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ORIGINAL PAPER

# Antibacterial action of (5-nitrofurfuryl)-derived aminophosphonates and their parent imines

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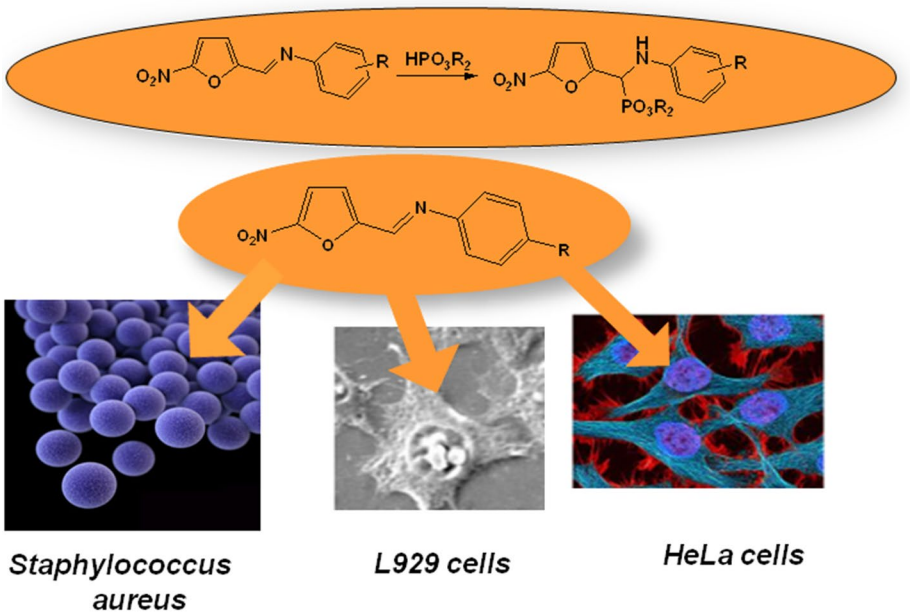
## Abstract

Ten aminophosphonates bearing 2-nitrofuran moiety and two *N*-aryl 5-nitro-furfuralaldimines, namely, *N*-(2-nitrofurfurylidene)-*p*-toluidine and *N*-(2-nitrofurfurylidene)-*p*-anisidine, were tested in aspect of their antibacterial action. *O,O′*-diphenyl derivatives were found inactive, while *O,O′*-dimethyl and *O,O′*-diethyl derivatives were revealed to act moderately efficiently against clinical isolates of *S. aureus*, especially against methicillin-resistant (MRSA) strains. A high activity against these strains was found for *N*-(4-methylphenyl)-5-nitrofurfuralaldimine and *N*-(4-methoxyphenyl)-5-nitro-furfuralaldimine, but they showed the cytotoxicity at a dangerous level.

## Graphical abstract




**Keywords** 5-Nitrofurfural-derived aminophosphonates · *S. aureus* · Antibacterial activity · MRSA strains · Cytotoxicity

## Introduction

Azomethine derivatives of 5-nitrofurfural are known for their strong bactericidal action, and therefore, some of them constitute active components of various drugs. Despite some controversies around their safety (Goemaere et al.

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Extended author information available on the last page of the article

2008; Huttner et al. 2015; Williams and Triller 2006; Tan et al. 2012; Stricker et al. 1988), they are still authorized to use in many countries, the most probably due to their significant usefulness. Nitrofurantoin (Garau 2008), nifuratel (Grüneberg and Leakey 1976) as well as furazidine are often applied in cases of various urinary tract infections, while nifuroxazide (Carron 1966; Cagliero 1978) and furazolidine (Machado et al. 2008) for intestinal infections. Although they are suspected to cause various toxicities, i.e., pulmonary toxicity (including pulmonary fibrosis caused by nitrofurantoin) (Goemaere et al. 2008; Huttner et al. 2015; Williams and Triller 2006), hepatotoxicity (Tan et al. 2012) and neurotoxicity (Stricker et al. 1988), they are still considered as efficient antibacterial and antiprotozoal agents (Jackson et al. 2010; Priotto et al. 2009) and are not withdrawn from official lists of pharmaceutical products (not in all countries). It is not unreasonable action, because due to the unique mode of action, nitrofurans together with nitroimidazole derivatives constitute an important and precious group of antiprotozoal and antibacterial agents. Their most important potential is related to their antiprotozoal action against *Trypanosoma brucei gambiense* and *Trypanosoma cruzii* causing the sleeping sickness and Chagas' disease; therefore, studies on action of nitrofuran derivatives on the above parasites are performed continuously (Jackson et al. 2010; Priotto et al. 2009; Stewart et al. 2004; Zhou et al. 2013).

Their mechanism of action is complex; nitroreductases (particularly nitrofuran reductase) catalyze reduction of their nitro groups inside the bacterial cell to multiple reactive intermediates, which react with ribosomal proteins, DNA, and other macromolecules within the cell, and affect pyruvate metabolism (Tu and McCalla 1975). The complex mechanism of action is the most probably responsible for the low development of resistance to their effects, as drugs affect many different processes important to the bacterial cell.

Microbial resistance to antibiotics comprises a significant problem in the treatment of bacterial infections. Although antibiotics are an effective way to control such infections, their excessive use can lead to faster spread of resistant strains in the population. Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most common and widespread antibiotic-resistant species. Until the mid-1990s, infections caused by MRSA were limited to hospitals (healthcare-associated MRSA, HA-MRSA); however, over the past 20 years, MRSA has been reported for healthy individuals who were not subject to prior hospitalization and were not in contact with the medical staff. These community-associated MRSA (CA-MRSA) strains have methicillin resistance as well as enhanced virulence and fitness (Otto 2013; Peacock and Paterson 2015).

*Staphylococcus aureus* colonizes about a third of the population and may cause moderately severe-to-severe infections and it is a leading cause of death in hospitalized patients (Hiramatsu et al. 2014). *S. aureus* causes disease through tissue damage or through the action of toxins. The multiplication of bacteria in the human body leads to the formation of abscesses as well as the destruction of tissues. More serious infections include, pneumonia, bacteremia, bone and joint infections, toxic shock syndrome, and endocarditis. Moreover, staphylococci produce enterotoxin which is not destroyed even at high temperature and its action is as dangerous as the bacterial infection itself (Peacock and Paterson 2015).

