $^{2}J_{HH}$ = 11.8 Hz, POCH, 1H); 3.73 (s, OCH_3, 3H); 2.27 (s, CH_3, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 159.49 (d, $^{2}J_{PC}$ = 2.1 Hz, C_{arom}); 142.82 (C_{arom}); 137.85 (C_{arom}); 135.95 (d, $^{3}J_{PC}$ = 6.3 Hz, C_{arom}); 135.70 (d, $^{3}J_{PC}$ = 5.7 Hz, C_{arom}); 129.49 (d, $^{3}J_{PC}$ = 6.3 Hz, C_{arom}); 129.06 (C_{arom}); 128.56 (C_{arom}); 12.44 (d, $^{3}J_{PC}$ =

6.2Hz, C_{arom}); 128.34 (C_{arom}); 127.82 (C_{arom}); 127.12 (C_{arom}); 125.52 (C_{arom}); 113.80 (C_{arom}); 69.09 (d, $^{2}J_{PC}$ = 7.2Hz, POC); 68.70 (d, $^{2}J_{PC}$ = 7.0 Hz, POC); 55.24 (OCH_3); 54.77 (d, $^{1}J_{PC}$ = 157.0 Hz, CPN); 21.34 (CH_3). ^{31}P NMR (243 MHz, $CDCl_3$): δ 20.70. IR (KBr): 3432 (NH); 3115 (C-H_{arom}), 1610, 1512 (C=C_{arom}); 1308 (S=O); 1233 (P=O); 1161 (S=O); 1032, 1010 (P-O-C). ESI-MS m/z 550 ($M-H^{+}$). Elem. anal. Calcd for $C_{29}H_{30}NO_6PS$: C 63.15; H 5.48; N 2.54. Found: C 62.85; H 5.28; N 2.48.

Dibenzyl (2-methoxyphenyl)(4-methylphenylsulfonamido)-methylphosphonate (2bC).

Yield = 0.34 g (62%). m.p. 121-122°C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.48 (d, $^{3}J_{HH}$ = 8.3 Hz, ArH, 2H); 7.36-7.30 (m, ArH, 5H); 7.25-7.19 (m, ArH, 4H); 7.10-7.07 (m, ArH , 1H); 7.00 (d, $^{3}J_{HH}$ = 6.7Hz, ArH, 2H); 6.91 (d, $^{3}J_{HH}$ = 8.0Hz, ArH, 2H); 6.71-6.69 (m, ArH, 1H); 6.59 (dd, $^{3}J_{HH}$ = 10.3Hz, $^{3}J_{PH}$ = 2.7Hz, NH, 1H); 6.56 (d, $^{3}J_{HH}$ = 8.3Hz, ArH, 1H), 5.39 (dd, $^{2}J_{PH}$ = 24.9Hz, $^{3}J_{HH}$ = 10.3Hz, CHP, 1H); 5.18-5.09 (Part of ABX system, $^{2}J_{HH}$ = 11.7 Hz, $^{3}J_{PH}$ = 7.0 and 8.7 Hz, POCH, 2H); 4.80 (Part of ABX system, $^{2}J_{HH}$ = 11.8 Hz, $^{3}J_{PH}$ = 6.8 Hz, POCH, 1H); 4.55 (Part of ABX system, $^{2}J_{HH}$ = 11.8 Hz, $^{3}J_{PH}$ = 8.0 Hz, POCH, 1H); 3.60 (s, OCH_3, 3H); 2.23 (s, CH_3, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 156.54 (d, $^{3}J_{PC}$ = 5.7Hz, C_{arom}); 142.75 (C_{arom}); 137.46 (C_{arom}); 136.22 (d, $^{3}J_{PC}$ = 5.9Hz, C_{arom}); 135.94 (d, $^{3}J_{PC}$ = 6.4Hz, C_{arom}); 129.50 (d, $^{3}J_{PC}$ = 4.5Hz, C_{arom}); 129.16 (d, $^{4}J_{PC}$ = 2.3Hz, C_{arom}); 128.8 (C_{arom}); 128.51 (C_{arom}); 128.34 (C_{arom}); 128.31 (C_{arom}); 128.12 (C_{arom}); 127.56 (C_{arom}); 127.04 (C_{arom}); 121.96 (C_{arom}); 120.67 (d, $^{4}J_{PC}$ = 1.9Hz, C_{arom}); 110.53 (C_{arom}); 69.08 (d, $^{2}J_{PC}$ = 7.3Hz, POC); 68.39 (d, $^{2}J_{PC}$ = 6.9Hz, POC); 55.36 (OCH_3); 49.71 (d, $^{1}J_{PC}$ = 161.3Hz, PC); 21.32 (CH_3). ^{31}P NMR (243 MHz, $CDCl_3$): δ 20.67. IR (KBr): 3432 (NH); 3153 (C-H_{arom}); 1051, 1495 (C=C_{arom}); 1334 (S=O); 1247 (P=O); 1167 (S=O); 1017 (P-O-C). ESI-MS m/z 574 (M+Na); 590 (M+K). Elem. anal. Calcd for $C_{29}H_{30}NO_6PS$: C 63.15; H 5.48; N 2.54. Found: C 63.49; H 5.31; N 2.45.

