Med Chem Res (2013) 22:852–860
DOI 10.1007/s00044-012-0065-3

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of new *N*-arylamino(2-furyl)methylphosphonic acid diesters, and in vitro evaluation of their cytotoxicity against esophageal cancer cells

Anna Agnieszka Klimczak · Agnieszka Kuropatwa · Jarosław Lewkowski · Janusz Szemraj

Received: 4 November 2011 / Accepted: 17 April 2012 / Published online: 15 May 2012

Abstract *N*-Furfurylideneanilines and *N*-arylamino(2-furyl)methylphosphonates with tolyl and anisyl moieties were synthesized by the addition of phosphites to azomethine bond of corresponding Schiff bases and their NMR spectroscopic properties were investigated. Then, they were analyzed in the point of view of their influence on KYSE 30, KYSE 150, and KYSE 270 esophageal cancer cell lines and on immortalized esophageal cell line HET 1 A as a control group. Toxicity was evaluated by MTT assay. Among 11 compounds, a few of them demonstrated influence on cancer cells being neutral toward the control, but only one aminophosphonate had IC_{50} lower than 100 μM and acted as a potential anticancer drug. Some approaches to structure–activity relation were performed.

Keywords *N*-Arylamino(2-furyl)methylphosphonate · Furfural Schiff base · Esophageal cancer cells · Cytotoxicity

Introduction

Substituted furan derivatives are important compounds (Bosshard and Eugster, 1967), as exemplified by a series of nitrofural-derived drugs (Merck, 2001) the efficient antihistamine agent ranitidine (Bradshaw *et al*., 1981) and 2,5-diphenylfuran derivatives with strong cytotoxicity toward *Pneumocystis carinii* (Boykin *et al*., 1998). Their importance derives also from their role as versatile synthetic intermediates (Keay and Dibble, 1996).

The first preparation of the phosphonic analogs of natural amino acids (Fields, 1952; Saito *et al*., 2007; Matveeva *et al*., 2006) stimulated biochemical studies (Mastalerz and Kafarski, 2000), which confirmed that aminophosphonic and aminophosphonous acids also belong to the group of biologically active compounds. Aminophosphonic acids are considered to be an interesting class of compounds for scientists in various fields of chemistry, biochemistry (Kafarski and Lejczak, 2001), pharmacology (Kafarski and Lejczak, 1991), and agriculture (Maier, 1999). They show interesting influence on fungi and plant growth in agriculture; they also show a high toxicity toward some cancers (Kafarski and Lejczak, 2001). Recently, the synthesis and the strong anti-leukemic action of several aminophosphonates-bearing furan moieties was reported (Kraicheva *et al*., 2009).

Esophageal carcinoma causes serious problems over the world as it is the sixth deadliest cancer in the world (Bernard and Stewart, 2003; Kordek *et al*., 2007) as the disease is very difficult to be treated because of the location of the esophagus. Inefficient prophylaxis and detection of this cancer is an additional problem. The treatment of most patients with cancer is very difficult because it is usually detected in later stages of development. High risk factors cause this disease to appear in very specific places around the globe. These areas include the "Asian Esophageal Cancer Belt" that spreads from eastern Turkey through Kazakhstan, Turkmenistan, Uzbekistan, Tajikistan, Iraq, Iran, western and northern China, and Japan. Other regions of the world with high esophageal cancer risk include south western Africa, France, Bermudas and South America,

A. Kuropatwa · J. Lewkowski (✉)
Department of Organic Chemistry, Faculty of Chemistry, University of Łódź, Tamka 12, 91-403 Lodz, Poland
e-mail: jlewkow@uni.lodz.pl

A. A. Klimczak (✉) · J. Szemraj
Department of Medicinal Biochemistry, Medical University of Łódź, Mazowiecka 6/8, 92-215 Lodz, Poland
e-mail: a.a.klimczak@gmail.com

mainly with Brazil (Muñoz, 1993; Coleman *et al*., 1993; Reed and Johnston, 1993).

In treatment of esophageal cancer, chemotherapy or chemoradiotherapy is important to be used simultaneously with surgery. These treatments increase the patient's overall survivability and decrease tumor conditions. Drug toxicity against healthy cells is also a very important issue in chemotherapy. It is important to find new drugs that are not toxic for normal cells and our research gives us a high chance of finding one.

Considering the above mentioned, in studies on various aspects of aminophosphonic systems, we have focused our attention on the synthesis of arylaminomethylphosphonates-containing furan moiety, and evaluation of their potential cytostatic action against esophageal cancer.

Results and discussion

Chemistry

Schiff bases **2a–f** were synthesized by the previously published (Cottier *et al*., 1996a, b) and commonly known procedure by simply mixing furfural with appropriate amine in methanol and stirring them at room temperature for 24 h. They were obtained in quantitative yield. Their identity was confirmed by melting point measurement (Grammaticakis and Texier, 1971; Kraicheva *et al*., 2009; Ojima *et al*., 1992) and the ^{1}H NMR spectroscopy (Kiepo and Jakopcic, 1985; Saito *et al*., 2001; Zubkov *et al*., 2004) and comparing the obtained measurements with literature data. The ^{1}H NMR revealed the diagnostic singlet of a proton of the azomethine group (-CH=N–) and signals of furan protons.

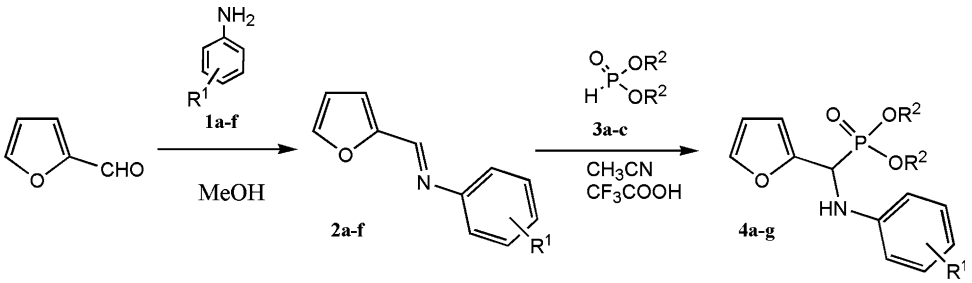
(2-Furyl)-*N*-arylaminomethylphosphonic acid esters (**4a–g**) were prepared based on the previously published procedures (Cottier *et al*., 1996a, b; Lewkowski *et al*., 2000). Reactions of Schiff bases **2a–f** with phosphites **3a–c** were carried out in acetonitrile with a catalytic amount (2 drops) of trifluoroacetic acid. This catalytic amount of trifluoroacetic acid acts in the case of furan derivatives (Cottier *et al*., 1996a, b) much better than Lewis acid catalysts such as $TaCl_5$ (Chandrasekhar *et al*., 2001) or $MgClO_4$ (Wu *et al*., 2006) (Scheme 1).