Until recently, glycopeptides such as vancomycin have been effective in the treatment of MRSA. However, the occurrence of VISA (vancomycin–intermediate *S. aureus*), and VRSA (vancomycin-resistant *S. aureus*) strains (Hiramatsu et al. 2014) caused possible variants of antibiotic therapy in hospitals to be very limited. One of them is linezolid belonging to the family of oxazolidinones (Meka and Gold 2004). Linezolid proved to be a suitable alternative to vancomycin in the treatment of MRSA infection and reduced the pressure on the use of vancomycin (Stevens et al. 2002). Apart from it, oxadiazoles were recognized as a new class of antibiotics, which inactivate PBP2a and inhibit cell-wall biosynthesis of MRSA (O'Daniel et al. 2014). Moreover, some newer cephalosporins, in particular, ceftaroline and ceftobiprole are active against MRSA (Holmes and Howden 2014).

Interesting scope of action of nitrofuran derivatives prompted us to perform tests with 5-nitrofurfural-derived aminophosphonates **2b–c, 2f, 3b–c, 3f, 4a–c, 4f** and some of their parent Schiff bases (**1c** and **1f**) in aspect of their action against *S. aureus* clinical strains including MRSA. In the course of the ecotoxicological evaluation of 5-nitrofuran-derived aminophosphonates, their harmful action on *Aliivibrio fischeri* bacteria was observed and it was a driving force for performing studies described herein. The choice of nitrofuran derivatives was driven also by results obtained for nitrofuran- and nitrothiophene-derived benzoic acid hydrazones (Osório et al. 2012), which showed antibacterial potential of these compounds.

## Experimental

### General

All solvents (POCh, Gliwice, Poland) were routinely distilled and dried prior to use. Amines, diphenyl phosphite, dimethyl phosphite, diethyl phosphite, and 5-nitrofurfural (Aldrich, Poznań, Poland) were used as received. Melting points were measured on a MelTemp II apparatus, and NMR spectra were recorded on a Bruker Avance III 600 MHz operating at 600 MHz ($^{1}$H NMR), 150 MHz ($^{13}$C NMR),

and 243 MHz ($^{31}$P NMR) or on a Varian Gemini 2000 BB operating at 81 MHz ($^{31}$P NMR). IR spectra were recorded on Nexus FT-IR (Thermo Nicolet). Elemental analyses were carried out at the Laboratory of Microanalysis, Faculty of Chemistry, University of Łódź, Poland.

Imines were synthesized following the previously published procedure (Csaszar 1984; Matusiak et al. 2013; Saikachi and Shimamura 1960).

## Synthesis of Schiff bases 1a–c and 1f

Furfural derivative (0.24 g, 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, and then, solvent was evaporated and residue dried on vacuum to obtain pure Schiff base **1c** and **1f**. *N*-methylphenyl derivative **1c** was compared to a previously obtained sample (Matusiak et al. 2013) and was found to be identical. Its data are given in our previous paper (Matusiak et al. 2013). Imines **1a** and **1b,** after isolation as above, and confirming their identity (Saikachi and Shimamura 1960) ($^1$H NMR spectra) were used for further conversion without any purification. Routine $^1$H NMR spectra of crude imines **1a–b** are in Supplementary Material, Figs. S6a and S6b.

### *N*-(2-Nitrofurfurylidene)-*p*-anisidine 1f

Quantitative yield (0.61 g), mp = 124–125 °C, lit (Csaszar 1984) 124–126 °C

$^1$H NMR ($CDCl_3$, 600 MHz): δ 8.42 (s, CH=N, 1H); 7.41 (dd, $^3J_{HH}$ = 3.8 and $^5J_{HH}$ = 0.6 Hz, $H_4^{fur}$, 1H); 7.32 (A part of AA'XX' system, $^3J_{HH}$ = 10.1, $^3J_{HH}$ = 10.1, $^4J_{HH}$ = 2.8 Hz, *p*-$C_6H_4$, 2H); 7.16 (dd, $^3J_{HH}$ = 3.8 and $^4J_{HH}$ = 0.5 Hz, $H_3^{fur}$, 1H); 6.96 (X part of AA'XX' system, $^3J_{HH}$ = 10.1, $^3J_{HH}$ = 10.1, $^4J_{HH}$ = 2.8 Hz, *p*-$C_6H_4$, 2H); 3.85 (s, $OCH_3$, 3H).

### Preparation of aminophosphonates 2a–f, 3a–f, and 4a–f

To a mixture of imine (5 mmol) and phosphite (5 mmol) in THF, $BF_3$•$OEt_2$ (1 mmol) was added via syringe. The mixture was stirred at room temperature over 24 h under a nitrogen atmosphere. A solution was purified by extraction and crystallization. Detailed description is to be found in our recent paper (Lewkowski et al. 2017) as well as detailed data concerning aminophosphonates **2c**, **2f**, **3c**, **3f**, **4c,** and **4f** (Lewkowski et al. 2017).