Dibenzyl (2-thienyl)(4-methylphenylsulfonamido)-methylphosphonate (2cC).

Yield = 0.30 g (55%). m.p. 115-117°C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.55 (d, $^{3}J_{HH}$ = 8.2 Hz, ArH, 2H); 7.34-7.24 (m, ArH, 7H); 7.11-7.09 (m, ArH, 3H); 7.03 (d, $^{3}J_{HH}$ = 8.2 Hz, ArH, 2H); 6.96-6.95 (m, H_{thioph}, 1H); 6.76 (dd, $^{2}J_{PH}$ = 4.9Hz, $^{3}J_{HH}$ = 3.7Hz, H_{thioph}, 1H); 6.28 (dd, $^{3}J_{HH}$ = 9.4Hz, $^{3}J_{PH}$ = 4.0Hz, NH, 1H); 5.15 (d, $^{2}J_{PH}$ = 23.9 and $^{3}J_{HH}$ = 9.5 Hz, CHP, 1H); 5.11 (Part of ABX system, $^{2}J_{HH}$ = 11.6 Hz, $^{3}J_{PH}$ = 7.3 Hz, POCH, 1H); 5.03 (Part of ABX system, $^{2}J_{HH}$ = 11.6 Hz, $^{3}J_{PH}$ = 9.2Hz, POCH, 1H); 4.87 (Part of ABX system, $^{2}J_{HH}$ = 11.7Hz, $^{3}J_{PH}$ = 7.3Hz, POCH, 1H); 4.62 (Part of ABX system, $^{2}J_{HH}$ = 11.6Hz, $^{3}J_{PH}$ = 8.9Hz, POCH, 1H); 2.30 (CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 143.05 (C_{arom}); 137.79 (C_{arom}); 137.86 (C_{arom}); 135.99 (C_{arom}); 135.85 (d, $^{3}J_{PC}$ = 6.1Hz, C_{arom}); 135.62 (d, $^{3}J_{PC}$ = 5.7Hz, C_{arom}); 129.18 (C_{arom}); 128.57 (C_{arom}); 128.49 (d, $^{4}J_{PC}$ = 3.2Hz, C_{arom}); 128.43 (C_{arom}); 128.14 (C_{arom}); 127.88 (d, $^{4}J_{PC}$ = 4.5Hz, C_{arom}); 127.09 (C_{arom}); 126.81 (d, $^{4}J_{PC}$ = 1.8Hz, C_{arom}); 126.10 (d, $^{4}J_{PC}$ = 3.1Hz, C_{arom}); 69.43 (d, $^{2}J_{PC}$ = 6.7Hz, POC); 69.02 (d, $^{2}J_{PC}$ = 6.7Hz, POC); 50.78 (d, $^{1}J_{PC}$ = 163.0Hz, CPN); 21.42 (CH_3). ^{31}P NMR (243 MHz, $CDCl_3$): δ 18.85. IR (KBr): 3432 (NH); 3152 (C-H_{arom}), 1599, 1451 (C=C_{arom}); 1322 (S=O); 1237 (P=O); 1157 (S=O); 1061, 995 (P-O-C). ESI-MS m/z 550 (M+Na); 566 (M+K). Elem. anal. Calcd for $C_{26}H_{26}NO_5PS_2$: C 59.19; H 4.97; N 2.65. Found: C 59.08; H 4.84; N 2.64.

Dibenzyl (ferrocenyl)(4-methylphenylsulfonamido)-methylphosphonate (2dC).