(2-Furyl)-*N*-arylaminomethylphosphonic acid esters (**4a–g**) were purified by the column chromatography on silica gel eluting with hexane–ethyl acetate (1:4) mixture to receive pure products. Diphenyl (2-furyl)-(3-methylphenyl)aminomethylphosphonate (**4e**) was contaminated with a 3 % amount of unreacted diphenyl phosphite; therefore, the product was dissolved in dichloromethane and washed with 10 % $NaHCO_3$ in water to remove a phosphite. That is why, aminophosphonate **4e** was crystallized with an occluded molecule of dichloromethane, which was demonstrated by results of elemental analysis.

The identity of aminophosphonates **4a–g** was confirmed by elemental analysis and ^{1}H and ^{31}P NMR spectroscopy. ^{1}H NMR spectra demonstrated several diagnostic peaks, the signal of a proton situated at the asymmetric carbon (CHP) appearing as a doublet of doublets as this proton couples with a phosphorus atom as well as with the proton of a secondary amino group (NH). The NH proton appears as a doublet. *O*-methyl groups of phosphoryl moieties appear as two doublets each for one OMe group demonstrating their magnetic non-equivalence.

Biological activity

Then, four 2-furaldimines **2a**, **2c**, **2e**, and **2f** and seven (2-furyl)-*N*-arylaminomethylphosphonic acid esters **4a–g** have been analyzed. We would like to test the cytotoxicity



Scheme 1 Preparation of imines **2a–f** and *N*-arylamino(2-furyl)methylphosphonates **4a–g**

1a, **2a**: R^1 = *o*-OCH_3; **1b**, **2b**: R^1 = *m*-OCH_3; **1c**, **2c**: R^1 = *p*-OCH_3;
1d, **2d**: R^1 = *o*-CH_3; **1e**, **2e**: R^1 = *m*-CH_3; **1f**, **2f**: R^1 = *p*-CH_3.

3a: R^2 = CH_3; **3b**: R^2 = Ph; **3c**: R^2 = CH_2Ph

4a: R^1 = *o*-OCH_3, R^2 = CH_3; **4b**: R^1 = *p*-OCH_3, R^2 = CH_3; **4c**: R^1 = *p*-OCH_3, R^2 = Ph;
4d: R^1 = *o*-CH_3, R^2 = Ph; **4e**: R^1 = *m*-CH_3, R^2 = Ph; **4f**: R^1 = *p*-CH_3, R^2 = Ph;
4g: R^1 = *m*-OCH_3, R^2 = CH_2Ph,

of these compounds toward esophageal cancer cell lines compared to the control.

To perform this biological assay, four different cell lines were taken: esophageal squamous cancer cell lines: KYSE 30, KYSE 150, KYSE 270, and SV-40. Immortalized human esophageal epithelial cell line Het-1A was taken as a control. All these lines were described in literature (Hou *et al.*, 2005; Chiu, 2008; Ji *et al.*, 2010; Tanaka *et al.*, 1998, 2007). The cell cytotoxicity was measured by means of standard MTT assay (Mosmann, 1983; Sieuwerts *et al.*, 1995).

The investigation demonstrated that none of the analyzed chemical compounds had IC_{50} less than 400 μM for Het-1A, so they could be considered as non toxic for immortalized human esophageal epithelial cell line. Unfortunately, testing esophageal cancer cell lines with *N*-furfurylidene-*p*-anisidine (**2c**), *N*-furfurylidene-*o*-anisidine (**2a**), and diphenyl (2-furyl)-*N*-(4-methylphenyl)aminomethylphosphonate (**4f**) demonstrated IC_{50} to be over 400 μM for all three cell lines. Similar results were obtained for *N*-furfurylidene-*p*-toluidine (**2f**) and diphenyl (2-furyl)-*N*-(3-methylphenyl)aminomethylphosphonate (**4e**), so they cannot be considered as cytotoxic for any of studied cancer cell lines. No highly promising results were obtained for diphenyl (2-furyl)-*N*-(2-methylphenyl)aminomethylphosphonate (**4d**) and diphenyl (2-furyl)-*N*-(4-methoxyphenyl)-aminomethylphosphonate (**4c**) because aminophosphonate **4c** turned out not to be cytotoxic for the KYSE 30 line, but for KYSE 150 and KYSE 270, the IC_{50} was evaluated to be slightly over 200 μM (Table 1). The aminophosphonate **4d** demonstrated the IC_{50} to be less than 300 μM only for KYSE 30.

For other compounds, results were much more interesting. Dimethyl (2-furyl)-*N*-(4-methoxyphenyl)aminomethyl-phosphonate (**4b**) (Table 1; Figs. 1, 2, 3) and dibenzyl (2-furyl)-*N*-(3-methoxyphenyl)-aminomethylphosphonate **4g**, where the IC_{50} was found to be less than 300 μM but higher than 100 μM (Table 1), which cannot be considered as significantly cytotoxic.

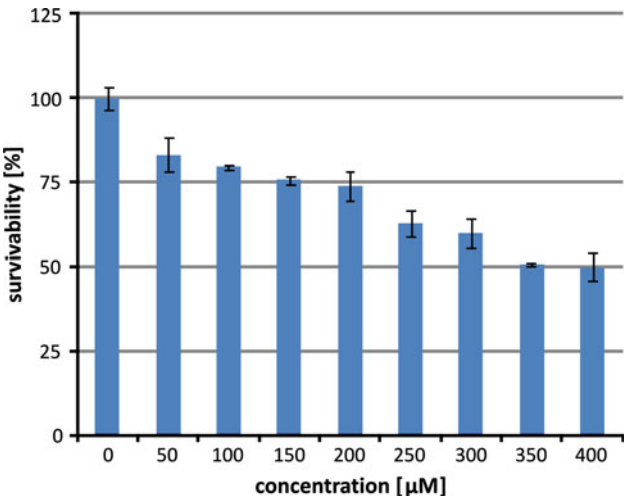


Fig. 1 Effect of aminophosphonate **4b** on the survival of KYSE 30. Cells were incubated with different concentrations of aminophosphonate **4a** for 24 h and their survival was estimated by MTT test (mean SD; $n \geq 3$)

Much more interesting results were obtained for *N*-furfurylidene-m-toluidine (**2e**), which showed IC_{50} much less than 200 μM and for KYSE 150 even 50.12 ± 1.84 μM (Table 1; Figs. 4, 5, 6).