### rac-Dimethyl *N*-(3-methylphenyl)amino(5-nitro-2-furyl) methylphosphonate 2b

*Y* = 1.7 g (56%). mp = 124–126 °C (yellow crystals). $^1$H NMR ($CDCl_3$, 600 MHz): δ 7.24 (dd, $^3J_{HH}$ = 3.8 and $^4J_{HH}$ = 0.9 Hz, $H_{fur}$, 1H), 7.07 (t, $^3J_{HH}$ = 7.8 Hz, *m*-$C_6H_4$, 1H); 6.63 (d, $^3J_{HH}$ = 7.5 Hz, $H_{fur}$, 1H); 6.60 (t, $^4J_{HH}$ = 3.5 Hz, *m*-$C_6H_4$, 1H); 6.50–6.49 (m, *m*-$C_6H_4$, 1H); 6.46 (dd, $^3J_{HH}$ = 8.1 and $^4J_{HH}$ = 2.4 Hz, *m*-$C_6H_4$, 1H); 4.97 (d, $^2J_{PH}$ = 25.0 Hz, CHP, 1H); 3.88 (d, $^3J_{PH}$ = 10.8 Hz, $POCH_3$, 1H); 3.77 (d, $^3J_{PH}$ = 10.8 Hz, $POCH_3$, 1H); 2.26 (s, $CH_3$, 1H). $^{13}$C NMR (150 MHz, $CDCl_3$): δ 153.8 ($C_{ar}$); 152.0 ($C_{fur}$); 145.1 (d, $^2J_{PC}$ = 12.6 Hz, $C_{fur}$); 139.4 ($C_{ar}$); 129.4 ($C_{ar}$); 120.8 ($C_{ar}$); 114.8 ($C_{ar}$); 112.6 (d, $^4J_{PC}$ = 3.1 Hz, $C_{fur}$); 111.9 (d, $^3J_{PC}$ = 6.6 Hz, $C_{fur}$); 110.9 ($C_{ar}$); 54.6 (d, $^3J_{PC}$ = 6.7 Hz, PO*C*); 54.0 (d, $^3J_{PC}$ = 7.2 Hz, PO*C*); 50.3 (d, $^1J_{PC}$ = 157.3 Hz, PC); 21.5 ($C_{ar}$-*C*). $^{31}$P NMR ($CDCl_3$, 600 MHz): δ 20.25. IR (KBr): 3298 (νNH); 1533, 1388 (νArNO$_2$); 3108 (νCH$_{arom}$); 1609, 1592, 1585, 1490 (νCC$_{arom}$); 1237 (νP-O); 872 (CH$_{arom}$). Elem. anal. Calcd. for $C_{14}H_{17}N_2O_6P$: C, 49.42; H, 5.04; N, 8.23. Found: C, 49.54; H, 5.12; N, 8.21.

### rac-Diethyl *N*-(3-methylphenyl)amino(5-nitro-2-furyl) methylphosphonate 3b

*Y* = 1.4 g (46%). mp = 111–112 °C (yellow crystals). $^1$H NMR ($CDCl_3$, 600 MHz): δ 7.24(dd, $^3J_{HH}$ = 3.7 and $^4J_{HH}$ = 0.8 Hz, $H_{fur}$, 1H); 7.06 (t, $^3J_{HH}$ = 7.8 Hz, *m*-$C_6H_4$, 1H); 6.62 (d, $^3J_{HH}$ = 7.5 Hz, $H_{fur}$, 1H); 6.59 (t, $^4J_{HH}$ = 3.4 Hz, *m*-$C_6H_4$, 1H); 6.49–6.48 (m, *m*-$C_6H_4$, 1H); 6.44 (dd, $^3J_{HH}$ = 8.0 and $^4J_{HH}$ = 2.4 Hz, *m*-$C_6H_4$, 1H); 4.93 (d, $^2J_{PH}$ = 25.0 Hz, CHP, 1H); 4.27–4.22 (m, $POCH_2CH_3$, 2H); 4.18–4.14 (m, $POCH_2CH_3$, 1H); 4.10–4.05 (m, $POCH_2CH_3$, 1H); 2.26 (s, $CH_3$, 3H); 1.34 (t, $^3J_{PH}$ = 7.1 Hz, $POCH_2CH_3$, 3H); 1.28 (t, $^3J_{PH}$ = 7.1 Hz, $POCH_2CH_3$, 3H). $^{13}$C NMR (150 MHz, $CDCl_3$): δ 154.4 (d, $^4J_{PC}$ = 1.7 Hz, $C_{ar}$); 151.9 ($C_{fur}$); 145.3 (d, $^2J_{PC}$ = 12.6 Hz, $C_{fur}$); 139.5 ($C_{ar}$); 129.4 ($C_{ar}$); 120.7 ($C_{ar}$); 114.9 ($C_{ar}$); 112.6 (d, $^4J_{PC}$ = 3.2 Hz, $C_{fur}$); 111.8 (d, $^3J_{PC}$ = 6.4 Hz, $C_{fur}$); 111.0 ($C_{ar}$); 64.3 (d, $^3J_{PC}$ = 7.0 Hz, POC); 63.8 (d, $^3J_{PC}$ = 7.2 Hz, POC); 50.7 (d, $^1J_{PC}$ = 156.0 Hz, PC); 21.6 ($C_{ar}$-*C*); 16.4 (d, $^3J_{PC}$ = 5.6 Hz, POC*C*); 16.3 (d, $^3J_{PC}$ = 5.9 Hz, POC*C*). $^{31}$P NMR ($CDCl_3$, 600 MHz): δ 17.64. IR (KBr): 3316 (νNH); 3110, 3050, 2982, 2910 (νCH$_{arom}$); 1530, 1353 (νArNO$_2$); 1607,1589, 1492 (νCC$_{arom}$), 1237 (νP-O); 768 (δCH$_{arom}$). Elem. anal. Calcd. for $C_{16}H_{21}N_2O_6P$: C, 52.17; H, 5.75; N, 7.61. Found: C, 52.12; H, 5.91; N, 7.65.

### rac-Diphenyl *N*-(2-methylphenyl)amino(5-nitro-2-furyl) methylphosphonate 4a

*Y* = 2.1 g (70%). mp = 131–133 °C (yellow crystals). $^1$H NMR ($CDCl_3$, 600 MHz): δ 7.33–7.29 (m, $C_6H_5$, 4H); 7.22-7.15 (m, $H_{fur}$, $H_{Ph}$, 7H); 7.12–7.07 (m, *o*-$C_6H_5$, 2H); 6.80–6.77 (m, *o*-$C_6H_5$, 1H); 6.65 (t, $^3J_{HH}$ = 3.6 Hz, $H_{fur}$, 1H); 6.56 (d, $^3J_{HH}$ = 7.9 Hz, *o*-$C_6H_5$, 1H); 5.33 (d, $^2J_{PH}$ = 25.3 Hz, CHP, 1H); 4.54 (s, NH, 1H); 2.19 (s, $CH_3$, 3H). $^{13}$C NMR (150 MHz, $CDCl_3$): δ 152.7 ($C_{ar}$); 152.1 ($C_{fur}$); 150.1 (d, $^2J_{CP}$ = 9.4 Hz, $C_{ar}$); 150.0 (d, $^2J_{CP}$ = 9.2 Hz, $C_{ar}$); 142.8 (d,