Yield = 0.48 g (77%). m.p. 173-175°C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.80 (d, $^{3}J_{HH}$ = 8.3Hz, ArH, 2H); 7.29-7.26 (m, ArH, 6H); 7.20 (d, $^{3}J_{HH}$ = 8.1Hz, ArH, 2H); 7.14-7.11 (m, ArH, 4H); 5.22 (dd, $^{3}J_{PH}$ = 3.7Hz, $^{3}J_{HH}$ = 8.9Hz, NH, 1H); 4.82 (Part of ABX system, $^{2}J_{HH}$ = 11.8Hz, $^{3}J_{PH}$ = 7.8Hz, POCH, 1H); 4.77-4.71 (m, POCH, CHP, 2H); 4.55 (Part of ABX system, $^{2}J_{HH}$ = 11.6Hz, $^{3}J_{PH}$ = 8.9Hz, POCH, 1H);

4.46 (Part of ABX system, $^{2}J_{HH}$ = 11.6 Hz, $^{3}J_{PH}$ = 10.0Hz, POC, 1H); 4.24 (s, C_5H_5, 5H); 4.24-4.23 (m, CH_{fer}, 1H); 4.19 (m, CH_{fer}, 1H); 4.16-4.15 (m, CH_{fer}, 2H); 2.36 (s, CH_3, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 143.16 (C_{arom}); 138.82 (C_{arom}); 136.00 (d, $^{3}J_{PC}$ = 5.3Hz, C_{arom}); 135.87 (d, $^{3}J_{PC}$ = 5.5Hz, C_{arom}); 129.44 (C_{arom}); 128.47 (C_{arom}); 128.39 (d, $^{3}J_{PC}$ = 5.2Hz, C_{arom}); 128.09 (C_{arom}); 127.97 (C_{arom}); 127.10 (C_{arom}); 84.09 (d, $^{2}J_{PC}$ = 6.5Hz, C_{fer}); 69.30 (C_{fer}); 68.42 (d, $^{2}J_{PC}$ = 7.3Hz, POC); 68.30 (d, $^{3}J_{PC}$ = 4.3Hz, C_{fer}); 68.14 (d, $^{2}J_{PC}$ = 7.6Hz, POC); 66.96 (d, $^{3}J_{PC}$ = 3.4Hz, C_{fer}); 50.81 (d, $^{1}J_{PC}$ = 158.8 Hz, CP); 21.51 (CH_3). ^{31}P NMR (243 MHz, $CDCl_3$): δ 20.52. IR (KBr): 3432 (NH); 3104 (C-H_{arom}), 1625, 1599 (C=C_{arom}); 1327 (S=O); 1234 (P=O); 1162 (S=O); 1020, 992 (P-O-C). ESI-MS m/z 652 (M+Na); 668 (M+K). Elem. anal. Calcd for $C_{32}H_{32}FeNO_5PS$: C 61.06; H 5.12; N 2.23. Found: C 61.12; H 5.19; N 2.10.

Evaluation of cytotoxicity of compounds 2aB-2cB, 2aC-2cC, 2bA and 2dA

Preparation of drug stock and working solutions

Stock solutions of phosphonates were prepared at the concentration of 1 mM in DMSO. The stock solutions of compounds were diluted in culture medium with the final concentration of DMSO in culture medium lower than 1%.

Cell Culture

PBMCs were isolated by density gradient centrifugation over Histopaque 1077 (Sigma-Aldrich, Poznan, Poland) from leukocyte buffy coat obtained from the Blood Donation Center in Lodz, Poland as described by Böyum.[46] A549 cell line was purchased from American Type Culture Collection (ATCC, Rockville, MD, USA) and U87 from HPA Culture Collections (Sigma-Aldrich,

Poznan, Polska). HL-60 was a kind gift from dr Adrianna Nowak from Łódź University of Technology.

PBMCs were cultured in RPMI 1640 medium (Lonza, Basel, Switzerland) with 10% foetal bovine serum (FBS, Biowest, Nuaillé, France), A549 cells were cultured in DMEM:F-12 1:1 mixture medium (Lozna) with 10% FBS, HL-60 cells were maintained in IMDM medium (Lonza) with 15% FBS and U87 cells were grown in EMEM (Sigma-Aldrich) with 10% FBS, 1% non-essential amino acids (Sigma-Aldrich) and 1 mM sodium pyruvate (Sigma-Aldrich). All media were supplemented with 100 units/ml penicillin (Lonza) and 100 µg/ml streptomycin (Lonza). Cultures were maintained in an incubator with 5% CO_2 at 100% humidity and 37 °C.

Resazurin cell viability assay

Cancer cells and PBMCs were plated in 96-well plates at a density of 1×10^4 or 5×10^4, respectively, in 100 µl of culture medium. After overnight adaptation, the cells were exposed in triplicate to different concentrations of studied compounds for subsequent 24 h. All experiments included a negative control and additionally a solvent control.

The cytotoxic effect of studied compounds was evaluated with a system based on the weakly fluorescent blue dye, resazurin, which upon reduction by living cells becomes highly red fluorescent resorufin using In Vitro Toxicology Assay Kit (Sigma-Aldrich, Poznan, Poland).[24] After the incubation, resazurin dye solution was added in an amount equal to 10% of the culture medium volume and the cells were incubated for another 3 h. Then the fluorescence intensity was measured with the excitation and emission set at 530/25 nm and 590/35 nm, respectively using a Synergy HT spectrophotometer (Biotek, Bad Friedrichshall, Germany). Relative cell

viability (%) was expressed as a percentage relative to the untreated cells. GraphPad Prism (version 5.01, GraphPad Software Inc., San Diego, CA, USA) was employed to produce graphs. All results were presented as mean ± SEM.