The highest cytotoxic influence on cancer cell lines was demonstrated by dimethyl (2-furyl)-*N*-(2-methoxyphenyl) amino-methylphosphonate (**4a**), where IC_{50} was found to be much less than 100 μM, i.e., 38.02 ± 2.39 μM for the KYSE 30, 51.29 ± 6.97 μM for the KYSE 150, and finally 77.43 ± 3.68 μM for the KYSE 270 cell line (Table 1; Figs. 7, 8, 9).

Considering that a limited number of compounds were used for this investigation, it is too early to perform the real

Table 1 The fractions of living cells after 24 h incubation with imines **2a**, **2c**, **2e–f,** and aminophosphonates **4a–g**

Comp.	R^1	R^2	IC_{50} (μM)			
			KYSE-30	KYSE-150	KYSE-270	Het-1A
2a	*o*-OCH_3	–	>400	>400	>400	>400
2c	*p*-OCH_3	–	>400	>400	>400	>400
2e	*m*-CH_3	–	158.49 ± 17.23	50.12 ± 1.84	111.2 ± 7.48	>400
2f	*p*-CH_3	–	>400	380.19 ± 15.12	398.11 ± 23.40	>400
4a	*o*-OCH_3	CH_3	38.02 ± 2.39	51.29 ± 6.97	77.43 ± 3.68	>400
4b	*p*-OCH_3	CH_3	316.23 ± 21.03	166 ± 17.28	223.87 ± 15.51	>400
4c	*p*-OCH_3	Ph	>400	208.93 ± 17.33	204.84 ± 8.90	>400
4d	*o*-CH_3	Ph	251.19 ± 15.41	>400	>400	>400
4e	*m*-CH_3	Ph	380 ± 21.35	>400	>400	>400
4f	*p*-CH_3	Ph	>400	>400	>400	>400
4g	*m*-OCH_3	CH_2Ph	251.19 ± 22.09	199.52 ± 16.85	251.19 ± 12.94	>400

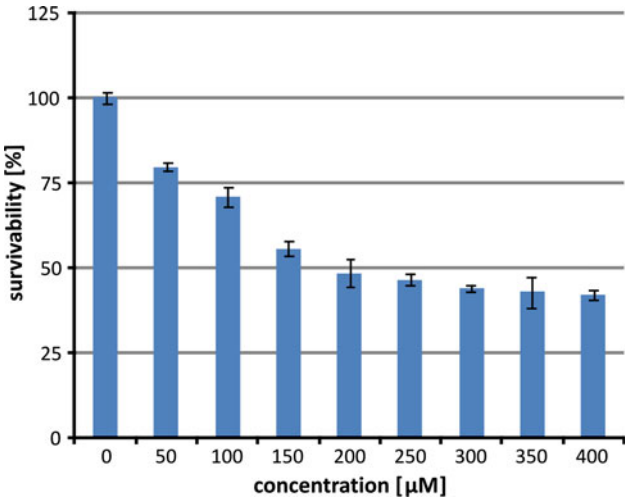


Fig. 2 Effect of aminophosphonate **4b** on the survival of KYSE 150. Cells were incubated with different concentrations of aminophosphonate **4a** for 24 h and their survival was estimated by MTT test (mean SD; $n \geq 3$)

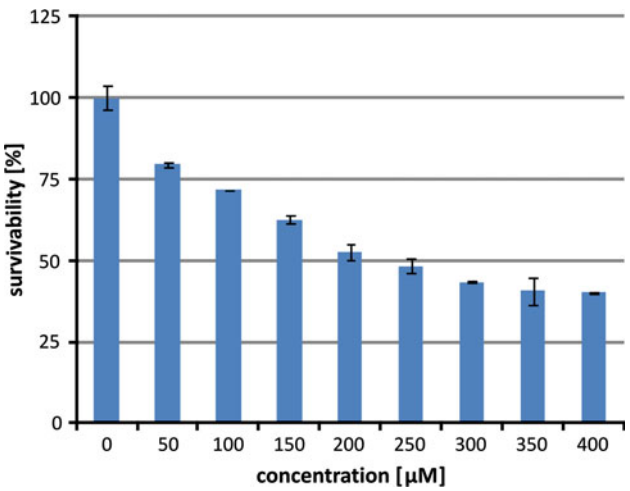


Fig. 3 Effect of aminophosphonate **4b** on the survival of KYSE 270. Cells were incubated with different concentrations of aminophosphonate **4a** for 24 h and their survival was estimated by MTT test (mean SD; $n \geq 3$)

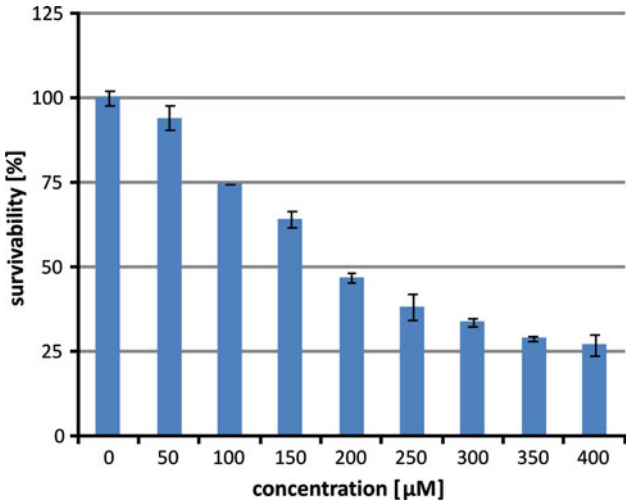


Fig. 4 Effect of imine **2e** on the survival of KYSE 30. Cells were incubated with different concentrations of imine **2e** for 24 h and their survival was estimated by MTT test (mean SD; $n \geq 3$)

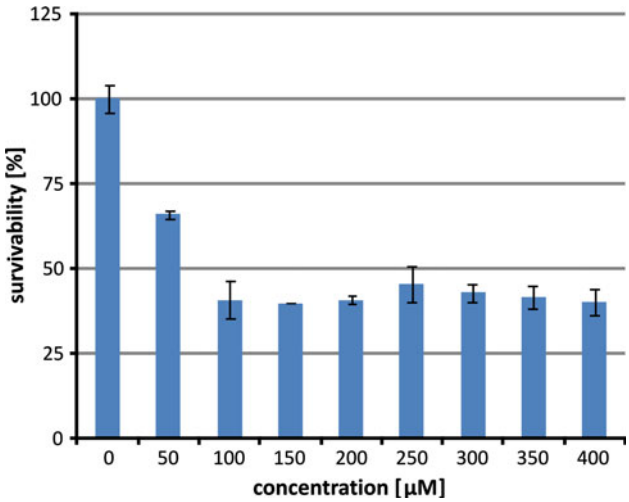


Fig. 5 Effect of imine **2e** on the survival of KYSE 150. Cells were incubated with different concentrations of imine **2e** for 24 h and their survival was estimated by MTT test (mean SD; $n \geq 3$)

structure vs. activity relationship (SAR) analysis. But, some tendencies are visible. Tests carried out for four diphenyl (2-furyl)-phenylaminomethylphosphonates **4c–f** demonstrated no antiproliferative activity (**4e** and **4f**) or very slight action (**4c** and **4d**) toward esophageal squamous cancer cell lines. It would allow us to suggest that aminophosphonic acid phenyl esters do not have the important cytotoxic action, maybe due to their chemical instability.