$^{2}J_{CP}$ = 12.6 Hz, $C_{fur}$); 130.8 ($C_{ar}$); 129.9 (d, $^{4}J_{CP}$ = 6.6 Hz, $C_{ar}$); 127.2 ($C_{ar}$); 125.9 ($C_{ar}$); 125.7 ($C_{ar}$); 123.9 ($C_{ar}$); 120.4 (d, $^{3}J_{CP}$ = 4.1 Hz, $C_{ar}$); 120.0 (d, $^{3}J_{CP}$ = 4.7 Hz, $C_{ar}$); 119.9 ($C_{ar}$); 112.5 (d, $^{3}J_{CP}$ = 6.7 Hz, $C_{fur}$); 112.43 (d, $^{4}J_{CP}$ = 3.3 Hz, $C_{fur}$); 111.3 ($C_{ar}$); 50.7 (d, $^{1}J_{CP}$ = 159.0 Hz, PC); 17.3 ($C_{ar}$-*C*). $^{31}$P NMR ($CDCl_3$, 600 MHz): δ 10.09. IR (KBr): 3387 (νNH); 3129 (ν$CH_{arom}$); 1530, 1355 (ν$ArNO_2$); 1588, 1489, 1452 (ν$CC_{arom}$); 1199 (νP-O); 953 ($CH_{arom}$). Elem. anal. Calcd. for $C_{24}H_{21}N_2O_6P$: C, 62.07; H, 4.56; N, 6.03. Found: C, 62.10; H, 4.70; N, 6.03.

### rac-Diphenyl *N*-(3-methylphenyl)amino(5-nitro-2-furyl) methylphosphonate **4b**

*Y* = 1.9 g (66%). mp = 131–133 °C (pale yellow powder). $^{1}$H NMR ($CDCl_3$, 600 MHz): δ 7.33–7.28 (m, $C_6H_5$, 4H); 7.22–7.13 (m, $H_{fur}$, $H_{Ph}$, 7H); 7.09 (t, $^{3}J_{HH}$ = 7.9 Hz, *m*-$C_6H_5$, 1H); 6.67–6.65 (m, *o*-$C_6H_5$, 2H); 6.51 (m, $H_{fur}$, 1H); 6.48 (dd, $^{3}J_{HH}$ = 2.5 Hz, $^{4}J_{HH}$ = 8.0 Hz, *o*-$C_6H_5$, 1H); 5.29 (d, $^{2}J_{PH}$ = 25.5 Hz, CHP, 1H); 2.27 (s, $CH_3$, 3H). $^{13}$C NMR (150 MHz, $CDCl_3$): δ 152.8 ($C_{arr}$); 152.1 ($C_{fur}$); 150.1 (d, $^{2}J_{CP}$ = 9.8 Hz, $C_{ar}$); 150.0 (d, $^{2}J_{CP}$ = 9.4 Hz, $C_{ar}$); 144.8 (d, $^{2}J_{CP}$ = 13,2 Hz, $C_{fur}$); 139.5 ($C_{ar}$); 129.9 (d, $^{4}J_{CP}$ = 7.6 Hz, $C_{ar}$); 129.4 ($C_{ar}$); 125.9 ($C_{ar}$); 125.7 ($C_{ar}$); 121.1 ($C_{ar}$); 120.6 (d, $^{3}J_{CP}$ = 4.3 Hz, $C_{ar}$); 120.0 (d, $^{3}J_{CP}$ = 4.7 Hz, $C_{ar}$); 115.1 ($C_{ar}$); 112.7 (d, $^{3}J_{CP}$ = 6.8 Hz, $C_{fur}$); 112.5 (d, $^{4}J_{CP}$ = 3,2 Hz, $C_{fur}$); 111.1 ($C_{ar}$); 50.53 (d, $^{1}J_{CP}$ = 160.4 Hz, PC); 21.5 ($C_{ar}$-*C*). $^{31}$P NMR ($CDCl_3$, 600 MHz): δ 10.10. IR (KBr): 3321 (νNH); 3141 (ν$CH_{arom}$); 1531, 1358 (ν$ArNO_2$); 1605, 1588, 1489 (ν$CC_{arom}$); 1176 (νP-O); 939 ($CH_{arom}$). Elem. anal. Calcd. for $C_{24}H_{21}N_2O_6P$: C, 62.07; H, 4.56; N, 6.03. Found: C, 62.19; H, 4.70; N, 6.08.

### Antibacterial assay

The in vitro antimicrobial activity of tested compounds was evaluated against the reference strains of Gram-negative (*Escherichia coli* NCTC 8196, *Proteus vulgaris* ATCC 49990, *Proteus mirabilis* ATCC 29906, *Pseudomonas aeruginosa* NCTC 6749), and Gram-positive (*Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 29213, and *Staphylococcus epidermidis* ATCC 12228) bacterial species. Due to satisfying results for *S. aureus,* selected compounds were also examined against a set of twelve clinical isolates of *S. aureus* (including two MRSA) from the collection of the Chair of Immunology and Infectious Biology, University of Łódź. These strains were isolated from the following three sources: naso-pharynx of young patients hospitalized at Children's Hospital in Łódź (*n* = 4), ulcers and furuncles from adult patients of Dermatological Clinic in Łódź (*n* = 4), and from infected bones of patients hospitalized at Oncological Hospital in Łódź (*n* = 4). All strains were kept frozen at − 80 °C on Tryptic Soy Broth with 15% of glycerol until testing. Before using, *S. aureus* strains were subcultured on blood agar and identified by routine methods (catalase, coagulase and clumping factor). Minimal inhibitory concentration (MIC) was determined as the lowest concentration of the compound preventing growth of the tested microorganism using microdilution method according to EUCAST guidelines [ISO 20776-1 (2006)]. The 96-well microplates were used; 50 µl of recommended Mueller–Hinton broth with a series of twofold dilutions of the tested compound in the range of the final concentrations from 2 to 128 µg/mL was inoculated with 50 µl of microbial suspension with a final cell number concentration approximately 5 × $10^5$ CFU/mL. All of the tested compounds were dissolved in dimethyl sulfoxide (DMSO) and its final concentration on plate (1%) had no influence on growth of microorganisms. The incubation was carried out at 37 °C for 18 h and optical density ($OD_{600}$) was measured. Ampicillin, oxacillin, nitrofurantoin, and streptomycin were used as control antimicrobials. All evaluations were performed in triplicates.