Evaluation of ecotoxicity of dibenzyl (ferrocenyl)(4-methylphenylsulfonamido)-methylphosphonate (2dC)

Phytotoxicity

The evaluation of phytotoxicity of the investigated aminophosphonate **2dC** at applied concentrations was made by comparing the germination, dry weight of control plants sprouts (seedlings) with germination and of dry and fresh plants sprouts grown in the soil with an admixture of given amounts of the tested compounds. In order to determine growth inhibition of root and shoot of selected plants, height of shoot and length of root were measured as described by Wang et al.[47] The length of plants is defined as the length the tip of the longest leaf to the base of culms, while root length is measured from the tip of longest root to the root-shoot junction.

Inhibition ratio was calculated according to method given by Pawłowska and Biczak,[48] namely:

<img class="ee_img tr_noresize" alt="Inhibition\ ratio = \frac{{length\ in\ control\ group - length\ in\ treatment\ group}}{{length\ of\ control\ group}}100\% " src="http://chart.apis.google.com/chart?cht=tx&chs=1x0&chf=bg,s,FFFFFF00&chco=000000&chl=Inhibition%5C%20ratio%20%3D%20%5Cfrac%7B%7Blength%5C%20in%5C%20control%5C%20group%20-%20length%5C%20in%5C%20treatment%5C%20group%7D%7D%7B%7Blength%5C%20of%5

C%20control%5C%20group%7D%7D100%5C%25%20" eeimg="1" style="vertical-align: middle; " longdesc="MathType!MTEF!2!1!+-
feaagKart1ev2aqatCvAUfeBSjuyZL2yd9gzLbvyNv2CaerbuLwBLn
hiov2DGi1BTfMBaeXatLxBI9gBaerbd9wDYLwzYbItLDharqqtubsr
4rNCHbGeaGqiVCI8FfYJH8YrFfeuY-Hhbbf9v8qqaqFr0xc9pk0xbb
a9q8WqFfeaY-biLkVcLq-JHqpepeea0-as0Fb9pgeaYRXxe9vr0-vr
0-vqpWqaaeaabiGaciaacaqabeaadaqaaqaaaOqaaabaaaaaaaaape
GaamysaiaadGacaWGObGaamyAaiaadkgacaWGPbGaamiDaiaadMga
caWGVbGaamOBaiaacckacaWGYbGaamyyaiaadshacaWGPbGaam4Bai
abg2da9maalaaapaqaa8qacaWGSbGaamyzaiaadGacaWGNbGaamiD
aiaadIgacaGGGcGaamyAaiaadGacaGGGcGaam4yaiaad+gacaWGUb
GaamiDaiaadkhacaWGVbGaamiBaiaacckacaWGNbGaamOCaiaad+ga
caWG1bGaamiCaiabgkHiTiaadYgacaWGLbGaamOBaiaadEgacaWG0b
GaamiAaiaacckacaWGPbGaamOBaiaacckacaWG0bGaamOCaiaadwga
caWGHbGaamiDaiaad2gacaWGLbGaamOBaiaadshacaGGGcGaam4zai
aadkhacaWGVbGaamyDaiaadchaa8aabaWdbiaadYgacaWGLbGaamOB
aiaadEgacaWG0bGaamiAaiaacckacaWGVbGaamOzaiaacckacaWGJb
Gaam4Baiaad6gacaWG0bGaamOCaiaad+gacaWGSbGaaiiOaiaadEga
caWGYbGaam4BaiaadwhacaWGWbaaaiaaigdacaaIWaGaaGimaiaacw
caaaa!8F20!
">

Effective concentration EC_{50} for fresh matter of plants was calculated using GraphPad Prism software (Version 7, GraphPad Software, Inc., La Jolla, CA 92037, USA).

The dry weights of tested plants were measured after drying at 75^{o}C until the constant weight.

Values of the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) of the compound under study **2dC** was performed. The visual evaluation of phytotoxicity of the aminophosphonates **2dC** at applied concentrations was performed by digital photography. Obtained pictures were analyzed in terms of any type of damage to tested plants, such as their growth inhibition, chlorosis, and necrosis. Tests were carried out three times for each sample.