Three tested Schiff bases *N*-furfurylidene-*o*-anisidine (**2a**), *N*-furfurylidene-*p*-anisidine (**2c**), and *N*-furfurylidene-*p*-toluidine (**2f**) have no cytotoxic influence on investigated cells. The negligible activity of imine **2f** which surprised us a bit in the light of Kraicheva's paper (Kraicheva *et al.*, 2009), who has proven its high cytotoxicity toward human leukemia cells. But, it cannot be stated that *N*-phenyl furfural Schiff bases are inactive for this cancer cell lines because *N*-furfurylidene-*m*-toluidine (**2e**) showed moderately interesting properties. Possibly, the *meta*-methyl substitution plays a key role in its cytotoxicity, but it is too early to draw any conclusion. It is to stress that the *O*,*O*′-dimethyl and *O*,*O*′-dibenzyl aminophosphonic esters **4a–b** and **4g** have interesting cytotoxic action and that *ortho*-substitution seem to improve greatly the cytotoxicity of aminophosphonates in study.

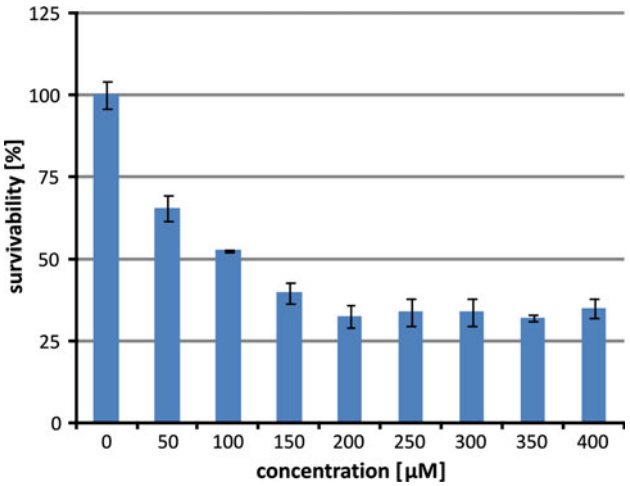


Fig. 6 Effect of imine **2e** on the survival of KYSE 270. Cells were incubated with different concentrations of imine **2e** for 24 h and their survival was estimated by MTT test (mean SD; $n \geq 3$)

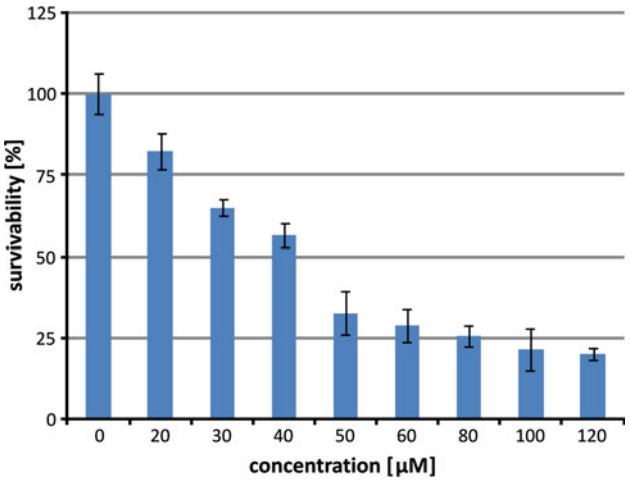


Fig. 7 Effect of aminophosphonate **4a** on the survival of KYSE 30. Cells were incubated with different concentrations of aminophosphonate **4a** for 24 h and their survival was estimated by MTT test (mean SD; $n \geq 3$)

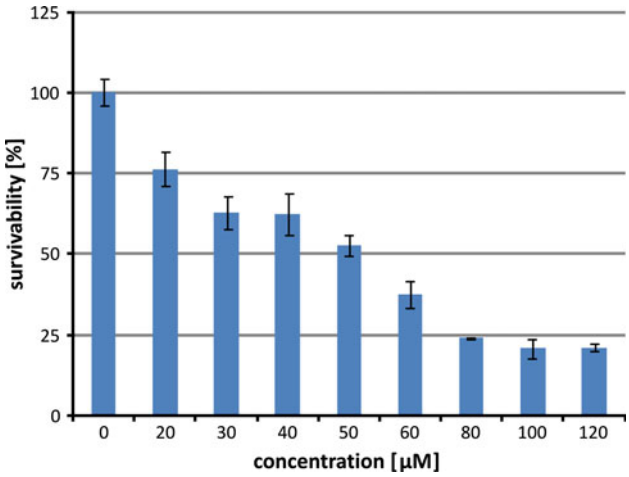


Fig. 8 Effect of aminophosphonate **4a** on the survival of KYSE 150. Cells were incubated with different concentrations of aminophosphonate **4a** for 24 h and their survival was estimated by MTT test (mean SD; $n \geq 3$)

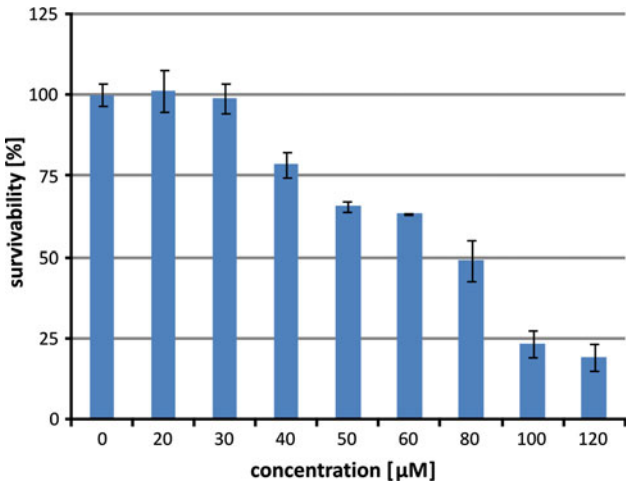


Fig. 9 Effect of aminophosphonate **4a** on the survival of KYSE 270. Cells were incubated with different concentrations of aminophosphonate **4a** for 24 h and their survival was estimated by MTT test (mean SD; $n \geq 3$)

Conclusion

In conclusion, the obtained results showed that none of the studied compounds are toxic for immortalized control, human esophageal epithelial cell line HET-1A.