### Cell viability assay

The effect of tested compounds on host cells was detected by determining cellular viability using MTT reduction assay. Murine fibroblasts L929 cells (ATTC®—CCL-1, mouse fibroblasts) or human tumor HeLa cells (ATTC®—CCL-2™, human epithelial cells) were plated in 96-well microplates at density of 1 × $10^4$ cells/100 µL according to international standards: ISO 10993-5:2009(E) (American National Standard 2009) and cultivated in Iscove's modified Dulbecco's medium (IMDM), supplemented with 10% fetal bovine serum (FBS). 100 U/mL of penicillin and 100 µg/mL of streptomycin were added to the media. The cell cultures were incubated at 37 °C in a humidified atmosphere with 10% $CO_2$. After overnight incubation, the growth medium was removed and 100 µL of medium supplemented with twofold dilutions of tested compounds in the range of concentration 2–128 µg/mL were added. All of the tested compounds were dissolved in dimethyl sulfoxide (DMSO), in which the final concentration on plate (1%) had no influence on cells viability. Cisplatin was used as known, toxic, control antitumor agent, and nitrofurantoin as control antibacterial drug. After 24 h incubation, the medium was removed and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well at a final concentration of 0.5 mg/mL and plates were incubated for the next 2 h at 37 °C, 10% $CO_2$. Then, formazan crystals were solubilized in 150 µL DMSO. The optical density was measured at 550 nm. The results of the experiments were shown as mean arithmetic values from 3 repeats in each of two independent experiments and the percentage of viability inhibition in comparison with untreated control was calculated for each concentration of the tested compounds and $IC_{50}$ values were

determined with the Prism GraphPad 7 software using non-linear regression.

## Results and discussion

### Synthesis of imines 1 and aminophosphonates 2, 3, and 4

This work is to some extend a continuation of our previous study, where we reported the synthesis of new aminophosphonates bearing a 5-nitrofuran moiety. This group of compounds has never been described before our recent paper (Lewkowski et al. 2017) (except for *N*-benzylamino-(5 nitrofurfuryl)methylphosphonic acid, which was reported by Boduszek 2001; Boduszek et al. 2001).

Schiff bases **1a–c** and **1f** were synthesized following the previously published procedure and their identity was confirmed by melting point measurement and the $^{1}$H NMR spectroscopy and comparing the obtained data with literature reports (Csaszar 1984; Matusiak et al. 2013; Saikachi and Shimamura 1960). Imines **1c** and **1f** were isolated and purified before undergoing the biological evaluation, while **1a** and **1b** were used for further conversion without any purification. Antibacterial action of imines **1a–b** was not evaluated.

*N*-arylamino(5-nitrofurfuryl)methylphosphonic acid esters (**2b–c, 2f, 3b–c, 3f, 4a–c, 4f**) were prepared based on the recently published procedure (Lewkowski et al. 2017) by the aza-Pudovik reaction as it was described (Lewkowski et al. 2017). (Scheme 1) The aminophosphonates **2b**, **3b**, **4a,** and **4b** were synthesized for the first time and have never been described in the literature. Their identities were confirmed by elemental analysis as well as by $^{1}$H, $^{13}$C, and $^{31}$P NMR spectroscopy. NMR spectra demonstrated diagnostic peaks, which were discussed previously (Lewkowski et al. 2017).

Aminophosphonates **2c, 2f, 3c, 3f, 4c,** and **4f** were obtained following the reported procedure (Lewkowski et al. 2017) and their identities were confirmed by comparison to authentic samples.

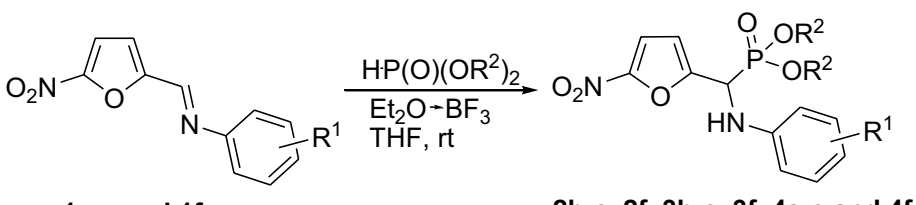
Spectra of new aminophosphonates **2b**, **3b**, **4a,** and **4b** (FT-IR, $^{1}$H, $^{13}$C, and $^{31}$P NMR) as well as $^{1}$H NMR spectrum of a Schiff base **1f** are collected in Supplemental Materials.

### Evaluation of antibacterial action of studied compounds

Ten nitrofuran-derived racemic aminophosphonates (**2b, 2c, 2f, 3b, 3c, 3f, 4a, 4b, 4c, 4f**) and some of their parent imines **1c** and **1f** (Fig. 1) were examined for its antimicrobial activity against a representative panel of bacteria, i.e., *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis*, *Proteus vulgaris, Proteus mirabilis,* and *Pseudomonas aeruginosa* using ampicillin, nitrofurantoin, and streptomycin as control antibacterial drugs. The in vitro antimicrobial activity of the tested compounds at concentrations ranging from 0.5 to 128 µg/mL was screened using the microdilution method. The results showed that diphenyl aminophosphonic acid esters **4a–c, 4f** were inactive against all tested strains and, therefore, were excluded from further studies. The remaining eight derivatives were only active against *S. aureus* and *S. epidermidis.* Aminophosphonates **2b, 2c, 2f, 3b, 3c,** and **3f** showed weak antimicrobial activity against both strains (MIC = 128 µg/mL). The most effective against these strains were Schiff bases **1c** and **1f** with MIC values of 16 µg/mL. The strong antibacterial activity of **1c** and **1f** against *S. aureus* was equal to nitrofurantoin; however, their activity was lower than exhibited by ampicillin and streptomycin—antibiotics commonly used in the therapy of *staphylococcal* infections. In addition, **1c** and **1f** showed good activity against *E. coli* (MIC = 64 µg/mL). The in vitro results of antibacterial activity of these compounds are presented in Table 1 as a minimal inhibitory concentration (MIC).