Pigment assay

Photosynthetic pigments content was determined according to method reported by Oren et al.[49] Fresh leaves (200 mg) were thoroughly homogenized in 20 mL of 80% acetone in a cooled mortar and centrifuged afterward. The content of chlorophyll a, chlorophyll b and carotenoids was calculated based on the absorbance at wavelength 470, 647 and 664 nm. The content of photosynthetic pigments were expressed in mg/g of dry weight.

Microtox® Toxicity Assay

Detailed procedure of Microtox Toxicity Assay was described previously by Lewkowski et al.[50] Method is based on the analysis of light emission reduction of luminescent bacteria (*Aliivibrio fischeri*) under toxic stress. The tests were carried out in a Microtox® M500 analyzer according

to the 1992 Microtox® Manual. The Microtox® Solid-Phase Test (MSPT) was adopted to report of Doe et al.[51]

The MSPT procedure allows the test organisms to come direct contact with the solid sample in an aqueous suspension of the test sample. Thus it is possible to detect toxicity which is due to the insoluble solids that are not in the solution. All materials and reagents were purchased from MODERNWATER (New Castle, DE, USA). Toxicity was determined by using the marine luminescent bacteria *Aliivibrio fischeri*, naturally adapted to a saline environment. Bacteria were regenerated with 1 mL of Reconstitution Solution (0.01%) and placed in the reagent well of the Microtox®. A suspension of 7 g of the sediment was prepared in 35 mL of a Solid Phase Diluent (3.5% NaCl) and was magnetically stirred for 10 min. Then a series of dilutions were made and bacteria (approx. $1x10^6$ cell/mL per assay) were exposed to these dilutions and to a blank (3.5% NaCl solution) for 20 min. Next, after filtration, the light output of supernatants containing exposed bacteria was measured after 5 min with a Microtox® Analyzer 500. Inhibition was calculated as the concentration of compound loaded to sediment (mg/L) that caused a 50% reduction in the light emitted by the bacteria, and EC_{50} along with 95% confidence limit determined by the software provided with the Analyzer.

Ostracod Test Kit

Ecotoxicity evaluation of synthesized compounds was performed in a short term contact test using Ostracodtoxkit F™ provided by MicroBiotests Inc. (Gent, Belgium). This direct sediment contact bioassay was performed in multiwell test plates using neonates of the benthic ostracod crustacean *Heterocypris incongruens* hatched from cysts.[52]

After 6 days of contact with the sediment (or soil) the percentage mortality and the growth of the crustaceans were determined and compared to the results obtained in a non-treated reference sediment (soil). Briefly, according to manual of Ostracodtoxkit test, the cysts (*Heterocypris incongruens*) were transferred into a Petri dish filled with 10 mL standard fresh water (reconstituted water) and were incubated at 25^{o}C for 52 h under continuous illumination (approx. 3000–4000 lux). After 48 h of cysts incubation, pre-feeding of the freshly hatched ostracods was performed with algae (spirulina-powder) provided in the test kit. Next, after hatching, before feeding with algal food suspension, the length measurements of ostracod neonates has been done. Algae (*Selenastrum capricornutum*) used as feed in the test plate were reconstituted according to the manufacturer's procedure. Each well of a test plate was filled in the following order: 2 mL standard freshwater, 2500 µL of sediment (soil) treated and non-treated for comparison (blank), 2 mL already prepared algal suspension, 10 ostracods. The test plates were covered with Parafilm® and closed by a lid. Then multi wall plates were incubated at 25^{o}C in darkness for 6 d. After 6 d of exposure, the ostracods have been recovered from the multiwells to determine the percentage mortality. To calculate the growth inhibition of survived organisms, their length measurements have been also done. Mortality of test organisms was determined in six replicates. The measurement of length was carried out by means of a micrometric strip placed on the bottom of a glass microscope plate. Growth inhibition (GI) of *H. incongruens* in the test sediment was calculated as follows:

Statistical analysis

The significance of the obtained results was evaluated using the analysis of variance (ANOVA). The least significant difference (LSD) values at a confidence level of 95% were computed using the Tukey test. Moreover, the mean standard deviation were determined and plotted in diagrams.

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Table 1. Effect of **2dC** on the shoot height, root length and fresh matter of oat seedlings plant (mean ± SD, n = 15).