The Schiff base **2e** as well as dimethyl aminophosphonates **4a**, **4b** and dibenzyl one **4g** show some cytotoxicity for all three esophageal squamous cancer cell lines (KYSE 30, KYSE 150, and KYSE 270). It is noteworthy that dimethyl (2-furyl)-(2-methoxyphenyl)aminomethylphosphonate (**4a**) presented the best results (IC_{50} much less than 100 μM and in one case less than 40 μM) and it seems to be a perfect candidate for further in vivo analysis, which is planned to be done. The SAR study of (2-furyl) aminomethylphosphonates bearing *O*,*O*′-dialkyl and *O*,*O*′-dibenzyl groups will be also performed.

Experimental

Chemistry

All solvents (POCh, Poland) were routinely distilled and dried before use. Amines, dimethyl, diphenyl and dibenzyl phosphites, and furfural (Aldrich) were used as received. NMR spectra were recorded on a Bruker Avance III 600 MHz operating at 600 MHz (^{1}H NMR) and 243 MHz

(^{31}P NMR). TMS was used as the internal standard for ^{1}H NMR and phosphoric acid was used as the external standard for ^{31}P NMR. Elemental analyses were carried out at the Centre for Molecular and Macromolecular Science of the Polish Academy of Science in Łódź, Poland.

General procedure for the synthesis of ***2a–f***

Furfural (2.5 mmol, 0.24 g) was dissolved in methanol (15 ml) and to this solution aniline derivative **1a–f** (2.5 mmol) was added. The mixture was stirred at room temperature for 24 h, then solvent was evaporated to obtain almost pure Schiff base (**2a–f**).

N-Furfurylidene-o-anisidine ***2a*** *(Saito et al., 2001)* Quantitative yield (0.50 g), dark yellow oil.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.36 (s, CH=N, 1H), 7.63 (d, $J = 1.8$ Hz, H_5^{fur}, 1H), 7.21 (ddd, $J = 9.0$, 7.8 and 1.8 Hz, *o*-C_6H_4, 1H), 7.07 (dd, $J = 7.2$ and 1.8 Hz, *o*-C_6H_4, 1H), 6.98 (m, *o*-C_6H_4, H_3^{fur}, 3H), 6.57 (dd, $J = 3.6$ and 1.8 Hz, H_3^{fur}, 1H), 3.91 (s, OCH_3, 3H).

N-Furfurylidene-m-anisidine ***2b*** *(Kiepo and Jakopcic, 1985)* Quantitative yield (0.50 g), yellow oil

^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.32 (s, CH=N, 1H), 7.64 (d, $J = 1.8$ Hz, H_5^{fur}, 1H), 7.30 (dd, $J = 8.4$ and 7.2 Hz, *m*-C_6H_4, 1H), 6.98 (d, $J = 3.6$ Hz, H_3^{fur}, 1H), 6.86 (d, $J = 7.2$ Hz, *m*-C_6H_4, 1H), 6.84 (s, *m*-C_6H_4, 1H), 6.82 (d, $J = 7.2$ Hz, *m*-C_6H_4, 1H), 6.58 (dd, $J = 3.6$ and 1.8 Hz, H_3^{fur}, 1H), 3.83 (s, OCH_3, 3H).

N-Furfurylidene-p-anisidine ***2c*** Quantitative yield (0.50 g), mp = 60–64 °C (Ojima *et al.*, 1992), 68–70 °C.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.30 (s, CH=N, 1H), 7.59 (d, $J = 1.8$ Hz, H_5^{fur}, 1H), 7.25 (d, $J = 9.0$ Hz, *p*-C_6H_4, 2H), 6.92 (d, $J = 9.0$ Hz, *p*-C_6H_4, 2H), 6.91 (d, $J = 3.6$ Hz, H_3^{fur}, 1H), 6.54 (dd, $J = 3.6$ and 1.8 Hz, H_3^{fur}, 1H), 3.82 (s, OCH_3, 3H).

N-Furfurylidene-o-toluidine ***2d*** Quantitative yield (0.46 g), mp = 50–53 °C (Grammaticakis and Texier, 1971), 58 °C.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.15 (s, CH=N, 1H), 7.60 (dd, $J = 1.8$ and 0.6 Hz, H_5^{fur}, 1H), 7.20 (ddd, $J = 9.0$, 7.8 and 1.8 Hz, *o*-C_6H_4, 1H), 7.18 (dd, $J = 7.8$ and 1.8 Hz, *o*-C_6H_4, 1H), 7.11 (ddd, $J = 9.0$, 7.8 and 1.8 Hz, *o*-C_6H_4, 1H), 6.94 (dd, $J = 3.6$ and 0.6 Hz, H_3^{fur}, 1H), 6.89 (dd, $J = 9.0$ and 1.8 Hz, *o*-C_6H_4, 1H), 6.54 (dd, $J = 3.6$ and 1.8 Hz, H_3^{fur}, 1H), 2.38 (s, OCH_3, 3H).

N-Furfurylidene-m-toluidine ***2e*** *(Zubkov et al., 2004)* Quantitative yield (0.46 g), dark yellow oil.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.29 (s, CH=N, 1H), 7.61 (d, $J = 1.8$ Hz, H_5^{fur}, 1H), 7.27 (d, $J = 7.8$ Hz, *m*-C_6H_4, 1H), 7.07–7.04 (m, *m*-C_6H_4, 3H), 6.95 (d, $J = 3.6$ Hz, H_3^{fur}, 1H), 6.55 (dd, $J = 3.6$ and 1.8 Hz, H_3^{fur}, 1H), 2.38 (s, OCH_3, 3H).

N-Furfurylidene-p-toluidine ***2f*** Quantitative yield (0.46 g), mp = 35–38 °C (Kraicheva *et al.*, 2009), 41–42 °C.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.30 (s, CH=N, 1H), 7.59 (d, $J = 1.8$ Hz, H_5^{fur}, 1H), 7.19–7.15 (AA′BB′ system, $J = 9.0$ Hz, *p*-C_6H_4, 4H), 6.92 (d, $J = 3.6$ Hz, H_3^{fur}, 1H), 6.54 (dd, $J = 3.6$ and 1.8 Hz, H_3^{fur}, 1H), 2.36 (s, CH_3, 3H).