Such properties are closely related to structures of investigated compounds. Diphenyl *N*-arylamino(5-nitro-2-furyl)methylphosphonates **4a–c, 4f** having a phosphonic group substituted with phenyl rings is undoubtedly more

**Scheme 1** Synthesis of studied aminophosphonates **2b–c**, **2f, 3b–c, 3f, 4a–c, 4f**




**1a-c and 1f** **2b-c, 2f, 3b-c, 3f, 4a-c and 4f**

**a**: $R^1$ = 2-$CH_3$; **b**: $R^1$ = 3-$CH_3$; **c**: $R^1$ = 4-$CH_3$; **d**: $R^1$ = 2-$OCH_3$;
**e**: $R^1$ = 3-$OCH_3$; **f**: $R^1$ = 4-$OCH_3$;
**2**: $R^2$ = $CH_3$; **3**: $R^2$ = $CH_2CH_3$; **4**: $R^2$ = Ph

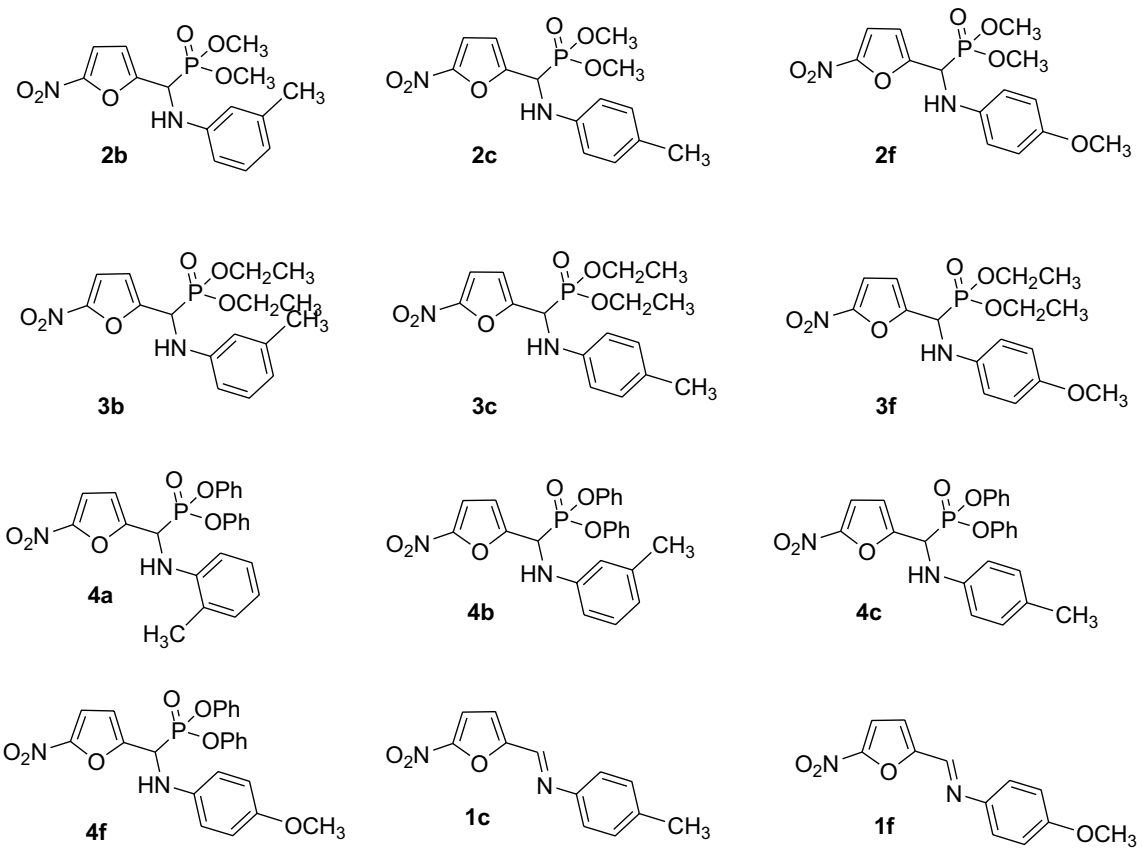



**Fig. 1** Structures of aminophosphonates and imines under study

**Table 1** In vitro antibacterial activity of **2b–c, 2f, 3b–c, 3f, 1c,** and **1f** expressed as a minimal inhibitory concentration (MIC) [μg/mL]

| Compound | MIC [μg/mL] | | | | | | |
|---|---|---|---|---|---|---|---|
| | *E. coli NCTC 8196* | *S. aureus* ATCC 6538 | *S. aureus* ATCC 29213 | *P. vulgaris* ATCC 49990 | *P. mirabilis* ATCC 29906 | *S. epidermidis* ATCC 12228 | *P. aeruginosa* NCTC 6749 |
| **3b** | na | 128 | 128 | na | na | 128 | na |
| **3c** | na | 128 | 128 | na | na | 128 | na |
| **3f** | na | 128 | 128 | na | na | 128 | na |
| **2b** | na | 128 | 128 | na | na | 128 | na |
| **2c** | na | 128 | 128 | na | na | 128 | na |
| **2f** | na | 128 | 128 | na | na | 128 | na |
| **1c** | 64 | 16 | 16 | na | na | 16 | na |
| **1f** | 64 | 16 | 16 | na | na | 16 | na |
| **AMP** | 4 | 1 | 2 | nd | nd | 1 | nd |
| **NFT** | 8 | 16 | 16 | nd | nd | 8 | nd |
| **STR** | 1 | 1 | 1 | nd | nd | na | nd |

*AMP* ampicillin, *NFT* nitrofurantoin, *STR* streptomycin, *na* no activity, *nd* not determined

hydrophobic than all other aminophosphonates **2** and **3**, which may decrease the ability of their molecules to penetrate bacterial cells. Apart from this, the presence of two large, electron-rich phenyl rings does not improve it either.

Comparison of antibacterial action of aminophosphonates **2b–c, 2f, 3b–c,** and **3f** with Schiff bases **1c** and **1f**, shows that although an active center ($NO_2$ group) is in each tested compound, the presence of an azomethine bond

is a necessary condition for a molecule to penetrate efficiently bacterial cells. It is not surprising, since all approved nitrofuran-derived drugs bear azomethine groups in their molecules.