Conc. of 2dC [mg/kg soil d.w.]	Inhibition biomarkers [%]					
	Shoot height		Root height		Fresh Matter	
	Oat	Radish	Oat	Radish	Oat	Radish
Control	0	0	0	0	0	0
1	0.21±0.06	0.32±0.11	2.43±0.45	1.35±0.49	5.03±7.05	1.26±8.15
10	1.45±1.39	3.42±0.09	2.57±0.65	4.55±1.34	6.25±0.17	2.95±0.13
100	8.47±1.43	5.17±1.45	3.05±1.35	5.67±3.21	16.45±0.08	4.02±0.18
200	16.14±2.09	6.74±2.54	3.95±0.42	16.45±5.35	18.72±0.10	15.74±0.3
400	18.21±4.55	34.46±5.44	7.83±1.33	21.83±3.43	27.32±8.92	24.94±5.2
800	21.52±1.54	39.40±1.88	11.04±0.55	29.43±4.66	29.11±9.93	53.20±5.5
1000	27.62±2.32	43.42±5.32	13.88±2.21	32.35±6.23	35.04±1.70	62.16±5.0

Table 2. Average changes (mean of three replicates) of germination of oat (*Avena sativa)* and common radish *(Raphanus sativus*) treated with **2dC**. Least significant difference for samples (LSD_S) and concentration (LSD_C) is given for each tested parameter. % Germination refers to number of emerged plants expressed as a percent of control plants.

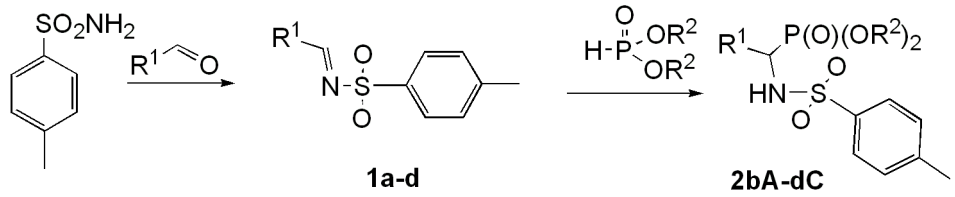
Concentration of 2dC in soil (mg/kg s.d.w.)	Number of Emerged Seedlings		Germination [%]	
	Oat	Radish	Oat	Radish
Control	19	19	100	100
1	19	19	100	97

10	19	18	100	95
100	19	18	98	95
200	19	18	98	95
400	18	18	96	93
800	18	18	96	91
1000	18	17	96	90
		$LSD_S = 1$		$LSD_S = 1$
		$LSD_C = 2$		$LSD_C = 2$

Table 3. Microtox® EC_{50} values (mg/kg of soil dry weight) of exposure of the luminescent marine bacteria *Aliivibrio fischeri* to tested **2dC** respective 95% confidence limits (in brackets) obtained in the fit of the data.

EC_{50} (lower limit; upper limit) [mg/L)	EC_{50} (lower limit; upper limit) [mg/kg s.d.w.)	Coefficient of determination (R^2)
581.0 (478.0; 706.3)	767.5 (631.0; 932.3)	0.9661

Caption to figures



a: R^1 = 4-CH_3O-C_6H_4; **b**: R^1 = 2-CH_3O-C_6H_4, **c**: R^1 = 2-thienyl; **d**: R^1 = ferrocenyl
A: R^2 = CH_2CH_3; **B:** R^2 = $CH(CH_3)_2$; **C:** R^2 = CH_2Ph

Figure 1. Preparation of N-sulfonylaminophosphonates **2bA-dC**

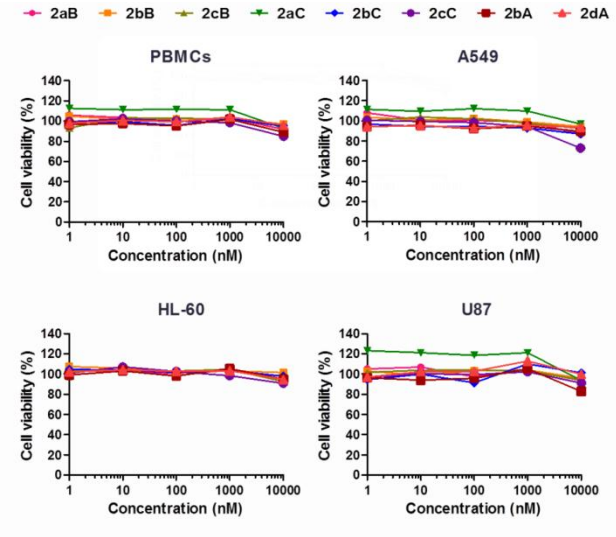


Figure 2. The cytotoxicity of compounds **2aB-2cB, 2aC-2cC, 2bA** and **2dA** against normal human peripheral blood mononuclear cells (PBMCs) and human cancer cells: non-small cell lung cancer cells (A549), promyelocytic leukemia cells (HL-60) and glioblastoma astrocytoma cells (U87).