General procedure for the synthesis of (2-furyl)-arylaminomethylphosphonic acid esters ***4a–g***

A Schiff base **2a–f** (4.5 mmol) was dissolved in acetonitrile and then phosphite **3a–c** (4.5 mmol) was added followed by the addition of 2 drops of trifluoroacetic acid. The mixture was stirred at 80 °C during the day and at room temperature overnight. Reactions lasted for 72 h. Then, solvent was evaporated and crude products were chromatographed on silica gel (AcOEt–hexane 4:1) to give pure aminophosphonates (**4a–g**).

Dimethyl (2-furyl)-N-(2-methoxyphenyl)aminomethylphosphonate ***4a*** Y = 71 % (1.08 g), dark yellow oil.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.38 (d, $J = 1.8$ Hz, H_5^{fur}, 1H), 6.71 (m, *o*-C_6H_4, H_3^{fur}, 3H), 7.07 (dd, $J = 7.8$ and 1.8 Hz, *o*-C_6H_4, 1H), 6.39 (m, *o*-C_6H_4, 1H), 6.57 (dd, $J = 3.6$ and 1.8 Hz, H_4^{fur}, 1H), 5.04 (d, $J = 7.8$ Hz, NH, 1H), 4.94 (dd, $^{2}J_{PH} = 23.4$ Hz, $^{3}J_{HH} = 7.8$ Hz, CHP, 1H), 3.84 (s, OCH_3, 3H), 3.80 (d, $^{3}J_{PH} = 10.8$ Hz, $POCH_3$, 3H), 3.65 (d, $^{3}J_{PH} = 10.8$ Hz, $POCH_3$, 3H). ^{31}P NMR (243 MHz, $CDCl_3$): δ 22.60.

Elemental analysis: Calcd for $C_{14}H_{18}NO_5P$: C, 54.02, H, 5.83, N, 4.50. Found: C, 54.29, H, 5.68, N, 4.78.

Dimethyl (2-furyl)-N-(4-methoxyphenyl)aminomethylphosphonate ***4b*** Y = 63 % (0.98 g), dark yellow oil.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.38 (d, $J = 1.8$ Hz, H_5^{fur}, 1H), 6.73 (d, $J = 9.0$ Hz, *p*-C_6H_4, 2H), 6.62 (d, $J = 9.0$ Hz, *p*-C_6H_4, 2H), 6.35 (d, $J = 3.6$ Hz, H_3^{fur}, 1H), 6.31 (dd, $J = 3.6$ and 1.8 Hz, H_3^{fur}, 1H), 4.94 (dd, $^{2}J_{PH} = 24.0$ Hz, $^{3}J_{HH} = 8.4$ Hz, CHP, 1H), 4.54 (d, $J = 8.4$ Hz, NH, 1H), 3.81 (d, $^{3}J_{PH} = 10.8$ Hz, $POCH_3$, 3H), 3.70 (s, OCH_3, 3H), 3.62 (d, $^{3}J_{PH} = 10.8$ Hz, $POCH_3$, 3H). ^{31}P NMR (243 MHz, $CDCl_3$): δ 22.76.

Elemental analysis: Calcd for $C_{14}H_{18}NO_5P$: C, 54.02, H, 5.83, N, 4.50. Found: C, 54.35, H, 5.98, N, 4.80.

Diphenyl (2-furyl)-N-(4-methoxyphenyl)aminomethylphosphonate ***4c*** Y = 96 % (2.08 g), mp = 77–79 °C.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.36 (m, H_5^{fur}, 1H), 7.29–7.24 (m, PhH, 4H), 7.22 (t, $J = 7.8$ Hz, PhH, 1H),

7.16 (t, $J = 7.8$ Hz, PhH, 1H), 7.14–7.11 (m, PhH, 2H), 7.02 (dd, $^3J_{HH} = 7.8$ Hz and $^4J_{HH} = 0.6$ Hz, PhH, 2H), 6.75 and 6.66 (AA′XX′ system, $J = 9.0$ and 2.4 Hz, *p*-C_6H_4, 4H), 6.44 (m, H_3^{fur}, 1H), 6.32 (t, $^3J_{HH} = 2.4$ Hz, H_4^{fur}, 1H), 5.15 (d, $^2J_{PH} = 24.0$ Hz, CHP, 1H), 3.72 (s, OCH_3, 3H). ^{31}P NMR (243 MHz, $CDCl_3$): δ 12.73.

Elemental analysis: Calcd for $C_{24}H_{22}NO_5P$: C, 66.20, H, 5.09, N, 3.22. Found: C, 66.15, H, 4.95, N, 3.27.

Diphenyl (2-furyl)-N-(2-methylphenyl)aminomethylphosphonate ***4d*** Y = 99 % (2.07 g), dark yellow oil.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.42 (dd, $^3J_{HH} = 1.8$ Hz and $^4J_{HH} = 0.6$ Hz, H_5^{fur}, 1H), 7.32–7.28 (m, PhH, 4H), 7.20–7.15 (m, PhH, 4H), 7.11–7.09 (m, *o*-C_6H_4, 2H), 7.07–7.05 (m, PhH, 2H), 6.76 (ddd, $^3J_{HH} = 7.8$ and 8.4 and $^4J_{HH} = 0.6$ Hz, *o*-C_6H_4, 1H), 6.69 (d, $J = 7.8$ Hz, *o*-C_6H_4, 1H), 6.49 (m, H_3^{fur}, 1H), 6.36 (dd, $^3J_{HH} = 3.6$ and 1.8 Hz, H_4^{fur}, 1H), 5.33 (dd, $^2J_{PH} = 24.6$ and $^3J_{HH} = 3.0$ Hz, CHP, 1H), 4.47 (large s, NH, 1H), 2.19 (s, CH_3, 3H). ^{31}P NMR (243 MHz, $CDCl_3$): δ 12.55.

Elemental analysis: Calcd for $C_{24}H_{22}NO_4P$: C, 68.73, H, 5.29, N, 3.34. Found: C, 68.48, H, 5.02, N, 3.55.

Diphenyl (2-furyl)-N-(3-methylphenyl)aminomethylphosphonate ***4e*** Y = 96 % (2.10 g), mp = 36–38 °C.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.37 (m, H_5^{fur}, 1H), 7.29–7.22 (m, PhH, 4H), 7.17–7.10 (m, PhH, 4H), 7.06 (t, $^3J_{HH} = 7.8$ Hz, *m*-C_6H_4, 1H), 7.03–7.01 (m, PhH, 2H), 6.61 (d, $^3J_{HH} = 7.2$ Hz, *m*-C_6H_4, 1H), 6.52 (m, *m*-C_6H_4, 2H), 6.46 (d, $^3J_{HH} = 3.6$ Hz, H_3^{fur}, 1H), 6.32 (m, H_4^{fur}, 1H), 5.28 (d, $^2J_{PH} = 24.0$ Hz, CHP, 1H), 3.81 (large s, NH, 1H), 2.53 (s, CH_3, 3H). ^{31}P NMR (243 MHz, $CDCl_3$): δ 12.61.