Considering good antibacterial activity of aminophosphonates **2b–c, 2f, 3b–c,** and **3f**, and especially **1c** and **1f**, against *Staphylococcus spp*., a set of 12 *S. aureus* clinical strains were used for further antimicrobial assays. These strains were isolated from patients from two typical sources such as naso-pharynx (carrier state) and ulcers/furuncles (skin and soft tissue infections), as well as those from infected bones (invasive infections) with two representatives of MRSA (*S. aureus* D15 and D17). Similar to the reference *S. aureus* strains, all clinical isolates displayed high level of susceptibility to Schiff bases **1c** and **1f** (MIC ranging from 16 to 64 µg/mL, Table 2). In most cases, except F7 strain, they had better activity than ampicillin. Especially, these two imines showed higher antibacterial activity against both MRSA strains than all control antibiotics, with MIC being equal to 16 µg/mL. Other derivatives also displayed good activity especially against MRSA strains, better than oxacillin, ampicillin, and streptomycin (MIC = 64–128 µg/mL); however, some clinical isolates were not sensitive to all tested compounds in analyzed concentrations. It is to note that dimethyl aminophosphonates **2b–c, 2f** were generally more efficient against both MRSA strains than diethyl derivatives **3b–c** and **3f** (MIC = 64 µg/mL vs. 128 µg/mL, respectively).

*N*-(2-nitrofurfurylidene)-*p*-anisidine **1f** turned out to be less toxic than *N*-(2-nitrofurfurylidene)-*p*-toluidine **1c** for seven of *S. aureus* strains. It suggests that the presence of a methyl group increases a harmful action of the imine **1c** in comparison with a methoxy group in **1f** and plays an important role in toxicity. These results coincide well with our recently reported observations (Lewkowski et al. 2017), where we demonstrated that *N*-methoxyphenyl aminophosphonates are less harmful for *Aliivibrio fischeri* bacteria than *N*-methylphenyl derivatives.

In addition to study antibacterial activity of tested compounds, we also evaluated their safety for mammalian cell lines. The cytotoxic activity was assessed using L929 murine cell line (recommended by the International Standard ISO 10993:2009 for evaluation of in vitro cytotoxicity) as well as HeLa human tumor cell line. The percentage of cells viability inhibition compared to the untreated control was estimated for concentrations of compounds ranging from 2 to 128 µg/mL and $IC_{50}$ values were determined. Cisplatin, as known cytotoxic, antitumor agent, and nitrofurantoin as control antibiotic was used for comparison. Our results demonstrated that Schiff bases **1c** and **1f** showing the highest antibacterial activity were the most toxic as well with $IC_{50}$ approximately 12 µg/mL, which was only twofold higher than $IC_{50}$ for cisplatin. *N*-methoxyphenylamino(5-nitrofurfuryl)methylphosphonates **2f** and **3f** were found to have the lowest cytotoxicity, but inhibitory concentrations for all analyzed compounds were lower than MIC values. The HeLa cells seemed to be a little less sensitive to tested compounds than L929 cells. The in vitro results of viability assay are presented in Table 3 and Fig. 2.

This results coincide with some extent with our observation described in our recent work (Lewkowski et al. 2017), where we found that *N*-methoxyphenylamino(5-nitrofurfuryl)methylphosphonates were significantly less toxic for *Aliivibrio fischeri* bacteria and for *Heterocypris incongruens* crustaceans than *N*-methylphenylamino derivatives. It is to state, however, that methoxyphenyl-substituted nitrofurfuryl

**Table 2** In vitro antibacterial activity of **2b–c, 2f, 3b–c, 3f, 1c, 1f** against clinical isolates of *S. aureus* expressed as the minimal inhibitory concentration (MIC) [µg/mL]

| *S. aureus* | MIC [µg/mL] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | naso-pharynx isolates | | | | ulcers/furuncles isolates | | | | bone isolates | | | |
| Compound | C4 | C7 | C8 | C19 | F1 | F7 | F12 | D12 | D14 | D15 | D17 | D20 |
| **3b** | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 |
| **3c** | na | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 |
| **3f** | na | na | na | na | na | na | na | na | 128 | 128 | 128 | na |
| **2b** | 128 | 128 | na | 128 | 128 | 128 | 128 | na | 128 | 64 | 64 | na |
| **2c** | 128 | 128 | na | 128 | 128 | 128 | 128 | na | 128 | 64 | 64 | 128 |
| **2f** | 128 | 128 | na | 128 | na | na | 128 | na | 128 | 64 | 64 | 128 |
| **1c** | 32 | 32 | 32 | 16 | 32 | 32 | 16 | 32 | 64 | 16 | 16 | 32 |
| **1f** | 32 | 64 | 64 | 64 | 64 | 64 | 32 | 64 | 64 | 16 | 16 | 16 |
| **OX** | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.5 | 0.5 | na | na | 0.5 |
| **AMP** | 512 | 64 | 512 | 64 | 128 | 4 | 64 | 256 | 256 | na | na | 256 |
| **NFT** | 16 | 16 | 16 | 32 | 32 | 16 | 16 | 32 | 32 | 32 | 32 | 32 |
| **STR** | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 256 | 256 | 8 |

*OX* oxacillin, *AMP* ampicillin, *NFT* nitrofurantoin, *STR* streptomycin, *na* no activity

**Table 3** Effect of **2b–c, 2f, 3b–c, 3f, 1c, 1f** on viability of L929 and HeLa cell lines expressed as compound concentration inhibiting cell growth by 50% ($IC_{50}$) [μg/mL]

| | $IC_{50}$ [μg/mL] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **3b** | **3c** | **3f** | **2b** | **2c** | **2f** | **1c** | **1f** | CIS | NFT |
| **L929** | 20.14 | 14.40 | 44.25 | 31.68 | 31.97 | 44.00 | 12.01 | 11.93 | 6.48 | 44.32 |
| **HeLa** | 28.12 | 25.96 | 71.66 | 33.86 | 48.67 | 77.04 | 11.32 | 16.23 | 6.80 | 150.90 |