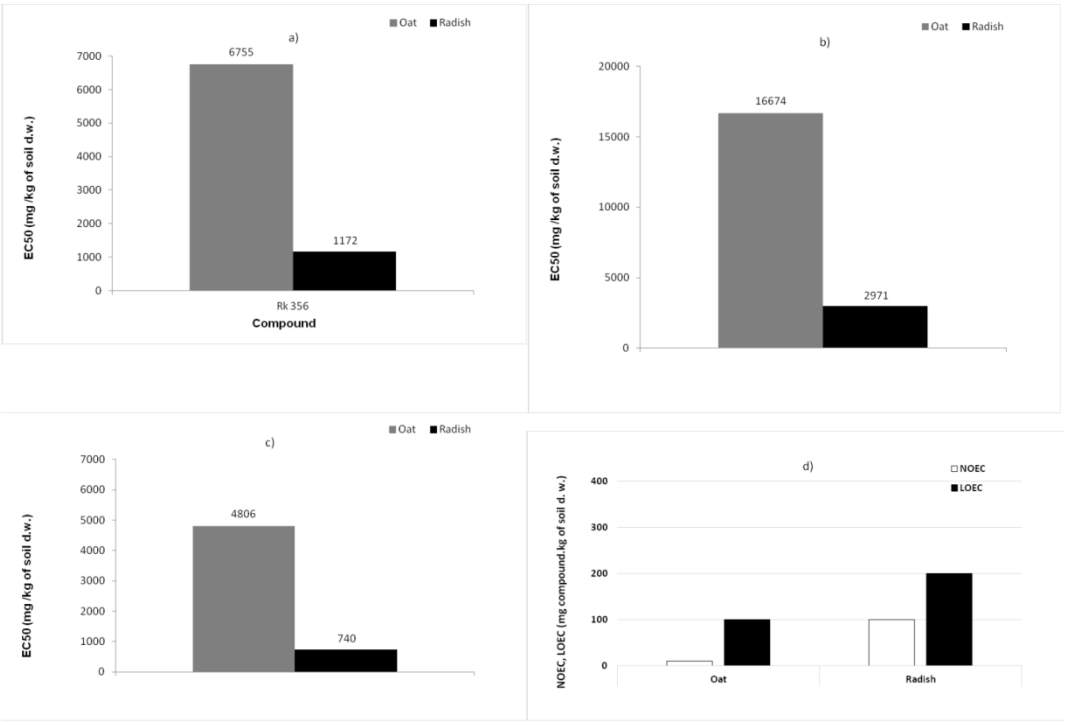


Figure 3. The EC_{50} values of growth inhibition of shoot (a), root (b) and fresh matter (c) as well as NOEC and LOEC values related to fresh matter (d) following exposure to the aminophosphonate **2dC**.

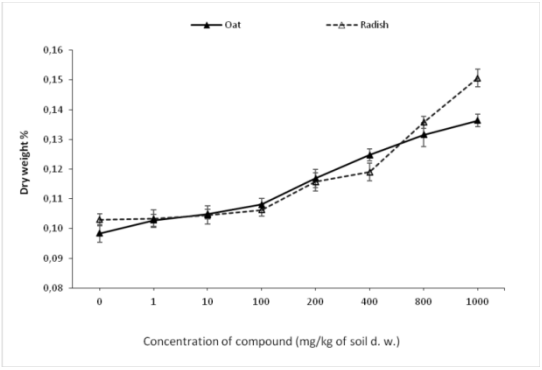


Figure 4. Changes of dry weight of treated plants with **2dC** expressed as percent to the value in untreated plants (control plants = 100% of dry weight).