Elemental analysis: Calcd for $C_{24}H_{22}NO_4P \bullet {}^2/_3CH_2Cl_2$: C, 62.24, H, 4.94, N, 2.94. Found: C, 62.13, H, 5.11, N, 3.63.

Diphenyl (2-furyl)-N-(4-methylphenyl)aminomethylphosphonate ***4f*** Y = 99 % (2.08 g), mp = 118–119 °C.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.38 (dd, $^3J_{HH} = 1.8$ Hz and $^4J_{HH} = 0.6$ Hz, H_5^{fur}, 1H), 7.29–7.25 (m, PhH, 4H), 7.18–7.12 (m, PhH, 4H), 7.03–7.01 (m, PhH, 2H), 6.98 (d, $J = 9.0$ Hz, *p*-C_6H_4, 2H), 6.62 (d, $J = 9.0$ Hz, *p*-C_6H_4, 2H), 6.44 (m, H_3^{fur}, 1H), 6.32 (m, H_4^{fur}, 1H), 5.22 (d, $^2J_{PH} = 24.6$ Hz, CHP, 1H), 4.47 (large s, NH, 1H), 2.23 (s, CH_3, 3H). ^{31}P NMR (243 MHz, $CDCl_3$): δ 12.70.

Elemental analysis: Calcd for $C_{24}H_{22}NO_4P$: C, 68.73, H, 5.29, N, 3.34. Found: C, 69.03, H, 5.32, N, 3.58.

Dibenzyl (2-furyl)-N-(3-methoxyphenyl)aminomethylphosphonate ***4g*** Y = 98 % (2.12 g), mp = 42–44 °C.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.36 (m, PhH, 1H), 7.29 (m, PhH, H_5^{fur}, 9H), 7.21 (m, PhH, 1H), 7.03 (t, $^3J_{HH} = 7.8$ Hz, *m*-C_6H_4, 1H), 6.36 (d, $^3J_{HH} = 3.6$ Hz, H_3^{fur}, 1H), 6.32 (d, $^4J_{HH} = 3.6$ Hz, *m*-C_6H_4, 1H), 6.30 (dd, $^3J_{HH} = 7.8$ Hz and $^4J_{HH} = 3.6$ Hz, *m*-C_6H_4, 1H), 6.22 (dd, $^3J_{HH} = 7.8$ Hz and $^4J_{HH} = 3.6$ Hz, *m*-C_6H_4, 1H), 6.16 (m, H_4^{fur}, 1H), 5.07 (part of ABX system, $^2J_{HH} = 12.0$ Hz, $^3J_{PH} = 9.0$ and 7.8 Hz, OCH_2Ph, 2H), 4.97 and 4.79 (part of AMX system, $^2J_{HH} = 12.0$ Hz, $^3J_{PH} = 8.7$ and 7.8 Hz, OCH_2Ph, 2H), 4.91 (d, $^2J_{PH} = 24.0$ Hz and $^3J_{HH} = 9$ Hz, CHP, 1H), 4.49 (d, $J = 9.0$ Hz, NH, 1H), 3.71 (s, OCH_3, 3H). ^{31}P NMR (243 MHz, $CDCl_3$): δ 21.67.

Elemental analysis: Calcd for $C_{26}H_{26}NO_5P$: C, 67.38, H, 5.65, N, 3.02. Found: C, 67.48, H, 5.77, N, 3.02.

Biological assays

Cell line and culture

The esophageal squamous cancer cell lines KYSE 30, KYSE 150, and KYSE 270 were obtained from the European Collection of Cell Cultures (ECACC, UK). Cells were grown in RPMI 1640 and Ham F12 in 1:1 ratio with 2 mM L-glutamine and 2 % fetal bovine serum (FBS). Het-1A (SV-40 immortalized human esophageal epithelial cell line) was used as control. This cell line was obtained from American Type Culture Collection (ATCC, USA) and was cultured in Quantum 286. Cells were incubated in standard conditions (37 °C, v/v 5 % CO_2, relative humidity 100 %) without antibiotics.

Materials

All mediums and chemicals for cell growth were bought from PAA. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were from Sigma Aldrich. Tested compounds were freshly prepared in DMSO and diluted in medium with the final concentration lower than 0.5 %.

MTT assay

The cell cytotoxicity was measured with the MTT assay (Mosmann, 1983; Sieuwerts *et al.*, 1995). 100 µl of a 10,000 cells/ml solution was added to each well of the 96-well plate. The plate was incubated at 37 °C in the dark. After 24 h, the cells were incubated for another day with eight different chemical concentrations. Wells on the edge of the plate were filled with 100 µl of phosphate-buffered saline (PBS). After incubation, the wells were washed with PBS and 100 µl 0.5 mg/ml MTT solution was added for 3 h. Afterward the medium with MTT was removed, and 100 µl of pure DMSO was added. After 20 min, a colorimetric reading was made with 580-nm light. Each experiment repeat was performed on a different day. There were at least three repeats of each experiment. IC_{50} was

calculated based on the control without chemicals added. An additional control of medium with 1 % DMSO was used to test the solvent.

Statistical analysis

To analyze cytotoxicity, IC_{50} values were used. All experiments were examined by non-liner regression analysis (GraphPad Prism Software). A probability level of 0.05 was used for statistical validity.

Acknowledgments Authors wish to express special thanks to Mr Jan Bitner from the University of Łódź for his great assistance in preparation of the final version of this manuscript.