*CIS* cisplatin, *NFT* nitrofurantoin

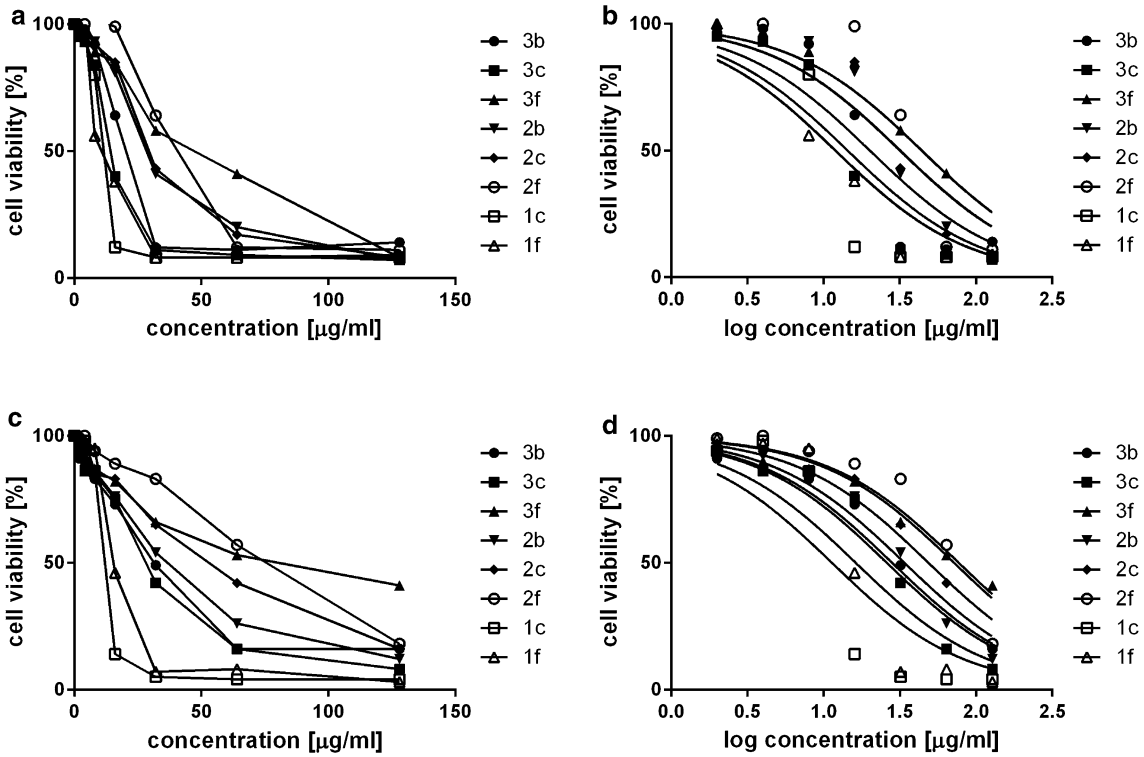



**Fig. 2** Concentration-dependent cytotoxic activity of **2b–c**, **2f**, **3b–c**, **3f**, **1c**, **1f**. Figures show the viability of L929 (**a**, **b**) and HeLa cells (**c**, **d**) as a function of the compound concentration [μg/mL]. **b**, **d** Concentrations in logarithmic scale with nonlinear regression used for IC50 determination

aminophosphonates showed higher phytotoxicity than methylphenyl derivatives. There is no possibility to compare cytotoxicological behavior of amino(5-nitrofurfuryl)-methylphosphonates to literature data, because our team synthesized this group of compounds for the first time (except for *N*-benzylamino(5-nitrofurfuryl)methylphosphonic acid, which was reported by Boduszek 2001 and Boduszek et al. 2001, and which was not biologically tested).

Cytotoxicity of 5-nitrofuraldimines **1c** and **1f** was found to be significant. These data coincide with previously reported observations, where we found the Schiff base **1c** to be significantly phytotoxic (Matusiak et al. 2013).

However, it is to stress that such high values of antibacterial action of 5-nitrofuraldimines **1c** and **1f** against MRSA strains should be an impulse for testing various azomethine derivatives of 5-nitrofuraldehyde in this aspect to find another efficient anti-MRSA agents.

## Conclusions

*N*-arylamino(5-nitrofurfuryl)methylphosphonic acid esters (**2b–c, 2f, 3b–c, 3f, 4a–c, 4f**) were tested in aspect of their antibacterial action. Synthesis of *p*-phenyl derivatives (**2c**, **2f**, **3c**, **3f**, **4c,** and **4f**) was recently published by us (Lewkowski et al. 2017), while the preparation of the rest (**2b, 3b, 4a–b**) is reported herein for the first time. Diphenyl aminophosphonic esters (**4a–c, 4f**) were found to be inactive for tested bacteria, while the rest was moderately active against *Staphylococcus aureus*. Dimethyl esters (**2b–c, 2f**) revealed their interesting activity against methicillin-resistant strains of *S. aureus* (MIC = 64 μg/mL), and this activity was much greater than its values for oxacillin, ampicillin, and streptomycin. Unfortunately, measured cytotoxicity against L929 and HeLa cell lines

was found to be high, with $IC_{50}$ values twice less than MIC (Table 3). Such activity and toxicity of nitro compounds has been recently reported by del Casino et al. (2018) in aspect of their action against *Plasmodium falciparum*.

It is to state that all tested aminophosphonates (**2b–c, 2f, 3b–c, 3f, 4a–c, 4f**) were studied as racemates, and obviously, the biological activity of their enantiomers can be very different. The unacceptably close concentrations for antibacterial efficacy and for cytotoxicity might diverge following resolution and separate evaluation for the enantiomers. Therefore, the problem is still open and further studies on biological activity of aminophosphonates (**2b–c, 2f, 3b–c, 3f, 4a–c, 4f**) will be performed.

Schiff bases **1c** and **1f** were also evaluated in this aspect, and although their antibacterial action was high, especially their impact against methicillin-resistant strains of *S. aureus*, which was found higher than four tested commercial antibiotics (MIC = 16 µg/mL), their cytotoxicity against abovementioned cell lines was dangerously high (ca. 12–13 µg/mL).

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