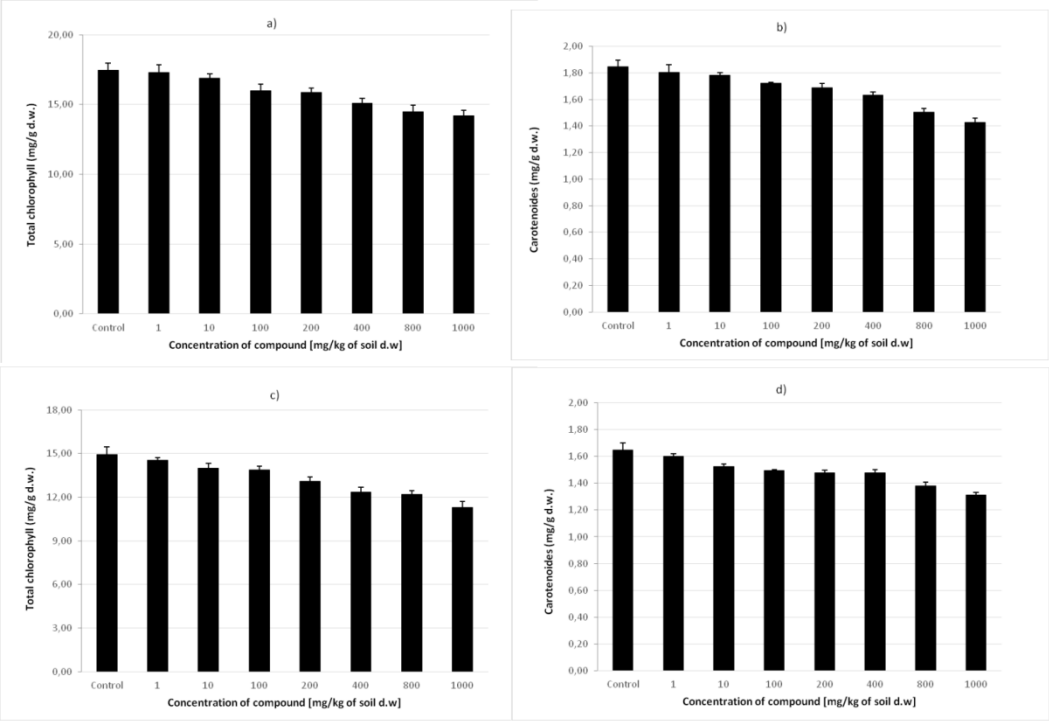


Figure 5. Effect of **2dC** on the content of total chlorophyll (a) and carotenoids (b) in oat seedlings and the content of total chlorophyll (c) and carotenoids (d) in radish leaves, Data are expressed as a mean± SD of three replicates for each concentration.

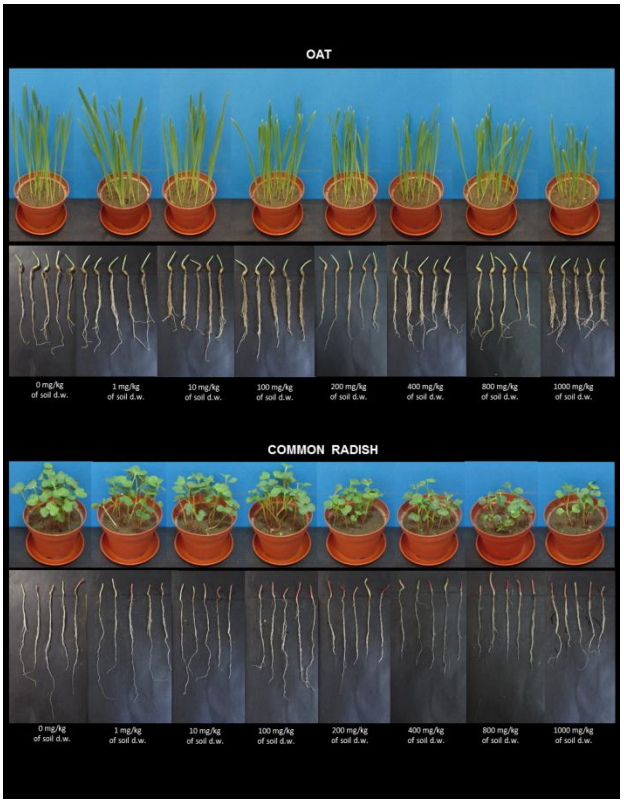


Figure 6. Digital photographs of oat and common radish: a) shoots, b) roots treated with the compound **2dC** (concentration in mg/kg of soil dry weight) on the 14th day of growth

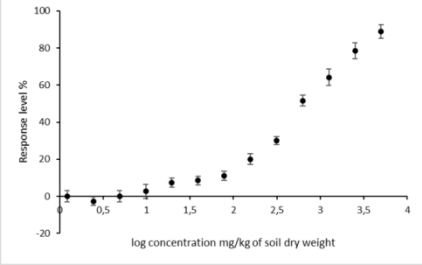


Figure 7. The EC_{50} graded dose-response curved for the tested compound **2dC**. Overlapped curves are plotted by Microtox® Analyzer 500 software. Error bars represent standard deviation errors (SD, n=3 determinations)

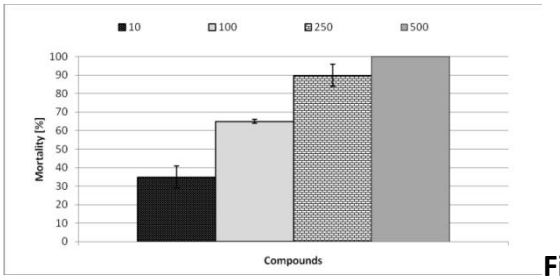


Figure 8. The mortality (%) of *Heterocypris incongruens* living in soil contaminated with **2dC** at concentrations: 10, 50, 100 and 250 mg/kg of soil dry weight. Error bars represent standard deviation error (SD, n = 6 determinations).