References

Bernard W, Stewart PK (2003) World cancer report. IARC Press, International Agency for Research on Cancer, Lyon

Bosshard P, Eugster CH (1967) The development of the chemistry of furans. Adv Heterocycl Chem 7:377–478

Boykin DW, Kumar A, Xiao G, Wilson WD, Bender BC, Curdy DR, Hall JE, Tidwell RR (1998) 2,5-Bis[4-(*N*-alkylamidino)phenyl] furans as anti-*Pneumocystis carinii* agents. J Med Chem 41: 124–129

Bradshaw J, Butcher ME, Clitherow JW, Dowle MD, Hayes R, Judd DB, McKinnon JM, Price BJ (1981) Aminoalkylfurans as H2-receptor antagonist. In: Creighton AM, Turner S (eds) The chemical regulation of biological mechanisms. The Royal Society of Chemistry, London

Chandrasekhar S, Jaya Prakash S, Jagadeshwar V, Narsihmulu C (2001) Three component coupling catalyzed by $TaCl_5$–SiO_2: synthesis of α-amino phosphonates. Tetrahedron Lett 42:5561–5563

Chiu PK (2008) Characterization of the anti-cancer effect of *Gleditsia sinensis* in esophageal squamous cell carcinoma. Dissertation, Hong Kong Polytechnic University

Coleman MP, Esteve J, Damiecki P et al (1993) Trends in cancer incidence and mortality. IARC Press, International Agency for Research on Cancer, Lyon

Cottier L, Descotes G, Gonera G, Grabowski G, Lewkowski J, Skowroński R (1996a) Synthesis of 1-aminoalkanephosphonic acid derivatives containing furan moiety. Part II: First synthesis of (2-furyl)aminomethane phosphonic acid. Phosphorus Sulfur Silicon 118:181–188

Cottier L, Descotes G, Lewkowski J, Skowroński R (1996b) Synthesis and its stereochemistry of aminophosphonic acids derived from 5-hydroxymethylfurfural. Phosphorus Sulfur Silicon 116:93–100

Fields EK (1952) The synthesis of esters of substituted amino phosphonic acids. J Am Chem Soc 74:1528–1531

Grammaticakis P, Texier H (1971) Contributions a l'etude de l'absorption dans l'ultraviolet moyen et le visible de derives fonctionnels azotes de quelques aldehydes et cetones aromatiques. Bull Soc Chim Fr 38:1323–1330

Hou Z, Sang S, You H, Lee M-J, Hong J, Chin K-V, Yang CS (2005) Mechanism of action of (–)-epigallocatechin-3-gallate: auto-oxidation-dependent inactivation of epidermal growth factor receptor and direct effects on growth inhibition in human esophageal cancer KYSE 150 cells. Cancer Res 65:8049–8056

Ji J, Wu K, Wu M, Zhan Q (2010) p53 functional activation is independent of its genotype in five esophageal squamous cell carcinoma cell lines. Front Med Chin 4:412–418

Kafarski P, Lejczak B (1991) Biological activity of aminophosphonic acids. Phosphorus Sulfur 63:193–215

Kafarski P, Lejczak B (2001) Aminophosphonic acids of potential medical importance. Curr Med Chem Anti-Cancer Agents 1:301–312

Keay BA, Dibble PW (1996) Furans and their benzo derivatives: applications. In: Katritzky AR, Rees CW, Scriven EFV (eds) Comprehensive heterocyclic chemistry 2, vol 2, chap 2.08. Pergamon Press, Oxford, pp 395–436

Kiepo Z, Jakopcic K (1985) Studies in the furan series. 22. *N*-Arylfurfuryl- and *N*-aryl-5-methylfurfurylamines and their *N*-allyl derivatives. J Chem Eng Data 30:235–237

Kordek R, Jassem J, Jeziorski A, Kornafel J, Krzakowski M, Pawlęga J (2007) Onkologia podręcznik dla studentów i lekarzy. Via Medica, Gdańsk

Kraicheva I, Bogomilova A, Tsacheva I, Momekov G, Troev K (2009) Synthesis, NMR characterization and in vitro antitumor evaluation of new aminophosphonic acid diesters. Eur J Med Chem 44:3363–3367

Lewkowski J, Rzeźniczak M, Skowroński R (2000) Synthesis of new furan-derived *N*-substituted aminomethanephosphonates. Org Prep Proced Int 32:453–460

Maier L (1999) What are the requirements in the glyphosate molecule in order to be herbicidally active? Phosphorus Sulfur Silicon 144–146:429–432

Mastalerz P, Kafarski P (2000) Naturally occurring aminophosphonic and aminophosphinic acids. In: Kukhar VP, Hudson HR (eds) Aminophosphonic and aminophosphinic acids. Wiley, New York, pp 1–31

Matveeva ED, Podrugina TA, Prisyajnoy MV, Zefirov NS (2006) Ketones in the catalytic three-component "one-pot" Kabachnik-Fields synthesis of α-amino phosphonates. Russ Chem Bull Int Ed 55:1209–1214

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Immunol J Methods 65:55–63

Muñoz N (1993) Epidemiological aspects of oesophageal cancer. Endoscopy 25:609–612

Ojima I, Habus I, Zhao M, Zucco M, Park YH, Sun CM, Brigaud T (1992) New and efficient approaches to the semisynthesis of taxol and its C-13 side chain analogs by means of β-lactam synthon method. Tetrahedron 48:6985–7012

Reed PI, Johnston BJ (1993) The changing incidence of oesophageal cancer. Endoscopy 25:606–608

Saito S, Hatanaka K, Yamamoto H (2001) Asymmetric Mannich-type reactions with a chiral acetate: effect of Lewis acid on activation of aldimine. Tetrahedron 57:875–887

Saito JB, Egami H, Katsuki T (2007) Synthesis of an optically active Al(salalen) complex and its application to catalytic hydrophosphonylation of aldehydes and aldimines. J Am Chem Soc 129: 1978–1986

Sieuwerts A, Klijn J, Peters H, Foekens J (1995) The MTT tetrazolium salt assay scrutinized: how to use this assay reliably to measure metabolic activity of cell cultures in vitro for the assessment of growth characteristics, IC50-values and cell survival. Eur J Clin Chem Clin Biochem 33:813–823

Tanaka H, Shimada Y, Harada H, Shinoda M, Hatooka S, Imamura M, Ishizaki K (1998) Methylation of the 5′ CpG island of the FHIT gene is closely associated with transcriptional inactivation in esophageal squamous cell carcinomas. Cancer Res 58: 3429–3434

Tanaka E, Hashimoto Y, Ito T, Kondo K, Higashiyama M, Tsunoda S, Ortiz C, Sakai Y, Inazawa J, Shimada (2007) The suppression of aurora-A/STK15/BTAK expression enhances chemosensitivity to docetaxel in human esophageal squamous cell carcinoma. Clin Cancer Res 13:1331–1340

The Merck Index (2001) Merck & Co. Inc., New York, pp 848–851
Wu J, Sun W, Xia HG, Sun X (2006) A facile and highly efficient route to α-amino phosphonates via three-component reactions catalyzed by $Mg(ClO_4)_2$ or molecular iodine. Org Biomol Chem 4:1663–1666
Zubkov FI, Boltukhina EV, Nikitina EV, Varlamov AV (2004) Study of regioselectivity of intramolecular cyclization of *N*-(*m*-R-phenyl)- and *N*-(α-naphthyl)-2-allyl(methallyl)-6-carboxy-4-oxo-3-aza-10-oxatricyclo[5.2.1.$0^{1,5}$]dec-8-enes. Russ Chem Bull Int Ed 53:2816–2829